Cuic – H067.18 – 195 – R p326

NATURE

A WEEKLY JOURNAL OF SCIENCE

"To the solid ground
Of nature trusts the Mind that builds for aye"—Wordsworth

195

Volume 191

JULY 1, 1961, to SEPTEMBER 30, 1961

LONDON
MACMILLAN AND CO., LTD.
NEW YORK: ST. MARTIN'S PRESS, INC.

DATES and PAGINATION of WEEKLY ISSUES

Serial No.		*Date of issue*		*Pagination*
4783	...	July	1	1–102
4784	...	,,	8	103–204
4785	...	,,	15	205–310
4786	...	,,	22	311–420
4787	...	,,	29	421–526
4788	...	August	5	527–630
4789	...	,,	12	631–736
4790	...	,,	19	737–836
4791	...	,,	26	837–942
4792	...	September	2	943–1026
4793	...	,,	9	1027–1128
4794	...	,,	16	1129–1228
4795	...	,,	23	1229–1330
4796	...	,,	30	1331–1422

RB326

INDEX

AARONS, Dr. Jules, and others : Very-Low-Frequency Modulation of Discrete Frequency Solar Noise Bursts, 56
Abel-Smith, Dr. Brian : Appointed to the University of London readership in social administration at the London School of Economics and Political Science, 651
Ability (Regenerative) : Adaptive Value of (Dr. A. E. Needham), 720
Ability (Swimming), of Non-aquatic Reptiles (R. R. Tercafs), 125
Abnormal Conditions : Electrical Activity of a Giant Nerve Cell under (Dr. Yutaka Oomura, Sachio Ozaki and Takashi Maéno), 1265
Abnormal Foetal Haemoglobins (Prof. E. Silvestroni and I. Bianco), 397
Abnormal Form of Female Rat Flea, *Xenopsylla cheopis* Roths. (M. I. D. Sharma and G. C. Joshi), 727
Abnormal Human Haemoglobins : Thalassaemia and the (Dr. Harvey A. Itano and Prof. Linus Pauling), 398
Abood, Dr. L. G., and others : Distribution of Piperidine in the Brain and its Possible Significance in Behaviour, 201
  Outflux of Inorganic and Organic Phosphate during Membrane Depolarization of Excitable Tissues, 395
Aborigines (Groote Eylandt) : Classification of Kin, Age Structure and Marriage amongst the (Dr. Frederick G. G. Rose), *review* by Dr. R. Needham, 6
Abrahamson, Seymour : Chromosome Rearrangements induced by X-Rays in Immature Germ Cells of *Drosophila*, 523
Absence of Lysogeny in *Pasteurella pestis* (Dr. D. A. Smith), 522
Absence (Virtual), of Glutathione Instability of the Erythrocytes among Armenians in Iran (Dr. J. E. Bowman and Dr. D. G. Walker), 221
Absorbing Object (Homogeneously) : The Feulgen-Stained Somatic Cell Nucleus as a (Dr. Simon Iversen), 150
Absorption (Infra-Red) : Quantitative Analysis of Milk and other Emulsions by (Dr. J. D. S. Goulden), 905
Absorption (Intestinal) : Enhancement of Inhibition of, by Cetrimide–Phloridzin Combination (J. A. Nissim), 37
Absorption (Ultrasonic), of Polymer Solutions (Dr. G. Gooberman), 693
Absorption Correction : Elimination of the, in the Viscometry of Dilute Solutions of High Polymers (H. van Oene and Prof. L. H. Cragg), 1160
Absorption Heights (Ionospheric) : A Method of Estimating, from Recovery Times (Syed Ziauddin), 1084
Absorption and Distribution of Calcium-45 in the Rat : Effect of Serotonin on (Prof. S. Garattini, E. Grossi, P. Paoletti, R. Paoletti and M. Poggi), 185
Absorption Bands of Lead Monoxide : Rotational Analysis of (Dr. R. F. Barrow, J. L. Deutsch and D. N. Travis), 374
Absorption Spectra of γ-Irradiated Lead Silicate Glass (R. S. Barker, T. K. Harrison, D. A. Richardson and R. Rimmer), 374
Absorption Spectra (Ultra-violet), of 3,4-Benzopyrene : Effect of Ozone on the Fluorescence and (Z. Morlin and K. M. Sáringer), 907
Absorption Spectra (Ultra-violet), of Proteins in Deuterium Oxide (Dr. G. H. Beaven and Elizabeth Bailey), 914
Absorption Spectroscopy (Atomic) : Determination of Lead in Urine by (J. B. Willis), 381
Absorptions (Electron Spin Resonance), induced by Light in Adsorbed Layers of Cyanine Dyes in Silver Bromide Microcrystals (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Abstract Set Theory (Prof. Abraham Fraenkel), second edition, *review* by Dr. R. G. Cooke, (June 17, 1047), *erratum*, 126
Abstracts : Gas Chromatography, 1958 (edited by C. E. H. Knapman, assisted by C. G. Scott), *review* by A. Goldup, 1135
  Gas Chromatography, 1959 (edited by C. E. H. Knapman, assisted by C. G. Scott), *review* by A. Goldup, 1135
Abstracts of Efficiency in the Hospital Service (Ministry of Health), 441
Abstracts (Biological and Medical), Ltd. : Publication of *Index Biologicus*, 544
Abstracts (Technical Education), from British Sources (National Foundation for Educational Research in England and Wales), Vol. 1, No. 1, September–December, 1960, 21
Academy of Sciences of the U.S.S.R. : Mathematical Tables, Vol. 12, A Collection of Tables and Nomograms for the Processing of Observations made on Artificial Earth Satellites (I. D. Zhongolovich and V. M. Amelin), *review* by D. G. King-Hele, 1135
Acarapis woodi (Rennie) : Distribution of (Prof. J. E. Eckert), 933
Acaricidal Properties of Di-(*p*-chloro-phenyl)-trifluoromethylcarbinol (K. R. S. Ascher and R. Cwilich), 1322
Accelerators (Electron Linear) : Pulse-shortening in (Dr. M. C. Crowley-Milling, T. R. Jarvis, C. W. Miller and G. Saxon), 483
Acceptance Sampling : Statistical Quality Control and (Birmingham College of Advanced Technology Course on), 337
Acceptor Molecule : Electron Affinity of the, in Charge-Transfer Complexes (Dr. M. E. Peover), 702
Accessory Respirator Organs in Air-breathing Fishes : A Hydrostatic Function of the (Dr. S. Z. Qasim and R. Hasan), 396
Accretion (Terrestrial), from the Solar Wind (Dr. Ronald Frith), 1182 ; (Dr. E. R. Harrison), 1182 ; (C. M. de Turville), 1183
Acetate : Synthesis of Succinate from (D. D. Davies), 1093
Acetate (Cellulose) Membranes : Use of, for Detecting Penicillinase-producing Organisms (Prof. R. Knox and J. T. Smith), 926
Acetic Acid (Glacial) : Use of, for Killing Parasitic Nematodes for Collection Purposes (Bjørn Berland), 1320
Acetone in the Blood and the Exhaled Air of Diabetic Patients : Rapid Infra-Red Determination of (Dr. Richard S. Stewart, Edward A. Boettner and Bruce T. Stubbs), 1008
Acheson, R. M. : An Introduction to the Chemistry of Heterocyclic Compounds, *review*, 320
Achievements in Physics, *review* by Prof. W. E. Burcham, 423
Achievements (The Major), of Science (A. E. E. McKenzie), *review* by J. M. Eyles, 110
Acid : Thyroglobulin—Effect of (Dr. A. D. Litonjua), 356
Acid (Mild), and Alkaline Hydrolysis of *M*- and *N*-Blood Group Substances: Products of (Dr. Elzbieta Romanowska), 1408
Acid Buffers : Solubility of Denatured Haemoglobin Variants in (I. D. Fraser and Dr. A. B. Raper), 355
Acid-Hydrolysis (Partial), of a Mucopolysaccharide without Appreciable *N*-Deacetylation of Hexosamine (Dr. T. J. Painter and Prof. W. T. J. Morgan), 39
Acid Mucopolysaccharide in Cockroach Ganglia (Doreen E. Ashhurst), 1224
Acid–Sulphate–Chloride Waters : Hot Spring Areas with (Dr. A. J. Ellis and S. H. Wilson), 696
Acid-stable Penicillins : Some Novel (F. P. Doyle, J. H. C. Nayler, H. Smith and E. R. Stove), 1091
Acoustical Training : A Take-Over Bid in (Prof. E. J. Richards), 748
Acoustics (Underwater) : Advanced Study Institute on (Imperial College of Science and Technology, London), 229
N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds : N,N'-Alkylene-*bis*(Acrylamides),—a New Series of Anti-tumour Agents (A. S. Tomcufcik, S. D. Willson, A. W. Vogel and A. Sloboda), 611
Actinomyces : Manual of Bacteria and, *review* by Dr. K. Zinnemann, 1333
Actinomycete : Prodigiosin in an (Jerome J. Perry), 77
Actinomyceten : Diagnostik der Bakterien und (Prof. N. A. Krassilnikov), *review* by Dr. K. Zinnemann, 1333
Action of Antigen–Antibody Complexes : Mechanism of—their Effect on Mast Cells (Dr. Ivan Mota), 572
Action of Calcium Chloride on Mortar and Concrete (M. Lawrence and H. E. Vivian), 125, *erratum*, 1145
Action of Carbon Dioxide on 6-Aminopenicillanic Acid (F. R. Batchelor, D. Gazzard and J. H. C. Nayler), 910
Action of the Hypothalamus (John Erskine Malcolm), 606
Action of Influenza Viruses, Receptor-destroying Enzyme and Proteases on Blood Group Agglutinogen M^g. (Prof. Georg F. Springer and Dr. Karlheinz Stalder), 187
Action of Neuraminidase on Haptoglobin (Prof. Max E. Rafelson, jun., Lise Cloarec, Jean Moretti and Max F. Jayle), 279
Action of Plasmin in Cartilage Matrix *in vivo* (Dr. C. H. Lack, A. J. Anderson and S. Y. Ali), 1402
Action of the Sucker of *Echeneis* : Structure and (Prof. B. Bonnell), 403
Action of Tetracycline on the Level of Properdin (Vl. Kubelka and M. Rybák), 820
Action (Auxin) : Improbability of the Chelation Hypothesis of (Dr. D. D. Perrin), 253
Action (Epinephrine) : Potentiation of (Prof. R. Imaizumi, Y. Hashimoto and M. Oka), 186
Action (Mutagenic), of the Alkylating Agents : Specificity of the (Prof. Bernard S. Strauss), 730
Action (Thyroid Protease) : Thyroglobulin—Evidence of (Dr. A. D. Litonjua), 357
Action Potential in Cells of *Chara* (A. B. Hope), 811
Actions (Potent), on Mammalian Neurones : Analogues of Glutamic and γ-Amino-*n*-butyric Acids having (Dr. J. C. Watkins and Dr. D. R. Curtis), 1010
Activation of Amino-Acids in Micro-organisms which produce Antibiotics (Dr. O. Ciferri, Dr. M. Di Girolamo and Dr. A. Bendicenti Di Girolamo), 411
Activation (Amino-Acid), in Tissues of Early Embryos (Dr. Elizabeth M. Deuchar), 1006
Activation (Neutron) : Rapid Determination of Ytterbium in Aqueous Solutions and in Thulium Oxide by (Minoru Okada), 1090
Active Cation Transport as a Pace-maker of Respiration (Dr. R. Whittam), 603
Active Components (Biologically), from Commercial Trypan Blue : Chromatographic Separation of (J. Dijkstra and J. Gillman), 803
Active Muscle Fibre : Ionic Currents in Membrane of (Dr. H. Jenerick), 1074
Active Substances (Pharmacologically), present in the Central Nervous System (Dr. Paul V. Buday), 245
Active Trisaccharides (Two Serologically), isolated from Human Blood-Group *A* Substance (I. A. F. Lister Cheese and Prof. W. T. J. Morgan), 149
Active, Sleeping and Torpid Hummingbirds : Thyroid Function in (Prof. C. J. Shellabarger, R. C. Lasiewski and G. E. Hyncik), 1318
Activities (Biological), of Steroids in relation to Cancer—Proceedings of a Conference sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, United States Department of Health, Education and Welfare (edited by Gregory Pincus and Erwin P. Vollmer), *review* by E. Reid, 1235
Activity of Enzymes of the Urea Cycle in Rat Liver : Effect of Adrenalectomy on the (Patricia McLean), 1302
Activity of Glutamic Acid Decarboxylase in Insect Nerve Tissue (Dr. N. Frontali), 178
Activity of Nitrate-Reductase in *Aerobacter aerogenes* : Inhibition by Oxygen of Biosynthesis and (Francis Pichinoty and Liliane D'Ornano), 879

Activity of the Reticulo-Endothelial System : Sensitivity of Mice to Bacterial Lipopolysaccharide following Alteration of (Dr. G. N. Cooper and Dr. A. E. Stuart), 294
Activity (Anthelmintic) : Pyridine Derivatives with (A. P. Phillips and R. B. Burrows), 707
Activity (L-Asparaginase), of Guinea Pig Serum : Evidence that the, is responsible for its Antilymphoma Effects (J. D. Broome), 1114
Activity (Biological), of a Tri-o-Cresyl Phosphate Metabolite (Prof. John E. Casida, Morifusa Eto and Ronald L. Baron), 1396
Activity (Circulating Thyrotrophic) : Increase of the Rate of Release of Thyroidal Iodine-131 and of, at Early Stages of Propylthiouracil Treatment in the Rat (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171
Activity (Electrical), of a Giant Nerve Cell under Abnormal Conditions (Dr. Yutaka Oomura, Sachio Ozaki and Takashi Maéno), 1265
Activity (Enzymic), of the Central Nervous System (Neurochemical Commission of the World Federation of Neurology International Symposium on), 1349
Activity (Glucose-6-phosphatase), in Liver and Kidney at Birth : Changes in (M. J. R. Dawkins), 72
Activity (Indoleacetic Acid Oxidase), of *Lupinus albus* L. exposed to Different Photoperiods : Effect of Gibberellic Acid on Growth and (F. Sági and A. S. Garay), 824
Activity (Mechanical), of Muscle and that of Blood Coagulation : Controlling Mechanism of the (Dr. Masahiro Fujino, Saburo Yamaguchi, Masa Takauji, Kohki Uchida, Masahide Yasuda and Tatsuaki Matsushima), 80
Activity (Mitochondrial α-Glycerophosphate Dehydrogenase), in Skeletal Muscle : Direct Relationship of Phosphorylase and (Dr. A. G. E. Pearse), 504
Activity (Oxytocinase), of Plasma of Pregnant Women (Stig E. J. Melander), 176
Activity (Phagocytic), of the Reticulo-Endothelial System of Mice : Effect of Added Vitamins on the (J. Cordingley and Prof. T. Nicol), 82
Activity (Thromboplastic), of Red Cells (Dr. James A. Inglis and Dr. June W. Halliday), 821
Activity (Transaminase), in the Cactus Plant : Diurnal Variation in (S. K. Mukerji, G. G. Sanwal and Prof. P. S. Krishnan), 516
Activity (Uterine) : Effect of Bradykinin on (Dr. B. Berde and Dr. K. Saameli), 83
β-Activity (Gross), in the Thames River (Conn.), (D. M. Skauen, T. Hatfield, D. Hanlon and J. S. Rankin, jun.), 1017
Acute Experimental Uraemia : Elimination of Bovine Serum Albumin in (Dr. G. Gowland, F. G. Smiddy, Dr. R. G. Burwell and F. M. Parsons), 1210
Acute Myeloid Leukaemia (Transplantable) : Effect of Calf Thymus Histone on Rats bearing (Dr. Lubomir Hnilica and Dr. Viktor Holoubek), 922
Adam, Dr. H. M. : Appointed reader in pharmacology at the University of Edinburgh, 443
Adaptive Value of Regenerative Ability (Dr. A. E. Needham), 720
Added Vitamins : Effect of, on the Phagocytic Activity of the Reticulo-Endothelial System of Mice (J. Cordingley and Prof. T. Nicol), 82
Addison, Prof. C. C. : Inorganic Polymers, 1047
Additional Anaphylactic Site : Pinnal Anaphylaxis, an (Dr. J. G. Feinberg), 712
Adductor Muscles ('Quick' and 'Catch'), in Brachiopods (Dr. M. J. S. Rudwick), 1021
Adducts of Urea and Thiourea obtained by means of a Sublimation Technique (B. Casu), 802
Adelberg, Edward A. (selected by) : Papers on Bacterial Genetics, *review* by L. B. Quesnel, 320
Adenine from Hydrogen Cyanide : Mechanism of Synthesis of, under Possible Primitive Earth Conditions (Prof. J. Oró), 1193
Adenosine Triphosphate : Electron Spin Resonance of (D. B. Russell and S. J. Wyard), 65
Adenosine Triphosphate Synthesis in Erythrocytes : A Positive Feedback Mechanism of (Makoto Nakao, Takashi Motegi, Toshiko Nakao, Saburo Yamazoe and Haruhisa Yoshikawa), 283
Adhikari, S., and others : Lipids of the Common Pea (*Pisum sativum* L.) with special reference to the Occurrence of Galactolipids, 1301
Adjuvants : Effectiveness of Lipid and Lipidophilic Substances as (D. W. Dresser), 1169
Adler, Saul : Immunity to Protozoa (Immunity in Leishmaniasis), 552
Administration of Endotoxin : Increase in Specific Bactericidal Antibodies after (J. Gabriel Michael, James L. Whitby and Maurice Landy), 296
Administration of Lysergic Acid Diethylamide : Effect of, on Serotonin-Levels in the Body (Dr. D. V. Siva Sankar, D. Barnara Sankar, Edward Phipps and Eleanor Gold), 499
Administration (Oral), of D-Glucosaccharo-1,4-Lactone : Inhibition of β-Glucuronidase by (S. Akamatsu, Dr. A. Kiyomoto, S. Harigaya and S. Ohshima), 1298
Administration (Parenteral), of the Vitamin : Metabolites of Vitamin B_{12} in Bile after (Dr. John B. Stokes), 807
Adrenalectomy : Effect of, on the Activity of Enzymes of the Urea Cycle in Rat Liver (Patricia McLean), 1302
Adrenalectomy and Corticosteroids : Effect of, on Distribution of Radioactivity in Protein of Cell Fractions from Myocardial Slices (Dr. Edward I. Weinshelbaum and Dr. Ira G. Wool), 1401
Adrenergic Neurone : The (Sir Lindor Brown), 1143
Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine (A. L. A. Boura, F. C. Copp, A. F. Green, H. F. Hodson, G. K. Ruffell, M. F. Sim, E. Walton and E. M. Grivsky), 1312
Adsorbed Layers of Cyanine Dyes on Silver Bromide Microcrystals : Electron Spin Resonance Absorptions induced by Light in (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Adsorbents of High Surface Area : Measurements of Heats of Adsorption of Organic Vapours on, using a Continuous Flow Calorimeter (G. H. Bell and A. J. Groszek), 1184
Adsorption (Heats of), of Organic Vapours on Adsorbents of High Surface Area using a Continuous Flow Calorimeter : Measurements of (G. H. Bell and A. J. Groszek), 1184
Adsorption Studies by Electron Bombardment : Evaporated Carbon Films of Large Area for (A. E. Bell, J. Pritchard and Prof. K. W. Sykes), 487
Adult Birds : Comparison of the Serum Protein Fractions of the Newly Hatched Chick with those of, using Starch-gel Electrophoresis (A. Amin), 703
Adult Education, Universities Council for : Report for 1959-60, 861
Adult Rats (New-born and) : Collagen Fractions in the Skin of (Nestor Gonzalez Cadavid, Berta Denduchis and Roberto E. Mancini), 1297
Adult Rats (Normal) : Stimulation by Heparin of Parenchymal Liver Cell Proliferation in (Dr. M. Zimmerman and Evemarie Celozzi), 1014
Advanced Research Projects Agency, and others (sponsored by the) : A Symposium on Space Trajectories (edited by the Technical Staff, Research Division, Radiation Incorporated), *review* by D. G. King-Hele, 1334
Advanced Study Institute Programme, 1962 (NATO), 442
Advanced Study Institute on Underwater Acoustics at the Imperial College of Science and Technology, London, 229
Advanced Technology, Birmingham College of : Course on Statistical Quality Control and Acceptance Sampling, 337
Advanced Technology, Welsh College of (Dr. A. Harvey), 127
Advanced Technology in Britain : Control of Colleges of, 20
Advancement of Basic Science : Disease and the (edited by Dr. Henry K. Beecher), *review* by Sir George Pickering, 530
Advancements in Space Research : Further, 631
Advances in Agronomy (edited by A. G. Norman), Vol. 12, *review* by G. V. Dyke, 213
Advances in Fluorine Chemistry (edited by Prof. M. Stacey, Prof. J. C. Tatlow and Dr. A. G. Sharp), Vol. 1, *review* by Dr. Richard Dresdner, 319
Advances in Processing Cereals : Recent (Food Group of the Society of Chemical Industry Symposium on), 755
Advisory Council (Regional), for Technological Education, London and Home Counties : Regional Development Plan for Courses for the Diploma in Technology, 1044
Aedes aegypti : Experimental Transmission of *Borrelia anserina* (Sakharoff 1891) by (J. A. Roberts), 1225
Aedes aegypti var. *Queenslandensis* : Blood-Feeding, Fecundity and Ageing in (Dr. M. M. J. Lavoipierre), 575
Aerobacter aerogenes : Induced Enzyme Synthesis in Aqueous Suspensions of Starved Stationary Phase (R. E. Strange), 1272
  Inhibition by Oxygen of Biosynthesis and Activity of Nitrate-Reductase in (Francis Pichinoty and Liliane D'Ornano), 879
Aerodynamics : Incompressible (edited by Bryan Thwaites), *review* by Prof. Leon Trilling, 315
Aeronautical Research in Asia and Africa, 1043
Aeronautics, College of (Cranfield) : Appointment of Prof. A. H. Lefebvre to the chair of aircraft propulsion, 1042
Affinity (Electron), of the Acceptor Molecule in Charge-Transfer Complexes (Dr. M. E. Peover), 702
Africa : A Poikilohydrous, Poikilochlorophyllous Angiosperm from (Dr. D. J. Hambler), 1415
Africa's First Science Museum (Murray Schoonraad), 848
Africa (East) : Medical Research in, 226
Africa (Eastern and North-Eastern) : Birds of (C. W. Mackworth-Praed and C. H. B. Grant), second edition, *review* by Sir Landsborough Thomson, 9
Africa (Asia and) : Aeronautical Research in, 1043
African Bibliographies : A Bibliography of, covering Territories South of the Sahara (South African Public Library, Grey Bibliographies No. 7), fourth edition, 959
African Jurisprudence (Prof. M. Gluckman), 991
African States (Modern) : Convervation of Nature and Natural Resources in (International Union for the Conservation of Nature and the Commission for Technical Co-operation in Africa Conference on), 958
African Wildlife : New Hope for, 958
African (East) Council for Medical Research : Report for 1959-60, 226
African (East and West) Stocks of *Papilio dardanus* (Lep.) : Genetics of a Difference in the Male Genitalia of (J. R. G. Turner, C. A. Clarke and P. M. Sheppard), 935
African (South) Animal Life—Results of the Lund University Expedition in 1950-1951, Vol. 7 (edited by Bertil Hanstrom, Per Brinck and Gustaf Rudebeck), *review* by Prof. O. Lowenstein, 1135
African (South) Museum : Annual Report for 1959-60, 1044
African (South) Public Library : Grey Bibliographies No. 7, A Bibliography of African Bibliographies, covering the Territories South of the Sahara, fourth edition, 959
African (West) Estuary : Seasonal Fluctuations and Distribution of Nitrite in a Tropical (J. C. D. Watts), 929
Africans in Northern Rhodesia : Blindness in (Dr. C. M. Phillips), 753
Africans in Rhodesia : Diabetes Mellitus in (Dr. M. Gelfand and W. R. Carr), 227
Afterglow Emission (New), associated with Metastable Nitrogen Atoms (L. Herman, and R. Herman), 346
Aftergut, S., and Brown, G. P. : Electronic Properties of Imidazole, 379
Afzelius, Björn A. : Flimmer-flagellum of the Sponge, 1318
Agammaglobulinaemia : Immunoelectrophoretic Examination of (Fred Rosen, Charles A. Janeway and Prof. David Gitlin), 1109
Agar Disk Method for the Direct Observation of Nematode-trapping Fungi in the Soil (Roderic C. Cooke), 1411
Agarwala, Dr. S. C., and others : Effect of Excess Supply of Heavy Metals on Barley during Germination, with special reference to Catalase and Peroxidase, 726
Age : Distribution of Human Chromosome Counts in relation to (Patricia A. Jacobs, W. M. Court Brown and Dr. Richard Doll), 1178
  Frequency of the 'Alpha' Rhythm, Reaction Time and (Dr. Walter W. Surwillo), 823
Age of Bed I, Olduvai Gorge, Tanganyika (Dr. L. S. B. Leakey, Dr. J. F. Evernden and Dr. G. H. Curtis), 478
Age of the Orogeny and Granites in South-West England (T. R. Owen), 486 ; (N. E. Butcher), 486
Age (A Great), for Science (Dr. Warren Weaver), 312
Age (The New), in Physics (Prof. Sir Harrie Massey), *review* by Prof. W. E. Burcham, 423
Age-Dependent Mortality in Fish : Expected Rate of Senescence and (Dr. Alex Comfort), 822
Age-Determinations (Preliminary), in the Lizard Area (J. A. Miller and D. H. Green), 159
Age Measurements on Antarctic Rocks (Queen Maud Land), (Sarah Deutsch, Prof. E. E. Picciotto and M. Reinharz), 1286

Age Structure and Marriage amongst the Groote Eylandt Aborigines: Classification of Kin, (Dr. Frederick G. G. Rose), *review* by Dr. R. Needham, 6
Age and Kinship in Australia, *review* by Dr. R. Needham, 6
Ageing: Research into Problems of, 1145
  Research Highlights in (United States Department of Health, Education and Welfare, Public Health Service Publication No. 854), 1145
Ageing and the Semi-skilled—a Survey in Manufacturing Industry on Merseyside (Alistair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine Cunningham), 1249
Ageing (Blood-Feeding, Fecundity and), in *Aedes aegypti* var. *Queenslandensis* (Dr. M. M. J. Lavoipierre), 575
Agents (Adrenergic Neurone-blocking), related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine (A. L. A. Boura, F. C. Copp, A. F. Green, H. F. Hodson, G. K. Ruffell, M. F. Sim, E. Walton and E. M. Grivsky), 1312
Agents (Alkylating): Specificity of the Mutagenic Action of the (Prof. Bernard S. Strauss), 730
Agents (Anabolic), and Histamine Metabolism (Dr. K. S. Kim), 1368
Agents (Antimicrobial), and Chemotherapy (American Society for Microbiology Interscience Conference on), 651, 1250
Agents (Anti-tumour): N,N'-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds—a New Series of (A. S. Tomcufcik, S. D. Willson, A. W. Vogel and A. Sloboda), 611
Agents (Chemical): Induction of Tumours by Polyoma Virus—Enhancement by (K. E. K. Rowson, F. J. C. Roe, J. K. Ball and Dr. M. H. Salaman), 893
Ages: The Frequency of Meteorite Falls throughout the (Prof. Herbert Dingle), 482; (Prof. Hans Pettersson), 482
Agglutinogen M^g (Blood Group): Action of Influenza Viruses, Receptor-destroying Enzyme and Proteases on (Prof. Georg J. Springer and Dr. Karlheinz Stalder), 187
Aggregates (Natural Soil): Role of Polysaccharides in Stabilization of (Dr. D. J. Greenland, G. R. Lindstrom and Dr. J. P. Quirk), 1283
Agrell, I. P. S., and Christensson, E. G.: Influence of Polyvalent Ions on Histones isolated from Different Tissues, 284
Agricultural Botany, National Institute of: Appointment of D. B. MacKay as chief officer of the Official Seed Testing Station, 856
Agricultural Materials: Strontium-90 in Milk and, in the United Kingdom, 1959-1960 (Agricultural Research Council, Report No. 4), 20
Agricultural Problems in Developing Countries (Nutrition Society Symposium on), 1250
Agricultural Research Council: Report No. 4, Strontium-90 in Milk and Agricultural Materials in the United Kingdom, 1959-1960, 20
  Survey of Farm Buildings in Britain, 440
  Bibliography of Research Publications on Farm Buildings, Section 5, Buildings for the Drying and Storage of Grain, 440
Agricultural Science, Australian Institute of: *Australian Journal of Experimental Agriculture and Animal Husbandry*, No. 1, 544
Agricultural Science, Easter School in: Proceedings of the University of Nottingham Seventh (1960)—Digestive Physiology and Nutrition of the Ruminant (edited by Dr. D. Lewis), *review* by Dr. I. W. McDonald, 1334
Agriculture at Bangor, 439
Agriculture: The Revolution in (Sir William Slater), 991
Agriculture, Ministry of (in the Government of Northern Ireland): Leaflets on the Raising of Crops and Animals, 959
Agriculture and Horticulture in Northern Ireland: Aids to, 959
Agriculture (Experimental), and Animal Husbandry (Australian Journal of), No. 1 (Australian Institute of Agricultural Science), 544
Agronomy: Advances in (edited by A. G. Norman), Vol. 12, *review* by G. V. Dyke, 213
Ahmed, K., and others: Electron Transport System and Phosphoproteins of Mitochondria, 1309
Aids to Agriculture and Horticulture in Northern Ireland, 959
Aims, Organization and Methods of University Extra-Mural Libraries in Great Britain (E. P. Pritchard), 649
Ainsworth, Dr. G. C.: Storage and Retrieval of Biological Information, 12
  Ainsworth and Bisby's Dictionary of the Fungi, fifth edition, *review* by Prof. John Colhoun, 109
Ainsworth and Bisby's Dictionary of the Fungi, fifth edition, by Dr. G. C. Ainsworth, *review* by Prof. John Colhoun, 109
Air at Ground Level: Seasonal Variations of Caesium-137 in (K. G. Vohra, V. S. Bhatnagar and C. Rangarajan), 747
Air at a Reduced Pressure: Bio-Assay of Erythropoietin in Mice made Polycythaemic by Exposure to (Dr. P. Mary Cotes and Dr. D. R. Bangham), 1065
Air within the Stratosphere: World-Wide Circulation of (P. Goldsmith and F. Brown), 1033
Air (Clean), National Society for: Fumifugium, or the Smoake of London Dissipated (John Evelyn), reprint, 752
  Twenty-eighth Conference, 1250
Air (Exhaled), of Diabetic Patients: Rapid Infra-Red Determination of Acetone in the Blood and the (Dr. Richard D. Stewart, Edward A. Boettner and Bruce T. Stubbs), 1008
Air (Ground-Level): Comparison of Beryllium-7 and Caesium-137 Radioactivity in (Dr. P. F. Gustafson, M. A. Kerrigan and S. S. Brar), 454
Air-breathing Fishes: A Hydrostatic Function of the Accessory Respiratory Organs in (Dr. S. Z. Qasim and R. Hasan), 396
Air Force Cambridge Research Laboratories, United States (Thermal Radiation Laboratory of the Geophysical Research Directorate): Conference on Electrically Exploded Wires, 1250
Air Showers (Extensive), in the Atmosphere: Photography of Cerenkov Light from (D. A. Hill and N. A. Porter), 690
Air-Swallowing, a Mechanism in Photic Reversal of the Beetle *Trypodendron* (Prof. Kenneth Graham), 519
Aira Caldera, Kyusyu, Tokyo: Gravity Survey on the (Izumi Yokoyama), 966
Aircraft Establishment (Royal), Farnborough: Open Days (Peter Sanderson), 232
Aircraft Propulsion at the College of Aeronautics, Cranfield, 1042
Aisenberg, Dr. Alan C.: The Glycolysis and Respiration of Tumours, *review* by Prof. F. Dickens, 1335
Aisenberg, Dr. Alan C., and Morris, Harold P.: Energy Pathways of Hepatoma No. 5123, 1314
Aitken, Prof. A. C.: *Obituary* of Prof. H. W. Turnbull, 223
Aizawa, Kazuyuki, and others: Isolation of Anthraquinone from Humus, 415
Akamatsu, A., and others: Inhibition of β-Glucoronidase by Oral Administration of D-Glucosaccharo-1,4-Lactone, 1298
Albe-Fessard, Prof. D. (Bowsheer, Dr. D. R., and): Assessment of Pain (Somatic Sensations), 1353
Albert, Prof. Z., and others: Histochemical Demonstration of Gamma-Glutamyl Transpeptidase, 767
Alberta, Research Council of: Information Series No. 23, Alberta Sub-bituminous Coals as Drilling Fluid Thinners (E. J. Jensen), 1144
Alberta Sub-bituminous Coals as Drilling Fluid Thinners (E. J. Jensen), 1144
Albumin (Bovine Serum): Elimination of, in Acute Experimental Uraemia (Dr. G. Gowland, F. G. Smiddy, Dr. R. G. Burwell and F. M. Parsons), 1210
Albumin (Serum): Splitting of, during Electrophoresis on Paper (J. V. McLoughlin), 1305
Alcock, Dr. C. B.: Appointed reader in chemical metallurgy at the Imperial College of Science and Technology, London, 443, 1349
Alchohol (Phenethyl), and Tyrosol: Formation of, during Fermentation of a Synthetic Medium lacking Amino-acids (R. Stevens), 913
Alcohol (Polyvinyl): Effect of, on the Distribution of Iodine-131 in the Internal Organs, Fluids and Excreta of Rabbits (H. F. Nowak and J. Kucharski), 665, *erratum*, 1045
Alcohol Consumption (Water and): Comparison between, in Six Animal Species in Free-choice Experiments (Dr. Alpo Arvola and Dr. Olaf Forsander), 819
Alcohol Dehydrogenase (Liver): An Inhibitor of, in Preparations of Reduced Diphosphopyridine Nucleotide (Dr. Keith Dalziel), 1098
Alcohol and Sodium Chloride: Serum Lipids in relation to the Intake of (Erling Lund, Torben Geill, Prof. Henrik Dam and Gunhild Kristensen), 1095
Alcohols: Peak-Shift Technique in Gas–Liquid Chromatography—Trimethylsilyl Ether Derivatives of (Stanley H. Langer and Peter Pantages), 141
Alcohols (Long-Chain), and Glycol Monoalkyl Ethers: Compressibility and Specific Evaporation Resistance of the Monolayers of (A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas), 378
Alcohols and Sugars: Regulation of Pyrogallol Oxidations by (B. Z. Siegel, S. M. Siegel and N. S. Goodman), 180
Alderson, Dr. Thomas: Mechanism of Mutagenesis induced by Formaldehyde—the Essential Role of the 6-Amino Group of Adenylic Acid (or Adenosine) in the Mediation of the Mutagenic Activity of Formaldehyde, 251
Aldosterone: Stimulation by, of Sodium Transport in the Loop of Henle (Dr. J. Crabbé), 817
Aldosterone Excretion and Potassium Retention in Subjects living at High Altitude (P. J. Ayres, Rona C. Hurter, Dr. E. S. Williams and J. Rundo), 78
Aldosterone (Anti-) *SC* 5233: Electrolyte Retention produced by the (J. B. Farmer and B. Petch), 1308
Aldred, Cyril: Appointed keeper of the Department of Art and Ethnography of the Royal Scottish Museum, Edinburgh, 648; work of, 648
Aldridge, D. D.: Norwich Meeting of the British Association for the Advancement of Science, 16
Alexander, Dr. P.: Effects of Ionizing Radiation on Organic and Biological Solids (Sulphur Compounds as Protective Agents), 1151
Alexander, Dr. P., and Block, R. J. (edited by): A Laboratory Manual of Analytical Methods of Protein Chemistry (including Polypeptides), Vol. 1, The Separation and Isolation of Proteins, *review* by Dr. D. T. Elmore, 320
Alexander, Pauline, and Nixon, Dr. D. A.: Foetal and Neonatal Physiology (The Foetal Kidney), 344
Algae (Marine), of the Eastern Tropical and Subtropical Coasts of the Americas (Prof. William Randolph Taylor), *review* by Prof. Lily Newton, 1234
Algological Study on the Eastern Coasts of the Americas, *review* by Prof. Lily Newton, 1234
Ali, Dr. M. Asgar: Appointed to an I.C.I. research fellowship in the University College of North Staffordshire, 229
Ali, Prof. M. A.: Effect of Thyroxine plus Thiourea on the Early Development of the Chum Salmon (*Oncorhynchus keta*), 1214
Ali, S. Y., and others: Action of Plasmin on Cartilage Matrix *in vivo*, 1402
Aliens: Weeds and (Sir Edward Salisbury), *review* by Dr. Alec Lazenby, 842
Alkaline Hydrolysis of *M*- and *N*-Blood Group Substances: Products of Mild Acid and (Dr. Elzbieta Romanowska), 1408
Alkaline Phosphatase: Distribution of (Phosphatases in *Schistosoma mansoni*), (Dr. D. L. H. Robinson), 473
Alkaloidal Fraction from the Bark of the Jamaican Shade Tree *Pithecolobium samath* Benth.: Pharmacology of the (B. E. Leonard and Dr. H. S. A. Sherratt), 287
Alkaloids (Heat-Labile), from Plants: Extraction of (Dr. A. R. Mattocks), 1281
Alkylated Derivatives (Reduced), of some Myeloma Globulins and Bence-Jones Proteins: Comparison of (Dr. M. D. Poulik and Dr. G. M. Edelman), 1274
Alkylating Agents: Specificity of the Mutagenic Action of the (Prof. Bernard S. Strauss), 730
N,N'-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds—a New Series of Anti-tumour Agents (A. S. Tomcufcik, S. D. Willson, A. W. Vogel and A. Sloboda), 611
Allam, D. S., and Lee, W. H.: Ultrasonic Velocity in Aqueous Silver Nitrate Solution, 1388
Allan, Dr. D. A.: At the sixty-seventh Annual Conference of the Museums Association (Ideal Display of Natural History Objects should include the Clinical and the Artistic), 553
  Retirement from the directorship of the Royal Scottish Museum, Edinburgh, 648; work of, 648
Allanson, J. T.: Appointed to the second chair of electrical engineering at the University of Birmingham, 647; work of, 647
Allard, Prof. R. W.: Principles of Plant Breeding, *review* by Dr. Kenneth S. Dodds, 636
Alleged Anticomplementary Effect of Aspirin (K. W. Anderson), 1012
'Alleles' (Amino-Acid): Non-Randomness in (Richard V. Eck), 1284

Allelic Series : Limitations of Geometrical Models for Complementation Mapping of (Prof. Elof Axel Carlson), 788
Allen, Dr. Clifford : *Obituary* of Prof. C. G. Jung, 223
  Jungian Psychology, *review*, 1235
Allen, C. P. : Blood Groups in Infra-human Species (Blood Groups of Chickens), 654
Allen, Prof. G. C. : The Structure of Industry in Britain, 1332
Allen, jun., Dr. Harry C. : Appointed chief of the Analytical and Inorganic Chemistry Division of the United States National Bureau of Standards, 1346 ; work of, 1346
Allen, Dr. J. A. : A Biology of Crustacea, *review*, 111
Allen, Dr. P. E. M., and Patrick, C. R. : Generation of Radicals in Pairs and the Kinetics of Radical Chain Reactions in Solution, 1194
Allen, Richard, and others : Very-Low-Frequency Modulation of Discrete Frequency Solar Noise Bursts, 56
Allen, Dr. T. K., and Bickerton, Dr. R. J. : Experiments on the Compression of Plasma in Cusp Geometry, 794
Alletson, D. L., and others : Inorganic Polymers (Dialkyl Tin Oxides), 1047
Allogibberic Acid : Effects of Gibberellic Acid and, on Flowering and Internode-length in *Pisum sativum* (I. C. Murfet and H. N. Barber), 514
Alloy (Magnesium) : Effect of Chloride Concentration on the Aqueous Corrosion of a (D. de G. Jones and H. G. Masterson), 165
α-Amylase : Effect of, on the Ehrlich Mouse Ascites Tumour (Dr. F. Steckerl, A. Ofodile, R. R. Campbell and G. H. Friedell), 1113
α-Glycerophosphate Dehydrogenase (Mitochondrial), in the Nervous System : Histochemical and Homogenization Studies of (R. Hess and Dr. A. G. E. Pearse), 718
α-Glycerophosphate Dehydrogenase Activity (Mitochondrial), in Skeletal Muscle : Direct Relationship of Phosphorylase and (Dr. A. G. E. Pearse), 504
α-Keto-Acid Hydrazones : Application of the Nickel Reaction and Thermal Isomerization of, for their Chromatographic Analysis (A. M. Tokushige and Dean E. S. Cook), 797
α-Particles (Polonium-210) : Circumscribing Irradiation of Surface Structures of Cylindrical Animal and Plant Cells with (R. M. Bergström, R. F. Blåfield and M. W. Brenner), 1165
'Alpha' Rhythm, Reaction Time and Age : Frequency of the (Dr. Walter W. Surwillo), 823
α-Tocopherol : Myopathy affecting the Rottnest Quokka (*Setonix brachyurus*) reversed by (B. A. Kakulas), 402
α-Virginis (Spica) Nebula (Walter A. Feibelman), 1083
α- and β-Forms of Selected Sugars : Relative Sweetness of (Rose Marie Pangborn and Sandra C. Gee), 810
Alteration of Activity of the Reticulo-Endothelial System : Sensitivity of Mice to Bacterial Lipopolysaccharide following (Dr. G. N. Cooper and Dr. A. E. Stuart), 294
Alterations of Conscious Awareness : Spike and Wave Discharges and (L. Goldie and J. M. Green), 200
Altitude (High): Aldosterone Excretion and Potassium Retention in Subjects living at (P. J. Ayres, Rona C. Hurter, Dr. E. S. Williams and J. Rundo), 78
Ambrózi, A. : At the Second International Measurement Conference (An Electronic Statistical Quality Control Apparatus which indicates Average and Variance Directly), 1350
Amelin, V. M. (Zhongolovich, I. D., and) : A Collection of Tables and Nomograms for the Processing of Observations made on Artificial Earth Satellites, *review* by D. G. King-Hele, 1135
America, Geological Society of : Special Paper 65, The Floors of the Oceans, I, The North Atlantic—Text to accompany the Physiographic Diagram of the North Atlantic (Bruce C. Heezen, Marie Tharp and Maurice Ewing), *review* by Dr. G. E. R. Deacon, 531
America (Latin) : Nutrition in (Food and Agriculture Organization of the United Nations Meetings on), 959
American : The Victorian—Lithographs from the Harry T. Peters 'American on Stone' Collection (Antony N. B. Garvan and Peter C. Welsh), 226
'American on Stone' Collection (Harry T. Peters) : The Victorian American —Lithographs from the (Antony N. B. Garvan and Peter C. Welsh), 226
American Academy of Arts and Sciences : Monograph Prizes, 442
American Academy of Neurology (Section on Neurochemistry) : Symposium on the Neurochemistry of Nucleotides and Amino Acids (edited by Roscoe O. Brady and Donald B. Tower), *review* by Dr. R. V. Coxon, 318
American Association of Clinical Chemists (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
American Astronautical Society, and others (sponsored by the) : A Symposium on Space Trajectories (edited by the Technical Staff, Research Division, Radiation Incorporated), *review* by D. G. King-Hele, 1334
American Bacteriologists, Society of (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
American Chemical Society (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
American Grants for Nuclear Equipment in Argentina, Brazil and Israel, 442
American Institute of Mining, Metallurgical and Petroleum Engineers (Iron and Steel Institute and the Metallurgical Society of the) : Special Meeting in the United States and Canada, 651
American Mathematical Society : Proceedings of Symposia in Applied Mathematics, Vol. 10, Combinatorial Analysis (edited by Richard Bellman and Marshall Hall, Jr.), *review* by Prof. L. S. Goddard, 321
American Orchid Society, and others : Third World Orchid Conference, London, 1960—Proceedings of the, *review* by V. S. Summerhayes, 213
American Philosophical Society : Year Book 1960, 335
  History of the American Philosophical Society (E. G. Conklin), 335
American Revolution : Drug Supplies during the (George B. Griffenhagen), 761
American Society for Cell Biology : Symposium on Cell Continuity, 1045
  Symposium on Cell Diversification, 1045
  Symposium on Characteristics of Cell Interfaces, 1045
American Society for Microbiology : Inter-Science Conference on Antimicrobial Agents and Chemotherapy, 651, 1250
American Society for Testing Materials (Committee *E*-14), (Mass Spectrometry Group of the Institute of Petroleum and the) : Conference on Mass Spectrometry, 24
American Electricity Plant : New, 22
Americas : Algological Study on the Eastern Coasts of the, *review* by Prof. Lily Newton, 1234
  Marine Algae of the Eastern Tropical and Subtropical Coasts of the (Prof. William Randolph Taylor), *review* by Prof. Lily Newton, 1234
Amides (Primary) : A New Relation between the N–H Stretching Frequencies of (Prof. P. G. Puranik and K. Venkata Ramiah), 796
Amiel, Dr. J. L. : Awarded a grant from the Lady Tata Memorial Trust, for research on leukaemia and allied diseases, 228
Amin, A. : Comparison of the Serum Protein Fractions of the Newly Hatched Chick with those of Adult Birds using Starch-gel Electrophoresis, 708
Amines in Rumen Liquid of Cattle : Keto-Acids and (Dr. C. J. G. van der Horst), 73
Amines (Catechol) : Interaction of Hydrocortisone, the Sulphydryl Group, and the (Dr. Sidney Roston), 490
Amino-Acid Activation in Tissues of Early Embryos (Dr. Elizabeth M. Deuchar), 1006
Amino-Acid 'Alleles' : Non-Randomness in (Richard V. Eck), 1284
Amino-Acid Composition of Serum Mucoprotein in Rheumatic Fever (Prof. D. O'Brien, C. H. M. Walker and D. Walker), 922
Amino-Acid Esters : Effect of, on the Growth of Fibroblast Cultures (Dr. I. Fehér, A. Döklen and V. Selmeci), 494
Amino-Acid Liberation from Proteins by Enzyme or 'Heat Catalysed' Hydrolysis (S. M. Hardy), 1061
Amino-Acid Mixtures from Protein Hydrolysates : A Simplified Method for separating, on One Ion-Exchange Column during Preparation (J. Liebster, K. Kopoldová and M. Dobiášová), 1198
Amino-Acid Replacements : Non-Random Occurrence of (C. R. Woese), 1196
Amino-Acid Sequences : Immunochemical Distinction between Insulins with Identical (Dr. Solomon A. Berson and Dr. Rosalyn S. Yalow), 1392
Amino-Acids, *review* by Sir Hans Krebs, 1132
Amino Acids : Chemistry of the (Dr. Jesse P. Greenstein and Dr. Milton Winitz), *review* by Sir Hans Krebs, 1132
  Complexes of Vermiculite with (G. F. Walker and W. G. Garrett), 1389
  Formation of Phenethyl Alcohol and Tyrosol during Fermentation of a Synthetic Medium lacking (R. Stevens), 913
  The Neurochemistry of Nucleotides and—a Symposium of the Section on Neurochemistry, American Academy of Neurology (edited by Roscoe O. Brady and Donald B. Tower), *review* by Dr. R. V. Coxon, 318
Amino-Acids concerned with the Biosynthesis of Urea : Prevention of Ammonia Toxicity by (Dr. Francesco Salvatore and Dr. Vincenzo Bocchini), 705
Amino-Acids in Micro-organisms which produce Antibiotics: Activation of (Dr. O. Ciferri, Dr. M. Di Girolamo and Dr. A. Bendicenti Di Girolamo), 411
Amino-Acids (Iodinated) : A Spray Method for the Simultaneous Detection of Iodide and, on Filter Paper Chromatograms (Dr. A. Van Zyl and M. Bhaga), 275
Amino-Acids (Sulphur) : Metabolism of the, and of Sulphate in *Blattella germanica* (Richard J. Block and S. Mark Henry), 392
Amino-Acids and Globin in Duck Blood *in vitro* : Intermediate Pool between (L. Bianco, G. Giustina and E. Lazzarini), 40
γ-Amino-*n*-butyric Acids (Glutamic and) : Analogues of, having Potent Actions on Mammalian Neurones (Dr. J. C. Watkins and Dr. D. R. Curtis), 1010
Amino-derivatives of 11β, 17α, 21-Trihydroxy-3,20-Dioxo-1,4-Pregnadiene (J. Tóth, Z. Tuba and L. Szporny), 607
2-Aminoethanol : Decreased Urinary Excretion of, by Irradiated Human Beings (Dr. Ralph R. Cavalieri, M. T. Van Metre and W. I. Sivertsen), 1303
6-Aminopenicillanic Acid : Action of Carbon Dioxide on (F. R. Batchelor, D. Gazzard and J. H. C. Nayler), 910
  Identification of a Compound related to, isolated from Culture Media of *Penicillium chrysogenum* (A. Ballio, Prof. E. B. Chain, F. Dentice di Accadia, M. Mauri, K. Rauer, M. J. Schlesinger and Sondra Schlesinger), 909
  Simple, Quick Methods for Detection of (J. Uri and F. Sztaricskai), 1223
6-Aminopenicillanic Acid from Benzylpenicillin : A Method for Isolating Bacteria capable of producing (Prof. Yukio Kameda, Yukio Kimura, Etsuko Toyoura and Tomoo Omori), 1122
Aminopeptidase (Human Serum Leucine) : Sub-fractionation of (Prof. Clyde A. Dubbs, Charlotte Vivonia and Jean M. Hilburn), 1203
Aminopeptidase (Leucine), in the Mammary Gland of the Cow and Rat (Prof. S. Talanti and Prof. Väinö K. Hopsu), 86
Amitotically : Deoxyribonucleic Acid Content of Nuclei dividing (Prof. K. W. Cleland), 504
Ammocoete Species : Oocyte Numbers as a Diagnostic Character for the Identification of (Dr. M. W. Hardisty), 1215
Ammonia Toxicity : Prevention of, by Amino-acids concerned in the Biosynthesis of Urea (Dr. Francesco Salvatore and Dr. Vincenzo Bocchini), 705
Amos, Dr. D. B. : Blood Groups in Infra-human Species (The Classical *H*-2 System of Tissue and Red Cell Antigens), 654
Amounts of Material in the Neurosecretory System of the Desert Locust : Induced Changes in the (Dr. K. C. Highnam), 199
α-Amylase: Effect of, on the Ehrlich Mouse Ascites Tumour (Dr. F. Steckerl, A. Ofodile, R. R. Campbell and G. H. Friedell), 1113
Anabolic Agents and Histamine Metabolism (Dr. K. S. Kim), 1368
Analogues of Glutamic and γ-Amino-*n*-butyric Acids having Potent Actions on Mammalian Neurones (Dr. J. C. Watkins and Dr. D. R. Curtis), 1010
Analysis : Combinatorial—Proceedings of the American Mathematical Society Symposia in Applied Mathematics, Vol. 10 (edited by Richard Bellman and Marshall Hall, Jr.), *review* by Prof. L. S. Goddard, 321
  Job (H. E. Roff and T. E. Watson), 336
  Organic (edited by John Mitchell, Jr., I. M. Kolthoff, E. S. Proskauer and A. Weissberger), Vol. 4, *review* by Dr. D. T. Lewis, 741
  Use of the Rotating Hanging Mercury Drop in—Anodic Stripping using a Rotating Mercury Drop (Dr. J. J. Engelsman and A. M. J. M. Claassens), 240 ; Trace Analysis with a Rotating Hanging Mercury Drop (Dr. M. van Swaay and R. S. Deelder), 1241

Analysis by Gas Chromatography : Detection by Ionization of Atmospheric Gases during (Dr. Arthur Karmen, Laura Giuffrida and Robert L. Bowman), 906
Analysis of Organic Compounds, *review* by Dr. D. T. Lewis, 741
Analysis of Polarized Light by the Bee's Eye (Prof. Donald Kennedy and Dr. Edward R. Baylor), 34
Analysis of Satellite Data (Prof. D. G. Parkyn), 896
Analysis of the Thymus Influence in Leukaemogenesis (Dr. J. F. A. P. Miller), 248
Analysis (Chromatographic) : Application of the Nickel Reaction and Thermal Isomerization of Alpha-Keto-Acid Hydrazones for their (A. M. Tokushige and Dean E. S. Cook), 797
Analysis (Chromatographic), of Infectious Ribonucleic Acid from Poliovirus (C. Cocito, A. Prinzie and P. De Somer), 573
Analysis (Comparative), of Inactivating Efficiency of Radiation on Different Organisms (Dr. M. Terzi), 461
Analysis (Discriminant), in Biometrical Genetics (Dr. S. K. Jain), 1420
Analysis (Inorganic) : The Use of Organic Reagents in (A. I. Busev and N. G. Polianskii), translated from the Russian by J. T. Greaves, *review* by Dr. D. T. Lewis, 1336
Analysis (Mathematical) : Modern Style in, *review* by Prof. L. S. Goddard, 6
Analysis (Modern) : Foundations of (Prof. J. Dieudonné), *review* by Prof. L. S. Goddard, 6
Analysis (Nodal), of Associated Populations (Prof. W. T. Williams and J. M. Lambert), 202
Analysis (Quantitative), of Milk and other Emulsions by Infra-Red Absorption (Dr. J. D. S. Goulden), 905
Analysis (Rotational), of Absorption Bands of Lead Monoxide (Dr. R. F. Barrow, J. L. Deutsch and D. N. Travis), 374
Analysis (Structural) : Basic Approaches to, *review* by Prof. R. C. Coates, 1134
Analysis (Trace), with a Rotating Hanging Mercury Drop (Use of the Rotating Hanging Mercury Drop in Analysis), (Dr. M. van Swaay and R. S. Deelder), 241
Analysis (Urine) : Determination of Caesium-137/Potassium Ratios in Diet and in the Human Body by (A. Morgan and Gloria M. Arkell), 1100
Analysis (X-Ray), of Diamond : Synthesis and (H. Ll. D. Pugh, J. Lees and J. A. Bland), 865
Analytical Chemistry in Nuclear Reactor Technology (Oak Ridge National Laboratory Fifth Conference on), 651
Analytical Methods of Protein Chemistry, including Polypeptides : A Laboratory Manual of (edited by Dr. P. Alexander and R. J. Block), Vol. 1, The Separation and Isolation of Proteins, *review* by Dr. D. T. Elmore, 320
Anaphylactic Sensitivity : A New Test for detecting—the Basophil Reaction (Prof. Walter B. Shelley and Lennart Juhlin), 1056
Anaphylactic Site : Pinnal Anaphylaxis, an Additional (Dr. J. G. Feinberg), 712
Anaphylactoid Reaction in Rats (J. M. Harris and Dr. G. B. West), 399
Anaphylaxis : Pinnal—an Additional Anaphylactic Site (Dr. J. G. Feinberg), 712
Anatomy at Oxford, 957
Anatomy (Human) : Atlas of (edited by Prof. Ferenc Kiss and Prof. János Szentágothai), Vol. 1, Osteology, Syndesmology, Myology ; Vol. 2, Splanchnology, Endocrine Glands, Heart ; Vol. 3, Nervous System, Vascular System, Lymphatic System, Sense Organs, *review* by Prof. J. M. Yoffey, 1232
    Illustrations of, *review* by Prof. J. M. Yoffey, 1232
Anatomy (Veterinary), at Liverpool, 648
Ancient Mesopotamia : Ecological Aspects of Irrigation in (Hans Helback), 227
Andersen, A. C., and others : Failure of a Dog to Discriminate between Strontium-90 and Calcium given Orally, 713
Anderson, A. J., and others : Action of Plasmin on Cartilage Matrix *in vivo*, 1402
Anderson, Dr. D. R. (Owen, Dr. R. D., and) : Blood Groups in Infra-human Species (A Study of Blood Groups in the Rhesus Monkey), 653
Anderson, Hugh C. : Differential Thermogravimetry versus Differential Thermometry in Studying the Pyrolysis of Polymers, 1088
Anderson, Dr. J. S. : Inorganic Polymers (What is an Inorganic Polymer ?), 1046
Anderson, Dr. J. S. (Emeléus, Prof. H. J., and) : Modern Aspects of Inorganic Chemistry, third edition, *review* by Prof. J. R. Partington, 637
Anderson, K. W. : Alleged Anticomplementary Effect of Aspirin, 1012
Anderson, Prof. M. L. : Four Decades of State Forestry in Great Britain, 656
    Forest Products Research in Britain, 760
    Forest Insect Pests and Diseases in Canada, 1260
Andresen, Dr. E.: Blood Groups in Infra-human Species (Blood Groups of Pigs), 654
Andrew, Prof. J. H. : *Obituary* by Dr. W. R. Maddocks, 121
Andrews, J. R., and Berry, R. T. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Survival Curves of Mouse Leukaemic Cells which grow normally under Anoxic Conditions in the Animal), 341
Andrews, W. H. H., and others : Fructose and Lactic Acid Metabolism in the Perfused Liver of Premature Lambs, 1307
Andrianov, Prof. K. A. : Inorganic Polymers, 1046
Angiosperm from Africa : A Poikilohydrous, Poikilochlorophyllous (Dr. D. J. Hambler), 1415
Angiosperm Fruit : An Early Cretaceous (Hanterivian), from California (M. E. J. Chandler and D. I. Axelrod), 442
Angiosperms ? : How Old are the (D. I. Axelrod), 442 ; (—. Scott, —. Barghoorn, and —. Leopold), 442
Angle (Low-) Light Scattering and the Molecular Weight of Deoxyribonucleic Acid (Dr. Sidney Katz), 280
Anglesey : Littoral Interstitial Species from, representing Three Families New to Britain (P. J. S. Boaden), 512
Anglo-American Corporation of South Africa, Ltd. : Grant of £15,000 a year for seven years, to continue the work of the Research Institute of African Geology in the University of Leeds, 650
Aniline Oxidation initiated by Ultrasonic Waves (Dr. Wiktor Wawrzyczek), 701
Animal Biology and the Medical Sciences : The Use of Radioisotopes in (International Atomic Energy Agency, the Food and Agriculture Organization and the World Health Organization of the United Nations, Conference on), 959
Animal Cell, Tissue and Organ Cultures in Radiobiology : Use of (New York Academy of Sciences Symposium on), 340
Animal Diseases : Animal Experiments in the Control of (Research Defence Society Pamphlet on), 226
Animal Ecology (Tropical), Nuffield Unit of : Appointment of Dr. R. M. Laws as senior investigator, 651
Animal Experiments in the Control of Animal Diseases (Research Defence Society Pamphlet on), 226
Animal Health Trust : Research Awards, 1250
Animal House : Hazards of the (Laboratory Animals Centre Discussion on), 339
Animal Husbandry (*Experimental Agriculture and*), *Australian Journal of*, No. 1 (Australian Institute of Agricultural Science), 544
Animal Life : South African—Results of the Lund University Expedition in 1950-1951, Vol. 7 (edited by Bertil Hanstrom, Per Brinck and Gustaf Rudebeck), *review* by Prof. O. Lowenstein, 1135
Animal Origin (Sea Cucumber) : A New Triterpenoid Sapogenin of (Takao Matsuno and Toshihiko Yamanouchi), 75
Animal Sounds and Communication (edited by Dr. W. E. Lanyon and Dr. W. N. Tavolga), *review* by Dr. W. H. Thorpe, 741
Animal Species (Six), in Free-choice Experiments : Comparison between Water and Alcohol Consumption in (Dr. Alpo Arvola and Dr. Olof Forsander), 819
Animal Welfare, Universities Federation for : Annual Summer Congress, 755
    Symposium on the Assessment of Pain, 1353
Animal and Plant Cells (Cylindrical) : Circumscribing Irradiation of Surface Structures of, with Polonium-210 α-Particles (R. M. Bergström, R. F. Blåfield and M. W. Brenner), 1165
Animals : Calories/gm. in Species of (Prof. L. B. Slobodkin and S. Richman), 299
    Sounds used by, *review* by Dr. W. H. Thorpe, 741
Animals other than Man : Blood Groups of (Dr. D. G. Gilmour), 653
Animals (Domestic), and Poultry in India : Occurrence of *Salmonellae* in (V. K. Sharma and Prof. C. M. Singh), 622
Animals (Experimental) : Demonstration of Anti-Depressant or Stimulant Properties of Imipramine in (Dr. D. R. Maxwell and Helen T. Palmer), 84
Animals (Man and) : Assessment of Pain in, 1353
Anisotropic Elastic Media : Surface Waves in (V. T. Buchwald and A. Davis), 899
Anisotropy Axis Dispersion : Variation of, with Thickness of Nickel-Iron Films (H. Clow), 996
Annual of the British School at Athens : Nos. 53-54, 1958-1959, *review* by Dr. Hugh Plommer, 212
Annual Reports on the Progress of Chemistry for 1959 (edited by Dr. R. S. Cahn), Vol. 61, *review* by Prof. J. R. Partington, 637
Anodic Stripping using a Rotating Mercury Drop (Use of the Rotating Hanging Mercury Drop in Analysis), (Dr. J. J. Engelsman and A. M. J. M. Claassens), 240
Anomalies (Resistivity), in Palladium Hydride : Heat Capacity and (Paul Mitacek, jun., and Prof. J. G. Aston), 271
Anomalous Branched Tapeworms, *Hymenolepis nana* (Cestoda ; Hymenolepididae), in a Colony of *DBA/1* Mice : A Natural Population of (Dr. Donald Heyneman), 297
Anopheles gambiae Giles : Maxillary Index in (L. K. H. Goma), 405
Ansell, W. F. H. : Mammals of Northern Rhodesia, *review* by R. W. Hayman, 8
Anstis, S. M., and others : Measuring Visual Constancy for Stationary or Moving Objects, 416
Antarctic Meteorology : Melbourne Symposium on, *review* by H. H. Lamb, 210
Antarctic Meteorology—Proceedings of the Symposium held in Melbourne, 1959 (Commonwealth of Australia, Bureau of Meteorology), *review* by H. H. Lamb, 210
Antarctic Rocks (Queen Maud Land) : Age Measurements on (Sarah Deutsch, Prof. E. E. Picciotto and M. Reinharz), 1286
Antenna (Cross-) of the Proposed Benelux Radio Telescope (Prof. W. N. Christiansen and Dr. J. A. Högbom), 215
Anthelmintic (Highly Effective), for Sheep ; Thiabendazole, a (Hugh McL. Gordon), 1409
Anthelmintic Activity : Pyridine Derivatives with (A. P. Phillips and R. B. Burrows), 707
Antheridium of *Pythium debaryanum* Hesse : Meiosis in the Oogonium and (Dr. Eva Sansome), 827
Anthocyanins (The First), appearing during the Ripening of Blueberries (Dr. Heikki Suomalainen and A. J. A. Keränen), 498
Anthony, Prof. A., and others : Oxygen Electrode for Measurement of Tissue Slice Respiration, 969
Anthraquinone : Isolation of, from Humus (Kyoichi Kumada, Akio Suzuki and Kazuyuki Aizawa), 415
Antialdosterone *SC* 5233 : Electrolyte Retention produced by the (J. B. Farmer and B. Petch), 1308
Antibiotics : Activation of Amino-Acids in Micro-organisms which produce (Dr. O. Ciferri, Dr. M. Di Girolamo and Dr. A. Bendicenti Di Girolamo), 411
Antibodies to Trophoblasts during the Post-Partum Period (J. F. Hulka, K. C. Hsu and S. M. Beiser), 510
Antibodies (Specific Bactericidal) : Increase in, after Administration of Endotoxin (J. Gabriel Michael, James L. Whitby and Maurice Landy), 296
Antibody (Anti-*D*) : Specific Inhibition of, by Colominic Acid (Prof. William C. Boyd and Egli Reeves), 511
Antibody (Antigen–) Complexes : Mechanism of Action of—their Effect on Mast Cells (Dr. Ivan Mota), 572
Antibody Response in Rabbits following Injection of Sheep Erythrocytes into Lateral Ventricle of Brain (Prof. B. D. Janković, Dr. M. Draškoci and Dr. K. Isaković), 288
Antibody Titre : Rise of, in Cell-free Spleen Preparations Incubated *in vitro* (Dr. Dieter H. Sussdorf), 770
Anticancer Properties of Thio-diglycollic Acid (M. B. Sahasrabudhe, M. V. Narurkar, L. B. Kotnis, B. D. Tilak, L. C. Shah and V. N. Gogte), 388
Anticomplementary Effect (Alleged), of Aspirin (K. W. Anderson), 1012
Anti-*D* Antibody : Specific Inhibition of, by Colominic Acid (Prof. William C. Boyd and Egli Reeves), 511

Anti-Depressant or Stimulant Properties of Imipramine : Demonstration of, in Experimental Animals (Dr. D. R. Maxwell and Helen T. Palmer), 84
Anti-Diego in Monozygotic Twins : The Diego Factor in a Puerto Rican Family—a Case of (Dr. Hubert J. Van Peenan, Dr. John Scudder, James A. Jack and Erika Awer), 188
Antifungal Substance from *Pisum sativum* L. : Pisatin, an (Dawn R. Perrin and W. Bottomley), 76
Antigen in Lesions of Molluscum Contagiosum (Dr. William L. Epstein, Irene P. Senecal and Dr. Alan M. Massing), 509
Antigen (*Rh*-like), in Red Cells of the Sheep : Occurrence of an (B. Urbaschek and Prof. K. Trautwein), 193
Antigen–Antibody Complexes : Mechanism of Action of—their Effect on Mast Cells (Dr. Ivan Mota), 572
Antiglobulin Consumption Test (Positive) : Nature of the Coating Globulin causing a (D. Nelken), 1110
Antiglobulin Tests : A Relation between the Reactivity of Human Red Cells to, and their Surface Structure (Judith E. Davis, Prof. H. N. Green and Pamela W. Tymms), 923
Anti-Haemophilic Globulin (Freeze-dried): Stability of (F. Nour-Eldin), 503
Anti-Leucocyte Cytoplasm, Anti-Leucocyte Nuclei and Anti-Platelets in Disseminated Lupus Erythematosis (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Anti-Leucocyte Nuclei and Anti-Platelets (Anti-Leucocyte Cytoplasm,), in Disseminated Lupus Erythematosis (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Antilymphoma Effects : Evidence that the L-Asparaginase Activity of Guinea Pig Serum is responsible for its (J. D. Broome), 1114
Antimicrobial Agents and Chemotherapy (American Society for Microbiology Interscience Conference on), 651, 1250
Antimicrobial Substances from Ferns (Prof. Jasper C. Maruzzella), 518
Antimonide (Indium), at High Pressures : Melting and Polymorphism of (A. Jayaraman, R. C. Newton and Dr. G. C. Kennedy), 1288
Antioxidant : Selenomethionine as an (H. S. Olcott, W. Duane Brown and J. Van der Veen), 1201
Antioxidant (New), from Oats : Isolation of a (Dr. D. G. H. Daniels and H. F. Martin), 1302
Anti-Platelets (Anti-Leucoyte Cytoplasm, Anti-Leucocyte Nuclei and), in Disseminated Lupus Erythematosis (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Anti-Sporulation Effect of Copper Fungicides : Significance of the, in the Control of Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476
Anti-Tumour Agents : N,N'-Alkylene-*bis* (Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds—a New Series of (A. S. Tomcufcik, S. D. Willson, A. W. Vogel and A. Sloboda), 611
Antony, T. Therattil : Inactivation and Reactivation of Chymotrypsin, 178
Aorta (Human), during Growth : Increase in Elastin Content of the (Dr. V. Scarselli), 710
Aortic Tissue (Human) : Degree of Polymerization of Hyaluronic Acid isolated from (Dr. Sv. Bertelsen and K. Marcker), 386
Apennines (Northern) : Structure of the (Sir Edward Bailey and Prof. W. J. McCallien), 1136
Apertures : Minimum Energy Theorem for Flow of Dry Granules through (R. L. Brown), 458
Aplysia depilans : The Path of the Giant Cell Axons in (Dr. G. M. Hughes and L. Tauc), 404
Apothecaries of London, Worshipful Society of : Second Annual Report and List of Members (1960-61) of the Faculty of the History of Medicine and Pharmacy, 21
  Maccabaean Prize and Medal for 1962, 650
Apparatus (An Experimental), for Contact Microradiography at 200-500 V. (Dr. A. Recourt and G. H. F. de Vries), 1185
Apparent Cytoplasmic Inheritance of Tyrosinase Competence in *Streptomyces scabies* (Prof. K. F. Gregory and Wei-jen Shyu), 465
Apparent Recession of Nebulae and the Nature of Radio Stars (Dr. N. S. Japolsky), 480
Appearance of the *T*-Phase (R. Green), 997
Apple Seedlings : Shortening the Juvenile Phase of (A. I. Campbell), 517
Appleby, Dr. Robert M. : Continuation of the Great Glen Fault beyond the Moray Firth, 1190
Apples : Determination by Mass Spectrometry of the Structure of Proline Derivatives from (Dr. K. Biemann, G. G. J. Deffner and Prof. F. C. Steward), 380
  Preparation of Mitochondria from the Peel of (Dr. J. D. Jones and Dr. A. C. Hulme), 370
Application to Cosmic Rays : Fission and Spallation Phenomena and their (European Organization for Nuclear Research and the Max Planck Institute, Heidelberg, Conference on), 24
Application of Current Steps in Motoneurones : Potential Changes produced by (T. Araki, Dr. M. Ito and T. Oshima), 1104
Application of the Nickel Reaction and Thermal Isomerization of Alpha-Keto-Acid Hydrazones for their Chromatographic Analysis (A. M. Tokushige and Dean E. S. Cook), 797
Applications of Solar Energy, 228
Applications (Practical), of Nuclear Energy (International Atomic Energy Agency Courses on), 1143
Applied Bacteriology, Society for : Election of Officers, 754
  Symposium on Bacteria of the Intestine, 1151
Applied Biologists, Association of : Provincial Meeting at the University College of North Wales, Bangor, 856
Applied Chemical Kinetics : Inversion of a Laplace Transform arising from a Problem in (T. Wood), 589
Applied Mathematics at Belfast, 19
Applied Mathematics at Manchester, 647
Applied Mechanics (Theoretical and) : Proceedings of the Fifth Congress on, 855
Applied Mechanics (Theoretical and), Indian Society of, and Unesco : Symposium on Non-Linear Physical Problems, 855
Applied Science (Museum of), of Victoria, Melbourne : Report for 1959-60, 124
Applied Spectroscopy, Society of (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
Applied Statics : Energy Principles in (T. M. Charlton), *review* by Prof. R. C. Coates, 1134
Appraisal of Phenomenology : An, *review* by F. I. G. Rawlins, 5
Appreciable *N*-Deacetylation of Hexosamine : Partial Acid-Hydrolysis of a Mucopolysaccharide without (Dr. T. J. Painter and Prof. W. T. J. Morgan), 39
Apprenticeship and Technical Education, 737
Apprenticeship and Training (House of Commons Debate on), 737
Approach (Holistic), to Selenology (Brian Warner), 586
Approach (A New), to Biology Teaching (Dr. L. H. Finlayson), 554
Approach (A New), to General Relativity, *review* by Dr. G. J. Whitrow, 424
Approaches (Basic), to Structural Analysis, *review* by Prof. R. C. Coates, 1134
Aprovechamiento de la Tierra en Nicaragua : Estudios Ecologicos para el (Dr. B. W. Taylor), 1259
Aptekman, Dr. P. M. (Bogden, Dr. A. E., and) : Blood Groups in Infrahuman Species (Inherited Haemagglutinogens of Rats and their Relationship to Histocompatability Factors), 654
Aqueous Corrosion of a Magnesium Alloy : Effect of Chloride Concentration on the (D. de G. Jones and H. G. Masterson), 165
Aqueous Silver Nitrate Solution : Ultrasonic Velocity in (D. S. Allam and W. H. Lee), 1388
Aqueous Solution of 5-Hydroxytryptophan treated with X-Rays : Formation of 5-Hydroxytryptamine in an (M. Jović and Z. Supek), 383
Aqueous Solutions : Ultrasonic Velocity in, of some Electrolytes (M. G. Seshagiri Rao and Prof. R. Ramachandra Rao), 164
Aqueous Solutions and in Thulium Oxide : Rapid Determination of Ytterbium in, by Neutron Activation (Minoru Okada), 1090
Aqueous Suspensions of Starved Stationary Phase *Aerobacter aerogenes* : Induced Enzyme Synthesis in (R. E. Strange), 1272
Araki, T., and others : Potential Changes produced by Application of Current Steps in Motoneurones, 1104
Arbiter, Prof. Nathaniel, and Harris, Prof. Colin C. : Power Requirements and Scale-up in Flotation, 268
Arbor Virus Group B : Infectious Ribonucleic Acids derived from Mouse Brains infected with Two Kinds of (Prof. Masahiro Nakamura), 624
Archaeology of Old Smyrna, *review* by Dr. Hugh Plommer, 212
Archer, G. T. : Release of Heparin from the Mast Cells of the Rat, 90
Archibald, Dr. A. R., and others : Teichoic Acids and the Structure of Bacterial Walls, 570
Area (High Surface) : Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of, using a Continuous Flow Calorimeter (G. H. Bell and A. J. Groszek), 1184
Areas of Isolated or Overlapping Normal Probability Curves : Calculation of the (Dr. D. Morison Smith and John C. Bartlet), 688
Argemone mexicana L. and *A. ochroleuca* Sweet : Intra-ovarian Pollination in *Eschscholzia californica* Cham., (Prof. P. Maheshwari and Kusum Kanta), 304
Argentina, Brazil and Israel : Grants from the United States to the International Atomic Energy Agency for Nuclear Equipment in, 442
Argersinger, Jr., Dr. William J., and others: Inorganic Chemistry, *review* by Dr. M. F. Lappert, 1133
Arkell, Gloria M. (Morgan, A., and): Determination of Caesium-137/Potassium Ratios in Diet and in the Human Body by Urine Analysis, 1100
Arm Bone (Australopithecine Upper) : An Australopithecine Scoop made from a Right (Prof. Raymond A. Dart), 372
Armed Forces (United States) : Technical Reports of the, 1347
Armenians in Iran : Virtual Absence of Glutathione Instability of the Erythrocytes among (Dr. J. E. Bowman and Dr. D. G. Walker), 221
Arms and Security, a Mathematical Study of the Causes and Origins of War (Dr. Lewis F. Richardson), edited by Nicolas Rashevsky and Ernesto Trucco, *review* by Sir Cyril Burt, 427
Armstrong, J., and Quilliam, Dr. T. Andrew : Nerve Endings in the Mole's Snout, 1379
Armstrong, J. J., and others : Teichoic Acids and the Structure of Bacterial Walls, 570
Armstrong, L. D., and Christensen, G. N. : Influence of Moisture Changes on Deformation of Wood under Stress, 869
Armstrong, Dr. Terence : Soviet Polar Research, *review*, 841
Armytage, Prof. W. H. G. : A Social History of Engineering, *review* by Prof. M. Postan, 946
Arnstein, H. R. V., and White, A. M. : Vitamin B_{12} and Intrinsic Factor (Biological Function of Vitamin B_{12}), 1155
Aromatic Hydrocarbons (Condensed Ring) : π-Electronic Excitation and Ionization Energies of (Dr. J. B. Birks and M. A. Slifkin), 761
Aromatic Hydrocarbons (Polycyclic) : Non-Enzymic Hydroxylation of (F. Dewhurst and G. Calcutt), 808
Aromatic Keto-Acids : Side-Reactions during Hydrogenolysis of 2,4-Dinitrophenylhydrazones of (Dr. John H. Menkes), 285
Arrangement (Number and), of Osteons in Corresponding Regions of Homotypic Long Bones (Dr. G. Marotti), 1400
Art : Australian Rock, 854
Art and the Engineer (Prof. Misha Black), 949
Art of Interviewing, 228
Art (Cave), of the Conjola District, New South Wales (F. D. McCarthy), 854
Artefact (Tissue Dilution)—a Re-interpretation of Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogenesis (G. R. N. Jones, L. Bitensky, J. Chayen and Prof. G. J. Cunningham), 1203
Arthrobacter : Auxin Production by Species of (Dr. H. Katznelson and Dr. J. C. Sirois), 1323
Arthropod Transmission of Micro-organisms (Dr. John Macleod), 885
Arthur, D. W. G., and Whitaker, E. A. (compiled by) : Orthographic Atlas of the Moon (edited by Gerard P. Kniper), Edition 'A' showing the Standard Orthographic Coordinate Grid, Part I, Central Area, *review* by D. H. P. Jones, 424
Artificial Earth Satellites : A Collection of Tables and Nomograms for the Processing of Observations made on (I. D. Zhongolovich and V. M. Amelin), *review* by D. G. King-Hele, 1135
Artificial Haematites (Natural and) : Hydrogen Reduction Characteristics of (John Henderson), 268
Artificial Production of Buds from the Embryos of *Cuscuta reflexa* (Prof. P. Maheshwari and B. Baldev), 197
Artificial Rations Low in Magnesium : Rapid Development of Hypomagnesaemia in Lactating Cows given (J. A. F. Rook), 1019
Arts : Infra-Red, *review* by Dr. E. S. Waight, 315
Arts and Sciences, American Academy of : Monograph Prizes, 442

Arvola, Dr. Alpo, and Forsander, Dr. Olof : Comparison between Water and Alcohol Consumption in Six Animal Species in Free-choice Experiments, 819
Ärzte : Parasitologie für (Prof. Otto Jírovec), *review* by Prof. G. Lapage, 107
Asahina, E. : Intracellular Freezing and Frost Resistance in Egg-Cells of the Sea Urchin—Intracellular Freezing as a Cause of Frost-killing in relatively Rapidly Frozen Cell Suspensions, 1263 ; A Mechanism to Prevent the Seeding of Intracellular Ice in Freezing Living Cells, 1264
Asbestos Filter Pads for Virus Filtration : A Method of treating (N. St. G. Hyslop), 305
Ascarylose : Dideoxysugars of *Pasteurella pseudotuberculosis*-specific Polysaccharides, and the Occurrence of (Dr. D. A. L. Davies), 43
Ascher, K. R. S., and Cwilich, R. : Acaricidal Properties of Di-(*p*-chlorophenyl)-trifluoromethylcarbinol, 1322
Ascidian Tadpole : Electron Microscope Observations of the Receptors in the Sensory Vesicle of the (Noël Dilly), 786
Ascites Tumour (Ehrlich Mouse) : Effect of Alpha-Amylase on the (Dr. F. Steckerl, A. Ofodile, R. R. Campbell and G. H. Friedell), 1113
Ascitic Hepatoma : Comparison of Pyridine Nucleotides in the Liver and in an (M. Wintzerith N. Klein, L. Mandel and Prof. P. Mandel), 467
L-Ascorbic Acid : Conversion of L-Galactono-γ-lactone into, by Plants (Dr. G. A. D. Jackson, R. B. Wood and M. V. Prosser), 282
L-Ascorbic Acid formed from D-Glucose by the Strawberry (*Fragaria*) : Identity of (Dr. Frank A. Loewus and Stanley Kelly), 1059
Ashcroft, N. W. : Elected to a Research Studentship in physics at Churchill College, Cambridge, 856
Ashhurst, Doreen E. : An Acid Mucopolysaccharide in Cockroach Ganglia, 1224
Asia (South-East) : Ecto- and Endo-Parasites of, *review* by P. F. Mattingly, 948
Asia and Africa : Aeronautical Research in, 1043
Aso, Prof. Kiyoshi, and others : Isolation of Nigerose and Kojibiose from Dextrans, 278
L-Asparaginase Activity of Guinea Pig Serum : Evidence that the, is responsible for its Antilymphoma Effects (J. D. Broome), 1114
Aspects of Haematology : Some, *review* by Dr. M. E. Nutt, 7
Aspects of Optical Lens Performance : Some, Part 1, Secondary Flare in Lenses (K. Hacking) ; Part 2, The Design of Lens Hoods (W. N. Sproson), 1355
Aspects of the Physics of Space Research : Some (Institute of Physics and the Physical Society Residential Symposium on), 229
Aspects (Biochemical), of Resistance to 6-Mercaptopurine in Human Epidermoid Carcinoma Cells in Culture (R. W. Brockman, George G. Kelley, Patricia Stutts and Virginia Copeland), 469
Aspects (Chemical), of the Structure of Small Peptides (Dr. Dorothy Wrinch), *review* by Prof. H. N. Rydon, 105
Aspects (Ecological), of Irrigation in Ancient Mesopotamia (Hans Helback), 227
Aspects (Modern), of Inorganic Chemistry (Prof. H. J. Emeléus and Dr. J. S. Anderson), third edition, *review* by Prof. J. R. Partington, 637
Aspirin : Alleged Anticomplementary Effect of (K. W. Anderson), 1012
Assay (Bio-) of Erythropoietin in Mice made Polycythaemic by Exposure to Air at a Reduced Pressure (Dr. P. Mary Cotes and Dr. D. R. Bangham), 1065
Assay (Urinary): Assessment of Chronic Exposure to Radio-strontium by (Dr. C. L. Comar and Jay Georgi), 390
Assay Procedure : A Nemin (E. J. Winkler, Shimpei Kuyama and Prof. David Pramer), 155
Assessing the Damage caused by *Heterodera rostochiensis* Woll. (E. B. Brown), 937
Assessment of Chronic Exposure to Radio-strontium by Urinary Assay (Dr. C. L. Comar and Jay Georgi), 390
Assessment of Pain (Universities Federation for Animal Welfare Symposium on), 1353
Assignment Problem : A Combinatorial (Prof. C. Radhakrishna Rao), 100
Assistance (Technical), to Overseas Countries (June 17, 1039), *errata*, 24
Associated Electrical Industries, Ltd. : Appointment of L. J. Davies as responsible for the direct supervision of the four Research Laboratories at Aldermaston, Harlow, Manchester and Rugby, 1142
  Appointment of Dr. J. E. Stanworth as director of research, 1142 ; *erratum*, 1349
Associated Populations : Nodal Analysis of (Prof. W. T. Williams and J. M. Lambert), 202
Association of Haemoglobin *N* and Microcythaemia in a Sardinian Family (Prof. E. Silvestroni and I. Bianco), 1208
Associations (The Biotic), of Cockroaches (Dr. Louis M. Roth and Dr. Edwin R. Willis), *review* by David R. Ragge, 106
Astbury, Prof. W. T. : *Obituary* by Dr. I. MacArthur, 331 ; *obituary* by Dame Kathleen Lonsdale, 332
Astbury, the late Prof. W. T., and Beighton, E. : Structure of Feather Keratin, 171
Aston, Prof. J. G. (Mitacek, jun., Paul, and) : Heat Capacity and Resistivity Anomalies in Palladium Hydride, 271
Astronautical Society (American), and others (sponsored by the) : A Symposium on Space Trajectories (edited by the Technical Staff, Research Division, Radiation Incorporated), *review* by D. G. King-Hele, 1334
Astronautics, International Academy of : Daniel and Florence Guggenheim International Astronautics Award to be given to Sir Bernard Lovell, 856
  Symposium on Space-Flight and Re-entry Trajectories, 960
Astronomy : Background to, *review* by Dr. M. A. Hoskin, 840
Astronomy in the Making, *review* by Colin A. Ronan, 314
Athens, British School at : The Annual of the, Nos. 53-54, 1958-1959, *review* by Dr. Hugh Plommer, 212
Atherosclerosis in Rabbits: Effects of Carrageenin on Serum Lipids and (Dr. Katsumi Murata), 189
Atlantic : Evidence of an Eastward Equatorial Undercurrent in the, from Measurements of Current Shear (Arthur D. Voorhis), 157
Atlantic (North) : The (The Floors of the Oceans, I, Text to accompany the Physiographic Diagram of the North Atlantic), (Bruce C. Heezen, Marie Tharp and Maurice Ewing), (Geological Society of America, Special Paper 65), *review* by Dr. G. E. R. Deacon, 531
Atlas of Human Anatomy (edited by Prof. Ferenc Kiss and Prof. János Szentágothai), Vol. 1, Osteology, Syndesmology, Myology ; Vol. 2, Splanchnology, Endocrine Glands, Heart ; Vol. 3, Nervous System, Vascular System, Lymphatic System, Sense Organs, *review* by Prof. J. M. Yoffey, 1232
Atlas (Orthographic), of the Moon (compiled by D. W. G. Arthur and E. A. Whitaker), edited by Gerard P. Kuiper, Edition 'A' showing the Standard Orthographic Coordinate Grid, Part 1, Central Area, *review* by D. H. P. Jones, 424
Atmosphere : Photography of Cerenkov Light from Extensive Air Showers in the (D. A. Hill and N. A. Porter), 690
Atmosphere (Upper): Density of the, determined by Satellite Orbits, and its Variation during 1957-61 (D. G. King-Hele and D. M. C. Walker), 114
Atmospheric Gases : Detection by Ionization of, during Analysis by Gas Chromatography (Dr. Arthur Karmen, Laura Giuffrida and Robert L. Bowman), 906
Atom (Recoil-) Reactions at Low Temperature (Slobodanka Veljković and Dr. Garman Harbottle), 1287
Atomic Absorption Spectroscopy : Determination of Lead in Urine by (J. B. Willis), 381
Atomic Energy, Soviet Journal of : Supplement No. 6, Fast-Neutron Spectroscopy (B. V. Rybakov and V. A. Sidorov), (edited by N. A. Vlasov), translated from the Russian, *review* by Dr. P. A. Egelstaff, 8
Atomic Energy Agency (International) : Appointment of Dr. Arno Sigvard Eklund as director-general, 338
  Grants from the United States for Nuclear Equipment in Argentina, Brazil and Israel, 442
  International Conference on Plasma Physics and Controlled Nuclear Fission Research, 443
  Purpose, Origin, Structure, Growth and Achievements, 1143
  Courses on Practical Applications of Nuclear Energy, 1143
Atomic Energy Agency (International), and others : Conference on the Use of Radioisotopes in Animal Biology and the Medical Sciences, 959
Atomic Energy Authority (United Kingdom) : Progress on the Advanced Gas-cooled Reactor at Windscale, 853
  Seventh Annual Report (1960-61), 857
Atomic Energy Commission (United States), and others : Conference on Transfer of Calcium and Strontium across Biological Membranes, 1250
Atomic Energy Levels in Crystals (J. L. Prather), 1348
Atomic Energy Research Establishment (United Kingdom) : Course on the Principles of Radiation Protection, 856
Atomic Energy Research and Development in Britain, 857
Atomic Existence : A Concept of (Prof. W. H. Watson), 658
Atomic Problem : The—a Challenge to Physicists and Mathematicians (Lancelot Law Whyte), *review* by Prof. R. Eisenschitz, 214
Atomic Radiations and Living Organisms (R. H. Mole), 556
Atomic Research : New Trends in, and their International Significance (Dr. L. Kowarski), 853
Atomic Structure of Graphite and Graphitization (Dr. G. E. Bacon), 27
Atomic and Nuclear Physics (Prof. Robert S. Shankland), second edition, *review* by Dr. E. B. Paul, 8
Atoms (Bridgehead Nitrogen) : Heterocyclic Systems with (William L. Mosby), Part 1, *review* by Dr. G. L. Buchanan, 425
Atoms (Metastable Nitrogen) : New Afterglow Emission associated with (L. Herman and R. Herman), 346
Atractyloside : Inhibition of Phosphorylation by (Dr. A. Bruni and A. R. Contessa), 818
Attention in Peripheral Vision : Effect of (Arthur Thomson), 732 ; (B. Babington Smith), 732
Attitude (Scientific), to Management, *review* by T. H. Hawkins, 209
Audiogenic Seizure : Psychophysiology, Neuropharmacology and Biochemistry of (Centre National de la Recherche Scientifique International Meeting on), 959
Auerbach, Dr. Charlotte : Chemical Genetics, *review*, 841
Augmentation of Toxicity of Monoamine Oxidase Inhibitor by Thyroid Feeding (Richard N. Carrier and Dr. Paul V. Buday), 1107
Augustat, S. : At the International Starch Conference (Degradation of Starch by Physical Means), 236
Augustin, R. : Immunity to Protozoa (Local Immunity to Coccidiosis), 553
Auroral Zone : Electric Currents associated with Geomagnetic Disturbances in the (Dr. F. J. Scrase), 963
Austenite : Transformation of Cathodically Impregnated and Cooled (E. G. Ramachandran and C. Dasarathy), 998
Australia : Age and Kinship in, *review* by Dr. R. Needham, 6
  Future of Nuclear Physics in (Prof. E. W. Titterton), 639
Australia (Commonwealth of) Bureau of Meteorology: Antarctic Meteorology—Proceedings of the Symposium held in Melbourne, 1959, *review* by H. H. Lamb, 210
Australia (Commonwealth of) Scientific and Industrial Research Organization : Overall Management of the Chemical Research Laboratories, 23
  Post-Doctoral Fellowships in Protein Chemistry, 336
  Conference on Food Science, 337
  Retirement of N. A. Esserman as director of the National Standards Laboratory, 542
  Appointment of I. E. Newnham as chief of the Division of Mineral Chemistry, 543
  New Laboratories for the Division of Food Preservation, 546
  Division of Food Preservation Conference on Food Science, 546
Australia (South), Royal Society of : Verco Medal presented to Dr. R. L. Specht, 1247
Australian Institute of Agricultural Science : *Australian Journal of Experimental Agriculture and Animal Husbandry*, No. 1, 544
Australian Journal of Experimental Agriculture and Animal Husbandry, No. 1 (Australian Institute of Agricultural Science), 544
Australian Mammal Society : Election of Officers for 1961-62, 755
Australian Mathematical Society : Second Summer Research Institute, 1145
Australian National University, Canberra : Hon.D.Sc. degree awarded to Sir Rudolph Peters and to Dr. J. L. Pawsey, 443
Australian Rock Art, 854
Australian Schools : Promotion of Science in, 543
Australian (Western) Museum : Annual Report for 1959-60, 335
Australopithecine Scoop made from a Right Australopithecine Upper Arm Bone (Prof. Raymond A. Dart), 372
Australopithecine Upper Arm Bone : An Australopithecine Scoop made from a Right (Prof. Raymond A. Dart), 372
Author and Publisher (Dr. D. Richter), 131
Auto-Immunological Precipitin possibly due to Fibrinolysins : Demonstration by Paper Electrophoresis of an (Marion I. Cooper and J. R. Sykes), 1212

Automation and Computation : Conference on, 1030
Autoradiographic Effect : An (Stuart McLean), 690 ; (A. H. Meleka and W. Barr), 692
Autoxidation of Haemoglobin : Reactions of Haemoproteins with Hydrogen Peroxide and the Supposed Formation of Hydrogen Peroxide during the (Prof. D. Keilin), 769
Auxin Action : Improbability of the Chelation Hypothesis of (Dr. D. D. Perrin), 253
Auxin Production by Species of *Arthrobacter* (Dr. H. Katznelson and Dr. J. C. Sirois), 1323
Average Extinction Coefficient for Re-absorption of Fluorescence Radiation through Uranin Solution (Dr. K. K. Rohatgi and G. S. Singhal), 799
Avery, D. G. (Conn, G. K. T., and) : Infrared Methods, *review* by Dr. E. S. Waight, 315
Avian Lymphomatosis : Cell Transplants of, treated with Hydrogen Peroxide (Dr. V. L. Sanger), 1406
Aviation, Ministry of : Grant of £2,470 to the University of Leeds, for a research project in the Department of Metallurgy, Houldsworth School of Applied Science, 650
Aviation and Cosmonautical Medicine (C.E.R.M.A. International Congress on), 338
Avirulent Strain of *Salmonella typhimurium* : Resistance to Infection with *Salmonella paratyphi C* in Mice parasitized with a relatively (Dr. J. G. Howard), 87
Awa, A. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Effect of Radiation on the Chromosomes of Leucocytes cultured from the Human Buffy Coat), 340
Award for Blood Transfusion work (Oliver Memorial Fund), 336
Awards for Science Teachers in Training (Arthur Guinness Son and Co., Ltd.), 338
Awards (Grants and), for 1962 (Lalor Foundation), 1348
Awards (Technological), National Council for : Report for 1960-61, 754
Awareness (Conscious) : Spike and Wave Discharges and Alterations of (L. Goldie and J. M. Green), 200
Awer, Erika, and others : The Diego Factor in a Puerto Rican Family—a Case of Anti-Diego in Monozygotic Twins, 188
Axelrod, D. I. : How Old are the Angiosperms?, 442
Axelrod, D. I. (Chandler, M. E. J., and) : An Early Cretaceous (Hanterivian) Angiosperm Fruit from California, 442
Axenic Seedlings of *Drosera* (Richard M. Burger), 724
Axis Dispersion (Anisotropy) : Variation of, with Thickness of Nickel-Iron Films (H. Clow), 996
Axon, Dr. H. J. : Microstructural and Chemical Identity of the Bear Creek and Narraburra Iron Meteorites, 1287
Axons (Giant Cell), in *Aplysia depilans*: The Path of the (Dr. G. M. Hughes and L. Tauc), 404
Aylward, Francis, and Wills, Pamela : Isolation of Sterol Esters from Human Faeces, 1397
Ayres, P. J., and others : Aldosterone Excretion and Potassium Retention in Subjects living at High Altitude, 78
6-Aza-Uracil : Chromosomal Elimination induced by (M. Collette Mahoney, Dr. E. R. Witkus and Dr. C. A. Berger), 627
Azotobacter : Oxygen Inhibition of Respiration in (M. J. Dilworth and C. A. Parker), 520

BAARLI, Johan, and others : Radiocaesium and Potassium in Norwegians, 436
Babcock, G. E., and others : Ultracentrifugal Differences in Soybean Protein Composition, 1395
Bacharach, A. L. : At the Scientific Publications Council Meeting on the Publication of Scientific Books (Ownership of Copyright), 131 ; (Contractual Relationships), 132
Backeberg, Curt : Die Cactaceae, Band 5, Cereoideae (Boreocactinae), *review* by N. Y. Sandwith, 1336
Background to Astronomy, *review* by Dr. M. A. Hoskin, 840
Backscatter Observations (Trans-Equatorial), of Magnetically Controlled Ionization (J. A. Thomas), 792
Backward Children in Britain : Education of, 1052
Bacon, Dr. G. E. : Atomic Structure of Graphite and Graphitization, 27
Bacteria of the Intestine (Society for Applied Bacteriology Symposium on), 1151
Bacteria capable of Producing 6-Aminopenicillanic Acid from Benzylpenicillin : A Method for Isolating (Prof. Yukio Kameda, Yukio Kimura, Etsuko Toyoura and Tomoo Omori), 1122
Bacteria (Dextran-producing) : Intracellular Glycosidases of (Dr. R. W. Bailey and Prof. E. J. Bourne), 277
Bacteria (Knallgas), *Hydrogenomonas* : Formation and Utilization of Poly-β-Hydroxybutyric Acid by (Prof. H. G. Schlegel, G. Gottschalk and R. von Bartha), 463
Bacteria (Numbers and Types of), from the Rhizosphere of Pasture Plants—Possible Occurrence of *L*-Forms (G. Jagnow), 1220
Bacteria and Actinomyces : Manual of, *review* by Dr. K. Zinnemann, 1333
Bacteria or Sulphydryl Compounds for Normal Growth : Spontaneous Development of *L* Forms of Streptococci requiring Secretions of (Ayala Frenkel and Dr. Willy Hirsch), 728
Bacterial Genetics : Papers on (selected by Edward A. Adelberg), *review* by L. B. Quesnel, 320
Bacterial Lipopolysaccharide : Sensitivity of Mice to, following Alteration of Activity of the Reticulo-Endothelial System (Dr. G. N. Cooper and Dr. A. E. Stuart), 294
Bacterial Plant Diseases of Central Europe : Some, *review* by Dr. W. J. Dowson, 4
Bacterial Plant Pathogens (C. Stapp), translated by A. Schoenfeld, *review* by Dr. W. J. Dowson, 4
Bacterial Viruses : Papers on (selected by Gunther S. Stent), *review* by L. B. Quesnel, 320
  X-Ray Diffraction Studies of (Dr. A. C. T. North and Prof. Alexander Rich), 1242
Bacterial Walls : Teichoic Acids and the Structure of (Dr. A. R. Archibald, J. J. Armstrong, Prof. J. Baddiley and J. B. Hay), 570
Bactericidal Antibodies (Specific) : Increase in, after Administration of Endotoxin (J. Gabriel Michael, James L. Whitby and Maurice Landy), 296
Bacteriologists (American), Society of (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
Bacteriology (Applied), Society for : Election of Officers, 754
  Symposium on Bacteria of the Intestine, 1151
Baddeley, A. D. : Stimulus-Response Compatibility in the Paired-Associate Learning on Nonsense Syllables, 1327
Baddiley, Prof. J., and others : Teichoic Acids and the Structure of Bacterial Walls, 570
Bailar, Prof. J. C. : Inorganic Polymers (Co-ordination Polymers), 1047
Bailey, Sir Edward, and McCallien, Prof. W. J. : Structure of the Northern Apennines, 1136
Bailey, Elizabeth (Beaven, Dr. G. H., and) : Ultra-violet Absorption Spectra of Proteins in Deuterium Oxide, 914
Bailey, Dr. R. W., and Bourne, Prof. E. J. : Intracellular Glycosidases of Dextran-producing Bacteria, 277
Bainbridge, V. : Warm-water Species in the Plankton off Newfoundland during Winter Months, 1216
Baird, jun., S. L., and others : Spin Resonance and Electrical Conductivity of Indole–Iodine, 168
Baker, Dr. George : Awarded the Research Medal of the Royal Society of Victoria, 1247 ; work of, 1247
Baker, H., and others : Vitamin-Levels in Blood and Serum, 78
Baker, Dr. J. R., and Vickerman, K. : Parasite Biology (Significance of the Trypanosomatid Kinetoplast), 134
Baker, W. T. : Appointed health physicist at the University of Sheffield, 337
Baking Industries Research Association (British) : New Laboratory Wing (Dr. G. A. H. Elton), 233
Bakterien und Actinomyceten : Diagnostik der (Prof. N. A. Krassilnikov), *review* by Dr. K. Zinnemann, 1333
Balasubrahmanyan, K., and Jacob, Dr. J. : Occurrence of *Eutrichocheles modestus* (Herbst) in the Near-shore Waters of Porto Novo, South India, 830
Balchin, Prof. W. G. V. : Water Resources in Wales, 135
  Water Resources of the United States, 444
Baldev, B. (Maheshwari, Prof. P., and) : Artificial Production of Buds from the Embryos of *Cuscuta reflexa*, 197
Baldwin, Prof. Ernest : Memoir to mark the Centenary of the birth of Sir Frederick Gowland Hopkins (Van den Berghs, Ltd.), 439, *erratum*, 546
Ball, Dr. C. J. : Appointed lecturer in metallurgy at the University of Sheffield, 337
Ball, J. K., and others : Induction of Tumours by Polyoma Virus—Enhancement by Chemical Agents, 893
Ballio, A., and others : Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of *Penicillium chrysogenum*, 909
Ballistic Missile and Space Technology—Proceedings of the Fifth Symposium held at Los Angeles, 1960 (edited by Donald P. LeGalley), Vol. 1, Bioastronautics and Electronics, and Invited Addresses ; Vol. 2, Propulsion and Auxiliary Power Systems ; Vol. 3, Guidance, Navigation, Tracking and Space Physics ; Vol. 4, Re-entry and Vehicle Design, *review* by W. H. Stephens, 1131
Bamber, R. K. : Staining Reaction of the Pit Membrane of Wood Cells, 409
Bananas (Sigatoka Disease of), caused by *Mycosphaerella musicola* Leach : Significance of the Anti-Sporulation Effect of Copper Fungicides in the Control of (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476
Bangham, Dr. D. R. (Cotes, Dr. P. Mary, and) : Bio-Assay of Erythropoietin in Mice made Polycythaemic by Exposure to Air at a Reduced Pressure, 1065
Banks, Prof. E., and Robbins, M. : Fluoride-compensated Substitution of Bivalent Cations in the Magnetoplumbite Structure, 1387
Baradell, D. L., and others : Space-Flight and Re-entry Trajectories (Dynamics of Terminal Re-entry—Methods of Control for Re-entry from Escape Speeds), 960
Barber, H. N. (Murfet, I. C., and) : Effects of Gibberellic Acid and Allogibberic Acid on Flowering and Internode-Length in *Pisum sativum*, 514
Barber, M. E. : At the Scientific Publications Council Meeting on the Publication of Scientific Books (Ownership of Copyright), 131 ; (Contractual Relationships), 132
Barbiturates and Eye Vergence (Gerald Westheimer and Cyril Rashbass), 833
Barclay-Smith, Phyllis : International Council for Bird Preservation, 550
Bard, Dr. B. J. A. : Appointed chairman of Dracone Developments, Ltd., 443
Bardell, P. R. : Magnetic Materials in the Electrical Industry, second edition, *review* by A. C. Lynch, 428
Barghoorn, —., and others : How Old are the Angiosperms?, 442
Barium Orthotitanate : Infra-Red Spectroscopic Evidence of Four-fold Co-ordination of Titanium in (P. Tarte), 1002
Barium Oxide : Effect of Ultra-Violet Irradiation on the Electrical Conductivity of Cathodes coated with (Dr. C. H. B. Mee), 1383
Bark of the Jamaican Shade Tree *Pithecolobium saman* Benth. : Pharmacology of the Alkaloidal Fraction from the (B. E. Leonard and Dr. H. S. A. Sherratt), 287
Barker, Dr. H. A. : Vitamin B_{12} and Intrinsic Factor (Differences in Chemical Composition and Molecular Structure between Vitamin B_{12} and its Coenzyme), 1154 ; (B_{12} Coenzymes), 1154
Barker, Dr. S. A., and others : Mannosyl D-Glucosamine Hydrochloride Isomers—*N*-Mannosyl D-Glucosamine Hydrochloride, 142
  Mannosyl D-Glucosamine Hydrochloride Isomers—6-O-Mannosyl D-Glucosamine Hydrochloride, 143
Barker, S. K., and others : Absorption Spectra of γ-Irradiated Lead Silicate Glass, 374
Barile, M. F., and others : Immunofluorescence of Pleuropneumonia-like Organisms isolated from Tissue Cell Cultures, 190
Barium-140 in New Zealand Rainwater (G. S. McNaughton and R. N. Woodward), 1344
Barley : Stabilizing Selection for Heading Time in a Bulk-Hybrid Population of (Dr. S. K. Jain), 1123
Barley during Germination : Effect of Excess Supply of Heavy Metals to, with special reference to Catalase and Peroxidase (Dr. S. C. Agarwala, A. Kumar and C. P. Sharma), 726
Barley Leaf-Spot Disease : Production of Symptoms of, by Culture Filtrate of *Helminthosporium sativum* (S. K. Gayed), 725
Barnett, Dr. C. H., and Marsden, C. D. : Functions of the Mammalian Carotid Rete Mirabile, 88
Barnett, S. F. : Immunity to Protozoa (Immunity in Piroplasmosis), 552

Baron, Ronald L., and others : Biological Activity of a Tri-*o*-Cresyl Phosphate Metabolite, 1396
Baron, Dr. Samuel, and Isaacs, Alick : Mechanism of Recovery from Viral Infection in the Chick Embryo, 97
Barr, W. (Meleka, A. H., and) : An Autoradiographic Effect, 692
Barrier (Electrostatic Diffusion), and Ion Pump : Electrical Properties of Orientated Lipid on a Biological Membrane—an (Dr. J. W. L. Beament), 217
Barrow, Dr. R. F., and others : Rotational Analysis of Absorption Bands of Lead Monoxide, 374
Barson, Dr. C. A. : Appointed a senior Research Fellow in the Department of Chemistry, University of Birmingham, 337
Bartels, R., and others (edited by) : Proceedings of the Fourth Conference on Potato Virus Diseases, Braunschweig 1960 (Biologische Bundesanstalt für Land- und Forstwirtschaft), 657
Bartlett, John C. (Smith, Dr. D. Morison, and) : Calculation of the Areas of Isolated or Overlapping Normal Probability Curves, 688
Bartlett, Prof. M. S. : Stochastic Population Models in Ecology and Epidemiology, *review*, 843
Barton, Prof. D. H. R. : Stereochemistry of Natural Phenolic Compounds (Alkaloid Synthesis), 236
Basch, Dr. R., and Stetson, Dr. C. A. : Blood Groups in Infra-human Species (Assay Method for those Antigens of Mice which elicit Cytotoxin and Haemagglutinin Production), 654
Base Composition (Unusual), of the Ribonucleic Acid of Wild Cucumber Mosaic Virus (H. Yamazaki and Prof. Paul Kaesberg), 96
Bases, R. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Sensitizing Action of Actinomycin *D* and Deuterium Oxide in experiments in which Puck's Single-Cell Technique was employed), 341
Basic Approaches to Structural Analysis, *review* by Prof. R. C. Coates, 1134
Basic Dyestuffs in Clay Mineralogy (Dr. V. S. Ramachandran, K. P. Kacker and N. K. Patwardhan), 696
Basic Science : Disease and the Advancement of (edited by Dr. Henry K. Beecher), *review* by Sir George Pickering, 530
  What is ?, *review* by Sir George Pickering, 530
Basis of the Diploid-like Meiotic Behaviour of Polyploid Cotton (G. Kimber), 98
Basophil Reaction : A New Test for Detecting Anaphylactic Sensitivity—the (Prof. Walter B. Shelley and Lennart Juhlin), 1056
Basu, Santimay, and others : Very-Low-Frequency Modulation of Discrete Frequency Solar Noise Bursts, 56
Batchelor, F. R., and others : Action of Carbon Dioxide on 6-Aminopenicillanic Acid, 910
Batchelor, J. R. : *Obituary* of Dr. P. A. Gorer, 18
Bateman, Dr. Nigel : Simultaneous Eradication of Three Ectoparasitic Species from a Colony of Laboratory Mice, 721
Batra, B. P., and others : Electrical Effects during Dissolution and Precipitation of Electrolytes, 764
Battersea College of Technology : Summer School on Corrosion (Corrosion Problems in the Electrical Power Industry), 651
Baylor, Dr. Edward R. (Kennedy, Prof. Donald, and) : Analysis of Polarized Light by the Bee's Eye, 34
B.B.C. : Saturday Morning Television introduces Science on Saturday, 20
  Facts and Figures about Viewing and Listening, 1051
  Engineering Monograph No. 34, A Mobile Laboratory for U.H.F. and V.H.F. Television Surveys (E. W. Taylor and L. C. Munn), 1355
  Engineering Monograph No. 36, Some Aspects of Optical Lens Performance, Part 1, Secondary Flare in Lenses (K. Hacking), Part 2, The Design of Lens Hoods (W. N. Sproson), 1355
  Engineering Monograph No. 37, An Instrument for Measuring Television Signal-to-Noise Ratio (S. M. Edwardson), 1355
Beam (Supported) : Initial Vibrations of a (Dr. R. V. Sharman and D. White), 692
Beam Reactor (A Molecular), for Examining the Rate, and Primary Products, of Reactive Bimolecular Collisions (C. W. Nutt and A. J. Biddlestone), 798
Beament, Dr. J. W. L. : Electrical Properties of Orientated Lipid on a Biological Membrane—an Electrostatic Diffusion Barrier and Ion Pump, 217
Bean (Pinto) : Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the Rate of Lesion Production on (John Piacitelli and Prof. Vincent Santilli), 624
Bean (Soy-) Protein Composition : Ultracentrifugal Differences in (W. J. Wolf, G. E. Babcock and A. K. Smith), 1395
Bear Creek and Narraburra Iron Meteorites : Microstructural and Chemical Identity of the (Dr. H. J. Axon), 1287
Beard, Dr. David B. : The Dust Cloud about the Earth, 32
Beats between Optical Masers : A Proposal for a Tunable Source of Radiation for the Far Infra-Red using (Dr. D. C. Lainé), 795
Beattie, C. P. : Immunity to Protozoa (Toxoplasmosis), 552
Beatty (Chester) Research Institute (of the Institute of Cancer Research, Royal Cancer Hospital), (Prof. A. Haddow), 430
Beaven, Dr. G. H., and Bailey, Elizabeth : Ultra-violet Absorption Spectra of Proteins in Deuterium Oxide, 914
Beaver, Prof. Paul C. : Control of Soil-transmitted Helminths (United Nations, World Health Organization, Public Health Papers No. 10), 1249
Beaver, Prof. S. H. : Technology and Geography (Presidential Address to Section E (Geography) of the British Association for the Advancement of Science), 986
Beck, Dr. M. T., and Halmos, M. : Stability Constant of Copper (II) Complexes of Reduction Products of 2,2'-Dipyridyl, 1090
Becker, J. V., and others : Space-Flight and Re-entry Trajectories (Dynamics of Terminal Re-entry—Methods of Control for Re-entry from Escape Speeds), 960
Bed of the Sea : Life and Environment on the (Prof. C. M. Yonge), 986
Bee Venom : Effect of, on Concentration of Blood Glucose and Liver Glycogen in Rabbits (Prof. A. H. Mohamed and K. Zaki), 605
Bee's Eye : Analysis of Polarized Light by the (Prof. Donald Kennedy and Dr. Edward R. Baylor), 34
Bee (Honey-) Larval Foods : Chromatographic Investigation of the Methyl Alcohol/Chloroform-soluble Constituents of (N. G. Patel, M. H. Haydak and R. Lovell), 362
Beecher, Dr. H. K. : Assessment of Pain (Effect of Drugs on High-Level Intellectual Performance), 1353
Beecher, Dr. H. K. (edited by) : Disease and the Advancement of Basic Science, *review* by Sir George Pickering, 530
Beesley, W. N. : Parasite Biology (Parasites in relation to Natural Fauna), 135
Beetle *Trypodendron* : Air-Swallowing, a Mechanism in Photic Reversal of the (Prof. Kenneth Graham), 519
Beevers, Prof. H. : Metabolic Production of Sucrose from Fat, 433
  Respiratory Metabolism in Plants, *review* by Prof. W. O. James, 1031
Beevers, Prof. H. (Benedict, C. R., and) : Triphosphopyridine Nucleotide-linked Glyceraldehyde Phosphate Dehydrogenase in a Non-Photosynthetic Plant Tissue, 71
Behaviour : Distribution of Piperidine in the Brain and its Possible Significance in (Dr. L. G. Abood, F. Rinaldi and Virginia Eagleton), 201
  Sense Organs, Integration and (edited by Talbot H. Waterman), (The Physiology of Crustacea, Vol. 2), *review* by Dr. J. Green, 428
Behaviour (Diploid-like Meiotic), of Polyploid Cotton : Basis of the (G. Kimber), 98
Behaviour (Emotional) : Changes in Visual Gnostic Functions and, following Unilateral Temporal Pole Damage in the 'Split-Brain' Monkey (Dr. J. L. deC. Downer), 50
Behaviour (Stomatal), in *Xanthium pennsylvanicum* : Photoperiodic Effects on (Dr. T. A. Mansfield and Prof. O. V. S. Heath), 974
Beighton, E. (Astbury, the late Prof. W. T., and) : Structure of Feather Keratin, 171
Beilby, Hilda M. : Endowment of £800 made to the Royal College of Science and Technology, Glasgow, to be known as the Kelvin Prizes, 1349
Beilby Memorial Fund : Gold Medal and Prize awarded to Dr. Constantin Edeleanu and to Prof. Jack Nutting, 224
Beiser, S. M., and others : Antibodies to Trophoblasts during the Post-Partum Period, 510
Bell, A. E., and others : Evaporated Carbon Films of Large Area for Adsorption Studies by Electron Bombardment, 487
Bell, G. H., and Groszek, A. J. : Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of High Surface Area using a Continuous Flow Calorimeter, 1184
Bell, Dr. G. H., and others : Biorheology and Microcirculation (Capillary Strength Tests in Scurvy and their Reactions to Vitamins C and P Therapy), 758
Bell, G. M., and others : Discreteness-of-Charge Effect and the Stability Theory of Colloids, 699
Bell, P. R. : Failure of Nucleotides to Diffuse Freely into the Embryo of *Pteridium aquilinum*, 91
Bellman, Richard, and Hall, Jr., Marshall (edited by) : Combinatorial Analysis—Proceedings of the American Mathematical Society Symposia in Applied Mathematics, Vol. 10, *review* by Prof. L. S. Goddard, 321
Ben-Yair, Dr. M. (Heller, Dr. L., and) : Formation of Calcium Chloraluminate by Interaction of Portland Cement with Sea-Water and Chloride Solutions, 488
Bence-Jones Proteins : Comparison of Reduced Alkylated Derivatives of some Myeloma Globulins and (Dr. M. D. Poulik and Dr. G. M. Edelman), 1274
Bendicenti Di Girolamo, Dr. A., and others : Activation of Amino-Acids in Micro-organisms which produce Antibiotics, 411
Bendich, Dr. A., and others : Isolation and Properties of Deoxyribonucleic Acid from Mammalian Sperm, 1375
Bending of Rod-like Polyelectrolytes : Displacement Potentials and (Prof. J. A. Christiansen, the late Dr. C. E. Jensen and Dr. Th. Vilstrup), 484
Benedict, C. R., and Beevers, Dr. H. : Triphosphopyridine Nucleotide-linked Glyceraldehyde Phosphate Dehydrogenase in a Non-Photosynthetic Plant Tissue, 71
Benelux Radio Telescope : Cross-Antenna of the Proposed (Prof. W. N. Christiansen and Dr. J. A. Högbom), 215
Benfey, B. G., and Varma, D. R. : Stimulation of the Heart of the Spinal Dog by Phenoxybenzamine, 919
Bensasson, Dr. R. : Effects of Ionizing Radiation on Organic and Biological Solids (Use of Electron-Spin Resonance to Identify and Measure the Free Radicals produced by Irradiation), 1150
Bensley, Dr. E. H.: Elected president of the Nutrition Society of Canada, 335
Benson, Dr. F. A. : Appointed to a readership in electronics in the University of Sheffield, 229
Benson-Evans, Kathryn : Environmental Factors and Bryophytes, 255
Bentley, H. R., and Burgan, J. G.: Cigarette Smoke Condensate—Preparation and Routine Laboratory Estimation, second edition (Tobacco Manufacturers' Standing Committee, Research Paper No. 4), 336
3,4-Benzopyrene : Effect of Ozone on the Fluorescence and Ultra-violet Absorption Spectra of (Z. Morlin and K. M. Sáringer), 907
Benzopyridocarbazoles : A New Family of Potent Carcinogens— (Prof. A. Laccasagne, Prof. N. P. Buu-Hoï, F. Zajdela, F. Périn and P. Jacquignon), 1005
Benzylpenicillin : A Method for Isolating Bacteria capable of Producing 6-Amino-penicillanic Acid from (Prof. Yukio Kameda, Yukio Kimura, Etsuko Toyoura and Tomoo Omori), 1122
Berde, Dr. B., and Saameli, Dr. K. : Effect of Bradykinin on Uterine Activity, 83
Berg, Dr. H., and Schweiss, H. : Photo-Polarography with a Flash-Lamp, 1270
Berg, Dr. W. F. : Appointed professor of the Photographic Institute of the Federal Technical College, Zurich, 1043 ; work of, 1043
Bergel, Prof. F. : Progress Reports and Tumour Research, *review*, 1231
Berger, Dr. C. A., and others: Chromosomal Elimination induced by 6-Aza-Uracil, 627
Bergman, I. : Nomenclature of Silica, 270
Bergström, R. M., and others : Circumscribing Irradiation of Surface Structures of Cylindrical Animal and Plant Cells with Polonium-210 α-Particles, 1165
Berkeley, C. : Indigotin Content of Woad, 1414
Berkeley, E. : Swarming of the Polychaete *Odontosyllis phosphorea* Moore, var. *Nanaimoensis* Berkeley, near Nanaimo, B.C., 1321
Berl, W. G., and Wilson, W. E. : Formation of Boron Nitride in Diborane–Hydrazine Flames, 380
Berland, Bjørn : Copepod *Ommatokoita elongata* (Grant) in the Eyes of the Greenland Shark—a Possible Cause of Mutual Dependence, 829
  Use of Glacial Acetic Acid for Killing Parasitic Nematodes for Collection Purposes, 1320

Berlman, Isadore B. : Pyrene Dimers, 594
Bernardi, Dr. Giorgio, and Sadron, Prof. Charles : Kinetics of the Enzymic Degradation of Deoxyribonucleic Acid into Sub-Units, 809
Bernau, S. J. : Elected to a Research Studentship in mathematics at Churchill College, Cambridge, 856
Bernhart, Dr. F. W. : Correlation between Growth-Rate of the Suckling of various Species and the Percentage of Total Calories from Protein in the Milk, 358
Bernhauer, Prof. K. : Vitamin B_{12} and Intrinsic Factor (B_{12} Coenzymes), 1154 ; (Biosynthesis by Different Pathways), 1155
Berries (Blue-) : The First Anthocyanins appearing during the Ripening of (Dr. Heikki Suomalainen and A. J. A. Keränen), 498
Berry (David Anderson) Prize for 1951 of the Royal Society of Edinburgh awarded to (*a*) Dr. H. B. Hewitt and Dr. C. W. Wilson, and (*b*) to Dr. D. H. Sussdorf, 229
Berry, R. T. (Andrews, J. R., and) : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Survival Curves of Mouse Leukaemic Cells which grow normally under Anoxic Conditions in the Animal), 341
Berson, Dr. Solomon A., and Yalow, Dr. Rosalyn S. : Immunochemical Distinction between Insulins with Identical Amino-Acid Sequences, 1392
Bertelsen, Dr. Sv., and Marcker, K. : Degree of Polymerization of Hyaluronic Acid isolated from Human Aortic Tissue, 386
Bertram, Dr. D. S. : Parasite Biology (Role of Arthropods as Vectors of Parasitic Infections), 134
Beryllium Oxide (Irradiated) : Scattering of Long Wave-length Neutrons by (T. M. Sabine, A. W. Pryor and B. S. Hickman), 1385
Beryllium-7 and Caesium-137 Radioactivity in Ground-Level Air : Comparison of (Dr. P. F. Gustafson, M. A. Kerrigan and S. S. Brar), 454
β-Activity (Gross), in the Thames River (Conn.), (D. M. Skauen, T. Hatfield, D. Hanlon and J. S. Rankin, jun.), 1017
β-Forms (α- and), of Selected Sugars : Relative Sweetness of (Rose Marie Pangborn and Sandra C. Gee), 810
β-Glucuronidase : Inhibition of, by Oral Administration of D-Glucosaccharo-1,4-Lactone (S. Akamatsu, Dr. A. Kiyomoto, S. Harigaya and S. Ohshima), 1298
β-Mercaptoethanol : A Morphogenetic Effect of—Distribution of Water in the Embryo (Dr. Peter Tuft), 1072 ; Distribution of Water in the Chick Blastoderm (A. Jurand and Dr. Peter Tuft), 1073
β-Rays in Dry and Wet Condition : Radiation Sensitivity of *Escherichia coli* to (S. B. Bhattacharjee and N. N. Das Gupta), 1015
Bettelheim, Prof. F. A., and others : Interaction of Lipids and Trypsin in Dimethyl Sulphoxide–Water Systems, 565
Bevington, H. L. S., and others : Significance of the Anti-Sporulation Effect of Copper Fungicides in the Control of Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach, 476
Bewick, A. : General Fellowship in chemistry renewed for 1961-62 by the Ramsay Memorial Trust, 337
Bhaga, M. (Van Zyl, Dr. A., and) : A Spray Method for the Simultaneous Detection of Iodide and Iodinated Amino-Acids on Filter Paper Chromatograms, 275
Bhar, Prof. B. N. : Theoretical Expression for Solubility Product, 1004
Bhatnagar, V. S., and others : Seasonal Variations of Caesium-137 in Air at Ground-Level, 747
Bhattacharjee, S. B., and Das Gupta, N. N. : Radiation Sensitivity of *Escherichia coli* to β-Rays in Dry and Wet Condition, 1015
Bhuchar, V. M. : Chromium, Uranium, Tungsten and Nickel Complexes of Thioglycolic Acid, 489
Bianco, I., and others : Haemoglobin *P* in a Family of Southern Italian Extraction, 292
Bianco, I. (Silvestroni, Prof. E., and) : Abnormal Foetal Haemoglobins, 397
  Association of Haemoglobin *N* and Microcythaemia in a Sardinian Family, 1208
Bianco, L., and others : Intermediate Pool between Amino-Acids and Globin in Duck Blood *in vitro*, 40
Bibliographies (African) : A Bibliography of, covering Territories South of the Sahara (South African Public Library, Grey Bibliographies No. 7), fourth edition, 959
Bibliography of African Bibliographies, covering Territories South of the Sahara (South African Public Library, Grey Bibliographies No. 7), fourth edition, 959
Bibliography of Research Publications on Farm Buildings : Section 5, Buildings for the Drying and Storage of Grain (Agricultural Research Council), 440
Bickerton, Dr. R. J. (Allen, Dr. T. K., and) : Experiments on the Compression of Plasma in Cusp Geometry, 794
Bid (A Take-Over), in Acoustical Training (Prof. E. J. Richards), 748
Biddlestone, A. J. (Nutt, C. W., and) : A Molecular Beam Reactor for Examining the Rate, and Primary Products, of Reactive Bimolecular Collisions, 798
Biemann, Dr. K., and others : Determination by Mass Spectrometry of the Structure of Proline Derivatives from Apples, 380
Bihler, Dr. I. : Appointed I.C.I. Research Fellow at the University of Edinburgh, 337
Bilateral Section of the Splanchnic Nerve : Effect of, on Erythropoiesis (Fumimaro Takaku, Kunitake Hirashima and Prof. Shigeo Okinaka), 500
Bile : Metabolites of Vitamin B_{12} in, after Parenteral Administration of the Vitamin (Dr. John B. Stokes), 807
Bimolecular Collisions (Reactive): A Molecular Beam Reactor for Examining the Rate, and Primary Products, of (C. W. Nutt and A. J. Biddlestone), 798
Binding of Tetracycline to Macromolecules : Mediation of Divalent Metal Ions in the (Dr. Kurt W. Kohn), 1156
Binding (Enzymic), of Hydroxylamine by Fumaric Acid (Prof. Nathan Grossowicz and the late Yehudith Lichtenstein), 412
Bio-Assay of Erythropoietin in Mice made Polycythaemic by Exposure to Air at a Reduced Pressure (Dr. P. Mary Cotes and Dr. D. R. Bangham), 1065
Biochemical Aspects of Resistance to 6-Mercaptopurine in Human Epidermoid Carcinoma Cells in Culture (R. W. Brockman, George G. Kelley, Patricia Stutts and Virginia Copeland), 469
Biochemical Changes in Lethal Staphylococcal Infections in Mice (George W. Counts, Dr. Ian M. Smith, Dr. Joseph I. Routh, Dr. Elia Ch. Hazard and J. F. McTavish), 783
Biochemical Disturbances (Nervous and), following Hexachlorobenzene Intoxication (Dr. F. De Matteis, B. E. Prior and Prof. C. Rimington), 363
Biochemical Effect of Deuterium (Dr. Howard J. Ringold, Dr. Sumner Burstein and Dr. Ralph I. Dorfman), 1294
Biochemical Society : Colloquium on the Training of Biochemists, 24, 955
Biochemistry at the University of Melbourne (Prof. V. M. Trikojus), 1238
Biochemistry of Steroids (Erich Heftmann and Erich Mosettig), *review* by Dr. D. T. Elmore, 111
Biochemistry (Psychophysiology, Neuropharmacology and), of Audiogenic Seizure (Centre National de la Recherche Scientifique International Meeting on), 959
Biochemists : A Reference Book for, *review* by Dr. D. Recaldin, 635
  The Training of (Biochemical Society Colloquium on), 24, 955
Biochemists' Handbook (edited by Dr. Cyril Long), *review* by Dr. D. Recaldin, 635
Biological Activities of Steroids in relation to Cancer—Proceedings of a Conference sponsored by the Cancer Chemotherapy National Service Centre, National Cancer Institute, National Institutes of Health, United States Department of Health, Education and Welfare (edited by Gregory Pincus and Erwin P. Vollmer), *review* by E. Reid, 1235
Biological Activity of a Tri-o-Cresyl Phosphate Metabolite (Prof. John E. Casida, Morifusa Eto and Ronald L. Baron), 1396
Biological Communications in the United States : Improving, 23
Biological Control of Conception (Dr. A. S. Parkes), 1256
Biological Incorporation of Implant Material in the Rat (James Calnan), 1017
Biological Information : Storage and Retrieval of (Dr. G. C. Ainsworth), 12
Biological Membrane : Electrical Properties of Orientated Lipid on a—an Electrostatic Diffusion Barrier and Ion Pump (Dr. J. W. L. Beament), 217
Biological Membranes : Transfer of Calcium and Strontium across (United States Atomic Energy Commission, United States National Institutes of Health, United States National Science Foundation and the New York State Veterinary College Conference on), 1250
Biological Photoreceptor : Transfer Function of a (Dr. Lawrence Stark and Dr. Howard T. Hermann), 1173
Biological Serials Record Centre : Establishment of a, 23
Biological Solids (Organic and) : Effects of Ionizing Radiation on (Société de Chimie Physique and the British Biophysical Society Discussion on), 1150
Biological Work : A Sealing Compound for Use in (G. Stotzky, W. Culbreth and Dr. L. B. Mish), 410
Biological Work in Burma : Use of Radiosiotopes in (Dr. Maung Mya Thaung), 875
Biological and Medical Abstracts, Ltd. : Publication of *Index Biologicus*, 544
Biologically Active Components from Commercial Trypan Blue : Chromatographic Separation of (J. Dijkstra and J. Gillman), 803
Biologicus (*Index*) : Publication of (Biological and Medical Abstracts, Ltd.), 544
Biologische Bundesanstalt für Land- und Forstwirtschaft : Proceedings of the Fourth Conference on Potato Virus Diseases, Braunschweig, 1960 (edited by J. Brandes, R. Bartels, V. Völk and C. Wetter), 657
Biologists (Applied), Association of : Provincial Meeting at the University College of North Wales, Bangor, 856
Biology in Britain (Dr. H. V. Wyatt), 960
Biology : Experimental Method in, from the Time of Antonio Vallisneri to the Present (University of Padua Symposium on), 546
  Parasite (Parasitology Group of the Institute of Biology Meeting on), 134
  Pigment Cell (New York Academy of Sciences fifth International Conference on), 1045
Biology, Institute of (Parasitology Group) : Spring Meeting (Parasite Biology), 134
Biology of Crustacea (Dr. J. Green), *review* by Dr. J. A. Allen, 111
Biology for Grammar Schools (Science Masters' Association and the Association of Women Science Teachers), 555
Biology of Micro-organisms, *review* by Prof. B. C. J. G. Knight, 1032
  An Introduction to the (Lilian E. Hawker, A. H. Linton, B. F. Folkes and M. J. Carlile), *review* by Prof. B. C. J. G. Knight, 1032
Biology of Reproduction in *Rubus* (Dr. G. J. Dowrick), 680
Biology of Stentor (Vance Tartar), *review* by Dr. J. A. Kitching, 1133
Biology and Medicine (Experimental), Society for (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
Biology Syllabus for Sixth Forms (Gulbenkian Foundation Conference on, at the University of Brimingham), 554
Biology Teaching : A New Approach to (Dr. L. H. Finlayson), 554
Biology (Animal), and the Medical Sciences : The Use of Radioisotopes in (International Atomic Energy Agency, the Food and Agriculture Organization and the World Health Organization of the United Nations, Conference on), 959
Biology (Cell), American Society for : Symposium on Cell Continuity, 1045
  Symposium on Cell Diversification, 1045
  Symposium on Characteristics of Cell Interfaces, 1045
Biology (Marine), or Oceanography : Research in (Challenger Society Grants for), 24
Biometrical Genetics : Discriminant Analysis in (Dr. S. K. Jain), 1420
Biophysical Society, British (Société de Chimie Physique and the) : Discussion on the Effects of Ionizing Radiation on Organic and Biological Solids, 1150
Biörck, Prof. Gunnar, and Paléus, Dr. Sven : Cytochrome c in the Heart of the Turtle, 712
Biorheology and Microcirculation (Medical School of the Hebrew University Seminar on), 757
Biosynthesis of Hydrastine (J. R. Gear and Prof. I. D. Spenser), 1393
Biosynthesis of Isoleucine : Release from the Feedback Inhibition controlling the (Masaya Hayashibe and Teijiro Uemura), 1417
Biosynthesis of Polyenoic Fatty Acids in Human Whole Blood (Dr. F. Leupold and G. Kremer), 805
Biosynthesis of the Pyrethrins (Dr. Mary P. Crowley, Helene S. Inglis, M. Snarey and Dr. E. M. Thain), 281
Biosynthesis of Riboflavin in Cell-free Systems (Prof. T. W. Goodwin and Dr. A. A. Horton), 772
Biosynthesis of Urea : Prevention of Ammonia Toxicity by Amino-Acids concerned in the (Dr. Francesco Salvatore and Dr. Vincenzo Bocchini), 705

Biosynthesis and Activity of Nitrate-Reductase in *Aerobacter aerogenes* : Inhibition by Oxygen of (Francis Pichinoty and Liliane D.Ornano), 879
Biotic Associations of Cockroaches (Dr. Louis M. Roth and Dr. Edwin R. Willis), *review* by David R. Ragge, 106
Birch, H. F., and Friend, M. T. : Resistance of Humus to Decomposition, 731
Bird, Lieut.-Gen. G. W. G. : Haemagglutinins from *Clerodendrum viscosum* Vent, 292
Bird (Oliver) Lecture : Fifth (Dr. A. S. Parkes, Biological Control of Conception), 1256
Bird, R. G. : Parasite Biology (Role of Arthropods as Vectors of Parasitic Infections), 134
Bird, R. G. : Appointed lecturer in the Department of Civil Engineering, University of Birmingham, 337
Bird Preservation, International Council for : Seventh Conference of the European Continental Section (Phyllis Barclay-Smith), 550
Bird Preservation, International Council for (British Section) : Annual Report for 1960, 124
Birds : Effects of Insecticides on, 440
Birds in a Changing World (Phyllis Bond), 133
Birds of Eastern and North-Eastern Africa (C. W. Mackworth-Praed and C. H. B. Grant), second edition, *review* by Sir Landsborough Thomson, 9
Birds (Adult) : Comparison of the Serum Protein Fractions of the Newly Hatched Chick with those of, using Starch-gel Electrophoresis (A. Amin), 708
Birds (European), for Caging : Trade in, 124
Birds (Humming-) : Thyroid Function in Active, Sleeping and Torpid (Prof. C. J. Shellabarger, R. C. Lasiewski and G. E. Hyncik), 1318
Birkbeck College, London : Title of professor emeritus of psychology conferred on Prof. C. A. Mace, 546
Birks, Dr. J. B., and Slifkin, M. A. : π-Electronic Excitation and Ionization Energies of Condensed Ring Aromatic Hydrocarbons, 761
Birmingham College of Advanced Technology : Course on Statistical Quality Control and Acceptance Sampling, 337
Birth : Changes in Glucose-6-phosphatase Activity in Liver and Kidney at (M. J. R. Dawkins), 72
Bisby's (Ainsworth and) Dictionary of the Fungi, fifth edition by Dr. G. C. Ainsworth, *review* by Prof. John Colhoun, 109
Bishop, Dr. J., and others : Control of Specificity in Haemoglobin Synthesis, 1365
Biswas, A. B., and others : Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers, 378
Bitensky, L., and others : Tissue Dilution Artefact—a Re-Interpretation of Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogenesis, 1203
Bivalent Cations in the Magnetoplumbite Structure : Fluoride-compensated Substitution of (Prof. E. Banks and M. Robbins), 1387
Black, Prof. Nisha : Art and the Engineer, 949
Black Shale, Oil and Sulphide Ore (Prof. K. C. Dunham), 985
Bladders (Rat) : Incorporation of Phosphorus-32 into Urinary Concretions forming on Zinc Implants in (Dr. Richard F. Keeler and Dr. Stuart A. Lovelace), 715
Blåfield, R. F., and others : Circumscribing Irradiation of Surface Structures of Cylindrical Animal and Plant Cells with Polonium-210 α-Particles, 1165
Blair, Dr. G. W. Scott : Biorheology and Microcirculation, 757
Blair, Dr. G. W. Scott, and Oosthuizen, J. C. : An Invariant Casein Substrate for Rennet Standardization, derivable from Diverse Sources of Cows' Milk, 697
Blake, C. C. F. : Effects of Ionizing Radiation on Organic and Biological Solids (Chemical Changes on Irradiation), 1151
Blake, C. D. : Nematology (*Radopholus similis* causing Root-rot in Banana in New South Wales), 1048
Bland, B. M. : Appointed to the University librarianship in the University of Southampton, 443
Bland, J. A., and others : Synthesis and X-Ray Analysis of Diamond, 865
Blastoderm (Chick) : Distribution of Water in the (A Morphogenetic Effect of Beta-Mercaptoethanol), (A. Jurand and Dr. Peter Tuft), 1073
Blattella germanica : Metabolism of the Sulphur Amino-Acids and of Sulphate in (Richard J. Block and S. Mark Henry), 392
Blight (Leaf) : Susceptibility to, caused by *Cochliobolus heterostrophus*, in Nigerian Varieties of Maize (C. L. M. van Eijnatten), 515
Blin-Stoyle, Dr. R. J. : Appointed professor of theoretical physics at the University of Sussex, 1349
Blindness in Africans in Northern Rhodesia (Dr. C. M. Phillips), 753
Bloch, A. : Damping without Influence on Frequency, 1086
Block, R. J. (edited by Dr. P. Alexander and) : A Laboratory Manual of Analytical Methods of Protein Chemistry (including Polypeptides), Vol. I, The Separation and Isolation of Proteins, *review* by Dr. D. T. Elmore, 320
Block, R. J., and Henry, S. Mark : Metabolism of the Sulphur Amino-Acids and of Sulphate in *Blattella germanica*, 392
Blockage of Transformation of Tryptophan to Nicotinic Acid by Experimental Hepatitis in Mice (M. Piazza, F. Tancredi and Prof. E. Quagliariello), 601
Bloemendal, Dr. H., and others : Modified Cellulose Ion Exchangers and their Use in Ribonucleic Acid Fractionation, 349
Blood : Precorticotrophin in (Dr. P. R. Dasgupta), 1106
Blood and the Exhaled Air of Diabetic Patients : Rapid Infra-Red Determination of Acetone in the (Dr. Richard D. Stewart, Edward A. Boettner and Bruce T. Stubbs), 1008
Blood (Cord) Serums : Haptoglobin Patterns in (A. R. Rausen, P. S. Gerald and Dr. L. K. Diamond), 717
Blood (Duck), *in vitro* : Intermediate Pool between Amino-Acids and Globin in (L. Bianco, G. Giustina and E. Lazzarini), 40
Blood (Human Whole) : Biosynthesis of Polyenoic Fatty Acids in (Dr. F. Leupold and G. Kremer), 805
Blood Cell (Red) : Dimer Formation and Glucose Transfer across the Membrane of the (Dr. W. D. Stein), 1277
Blood Coagulation : Controlling Mechanism of the Mechanical Activity of Muscle and that of (Dr. Masahiro Fujino, Saburo Yamaguchi, Masa Takauji, Kohki Uchida, Masahide Yasuda and Tatsuaki Matsushima), 80
Blood-Feeding, Fecundity and Ageing in *Aedes aegypti* var. *Queenslandensis* (Dr. M. M. J. Lavoipierre), 575
Blood Glucose and Liver Glycogen in Rabbits : Effect of Bee Venom on Concentration of (Prof. A. H. Mohamed and K. Zaki), 605
Blood Group Agglutinogen M^{g} : Action of Influenza Viruses, Receptor-destroying Enzyme and Proteases on (Prof. Georg F. Springer and Dr. Karlheinz Stalder), 187
Blood-Group *A* Substance (Human) : Two Serologically Active Trisaccharides isolated from (I. A. F. Lister Cheese and Prof. W. T. J. Morgan), 149
Blood Group (*M*- and *N*-) Substances : Products of Mild Acid and Alkaline Hydrolysis of (Dr. Elzbieta Romanowska), 1408
Blood Groups of Animals other than Man (Dr. D. G. Gilmour), 653
Blood Groups in Infra-human Species (New York Academy of Sciences Conference on), 653
Blood Sera : Emulsifying Capacity of (Prof. B. Breyer), 174
Blood Serotonin : Changes in, during Exposure to Cold (Dr. Paul Gordon), 183
Blood Transfusion work : Award for (Oliver Memorial Fund), 336
Blood-Typing Compact Human Bone Tissue (H. E. Ezra-Cohn and Prof. S. F. Cook), 1267
'Blood Waves' : Brain Waves and (Henry R. Pantek), 577
Blood and Serum : Vitamin-Levels in (H. Baker, O. Frank, I. Pasher, Dr. H. Sobotka and S. H. Hutner), 78
Bloom, Dr. Joyce L. : Pulmonary Tumours and Group VII in the House-Mouse, 1124
Bloom, Dr. Joyce L. (Falconer, Dr. D. S., and) : Inheritance of Susceptibility to Induced Pulmonary Tumours in Mice, 1070
Blowfly, *Phormia regina* Meigen : Response of the Labellar Hairs of the, to Protein (D. I. Wallis), 917
Blueberries : The First Anthocyanins appearing during the Ripening of (Dr. Heikki Suomalainen and A. J. A. Keränen), 498
Blumer, Max, and Omenn, Gilbert : Fossil Chlorins in a Triassic Sediment, 161
Boaden, P. J. S. : Littoral Interstitial Species from Anglesey representing Three Families New to Britain, 512
Board Makers' Association, British Paper and (Technical Section) : Conference on the Formation and Structure of Paper, 126
Bocchini, Dr. Vincenzo (Salvatore, Dr. Francesco, and) : Prevention of Ammonia Toxicity by Amino-acids concerned in the Biosynthesis of Urea, 705
Böcher, Prof. T. W. : Flora Europaea, 447
Bodard, Dr. M. : Effects of Ionizing Radiation on Organic and Biological Solids (Use of Electron-Spin Resonance to Identify and Measure the Free Radicals produced by Irradiation), 1150
Bodies (Pigment), of the Spinal Neurones of some Teleosts (Prof. A. Stolk), 924
Body : Effect of Administration of Lysergic Acid Diethylamide on Serotonin-Levels in the (Dr. D. V. Siva Sankar, D. Barbara Sankar, Edward Phipps and Eleanor Gold), 499
Body Constituents: Metabolic Stability of (Dr. A. N. Davison and Dr. J. Dobbing), 844
Boeri, Prof. Enzo : *Obituary* by Prof. Britton Chance, 1247
Boettner, Edward A., and others : Rapid Infra-Red Determination of Acetone in the Blood and the Exhaled Air of Diabetic Patients, 1008
Bogden, Dr. A. E., and Aptekman, Dr. P. M. : Blood Groups in Infra-human Species (Inherited Haemagglutinogens of Rats and their Relationship to Histocompatability Factors), 654
Bollard, E. G. (Letham, D. S., and) : Stimulants of Cell Division in Developing Fruits, 1119
Boman, Hans G., and Svensson, Ingvar : Evidence for a New Enzyme connected with Protein Synthesis, 674
Bombardment (Electron) : Evaporated Carbon Films of Large Area for Adsorption Studies by (A. E. Bell, J. Pritchard and Prof. K. W. Sykes), 487
Bombyx mori (Silkworm) : Brain Hormone of the (Mamori Ichikawa and Hironori Ishizaki), 933
    Role of Lipid in the Hardening of the Cuticle in the (Shigemi Kawase), 279
    Sterol Requirements of the (Dr. Toshio Ito), 882
Bond, Dr. Joan, and Cooper, J. G. : Effect of Polyamino Carboxylic Acids on Catalase, 385
Bond, Phyllis : At the Annual Congress of the South-Eastern Union of Scientific Societies (Birds in a Changing World), 133
Bond, T. J., and others : Enzymic Conversion of Tetrahydrofolic Acid to Folinic Acid by Plant Protein, 179
Bond, V. P., and others : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture, 1398
Bond-Lengths in Solid Helium (Dr. A. F. Schuch), 591
Bondi, Prof. H. : Cosmology, second edition, *review* by Prof. W. H. McCrea, 1336
Bone (Australopithecine Upper Arm) : An Australopithecine Scoop made from a Right (Prof. Raymond A. Dart), 372
Bone Tissue (Human) : Blood-Typing Compact (H. E. Ezra-Cohn and Prof. S. F. Cook), 1267
Bones (Homotypic Long) : Number and Arrangement of Osteons in Corresponding Regions of (Dr. G. Marotti), 1400
Bonnell, Prof. B. : Structure and Action of the Sucker of *Echeneis*, 403
Bonner, Dr. T. G. : Title of reader in organic chemistry in the University of London (at Royal Holloway College) conferred on, 229
Bonner, jun., Dr. Walter D., and Voss, Dinor O. : Some Characteristics of Mitochondria Extracted from Higher Plants, 682
Bonnington, S. T. : The British Hydromechanics Research Association, 652
Bonnycastle, Dr. D. D. : Assessment of Pain (Testing of Analgesic Drugs on Animals), 1354
Book (Reference) : Civil Engineering (edited by J. Comrie), *review* by T. M. Charlton, 1032
Book (Reference), for Biochemists, *review* by Dr. D. Recaldin, 635
Books : Recent Scientific and Technical, Supp. iv, July 29 ; Supp. iv, August 26 ; ccccxciii, September 30
Books on the Chemical and Allied Industries (Science Museum Library, London), 22
Books (Scientific) : Publication of (Scientific Publications Council Meeting on), 131

Borden Award of the Nutrition Society of Canada awarded to Dr. Donald Fraser, 335
Borek, Dr. Felix : A New Two-Stage Method for Cross-linking Proteins, 1293
Borenfreund, Dr. E., and others : Isolation and Properties of Deoxyribonucleic Acid from Mammalian Sperm, 1375
Borghese, E. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Film showing the Growth of Mouse Embryonic Lung explanted by the Organ Culture Technique and the Effects of γ-Rays on this Tissue), 342
Borghese, E., and others : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Action of Radiation on Freshly Explanted Cultures of Organized Tissues), 341
Boric Acid: Effect of, on the Phosphoglucomutase of Pea Seeds (Dr. B. C. Loughman), 1399
Born, Prof. G. V. R. : At the Scientific Publications Council Meeting on the Publication of Scientific Books (Contractual Relationships), 132
Borneman, I. D., *et al.* (edited by) : English–Russian Geological Dictionary (T. A. Sofiano), *review* by Dr. S. I. Tomkeieff, 9
Borneo (North), Government of (Royal Society of London in co-operation with the) : North Borneo Expedition 1961, 26
Borneo (North) Expedition 1961 (Royal Society of London in co-operation with the Government of North Borneo), 26
Boron (Polycrystalline) : Electrical Effects observed in (C. H. Carmichael and M. Dore), 485
Boron Nitride : Formation of, in Diborane–Hydrazine Flames (W. G. Berg and W. E. Wilson), 380
Borough Polytechnic, London : Symposium on the Freeze-drying of Foodstuffs, 651
  Lectures on the Technique of Technical Writing, 755
Borrelia anserina (Sakharoff 1891) : Experimental Transmission of, by *Aedes aegypti* (J. A. Roberts), 1225
Borza, Prof. A. : Flora Europaea, 449
Bosch, Dr. L., and others : Modified Cellulose Ion Exchangers and their Use in Ribonucleic Acid Fractionation, 349
Botanical Congress (International) : Symposium on Forest Ecosystem (Montreal, 1959)—Proceedings of the, 755
Botany (Agricultural), National Institute of : Appointment of D. B. MacKay as chief officer of the Official Seed Testing Station, 856
Bottomley, W. (Perrin, Dawn R., and) : Pisatin, an Antifungal Substance from *Pisum sativum* L., 76
Bouillon, A., and Lekie, R. : Cycle and Rhythm in the Ovulation of the Spider *Latrodectus geometricus* C. Koch, 620
Bouldin, D. R. (Hauck, R. D., and) : Distribution of Isotopic Nitrogen in Nitrogen Gas during Denitrification, 871
Boulton, V. R. : At the Second International Measurement Conference (Measuring Systems for Flight Testing of Aircraft), 1350
Bound Forms (Free and), of Vitamin B_{12} : Gel Filtration of Sea-Water—Separation of (Dr. K. W. Daisley), 868
Boura, A. L. A., and others : Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine, 1312
Bourne, Prof. E. J. (Bailey, Dr. R. W., and) : Intracellular Glycosidases of Dextran-producing Bacteria, 277
Bourne, Dr. G. H. (edited by) : The Structure and Function of Muscle, Vol. 2, Biochemistry and Physiology ; Vol. 3, Pharmacology and Disease, *review* by Dr. D. H. Sproull, 948
Bovine Insulins (Cod and) : A Comparison of (Dr. S. Wilson and Dr. G. H. Dixon), 876
Bovine Serum Albumin : Elimination of, in Acute Experimental Uraemia (Dr. G. Gowland, F. G. Smiddy, Dr. R. G. Burwell and F. M. Parsons), 1210
Bowden, Dr. F. P., and Montagu-Pollock, H. M. : Slow Decomposition of Explosive Crystals and their Damage by Fission Fragments, 556
Bowen, G. D., and Rovira, A. D. : Plant Growth in Irradiated Soil, 936
Bowen, Trevor : New Laboratory Wing of the British Baking Industries Research Association opened by, 233
Bowers, R. (Stride, A. H., and) : Two Unexplored Records of Quaternary History, 1001
Bowles Memorial Travel Scholarship (Royal Horticultural Society), 1349
Bowman, Dr. J. E., and Walker, Dr. D. G. : Virtual Absence of Glutathione Instability of the Erythrocytes among Armenians in Iran, 221
Bowman, Robert L., and others : Detection by Ionization of Atmospheric Gases during Analysis by Gas Chromatography, 906
Bowsheer, Dr. D. R., and Albe-Fessard, Prof. D. : Assessment of Pain (Somatic Sensations), 1353
Boyd, Prof. William C., and Reeves, Egli : Specific Inhibition of Anti-*D* Antibody by Colominic Acid, 511
Boys (School) : Research by Sixth-Form (J. Heywood), 860
Braams, Dr. R. A. : Effects of Ionizing Radiation on Organic and Biological Solids (Dry Solids are more Sensitive than Wet Solids or Solutions), 1151
Brachiopods : 'Quick' and 'Catch' Adductor Muscles in (Dr. M. J. S. Rudwick), 1021
Bradford Institute of Technology : Course on Recent Developments in the Chemistry and Technology of Polymers, 1349
Bradley, Prof. D. C., and Westlake, A. H. : Structures of Polymeric Titanium Ethoxide and Titanium Oxide Ethoxides, 273
Bradley, Dr. P. B., and others : Tryptamine Receptors in the Central Nervous System, 1068
Brady, Roscoe O., and Tower, Donald B. (edited by) : The Neurochemistry of Nucleotides and Amino Acids—a Symposium of the Section on Neurochemistry, American Academy of Neurology, *review* by Dr. R. V. Coxon, 318
Bradykinin : Effect of, on Uterine Activity (Dr. B. Berde and Dr. K. Saameli), 83
Bragiński, A., and Merceron, Th. : Magnetic Relaxation on Magnetite and in its Solid Solutions with Manganese and Nickel Ferrites, 898
Braganca, Dr. B. M. : Awarded a grant from the Lady Tata Memorial Trust, for research on leukaemia and allied diseases, 228
Brahma, S. K., and Kundu, N. : Effects of *Grewia populifolia* Extracts on the Development of Chick Embryos, 506
Brahmachari, Dr. H. D., and Sarma, G. Raghupathy : Effects of Injections of some Intermediary Metabolites on the 'Insulin Response' of Normal Rabbits, 491, *erratum*, 755
Brain, Sir Russell : Speech, Handedness and Cerebral Dominance, *review*, 840
  Presidential Address at the Universities Federation for Animal Welfare Symposium on the Assessment of Pain, 1353
Brain : Antibody Response in Rabbits following injection of Sheep Erythrocytes into Lateral Ventricle of (Prof. B. D. Janković, Dr. M. Draškoci and Dr. K. Isaković), 288
  Distribution of Piperidine in the, and its Possible Significance in Behaviour (Dr. L. G. Abood, F. Rinaldi and Virginia Eagleton), 201
  Phosphorus Metabolism of (Dr. P. J. Heald), *review* by Dr. R. V. Coxon, 318
Brain of the Leech : Neurosecretion and Granules in Neurones of the (Irvine R. Hagadorn and Richard S. Nishioka), 1013
Brain of the Rat during 'Metrazol' Convulsions : Changes in the Pentose–Nucleic Acid Content of Sub-Cellular Fractions of the (Prof. G. P. Talwar, B. Sadasivudu and V. S. Chitre), 1007
Brain (Living Rat) : Urea Synthesis in the (R. K. Davies, A. J. Defalco, D. Shander, A. Kopelman and Prof. J. Kiyasu), 288
Brain Hormone of the Silkworm, *Bombyx mori* (Mamori Ichikawa and Hironori Ishizaki), 933
Brain Waves and 'Blood Waves' (Henry R. Pantek), 577
Brains : The Economic Use of (Prof. C. F. Carter), 987
Brains (Mouse), infected with Two Kinds of Arbor Virus Group *B* : Infectious Ribonucleic Acids derived from (Prof. Mashiro Nakamura), 624
Brancati, C., and others : Haemoglobin *P* in a Family of Southern Italian Extraction, 292
Branched Polysaccharides : Separation of Linear from, by Precipitation as Iodine Complexes (Blanche D. E. Gaillard), 1295
Branched Tapeworms, *Hymenolepis nana* (Cestoda : Hymenolepididae) : A Natural Population of Anomalous, in a Colony of *DBA*/1 Mice (Dr. Donald Heyneman), 297
Brandes, J., and others (edited by) : Proceedings of the Fourth Conference on Potato Virus Diseases, Braunschweig, 1960 (Biologische Bundesanstalt für Land- und Forstwirtschaft), 657
Brar, S. S., and others : Comparison of Beryllium-7 and Caesium-137 Radioactivity in Ground-Level Air, 454
Bray, A. : At the Second International Measurement Conference (A Novel Technique for Re-using Resistance Strain Gauges), 1351
Brazil and Israel (Argentina,) : Grants from the United States to the International Atomic Energy Agency for Nuclear Equipment in, 442
Breakdown of [2-^{14}C]-Glycine during Paper Chromatography (A. K. Huggins and V. Moses), 668
Breakdown (Enzymic), of Stigmatic Cuticula of Flowers (W. Heinen and H. F. Linskens), 1416
Breaking Wires at High Speed : Ignition of Gas by Sparks produced by (Dr. R. L. Gordon, Dr. H. Lord and D. W. Widginton), 1085
Breeding (Plant) : Principles of (Prof. R. W. Allard), *review* by Dr. Kenneth S. Dodds, 636
Breeds (Rajasthan Sheep) : Composition of the Skin Follicle Population in seven (Sunanda Narayan), 1115
Bremner, P. M., and others : Tuber Bulking in the Potato Crop, 782
Brenner, M. W., and others : Circumscribing Irradiation of Surface Structures of Cylindrical Animal and Plant Cells with Polonium-210 α-Particles, 1165
Bretylium and Guanethidine : Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), (A. L. A. Boura, F. C. Copp, A. F. Green, H. F. Hodson, G. K. Ruffell, M. F. Sim, E. Walton and E. M. Grivsky), 1312
Breuil, l'Abbé H. : *Obituary* by Miles Burkitt, 1246
Brewer, Dr. D. F., and Keyston, J. R. G. : Specific Heat and Expansion Coefficient of Liquid Helium-3 under Pressure below 0·1° K., 1261
Brewer, Dr. F. M. : Determination of Uranium as Pyrophosphate, 1290
Breyer, Prof. B. : Emulsifying Capacity of Blood Sera, 174
Bridgehead Nitrogen Atoms : Heterocyclic Systems with (William L. Mosby), Part I, *review* by Dr. G. L. Buchanan, 425
Bridgewater, T. H. : *Obituary* of Geoffrey Parr, 332
Briggs, Michael H. : Organic Constituents of Meteorites, 1137
Briggs, Michael H., and Duncan, R. B. : Odour Receptors, 1310
Brightman, R. : *Obituary* of the Rt. Hon. the Lord McGowan, 1041
Brightness in Extragalactic Radio Sources : Distribution of (A. T. Moffet and P. Maltby), 453
Briles, Dr. W. E. : Blood Groups in Infra-human Species (Blood Groups of Chickens), 654
Brillouin, Dr. Léon : Wave Propagation and Group Velocity, *review* by Prof. M. H. L. Pryce, 104
Brimelow, T. : Solar Energy Technology, 1954-1959 (Library Association, Special Subject List No. 34), 228
Brinck, Per, and others (edited by) : South African Animal Life—Results of the Lund University Expedition in 1950-1951, Vol. 7, *review* by Prof. O. Lowenstein, 1135
Britain : Atomic Energy Research and Development in, 857
  Biology in (Dr. H. V. Wyatt), 960
  Control of Colleges of Advanced Technology in, 20
  Crisis in Mathematical Education in, 103
  Education of Backward Children in, 1052
  Forest Products Research in (Prof. M. L. Anderson), 760
  Hospital In-Patients in, 345
  Industrial Research in, 1252
  Littoral Interstitial Species from Anglesey representing Three Families new to (P. J. S. Boaden), 512
  Management in (T. McGivering, D. Matthews and W. H. Scott), 1332
  Management Studies in, 1251
  Production of Gas from Coal in, 225
  Science and the Future of (Labour Party), 837
  Shipbuilding Research in, 543
  Shortage of Mathematics Teachers in, 127
  Social Studies in, 1354
  The Structure of Industry in (Prof. G. C. Allen), 1332
  Survey of Farm Buildings in (Agricultural Research Council), 440
  Training for Management in, 1331
  Universities in, 1027
  Weeds in, *review* by Dr. Alec Lazenby, 842
  See also under Great Britain

British Association for the Advancement of Science : Norwich Meeting of the (D. D. Aldridge), 16
  Election of Sir John Cockcroft as president for 1962, 956
  Norwich and its Region, 956
  Presidential Address, The Humanity of Man (Sir Wilfrid Le Gros Clark), 977
  Summaries of Addresses of Presidents of Sections, 983
  Presidential Address to Section A (Mathematics and Physics), The Interior of the Earth (Dr. R. Stoneley), 983
  Presidential Address to Section B (Chemistry), Chemistry and the Spectroscope (Prof. R. G. W. Norrish), 984
  Presidential Address to Section C (Geology), Black Shale, Oil and Sulphide Ore (Prof. K. C. Dunham), 985
  Presidential Address to Section D (Zoology), Life and Environment on the Bed of the Sea (Prof. C. M. Yonge), 986
  Presidential Address to Section E (Geography), Technology and Geography (Prof. S. H. Beaver), 986
  Presidential Address to Section F (Economics), The Economic Use of Brains (Prof. C. F. Carter), 987
  Presidential Address to Section G (Engineering), World Communications (Sir Gordon Radley), 987
  Presidential Address to Section H (Anthropology), Dating Man's Emergence (Dr. K. P. Oakley), 988
  Presidential Address to Section I (Physiology and Biochemistry), The Senses and the Sciences (Sir Bryan Matthew), 989
  Presidential Address to Section J (Psychology), From Galton to Crowther (Prof. George Seth), 989
  Presidential Address to Section K (Botany), Plant Taxonomy as a Scientific Discipline (Dr. R. E. Holttum), 990
  Presidential Address to Section L (Education), Education in Uganda (Dr. B. E. Lawrence), 990
  Presidential Address to Section M (Agriculture), The Revolution in Agriculture (Sir William Slater), 991
  Presidential Address to Section N (Sociology), African Jurisprudence (Prof. M. Gluckman), 991
  Presidential Address to Section X (General), Science, Politics and Social Service (Earl of Cranbrook), 992
  Annual Report for 1960-61, 1043
British Association for Commercial and Industrial Education : Interviewing in Twenty-six Steps (John S. Gough), 228
  New Routes to Further Education—Papers presented at the B.A.C.I.E. Spring Conference, London (1961), 739
British Baking Industries Research Association : New Laboratory Wing (Dr. G. A. H. Elton), 233
British Biophysical Society (Société de Chimie Physique and the) : Discussion on the Effects of Ionizing Radiation on Organic and Biological Solids, 1150
British Broadcasting Corporation : Saturday Morning Television introduces Science on Saturday, 20
  Facts and Figures about Viewing and Listening, 1051
  Engineering Monograph No. 34, A Mobile Laboratory for U.H.F. and V.H.F. Television Surveys (E. W. Taylor and L. C. Munn), 1355
  Engineering Monograph No. 36, Some Aspects of Optical Lens Performance, Part I, Secondary Flare in Lenses (K. Hacking), Part 2, The Design of Lens Hoods (W. N. Sproson), 1355
  Engineering Monograph No. 37, An Instrument for Measuring Television Signal-to-Noise Ratio (S. M. Edwardson), 1355
British Ceramic Research Association : A. T. Green and the, *review* by Prof. R. W. Douglas, 207
British Columbia : A Lake in, containing Old Sea-Water (Dr. P. M. Williams, Prof. W. H. Matthews and Prof. G. L. Pickard), 830
British Commonwealth, Association of Universities of the : Commonwealth Universities Yearbook 1961, *review*, 843
British Council : *British Medical Bulletin*, Vol. 17, No. 2, Foetal and Neonatal Physiology, 344
British Council (Science Department) : Nuclear Studies, 125
British Dietetic Association and an International Committee of Dietitians : Third International Congress of Dietetics, 1343
British Electrical and Allied Industries Research Association : Annual Report, 450
  New Creep Laboratory, 1049
British Empire Cancer Campaign (Yorkshire Council) : Grant of £1,000 to the Department of Physical Chemistry in the University of Leeds, 650
British Gelatine and Glue Research Association : Twenty-first Meeting of the Research Panel (Dr. D. A. Sutton), 857
British Hydromechanics Research Association : Open Days (S. T. Bonnington), 652
British Institution of Radio Engineers : Grant of a Charter to the, 651
British Insular Races of Small Mammals : Origin of the, and of the 'Lusitanian' Fauna (G. B. Corbet), 1037
British Iron and Steel Research Association : Open Days at the Sheffield Station, 548
  Computer Applications Section of the Operational Research Department renamed the Systems Evaluation Section, 1249
British Isles : Mammals of the (Philip Street), *review* by B. J. G. Maitland, 110
British Libraries : A Soviet View of (V. I. Shunkov, G. G. Firsov and N. I. Tyulina), translated by A. Thompson (Library Association, Pamphlet No. 22), 752
British Medical Bulletin : Vol. 17, No. 2, Foetal and Neonatal Physiology (British Council), 344
British Microlepidoptera (Collection of), belonging to the late L. T. Ford acquired by the British Museum (Natural History), 124
British Museum (Natural History), London : Bulletin, Vol. 5, No. 4, Specimens of very early Pleistocene Age from certain beds in Israel (Dr. Desmond Clark), 23
  Recent Acquisitions, 124
  The Lower Tertiary Floras of Southern England, I, Palaeocene Floras, London Clay Flora (Supplement), (Marjorie Elizabeth Jane Chandler), *review* by Prof. John Walton, 209
British Occupational Hygiene Society (Ergonomics Research Society and the) : Discussion on the Design and Use of Respirators, 655
British Orchid Growers' Association, and others : Third World Orchid Conference, London, 1960—Proceedings of the, *review* by V. S. Summerhayes, 213
British Paper and Board Makers' Association (Technical Section) : Conference on the Formation and Structure of Paper, 126
British Pharmaceutical Conference : Symposium on Skin Medication, 755
British Radio Spectroscopy Group (Institute of Physics and the Physical Society and the) : Joint Conference on Radio Spectroscopy of Solids, 126
British Regional Development Plan for Courses for the Diploma in Technology, 1044
British School at Athens : The Annual of the, Nos. 53-54, 1958-1959, *review* by Dr. Hugh Plommer, 212
British Society for Immunology : Symposium on Immunity to Protozoa, 551
British Society for the Philosophy of Science : Sixth Annual Conference, 338
British Society of Rheology and the Non-Destructive Testing Group of the Institute of Physics and the Physical Society : Joint Conference on Rheological Techniques, 24
British Space Development Co., Ltd. : Aims of the, 230
  Appointment of Sir Bernard Lovell as scientific director, 230
British Surface and Underground Waters : Uranium in (J. D. Peacock), 1189
British Television : Isaac Shoenberg—Silver Jubilee of, 751
British Universities : Scientific Research in, 1960-61 (Department of Scientific and Industrial Research), 228
British Welding Research Association : New Laboratory (R. P. Newman), 1147
Britton, H. G., and others : Fructose and Lactic Acid Metabolism in the Perfused Liver of Premature Lambs, 1307
Briziarelli, Prof. Giuliano : Induction of Cancer in Suckled and Unsuckled Female Rats, 921
Broadcasting (International Telecommunication Union European Conference on), 449
Broadhurst, Dr. J. H. : Appointed senior research associate in nuclear physics in the University of Birmingham, 337
Brock, Sir Russell : Doctors and Disease in Tudor Times, *review*, 428
Brockman, R. W., and others : Biochemical Aspects of Resistance to 6-Mercaptopurine in Human Epidermoid Carcinoma Cells in Culture, 469
Broglio, L. : Space-Flight and Re-entry Trajectories (Dynamics of Terminal Re-entry—Errors in Re-entry Guidance), 960
Bromer, W. W. : Vitamin B_{12} and Intrinsic Factor (Efforts to Purify and Isolate Intrinsic Factor), 1155
Bronmelle, Norman : At the sixty-seventh Annual Conference of the Museums Association (Problems of Conservation), 553
Brook, Dr. P. R. : Appointed lecturer in the Department of Organic Chemistry in the University of Leeds, 650
Brooksby, Dr. J. B. : Hazards of the Animal House, 339
Broome, J. D. : Evidence that the L-Asparaginase Activity of Guinea Pig Serum is responsible for its Anti-lymphoma Effects, 1114
Brothwell, D. R. : The Whaley Skull, 854
Brown, Allan, and Norreys, J. J. : Uranium Disilicide, 61
Brown, Dr. B. R. : Stereochemistry of Natural Phenolic Compounds (Determination of Relative Stereochemistry), 234
Brown, E. B. : Assessing the Damage caused by *Heterodera rostochiensis* Woll., 937
Brown, F. (Goldsmith, P., and) : World-Wide Circulation of Air within the Stratosphere, 1033
Brown, F., and others : Effect of *p*-Fluorophenylalanine on the Multiplication of Foot-and-Mouth Disease Virus, 414
Brown, Prof. Frank A. : Diurnal Rhythm in Cave Crayfish, 929
Brown, G. P. (Aftergut, S., and) : Electronic Properties of Imidazole, 379
Brown, K. N. : Immunity to Protozoa (Immunity in Trypanosomiasis), 552
Brown, Sir Lindor : Awarded a Prize by the Feldberg Foundation for German–English Scientific Exchange for Experimental Medical Research, 1143
  The Adrenergic Neurone, 1143
Brown, P. E. : Appointed senior lecturer in preventive medicine and public health at the University of Sheffield, 337
Brown, Dr. P. P. : Appointed lecturer in bacteriology at the University of Edinburgh, 443
Brown, R. L. : Minimum Energy Theorem for Flow of Dry Granules through Apertures, 458
Brown, W. Duane, and others : Selenomethionine as an Antioxidant, 1201
Brown, W. M. Court, and others : Distribution of Human Chromosome Counts in relation to Age, 1178
Brown, Prof. W. Spencer : Fracture and Fusion of Coccid Chromosomes, 1419
Brown, Picton and Hornby Libraries (Liverpool Libraries, Museums and Arts Committee), 21
Brown-Grant, Dr. K. : Enlargement of Salivary Gland in Mice Treated with *Iso*propylnoradrenaline, 1076
Browne, Hilda M. : The World University Service, 1140
Bruce-Chwatt, L. J. : Immunity to Protozoa (Variability of Immunity in Malaria inherited by an Infant from its Mother), 552
Bruce-Preller Lecture of the Royal Society of Edinburgh (Dr. V. A. Eyles, The Life and Scientific Work of Sir James Hall), 345
Brues, A. M. (Stroud, A. N., and) : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Differences in Synthesis of DNA Response in Two Strains of Pig- and Monkey- Kidney Cells), 341
Bruni, Dr. A., and Contessa, A. R. : Inhibition of Phosphorylation by Atractyloside, 818
Brunn, P., and Gerritsen, F. : Stability of Coastal Inlets, *review* by W. J. Reid, 426
Brustad, Dr. T. : Effect of Ionizing Radiation on Organic and Biological Solids (Role of Oxygen in Radiation Damage), 1150
Bryophyllum fedtschenkoi : An Endogenous Rhythm in the Rate of Dark-Fixation of Carbon Dioxide in Leaves of (Diana M. Warren and Malcolm B. Wilkins), 686
Bryophytes : Environmental Factors and (Kathryn Benson-Evans), 255
Buchanan, Dr. G. L. : Heterocyclic Chemistry, *review*, 425
Buchanan, Prof. R. O. : Title of professor emeritus of geography (at the London School of Economics and Political Science) conferred on, 546
Buchheit, R. G., and others : Recovery and *in vivo* Survival of Rabbit Erythrocytes, 1405
Buchi, Prof. G. : Stereochemistry of Natural Phenolic Compounds (Absolute Configuration of Rotenone and its Derivatives), 235
Buchwald, V. T., and Davis, A. : Surface Waves in Anisotropic Elastic Media, 899

Buday, Dr. Paul V. : Pharmacologically Active Substances present in the Central Nervous System, 245
Buday, Dr. Paul V. (Carrier, Richard N., and) : Augmentation of Toxicity of Monoamine Oxidase Inhibitor by Thyroid Feeding, 1107
Buds from the Embryos of *Cuscuta reflexa* : Artificial Production of (Prof. P. Maheshwari and B. Baldev), 197
Buettner-Janusch, Dr. J. : Blood Groups in Infra-human Species (Reactions of the Red Cells of various Primates with various Antisera prepared to Agglutinate Human *A* or *B* Red Cells), 654
Buffer System : A New Universal (Dr. D. A. Ellis), 1099
Buffers (Acid) : Solubility of Denatured Haemoglobin Variants in (I. D. Fraser and Dr. A. B. Raper), 355
Buffers (Veronal): Use of, in Formalin Fixatives (Dr. S. J. Holt and Dr. R. Marian Hicks), 832
Building Research Station : Open Days, 1053
Buildings for the Drying and Storage of Grain (Bibliography of Research Publications on Farm Buildings, Section 5), (Agricultural Research Council), 440
Buildings (Farm) : Bibliography of Research Publications on, Section 5, Buildings for the Drying and Storage of Grain (Agricultural Research Council), 440
Buildings (Farm), in Britain : Survey of (Agricultural Research Council), 440
Bukovsky, Josef (Eicher, Dr. Herbert J., and) : Intracellular Distribution Pattern of Rat Liver Glutamic-Oxalacetic Transaminase, 243
Bulk-Hybrid Population of Barley : Stabilizing Selection for Heading Time in a (Dr. S. K. Jain), 1123
Bulking (Tuber), in the Potato Crop (R. W. Radley, M. A. Taha and P. M. Bremner), 782
Bullen, Prof. K. E. : Elected a foreign associate of the United States National Academy of Sciences, 18 ; work of, 18
Bullion : From Buttons to, *review* by Prof. R. W. K. Honeycombe, 108
Buluk, K., and others : Conversion of Fibrin to Desmofibrin, 1093
Bunting, Prof. A. H. (Hutchinson, Sir Joseph, and) : A Strategy for the War on Hunger, 539
Burch, Dr. P. R. J. : Radiological Units and Measurements, 28
Burcham, Prof. W. E. : Achievements in Physics, *review*, 423
Burg, Prof. A. B. : Inorganic Polymers (Phosphonitrilic Compounds), 1047
Burg, Ellen A. (Burg, Prof. Stanley P., and) : Ethylene Evolution and Sub-Cellular Particles, 967
Burg, Prof. Stanley P., and Burg, Ellen A. : Ethylene Evolution and Sub-Cellular Particles, 967
Burgan, J. G. (Bentley, H. R., and): Cigarette Smoke Condensate—Preparation and Routine Laboratory Estimation, second edition (Tobacco Manufacturers' Standing Committee, Research Paper No. 4), 336
Burger, Dr. M. (Lacko, Dr. L., and) : Common Carrier System for Sugar Transport in Human Red Cells, 881
Burger, Richard M. : Axenic Seedlings of *Drosera*, 724
Burges, Prof. N. A. : Flora Europaea (Problems of Abstracting and Indexing European Taxonomic Literature), 448
Burgess, Paul R. (Wilson, Dr. Victor J., and) : Changes in the Membrane during Recurrent Disinhibition of Spinal Motoneurones, 918
Burkin, A. R., and Halsey, G. : Chemisorption at Solid–Liquid Interfaces, 348
Burkitt, Miles : *Obituary* of l'Abbé H. Breuil, 1246
Burma : Use of Radio-isotopes in Biological Work in (Dr. Maung Mya Thaung), 875
Burman, Dr. N. P. : Bacteria of the Intestine (Optimum Growth Temperature Characteristics of Coli-aerogenes), 1154
Burns, Dr. James : Elected president of the Institute of Fuel, 23
Burrows, R. B. (Phillips, A. P., and) : Pyridine Derivatives with Anthelmintic Activity, 707
Bursaries (Commonwealth) Scheme, Royal Society and Nuffield Foundation : Awards, 126
Burstein, Dr. Sumner, and others : A Biochemical Effect of Deuterium, 1294
Burt, Sir Cyril : Mathematical Studies in the Psychology of War, *review*, 427
Burwell, Dr. R. G., and others : Elimination of Bovine Serum Albumin in Acute Experimental Uraemia, 1210
Busev, A. I., and Polianskii, N. G. : The Use of Organic Reagents in Inorganic Analysis (translated from the Russian by J. T. Greaves), *review* by Dr. D. T. Lewis, 1336
Business Machines (International), United Kingdom, Ltd. : IBM Research Fellowships, 442
  Endowed Research Time, 442
Butcher, N. E. : Age of the Orogeny and Granites in South-West England, 486
Butler, Prof. J. A. V. : Effects of Ionizing Radiation on Organic and Biological Solids (Need for a Re-appraisal of such terms as 'Direct' and 'Indirect' Effect, 'Target', etc.), 1150 ; (Role of Oxygen in Radiation Damage), 1150
Butler, Dr. J. R. : Appointed reader in pure geochemistry at the Imperial College of Science and Technology, London, 1349
Butterfat (New Zealand) : Growth of Ryegrass-dominant Pasture grazed by Cows, and Properties of (F. H. McDowall, W. A. McGillivray and J. C. Hawke), 303
Buttiaux, Dr. R., and Mossel, Dr. D. A. A. : Bacteria of the Intestine (The Significance of Organisms of Supposed Faecal Origin in Food and Water), 1153
Buttons to Bullion : From, *review* by Prof. R. W. K. Honeycombe, 108
Buu-Hoi, Prof. N. P., and others : A New Family of Potent Carcinogens—Benzopyridocarbazoles, 1005
Byers, E. H., and others : Enzymic Conversion of Tetrahydrofolic Acid to Folinic Acid by Plant Protein, 179
Byrne, F. N. (Poole, Prof. J. H. J., and) : Measurement of the Thorium Content of Natural Materials by a γ-Ray Counting Method, 62
Bywater, –. : Hazards of the Animal House, 339

CACTACEAE : Die (Curt Backeberg), Band 5, Cereoideae (Boreocactinae), *review* by N. Y. Sandwith, 1336
Cactus Plant : Diurnal Variation in Transaminase Activity in the (S. K. Mukerji, G. G. Sanwal and Prof. P. S. Krishnan), 516
Cactus Virus in the United States (the late Irene M. Sammons and Prof. M. Chessin), 517
Cadavid, Nestor Gonzalez, and others : Collagen Fractions in the Skin of New-born and Adult Rats, 1297
Caesium-137 in Air at Ground-Level : Seasonal Variations of (K. G. Vohra, V. S. Bhatnagar and C. Rangarajan), 747
Caesium-137 (Beryllium-7 and) Radioactivity in Ground-Level Air : Comparison of (Dr. P. F. Gustafson, M. A. Kerrigan and S. S. Brar), 454
Caesium 137/Potassium Ratios : Determination of, in Diet and in the Human Body by Urine Analysis (A. Morgan and Gloria M. Arkell), 1100
Caging : Trade in European Birds for, 124
Cahn, Dr. R. S. (edited by) : Annual Reports on the Progress of Chemistry for 1959, Vol. 61, *review* by Prof. J. R. Partington, 637
Calcium-45 : Calcium Metabolism in Man with—Malabsorption Syndrome and Exudative Enteropathy (Dr. G. Milhaud and Dr. P. Vesin), 872
Calcium-45 in the Rat : Effect of Serotonin on Absorption and Distribution of (Prof. S. Garattini, E. Grossi, P. Paoletti, R. Paoletti and M. Poggi), 185
Calcium Chloraluminate : Formation of, by Interaction of Portland Cement with Sea-Water and Chloride Solutions (Dr. L. Heller and Dr. M. Ben-Yair), 488
Calcium Chloride : The Action of, on Mortar and Concrete (M. Lawrence and H. E. Vivian), 125, *erratum*, 1145
Calcium Metabolism of Human Beings : Effect of Sodium Fluoride on (Dr. Clayton Rich and John Ensinck), 184
Calcium Metabolism in Man with Calcium-45—Malabsorption Syndrome and Exudative Enteropathy (Dr. G. Milhaud and Dr. P. Vesin), 872
$CaO–Al_2O_3–MgO$: Ternary Compound Formation in the System (J. H. Welch), 559
Calcium Sulphate Hemihydrate : Reactivity of (M. J. Ridge, H. Surkevicius and Angela A. Milne), 704
Calcium (Plasma), in the Calf : Importance of Magnesium in the Control of (Dr. R. H. Smith), 181
Calcium (Strontium-90 and), given Orally : Failure of a Dog to Discriminate between (Dr. Betsy J. Stover, M. Goldman and A. C. Andersen), 713
Calcium and Strontium : Transfer of, across Biological Membranes (United States Atomic Energy Commission, United States National Institutes of Health, United States National Science Foundation and the New York State Veterinary College Conference on), 1250
Calculation of the Areas of Isolated or Overlapping Normal Probability Curves (Dr. D. Morison Smith and John C. Bartlet), 688
Calculations : Rapid Statistical (M. H. Quenouille), *review* by M. J. R. Healy, 1236
Calculations (Nuclear Reactor) : Digital Computers and (Ward C. Sangren), *review* by Dr. M. V. Wilkes, 9
Calcutt, G. (Dewhurst, F., and) : Non-Enzymic Hydroxylation of Polycyclic Aromatic Hydrocarbons, 808
Calder, D. M., and Cooper, J. P. : Effect of Spacing and Nitrogen-level on Floral Initiation in Cocksfoot (*Dactylis glomerata* L.), 195
Caldera (Aira), Kyusyu, Tokyo : Gravity Survey on the (Izumi Yokoyama), 966
Calf : Importance of Magnesium in the Control of Plasma Calcium in the (Dr. R. H. Smith), 181
Calf Thymus Histone : Effect of, on Rats bearing Transplantable Acute Myeloid Leukaemia (Dr. Lubomir Hnilica and Dr. Viktor Holoubek), 922
California : An Early Cretaceous (Hanterivian) Angiosperm Fruit from (M. E. J. Chandler and D. I. Axelrod), 442
Call, F., and Hague, N. G. M. : Nematology (Diffusion of Ethylene Dibromide through Soil), 1049
Callender, Charles (edited by Sol Tax and) : Evolution after Darwin, Vol. 3, Issues in Evolution, *review* by Dr. P. G. Fothergill, 425
Calnan, James : Biological Incorporation of Implant Material in the Rat, 1017
Calories/gm. in Species of Animals (Prof. L. B. Slobodkin and S. Richman), 299
Calories (Total), from Protein in the Milk : Correlation between Growth-Rate of the Suckling of various Species and the Percentage of (Dr. F. W. Bernhart), 358
Calorimeter (Continuous Flow) : Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of High Surface Area using a (G. H. Bell and A. J. Groszek), 1184
Calvert, D., and others : Discreteness-of-Charge Effect and the Stability Theory of Colloids, 699
Cameron, Margaret : At the Third International Congress of Dietetics (Protein Values of Foods, Meals and Mixtures as eaten by Man), 1344
Campbell, A. I. : Shortening the Juvenile Phase of Apple Seedlings, 517
Campbell, Dr. I. G. M. : Title of reader in chemistry in the University of Southampton conferred on, 443
Campbell, L. G. : Awarded a Royal Society and Nuffield Foundation Commonwealth Bursary, 126
Campbell, R. R., and others : Effect of Alpha-Amylase on the Ehrlich Mouse Ascites Tumour, 1113
Canada : Forest Insect Pests and Diseases in (Prof. M. L. Anderson), 1260
Canada, Department of Forestry (Forest Entomology and Pathology Branch): Annual Report of the Forest Insect and Disease Survey (1960), 1260
Canada, National Research Council of : Problems of the North (Translation of *Problemy Severa*), No. 1, 1958 (translated December 1960), *review* by Dr. Terence Armstrong, 841
Canada, Nutrition Society of : Election of Officers, 335
  Borden Award awarded to Dr. Donald Fraser, 335
  Symposium on Current Problems in Nutrition, 335
Canadian Embassy in Washington : Appointment of Dr. E. O. Hughes as scientific attaché, 542
Cancer : Biological Activities of Steroids in relation to—Proceedings of a Conference sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, United States Department of Health, Education and Welfare (edited by Gregory Pincus and Erwin P. Vollmer), *review* by E. Reid, 1235
  Eleanor Roosevelt Fellowships for Research on (International Union against Cancer), 125
  Induction of, in Suckled and Unsuckled Female Rats (Prof. Giuliano Briziarelli), 921
  Steroids and, *review* by E. Reid, 1235
Cancer, International Union against : Eleanor Roosevelt Fellowships for Research on Cancer, 125
  Grant from the Gulbenkian Foundation, 754

Cancer Campaign (British Empire) (Yorkshire Council) : Grant of £1,000 to Department of Physical Chemistry in University of Leeds, 650
Cancer Cells (HeLa) (Human), in Culture : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Cancer Hospital (Royal) : The Chester Beatty Research Institute of the Institute of Cancer Research (Prof. A. Haddow), 430
Cancer Institute (United States National), and others (sponsored by the) : Proceedings of a Conference on Biological Activities of Steroids in relation to Cancer (edited by Gregory Pincus and Erwin P. Vollmer), *review* by E. Reid, 1235
Cancer Problem : A Clinical Prospect of the (Prof. D. W. Smithers), *review*, 1336
Cancer (Anti-) Properties of Thio-diglycollic Acid (M. B. Sahasrabudhe, M. V. Narurkar, L. B. Kotnis, B. D. Tilak, L. C. Shah, V. N. Gogte), 388
Cannabis sativa : Essential Oil from Fresh, and its Use in Identification (L. Martin, Dr. D. Morison Smith and C. G. Farmilo), 774
Capacity (Emulsifying), of Blood Sera (Prof. B. Breyer), 174
Capacity (Heat), and Resistivity Anomalies in Palladium Hydride (Paul Mitacek, jun., and Prof. J. G. Aston), 271
Capellaro, David F., and others: A Hypodermic Probe using Fibre Optics, 927
Capillary Columns (Solid-coated) : Deterioration of, on Standing (George M. Rouayheb and William C. Hamilton), 801
Capillary Discharge (Direct-Current) : Pressure Differences in a (D. S. Smith), 265
Capital Expenditure of Colleges and Universities in the United States for research and development in the Natural and Social Sciences in 1958 (United States National Science Foundation), 545
Captive Weaver-Finches (*Quelea quelea*) : Melanism in (Dr. B. Lofts), 993
Capture, Carriage and Deposition (Wettability and the), of Particles by Raindrops (R. R. Davies), 616
Carbohydrate (Multi-) Media : A Micro-method for Performing the Phenylalanine Test on Cultures from (D. J. Stewart), 521
Carbon-14 from Nuclear Explosions as a Short-term Dating System—Use to determine the Origin of Heartwood (Dr. A. T. Wilson), 714
5,10,15 ^{14}C-Farnesol Pyrophosphate : Incorporation of, into *Phycomyces carotenoids* (H. Yamamoto, H. Yokoyama, K. Simpson, T. O. M. Nakayama and C. O. Chichester), 1299
[2-^{14}C]-Glycine : Breakdown of, during Paper Chromatography (A. K. Huggins and V. Moses), 668
^{14}C-Tetrachloroethylene in Mice : Urinary Metabolites in (Dr. Sven Yllner), 820
^{14}C-Uridine : Catalysis of Incorporation of, into Rat Lens Ribonucleic Acid by Rat Lens Enzyme System (Dr. Anima Devi and Dr. N. K. Sarkar), 1094
Carbon Compounds : Chemistry of (edited by Dr. E. H. Rodd), Vol. 4, Part C, Heterocyclic Compounds, *review* by Dr. James D. Loudon, 214
Carbon Dioxide : Action of, on 6-Amino-penicillanic Acid (F. R. Batchelor, D. Gazzard and J. H. C. Nayler), 910
  Irradiation Decomposition of (T. B. Copestake and N. S. Corney), 1192
Carbon Dioxide in Leaves of *Bryophyllum fedtschenkoi* : An Endogenous Rhythm in the Rate of Dark-Fixation of (Diana M. Warren and Malcolm B. Wilkins), 686
Carbon Dioxide and Ethanol as Factors controlling the Growth of Etiolated Oat Seedlings (Dr. C. L. Mer), 260
Carbon Films (Evaporated), of Large Area for Adsorption Studies by Electron Bombardment (A. E. Bell, J. Pritchard and Prof. K. W. Sykes), 487
Carbon Monoxide: Selectivity of Palladium Catalysts for the Oxidation of, in the Presence of Hydrogen (P. Mars and Th. van de Mond), 1003
Carbonates (Water-soluble) : Infra-Red Spectra of (Herman A. Szymanski and Richard Povinelli), 64
Carbonyls : Girard *T* Reagent for (A. M. Gaddis, Rex Ellis and G. T. Currie), 1391
Carboxylic Acids (Polyamino) : Effect of, on Catalase (Dr. Joan Bond and J. G. Cooper), 385
Carcinogenesis (Chemical) : Tissue Dilution Artefact—a Re-interpretation of Variations in Levels of Succinic Dehydrogenase during (G. R. N. Jones, L. Bitensky, J. Chayen and Prof. G. J. Cunningham), 1203
Carcinogenicity (Oxidative Demethylation and), of 4-N-Dimethylaminoazobenzene Derivatives (Prof. Morizo Ishidate and Akira Hanaki), 1198
Carcinogens : Kennaway and the (Prof. Antoine Lacassagne), 743
Carcinogens (Potent) : A New Family of—Benzopyridocarbazoles (Prof. A. Lacassagne, Prof. N. P. Buu-Hoï, F. Zajdela, F. Périn and P. Jacquignon), 1005
Carcinoma (Mouse) : Effect of Irradiated Normal Tissues on the Growth of (Dr. Arthur Compton and Prof. Charles A. Pannett), 1101
Carcinoma Cells (Human Epidermoid), in Culture : Biochemical Aspects of Resistance to 6-Mercaptopurine in (R. W. Brockman, George G. Kelley, Patricia Stutts and Virginia Copeland), 469
Cardiac Contraction and Size of the Heart : Relationship between Velocity of—a Hypothesis (Dr. H. Feinberg), 1373
Carlile, M. J., and others : An Introduction to the Biology of Micro-organisms, *review* by Prof. B. C. J. G. Knight, 1032
Carlsberg Foundation and the Wellcome Trust : Award of Carlsberg–Wellcome Research Fellowships for 1960-61, 754
Carlson, Prof. Elof Axel : Limitations of Geometrical Models for Complementation Mapping of Allelic Series, 788
Carlson, J. B. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Grasshopper Neuroblast Technique for Quantitative Radiation Studies), 342
Carlson, J. G., and others : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Action of Radiation on Freshly Explanted Cultures of Organized Tissues), 341
Carlson, Dr. K. D. : Awarded a United States fellowship in chemistry for 1961-62 by the Ramsay Memorial Trust, 337
Carman, P. C., and others : Kirkendall Effect in Gases, 375
Carmichael, C. H., and Dore, M. : Electrical Effects observed in Polycrystalline Boron, 485
Carnation : Flower Initiation in the, in Response to Photoperiod (Dr. G. P. Harris and Juliet E. Griffin), 614
Carnegie United Kingdom Trust : 47th Annual Report (1960), 756
Caro, Lucien G. : Proposed Use of Magnetic Fields in Electron Microscopic Radio-autography, 1188
Carotid Rete Mirabile (Mammalian) : Functions of the (Dr. C. H. Barnett and C. D. Marsden), 88
Carp, Dr. Richard I., and others : Effects of Heavy Water on Poliovirus Multiplication—Results and Speculations on Mechanism, 250
Carp : Function of Taste Fibres in the (Jihei Konishi and Prof. Yngve Zotterman), 286
Carpenter, E. W., and others : Possible Method for Increasing the Signal-Noise Ratio in the Detection of the First Motion of a Refracted *P*-Wave, 1382
Carr, J. R. : Design and Use of Respirators (Design of Underwater Breathing Apparatus), 656
Carr, W. R. (Gelfand, Dr. M., and) : Diabetes Mellitus in Africans in Rhodesia, 227
Carrageenin : Effects of, on Serum Lipids and Atherosclerosis in Rabbits (Dr. Katsumi Murata), 189
Carriage and Deposition (Wettability and the Capture,), of Particles by Raindrops (R. R. Davies), 616
Carrier, Richard N., and Buday, Dr. Paul V. : Augmentation of Toxicity of Monoamine Oxidase Inhibitor by Thyroid Feeding, 1107
Carrier System (Common), for Sugar Transport in Human Red Cells (Dr. L. Lacko and Dr. M. Burger), 881
Carroll, Dr. K. K. : Quantitative Estimation of Peak Areas in Gas–Liquid Chromatography, 377
Carroll, Patricia M. (Gigg, Dr. Roy, and) : A Convenient Synthesis of Muramic Acid and other 3-O-Ethers of D-Glucosamine, 495
Carrots : Occurrence of Mevalonic Acid in (Prof. V. V. Modi and D. K. Patwa), 1202
Carruthers, Dr. W. : Effects of Smoking, *review*, 108
Carter, Prof. C. F. : The Economic Use of Brains (Presidential Address to Section F (Economics) of the British Association for the Advancement of Science), 987
Cartilage Matrix *in vivo* : Action of Plasmin on (Dr. C. H. Lack, A. J. Anderson and S. Y. Ali), 1402
Casein Substrate (An Invariant), for Rennet Standardization, derivable from Diverse Sources of Cows' Milk (Dr. G. W. Scott Blair and J. C. Oosthuizen), 697
Casey, James P. : Pulp and Paper—Chemistry and Chemical Technology, Vol. 2, Papermaking, second edition, *review* by Dr. Julius Grant, 1135
Casida, Prof. John E., and others : Biological Activity of a Tri-o-Cresyl Phosphate Metabolite, 1396
Casson, Sir Hugh : At the sixty-seventh Annual Conference of the Museums Association (Display in Museums), 553
Castorseed and its Products (Council for Scientific and Industrial Research, India, Seminar on), 753
Casu, B.: Adducts of Urea and Thiourea obtained by means of a Sublimation Technique, 802
Cat Tissues : Effects of Drugs on Uptake of Isotopic Norepinephrine by (Hans J. Dengler, Herbert E. Spiegel and Elwood O. Titus), 816
Catalase : Effect of Polyamino Carboxylic Acids on (Dr. Joan Bond and J. G. Cooper), 385
Catalase and Peroxidase : Effect of Excess Supply of Heavy Metals to Barley during Germination, with special reference to (Dr. S. C. Agarwala, A. Kumar and C. P. Sharma), 726
Catalysis of Incorporation of ^{14}C-Uridine into Rat Lens Ribonucleic Acid by Rat Lens Enzyme System (Dr. Anima Devi and Dr. N. K. Sarkar), 1094
Catalysis (Enzyme): Dextransucrase *V* and the Role of Metal Ions in (W. Brock Neely and Julie Hallmark), 385
Catalysts (Palladium), for the Oxidation of Carbon Monoxide in the Presence of Hydrogen : Selectivity of (P. Mars and Th. van de Mond), 1003
Catalytic Chemical System : Sustained Oscillations in a (R. A. Spangler and F. M. Snell), 457
'Catch' ('Quick' and) Adductor Muscles in Brachiopods (Dr. M. J. S. Rudwick), 1021
Catchpole, Dr. Hubert R., and others : Distribution of Sodium and Potassium in certain Cells and Tissues, 1175
Catchpole, J. P. : Awarded the Student's Prize of the Institution of Chemical Engineers, 338
Catechol Amines : Interaction of Hydrocortisone, the Sulphydryl Group, and the (Dr. Sidney Roston), 490
Cathodes coated with Barium Oxide : Effect of Ultra-Violet Irradiation on the Electrical Conductivity of (Dr. C. H. B. Mee), 1383
Cathodically Impregnated and Cooled Austenite : Transformation of (E. G. Ramachandran and C. Dasarathy), 998
Cation Transport (Active), as a Pacemaker of Respiration (Dr. R. Whittam), 603
Cation Vacancies : Quenching of, in Doped Crystals of Sodium Chloride (the late Sir K. S. Krishnan and S. C. Jain), 162
Cations in Oxide Spinels : Dependence on Temperature of the Distribution of (Ranajit K. Datta and Prof. Rustum Roy), 169
Cations (Bivalent), in the Magnetoplumbite Structure : Fluoride-compensated Substitution of (Prof. E. Banks and M. Robbins), 1387
Catsch, Prof. A., and Schindewolf-Jordan, D. : Removal of Internally Deposited Radionuclides by Triethylenetetraamine-hexaacetic Acid, 715
Cattle : Keto-Acids and Amines in Rumen Liquid of (Dr. C. J. G. van der Horst), 73
Caulet, Dr. M., and others : Bacteria of the Intestine (Studies on the Bacterial Flora in the Intestinal Tract of Pigs, 2, Streptococci : Selective Enumeration and Differentiation of the Dominant Groups), 1152
Cause (Possible), of Mutual Dependence : Copepod *Ommatokoita elongata* (Grant) in the Eyes of the Greenland Shark—a (Bjørn Berland), 829
Causes and Origins of War : Arms and Security, a Mathematical Study of the (Dr. Lewis F. Richardson), edited by Nicolas Rashevsky and Ernesto Trucco, *review* by Sir Cyril Burt, 427
Cavalieri, Dr. Ralph R., and others : Decreased Urinary Excretion of 2-Aminoethanol by Irradiated Human Beings, 1303
Cave Art of the Conjola District, New South Wales (F. D. McCarthy), 854
Cave Crayfish : Diurnal Rhythm in (Prof. Frank A. Brown), 929
Caves (Wookey Hole) : Further Observations on a Filamentous Saprophyte from (Dr. A. J. Willis), 724
Cell (Animal), Tissue and Organ Cultures in Radiobiology : Use of (New York Academy of Sciences Symposium on), 340
Cell (Giant Nerve), under Abnormal Conditions : Electrical Activity of a (Dr. Yutaka Oomura, Sachio Ozaki and Takashi Maéno), 1265
Cell (Pigment) Biology (New York Academy of Sciences fifth International Conference on), 1045

Cell (Red Blood) : Dimer Formation and Glucose Transfer across the Membrane of the (Dr. W. D. Stein), 1277
Cell Axons (Giant), in *Aplysia depilans* : The Path of the (Dr. G. M. Hughes and L. Tauc), 404
Cell Biology, American Society for : Symposium on Cell Continuity, 1045
  Symposium on Cell Diversification, 1045
  Symposium on Characteristics of Cell Interfaces, 1045
Cell Continuity (American Society for Cell Biology Symposium on), 1045
Cell Cultures (Tissue) : Immunofluorescence of Pleuropneumonia-like Organisms isolated from (Major W. F. Malizia, M. F. Barile and D. B. Riggs), 190
Cell Dimensions of Layer Lattice Silicates : Surface Symmetry and (E. W. Radoslovich), 67
Cell Diversification (American Society for Cell Biology Symposium on), 1045
Cell Division : Stimulants of, in Developing Fruits (D. S. Letham and E. G. Bollard), 1119
Cell Fractions from Myocardial Slices : Effect of Adrenalectomy and Corticosteroids on Distribution of Radioactivity in Protein of (Dr. Edward I. Weinshelbaum and Dr. Ira G. Wool), 1401
Cell Interfaces : Characteristics of (American Society for Cell Biology Symposium on), 1045
Cell Metabolism : Multiple Inhibitions of, by a Single Substance (M. Harris and Dr. G. A. Morrison), 1276
Cell Nucleus (The Feulgen-Stained Somatic), as a Homogeneously Absorbing Object (Dr. Simon Iversen), 150
Cell Proliferation (Parenchymal Liver), in Normal Adult Rats : Stimulation by Heparin of (Dr. M. Zimmerman and Evemarie Celotti), 1014
Cell Surfaces (Mammalian Tissue) : Sialic Acid as a Structural Component of some (Dr. Leonard Weiss), 1108
Cell Transplants of Avian Lymphomatosis treated with Hydrogen Peroxide (Dr. V. L. Sanger), 1406
Cell-free Spleen Preparations Incubated *in vitro* : Rise of Antibody Titre in (Dr. Dieter H. Sussdorf), 770
Cell-free Systems : Biosynthesis of Riboflavin in (Prof. T. W. Goodwin and Dr. A. A. Horton), 772
Cell-stimulating Hormone (Human Pituitary Interstitial) : An Immunological Study of a (N. R. Moudgal and Prof. Choh Hao Li), 192
Cells : Hexosamine and Sialic Acid Contents in (Atsushi Kimuro, Yasushi Nagai, Dr. Koh-Iti Turumi, Yoshiko Kawashima and Haruo Sato), 596
Cells of *Chara* : The Action Potential in (A. B. Hope), 811
Cells from Liver Tissue : Separation and *in vitro* Culture of (Dr. Justine S. Garvey), 972
Cells (Cylindrical Animal and Plant) : Circumscribing Irradiation of Surface Structures of, with Polonium-210 α-Particles (R. M. Bergström, R. F. Blåfield and M. W. Brenner), 1165
Cells (Egg-) of the Sea Urchin : Intracellular Freezing and Frost Resistance in (E. Asahina)—Intracellular Freezing as a Cause of Frost-killing in relatively Rapidly Frozen Cell Suspensions, 1263 ; A Mechanism to Prevent the Seeding of Intracellular Ice in Freezing Living Cells, 1264
Cells (HeLa) (Human Cancer), in Culture : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Cells (Human Epidermoid Carcinoma), in Culture : Biochemical Aspects of Resistance to 6-Mercaptopurine in (R. W. Brockman, George G. Kelley, Patricia Stutts and Virginia Copeland), 469
Cells (Human Red) : Common Carrier System for Sugar Transport in (Dr. L. Lacko and Dr. M. Burger), 881
  A Relation between the Reactivity of, to Antiglobulin Tests and their Surface Structure (Judith E. Davis, Prof. H. N. Green qnd Pamela W. Tymms), 923
Cells (Human and Mouse Tumour) : Selective Uptake of Specifically Bound Cobalt-58 Vitamin B_{12} by (Dr. Bernard A. Cooper and Dr. William Paranchych), 393
Cells (Immature Germ), of *Drosophila* : Chromosome Rearrangements induced by X-Rays in (Seymour Abrahamson), 523
Cells (*L*1210) : Cytochemistry of (Dr. H. J. Woodliff), 1313
Cells (Living) : Estimation of Time of Generation of (R. J. M. Fry, S. Lesher and H. I. Kohn), 290
Cells (Mammalian), *in vitro* : Incorporation of Deoxyribonucleic Acid by (Prof. E. R. M. Kay), 387
Cells (Mast) : Mechanism of Action of Antigen–Antibody Complexes—their Effect on (Dr. Ivan Mota), 572
Cells (Mast), in Photosensitivity Reactions (V. J. McGovern), 90
Cells (Mast), of the Rat : Release of Heparin from the (G. T. Archer), 90
Cells (Neurosecretory) : Synaptic Transmission in (Hiromichi Morita, Takaaki Ishibashi and Satory Yamashita), 183
Cells (Normal Rat Hepatic) : Nucleolar Localization of Succinic Dehydrogenase in (P. De, R. Chatterjee and Dr. S. Mitra), 925
Cells (Parental Somatic) : Inclusion of, in Sponge Larvae (Frederick E. Warburton), 1317
Cells (Periodic Acid–Schiff Positive Giant), in the Mouse Thymus Cortex (D. Metcalf and M. Ishidate), 305
Cells (Red) : Thromboplastic Activity of (Dr. James A. Inglis and Dr. June W. Halliday), 821
Cells (Red), of the Sheep : Occurrence of an *Rh*-like Antigen in (B. Urbaschek and Prof. K. Trautwein), 193
Cells (Wood) : Staining Reaction of the Pit Membrane of (R. K. Bamber), 409
Cells and Tissues : Distribution of Sodium and Potassium in certain (Dr. Norman R. Joseph, Dr. Milton B. Engel and Dr. Hubert R. Catchpole), 1175
Cellular Material : Estimation of Protein in (Prof. David Racusen and D. R. Johnstone), 492
Cellulose Acetate Membranes : Use of, for Detecting Penicillinase-producing Organisms (Prof. R. Knox and J. T. Smith), 926
Cellulose Ion Exchangers (Modified), and their Use in Ribonucleic Acid Fractionation (Dr. L. Bosch, G. van der Wende and Dr. H. Bloemendal), 349
Cellulose Microfibrils : Constitution of (D. T. Dennis and Prof. R. D. Preston), 667
Cellulose Paper (Diethyl-amino-ethyl (DEAE)) : Chromatography of Proteins on (C. C. Curtain), 1269
Cellulose-decomposing Microflora on Soil Crumb Plates : Factors influencing the Composition of the (A. Keynan, Y. Henis and P. Keller), 307
Celozzi, Evemarie (Zimmerman, Dr. M., and) : Stimulation by Heparin of Parenchymal Liver Cell Proliferation in Normal Adult Rats, 1014
Cement (Portland) : Formation of Calcium Chloraluminate by Interaction of, with Sea-Water and Chloride Solutions (Dr. L. Heller and Dr. M. Ben-Yair), 488
Centaurus *A* (Radio Source) : Central Component of the (P. Maltby), 793
Central Component of the Radio Source Centaurus *A* (P. Maltby), 793
Central Europe : Some Bacterial Plant Diseases of, *review* by Dr. W. J. Dowson, 4
Central Europe (Western and) : Introduction of the Potato into (V. S. Lekhnovitch), 518
Central Nervous System : Enzymic Activity of the (Neurochemical Commission of the World Federation of Neurology International Symposium on), 1349
  Pharmacologically Active Substances present in the (Dr. Paul V. Buday), 245
  Tryptamine Receptors in the (Dr. J. R. Vane, Dr. H. O. J. Collier and Dr. S. J. Corne, and Dr. E. Marley and Dr. P. B. Bradley), 1068
Central Nervous System of an Insect (*Periplaneta americana* L.) : Exchanges of Sodium Ions in the (J. E. Treherne), 1223
Centrally Produced Geometrical Illusions (S. Papert), 733
Centre National de la Recherche Scientifique : International Meeting on Psychophysiology, Neuropharmacology and Biochemistry of Audiogenic Seizure, 959
Cephalosporins : Colorimetric Detection of Penicillins and, on Paper (Robert Thomas), 1161
Ceramic Research Association (British) : A. T. Green and the, *review* by Prof. R. W. Douglas, 207
Cereals (Processing) : Recent Advances in (Food Group of the Society of Chemical Industry Symposium on), 755
Cerebral Dominance : Speech, Handedness and, *review* by Sir Russell Brain, 840
Cerebral Dominance and its relation to Psychological Function (Prof. O. L. Zangwill), *review* by Sir Russell Brain, 840
Cerenkov Light from Extensive Air Showers in the Atmosphere : Photography of (D. A. Hill and N. A. Porter), 690
Cereoideae (Boreocactinae), (Curt Backeberg), (Die Cactaceae, Band 5), *review* by N. Y. Sandwith, 1336
C.E.R.M.A. : International Congress of Aviation and Cosmonautical Medicine, 338
CERN : International Conference on Elementary Particles, 126
Cetrimide–Phloridzin Combination : Enhancement of Inhibition of Intestinal Absorption by (J. A. Nissim), 37
Chachaty, Dr. C. : Effects of Ionizing Radiation on Organic and Biological Solids (Use of Electron-Spin Resonance to Identify and Measure the Free Radicals produced by Irradiation), 1150
Chadwick, Dr. A. : Appointed lecturer in zoology at the University of Leeds, 228
Chaetomium globosum (Kunze) : Spore Dispersal in (P. A. Dixon), 1418
Chain, Prof. E. B., and others : Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of *Penicillium chrysogenum*, 909
Chain Reactions (Radical), in Solution : Generation of Radicals in Pairs and the Kinetics of (Dr. P. E. M. Allen and C. R. Patrick), 1194
Chalcocite and Pyrrhotite in Cold Solution : Formation of Chalcopyrite by Reaction between (W. M. B. Roberts), 560
Chalcopyrite : Formation of, by Reaction between Chalcocite and Pyrrhotite in Cold Solution (W. M. B. Roberts), 560
Challenge to Physicists and Mathematicians : The Atomic Problem—a (Lancelot Law Whyte), *review* by Prof. R. Eisenschitz, 214
Challenger Society : Grants for Research in Marine Biology or Oceanography, 24
Challenger Society (Systematics Society and the) : Symposium on Speciation in the Sea, 229
Chamber Tombs (Prehistoric), of France (Dr. Glyn Daniel), *review* by Prof. C. F. C. Hawkes, 637
Chambers, Ll. G. : The Steady Universe with Charge Excess, 262
  An Unsteady Universe with Excess of Charge, 1082
Chance, Prof. Britton : *Obituary* of Prof. Enzo Boeri, 1247
Chance, Dr. M. R. A. : Assessment of Pain (Central Mechanisms of Pain—Audiogenic Seizures), 1353
Chandler, M. E. J. : The Lower Tertiary Floras of Southern England, 1, Palaeocene Floras, London Clay Flora (Supplement), *review* by Prof. John Walton, 209
Chandler, M. E. J., and Axelrod, D. I. : An Early Cretaceous (Hanterivian) Angiosperm Fruit from California, 442
Changes in Blood Serotonin during Exposure to Cold (Dr. Paul Gordon), 183
Changes in Glucose-6-phosphatase Activity in Liver and Kidney at Birth (M. J. R. Dawkins), 72
Changes in the Membrane during Recurrent Disinhibition of Spinal Motoneurones (Dr. Victor J. Wilson and Paul R. Burgess), 918
Changes in the Pentose-Nucleic Acid Content of Sub-Cellular Fractions of the Brain of the Rat during 'Metrazol' Convulsions (Prof. G. P. Talwar, B. Sadasivudu and V. S. Chitre), 1007
Changes of Permeability of Yeast with Sodium Deoxycholate (Dr. L. Kováč), 698
Changes in Visual Gnostic Functions and Emotional Behaviour following Unilateral Temporal Pole Damage in the 'Split-Brain' Monkey (Dr. J. L. deC. Downer), 50
Changes (Biochemical), in Lethal Staphylococcal Infections in Mice (George W. Counts, Dr. Ian M. Smith, Dr. Joseph I. Routh, Dr. Elia Ch. Hazard and J. F. McTavish), 783
Changes (Induced), in the Amounts of Material in the Neurosecretory System of the Desert Locust (Dr. K. C. Highnam), 199
Changes (Moisture) : Influence of, on Deformation of Wood under Stress (L. D. Armstrong and G. N. Christensen), 869
Changes (Potential), produced by Application of Current Steps in Motoneurones (T. Araki, Dr. M. Ito and T. Oshima), 1104
Changes (Resistivity), due to Formation of *G.P.* Zones (Clusters rich in Solute), (H. Herman and Prof. J. B. Cohen), 63
Changing Flora of South-East England (J. E. Lousley), 133
Changing World : Birds in a (Phyllis Bond), 133
Chapman, J. H., and Molozzi, A. R. : Interpretation of Cosmic Noise Measurements at 3·8 Mc./s. from Satellite 1960 η1, 480
Chapman, Dr. Ross A. : Research in the Food and Drug Directorate, 335

Chara : The Action Potential in Cells of (A. B. Hope), 811
Character (Chemical), of the Pigments of a New Nitrogen-fixing Micro-organism (Gy. Németh, F. Uresch, Prof. G. Fodor and L. Láng), 1413
Character (Diagnostic), for the Identification of Ammocoete Species : Oocyte Numbers as a (Dr. M. W. Hardisty), 1215
Character Recognition : Optical (Information Systems Branch of the Office of Naval Research Information Centre of the United States National Bureau of Standards Symposium on), 1145
Characteristics of Cell Interfaces (American Society for Cell Biology Symposium on), 1045
Characteristics of Mitochondria Extracted from Higher Plants : Some (Dr. Walter D. Bonner, jun., and Dinor O. Voss), 682
Characteristics (Hydrogen Reduction), of Natural and Artificial Haematites (John Henderson), 268
Characterization (Pharmacological), of the Oxytocic Peptides in the Pituitary of a Marine Teleost Fish (*Pollachius virens*), (Prof. H. Heller, B. T. Pickering, J. Maetz and F. Morel), 670
Chargaff, Erwin, and Davidson, J. N. (edited by) : The Nucleic Acids, Vol. 3, *review* by Dr. Roy Markham, 742
Charge : An Unsteady Universe with Excess of (Ll. G. Chambers), 1082
Charge ('Effective' Ionic), in Crystals (Dr. W. Cochran), 60
Charge Electrokinetic), of the Human Erythrocyte : Sialic Acids and the (G. M. W. Cook, Dr. D. H. Heard and Dr. G. V. F. Seaman), 44
Charge Excess : The Steady Universe with (Ll. G. Chambers), 262
Charge-Transfer Complexes : Electron Affinity of the Acceptor Molecule in (Dr. M. E. Peover), 702
Charlton, T. M. : Civil Engineering, *review*, 1032
    Energy Principles in Applied Statics, *review* by Prof. R. C. Coates, 1134
Chatt, Dr. J., and Rowe, G. A. : Complex Compounds of Zero-valent Platinum, 1191
Chatterjee, R., and others : Nucleolar Localization of Succinic Dehydrogenase in Normal Rat Hepatic Cells, 925
Chatterjee, S. : Elected to a Postgraduate Studentship (for a course in statistics) at Churchill College, Cambridge, 856
Chayen, J., and others : Tissue Dilution Artefact—a Re-interpretation of Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogenesis, 1203
Cheese, I. A. F. Lister, and Morgan, Prof. W. T. J. : Two Serologically Active Trisaccharides isolated from Human Blood-Group *A* Substance, 149
Cheeseman, Dr. E. A. : Appointed to a personal chair of medical statistics in the Queen's University, Belfast, 20 ; work of, 20
Chelation Hypothesis of Auxin Action : Improbability of the (Dr. D. D. Perrin), 253
Chemi-Luminescence of Luminol : Inhibition of, by means of Oximes (Prof. K. Weber, J. Matković and D. Fleš), 177
Chemi-Osmotic Type of Mechanism : Coupling of Phosphorylation to Electron and Hydrogen Transfer by a (Dr. Peter Mitchell), 144
Chemical Agents : Induction of Tumours by Polyoma Virus—Enhancement by (K. E. K. Rowson, F. J. C. Roe, J. K. Ball and Dr. M. H. Salaman), 893
Chemical Aspects of the Structure of Small Peptides (Dr. Dorothy Wrinch), *review* by Prof. H. N. Rydon, 105
Chemical Carcinogenesis : Tissue Dilution Artefact—a Re-interpretation of Variations in Levels of Succinic Dehydrogenase during (G. R. N. Jones, L. Bitensky, J. Chayen and Prof. G. J. Cunningham), 1203
Chemical Character of the Pigments of a New Nitrogen-fixing Micro-organism (Gy. Németh, F. Uresch, Prof. G. Fodor and L. Láng), 1413
Chemical Composition of Precipitable Water Vapour over the United States (Dr. Henry M. Papée and Dr. Alberto Montefinale), 136
Chemical Defence Experimental Establishment, Porton : Retirement of Dr. E. A. Perren as director, 750
    Appointment of E. E. Haddon as director, 751
Chemical Engineers, Institution of : Student's Prize awarded to J. P. Catchpole, 338
Chemical Factory : Quality Measurement in the (A. J. Clark and C. W. Munday), (Department of Scientific and Industrial Research), 752
Chemical Genetics, *review* by Dr. Charlotte Auerbach, 841
    An Outline of (Prof. Bernard S. Strauss), *review* by Dr. Charlotte Auerbach, 841
Chemical Identity (Microstructural and), of the Bear Creek and Narraburra Iron Meteorites (Dr. H. J. Axon), 1287
Chemical Industry, Society of : Formation of the Industrial Water and Effluents Group, 853
Chemical Industry, Society of (Food Group) : Symposium on Recent Advances in Processing Cereals, 755
Chemical Kinetics (Applied) : Inversion of a Laplace Transform arising from a Problem in (T. Wood), 589
Chemical Laboratory (National) : Report for 1960 ; Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for 1960 (Department of Scientific and Industrial Research), 342
Chemical Literature : Report on Computer Systems for Searching (E. C. Medam and H. R. Koller), (United States National Bureau of Standards Research Information Centre and Advisory Service on Information Processing), 23
Chemical Processing : Technology of, *review* by R. H. Villet, 1131
Chemical Society : Grants for Research in Chemistry, 651
    Symposium on Electron Spin Resonance, 755
Chemical Society (American), (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
Chemical Society (Czech) : Conference on Paper Chromatography, 759
Chemical System (Catalytic) : Sustained Oscillations in a (R. A. Spangler and F. M. Snell), 457
Chemical Technology : Encyclopedia of (edited by Raymond E. Kirk and Donald F. Othmer), second Supplement Volume edited by Anthony Standen, *review* by R. H. Villet, 1131
Chemical and Allied Industries : Books on the (Science Museum Library, London), 22
Chemie der Organischen Fluorverbindungen (Milos Hudlicky), *review* by Dr. Richard Dresdner, 319
Chemisorption at Solid–Liquid Interfaces (A. R. Burkin and G. Halsey), 348
Chemistry : Annual Reports on the Progress of, for 1959 (edited by Dr. R. W. Cahn), Vol. 61, *review* by Prof. J. R. Partington, 637
    Fellowships in (Ramsay Memorial Trust Awards for 1961-62), 337
    Fluorine, *review* by Dr. Richard Dresdner, 319
    Grants for Research in (Chemical Society), 651
    Heterocyclic, *review* by Dr. G. L. Buchanan, 425
    Inorganic (Dr. Jacob Kleinberg, Dr. William J. Argersinger, Jr., and Dr. Ernest Griswold), *review* by Dr. M. F. Lappert, 1133
    Nuclear Reactor (Oak Ridge National Laboratory Second Conference on), 651
    Progress in, *review* by Prof. J. R. Partington, 637
    Terpene, *review* by Prof. B. C. L. Weedon, 1234
    Transition-Metal, *review* by Prof. C. A. Coulson, 1233
Chemistry for Grammar Schools (Science Masters' Association and the Association of Women Science Teachers), 555
Chemistry in University College, Dublin, 439
Chemistry of the Amino Acids (Dr. Jesse P. Greenstein and Dr. Milton Winitz), *review* by Sir Hans Krebs, 1132
Chemistry of Carbon Compounds (edited by Dr. E. H. Rodd), Vol. 4, Part C, Heterocyclic Compounds, *review* by Dr. James D. Loudon, 214
Chemistry of Heterocyclic Compounds : An Introduction to the (R.M. Acheson), *review*, 320
Chemistry of a Mucopolysaccharide produced by Guinea Pig Lymphocytes (Helen Muir and A. H. E. Marshall), 706
Chemistry of the Terpenes (Dr. A. R. Pinder), *review* by Prof. B. C. L. Weedon, 1234
Chemistry and Industry : Radioactive Tracers in (Pascaline Daudel), translated by Dr. Ulli Eisner, *review* by Prof. J. R. Partington, 637
Chemistry and the Nervous System, *review* by Dr. R. V. Coxon, 318
Chemistry and the Spectroscope (Prof. R. G. W. Norrish), 984
Chemistry and Technology of Polymers : Recent Developments in the (Bradford Institute of Technology Course on), 1349
Chemistry (Analytical), in Nuclear Reactor Technology (Oak Ridge National Laboratory Fifth Conference on), 651
Chemistry (Co-ordination) : An Introduction to (Dr. D. P. Graddon), *review* by Prof. C. A. Coulson, 1233
Chemistry (Fluorine) : Advances in (edited by Prof. M. Stacey, Prof. J. C. Tatlow and Dr. A. G. Sharp), Vol. 1, *review* by Dr. Richard Dresdner, 319
Chemistry (Inorganic) : Modern Aspects of (Prof. H. J. Emeléus and Dr. J. S. Anderson), third edition, *review* by Prof. J. R. Partington, 637
Chemistry (Organic) : Physical Methods of (edited by Arnold Weissberger), third edition, Parts 3 and 4, *review* by Dr. W. A. Waters, 317
Chemistry (Organo-Phosphorus) : Russian Work in, *review* by Dr. F. G. Mann, 1232
Chemistry (Physical), in Trinity College, Dublin, 333
Chemistry (Protein) : Post-Doctoral Fellowships in (Division of Protein Chemistry of the Australian Commonwealth Scientific and Industrial Research Organization), 336
Chemistry (Protein), including Polypeptides : A Laboratory Manual of Analytical Methods of (edited by Dr. P. Alexander and R. J. Block), Vol. 1, The Separation and Isolation of Proteins, *review* by Dr. D. T. Elmore, 320
Chemistry (Radiation), of Organic Compounds (Dr. A. J. Swallow), *review* by Dr. S. J. Thomson, 317
Chemistry (Transition-Metal) : An Introduction to—Ligand-Field Theory (Dr. Leslie E. Orgel), *review* by Prof. C. A. Coulson, 1233
Chemists (Clinical), American Association of (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
Chemotherapy : Antimicrobial Agents and (American Society for Microbiology Interscience Conference on), 651, 1250
Chenkin, Dr. Theodore, and others : Lack of Effect of Coenzyme Q_{10} on Coumarin-induced Hypoprothrombinaemia, 711
Chepstow : Geology of the Country around Monmouth and (Memoirs of the Geological Survey of Great Britain—England and Wales), (Dr. F. B. A. Welch and Dr. F. M. Trotter), 227
Chessin, Prof. M. (Sammons, the late Irene M., and) : Cactus Virus in the United States, 517
Chew, Herman Wen-Geun : Appointed I.C.I. Research Fellow at the University of Edinburgh, 337
Chibcha Indian Tribe : New *Rh* Phenotype, $Dcce^{l}elf$, found in a (Dr. Miguel Layrisse, Zulay Layrisse, Esperanza García, Dr. Johannes Wilbert and Joaquín Parra R.), 503
Chichester, C. O., and others : Incorporation of 5,10,15 ^{14}C-Farnesol Pyrophosphate into *Phycomyces carotenoids*, 1299
Chick (Newly Hatched) : Comparison of the Serum Protein Fractions of the, with those of Adult Birds using Starch-gel Electrophoresis (A. Amin), 708
Chick Blastoderm : Distribution of Water in the (A Morphogenetic Effect of Beta-Mercaptoethanol), (A. Jurand and Dr. Peter Tuft), 1073
Chick Embryo : Mechanism of Recovery from Viral Infection in the (Dr. Samuel Baron and Alick Isaacs), 97
Chick Embryos : Effects of *Grewia populifolia* Extracts on the Development of (S. K. Brahma and N. Kundu), 506
Chicks : Effects of Morphine-like Drugs in (C. Schneider), 607
Chieco-Bianchi, Dr. L. : Awarded a scholarship by the Lady Tata Memorial Trust, for research on leukaemia and allied diseases, 228
Children (Backward), in Britain : Education of, 1052
Chimie Industrielle, Société de : Symposium on Corrosion in the Nuclear Energy Industry, 651
Chimie Physique, Société de, and the British Biophysical Society : Discussion on the Effects of Ionizing Radiation on Organic and Biological Solids, 1150
Chitin Columns : Chromatography of Tobacco Mosaic Virus (TMV) on (P. M. Townsley), 626
Chitre, V. S., and others : Changes in the Pentose-Nucleic Acid Content of Sub-Cellular Fractions of the Brain of the Rat during 'Metrazol' Convulsions, 1007
Chitwood, B. G. : Nematology (Holotype and Allotype Specimens of *Meloidogyne* Species and Sub-species), 1048
Chiyoda Kogaku Seiko K.K. : Minolta Planetarium Projectors, 753
Chloraluminate (Calcium) : Formation of, by Interaction of Portland Cement with Sea-Water and Chloride Solutions (Dr. L. Heller and Dr. M. Ben-Yair), 488
Chloride Concentration : Effect of, on the Aqueous Corrosion of a Magnesium Alloy (D. de G. Jones and H. G. Masterson), 165
Chloride Solutions : Formation of Calcium Chloraluminate by Interaction of Portland Cement with Sea-Water and (Dr. L. Heller and Dr. M. Ben-Yair), 488

Chloride (Calcium) : The Action of, on Mortar and Concrete (M. Lawrence and H. E. Vivian), 125, *erratum*, 1145
Chloride (Picryl), in Guinea Pigs : Passive Transfer of Contact Sensitivity to, with Subcellular Material (Dr. J. L. Turk), 915
Chloride (Sodium) : Quenching of Cation Vacancies in Doped Crystals of (the late Sir K. S. Krishnan and S. C. Jain), 162
  Serum Lipids in relation to the Intake of Alcohol and (Erling Lund, Torben Geill, Prof. Henrik Dam and Gunhild Kristensen), 1095
Chlorin (Metal) Complexes in Recent Sediments as Initial Precursors to Petroleum Porphyrin Pigments (G. W. Hodgson and Eric Peake), 766
Chlorins (Fossil), in a Triassic Sediment (Max Blumer and Gilbert Omenn), 161
Di-(*p*-chlorophenyl)-trifluoromethylcarbinol : Acaricidal Properties of (K. R. S. Ascher and R. Cwilich), 1322
Chlorophyll in the Leaves of Etiolated Pea Plants : Correlative Inhibition of Formation of (Jiří Ulehla), 613
Chloroplasts (Isolated) : Oxidation-Reduction Reactions of Plastoquinone in (Dr. E. R. Redfearn and J. Friend), 806
Chlorpromazine : Semiconductivity of (Dr. F. Gutmann and A. Netschey), 1390
Choaripour, R., and others : Serum Factor *Gm*^{a} among the Iranians, 717
Choice (Free-) Experiments : Comparison between Water and Alcohol Consumption in Six Animal Species in (Dr. Alpo Arvola and Dr. Olof Forsander), 819
Cholestasis (Extrahepatic) : Immunological Study of Experimentally Induced (Dr. Roy G. Shorter and R. G. Lack), 610
Cholesterogenesis : Effect of Pituitary Growth Hormone on (Dr. A. L. Green-Baum and R. S. Leal), 600
Cholesterol in the Tapetum Lucidum of the Eye of the Opossum, *Didelphis virginiana* (Antoinette Pirie), 708
Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine : Adrenergic Neurone-blocking Agents related to (A. L. A. Boura, F. C. Copp, A. F. Green, H. F. Hodson, G. K. Ruffell, M. F. Sim, E. Walton and E. M. Grivsky), 1312
Cholinergic Mechanism in Sympathetic Transmission : Lack of Evidence for a (Dr. J. E. Gardiner and Dr. J. W. Thompson), 86
Cholinergic Transmission in Fish Muscle (K. Krnjević), 1403
Cholinesterase (Human Serum), with Fluoride : Differential Inhibition of—Recognition of Two New Phenotypes (Prof. H. Harris and Mary Whittaker), 496
Chorover, S. L., and others : Cross-modal Transfer in Man, 1225
Chosen Few : The—an Examination of some Aspects of University Selection in Britain (W. D. Furneaux), *review* by Dr. W. H. King, 947
Chown, Sheila M. (Heron, Alistair, and), assisted by M. S. Featherstone and Catherine Cunningham : Ageing and the Semi-Skilled—a Survey in Manufacturing Industry on Merseyside (Medical Research Council, Memorandum No. 40), 1249
Christensen, G. N. (Armstrong, L. D., and) : Influence of Moisture Changes on Deformation of Wood under Stress, 869
Christensen, Dr. Tyge (Saltman, Dr. Paul, and) : Relative Volumes of Cytoplasm and Vacuole in *Nitella*, 825
Christiansen, Prof. J. A., and others : Displacement Potentials and Bending of Rod-like Polyelectrolytes, 484
Christiansen, Prof. W. N., and Högbom, Dr. J. A. : Cross-Antenna of the Proposed Benelux Radio Telescope, 215
Christiensson, E. G. (Agrell, I. P. S., and) : Influence of Polyvalent Ions on Histones isolated from Different Tissues, 284
Chromatid (Sub-) Structure and Chromosome Duplication in *Vicia faba* (W. J. Peacock), 832
Chromatin (Sex) : Peripheral Position of (Dr. Charles P. Miles), 626
Chromatograms (Filter Paper) : A Spray Method for the Simultaneous Detection of Iodide and Iodinated Amino-Acids on (Dr. A. Van Zyl and M. Bhaga), 275
Chromatographic Analysis : Application of the Nickel Reaction and Thermal Isomerization of Alpha-Keto-Acid Hydrazones for their (A. M. Tokushige and Dean E. S. Cook), 797
Chromatographic Analysis of Infectious Ribonucleic Acid from Poliovirus (C. Cocito, A. Prinzie and P. De Somer), 573
Chromatographic Fractionation of Hog Thyroglobulin (Prof. Nobuo Ui, Osamu Tarutani, Yoichi Kondo and Hiroko Tamura), 1199
Chromatographic Investigation of the Methyl Alcohol/Chloroform-soluble Constituents of Honeybee Larval Foods (N. G. Patel, M. H. Haydak and R. Lovell), 362
Chromatographic Separation of Biologically Active Components from Commercial Trypan Blue (J. Dijkstra and J. Gillman), 803
Chromatography : Paper (Czech Chemical Society Conference on), 759
Chromatography of Proteins on Diethyl-amino-ethyl (DEAE) Cellulose Paper (C. C. Curtain), 1269
Chromatography of Tobacco Mosaic Virus (TMV) on Chitin Columns (P. M. Townsley), 626
Chromatography (Gas) : Detection by Ionization of Atmospheric Gases during Analysis by (Dr. Arthur Karmen, Laura Giuffrida and Robert L. Bowman), 906
  Determination of Hydrogen in Gaseous Mixtures by (P. J. Kipping), 270
  Integration with Ionization Detectors in (P. M. E. M. van der Grinten and Dr. A. Dijkstra), 1195
  Role of Pressure Gradients in obtaining Minimum Time in (Prof. J. Calvin Giddings), 1291
Chromatography (Gas) Abstracts, 1958 (edited by C. E. H. Knapman, assisted by C. G. Scott), *review* by A. Goldup, 1135
Chromatography (Gas) Abstracts, 1959, (edited by C. E. H. Knapman, assisted by C. G. Scott), *review* by A. Goldup, 1135
Chromatography (Gas–Liquid) : Peak-Shift Technique in—Trimethylsilyl Ether Derivatives of Alcohols (Stanley H. Langer and Peter Pantages), 141
  Quantitative Estimation of Peak Areas in (Dr. K. K. Carroll), 377
Chromatography (Paper) : Breakdown of [2-^{14}C]-Glycine during (A. K. Huggins and V. Moses), 668
  R_M and Solvent Composition in (Dr. Edward Soczewiński), 68
Chromatography (Pyrolysis and Gas–Liquid), on the Microgram Scale (C. E. Roland Jones and A. F. Moyles), 663
Chromium, Uranium, Tungsten and Nickel Complexes of Thioglycolic Acid (V. M. Bhuchar), 489
Chromone-2-Carboxylic Acid : Some New Esters of (Juan X. de Vries and Kempis Vidal-Beretervide), 171
Chromosomal Elimination induced by 6-Aza-Uracil (M. Collette Mahoney, Dr. E. R. Witkus and Dr. C. A. Berger), 627
Chromosome Counts (Human), in relation to Age : Distribution of (Patricia A. Jacobs, W. M. Court Brown and Dr. Richard Doll), 1178
Chromosome Duplication in *Vicia faba* : Sub-Chromatid Structure and (W. J. Peacock), 832
Chromosome Rearrangements induced by X-Rays in Immature Germ Cells of *Drosophila* (Seymour Abrahamson), 523
X-Chromosome of *Drosophila melanogaster* : Recombination in the (C. N. Law), 1180
Chromosomes of *Neurospora*, 658
Chromosomes (Coccid) : Fracture and Fusion of (Prof. W. Spencer Brown), 1419
Chronic Copper Poisoning of Sheep : Methaemoglobin in (J. R. Todd and R. H. Thompson), 89
Chronic Exposure to Radiostrontium : Assessment of, by Urinary Assay (Dr. C. L. Comar and Jay Georgi), 390
Chubb, J. C. : Parasite Biology (Parasites in relation to Natural Fauna), 135
Chubb, R. C. : Awarded a Research Training Scholarship by the Animal Health Trust, 1250
Chum Salmon (*Oncorhynchus keta*) : Effect of Thyroxine plus Thiourea on the Early Development of the (Prof. M. A. Ali), 1214
Churchill College, Cambridge : Grant of £50,000 from the Gulbenkian Foundation, for three fellowships, 855
  Election of Dr. A. Hewish to a Fellowship, on appointment as College lecturer in physics, 856
  Election of J. A. Miller and R. M. Loynes to Junior Research Fellowships, 856
  Election of N. W. Ashcroft, S. J. Bernau, G. R. G. Lewison, D. McCormack Smyth and W. K. Stell to Research Studentships, 856
  Election of S. Chatterjee and E. A. Kohll to Postgraduate Studentships, 856
Chymotrypsin : Inactivation and Reactivation of (T. Therattil Antony), 178
Ciba Foundation : Symposium on Quinones in Electron Transport (edited by G. E. W. Wolstenholme and Cecilia M. O'Connor), *review* by A. I. Scott, 321
Ciferri, Dr. O., and others : Activation of Amino-Acids in Micro-organisms which produce Antibiotics, 411
Cigarette Smoke Condensate—Preparation and Routine Laboratory Estimation (H. R. Bentley and J. G. Burgan), second edition, 336
Cilia : An Example of Mechanical Co-ordination of (Dr. M. A. Sleigh), 931
Circulating Eosinophiles in Man : Effects of Corticotrophin, Hydrocortisone and Methopyrapone on the (Dr. N. J. Kothari and Dr. J. C. Saunders), 1105
Circulating Thyrotrophic Activity : Increase of the Rate of Release of Thyroidal Iodine-131 and of, at Early Stages of Propylthiouracil Treatment in the Rat (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171
Circulation (World-Wide), of Air within the Stratosphere (P. Goldsmith and F. Brown), 1033
Circumscribing Irradiation of Surface Structures of Cylindrical Animal and Plant Cells with Polonium-210 α-Particles (R. M. Bergström, R. F. Blåfield and M. W. Brenner), 1165
cis and *trans* Dichloro- and Dibromo-ethylenes : Ionization Potentials of (Dr. J. Momigny), 1089
Civil Aviation Organization (International), and others : To set up and operate an Aeronautical Research Laboratory and three Civil Aviation Training Centres, 1043
Civil Engineering, *review* by T. M. Charlton, 1032
Civil Engineering Reference Book (edited by J. Comrie), *review* by T. M. Charlton, 1032
Claassens, A. M. J. M. (Engelsman, Dr. J. J., and) : Use of the Rotating Hanging Mercury Drop in Analysis—Anodic Stripping using a Rotating Mercury Drop, 240
Claridge, Dr. M. F., and Lyon, A. G. : Lung-books in the Devonian Palaeocharinidae (Arachnida), 1190
Clark, A. J., and Munday, C. W. : Quality Measurement in the Chemical Factory (Department of Scientific and Industrial Research), 752
Clark, Dr. Desmond : Specimens of very early Pleistocene Age from certain beds in Israel (Bulletin of the British Museum (Natural History), Vol. 5, No. 4), 23
Clark, F. Le Gros : Growing Old in a Mechanized World, *review* by Prof. L. S. Hearnshaw, 532
  At the Third International Congress of Dietetics (History of Human Diet), 1343
Clark, M. A., and others : Production of Neon in Stars, 1381
Clark, R. H., and Fyfe, Prof. W. S. : Ultra-basic Liquids, 158
Clark, W. C. : Nematology (Variability and Accuracy of Linear Measurements used in Systematics, and of Ratios based on them), 1048
Clark, Sir Wilfrid Le Gros : Retirement from the chair of anatomy in the University of Oxford, 957 ; work of, 957
  The Humanity of Man (Presidential Address to the British Association for the Advancement of Science), 977
Clark-Lewis, Dr. J. W. : Stereochemistry of Natural Phenolic Compounds (Stereochemistry of Flavan Derivatives), 235
Clarke, C. A., and others : Genetics of a Difference in the Male Genitalia of East and West African Stocks of *Papilio dardanus* (Lep.), 935
Clarke, Dr. F. J. J. (Jones, Dr. O. C., and) : A New Photometric Technique using a Variable Shutter Device, 1290
Clarkson, Dr. T. W., and others : Dependence on Substrate of the Electrical Potential across the Isolated Gut, 501
Classics : Science and the (Prof. B. Farrington), 1337
Classification of Kin, Age Structure and Marriage amongst the Groote Eylandt Aborigines (Dr. Frederick G. G. Rose), *review* by Dr. R. Needham, 6
Classification of the Tea Plant (Dr. H. H. Mann), 409
Clay Mineralogy : Basic Dyestuffs in (Dr. V. S. Ramachandran, K. P. Kacker and N. K. Patwardhan), 696
Clays : Role of Interparticle Forces in Development of Upper Plastic Limit of (Dr. Raymond Yong and E. T. Selig), 938
Clean Air, National Society for : Fumifugium, or the Smoake of London Dissipated (John Evelyn), reprint, 752
  Twenty-eighth Conference, 1250
Clean Rhodium Film : Reaction of Ethane with a (Dr. Richard W. Roberts), 170
Clear Instance of Rheopectic Flow (J. B. Yannas and R. N. Gonzalez), 1384

Cleland, Prof. K. W. : Deoxyribonucleic Acid Content of Nuclei dividing Amitotically, 504
Clerodendrum viscosum Vent : Haemagglutinins from (Lieut.-Gen. G. W. G. Bird), 292
Climate and Weathered Karroo Dolerites (Dr. H. H. Weinert), 325
Climatology and Conservation in Museums (International Centre for the Study of the Preservation and the Restoration of Cultural Property: Works and Publications 3), 1050
Clinical Chemists, American Association of (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
Clinical Prospect of the Cancer Problem (Prof. D. W. Smithers), *review*, 1336
Cloarec, Lise, and others : Action of Neuraminidase on Haptoglobin, 279
Clopine, Marjorie S. : Resources and Treasures of the Library of the United States Naval Observatory, 22
Cloud (The Dust), about the Earth (Dr. J. H. Fremlin), 31; (Dr. David B. Beard), 32 ; (Dr. Fred L. Whipple), 33
Clougherty, E. V., and others : A New Phase formed by High-Pressure Treatment—Face-centred Cubic Molybdenum Monocarbide, 1194
Clover (Kent Wild White), *Trifolium repens* L. : Flower Initiation in, under Controlled Environmental Conditions (R. J. Haggar), 1120
Clow, H. : Variation of Anisotropy Axis Dispersion with Thickness of Nickel-Iron Films, 996
Clusters rich in Solute (*G.P.* Zones) : Resistivity Changes due to Formation of (H. Herman and Prof. J. B. Cohen), 63
Coagulation (Blood) : Controlling Mechanism of the Mechanical Activity of Muscle and that of (Dr. Masahiro Fujino, Saburo Yamaguchi, Masa Takauji, Kohki Uchida, Masahide Yasuda, Tatsuaki Matsushima), 80
Coal : Electronic Microscopic Observations of Structure of Ultra-Thin Sections of Vitrinite in (J. T. McCartney, Sabri Ergun and R. E. Walline), 1361
Coal in Britain : Production of Gas from, 225
Coal in Oxfordshire (Dr. C. J. Stubblefield and Dr. F. M. Trotter), 953
Coal Tar Research Association : *Review of Coal Tar Technology*, Vol. 12, Part 1, January–June 1960, 22
Coal Tar Technology, Review of (Coal Tar Research Association), Vol. 12. Part 1, January–June 1960, 22
Coalescence (Liquid/Liquid) : The Marangoni Effect and (G. D. M. MacKay and S. G. Mason), 488
Coals (Alberta Sub-bituminous), as Drilling Fluid Thinners (E. J. Jensen), 1144
Coastal Inlets, *review* by W. J. Reid, 426
  Stability of (P. Brunn and F. Gerritsen), *review* by W. J. Reid, 426
Coasts (Eastern), of the Americas : Algological Study on the, *review* by Prof. Lily Newton, 1234
Coasts (Eastern Tropical and Subtropical), of the Americas : Marine Algae of the (Prof. William Randolph Taylor), *review* by Prof. Lily Newton, 1234
Coates, Prof. G. E. : Organo-Metallic Compounds, second edition, *review* by Dr. D. S. Payne, 316
Coates, M. E. : Vitamin B_{12} and Intrinsic Factor (B_{12} Coenzymes), 1154
Coates, Prof. R. C. : Basic Approaches to Structural Analysis, *review*, 1134
Coating Globulin causing a Positive Antiglobulin Consumption Test : Nature of the (D. Nelken), 1110
Cobalt-58 Vitamin B_{12} (Specifically Bound) : Selective Uptake of, by Human and Mouse Tumour Cells (Dr. Bernard A. Cooper and Dr. William Paranchych), 393
Cobbett, W. G. : At the Twenty-first Meeting of the Research Panel of the British Gelatine and Glue Research Association (Rupture Properties of Gelatine Gels), 857
Coccid Chromosomes : Fracture and Fusion of (Prof. W. Spencer Brown), 1419
Cochliobolus heterostrophus : Susceptibility to Leaf Blight caused by, in Nigerian Varieties of Maize (C. L. M. van Eijnatten), 515
Cochran, Dr. W. : 'Effective' Ionic Charge in Crystals, 60
Cocito, A., and others : Chromatographic Analysis of Infectious Ribonucleic Acid from Poliovirus, 573
Cockcroft, Sir John : Elected president of the Institute of Physics and the Physical Society, 228
  Elected president for 1962 of the British Association for the Advancement of Science, 956 ; work of, 956
Cocking, C. G. : Properties of Isolated Plant Protoplasts, 780
Cockroach Ecology, *review* by David R. Ragge, 106
Cockroach Ganglia : An Acid Mucopolysaccharide in (Doreen E. Ashhurst), 1224
Cockroaches : The Biotic Associations of (Dr. Louis M. Roth and Dr. Edwin R. Willis), *review* by David R. Ragge, 106
Cocksfoot (*Dactylis glomerata* L.) : Effect of Spacing and Nitrogen-level on Floral Initiation in (D. M. Calder and J. P. Cooper), 195
Coconut (*Cocos nucifera* L.) : Possibilities of Vegetative Propagation in the (S. R. Gangolly and K. M. Pandalai), 1218
Cocos nucifera L. (Coconut) : Possibilities of Vegetative Propagation in the (S. R. Gangolly and K. M. Pandalai), 1218
Cod and Bovine Insulins : A Comparison of (Dr. S. Wilson and Dr. G. H. Dixon), 876
Code of Practice for the Display of Sources of Ionizing Radiations at Exhibitions, 1249
Coefficient (Average Extinction), for Re-absorption of Fluorescence Radiation through Uranin Solution (Dr. K. K. Rohatgi and G. S. Singhal), 799
Coefficient (Expansion) : Specific Heat and, of Liquid Helium-3 under Pressure below 0·1° K. (Dr. D. F. Brewer and J. R. G. Keyston), 1261
Coenzyme Q_{10} : Lack of Effect of, on Coumarin-induced Hypoprothrombinaemia (Prof. Murray Weiner, Dr. Theodore Chenkin and Dr. Peter G. Dayton), 711
Coffee Rust *Hemileia vastatrix* B. et Br. : Spore Liberation and Dispersal of (R. W. Rayner), 725
Cohen, Dr. C. : Blood Groups in Infra-human Species (The Rabbit in Comparative Research on Haemolytic Disease of the New-born), 653
Cohen, Prof. J. B. (Herman, H., and) : Resistivity Changes due to Formation of *G.P.* Zones (Clusters rich in Solute), 63
Cohen, Leonard, and Craven, Kenneth : Science Information Personnel—the New Profession of Information combining Science, Librarianship and Foreign Languages, 452
Cohen, S. : Immunity to Protozoa (Use of Gamma-Globulin in the Cure of Malaria), 553
Cold : Changes in Blood Serotonin during Exposure to (Dr. Paul Gordon), 183
Cold Solution : Formation of Chalcopyrite by Reaction between Chalcocite and Pyrrhotite in (W. M. B. Roberts), 560
Cole, Dr. A. J. : Digital Computers, their Use and Control (The Computer in the University), 554
Cole, Dr. A. J., and Iball, Dr. J. : Digital Computers, their Use and Control, 554
Cole, Dr. Anne S. : Soluble Material in the Gastro-intestinal Tract of Rats under Normal Feeding Conditions, 502
Cole, M., and others : Cross-modal Transfer in Man, 1225
Coleridge, J. C. G. : Appointed reader in physiology at the Royal Free Hospital School of Medicine, London, 1349
Colhoun, Prof. John : Dictionary of the Fungi, *review*, 109
Collagen Fractions in the Skin of New-born and Adult Rats (Nestor Gonzalez Cadavid, Berta Denduchis and Roberto E. Mancini), 1297
Collection Purposes : Use of Glacial Acetic Acid for Killing Parasitic Nematodes for (Bjørn Berland), 1320
Collee, Dr. J. G., and others : Bacteria of the Intestine (Studies on the Growth, Sporulation and Carriage of *Clostridium welchii* with special reference to Food Poisoning Strains), 1153
College of Advanced Technology (Birmingham) : Course on Statistical Quality Control and Acceptance Sampling, 337
College of Advanced Technology (Welsh), (Dr. A. Harvey), 127
College of Aeronautics, Cranfield : Appointment of Prof. A. H. Lefebvre to the chair of aircraft propulsion, 1042
College of Science and Technology (Manchester) : Appointment of Ronald Beresford Dew as visiting professor of Industrial Administration, 651
College of Technology (Liverpool) : Postgraduate Courses in Nuclear Studies, 338
Colleges of Advanced Technology in Britain : Control of, 20
Colleges and Universities in the United States : Capital Expenditure of, for research and development in the Natural and Social Sciences in 1958 (United States National Science Foundation), 545
  Report on Scientists and Engineers engaged in research and development in, for 1958 (United States National Science Foundation), 225
Collier, Dr. H. O. J. : Assessment of Pain (Testing of Analgesic Drugs on Animals), 1354
Collier, Dr. H. O. J., and others : Tryptamine Receptors in the Central Nervous System, 1068
Collinson, Dr. E. : Effects of Ionizing Radiation on Organic and Biological Solids (Scavenging of Radicals is more effective in the Solid and with Aromatic Compounds), 1150
Collisions (Reactive Bimolecular) : A Molecular Beam Reactor for Examining the Rate, and Primary Products, of (C. W. Nutt and A. J. Biddlestone), 798
Colloids : Discreteness-of-Charge Effect and the Stability Theory of (Dr. S. Levine, D. Calvert and G. M. Bell), 699
Colombani, J., and others : Anti-Leucocyte Cytoplasm, Anti-Leucocyte Nuclei and Anti-Platelets in Disseminated Lupus Erythematosis, 1209
Colombani, M., and others: Anti-Leucocyte Cytoplasm, Anti-Leucocyte Nuclei and Anti-Platelets in Disseminated Lupus Erythematosis, 1209
Colominic Acid : Specific Inhibition of Anti-*D* Antibody by (Prof. William C. Boyd and Egli Reeves), 511
Colonial Microbiological Research Institute, Trinidad : Closing of the, 20
Colony of *DBA/1* Mice : A Natural Population of Anomalous Branched Tapeworms, *Hymenolepis nana* (Cestoda : Hymenolepididae), in a (Dr. Donald Heyneman), 297
Colony of Laboratory Mice : Simultaneous Eradication of Three Ecto-parasitic Species from a (Dr. Nigel Bateman), 721
Color Council, Inter-Society (U.S.A.), (Colour Group (Great Britain)), and the : Maxwell Colour Centenary Conference, 10
Colorimetric Detection of Penicillins and Cephalosporins on Paper (Robert Thomas), 1161
Colour Centenary : The Maxwell (Prof. W. D. Wright), 10
Colour Group (Great Britain), and the Inter-Society Color Council (U.S.A.) : Maxwell Colour Centenary Conference, 10
Colour (Leaf) : Measurement of (J. Philip Grime), 614
Colouring in Factories (Factory Buildings Studies No. 8, Department of Scientific and Industrial Research), 336
Colwell, R. R., and Liston, Prof. J. : Taxonomy of *Xanthomonas* and *Pseudomonas*, 617
Colwyn Medal of the Institution of the Rubber Industry awarded to Dr. L. R. G. Treloar, 755
Comar, Dr. C. L., and Georgi, Jay : Assessment of Chronic Exposure to Radiostrontium by Urinary Assay, 390
Combination (Cetrimide–Phloridzin) : Enhancement of Inhibition of Intestinal Absorption by (J. A. Nissim), 37
Combinatorial Analysis—Proceedings of the American Mathematical Society Symposia in Applied Mathematics, Vol. 10 (edited by Richard Bellman and Marshall Hall, Jr.), *review* by Prof. L. S. Goddard, 321
Combinatorial Assignment Problem (Prof. C. Radhakrishna Rao), 100
Comets of 1618 : The Controversy on the (Galileo Galilei, Horatio Grassi, Mario Guiducci and Johann Kepler), translated by Stillman Drake and Dr. C. D. O'Malley, *review* by Colin A. Ronan, 314
Comfort, Dr. Alex : Expected Rate of Senescence and Age-dependent Mortality in Fish, 822
Commercial Trypan Blue : Chromatographic Separation of Biologically Active Components from (J. Dijkstra and J. Gillman), 803
Commercial and Industrial Education, British Association for : Interviewin in Twenty-six Steps (John S. Gough), 228
  New Routes to Further Education—Papers presented at the B.A.C.I.E. Spring Conference, London (1961), 739
Common Carrier System for Sugar Transport in Human Red Cells (Dr. L. Lacko and Dr. M. Burger), 881
Common Pea (*Pisum sativum* L.) : Lipids of the, with special reference to the Occurrence of Galactolipids (S. Adhikari, Dr. F. B. Shorland and R. O. Weenink), 1301
Commons, House of : Debate on Shipping and Shipbuilding, 543
  Debate on Apprenticeship and Training, 737
  Debate on Science, 837
  Adjournment Debate on the Research Work of the Nature Conservancy, 962
  Debate on the Social Sciences, 1354
Commonwealth Bursaries Scheme, Royal Society and Nuffield Foundation : Awards, 126
Commonwealth Fund, New York : Harkness Fellowships for 1962, 1045

Commonwealth Universities Yearbook 1961 (Association of Universities of the British Commonwealth), *review*, 843
Commonwealth of Australia Bureau of Meteorology : Antarctic Meteorology—Proceedings of the Symposium held in Melbourne, 1959, *review* by H. H. Lamb, 210
Commonwealth of Australia Scientific and Industrial Research Organization : Overall management of the Chemical Research Laboratories, 23
  Post-Doctoral Fellowships in Protein Chemistry, 336
  Conference on Food Science, 337
  Retirement of N. A. Esserman as director of the National Standards Laboratory, 542
  Appointment of I. E. Newnham as chief of the Division of Mineral Chemistry, 543
  New laboratories for the Division of Food Preservation, 546
  Division of Food Preservation Conference on Food Science, 546
Commonwealth (British), Association of Universities of the : Commonwealth Universities Yearbook 1961, *review*, 843
Communication : Animal Sounds and (edited by Dr. W. E. Lanyon and Dr. W. N. Tavolga), *review* by Dr. W. H. Thorpe, 741
Communication (Long-Range Radio), by Satellite Microwave Dipoles—the West Ford Project, 1237
Communications : World (Sir Gordon Radley), 987
Communications (Biological), in the United States : Improving, 23
Compact Human Bone Tissue : Blood-Typing (H. E. Ezra-Cohn and Prof. S. F. Cook), 1267
Comparative Analysis of Inactivating Efficiency of Radiation on Different Organisms (Dr. M. Terzi), 461
Comparison of Beryllium-7 and Caesium-137 Radioactivity in Ground-Level Air (Dr. P. F. Gustafson, M. A. Kerrigan and S. S. Brar), 454
Comparison of Cod and Bovine Insulins (Dr. S. Wilson and Dr. G. H. Dixon), 876
Comparison of Pyridine-Nucleotides in the Liver and in an Ascitic Hepatoma (M. Wintzerith N. Klein, L. Mandel and Prof. P. Mandel), 467
Comparison of Reduced Alkylated Derivatives of some Myeloma Globulins and Bence-Jones Proteins (Dr. M. D. Poulik and Dr. G. M. Edelman), 1274
Comparison of the Serum Protein Fractions of the Newly Hatched Chick with those of Adult Birds using Starch-gel Electrophoresis (A. Amin), 708
Comparison of Short-Circuit Current and Net Water Movement in Single Perfused Proximal Tubules of Rat Kidneys (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Comparison between Water and Alcohol Consumption in Six Animal Species in Free-choice Experiments (Dr. Alpo Arvola and Dr. Olof Forsander), 819
Compatibility (Stimulus-Response), in the Paired-Associate Learning of Nonsense Syllables (A. D. Baddeley), 1327
Competence (Tyrosinase), in *Streptomyces scabies* : Apparent Cytoplasmic Inheritance of (Prof. K. F. Gregory and Wei-jen Shyu), 465
Complementation Mapping of Allelic Series : Limitations of Geometrical Models for (Prof. Elof Axel Carlson), 788
Complex (1:1 Crystalline), between 1,3,7,9-Tetramethyluric Acid and Pyrene : Molecular Geometry of a (F. De Santis, E. Giglio, Dr. A. M. Liquori and A. Ripamonti), 900
Complex Compounds of Zero-valent Platinum (Dr. J. Chatt and G. A. Rowe), 1191
Complexes of Vermiculite with Amino-Acids (G. F. Walker and W. G. Garrett), 1389
Complexes (Antigen-Antibody) : Mechanism of Action of—their Effect on Mast Cells (Dr. Ivan Mota), 572
Complexes (Charge-Transfer) : Electron Affinity of the Acceptor Molecule in (Dr. M. E. Peover), 702
Complexes (Chromium, Uranium, Tungsten and Nickel), of Thioglycolic Acid (V. M. Bhuchar), 489
Complexes (Copper (II)), of Reduction Products of 2,2'-Dipyridyl: Stability Constant of (Dr. M. T. Beck and M. Halmos), 1090
Complexes (Iodine) : Separation of Linear from Branched Polysaccharides by Precipitation as (Blanche D. E. Gaillard), 1295
Complexes (Metal Chlorin), in Recent Sediments as Initial Precursors to Petroleum Porphyrin Pigments (G. W. Hodgson and Eric Peake), 766
Component (Central), of the Radio Source Centaurus A (P. Maltby), 793
Component (Structural), of some Mammalian Tissue Cell Surfaces : Sialic Acid as a (Dr. Leonard Weiss), 1108
Components (Biologically Active), from Commercial Trypan Blue : Chromatographic Separation of (J. Dijkstra and J. Gillman), 803
Components (Non-specific Staining), from Fluorescein-labelled Conjugates : Simplified Procedure for removing (G. D. Johnson), 70
Composition : Effect of Spraying with Gibberellic Acid on Yield and (Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid), (R. Stevens, J. B. Roberts and I. H. Williams), 360
Composition of the Cellulose-decomposing Microflora on Soil Crumb Plates : Factors influencing the (A. Keynan, Y. Henis and P. Keller), 307
Composition of the Skin Follicle Population in Seven Rajasthan Sheep Breeds (Sunanda Narayan), 1115
Composition (Amino-Acid), of Serum Mucoprotein in Rheumatic Fever (Prof. D. O'Brien, C. H. M. Walker and D. Walker), 922
Composition (Chemical), of Precipitable Water Vapour over the United States (Dr. Henry M. Papée and Dr. Alberto Montefinale), 136
Compositions (Exothermic) : Solid Propellant and (Dr. James Taylor), *review* by Dr. W. B. Littler, 3
Composition (Sex), of Lamprey Populations (Dr. M. W. Hardisty), 1116
Composition (Solvent), in Paper Chromatography : R_M and (Dr. Edward Soczewiński), 68
Composition (Soybean Protein) : Ultra-centrifugal Differences in (W. J. Wolf, G. E. Babcock and A. K. Smith), 1395
Composition (Unusual Base), of the Ribonucleic Acid of Wild Cucumber Mosaic Virus (H. Yamazaki and Prof. Paul Kaesberg), 96
Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of *Penicillium chrysogenum* : Identification of a (A. Ballio, Prof. E. B. Chain, F. Dentice di Accadia, M. Mauri, K. Rauer, M. J. Schlesinger and Sondra Schlesinger), 909
Compound (New), in the Metabolism of Indole-3-acetic Acid in Plants : 1-(Indole-3-acetyl)-β-D-Glucose, a (Dr. M. H. Zenk), 493
Compound (A Sealing), for Use in Biological Work (G. Stotzky, W. Culbreth and Dr. L. B. Mish), 410
Compound Formation (Ternary), in the System $CaO–Al_2O_3–MgO$ (J. H. Welch), 559
Compounds : N,N'-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related—a New Series of Anti-tumour Agents (A. S. Tomcufcik, S. D. Willson, A. W. Vogel and A. Sloboda), 611
  Gas-Phase Reactions of the Halogens and their (Dr. J. H. Thomas and Dr. E. Whittle), 129
  Heterocyclic (edited by Dr. E. H. Rodd), (Chemistry of Carbon Compounds, Vol. 4, Part C), *review* by Dr. James D. Loudon, 214
  Organo-Metal, *review* by Dr. D. S. Payne, 316
  Organo-Metallic (Prof. G. E. Coates), second edition, *review* by Dr. D. S. Payne, 316
  Sintered High Temperature (Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals Symposium on), 1250
Compounds (Carbon) : Chemistry of (edited by Dr. E. H. Rodd), Vol. 4, Part C, Heterocyclic Compounds, *review* by Dr. James D. Loudon, 214
Compounds (Complex), of Zero-valent Platinum (Dr. J. Chatt and G. A. Rowe), 1191
Compounds (Fungitoxic) : Transformation in Plants of Sodium Dimethyldithiocarbamate into other (H. M. Dekhuijzen), 198
Compounds (Heterocyclic) : An Introduction to the Chemistry of (R. M. Acheson), *review*, 320
Compounds (Inorganic) : Co-ordination of the Thiocyanate Group in (A. Turco and C. Pecile), 66
Compounds (Natural Phenolic) : Stereochemistry of (Plant Phenolics Group Symposium on), 234
Compounds (Organic) : Analysis of, *review* by Dr. D. T. Lewis, 741
  'Gel Filtration' of (B. Cortis-Jones), 272
  Radiation Chemistry of (Dr. A. J. Swallow), *review* by Dr. S. J. Thomson, 317
Compounds (Organo-Phosphorus) : Soviet Research on, 1949-1956 (in English translation), *review* by Dr. F. G. Mann, 1232
Compounds (Sulphydryl), for Normal Growth : Spontaneous Development of *L* Forms of Streptococci requiring Secretions of other Bacteria or (Ayala Frenkel and Dr. Willy Hirsch), 728
Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers (A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas), 378
Compression of Plasma in Cusp Geometry: Experiments on the (Dr. T. K. Allen and Dr. R. J. Bickerton), 794
Compton, Dr. Arthur, and Pannett, Prof. Charles A. : Effect of Irradiated Normal Tissues on the Growth of Mouse Carcinoma, 1101
Computation : Conference on Automation and, 1030
Computer Systems for Searching Chemical Literature : Report on (E. C. Medam and H. R. Koller), (United States National Bureau of Standards Research Information Centre and Advisory Service on Information Processing), 23
Computers (Digital), their Use and Control (Mathematics Department of Queen's College, St. Andrews, in collaboration with Standard Telephones and Cables, Ltd., Symposium on), 554
Computers (Digital), and Nuclear Reactor Calculations (Ward C. Sangren), *review* by Dr. M. V. Wilkes, 9
Computers and Tabulators (International), Ltd. : Appointment of B. L. J. Hart as principal of the Computer Application Training Centre, 24
Computing Centre : A New (IBM Co.), 23
Computing Systems : Redundancy Techniques for (Information Systems Branch of the United States Office of Naval Research Symposium on), 1349
Comrie, J. (edited by) : Civil Engineering Reference Book, *review* by T. M. Charlton, 1032
Concentration of Blood Glucose and Liver Glycogen in Rabbits : Effect of Bee Venom on (Prof. A. H. Mohamed and K. Zaki), 605
Concentration of Solutes : Cryoscopic Effect of Separation and (Dr. K. S. Rangappa), 1087
Concentration (Chloride) : Effect of, on the Aqueous Corrosion of a Magnesium Alloy (D. de G. Jones and H. G. Masterson), 165
Concentration (High), of (–)-Noradrenaline in *Portulaca oleracea* L. (Dr. P. C. Feng, Prof. L. J. Haynes and Dr. K. E. Magnus), 1108
Concentration (Tobacco Mosaic Virus (TMV), Lesion Number and) : Relationship of, to the Rate of Lesion Production on Pinto Bean (John Piacitelli and Prof. Vincent Santilli), 624
Concentrations (Different Iron) : Electrophoretic and Immuno-electrophoretic Patterns of Extracellular Diphtheria Proteins from Culture Filtrates of (Bertold J. Radola), 382
Concentrations (High Indole-3-Acetic Acid) : Growth Stimulation by (T. A. Truelsen), 1410
Concept of Atomic Existence (Prof. W. H. Watson), 658
Concept of Kind of Tea (W. Wight and R. C. J. H. Gilchrist), 14
Conception : Biological Control of (Dr. A. S. Parkes), 1256
Conchological Society of Great Britain and Ireland and the Malacological Society of London : First European Malacological Congress, 126
Concrete : The Action of Calcium Chloride on Mortar and (M. Lawrence and H. E. Vivian), 125, *erratum*, 1145
Concretions (Urinary), forming on Zinc Implants in Rat Bladders : Incorporation of Phosphorus-32 into (Dr. Richard F. Keeler and Dr. Stuart A. Lovelace), 715
Condensate : Cigarette Smoke—Preparation and Routine Laboratory Estimation (H. R. Bentley and J. G. Burgan), second edition, 336
Condensed Ring Aromatic Hydrocarbons : π-Electronic Excitation and Ionization Energies of (Dr. J. B. Birks and M. A. Slifkin), 761
Condition (Dry and Wet) : Radiation Sensitivity of *Escherichia coli* to β-Rays in (S. B. Bhattacharjee and N. N. Das Gupta), 1015
Conditions (Abnormal): Electrical Activity of a Giant Nerve Cell under (Dr. Yutaka Oomura, Sachio Ozaki and Takashi Maéno), 1265
Conditions (Controlled Environmental) : Flower Initiation in Kent Wild White Clover (*Trifolium repens* L.), under (R. J. Haggar), 1120
Conditions (External) : Effect of, on the Phase of Radio Signals (D. E. Watt-Carter and R. L. Corke), 1286
Conditions (Normal Feeding) : Soluble Material in the Gastro-intestinal Tract of Rats under (Dr. Anne S. Cole), 502
Conditions (Primitive Earth) : Mechanism of Synthesis of Adenine from Hydrogen Cyanide under Possible (Prof. J. Oró), 1193

Conditions (Tuberculous and Non-tuberculous) : Mycobacteriophages isolated from Persons with (Dr. E. Mankiewicz), 1416
Conductivity (Electrical), of Cathodes coated with Barium Oxide : Effect of Ultra-Violet Irradiation on the (Dr. C. H. B. Mee), 1383
Conductivity (Electrical), of Indole–Iodine : Spin Resonance and (B. Smaller, Dr. I. Isenberg and S. L. Baird, jun.), 168
Conductivity (Semi-) of Chlorpromazine (Dr. F. Gutmann and Z. Netschey), 1390
Conferences (Scientific and Technical) : Forthcoming International (Department of Scientific and Industrial Research, List No. 29), 959
Confinement (Magnetic Mirror), of Exploding Wire Plasma (E. J. Seykora), 995
Conflict : The Strategy of (Prof. Thomas C. Schelling), *review* by Sir Cyril Burt, 427
Conjola District, New South Wales : Cave Art of the (F. D. McCarthy), 854
Conjugates (Fluorescein-labelled) : Simplified Procedure for Removing Non-specific Staining Components from (G. D. Johnson), 70
Conklin, E. G. : History of the American Philosophical Society, 335
Conn, G. K. T., and Avery, D. G. : Infrared Methods, *review* by Dr. E. S. Waight, 315
Connexion between Micropulsations and the Ionosphere (C. M. Lock and P. J. Stevens), 584
Conscious Awareness : Spike and Wave Discharges and Alterations of (L. Goldie and J. M. Green), 200
Consden, Dr. R. : Paper Chromatography (Origin and Development of Paper Chromatography), 759
Conservation : Course in, by the Verein Naturpark in the Lüneburger Heide, Germany (International Youth Federation for the Study and Conservation of Nature), 1045
Conservation in Museums : Climatology and (International Centre for the Study of the Preservation and the Restoration of Cultural Property: Works and Publications 3), 1050
Conservation of Nature, International Union for the, and the Commission for Technical Co-operation in Africa : Conference on the Conservation of Nature and Natural Resources in Modern African States, 958
Conservation of Nature, International Youth Federation for the Study and : Course in Conservation, by the Verein Naturpark in the Lüneburger Heide, Germany, 1045
Conservation of Nature and Natural Resources in Modern African States (International Union for the Conservation of Nature and the Commission for Technical Co-operation in Africa, Conference on), 958
Conservation of Water in Great Britain, 421
Considerations (New), on Survival Curves and Target Theory (Dr. E. L. Powers), 1045
Constancy (Visual) : Measuring, for Stationary or Moving Objects (S. M. Anstis, C. D. Shopland and R. L. Gregory), 416
Constant (Planck's) : Theory of the Red Shift and the Nature of (L. Infeld), 1184
Constant (Stability), of Copper (II) Complexes of Reduction Products of 2,2'-Dipyridyl (Dr. M. T. Beck and M. Halmos), 1090
Constituents (Body) : Metabolic Stability of (Dr. A. N. Davison and Dr. J. Dobbing), 844
Constituents (Methyl Alcohol/Chloroform-soluble), of Honeybee Larval Foods : Chromatographic Investigation of the (N. G. Patel, M. H. Haydak and R. Lovell), 362
Constituents (Milk) : Removal of, from the Mammary Gland of the Ewe (A. K. Lascelles), 1404
Constituents (Organic), of Meteorites (Michael H. Briggs), 1137
Constitution of Cellulose Microfibrils (D. T. Dennis and Prof. R. D. Preston), 667
Consumption (Oil), in the United Kingdom during 1960, 123
Consumption (Oxygen), induced by Potassium in Muscle : Inhibition by Physostigmine of the Increase of (Dr. Ivan Novotny and František Vyskočil), 916
Consumption (Water and Alcohol) : Comparison between, in Six Animal Species in Free-choice Experiments (Dr. Alpo Arvola and Dr. Olof Forsander), 819
Consumption Test (Positive Antiglobulin) : Nature of the Coating Globulin causing a (D. Nelken), 1110
Consumption and Refinery Production : U.K. Petroleum Industry Statistics relating to, 1959 and 1960 (Petroleum Information Bureau), 123
Contact Microradiography at 200-500 V. : An Experimental Apparatus for (Dr. A. Recourt and G. H. F. de Vries), 1185
Contact Sensitivity to Picryl Chloride in Guinea Pigs : Passive Transfer of, with Subcellular Material (Dr. J. L. Turk), 915
Contact Stresses and Friction : Effective (Dr. D. H. Trollope), 376
Contensou, P. : Space-Flight and Re-entry Trajectories (Orbital Transfer and Rendezvous), 960
Content of Jobs : The, 336
Contessa, A. R. (Bruni, Dr. A., and) : Inhibition of Phosphorylation by Atractyloside, 818
Continuation of the Great Glen Fault beyond the Moray Firth (Dr. Derek Flinn), 589 ; (Dr. Robert M. Appleby), 1190
Continuity : Cell (American Society for Cell Biology Symposium on), 1045
Continuous Flow Calorimeter : Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of High Surface Area using a (G. H. Bell and A. J. Groszek), 1184
Contraction (Cardiac), and Size of the Heart : Relationship between Velocity of—a Hypothesis (Dr. H. Feinberg), 1373
Control : Digital Computers, their Use and (Mathematics Department of Queen's College, St. Andrews, in collaboration with Standard Telephones and Cables, Ltd., Symposium on), 554
Control of Animal Diseases : Animal Experiments in the (Research Defence Society Pamphlet on), 226
Control of Colleges of Advanced Technology in Britain, 20
Control of Health Hazards from Radioactive Materials : Introductory Manual on the (Medical Research Council, Memorandum No. 39), new edition, 1257
Control of Mitosis-rates in Generating Liver : Influence of Plasma Proteins in the (Dr. J. T. Hemingway), 706
Control of Plasma Calcium in the Calf : Importance of Magnesium in the (Dr. R. H. Smith), 181
Control of Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach : Significance of the Anti-Sporulation Effect of Copper Fungicides in the (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476
Control of Soil-transmitted Helminths (Prof. Paul C. Beaver), 1249
Control of Specificity in Haemoglobin Synthesis (Dr. J. Bishop, Dr. G. Favelukes, Prof. R. Schweet and Dr. E. Russell), 1365
Control (Biological), of Conception (Dr. A. S. Parkes), 1256
Control (Genetic), of Protein Structure : Haemoglobin *F* and the (Prof. V. M. Ingram), 608 ; (Dr. Wolf W. Zuelzer and Abner R. Robinson), 609
Control (Quality) : Handbook of Textile Testing and (Prof. Elliot B. Grover and Prof. D. S. Hamby), *review* by W. J. Onions, 106
Control (Spiracle), in Dragonflies (Dr. P. L. Miller), 621
Control (Statistical Quality), and Acceptance Sampling (Birmingham College of Advanced Technology Course on), 337
Control (Traffic) : Modern Methods of (Exhibition at the Science Museum, London, by the Westinghouse Brake and Signal Co., Ltd.), 1044
Controlled Environmental Conditions : Flower Initiation in Kent Wild White Clover (*Trifolium repens* L.), under (R. J. Haggar), 1120
Controlled Ionization (Magnetically) : Trans-Equatorial Backscatter Observations of (J. A. Thomas), 792
Controlled Nuclear Fission Research : Plasma Physics and (International Atomic Energy Agency International Conference on), 443
Controlling Mechanism of the Mechanical Activity of Muscle and that of Blood Coagulation (Dr. Masahiro Fujino, Saburo Yamaguchi, Masa Takauji, Kohki Uchida, Masahide Yasuda and Tatsuaki Matsushima), 80
Controversy on the Comets of 1618 (Galileo Galilei, Horatio Grassi, Mario Guiducci and Johann Kepler), translated by Stillman Drake and Dr. C. D. O'Malley, *review* by Colin A. Ronan, 314
Convenient Synthesis of Muramic Acid and other 3-O-Ethers of D-Glucosamine (Dr. Roy Gigg and Patricia M. Carroll), 495
Conversion of Fibrin to Desmofibrin (K. Buluk, T. Januszko and J. Olbromski), 1093
Conversion of L-Galactono-γ-lactone into L-Ascorbic Acid by Plants (Dr. G. A. D. Jackson, R. B. Wood and M. V. Prosser), 282
Conversion (Enzymic), of Tetrahydrofolic Acid to Folinic Acid by Plant Protein (E. H. Byers, J. Stewart and T. J. Bond), 179
Convulsions ('Metrazol') : Changes in the Pentose–Nucleic Acid Content of Sub-Cellular Fractions of the Brain of the Rat during (Prof. G. P. Talwar, B. Sadasivudu and V. S. Chitre), 1007
Cook, E. Fullerton, and others (edited by) : Remington's Practice of Pharmacy, twelfth edition, *review* by Dr. J. H. Gaddum, 8
Cook, Dean E. S. (Tokushige, A. M., and) : Application of the Nickel Reaction and Thermal Isomerization of Alpha-Keto-Acid Hydrazones for their Chromatographic Analysis, 797
Cook, G. M. W., and others : Sialic Acids and the Electrokinetic Charge of the Human Erythrocyte, 44
Cook, Margaret J., and Vlček, Augustin : Sex-Ratio in Mice, 89
Cook, Norman : At the sixty-seventh Annual Conference of the Museums Association (Display of Archaeological Exhibits), 553
Cook, Prof. S. F. (Ezra-Cohn, H. E., and) : Blood-Typing Compact Human Bone Tissue, 1267
Cooke, Roderic C. : Agar Disk Method for the Direct Observation of Nematode-trapping Fungi in the Soil, 1411
Cooled Austenite : Transformation of Cathodically Impregnated and (E. G. Ramachandran and C. Dasarathy), 998
Coombs, Dr. R. A. A., and Dodd, Barbara : Microscopical Technique to determine the Nature of a Stain on a Single Fibre of Clothing or other Material, 753
Cooper, Dr. : Hazards of the Animal House (Handling of Large-scale Experiments with Polio Virus), 339
Cooper, A. Y. : Digital Computers, their Use and Control, 554
Cooper, Dr. Bernard A., and Paranchych, Dr. William : Selective Uptake of Specifically Bound Cobalt-58 Vitamin B_{12} by Human and Mouse Tumour Cells, 393
Cooper, E. A. : Design and Use of Respirators (Ventilation), 655
Cooper, G. A., and Forsyth, D. C. T. : *Pneumodermopsis paucidens* in the North Sea, 1412
Cooper, Dr. G. N., and Stuart, Dr. A. E. : Sensitivity of Mice to Bacterial Lipopolysaccharide following Alteration of Activity of the Reticulo-Endothelial System, 294
  Non-Specific Immunity in Pneumococcal Infection of Mice, 295
Cooper, J. G. (Bond, Dr. Joan, and) : Effect of Polyamino Carboxylic Acids on Catalase, 385
Cooper, J. P. (Calder, D. M., and) : Effect of Spacing and Nitrogen-level on Floral Initiation in Cocksfoot (*Dactylis glomerata* L.), 195
Cooper, Marion I., and Sykes, J. R. : Demonstration by Paper Electrophoresis of an Auto-Immunological Precipitin possibly due to Fibrinolysins, 1212
Cooper, R. A., and Greenshields, R. N. : Sucrases in *Phaseolus vulgaris*, 601
Co-operation (International) : The Urgency of, 943
Co-operation (Technical), in Africa, Commission for (International Union for the Conservation of Nature and the) : Conference on the Conservation of Nature and Natural Resources in Modern African States, 958
Co-operation (Technical), Department of : Work of the, 440
Co-ordination of the Thiocyanate Group in Inorganic Compounds (A. Turco and C. Pecile), 66
Co-ordination (Four-fold), of Titanium in Barium Orthotitanate : Infra-Red Spectroscopic Evidence of (P. Tarte), 1002
Co-ordination (Mechanical), of Cilia : An Example of (Dr. M. A. Sleigh), 931
Co-ordination Chemistry : An Introduction to (Dr. D. P. Graddon), *review* by Prof. C. A. Coulson, 1233
Copeland, Virginia, and others : Biochemical Aspects of Resistance to 6-Mercaptopurine in Human Epidermoid Carcinoma Cells in Culture, 469
Copeman, W. S. C. : Doctors and Disease in Tudor Times, *review* by Sir Russell Brock, 428
Copepod *Ommatokoita elongata* (Grant) in the Eyes of the Greenland Shark—a Possible Cause of Mutual Dependence (Bjørn Berland), 829
Copestake, T. B., and Corney, N. S. : Irradiation Decomposition of Carbon Dioxide, 1192
Copp, F. C., and others : Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine, 1312
Copper in Isopods (Dr. W. Wieser), 1020
Copper Fungicides : Significance of the Anti-Sporulation Effect of, in the Control of Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476

Copper Poisoning of Sheep : Methaemoglobin in Chronic (J. R. Todd and R. H. Thompson), 89
Copper (II) Complexes of Reduction Products of 2,2'-Dipyridyl : Stability Constant of (Dr. M. T. Beck and M. Halmos), 1090
Co-precipitated Gels : Tetragonal Mullite-like Phase from (J. Ossaka), 1000
Corbet, G. B. : Origin of the British Insular Races of Small Mammals and of the 'Lusitanian' Fauna, 1037
Cord Blood Serums : Haptoglobin Patterns in (A. R. Rausen, P. S. Gerald and Dr. L. K. Diamond), 717
Cordes, S., and others : Some Properties of the Deoxyribonucleic Acid of Phage Alpha, 1097
Cordingley, J., and Nicol, Prof. T. : Effect of Added Vitamins on the Phagocytic Activity of the Reticulo-Endothelial System of Mice, 82
Coregonus pollan, Thompson (Lough Neagh Pollan) : Furunculosis in (K. U. Vickers and R. McClean), 930
Coremans, Dr. P. : At the sixty-seventh Annual Conference of the Museums Association (Problems of Conservation), 553
Corke, R. L. (Watt-Carter, D. E., and) : Effect of External Conditions on the Phase of Radio Signals, 1286
Corne, Dr. S. J., and others : Tryptamine Receptors in the Central Nervous System, 1068
Corney, N. S. (Copestake, T. B., and) : Irradiation Decomposition of Carbon Dioxide, 1192
Corradetti, A. : Immunity to Protozoa (True Residual Immunity in Rats which had recovered from *Plasmodium berghei* and *Trypanosoma lewisi* Infections), 551
Correction (Absorption) : Elimination of the, in the Viscometry of Dilute Solutions of High Polymers (H. van Oene and Prof. L. H. Cragg), 1160
Correlation between Growth-Rate of the Suckling of various Species and the Percentage of Total Calories from Protein in the Milk (Dr. F. W. Bernhart), 358
Correlative Inhibition of Formation of Chlorophyll in the Leaves of Etiolated Pea Plants (Jiří Úlehla), 613
Corresponding Regions of Homotypic Long Bones : Number and Arrangement of Osteons in (Dr. G. Marotti), 1400
Corrosion : Summer School on—Corrosion Problems in the Electrical Power Industry (Battersea College of Technology), 651
Corrosion in the Nuclear Energy Industry (Société de Chimie Industrielle Symposium on), 651
Corrosion Problems in the Electrical Power Industry (Battersea College of Technology Summer School on Corrosion), 651
Corrosion (Aqueous), of a Magnesium Alloy : Effect of Chloride Concentration on the (D. de G. Jones and H. G. Masterson), 165
Cortex (Mouse Thymus) : Periodic Acid–Schiff Positive Giant Cells in the (D. Metcalf and M. Ishidate), 305
Corticosteroids : Effect of Adrenalectomy and, on Distribution of Radioactivity in Protein of Cell Fractions from Myocardial Slices (Dr. Edward I. Weinshelbaum and Dr. Ira G. Wool), 1401
Corticotrophin, Hydrocortisone and Methopyrapone : Effects of, on the Circulating Eosinophiles in Man (Dr. N. J. Kothari and Dr. J. C. Saunders), 1105
Cortis-Jones, B. : 'Gel Filtration' of Organic Compounds, 272
Corundum (Synthetic) : A New Technique for the Production of (E. A. D. White), 901
Coryndon Memorial Museum, Nairobi : Annual Report for 1959, 335
Cosmic Noise Measurements at 3·8 Mc./s. from Satellite 1960 $\eta 1$: Interpretation of (J. H. Chapman and A. R. Molozzi), 480
Cosmic Rays : Fission and Spallation Phenomena and their Application to (European Organization for Nuclear Research and the Max Planck Institute, Heidelberg, Conference on), 24
Cosmology (Prof. H. Bondi), second edition, *review* by Prof. W. H. McCrea, 1336
Cosmonautical Medicine : Aviation and (C.E.R.M.A. International Congress on), 338
Cosslett, Dr. V. E., and Nixon, Dr. W. C. : X-Ray Microscopy, *review* by Prof. Arne Engström, 1332
Cotes, J. E. : Design and Use of Respirators (Physiological Aspects of Respirator Design), 655
Cotes, Dr. P. Mary, and Bangham, Dr. D. R.: Bio-Assay of Erythropoietin in Mice made Polycythaemic by Exposure to Air at a Reduced Pressure, 1065
Cotton : Leaf Curl Virus and the Flavonoid Content of Resistant and Susceptible Strains of (Dr. Esam M. Moustafa), 415
Cotton (Polyploid) : Basis of the Diploid-like Meiotic Behaviour of (G. Kimber), 98
Cotton, Silk and Man-made Fibres Research Association : Appointment of N. G. McCulloch as an honorary life governor, 443
Coulson, Prof. C. A. : Graphite (Electronic Band Structure of Graphite), 27
    Transition-Metal Chemistry, *review*, 1233
Coulson, C. B., and Lord, P. Mary : Isolation and Fractionation of some Damselfly Pigments, 1300
Coumarin-induced Hypoprothrombinaemia : Lack of Effect of Coenzyme Q_{10} on (Prof. Murray Weiner, Dr. Theodore Chenkin and Dr. Peter G. Dayton), 711
Counting Method (γ-Ray) : Measurement of the Thorium Content of Natural Materials by a (Prof. J. H. J. Poole and F. N. Byrne), 62
Countries (Developing) : Agricultural Problems in (Nutrition Society Symposium on), 1250
Countries (Overseas): Technical Assistance to (June 17, 1039), *errata*, 24
Country around Monmouth and Chepstow : Geology of the (Memoirs of the Geological Survey of Great Britain—England and Wales), (Dr. F. B. A. Welch and Dr. F. M. Trotter), 227
Countryside : The Impact of Industrial Development on the (Council for the Preservation of Rural England Conference on), 959
Counts, George W., and others : Biochemical Changes in Lethal Staphylococcal Infections in Mice, 783
Counts (Human Chromosome), in relation to Age : Distribution of (Patricia A. Jacobs, W. M. Court Brown and Dr. Richard Doll), 1178
Coupling of Phosphorylation to Electron and Hydrogen Transfer by a Chemi-Osmotic Type of Mechanism (Dr. Peter Mitchell), 144
Course in Conservation, by the Verein Naturpark in the Lüneburger Heide, Germany (International Youth Federation for the Study and Conservation of Nature), 1045
Course on the Principles of Radiation Protection (Harwell Reactor School of the Atomic Energy Research Establishment), 856
Course on Recent Developments in the Chemistry and Technology of Polymers (Bradford Institute of Technology), 1349
Course on Statistical Quality Control and Acceptance Sampling (Birmingham College of Advanced Technology), 337
Course (Fourteenth International Instructional), by Carl Zeiss, Jena, at the Imperial College of Science and Technology, London, on Fine Measuring Instruments, 1045
Courses for the Diploma in Technology : Regional Development Plan for (Regional Advisory Council for Technological Education, London and Home Counties), 1044
Courses on Practical Applications of Nuclear Energy (International Atomic Energy Agency), 1143
Courses (Postgraduate) in Nuclear Studies (Liverpool College of Technology), 338
Courses (United Kingdom), in Nuclear Energy, 125
Courtaulds, Ltd. : Grant of £1,000 to the Department of Textile industries in the University of Leeds, 650
Courts, Dr. A. : At the Twenty-first Meeting of the Research Panel of the British Gelatine and Glue Research Association (Structural Reorientation of Gelatine in Citrate), 857
    Eucollagen, 1097
Cow and Rat : Leucine Aminopeptidase in the Mammary Gland of the (Prof. S. Talanti and Prof. Väinö K. Hopsu), 86
Cows : Growth of Ryegrass-dominant Pasture grazed by, and Properties of New Zealand Butterfat (F. H. McDowall, W. A. McGillivray and J. C. Hawke), 303
Cows' Milk : An Invariant Casein Substrate for Rennet Standardization, derivable from Diverse Sources of (Dr. G. W. Scott Blair and J. C. Oosthuizen), 697
Cows (Lactating), given Artificial Rations Low in Magnesium : Rapid Development of Hypomagnesaemia in (J. A. F. Rook), 1019
Cox, E. V. : Vitamin B_{12} and Intrinsic Factor (Relationships between Vitamin B_{12} and Folic Acid), 1156
Coxon, Dr. R. V. : Chemistry and the Nervous System, *review*, 318
Crabbé, Dr. J. : Stimulation by Aldosterone of Sodium Transport in the Loop of Henle, 817
Cracks (Expansion), in Mortar : The Widening of, by Salt Solutions (J. A. Roberts and H. E. Vivian), 125, *erratum*, 1145
Cragg, Prof. L. H. (van Oene, H., and) : Elimination of the Absorption Correction in the Viscometry of Dilute Solutions of High Polymers, 1160
Craggs, Dr. J. W. : Appointed to the newly created chair of mathematics in the University of Leeds, 122 ; work of, 122
Cranbrook, Earl of : Science, Politics and Social Service (Presidential Address to Section X (General) of the British Association for the Advancement of Science), 992
Craven, Kenneth (Cohen, Leonard, and) : Science Information Personnel—the New Profession of Information combining Science, Librarianship and Foreign Languages, 452
Crawford, Dr. B. H. : At the Maxwell Colour Centenary Conference (Some New Results on Large-field Colour Matching), 10 ; (Colour Rendering), 11
Crayfish (Cave) : Diurnal Rhythm in (Prof. Frank A. Brown), 929
Creep : A New Laboratory for Research on, 1049
Cretaceous (Hanterivian) Angiosperm Fruit : An Early, from California (M. E. J. Chandler and D. I. Axelrod), 442
Crisis in Mathematical Education in Britain, 103
Crofton, Dr. H. D. : Parasite Biology (Influence of Temperature and Climatic Conditions on the Level of Helminthic Infestation in Domestic Animals), 134
Crombie, Dr. L. : Stereochemistry of Natural Phenolic Compounds (Absolute Configuration of Rotenone and its Derivatives), 235
Crook, Dr. E. M. : The Training of Biochemists (The M.Sc. Course in Biochemistry at University College, London), 956
Crookes Laboratories : Appointment of Dr. F. A. Robinson as chairman of the board of directors, 546
Crop (Potato) : Tuber Bulking in the (R. W. Radley, M. A. Taha and P. M. Bremner), 782
Crop Production : Importance of Nitrogen in (Prof. N. R. Dhar), 544
Cross, A. C., and others : Dependence on Substrate of the Electrical Potential across the Isolated Gut, 501
Cross, Dr. P. C. : Appointed president and chief executive officer of the Mellon Institute, 439
Cross-Antenna of the Proposed Benelux Radio Telescope (Prof. W. N. Christiansen and Dr. J. A. Högbom), 215
Cross-Linking in Ion-Exchange Polymers : Non-Uniformity of (Dr. E. Glueckauf and R. E. Watts), 904
Cross-Linking Proteins : A New Two-Stage Method for (Dr. Felix Borek), 1293
Cross-Modal Transfer in Man (M. Cole, S. L. Chorover and Dr. G. Ettlinger), 1225
Crosses (Sterile), between Mutations of *Tribolium confusum* Duv. (Prof. John Stanley), 934
Crowley, Dr. Mary P., and others : Biosynthesis of the Pyrethrins, 281
Crowley-Milling, Dr. M. C., and others : Pulse-shortening in Electron Linear Accelerators, 483
Crowther : From Galton to (Prof. George Seth), 989
Crustacea : A Biology of (Dr. J. Green), *review* by Dr. J. A. Allen, 111
    The Physiology of (edited by Talbot H. Waterman), Vol. 2, Sense Organs, Integration and Behaviour, *review* by Dr. J. Green, 428
Cryoscopic Effect of Separation and Concentration of Solutes (Dr. K. S. Rangappa), 1087
Crystal and Molecular Structure of Diphenyl (Dr. G. B. Robertson), 593
1:1 Crystalline Complex between 1,3,7,9-Tetramethyluric Acid and Pyrene : Molecular Geometry of a (F. De Santis, E. Giglio, Dr. A. M. Liquori and A. Ripamonti), 900
Crystallization of Isotactic Polypropylene using a Light Depolarization Technique (J. H. Magill), 1092
Crystallization in Wool Keratin (Dr. Allan Skertchly), 138
Crystallization Kinetics in Polyvinylchloride and Thermoelastic Relaxation induced by Ultrasonics (Dr. V. Nardi), 563
Crystallographic Society of Japan, and others : International Conference on Magnetism and Crystallography, 24
Crystallography : Magnetism and (Science Council, the Physical Society and the Crystallographic Society of Japan International Conference on), 24

Crystals : Atomic Energy Levels in (J. L. Prather), 1348
  'Effective' Ionic Charge in (Dr. W. Cochran), 60
  Epitaxial Growth of, on One-Degree Orientated Substrates (Dr. A. Goswami), 160
Crystals (Doped), of Sodium Chloride : Quenching of Cation Vacancies in (the late Sir K. S. Krishnan and S. C. Jain), 162
Crystals (Explosive) : Slow Decomposition of, and their Damage by Fission Fragments (Dr. F. P. Bowden and H. M. Montagu-Pollock), 556
Crystals (Silver Bromide Micro-) : Electron Spin Resonance Absorptions induced by Light in Adsorbed Layers of Cyanine Dyes on (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Crystals (Single), of Gallium : Thermo-electric Power of (P. Horner), 58
Csáky, Dr. T. Z., and Fernald, G. W. : Localization of the 'Sugar Pump' in the Intestinal Epithelium, 709
Csóka, R. (Füzi, Dr. M., and) : Leptospirosis of White Laboratory Rats, 1123
Cubic Molybdenum Monocarbide (Face-centred) : A New Phase formed by High-Pressure Treatment— (E. V. Clougherty, K. H. Lothrop and J. A. Kafalas), 1194
Cucumber (Wild) Mosaic Virus : Unusual Base Composition of the Ribonucleic Acid of (H. Yamazaki and Prof. Paul Kaesberg), 96
Culbreth, W., and others : A Sealing Compound for Use in Biological Work, 410
Cultural Property, International Centre for the Study of the Preservation and the Restoration of (Rome) : Works and Publications, 3, Climatology and Conservation in Museums, 1050
Culture : Biochemical Aspects of Resistance to 6-Mercaptopurine in Human Epidermoid Carcinoma Cells in (R. W. Brockman, George G. Kelley, Patricia Stutts and Virginia Copeland), 469
  Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Culture (Separation and *in vitro*), of Cells from Liver Tissue (Dr. Justine S. Garvey), 972
Culture (Sterile) : Growth of Fern Embryos in (Prof. Augustus E. DeMaggio and Prof. Ralph H. Wetmore), 94
Culture Filtrate of *Helminthosporium sativum* : Production of Symptoms of Barley Leaf-Spot Disease by (S. K. Gayed), 725
Culture Filtrates of Different Iron Concentrations : Electrophoretic and Immuno-electrophoretic Patterns of Extracellular Diphtheria Proteins from (Bertold J. Radola), 382
Culture Forms of *Trypanosoma rhodesiense* : Cyclical Infection of *Glossina mortisans* with (the late Prof. R. M. Gordon and J. K. Miller), 1317
Culture Media of *Penicillium chrysogenum* : Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from (A. Ballio, Prof. E. B. Chain, F. Dentice di Accadia, M. Mauri, K. Rauer, M. J. Schlesinger and Sondra Schlesinger), 909
Cultures from Multi-carbohydrate Media : A Micro-method for Performing the Phenylalanine Test on (D. J. Stewart), 521
Cultures (Animal Cell, Tissue and Organ), in Radiobiology : Use of (New York Academy of Sciences Symposium on), 340
Cultures (Fibroblast) : Effect of Amino-Acid Esters on the Growth of (Dr. I. Fehér, A. Döklen and V. Selmeci), 494
Cultures (Tissue Cell) : Immunofluorescence of Pleuropneumonia-like Organisms isolated from (Mayor W. F. Malizia, M. F. Barile and D. B. Riggs), 190
Cunningham, Catherine (Featherstone M. S. and), (Alistair Heron and Sheila M. Chown, assisted by) : Ageing and the Semi-Skilled—a Survey in Manufacturing Industry on Merseyside (Medical Research Council, Memorandum No. 40), 1248
Cunningham, Prof. G. J., and others : Tissue Dilution Artefact—a Re-interpretation of Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogenesis, 1203
Current (Short-Circuit), and Net Water Movement : Comparison of, in Single Perfused Proximal Tubules of Rat Kidneys (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Current Problems in Nutrition (Nutrition Society of Canada Symposium on), 335
Current Shear : Evidence of an Eastward Equatorial Undercurrent in the Atlantic from Measurements of (Arthur D. Voorhis), 157
Current Steps in Motoneurones : Potential Changes produced by Application of (T. Araki, Dr. M. Ito and T. Oshima), 1104
Currents (Ionic), in Membrane of Active Muscle Fibre (Dr. H. Jenerick), 1074
Currents (Ocean) : Dispersal of Tropical Seeds by (R. Mason), 408
Curriculum (School), in Mathematics (edited by Howard Fehr), (Organization for European Economic Co-operation Report on), 335
Currie, G. T., and others : Girard *T* Reagent for Carbonyls, 1391
Currie, Ronald, and Draper, Laurence : Time-integrated Measurements of Submarine Irradiance, 661
Curtain, C. C. : Chromatography of Proteins on Diethyl-amino-ethyl (DEAE) Cellulose Paper, 1269
Curtis, Dr. D. R., and Watkins, Dr. J. C. : Analogues of Glutamic and γ-Amino-*n*-butyric Acids having Potent Actions on Mammalian Neurones, 1010
Curtis, Dr. G. H., and others : Age of Bed I, Olduvai Gorge, Tanganyika, 478
Curves (Isolated or Overlapping Normal Probability) : Calculation of the Areas of (Dr. D. Morison Smith and John C. Bartlet), 688
Curves (Survival), and Target Theory : New Considerations on (Dr. E. L. Powers), 1045
Cuscuta reflexa : Artificial Production of Buds from the Embryos of (Prof. P. Maheshwari and B. Baldev), 197
Cusp Geometry : Experiments on the Compression of Plasma in (Dr. T. K. Allen and Dr. R. J. Bickerton), 794
Cuthbertson, Dr. W. F. J. : The Training of Biochemists (Industry as a whole not fully aware of the Potential Value of the Trained Biochemist), 955
Cuticle in the Silkworm, *Bombyx mori* : Role of Lipid in the Hardening of the (Shigemi Kawase), 279
Cuticula (Stigmatic), of Flowers : Enzymic Breakdown of (W. Heinen and H. F. Linskens), 1416
Cuttings of *Hevea brasiliensis* : Effect of Ferric Dimethyldithiocarbamate on the Rooting of (G. H. Tinley), 1217
Cwilich, R. (Ascher, K. R. S., and) : Acaricidal Properties of Di-(*p*-chlorophenyl)-trifluoromethylcarbinol, 1322
Cyanide (Hydrogen) : Mechanism of Synthesis of Adenine from, under Possible Primitive Earth Conditions (Prof. J. Oró), 1193
Cyanine Dyes on Silver Bromide Microcrystals : Electron Spin Resonance Absorptions induced by Light in Adsorbed Layers of (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Cycle and Rhythm in the Ovulation of the Spider *Latrodectus geometricus* C. Koch (A. Bouillon and R. Lekie), 620
Cyclical Infection of *Glossina mortisans* with Culture Forms of *Trypanosoma rhodesiense* (the late Prof. R. M. Gordon and J. K. Miller), 1317
Cyclol Revived : The, *review* by Prof. H. N. Rydon, 105
Cyclotrons : Sector-Focused—Proceedings of an Informal Conference, Sea Island, Georgia, 1959 (edited by F. T. Howard), (United States National Academy of Sciences—National Research Council, Nuclear Science Series, Report No. 26, Publication No. 656), *review* by M. Snowden, 532
Cylindrical Animal and Plant Cells : Circumscribing Irradiation of Surface Structures of, with Polonium-210 α-Particles (R. M. Bergström, R. F. Blåfield and M. W. Brenner), 1165
Cytochemistry of *L*1210 Cells (Dr. H. J. Woodliff), 1313
Cytochrome *c* in the Heart of the Turtle (Prof. Gunnar Biörck and Dr. Sven Paléus), 712
Cytology of some Genera of Polypodiaceae in Eastern India (G. Panigrahi and S. N. Patnaik), 1207
Cytoplasm (Anti-Leucocyte), Anti-Leucocyte Nuclei and Anti-Platelets in Disseminated Lupus Erythematosis (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Cytoplasm and Vacuole in *Nitella* : Relative Volumes of (Dr. Paul Saltman and Dr. Tyge Christensen), 825
Cytoplasmic Inheritance (Apparent), of Tyrosinase Competence in *Streptomyces scabies* (Prof. K. F. Gregory and Wei-jen Shyu), 465
Czaja, Dr. A. H. : At the International Starch Conference (Microphotographs showing the manner in which Starch Granules Gelatinize in Water), 236
Czech Chemical Society : Conference on Paper Chromatography, 759

D*ACTYLIS glomerata* L. (Cocksfoot) : Effect of Spacing and Nitrogen-level on Floral Initiation in (D. M. Calder and J. P. Cooper), 195
Dagnall, D., and others : Techniques in Tropical Pathology, *review* by Prof. P. C. C. Garnham, 1236
Dainton, Prof. F. S. : Effects of Ionizing Radiation on Organic and Biological Solids (Scavenging of Radicals is more effective in the Solid and with Aromatic Compounds), 1150
Dairying, National Institute for Research in : Annual Report for 1960, 237
Daisley, Dr. K. W. : Gel Filtration of Sea-Water—Separation of Free and Bound Forms of Vitamin B_{12}, 868
Dalgarno, Dr. A. : Appointed to a chair of quantum mechanics in the Queen's University, Belfast, 19 ; work of, 19
Dalton, Dr. G. C. J. : *Obituary* by Prof. C. N. Watson-Munro, 1142
Dalton's Irish Rival, *review* by Frank Greenaway, 533
Dalziel, Dr. Keith : An Inhibitor of Liver Alcohol Dehydrogenase in Preparations of Reduced Diphosphopyridine Nucleotide, 1098
Dam, Prof. Henrik, and others : Serum Lipids in relation to the Intake of Alcohol and Sodium Chloride, 1095
Damage by Fission Fragments : Slow Decomposition of Explosive Crystals and their (Dr. F. P. Bowden and H. M. Montagu-Pollock), 556
Damage caused by *Heterodera rostochiensis* Woll. : Assessing the (E. B. Brown), 937
Damage in Stainless Steel due to Fission Fragments (P. R. B. Higgins and A. C. Roberts), 1158
Damage (Radiation), to Proteins (U. S. Kumta and A. L. Tappel), 1304
Damage (Unilateral Temporal Pole), in the 'Split-Brain' Monkey : Changes in Visual Gnostic Functions and Emotional Behaviour following (Dr. J. L. deC. Downer), 50
Damping without Influence on Frequency (A. Bloch), 1086
Damselfly Pigments : Isolation and Fractionation of some (C. B. Coulson and P. Mary Lord), 1300
Daniel, Dr. Glyn : The Prehistoric Chamber Tombs of France, *review* by Prof. C. F. C. Hawkes, 637
Daniels, Dr. D. G. H., and Martin, H. F. : Isolation of a New Antioxidant from Oats, 1302
Danon, Dr. D. : Biorheology and Microcirculation (Phase-contrast Microcine Films illustrating Deformation, Shearing and Haemolysis of Normal Human Erythrocytes under Gradually Decreasing Salt Concentration), 758
Darby, Prof. H. C. : The West Riding of Yorkshire, *review*, 208
Darcy, Dr. D. A. : Estimation of Specific Proteins in Serum and other Mixtures, 1163
Dark-Fixation of Carbon Dioxide in Leaves of *Bryophyllum fedtschenkoi* : An Endogenous Rhythm in the Rate of (Diana M. Warren and Malcolm B. Wilkins), 686
Darkening of the Hair in Yellow A^{vy}/a Mice : 5-Methyltryptophan and (Dr. Vivianne T. Nachmias), 934
Darlow, Surg.-Comdr. : Hazards of the Animal House (Provision of Clean Air as a Factor in Preventing the Spread of Infection in the Animal House), 339
Dart, Prof. Raymond A. : An Australopithecine Scoop made from a Right Australopithecine Upper Arm Bone, 372
Darwin : Evolution after (edited by Sol Tax and Charles Callender), Vol. 3, Issues in Evolution, *review* by Dr. P. G. Fothergill, 425
Das, Dr. S. M. : Hydrogen Ion Concentration, Plankton and Fish in Freshwater Eutrophic Lakes of India, 511
Das Gupta, N. N. (Bhattacharjee, S. B., and) : Radiation Sensitivity of *Escherichia coli* to β-Rays in Dry and Wet Condition, 1015
Dasarathy, C. (Ramachandran, E. G., and) : Transformation of Cathodically Impregnated and Cooled Austenite, 998
Dasgupta, Dr. P. R. : Precorticotrophin in Blood, 1106
Dass, R. K., and others : Electrical Effects during Dissolution and Precipitation of Electrolytes, 764
Data : Organic Electronic Spectral, Vol. 1, 1946-1952 (edited by Mortimer J. Kamlet) ; Vol. 2, 1953-1955 (edited by Herbert E. Ungnade), *review* by Dr. E. G. Kellett, 107

Data on Research and Development : Reviews of, No. 27, Report on Scientists and Engineers engaged in research and development in Colleges and Universities in the United States for 1958 (United States National Science Foundation), 225
  Reviews of, No. 28, Capital Expenditure of Colleges and Universities in the United States for research and development in the Natural and Social Sciences in 1958 (United States National Science Foundation), 545
Data (Satellite) : Analysis of (Prof. D. G. Parkyn), 896
Data-processing Centre : A New (IBM Co.), 23
Dating Man's Emergence (Dr. K. P. Oakley), 988
Dating System (Short-term) : Carbon-14 from Nuclear Explosions as a—Use to determine the Origin of Heartwood (Dr. A. T. Wilson), 714
Datta, Ranajit K., and Roy, Prof. Rustum : Dependence on Temperature of the Distribution of Cations in Oxide Spinels, 169
Daudel, Pascaline : Radioactive Tracers in Chemistry and Industry (translated by Dr. Ulli Eisner), *review* by Prof. J. R. Partington, 637
Dauphinee, T. M., and others : At the Second Measurement Conference (Accurate Measurement of Temperature and Depth in the Ocean), 1351
Dausset, Prof. J., and others : Anti-Leucocyte Cytoplasm, Anti-Leucocyte Nuclei and Anti-Platelets in Disseminated Lupus Erythematosis, 1209
Davey, Prof. T. H. : *Obituary* of Prof. R. M. Gordon, 852
Davidson, J. N. (edited by Erwin Chargaff and) : The Nucleic Acids, Vol. 3, *review* by Dr. Roy Markham, 742
Davidson, R. S. : Appointed Brotherton Research Fellow in the Department of Organic Chemistry in the University of Leeds, 650
Davies, A. G., and others : Inorganic Polymers (Dialkyl Tin Oxides), 1047
Davies, C. N. : Design and Use of Respirators, 655
Davies, Dr. D. A. L. : Dideoxysugars of *Pasteurella pseudotuberculosis*-specific Polysaccharides, and the Occurrence of Ascarylose, 43
Davies, D. D. : Synthesis of Succinate from Acetate, 1093
Davies, Prof. J. N. P. : Appointed to the University of London readership in morbid anatomy at the Postgraduate Medical School of London, 229
Davies, L. J. : *Obituary* of Sir Hugh Warren, 1042
  Appointed responsible for the direct supervision of the four Research Laboratories of Associated Electrical Industries, Ltd., at Aldermaston, Harlow, Manchester and Rugby, 1142 ; work of, 1142
Davies, R. K., and others : Urea Synthesis in the Living Rat Brain, 288
Davies, R. R. : Wettability and the Capture, Carriage and Deposition of Particles by Raindrops, 616
Davis, A. (Buchwald, V. T., and) : Surface Waves in Anisotropic Elastic Media, 899
Davis, Bernard D., and others : Influence of Salts on the Uptake of Streptomycin by *Escherichia coli*, 1324
Davis, Dr. E. : Biorheology and Microcirculation (General Nature and Problems of Microcirculation as studied by Optical and Electron Microscopy). 757 ; (Effect of Adrenaline on the Small Blood Vessels of the Conjunctiva and Nailfold), 758 ; (Observations and Experiments on Vasomotion in Human Capillaries), 758
Davis, Judith E., and others : A Relation between the Reactivity of Human Red Cells to Antiglobulin Tests and their Surface Structure, 923
Davison, Dr. A. N., and Dobbing, Dr. J. : Metabolic Stability of Body Constituents, 844
Davison, P. S. : A National Spectra Index—Plans and Problems, 1250
Davisson, E. O. : Vitamin B_{12} and Intrinsic Factor (Efforts to Purify and Isolate Intrinsic Factor), 1155
Dawkins, M. J. R. : Changes in Glucose-6-phosphatase Activity in Liver and Kidney at Birth, 72
Dayton, Dr. Peter G., and others : Lack of Effect of Coenzyme Q_{10} on Coumarin-induced Hypoprothrombinaemia, 711
De, P., and others : Nucleolar Localization of Succinic Dehydrogenase in Normal Rat Hepatic Cells, 925
de Coninck, Prof. : Problems of Systematics and Taxonomy in Nematology Today, 1048
de Forest, Dr. Lee : *Obituary* by Dr. R. L. Smith-Rose, 749
de Haas-van Alphen Effect and the Electronic Structure of Metals (Dr. D. Shoenberg), 126
de Issaly, Inda M., and others : Role of the Pentose Phosphate Pathway in *Pasteurella multocida*, 727
de Körösy, Dr. Francis : Poisoning and Sign Reversal of Permselective Membranes, 1363
de Lamirande, Dr. Gaston : Effect of Enzyme Injections on Mitosis in Regenerating Liver, 400
de Maeseneer, J. : Nematology (Distribution of some of the chief Migratory Root Nematodes in Belgium), 1048
DeMaggio, Prof. Augustus E., and Wetmore, Prof. Ralph H. : Growth of Fern Embryos in Sterile Culture, 94
De Matteis, Dr. F., and others : Nervous and Biochemical Disturbances following Hexachlorobenzene Intoxication, 363
De Santis, F., and others : Molecular Geometry of a 1:1 Crystalline Complex between 1,3,7,9-Tetramethyluric Acid and Pyrene, 900
De Somer, P., and others : Chromatographic Analysis of Infectious Ribonucleic Acid from Poliovirus, 573
de Turville, C. M. : Terrestrial Accretion from the Solar Wind, 1183
de Vries, G. H. F. (Recourt, Dr. A., and) : An Experimental Apparatus for Contact Microradiography at 200-500 V., 1185
De Vries, Juan X., and Vidal-Beretervide, Kempis : Some New Esters of Chromone-2-Carboxylic Acid, 171
N-Deacetylation of Hexosamine : Partial Acid-Hydrolysis of a Mucopolysaccharide without Appreciable (Dr. T. J. Painter and Prof. W. T. J. Morgan), 39
Deacon, Dr. G. E. R. : The Ocean Floor, *review*, 531
Deadly Quarrels : Statistics of (Dr. Lewis F. Richardson), edited by Quincy Wright and C. C. Lienau, *review* by Sir Cyril Burt, 427
Deanesley, Dr. R. : Foetal and Neonatal Physiology (Present Position in Foetal Endocrinology), 344
Decades (Four), of State Forestry in Great Britain (Prof. M. L. Anderson), 656
Decades (Four), of State Forestry in New Zealand, 451
Decarboxylase (Glutamic Acid), in Insect Nerve Tissue : Activity of (Dr. N. Frontali), 178
Decarboxylase (Histidine), and Growth (D. Mackay, Joan D. Reid and Dr. D. M. Shepherd), 1311
Decker, Martin T. : Appointed chief of the Tropospheric Measurements Section of the United States National Bureau of Standards Laboratories at Boulder, Colorado, 334 ; work of, 334
Decolorization (Ultrasonic), of Rhodamine *B* : Inhibition of, in Presence of Organic Substances (Prof. Satya Prakash and Sheo Prakash), 1292
Decomposition : Resistance of Humus to (H. R. Birch and M. T. Friend), 731
Decomposition (Irradiation), of Carbon Dioxide (T. B. Copestake and N. S. Corney), 1192
Decomposition (Slow), of Explosive Crystals and their Damage by Fission Fragments (Dr. F. P. Bowden and H. M. Montagu-Pollock), 556
Decrease in Urinary Excretion of Histamine and the Initiation of the Labour Process : Relationship between the (Dr. Halina Wiskont-Buczkowska), 290
Decreased Urinary Excretion of 2-Aminoethanol by Irradiated Human Beings (Dr. Ralph R. Cavalieri, M. T. Van Metre and W. I. Sivertsen), 1303
Deelder, R. S. (van Swaay, Dr. M., and) : Use of the Rotating Hanging Mercury Drop in Analysis—Trace Analysis with a Rotating Hanging Mercury Drop, 241
Deer (Red) Commission : Report for 1959-1960, 29
Defalco, A. J., and others : Urea Synthesis in the Living Rat Brain, 288
Defects (Metabolic) : Nutrition and (Nutrition Society Symposium on), 125
Defence (Perceptual), and Filter Theory (Neville Moray), 940
Deffner, G. G. J., and others : Determination by Mass Spectrometry of the Structure of Proline Derivatives from Apples, 380
Deformation of Wood under Stress : Influence of Moisture Changes on (L. D. Armstrong and G. N. Christensen), 869
Degradation (Enzymic), of Deoxyribonucleic Acid into Sub-Units : Kinetics of the (Dr. Giorgio Bernardi and Prof. Charles Sadron), 809
Degradation (Phosphate-dependent), of Urea (R. C. Valentine and Prof. R. S. Wolfe), 925
Degree of Polymerization of Hyaluronic Acid isolated from Human Aortic Tissue (Dr. Sv. Bertelsen and K. Marcker), 386
Dehydrogenase (Liver Alcohol) : An Inhibitor of, in Preparations of Reduced Diphosphopyridine Nucleotide (Dr. Keith Dalziel), 1098
Dehydrogenase (Mitochondrial α-Glycerophosphate), in the Nervous System : Histochemical and Homogenization Studies of (R. Hess and Dr. A. G. E. Pearse), 718
Dehydrogenase (Succinic) : Nucleolar Localization of, in Normal Rat Hepatic Cells (P. De, R. Chatterjee and Dr. S. Mitra), 925
Dehydrogenase (Succinic), during Chemical Carcinogenesis : Tissue Dilution Artefact—a Re-interpretation of Variations in Levels of (G. R. N. Jones, L. Bitensky, J. Chayen and Prof. G. J. Cunningham), 1203
Dehydrogenase (Triphosphopyridine Nucleotide-linked Glyceraldehyde Phosphate), in a Non-Photosynthetic Plant Tissue (C. R. Benedict and Dr. H. Beevers), 71
Dehydrogenase Activity (Mitochondrial α-Glycerophosphate), in Skeletal Muscle : Direct Relationship of Phosphorylase and (Dr. A. G. E. Pearse), 504
Dehydrogenases (Heterogeneous Lactic), in Malignant Effusions : Diagnostic Significance of (R. Richterich, K. Zuppinger and E. Rossi), 507
Dekhuijzen, H. M. : Transformation in Plants of Sodium Dimethyldithiocarbamate into other Fungitoxic Compounds, 198
Delbouille, L., and others : Molecular Emission Spectroscopy from 2μ to 12μ by a Michelson Interferometer, 264
Delight : Mathematical, *review* by W. J. Langford, 638
Delorme, Dr. E. J. : Assessment of Pain (Arrangements at the Chester Beatty Research Institute for the Comfort of the Animals used), 1354
δ-Plutonium : Structure of (J. A. C. Marples and J. A. Lee), 1391
Demethylation (Oxidative), and Carcinogenicity of 4-N-Dimethylaminoazobenzene Derivatives (Prof. Morizo Ishidate and Akira Hanaki), 1198
Demographic Yearbook for 1960 (United Nations), 1346
Demonstration of Anti-Depressant or Stimulant Properties of Imipramine in Experimental Animals (Dr. D. R. Maxwell and Helen T. Palmer), 84
Demonstration by Paper Electrophoresis of an Auto-Immunological Precipitin possibly due to Fibrinolysins (Marion I. Cooper and J. R. Sykes), 1212
Demonstration (Histochemical), of Gamma-Glutamyl Transpeptidase (Prof. Z. Albert, Dr. Marian Orlowski and Dr. A. Szewczuk), 767
Demyelinating Diseases (Establishment by the Medical Research Council of a Research Group at the University of Durham on), 1143
Denatured Haemoglobin Variants in Acid Buffers : Solubility of (I. D. Fraser and Dr. A. B. Raper), 355
Denduchis, Berta, and others : Collagen Fractions in the Skin of New-born and Adult Rats, 1297
Denervation : Muscle Target Fibres, a Newly Recognized Sign of (Dr. W. K. Engel), 389
Dengler, Hans J., and others : Effects of Drugs on Uptake of Isotopic Norepinephrine by Cat Tissues, 816
Denitrification : Distribution of Isotopic Nitrogen in Nitrogen Gas during (R. D. Hauck and D. R. Bouldin), 871
Dennis, D. T., and Preston, Prof. R. D. : Constitution of Cellulose Microfibrils, 667
Density of the Upper Atmosphere determined from Satellite Orbits, and its Variation during 1957-61 (D. G. King-Hele and D. M. C. Walker), 114
Density (Population) : Sexual Differentiation in *Hydra* in relation to (Helen D. Park, Christyna Mecca and Anne Ortmeyer), 92
Dent, Prof. H. C. : Universities in Transition, 1027
Dentice di Accadia, F., and others : Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of *Penicillium chrysogenum*, 909
Deo, A. V., and others : Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers, 378
Deoxycholate (Sodium) : Changes of Permeability of Yeast with (Dr. L. Kováč), 698
Deoxyribonuclease : Effect of Ribonuclease and, on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Deoxyribonucleic Acid : Incorporation of, by Mammalian Cells *in vitro* (Prof. E. R. M. Kay), 387
  Kinetics of the Enzymic Degradation of, into Sub-Units (Dr. Giorgio Bernardi and Prof. Charles Sadron), 809
  Low-Angle Light Scattering and the Molecular Weight of (Dr. Sidney Katz), 280

Deoxyribonucleic Acid in *Drosophila melanogaster* : Induction of Mutations by (Dr. O. G. Fahmy and Dr. Myrtle J. Fahmy), 776

Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398

Deoxyribonucleic Acid from Mammalian Sperm : Isolation and Properties of (Dr. E. Borenfreund, E. Fitt and Dr. A. Bendich), 1375

Deoxyribonucleic Acid of Phage Alpha : Some Properties of the (S. Cordes, H. T. Epstein and Prof. J. Marmur), 1097

Deoxyribonucleic Acid Content of Nuclei dividing Amitotically (Prof. K. W. Cleland), 504

Deoxyribonucleic Acid Structures in the Nucleolus (Dr. Elsa H. J. O'Donnell), 1325

Dependence on Light of Geotropism in Plant Roots (J. V. Lake and G. Slack), 300

Dependence on Substrate of the Electrical Potential across the Isolated Gut (Dr. T. W. Clarkson, A. C. Cross and S. Toole), 501

Dependence on Temperature of the Distribution of Cations in Oxide Spinels (Ranajit K. Datta and Prof. Rustum Roy), 169

Dependence (Mutual) : Copepod *Ommatokoita elongata* (Grant) in the Eyes of the Greenland Shark—a Possible Cause of (Bjørn Berland), 829

Depolarization (Membrane), of Excitable Tissues : Outflux of Inorganic and Organic Phosphate during (Dr. L. G. Abood, Prof. K. Koketsu and I. Koyama), 395

Depolarization Technique (Light) : Crystallization of Isotactic Polypropylene using a (J. H. Magill), 1092

Deposited Radionuclides (Internally) : Removal of, by Triethylenetetraamine-hexaacetic Acid (Prof. A. Catsch and D. Schindewolf-Jordan), 715

Deposition (Wettability and the Capture, Carriage and), of Particles by Raindrops (R. R. Davies), 616

Depressant (Anti-) or Stimulant Properties of Imipramine : Demonstration of, in Experimental Animals (Dr. D. R. Maxwell and Helen T. Palmer), 84

Derivatives : Pyridine and its (edited by Erwin Klingsberg), *review* by Dr. G. L. Buchanan, 425

Derivatives (Amino-) of 11β, 17α, 21-Trihydroxy-3,20-Dioxo-1,4-Pregnadiene (J. Tóth, Z. Tuba and L. Szporny), 607

Derivatives (4-N-Dimethylaminoazobenzene) : Oxidative Demethylation and Carcinogenicity of (Prof. Morizo Ishidate and Akira Hanaki), 1198

Derivatives (Proline), from Apples : Determination by Mass Spectrometry of the Structure of (Dr. K. Biemann, G. G. J. Deffner and Prof. F. C. Steward), 380

Derivatives (Pyridine), with Anthelmintic Activity (A. P. Phillips and R. B. Burrows), 707

Derivatives (Reduced Alkylated), of some Myeloma Globulins : Comparison of, and Bence-Jones Proteins (Dr. M. D. Poulik and Dr. G. M. Edelman), 1274

Derivatives (Trimethylsilyl Ether), of Alcohols : Peak-Shift Technique in Gas–Liquid Chromatography— (Stanley H. Langer and Peter Pantages), 141

Desert Locust : Induced Changes in the Amounts of Material in the Neurosecretory System of the (Dr. K. C. Highnam), 199

Desertomycin Medium : Use of, in Routine Selective Isolation of Fungi Pathogenic to Man (J. Uri and Zs. Herpay), 1015

Design and Use of Respirators (Ergonomics Research Society and the British Occupational Hygiene Society Discussion on), 655

Desmofibrin : Conversion of Fibrin to (K. Buluk, T. Januszko and J. Olbromski), 1093

Detecting Anaphylactic Sensitivity : A New Test for—the Basophil Reaction (Prof. Walter B. Shelley and Lennart Juhlin), 1056

Detecting Penicillinase-producing Organisms : Use of Cellulose Acetate Membranes for (Prof. R. Knox and J. T. Smith), 926

Detection of 6-Amino-penicillanic Acid : Simple, Quick Methods for (J. Uri and F. Sztaricskai), 1223

Detection of the First Motion of a Refracted *P*-Wave : Possible Method for Increasing the Signal-Noise Ratio in the (F. A. Key, J. K. Wright, E. W. Carpenter and B. M. H. Stott), 1382

Detection by Ionization of Atmospheric Gases during Analysis by Gas Chromatography (Dr. Arthur Karmen, Laura Giuffrida and Robert L. Bowman), 906

Detection (Colorimetric), of Penicillins and Cephalosporins on Paper (Robert Thomas), 1161

Detection (Simultaneous), of Iodide and Iodinated Amino-Acids on Filter Paper Chromatograms : A Spray Method for the (Dr. A. Van Zyl and M. Bhaga), 275

Detector (Silicon Semiconductor Nuclear Particle) : Interaction of 14-MeV. Neutrons with a (Dr. W. M. Deuchars and G. P. Lawrence), 995

Detectors (Ionization), in Gas Chromatography : Integration with (P. M. E. M. van der Grinten and Dr. A. Dijkstra), 1195

Deterioration of Solid-coated Capillary Columns on Standing (George M. Rouayheb and William C. Hamilton), 801

Determination of Caesium-137/Potassium Ratios in Diet and in the Human Body by Urine Analysis (A. Morgan and Gloria M. Arkell), 1100

Determination of Hydrogen in Gaseous Mixtures by Gas-Chromatography (P. J. Kipping), 270

Determination of Indium in High-purity Zinc (Naîm Košaric and the late G. Leliaert), 703

Determination of Lead in Urine by Atomic Absorption Spectroscopy (J. B. Willis), 381

Determination by Mass Spectrometry of the Structure of Proline Derivatives from Apples (Dr. K. Biemann, G. G. J. Deffner and Prof. F. C. Steward), 380

Determination of Spontaneous Plasma Fibrinolysis by a Practical Procedure (Dr. H. Yatzidis), 604

Determination of Uranium as Pyrophosphate (Dr. F. M. Brewer), 1290 ; (J. S. Wright, T. J. Hayes and J. A. Ryan), 1291

Determination (Quantitative), of Metadrenaline and Normetadrenaline in Normal Human Urine (Dr. Kaoru Yoshinaga, Dr. Chuichi Itoh, Dr. Nozomu Ishida, Dr. Tatsuo Sato and Dr. Yasuo Wada), 599

Determination (Rapid), of Ytterbium in Aqueous Solutions and in Thulium Oxide by Neutron Activation (Minoru Okada), 1090

Determination (Rapid Infra-Red), of Acetone in the Blood and the Exhaled Air of Diabetic Patients (Dr. Richard D. Stewart, Edward A. Boettner and Bruce T. Stubbs), 1008

Determination (Simultaneous), of Dextran and Glucose in Serum (A. Rédei and S. Nagy), 173

Determinations (Age-), in the Lizard Area : Preliminary (J. A. Miller and D. H. Green), 159

Detonation : Theory of (Ia. B. Zeldovich and A. S. Kompaneets), *review* by Dr. W. L. Murray, 1236

Detonations (Nuclear) : Iron-55 from (Rama, Minoru Koide and Prof. Edward D. Goldberg), 162

Deuchar, Dr. Elizabeth M. : Amino-Acid Activation in Tissues of Early Embryos, 1006

Deuchars, Dr. W. M., and Lawrence, G. P. : Interaction of 14-MeV. Neutrons with a Silicon Semiconductor Nuclear Particle Detector, 995

Deuterium : A Biochemical Effect of (Dr. Howard J. Ringold, Dr. Sumner Burstein and Dr. Ralph I. Dorfman), 1294

Deuterium Oxide : Ultra-violet Absorption Spectra of Proteins in (Dr. G. H. Beaven and Elizabeth Bailey), 914

Deutsch, J. L., and others : Rotational Analysis of Absorption Bands of Lead Monoxide, 374

Deutsch, Sarah, and others : Age Measurements on Antarctic Rocks (Queen Maud Land), 1286

Deutsche Akademie der Naturforscher Leopoldina : Election of new Members, 122

Deutsche Akademie der Wissenschaften zu Berlin : Völkerkundliche Forschungen, Band 3, Classification of Kin, Age Structure and Marriage amongst the Groote Eylandt Aborigines (Dr. Frederick G. G. Rose), *review* by Dr. R. Needham, 6

Developing Countries : Agricultural Problems in (Nutrition Society Symposium on), 1250

Developing Fruits : Stimulants of Cell Division in (D. S. Letham and E. G. Bollard), 1119

Development : Effect on Growth and (Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid), (I. H. Williams), 361

Development of Chick Embryos : Effects of *Grewia populifolia* Extracts on the (S. K. Brahma and N. Kundu), 506

Development in Mice of Prolonged Non-specific Resistance to Sarcoma Implant and Staphylococcus Infection following Repository Injection of Lipopolysaccharide (Samuel J. Prigal), 1111

Development of the Neon Glow Lamp (1911-61), (Prof. H. E. Watson), 1040

Development of Upper Plastic Limit of Clays : Role of Interparticle Forces in (Dr. Raymond Yong and E. T. Selig), 938

Development (Atomic Energy Research and), in Britain, 857

Development (Early), of the Chum Salmon (*Oncorhynchus keta*) : Effect of Thyroxine plus Thiourea on the (Prof. M. A. Ali), 1214

Development (Industrial) : The Impact of, on the Countryside (Council for the Preservation of Rural England Conference on), 959

Development (Rapid), of Hypomagnesaemia in Lactating Cows given Artificial Rations Low in Magnesium (J. A. F. Rook), 1019

Development (Research and), in Scotland, 440

Development (Research and) : Reviews of Data on, No. 27, Report on Scientists and Engineers engaged in research and development in Colleges and Universities in the United States for 1958 (United States National Science Foundation), 225

  Reviews of Data on, No. 28, Capital Expenditure of Colleges and Universities in the United States for research and development in the Natural and Social Sciences in 1958 (United States National Science Foundation), 545

Development (Scientific Research and) : Government Responsibility in, 837

Development (Spontaneous), of *L* Forms of Streptocci requiring Secretions of other Bacteria or Sulphydryl Compounds during Normal Growth (Ayala Frenkel and Dr. Willy Hirsch), 728

Development Plan (Regional), for Courses for the Diploma in Technology (Regional Advisory Council for Technological Education, London and Home Counties), 1044

Developments in the Chemistry and Technology of Polymers : Recent (Bradford Institute of Technology Course on), 1349

Devi, Dr. Anima, and Sarkar, Dr. N. K. : Catalysis of Incorporation of ^{14}C-Uridine into Rat Lens Ribonucleic Acid by Rat Lens Enzyme System, 1094

Device (Variable Shutter) : A New Photometric Technique using a (Dr. O. C. Jones and Dr. F. J. J. Clarke), 1290

Devices (Gas Discharge) : Physics of (Electronics Group of the Institute of Physics and the Physical Society Conference on), 546

Devonian Palaeocharinidae (Arachnida) : Lung-books in the (Dr. M. F. Claridge and A. G. Lyon), 1190

Dew, Ronald Beresford : Appointed visiting professor of industrial administration at the Manchester College of Science and Technology, 651

Dewhurst, F., and Calcutt, G. : Non-Enzymic Hydroxylation of Polycyclic Aromatic Hydrocarbons, 808

Dewing, J. : Inorganic Polymers (BN Polymers), 1047

Dextran and Glucose in Serum : Simultaneous Determination of (A. Rédei and S. Nagy), 173

Dextran-producing Bacteria : Intracellular Glycosidases of (Dr. R. W. Bailey and Prof. E. J. Bourne), 277

Dextrans : Isolation of Nigerose and Kojibiose from (Kazuo Matsuda, Hiroshi Watanabe, Kentaroh Fujimoto and Prof. Kiyoshi Aso), 278

Dextransucrase *V* and the Role of Metal Ions in Enzyme Catalysis (W. Brock Neely and Julie Hallmark), 385

Dhar, Prof. N. R. : Importance of Nitrogen in Crop Production (Presidential Address to the forty-eighth Indian Science Congress), 544

Di Girolamo, Dr. M., and others : Activation of Amino-Acids in Micro-organisms which produce Antibiotics, 411

Di Marco, A. : Vitamin B_{12} and Intrinsic Factor (Biosynthesis by Different Pathways), 1155

Diabetes Mellitus in Africans in Rhodesia (Dr. M. Gelfand and W. R. Carr), 227

Diabetic Patients : Rapid Infra-Red Determination of Acetone in the Blood and the Exhaled Air of (Dr. Richard D. Stewart, Edward A. Boettner and Bruce T. Stubbs), 1008

Diagnostic Character for the Identification of Ammocoete Species : Oocyte Numbers as a (Dr. M. W. Hardisty), 1215

Diagnostic Significance of Heterogeneous Lactic Dehydrogenases in Malignant Effusions (R. Richterich, K. Zuppinger and E. Rossi), 507

Diagnostik der Bakterien und Actinomyceten (Prof. N. A. Krassilnikov), *review* by Dr. K. Zinnemann, 1333

Diamond, Dr. L. K., and others : Haptoglobin Patterns in Cord Blood Serums, 717
Diamond : Synthesis and X-Ray Analysis of (H. Ll. D. Pugh, J. Lees and J. A. Bland), 865
X-Ray Diffraction Spikes of (Y. Yoneda), 1187 ; (Prof. F. C. Frank), 1188
Diborane–Hydrazine Flames : Formation of Boron Nitride in (W. G. Berg and W. E. Wilson), 380
Dibromo-ethylenes (*cis* and *trans* Dichloro- and) : Ionization Potentials of (Dr. J. Momigny), 1089
Di-(*p*-chlorophenyl)-trifluoromethylcarbinol) : Acaricidal Properties of (K. R. S. Archer and R. Cwilich), 1322
Dichloro- and Dibromo-ethylenes (*cis* and *trans*) : Ionization Potentials of (Dr. J. Momigny), 1089
Dickens, David : At the Scientific Publications Council Meeting on the Publication of Scientific Books (Ownership of Copyright), 131
Dickens, Prof. F. : Tumour Metabolism, *review*, 1335
Dickinson, Dr. Anne, and Mocquot, Dr. G. : Bacteria of the Intestine (Studies on the Bacterial Flora in the Alimentary Tract of Pigs, 1, Enterobacteriaceae and other Gram-negative Bacteria), 1152
Dictionary : English–Russian Geological (T. A. Sofiano), (edited by I. D. Borneman, *et al.*), *review* by Dr. S. I. Tomkeieff, 9
Russian–English Geological (T. A. Sofiano), (edited by A. P. Lebedev and V. E. Khain), *review* by Dr. S. I. Tomkeieff. 9
Dictionary of the Fungi (Ainsworth and Busby's), fifth edition by Dr. G. C. Ainsworth, *review* by Prof. John Colhoun, 109
Didelphis virginiana (Opossum) : Cholesterol in the Tapetum Lucidum of the Eye of the (Antoinette Pirie), 708
Dideoxysugars of *Pasteurella pseudotuberculosis*-specific Polysaccharides, and the Occurrence of Ascarylose (Dr. D. A. L. Davies), 43
Diego Factor in a Puerto Rican Family—a Case of Anti-Diego in Monozygotic Twins (Dr. Hubert J. Van Peenen, Dr. John Scudder, James A. Jack and Erika Awer), 188
Diet and in the Human Body : Determination of Caesium-137/Potassium Ratios in, by Urine Analysis (A. Morgan and Gloria M. Arkell), 1100
Dietetic Association (British), and an International Committee of Dietitians : Third International Congress of Dietetics, 1343
Dietetics : Third International Congress of (British Dietetic Association and an International Committee of Dietitians), 1343
Tradition, Science and Practice in (D. F. Hollingsworth), 1343
Diethylamide (Lysergic Acid) : Effect of Administration of, on Serotonin-Levels in the Body (Dr. D. V. Siva Sankar, D. Barbara Sankar, Edward Phipps and Eleanor Gold), 499
Diethyl-amino-ethyl (DEAE) Cellulose Paper : Chromatography of Proteins on (C. C. Curtain), 1269
Dietitians, International Committee of (British Dietetic Association and an) : Third International Congress of Dietetics, 1343
Diets : The Effect of various, upon Sexual Maturity of Mice (J. Szepsenwol), 1112
Dieu, Dr. H. A. : Effects of Ionizing Radiation on Organic and Biological Solids (Chemical Changes on Irradiation), 1151
Dieudonné, Prof. J. : Foundations of Modern Analysis, *review* by Prof. L. S. Goddard, 6
Difference in the Male Genitalia of East and West African Stocks of *Papilio dardanus* (Lep.) : Genetics of a (J. R. G. Turner, C. A. Clarke and P. M. Sheppard), 935
Differences (Pressure), in a Direct-Current Capillary Discharge (D. S. Smith), 265
Differences (Ultracentrifugal), in Soybean Protein Composition (W. J. Wolf, G. E. Babcock and A. K. Smith), 1395
Different Iron Concentrations : Electrophoretic and Immuno-electrophoretic Patterns of Extracellular Diphtheria Proteins from Culture Filtrates of (Bertold J. Radola), 382
Different Organisms : Comparative Analysis of Inactivating Efficiency of Radiation on (Dr. M. Terzi), 461
Different Photoperiods : Effect of Gibberellic Acid on Growth and Indoleacetic Acid Oxidase Activity of *Lupinus albus* L. exposed to (F. Sági and A. S. Garay), 824
Different Tissues : Influence of Polyvalent Ions on Histones isolated from (I. P. S. Agrell and E. G. Christensson), 284
Different Type of Fluorescent Material as an Optical Maser : Use of a (Jordan J. Markham and Dickron Mergerian), 586
Differential Inhibition of Human Serum Cholinesterase with Fluoride—Recognition of Two New Phenotypes (Prof. H. Harris and Mary Whittaker), 496
Differential Staining of Nucleic Acids : Mechanism of (D. J. Goldstein), 407
Differential Thermogravimetry versus Differential Thermometry in Studying the Pyrolysis of Polymers (Hugh C. Anderson), 1088
Differential Thermometry : Differential Thermogravimetry versus, in Studying the Pyrolysis of Polymers (Hugh C. Anderson), 1088
Differentiation (Sexual), in *Hydra* in relation to Population Density (Helen D. Park, Christyna Mecca and Anne Ortmeyer), 92
Diffraction Conference : Annual Pittsburgh (Mellon Institute, Pittsburgh), 755
Diffraction Spikes (X-Ray), of Diamond (Y. Yoneda), 1187 ; (Prof. F. C. Frank), 1188
Diffraction Studies (X-Ray), of Bacterial Viruses (Dr. A. C. T. North and Prof. Alexander Rich), 1242
Diffuse Freely into the Embryo of *Pteridium aquilinum* : Failure of Nucleotides to (P. R. Bell), 91
Diffusion Barrier (Electrostatic), and Ion Pump : Electrical Properties of Orientated Lipid on a Biological Membrane—an (Dr. J. W. L. Beament), 217
2,6-Difluoropyridine (G. C. Finger and L. D. Starr), 595
Digby, Peter S. B. : Mechanism of Sensitivity to Hydrostatic Pressure in the Prawn, *Palaemonetes varians* Leach, 366
Digestion and Nutrition : Ruminant, *review* by Dr. I. W. McDonald, 1334
Digestive Physiology and Nutrition of the Ruminant—Proceedings of the University of Nottingham Seventh Easter School in Agricultural Science, 1960 (edited by Dr. D. Lewis), *review* by Dr. I. W. McDonald, 1334
Digital Computers, their Use and Control (Mathematics Department of Queen's College, St. Andrews, in collaboration with Standard Telephones and Cables, Ltd., Symposium on), 554
Digital Computers and Nuclear Reactor Calculations (Ward C. Sangren), *review* by Dr. M. V. Wilkes, 9
Dijkstra, Dr. A. (van der Grinten, P. M. E. M., and) : Integration with Ionization Detectors in Gas Chromatography, 1195
Dijkstra, J., and Gillman, J. : Chromatographic Separation of Biologically Active Components from Commercial Trypan Blue, 803
Dilly, Noël : Electron Microscope Observations of the Receptors in the Sensory Vesicle of the Ascidian Tadpole, 786
Dils, Dr. R. R. : Appointed lecturer in the Department of Medical Biochemistry and Pharmacology, University of Birmingham, 337
Dilute Solutions of High Polymers : Elimination of the Absorption Correction in the Viscometry of (H. van Oene and Prof. L. H. Cragg), 1160
Viscosity–Temperature Relationships for (Dr. W. R. Moore), 1292
Dilution Artefact (Tissue)—a Re-interpretation of Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogensis (G. R. N. Jones, L. Bitensky, J. Chayen and Prof. G. J. Cunningham), 1203
Dilworth, M. J., and Parker, C. A. : Oxygen Inhibition of Respiration in *Azotobacter*, 520
Dimensions of Electrical and Magnetic Quantities (Dr. A. E. Martin), 588
Dimensions (Cell), of Layer Lattice Silicates : Surface Symmetry and (E. W. Radoslovich), 67
Dimer Formation and Glucose Transfer across the Membrane of the Red Blood Cell (Dr. W. D. Stein), 1277
Dimer Formation and the Transfer of Glycerol across the Erythrocyte Membrane (Dr. W. D. Stein), 352
Dimers : Pyrene (Isadore B. Berlman), 594
Dimethyl Sulphoxide–Water Systems : Interaction of Lipids and Trypsin in (Prof. A. Lukton, H. Donohue and Prof. F. A. Bettelheim), 565
4-N-Dimethylaminoazobenzene Derivatives : Oxidative Demethylation and Carcinogenicity of (Prof. Morizo Ishidate and Akira Hanaki), 1198
Dimethyldithiocarbamate (Ferric) : Effect of, on the Rooting of Cuttings of *Hevea brasiliensis* (G. H. Tinley), 1217
Dimethyldithiocarbamate (Sodium) : Inhibition of Growth and Photosynthesis in Submerged Plants with Tetramethylthiuram Disulphide and, and Reversal of this Inhibition of Photosynthesis (P. E. Björn Lindahl), 51
Transformation in Plants of, into other Fungitoxic Compounds (H. M. Dekhuijzen), 198
Dineen, Dr. J. K., and Szenberg, Dr. Aleksander : Regeneration of Elements of Donor Origin in Orthotopically Grafted Skin following the Homograft Response, 153
Dingle, Prof. Herbert : The Frequency of Meteorite Falls throughout the Ages, 482
2,4-Dinitrophenol : Effect of Thyroxine and, on the Rate of Utilization of Glucose (Dr. L. Macho), 604
2,4-Dinitrophenylhydrazones of Aromatic Keto-Acids : Side-Reactions during Hydrogenolysis of (Dr. John H. Menkes), 285
Dioxide (Carbon) : Action of, on 6-Aminopenicillanic Acid (F. R. Batchelor, D. Gazzard and J. H. C. Nayler), 910
Irradiation Decomposition of (T. B. Copestake and N. S. Corney), 1192
Dioxide (Carbon), in Leaves of *Bryophyllum fedtschenkoi* : An Endogenous Rhythm in the Rate of Dark-Fixation of (Diana M. Warren and Malcolm B. Wilkins), 686
Dioxide (Carbon), and Ethanol as Factors controlling the Growth of Etiolated Oat Seedlings (Dr. C. L. Mer), 260
Diphenyl : Crystal and Molecular Structure of (Dr. G. B. Robertson), 593
Diphtheria Proteins (Extracellular), from Culture Filtrates of Different Iron Concentrations : Electrophoretic and Immuno-electrophoretic Patterns of (Bertold J. Radola), 382
Diploid-like Meiotic Behaviour of Polyploid Cotton : Basis of the (G. Kimber), 98
Diploma in Technology : Regional Development Plan for Courses for the (Regional Advisory Council for Technological Education, London and Home Counties), 1044
Diphosphopyridine Nucleotide (Reduced) : An Inhibitor of Liver Alcohol Dehydrogenase in Preparations of (Dr. Keith Dalziel), 1098
Dipoles (Satellite Microwave) : Long-Range Radio Communication by—the West Ford Project, 1237
2,2′-Dipyridyl : Stability Constant of Copper (II) Complexes of Reduction Products of (Dr. M. T. Beck and M. Halmos), 1090
Direct-Current Capillary Discharge : Pressure Differences in a (D. S. Smith), 265
Direct Observation of Nematode-trapping Fungi in the Soil : Agar Disk Method for the (Roderic C. Cooke), 1411
Direct Relationship of Phosphorylase and Mitochondrial α-Glycerophosphate Dehydrogenase Activity in Skeletal Muscle (Dr. A. G. E. Pearse), 504
Directions (Feasible) : Methods of—a Study in Linear and Non-Linear Programming (Dr. G. Zoutendijk), *review* by Dr. M. V. Wilkes, 9
Discharge (Direct-Current Capillary) : Pressure Differences in a (D. S. Smith), 265
Discharges (Spontaneous Spike-), of Vascular Smooth Muscle (Dr. S. Funaki), 1102
Discharges (Wave) : Spike and, and Alterations of Conscious Awareness (L. Goldie and J. M. Green), 200
Discipline (Scientific) : Plant Taxonomy as a (Dr. R. E. Holttum), 990
Discovery : Appointment of Gerald Adrian Leach to the editorship, 1043
Discrete Frequency Solar Noise Bursts : Very-Low-Frequency Modulation of (Dr. Jules Aarons, Santimay Basu, William Kidd and Richard Allen), 56
Discreteness-of-Charge Effect and the Stability Theory of Colloids (Dr. S. Levine, D. Calvert and G. M. Bell), 699
Discriminant Analysis in Biometrical Genetics (Dr. S. K. Jain), 1420
Discrimination (Shape) : Facts and Theories of (Dr. P. C. Dodwell), 578 ; (Dr. N. S. Sutherland), 581
Disease : Doctors and, in Tudor Times (W. S. C. Copeman), *review* by Sir Russell Brock, 428
Disease and the Advancement of Basic Science (edited by Dr. Henry K. Beecher), *review* by Sir George Pickering, 530
Disease (Barley Leaf-Spot) : Production of Symptoms of, by Culture Filtrate of *Helminthosporium sativum* (S. K. Gayed), 725
Disease (Sigatoka), of Bananas caused by *Mycosphaerella musicola* Leach : Significance of the Anti-Sporulation Effect of Copper Fungicides in the Control of (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476
Disease Virus (Foot-and-Mouth) : Effect of *p*-Fluorophenylalanine on the Multiplication of (F. Brown, D. N. Planterose and Doreen L. Stewart), 414

Diseases : Demyelinating (Establishment by the Medical Research Council of a Research Group at the University of Durham on), 1143
Diseases (Animal) : Animal Experiments in the Control of (Research Defence Society Pamphlet on), 226
Diseases (Bacterial Plant), of Central Europe : Some, *review* by Dr. W. J. Dowson, 4
Diseases (Forest Insect Pests and), in Canada (Prof. M. L. Anderson), 1260
Diseases (Potato Virus) : Proceedings of the Fourth Conference on (Braunschweig, 1960), edited by J. Brandes, R. Bartels, V. Völk and C. Wetter (Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig), 657
Diseases (Virus) : Research into (Pfizer Laboratories for), 20
Disilicide : Uranium (Allan Brown and J. J. Norreys), 61
Disinhibition (Recurrent), of Spinal Motoneurones : Changes in the Membrane during (Dr. Victor J. Wilson and Paul R. Burgess), 918
Disk Method (Agar), for the Direct Observation of Nematode-trapping Fungi in the Soil (Roderic C. Cooke), 1411
Dislikes (Food) : Taste Thresholds and (Dr. R. Fischer, F. Griffin, S. England and S. M. Garn), 1328
Dispersal of Tropical Seeds by Ocean Currents (R. Mason), 408
Dispersal (Spore), in *Chaetomium globosum* (Kunze), (P. A. Dixon), 1418
Dispersal (Spore Liberation and), of Coffee Rust *Hemileia vastatrix* B. et Br. (R. W. Rayner), 725
Dispersion (Anisotropy Axis) : Variation of, with Thickness of Nickel-Iron Films (H. Clow), 996
Displacement Potentials and Bending of Rod-like Polyelectrolytes (Prof. J. A. Christiansen, the late Dr. C. E. Jensen and Dr. Th. Vilstrup), 484
Display of Sources of Ionizing Radiations at Exhibitions : Code of Practice for the, 1249
Disseminated Lupus Erythematosis : Anti-Leucocyte Cytoplasm, Anti-Leucocyte Nuclei and Anti-Platelets in (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Dissolution and Precipitation of Electrolytes : Electrical Effects during (R. P. Rastogi, B. P. Batra and R. K. Dass), 764
Distinction (Immunochemical), between Insulines with Identical Amino-Acid Sequences (Dr. Solomon A. Berson and Dr. Rosalyn S. Yalow), 1392
Distribution of *Acarapis woodi* (Rennie), (Prof. J. E. Eckert), 933
Distribution of Alkaline Phosphatase (Phosphatases in *Schistosoma mansoni*), (Dr. D. L. H. Robinson), 473
Distribution of Brightness in Extra-galactic Radio Sources (A. T. Moffet and P. Maltby), 453
Distribution of Calcium-45 in the Rat : Effect of Serotonin on Absorption and (Prof. S. Garattini, E. Grossi, P. Paoletti, R. Paoletti and M. Poggi), 185
Distribution of Cations in Oxide Spinels : Dependence on Temperature of the (Ranajit K. Datta and Prof. Rustum Roy), 169
Distribution of Human Chromosome Counts in relation to Age (Patricia A. Jacobs, W. M. Court Brown and Dr. Richard Doll), 1178
Distribution of Iodine-131 in the Internal Organs, Fluids and Excreta of Rabbits: Effect of Polyvinyl Alcohol on the (H. F. Nowak and J. Kucharski), 665, *erratum*, 1045
Distribution of Isotopic Nitrogen in Nitrogen Gas during Denitrification (R. D. Hauck and D. R. Mouldin), 871
Distribution of Piperidine in the Brain and its Possible Significance in Behaviour (Dr. L. G. Abood, F. Rinaldi and Virginia Eagleton), 201
Distribution of Radioactivity in Protein of Cell Fractions from Myocardial Slices : Effect of Adrenalectomy and Corticosteroids on (Dr. Edward I. Weinshelbaum and Dr. Ira G. Wool), 1401
Distribution of Sodium and Potassium in certain Cells and Tissues (Dr. Norman R. Joseph, Dr. Milton B. Engel and Dr. Hubert R. Catchpole), 1175
Distribution of Water in the Chick Blastoderm (A Morphogenetic Effect of Beta-Mercaptoethanol), (A. Jurand and Dr. Peter Tuft), 1073
Distribution of Water in the Embryo (A Morphogenetic Effect of Beta-Mercaptoethanol), (Dr. Peter Tuft), 1072
Distribution of X-Ray- and Nitrous Acid-induced Mutations in the Genetic Fine Structure of the ad_7 Locus of *Schizosaccharomyces pombe* (Dr. Herbert Gutz), 1125
Distribution (Latitude), of Ionospheric Irregularities : Scintillations and the (D. G. Singleton), 482
Distribution (Seasonal Fluctuations and), of Nitrite in a Tropical West African Estuary (J. C. D. Watts), 929
Distribution (Spatial), of Free Radicals as studied by Electron Spin Resonance (Robert C. Smith and S. J. Wyard), 897
Distribution (Species), of Nineteen Phenotypes : Serum Transferrins in the Genus *Macaca*— (Prof. Morris Goodman and Emily Poulik), 1407
Distribution Pattern (Intracellular), of Rat Liver Glutamic-Oxalacetic Transaminase (Dr. Herbert J. Eichel and Josef Bukovsky), 243
Disturbance (Travelling), in the *F* Region of the Ionosphere : Group and Phase Velocities of a (Dr. L. H. Heisler and Dr. J. D. Whitehead), 157
Disturbances (Geomagnetic), in the Auroral Zone : Electric Currents associated with (Dr. F. J. Scrase), 963
Disturbances (Nervous and Biochemical), following Hexachlorobenzene Intoxication (Dr. F. De Matteis, B. E. Prior and Prof. C. Rimington), 363
Disulphide (Tetramethylthiuram), and Sodium Dimethyldithiocarbamate : Inhibition of Growth and Photosynthesis in Submerged Plants with, and Reversal of this Inhibition of Photosynthesis (P. E. Björn Lindahl), 51
Ditchburn, Prof. R. W. : At the Maxwell Colour Centenary Conference (Complexities of the Visual Process), 10
Diurnal Rhythm in Cave Crayfish (Prof. Frank A. Brown), 929
Diurnal Variation in Transaminase Activity in the Cactus Plant (S. K. Mukerji, G. G. Sanwal and Prof. P. S. Krishnan), 516
Divalent Metal Ions in the Binding of Tetracycline to Macromolecules : Mediation of (Dr. Kurt W. Kohn), 1156
Diverse Sources of Cows' Milk : An Invariant Casein Substrate for Rennet Standardization, derivable from (Dr. G. W. Scott Blair and J. C. Oosthuizen), 697
Diversification : Cell (American Society for Cell Biology Symposium on), 1045
Dividing Amitotically : Deoxyribonucleic Acid Content of Nuclei (Prof. K. W. Cleland), 504
Division (Cell) : Stimulants of, in Developing Fruits (D. S. Letham and E. G. Bollard), 1119
Dixon, Dr. G. H. (Wilson, Dr. S., and) : A Comparison of Cod and Bovine Insulins, 876
Dixon, P. A. : Spore Dispersal in *Chaetomium globosum* (Kunze), 1418
Djerassi, Prof. Carl : Stereochemistry of Natural Phenolic Compounds (Relative Configuration of the Rotenoids), 236
Dobbing, Dr. J. (Davison, Dr. A. N., and) : Metabolic Stability of Body Constituents, 844
Dobiášová, M., and others : A Simplified Method for Separating Amino-Acid Mixtures from Protein Hydrolysates on One Ion-Exchange Column during Preparation, 1198
Doctors and Disease in Tudor Times (W. S. C. Copeman), *review* by Sir Russell Brock, 428
Dodd, Barbara (Coombs, Dr. R. A. A., and) : Microscopical Technique to determine the Nature of a Stain on a Single Fibre of Clothing or other Material, 753
Dodds, Dr. Kenneth S. : Reviews of Genetics, *review*, 636
Dodgson, Dr. K. S. : The Training of Biochemists, 955
Dodwell, Dr. P. C. : Facts and Theories of Shape Discrimination, 578
Doebbler, Dr. G. F., and others : Recovery and *in vivo* Survival of Rabbit Erythrocytes, 1405
Dog : Failure of a, to Discriminate between Strontium-90 and Calcium given Orally (Dr. Betsy J. Stover, M. Goldman and A. C. Andersen), 713
Dog (Spinal) : Stimulation of the Heart of the, by Phenoxybenzamine (B. G. Benfey and D. R. Varma), 919
Döklen, A., and others : Effect of Amino-Acid Esters on the Growth of Fibroblast Cultures, 494
Dolerites (Weathered Karroo) : Climate and (Dr. H. H. Weinert), 325
Doll, Dr. Richard, and others : Distribution of Human Chromosome Counts in relation to Age, 1178
Domestic Animals and Poultry in India : Occurrence of *Salmonellae* in (V. K. Sharma and Prof. C. M. Singh), 622
Domestic Fowl : Effect of Varying Doses of Undiluted Semen on Fertility in the (G. C. Taneja and R. S. Gowe), 828
Dominance (Cerebral) : Speech, Handedness and, *review* by Sir Russell Brain, 840
Dominance (Cerebral), and its relation to Psychological Function (Prof. O. L. Zangwill), *review* by Sir Russell Brain, 840
Dominance (Social), in Inbred Mouse Strains (Prof. G. Lindzey, H. Winston and M. Manosevitz), 474
Donaldson, W. A. : Digital Computers, their Use and Control (Computers in Industry), 554
Doncaster, C. C. : Nematology (Feeding Mechanisms of Rhabditids and Tylenchids), 1048
Donohue, H., and others : Interaction of Lipids and Trypsin in Dimethyl Sulphoxide–Water Systems, 565
Donor Origin (Regeneration of Elements of), in Orthotopically Grafted Skin following the Homograft Response (Dr. J. K. Dineen and Dr. Aleksander Szenberg), 153
Doped Crystals of Sodium Chloride : Quenching of Cation Vacancies in (the late Sir K. S. Krishnan and S. C. Jain), 162
Dore, M. (Carmichael, C. H., and) : Electrical Effects observed in Polycrystalline Boron, 485
Dorfman, Dr. Ralph I., and others : A Biochemical Effect of Deuterium, 1294
Dorling, J. W. : New Research Laboratories for the Paints Division of Imperial Chemical Industries, Ltd., 25
Dorman, R. G. : Design and Use of Respirators (Fibrous Filters for Protection against Aerosol Particles), 655
D'Ornano, Liliane (Pichinoty, Francis, and) : Inhibition by Oxygen of Biosynthesis and Activity of Nitrate-Reductase in *Aerobacter aerogenes*, 879
Doses (Varying), of Undiluted Semen : Effect of, on Fertility in the Domestic Fowl (G. C. Taneja and R. S. Gowe), 828
Double and Single Spermathecae in Fleas (Siphonaptera), (Hon. Miriam Rothschild), 1322
Douglas, R. H. : Radar Reflectivities of Hail Samples, 266
Douglas, Prof. R. W. : A. T. Green and the British Ceramic Research Association, *review*, 207
Downer, Dr. J. L. deC. : Changes in Visual Gnostic Functions and Emotional Behaviour following Unilateral Temporal Pole Damage in the 'Split-Brain' Monkey, 50
Downie, Dr. C. : Palaeogeological Maps, *review*, 842
Dowrick, Dr. G. J. : Biology of Reproduction in *Rubus*, 680
Dowson, Dr. W. J. : Some Bacterial Plant Diseases of Central Europe, *review*, 4
Doyle, F. P., and others : Some Novel Acid-stable Penicillins, 1091
Doyle, Dr. W. P. : Appointed a lecturer in chemistry at the University of Edinburgh, 443
Dracone Developments, Ltd. : Appointment of Dr. B. J. A. Bard as chairman, 443
Dragonflies : Spiracle Control in (Dr. P. L. Miller), 621
Draper, Laurence (Currie, Ronald, and) : Time-integrated Measurements of Submarine Irradiance, 661
Draškoci, Dr. M., and others : Antibody Response in Rabbits following Injection of Sheep Erythrocytes into Lateral Ventricle of Brain, 288
Dresdner, Dr. Richard : Fluorine Chemistry, *review*, 319
Dresser, D. W. : Effectiveness of Lipid and Lipidophilic Substances as Adjuvants, 1169
Drew, R. M., and others : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture, 1398
Drilling Fluid Thinners : Alberta Sub-bituminous Coals as (E. J. Jensen), 1144
Drinking Water : European Standards for (United Nations, World Health Organization), 1261
Drinking-Water Standards (Nora H. Johnson), 1261
Drosera : Axenic Seedlings of (Richard M. Burger), 724
Drosophila : Chromosome Rearrangements induced by X-Rays in Immature Germ Cells of (Seymour Abrahamson), 523
Drosophila melanogaster : Induction of Mutations by Deoxyribonucleic Acid in (Dr. O. G. Fahmy and Dr. Myrtle J. Fahmy), 776
  Recombination in the X-Chromosome of (C. N. Law), 1180
Drug Directorate (Food and) : Research in the (Dr. Ross A. Chapman), 335
Drug Supplies during the American Revolution (George B. Griffenhagen), 761

Drugs : Effects of, on Uptake of Isotopic Norepinephrine by Cat Tissues (Hans J. Dengler, Herbert E. Spiegel and Elwood O. Titus), 816
Drugs (Morphine-like) : Effects of, in Chicks (C. Schneider), 607
Dry Granules through Apertures : Minimum Energy Theorem for Flow of (R. L. Brown), 458
Dry or Moist Surfaces: A Method of replicating, for Examination by Light Microscopy (Joan Sampson), 932
Dry and Wet Condition : Radiation Sensitivity of *Escherichia coli* to β-Rays in (S. B. Bhattacharjee and N. N. Das Gupta), 1015
Drying (Freeze-) of Foodstuffs (Borough Polytechnic, London, Symposium on), 651
Drying and Storage of Grain : Buildings for the (Bibliography of Research Publications on Farm Buildings, Section 5), (Agricultural Research Council), 440
Dubbs, Prof. Clyde A., and others : Subfractionation of Human Serum Leucine Aminopeptidase, 1203
Dubin, Donald T., and others : Influence of Salts on the Uptake of Streptomycin by *Escherichia coli*, 1324
Duck Blood *in vitro* : Intermediate Pool between Amino-Acids and Globin in (L. Bianco, G. Giustina and E. Lazzarini), 40
Ducts (Excretory), in the Genus *Notopterus* (Lacepede), (Madhu Sudan Sharma), 1118
Duncan, Dr. D. R. : At the Maxwell Colour Centenary Conference (Colour Printing), 11
Duncan, R. B. (Briggs, M. H., and) : Odour Receptors, 1310
Dunham, Prof. K. C. : Black Shale, Oil and Sulphide Ore (Presidential Address to Section C (Geology) of the British Association for the Advancement of Science), 985
Dunn, A. M. : Parasite Biology (Parasites in relation to Natural Fauna), 134
Dunn, Dr. F. L., and others : Isolation of *Plasmodium knowlesi* from Philippine Macaques, 1117
Dunsford, Dr. I., and others : Investigation into Blood Groups in a Paternity Case, 753
Duplication (Chromosome), in *Vicia faba* : Sub-Chromatid Structure and (W. J. Peacock), 832
Durbin, James : Appointed to the University of London chair of statistics at the London School of Economics and Political Science, 1247 ; work of, 1247
Dust Cloud about the Earth (Dr. J. H. Fremlin), 31 ; (Dr. David B. Beard), 32 ; (Dr. Fred L. Whipple), 33
Dux, Dr. E. : International Convention on Starch, 236
Dyes (Cyanine), on Silver Bromide Microcrystals : Electron Spin Resonance Absorptions induced by Light in Adsorbed Layers of (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Dyestuffs (Basic), in Clay Mineralogy (Dr. V. S. Ramachandran, K. P. Kacher and N. K. Patwardhan), 696
Dyke, G. V. : Advances in Agronomy, *review*, 213
Dynamics of the Psyche : The Structure and (C. G. Jung), translated by R. F. C. Hull, *review* by Dr. C. Allen, 1235
Dynkin, E. B. : Theory of Markov Processes (translated from the Russian by D. E. Brown ; edited by T. Köváry), *review* by D. G. Kendall, 321

EABORN, Dr. C. : Appointed professor of chemistry at the University of Sussex, 1349
Eagland, D., and Franks, F. : *p*H of Soap Solutions, 1003
Eagleton, Virginia, and others : Distribution of Piperidine in the Brain and its Possible Significance in Behaviour, 201
Earland, Dr. C., and Raven, D. J. : Lanthionine Formation in Keratin, 384
Early Cretaceous (Hanterivian) Angiosperm Fruit from California (M. E. J. Chandler and D. I. Axelrod), 442
Early Development of the Chum Salmon (*Oncorhynchus keta*) : Effect of Thyroxine plus Thiourea on the (Prof. M. A. Ali), 1214
Early Embryos : Amino-Acid Activation in Tissues of (Dr. Elizabeth M. Deuchar), 1006
Early Stages of Propylthiouracil Treatment in the Rat : Increase of the Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171
Earth : The Dust Cloud about the (Dr. J. H. Fremlin), 31 ; (Dr. David B. Beard), 32 ; (Dr. Fred L. Whipple), 33
  The Interior of the (Dr. R. Stoneley), 983
  The Rotation of the (Walter H. Munk and Gordon J. F. Macdonald), *review* by Prof. S. K. Runcorn, 313
Earth Conditions (Primitive) : Mechanism of Synthesis of Adenine from Hydrogen Cyanide under Possible (Prof. J. Oró), 1193
Earth Satellites (Artificial) : A Collection of Tables and Nomograms for the Processing of Observations made on (I. D. Zhongolovich and V. M. Amelin), *review* by D. G. King-Hele, 1135
Earthworm in India : Taxonomy of the (G. E. Gates), 227
East Africa : Medical Research in, 226
East African Council for Medical Research : Report for 1959-60, 226
East and West African Stocks of *Papilio dardanus* (Lep.) : Genetics of a Difference in the Male Genitalia of (J. R. G. Turner, C. A. Clarke and P. M. Sheppard), 935
Easter School in Agricultural Science : Proceedings of the University of Nottingham seventh (1960)—Digestive Physiology and Nutrition of the Ruminant (edited by Dr. D. Lewis), *review* by Dr. I. W. McDonald, 1334
Eastern Coasts of the Americas : Algological Study on the, *review* by Prof. Lily Newton, 1234
Eastern India : Cytology of some Genera of Polypodiaceae in (G. Panigrahi and S. N. Patnaik), 1207
Eastern Tropical and Subtropical Coasts of the Americas : Marine Algae of the (Prof. William Randolph Taylor), *review* by Prof. Lily Newton, 1234
Eastern and North-Eastern Africa : Birds of (C. W. Mackworth-Praed and C. H. B. Grant), second edition, *review* by Sir Landsborough Thomson, 9
Eastward Equatorial Undercurrent in the Atlantic : Evidence of an, from Measurements of Current Shear (Arthur D. Voorhis), 157
Eccles, Sir John C. : Elected a Member of the Physiology Section of the Leopoldina German Academy of Sciences, 122
Echeneis : Structure and Action of the Sucker of (Prof. B. Bonnell), 403
Eck, Richard V. : Non-Randomness in Amino-Acid 'Alleles', 1284
Eckert, Prof. J. E. : Distribution of *Acarapis woodi* (Rennie), 933
Ecological Aspects of Irrigation in Ancient Mesopotamia (Hans Helback), 227
Ecologicos (Estudios), para el Aprovechamiento de la Tierra en Nicaragua (Dr. B. W. Taylor), 1259
Ecology : Cockroach, *review* by David R. Ragge, 106
Ecology (Tropical), International Society for : Proceedings of the Symposium on Forest Ecosystem (Montreal, 1959), 755
Ecology (Tropical Animal), Nuffield Unit of : Appointment of Dr. R. M. Laws as senior investigator, 651
Ecology and Epidemiology : Stochastic Population Models in (Prof. M. S. Bartlett), *review*, 843
Economic Use of Brains (Prof. C. F. Carter), 987
Ecto- and Endo-Parasites of South-East Asia, *review* by P. F. Mattingly, 948
Ectoparasitic Species (Three) : Simultaneous Eradication of, from a Colony of Laboratory Mice (Dr. Nigel Bateman), 721
Edeleanu, Dr. Constantin : Awarded a Gold Medal and Prize from the Beilby Memorial Fund, 224 ; work of, 224
Edelman, Dr. G. M. (Poulik, Dr. M. D., and) : Comparison of Reduced Alkylated Derivatives of some Myeloma Globulins and Bence-Jones Proteins, 1274
Edelstam, Dr. C. : *Obituary* of Dr. H. Horn af Rantzien, 121
Edgar, Janice B., and Stocker, Dr. B. A. D. : Metabolic and Genetic Investigations of Nutritionally Exacting Strains of *Staphylococcus pyogenes*, 1121
Edozien, Dr. J. C., and Phillips, E. J. : Partition of Urine Nitrogen in Kwashiorkor, 47
Education : Science and—a Policy Statement (Science Masters' Association and the Association of Women Science Teachers), 555
Education of Backward Children in Britain, 1052
Education by Television, 226
Education in Uganda (Dr. B. E. Lawrence), 990
Education (Adult), Universities Council for : Report for 1959-60, 861
Education (Commercial and Industrial), British Association for : Interviewing in Twenty-six Steps (John S. Gough), 228
  New Routes to Further Education—Papers presented at the B.A.C.I.E. Spring Conference, London (1961), 739
Education (Further) : New Routes to—Papers presented at the B.A.C.I.E. Spring Conference, London, 1961 (British Association for Commercial and Industrial Education), 739
Education (Health) : Teacher Preparation for (United Nations, World Health Organization, Technical Report Series No. 193—Report of a joint WHO/Unesco Expert Committee), 441
Education (Management), Foundation for : Grant of £16,000 a year for seven years to the University of Leeds, 228
Education (Mathematical), in Britain : Crisis in, 103
Education (Technical) : Apprenticeship and, 737
Education (Technical) Abstracts from British Sources (National Foundation for Educational Research in England and Wales), Vol. 1, No. 1, September–December 1960, 21
Education (Technological), Regional Advisory Council for, London and Home Counties : Regional Development Plan for Courses for the Diploma in Technology, 1044
Educational Research in England and Wales, National Foundation for : *Technical Education Abstracts from British Sources*, Vol. 1, No. 1, September–December 1960, 21
Educational Television—a Survey and a Plan (John Wellens), 226
Edwards, Dr. D. K. : Influence of Electrical Field on Pupation and Oviposition in *Nepytia phantasmaria* Stkr. (Lepidoptera : Geometridae), 976
Edwardson, S. M. : An Instrument for Measuring Television Signal-to-Noise Ration (B.B.C. Engineering Monograph No. 37), 1355
Effect : An Autoradiographic (Stuart McLean), 690 ; (A. H. Meleka and W. Barr), 692
Effect of Acid : Thyroglobulin— (Dr. A. D. Litonjua), 356
Effect of Added Vitamins on the Phagocytic Activity of the Reticulo-Endothelial System of Mice (J. Cordingley and Prof. T. Nicol), 82
Effect of Administration of Lysergic Acid Diethylamide on Serotonin-Levels in the Body (Dr. D. V. Siva Sankar, D. Barbara Sankar, Edward Phipps and Eleanor Gold), 499
Effect of Adrenalectomy on the Activity of Enzymes of the Urea Cycle in Rat Liver (Patricia McLean), 1302
Effect of Adrenalectomy and Corticosteroids on Distribution of Radioactivity in Protein of Cell Fractions from Myocardial Slices (Dr. Edward I. Weinshelbaum and Dr. Ira G. Wool), 1401
Effect of α-Amylase on the Ehrlich Mouse Ascites Tumour (Dr. F. Steckerl, A. Ofodile, R. R. Campbell and G. H. Friedell), 1113
Effect of Amino-Acid Esters on the Growth of Fibroblast Cultures (Dr. I. Fehér, A. Döklen and V. Selmeci), 494
Effect of Attention in Peripheral Vision (Arthur Thomson), 732 ; (B. Babington Smith), 732
Effect of Bee Venom on Concentration of Blood Glucose and Liver Glycogen in Rabbits (Prof. A. H. Mohamed and K. Zaki), 605
Effect of Bilateral Section of the Splanchnic Nerve on Erythropoiesis (Fumimaro Takaku, Kunitake Hirashima and Prof. Shigeo Okinaka), 500
Effect of Boric Acid on the Phosphoglucomutase of Pea Seeds (Dr. B. C. Loughman), 1399
Effect of Bradykinin on Uterine Activity (Dr. B. Berde and Dr. K. Saameli), 83
Effect of Calf Thymus Histone on Rats bearing Transplantable Acute Myeloid Leukaemia (Dr. Lubomir Hnilica and Dr. Viktor Holoubek), 922
Effect of Chloride Concentration on the Aqueous Corrosion of a Magnesium Alloy (D. de G. Jones and H. G. Masterson), 165
Effect of various Diets upon Sexual Maturity of Mice (J. Szepsenwol), 1112
Effect of Enzyme Injections on Mitosis in Regenerating Liver (Dr. Gaston de Lamirand), 400
Effect of Ethylene Oxide on the Tapping of *Hevea brasiliensis* (D. H. Taysum), 1319
Effect of Excess Supply of Heavy Metals to Barley during Germination, with special reference to Catalase and Peroxidase (Dr. S. C. Agarwala, A. Kumar and C. P. Sharma), 726
Effect of External Conditions on the Phase of Radio Signals (D. E. Watt-Carter and R. L. Corke), 1286
Effect of Ferric Dimethyldithiocarbamate on the Rooting of Cuttings of *Hevea brasiliensis* (G. H. Tinley), 1217

Effect of *p*-Fluorophenylalanine on the Multiplication of Foot-and-Mouth Disease Virus (F. Brown, D. N. Planterose and Doreen L. Stewart), 414
Effect of 5-Fluorouracil on Hepatic Induced Increase of Tryptophan-Pyrrolase (Sancia Gaetani and M. Antonietta Spadoni), 1296
Effect of Gibberellic Acid on Growth and Indoleacetic Acid Oxidase Activity of *Lupinus albus* L. exposed to Different Photoperiods (F. Sági and A. S. Garay), 824
Effect of Growth and Development (Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid), (I. H. Williams), 361
Effect of Hybridization on the Primary Polydipsic Trait of an Inbred Strain of Mice (Dr. Emanuel Silverstein), 523
Effect of Irradiated Normal Tissues on the Growth of Mouse Carcinoma (Dr. Arthur Compton and Prof. Charles A. Pannett), 1101
Effect on Mast Cells : Mechanism of Action of Antigen–Antibody Complexes —their (Dr. Ivan Mota), 572
Effect of Ozone on the Fluorescence and Ultra-violet Absorption Spectra of 3,4-Benzopyrene (Z. Morlin and K. M. Sáringer), 907
Effect of Pituitary Growth Hormone on Cholesterogenesis (Dr. A. L. Greenbaum and R. S. Leal), 600
Effect of Polyamino Carboxylic Acids on Catalase (Dr. Joan Bond and J. G. Cooper), 385
Effect of Polyvinyl Alcohol on the Distribution of Iodine-131 in the Internal Organs, Fluids and Excreta of Rabbits (H. F. Nowak and J. Kucharski), 665, *erratum*, 1045
Effect of Population Movements on Malaria Eradication (R. Mansell Prothero), 863
Effect of Red and Far-red Irradiation on the Germination Process in Seeds of *Pinus sylvestris* L. (Bengt Nyman), 1219
Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Effect of Serotonin on Absorption and Distribution of Calcium-45 in the Rat (Prof. S. Garattini, E. Grossi, P. Paoletti, R. Paoletti and M. Poggi), 185
Effect of Sodium Fluoride on Calcium Metabolism of Human Beings (Dr. Clayton Rich and John Ensinck), 184
Effect of Spacing and Nitrogen-level on Floral Initiation in Cocksfoot (*Dactylis glomerata* L.), (D. M. Calder and J. P. Cooper), 195
Effect of Spraying with Gibberellic Acid on Yield and Composition (Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid), (R. Stevens, J. B. Roberts and I. H. Williams), 360
Effect of Thyroxine and 2,4-Dinitrophenol on the Rate of Utilization of Glucose (Dr. L. Macho), 604
Effect of Thyroxine plus Thiourea on the Early Development of the Chum Salmon (*Oncorhynchus keta*), (Prof. M. A. Ali), 1214
Effect of Ultra-Violet Irradiation on the Electrical Conductivity of Cathodes coated with Barium Oxide (Dr. C. H. B. Mee), 1383
Effect of Varying Doses of Undiluted Semen on Fertility in the Domestic Fowl (G. C. Taneja and R. S. Gowe), 828
Effect (Alleged Anticomplementary), of Aspirin (K. W. Anderson), 1012
Effect (Anti-Sporulation), of Copper Fungicides : Significance of the, in the Control of Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476
Effect (A Biochemical), of Deuterium (Dr. Howard J. Ringold, Dr. Sumner Burstein and Dr. Ralph I. Dorfman), 1294
Effect (Cryoscopic), of Separation and Concentration of Solutes (Dr. K. S. Rangappa), 1087
Effect (The de Haas-van Alphen), and the Electronic Structure of Metals (Dr. D. Shoenberg), 126
Effect (Discreteness-of-Charge), and the Stability Theory of Colloids (Dr. S. Levine, D. Calvert and G. M. Bell), 699
Effect (Kirkendall), in Gases (Prof. E. A. Mason, L. Miller and P. C. Carman), 375
Effect (Lack of), of Coenzyme Q_{10} on Coumarin-induced Hypoprothrombinaemia (Prof. Murray Weiner, Dr. Theodore Chenkin and Dr. Peter G. Dayton), 711
Effect (The Marangoni), and Liquid/Liquid Coalescence (G. D. M. MacKay and S. G. Mason), 488
Effect (A Morphogenetic), of Beta-Mercaptoethanol—Distribution of Water in the Embryo (Dr. Peter Tuft), 1072 ; Distribution of Water in the Chick Blastoderm (A. Jurand and Dr. Peter Tuft), 1073
Effect (Mutagenic), of Radiant Heat Shocks on Phanerogamous Plants (Dr. Bror Pettersson), 1167
Effective Anthelmintic (Highly), for Sheep : Thiabendazole, a (Hugh McL. Gordon), 1409
Effective Contact Stresses and Friction (Dr. D. H. Trollope), 376
'Effective' Ionic Charge in Crystals (Dr. W. Cochran), 60
Effectiveness of Lipid and Lipidophilic Substances as Adjuvants (D. W. Dresser), 1169
Effects of Carrageenin on Serum Lipids and Atherosclerosis in Rabbits (Dr. Katsumi Murata), 189
Effects of Corticotrophin, Hydrocortisone and Methopyrapone on the Circulating Eosinophiles in Man (Dr. N. J. Kothari and Dr. J. C. Saunders), 1105
Effects of Drugs on Uptake of Isotopic Norepinephrine by Cat Tissues (Hans J. Dengler, Herbert E. Spiegel and Elwood O. Titus), 816
Effects of Gibberellic Acid and Allogibberic Acid on Flowering and Internode-Length in *Pisum sativum* (I. C. Murfet and H. N. Barber), 514
Effects of *Grewia populifolia* Extracts on the Development of Chick Embryos (S. K. Brahma and N. Kundu), 506
Effects of Heavy Water on Poliovirus Multiplication—Results and Speculations on Mechanism (Dr. David Kritchevsky, Dr. Richard I. Carp and Dr. Hilary Koprowski), 250
Effects of Inhaled Radioactive Particles—Report of the Subcommittee on Inhalation Hazards, Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 848), 556
Effects of Injections of some Intermediary Metabolites on the 'Insulin Response' of Normal Rabbits (Dr. H. D. Brahmachari and G. Raghupathy Sarma), 491, *erratum*, 755
Effects of Insecticides on Birds, 440
Effects of Ionizing Radiation on the Human Haematopoietic System—Report of the Subcommittee on Haematologic Effects, Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 875), 556
Effects of Ionizing Radiation on Organic and Biological Solids (Société de Chimie Physique and the British Biophysical Society Discussion on), 1150
Effects of Kinetin and Indole-3-acetic Acid on Multiplication Rates of Paramecium (Mary Annunciata McManus and Kathleen Sullivan), 619
Effects of Light Intensity on Reproduction in S.48 Timothy (*Phleum pratense* L.), (Dr. G. J. A. Ryle), 196
Effects of Morphine-like Drugs in Chicks (C. Schneider), 607
Effects of Radioactivity in the Sea : Research on the, 122
Effects of Smoking, *review* by Dr. W. Carruthers, 108
Effects (Antilymphoma) : Evidence that the L-Asparaginase Activity o Guinea Pig Serum is responsible for its (J. D. Broome), 1114
Effects (Electrical), during Dissolution and Precipitation of Electrolytes (R. P. Rastogi, B. P. Batra and R. K. Dass), 764
Effects (Electrical), observed in Polycrystalline Boron (C. H. Carmichael and M. Dore), 485
Effects (Long-Term), of Ionizing Radiations from External Sources—Report of the Subcommittee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 849), 556
Effects (Photoperiodic), on Stomatal Behaviour in *Xanthium pennsylvanicum* (Dr. T. A. Mansfield and Prof. O. V. S. Heath), 974
Effects (Stress), on Thermoremanent Magnetization (Dr. P. M. Stott and Dr. F. D. Stacey), 585 ; (J. M. Hall), 586
Efficiency in the Hospital Service : Abstracts of (Ministry of Health), 441
Efficiency (Inactivating), of Radiation : Comparative Analysis of, on Different Organisms (Dr. M. Terzi), 461
Effusions (Malignant) : Diagnostic Significance of Heterogeneous Lactic Dehydrogenases in (R. Richterich, K. Zuppinger and E. Rossi), 507
Eftekhari, M., and others : Serum Factor Gm^a among the Iranians, 717
Egelstaff, Dr. P. A. : Fast-Neutron Spectroscopy, *review*, 8
Egg-Cells of the Sea Urchin : Intracellular Freezing and Frost Resistance in (E. Asahina)—Intracellular Freezing as a Cause of Frost-killing in relatively Rapidly Frozen Cell Suspensions, 1263 ; A Mechanism to Prevent the Seeding of Intracellular Ice in Freezing Living Cells, 1264
Ehrenberg, Dr. L. : Effects of Ionizing Radiation on Organic and Biological Solids (Role of Oxygen in Radiation Damage), 1150
Ehrlich Mouse Ascites Tumour : Effect of Alpha-Amylase on the (Dr. F. Steckerl, A. Ofodile, R. R. Campbell and G. H. Friedell), 1113
Eichel, Dr. Herbert J., and Bukovsky, Josef : Intracellular Distribution Pattern of Rat Liver Glutamic-Oxalacetic Transaminase, 243
Eisenschitz, Prof. R. : The Atomic Problem, *review*, 214
Eklund, Dr. Arno Sigvard : Appointed director-general of the International Atomic Energy Agency, 338
Elastic Media (Anisotropic): Surface Waves in (V. T. Buchwald and A. Davis), 899
Elastic Tube : Fatigue in a Fluid-filled Pulsating (Group-Capt. John Erskine Malcolm), 1012
Elastin Content of the Human Aorta during Growth : Increase in (Dr. V. Scarselli), 710
Elbers, Dr. P. F. : Fixation of Yeast Protoplasts for Electron Microscopy, 1022
Elder, H. Y., and Garrod, D. J. : A Natural Hybrid of *Tilapia nigra* and *Tilapia leucosticta* from Lake Naivasha, Kenya Colony, 722
Electric Currents associated with Geomagnetic Disturbances in the Auroral Zone (Dr. F. J. Scrase), 963
Electrical Activity of a Giant Nerve Cell under Abnormal Conditions (Dr. Yutaka Oomura, Sachio Ozaki and Takashi Maéno), 1265
Electrical Conductivity of Cathodes coated with Barium Oxide : Effect of Ultra-Violet Irradiation on the (Dr. C. H. B. Mee), 1383
Electrical Conductivity of Indole–Iodine : Spin Resonance and (B. Smaller, Dr. I. Isenberg and S. L. Baird, jun.), 168
Electrical Effects during Dissolution and Precipitation of Electrolytes (R. P. Rastogi, B. P. Batra and R. K. Dass), 764
Electrical Effects observed in Polycrystalline Boron (C. H. Carmichael and M. Dore), 485
Electrical Engineering at Birmingham, 647
Electrical Engineering at Glasgow, 224
Electrical Engineers, Institution of : Work to be conducted by three specialised Divisions, 544
Electrical Field : Influence of, on Pupation and Oviposition in *Nepytia phantasmaria* Stkr. (Lepidoptera : Geometridae), (Dr. D. K. Edwards), 976
Electrical Industries, Ltd. (Associated) : Appointment of L. J. Davies as responsible for the direct supervision of the four Research Laboratories at Aldermaston, Harlow, Manchester and Rugby, 1142
    Appointment of Dr. J. E. Stanworth as director of research, 1142 ; *erratum*, 1349
Electrical Industry : Magnetic Materials in the (P. R. Bardell), second edition, *review* by A. C. Lynch, 428
Electrical Potential across the Isolated Gut : Dependence on Substrate of the (Dr. T. W. Clarkson, A. C. Cross and S. Toole), 501
Electrical Power Industry : Corrosion Problems in the (Battersea College of Technology Summer School on), 651
Electrical Properties of Orientated Lipid on a Biological Membrane—an Electrostatic Diffusion Barrier and Ion Pump (Dr. J. W. L. Beament), 217
Electrical and Allied Industries Research Association (British) : Annual Report, 450
    New Creep Laboratory, 1049
Electrical and Magnetic Quantities : Dimensions of (Dr. A. E. Martin), 588
Electrically Exploded Wires (Thermal Radiation Laboratory of the Geophysics Research Directorate of the United States Air Force Cambridge Research Laboratories Conference on), 1250
Electricity Plant : New American, 22
Electrode (Oxygen), for Measurement of Tissue Slice Respiration (E. H. Strickland, F. D. Ziegler and Prof. A. Anthony), 969
Electrodialysis Systems : Thermo-electric Phenomena in (Dr. Lloyd H. Shaffer), 591
Electrokinetic Charge of the Human Erythrocyte : Sialic Acids and the (G. M. W. Cook, Dr. D. H. Heard and Dr. G. V. F. Seaman), 44

Electrolyte Retention produced by the Antialdosterone *SC* 5233 (J. B. Farmer and B. Petch), 1308
Electrolytes : Electrical Effects during Dissolution and Precipitation of (R. P. Rastogi, B. P. Batra and R. K. Dass), 764
  Ultrasonic Velocity in Aqueous Solutions of some (M. G. Seshagiri Rao and Prof. R. Ramachandra Rao), 164
Electron Affinity of the Acceptor Molecule in Charge-Transfer Complexes (Dr. M. E. Peover), 702
Electron Bombardment : Evaporated Carbon Films of Large Area for Adsorption Studies by (A. E. Bell, J. Pritchard and Prof. K. W. Sykes), 487
Electron Linear Accelerators : Pulse-shortening in (Dr. M. C. Crowley-Milling, T. R. Jarvis, C. W. Miller and G. Saxon), 483
Electron Microscope : The (M. E. Haine), *review* by Prof. D. Gabor, 316
Electron Microscope Observations of the Receptors in the Sensory Vesicle of the Ascidian Tadpole (Noël Dilly), 786
Electron Microscopic Observations of Structure in Ultra-Thin Sections of Vitrinite in Coal (J. T. McCartney, Sabri Ergun and R. E. Walline), 1361
Electron Microscopic Radioautography : Proposed Use of Magnetic Fields in (Lucien G. Caro), 1188
Electron Microscopy : Fixation of Yeast Protoplasts for (Dr. P. F. Elbers), 1022
Electron Spin Resonance (Chemical Society Symposium on), 755
  Spatial Distribution of Free Radicals as studied by (Robert C. Smith and S. J. Wyard), 897
Electron Spin Resonance of Adenosine Triphosphate (D. B. Russell and S. J. Wyard), 65
Electron Spin Resonance Absorptions induced by Light in Adsorbed Layers of Cyanine Dyes on Silver Bromide Microcrystals (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Electron Transport : Quinones in (Ciba Foundation Symposium on), (edited by G. E. W. Wolstenholme and Cecilia M. O'Connor), *review* by A. I. Scott, 321
Electron Transport System and Phosphoproteins of Mitochondria (K. Ahmed, J. D. Judah and C. H. Gallagher), 1309
Electron and Hydrogen Transfer : Coupling of Phosphorylation to, by a Chemi-Osmotic Type of Mechanism (Dr. Peter Mitchell), 144
π-Electronic Excitation and Ionization Energies of Condensed Ring Aromatic Hydrocarbons (Dr. J. B. Birks and M. A. Slifkin), 761
Electronic Properties of Imidazole (S. Aftergut and G. P. Brown), 379
Electronic Spectral Data : Organic, Vol. 1, 1946-1952 (edited by Mortimer J. Kamlet) ; Vol. 2, 1953-1955 (edited by Herbert E. Ungnade), *review* by Dr. E. G. Kellett, 107
Electronic Structure of Metals : The de Haas-van Alphen Effect and the (Dr. D. Shoenberg), 126
Electronics Industry : Glass in the, 1143
Electrophoresis on Paper : Splitting of Serum Albumin during (J. V. McLoughlin), 1305
Electrophoresis in a Thin Layer of Starch-Gel followed by a Plastifying Treatment (J. Groulade, Dr. J. M. Fine and C. Ollivier), 72
Electrophoresis (Paper) : Demonstration by, of an Auto-Immunological Precipitin possibly due to Fibrinolysins (Marion I. Cooper and J. R. Sykes), 1212
Electrophoresis (Paper), of Proteins while Preserving the Pattern of their Localization in Tissues (Dr. Zh. A. Medvedev), 1021
Electrophoresis (Starch-gel) : Comparison of the Serum Protein Fractions of the Newly Hatched Chick with those of Adult Birds using (A. Amin), 708
Electrophoretic and Immuno-electrophoretic Patterns of Extracellular Diphtheria Proteins from Culture Filtrates of Different Iron Concentrations (Bertold J. Radola), 382
Electrophysiology of Thermoreceptors (Prof. Herbert Hensel), 1143
Electrostatic Diffusion Barrier and Ion Pump : Electrical Properties of Orientated Lipid on a Biological Membrane—an (Dr. J. W. L. Beament), 217
Elementary Particles (CERN International Conference on), 126
Elements of Donor Origin in Orthotopically Grafted Spin following the Homograft Response : Regeneration of (Dr. J. K. Dineen and Dr. Aleksander Szenberg), 153
Elgindi, D. M. (Oteifa, B. A., and) : Nematology (Radiotracer Techniques using Phosphorus-32 applied to a study of Galling in Tomatoes by *Meloidogyne javanica* and its Effect on Uptake of Nutrient), 1049
Elimination of the Absorption Correction in the Viscometry of Dilute Solutions of High Polymers (H. van Oene and Prof. L. H. Cragg), 1160
Elimination of Bovine Serum Albumin in Acute Experimental Uraemia (Dr. G. Gowland, G. F. Smiddy, Dr. R. G. Burwell and F. M. Parsons), 1210
Elimination (Chromosomal), induced by 6-Aza-Uracil (M. Collette Mahoney, Dr. E. R. Witkus and Dr. C. A. Berger), 627
Ellenbogen, L. : Vitamin B_{12} and Intrinsic Factor (Efforts to Purify and Isolate Intrinsic Factor), 1155
Ellis, Dr. A. J., and Wilson, S. H. : Hot Spring Areas with Acid–Sulphate–Chloride Waters, 696
Ellis, Dr. D. A. : A New Universal Buffer System, 1099
Ellis, Rex, and others : Girard *T* Reagent for Carbonyls, 1391
Elmore, Dr. D. T. : Biochemistry of Steroids, *review*, 111
  A Laboratory Manual of Analytical Methods of Protein Chemistry, *review*, 320
Elton, Dr. G. A. H. : British Baking Industries Research Association—New Laboratory Wing, 233
Embassy (Canadian), in Washington : Appointment of Dr. E. O. Hughes as scientific attaché, 542
Embryo : Distribution of Water in the (A Morphogenetic Effect of Beta-Mercaptoethanol), (Dr. Peter Tuft), 1072
Embryo of *Pteridium aquilinum* : Failure of Nucleotides to Diffuse Freely into the (P. R. Bell), 91
Embryo (Chick) : Mechanism of Recovery from Viral Infection in the (Dr. Samuel Baron and Alick Isaacs), 97
Embryology : International Conference on (Editorial Board of the *Journal of Embryology and Experimental Morphology*), 24
Embryology and Experimental Morphology, Journal of (Editorial Board of the) : International Conference on Embryology, 24
Embryos of *Cuscuta reflexa* : Artificial Production of Buds from the (Prof. P. Maheshwari and B. Baldev), 197
Embryos (Chick) : Effects of *Grewia populifolia* Extracts on the Development of (S. K. Brahma and N. Kundu), 506
Embryos (Early) : Amino-Acid Activation in Tissues of (Dr. Elizabeth M. Deuchar), 1006
Embryos (Fern), in Sterile Culture : Growth of (Prof. Augustus E. DeMaggio and Prof. Ralph H. Wetmore), 94
Emeléus, Prof. H. J., and Anderson, Dr. J. S. : Modern Aspects of Inorganic Chemistry, third edition, *review* by Prof. J. R. Partington, 637
Emergence : Dating Man's (Dr. K. P. Oakley), 988
Emission (Galactic Radio) : Spectrum of (Dr. F. Graham Smith), 1381
Emission (New Afterglow), associated with Metastable Nitrogen Atoms (L. Herman and R. Herman), 346
Emission Spectra (Triplet→Singlet) : Polarization of (V. G. Krishna and Prof. Lionel Goodman), 800
Emission Spectroscopy (Molecular), from 2μ to 12μ by a Michelson Interferometer (H. A. Gebbie, G. Roland and L. Delbouille), 264
Emotional Behaviour : Changes in Visual Gnostic Functions and, following Unilateral Temporal Pole Damage in the 'Split-Brain' Monkey (Dr. J. L. deC. Downer), 50
Employment of Graduates—a Durham University Survey, 649
Emulsifying Capacity of Blood Sera (Prof. B. Breyer), 174
Emulsions : Quantitative Analysis of Milk and other, by Infra-Red Absorption (Dr. J. D. S. Goulden), 905
Encyclopedia of Chemical Technology (edited by Raymond E. Kirk and Donald F. Othmer), second Supplement Volume edited by Anthony Standen, *review* by R. H. Villet, 1131
Endings (Nerve), in the Mole's Snout (J. Armstrong and Dr. T. Andrew Quilliam), 1379
Endo-Parasites (Ecto- and), of South-East Asia, *review* by P. F. Mattingly, 948
Endogenous Rhythm in the Rate of Dark-Fixation of Carbon Dioxide in Leaves of *Bryophyllum fedtschenkoi* (Diana M. Warren and Malcolm B. Wilkins), 686
Endotoxin : Increase in Specific Bactericidal Antibodies after Administration of (J. Gabriel Michael, James L. Whitby and Maurice Landy), 296
Endowed Research Time (International Business Machines, United Kingdom, Ltd.), 442
Energies (Ionization), of Condensed Ring Aromatic Hydrocarbons : π-Electronic Excitation and (Dr. J. B. Birks and M. A. Slifkin), 761
Energy Pathways of Hepatoma No. 5123 (Dr. Alan C. Aisenberg and Harold P. Morris), 1314
Energy Transfer (Inter-Molecular), in Photo-sensitized Oxidation-Reduction : Paths of (N. Takeyama), 1359
Energy (High) Nuclear Physics (Institute of Physics and the Physical Society Symposium on), 1349
Energy (Solar) : Applications of, 228
Energy (Solar) Technology, 1954-1959 (T. Brimelow), 228
Energy Principles in Applied Statics (T. M. Charlton), *review* by Prof. R. C. Coates, 1134
Energy Theorem (Minimum), for Flow of Dry Granules through Apertures (R. L. Brown), 458
Engel, Dr. Milton B., and others : Distribution of Sodium and Potassium in certain Cells and Tissues, 1175
Engel, Dr. W. K. : Muscle Target Fibres, a Newly Recognized Sign of Denervation, 389
Engelsman, Dr. J. J., and Claassens, A. M. J. M. : Use of the Rotating Hanging Mercury Drop in Analysis—Anodic Stripping using a Rotating Mercury Drop, 240
Engineer : Art and the (Prof. Misha Black), 949
Engineering : Civil, *review* by T. M. Charlton, 1032
  A Social History of (Prof. W. H. G. Armytage), *review* by Prof. M. Postan, 946
  Timber (University of Southampton first International Conference on), 338
Engineering (Civil) Reference Book (edited by J. Comrie), *review* by T. M. Charlton, 1032
Engineering (Electrical), at Birmingham, 647
Engineering (Electrical), at Glasgow, 224
Engineering (Machine Tool), at Manchester, 19
Engineering (Systems) : Operations Research and (Charles D. Flagle, William H. Huggins and Robert H. Roy), *review* by Prof. L. S. Goddard, 105
Engineering (Textile), at Leeds, 1346
Engineers (Chemical), Institution of : Student's Prize awarded to J. P. Catchpole, 338
Engineers (Electrical), Institution of : Work to be conducted by three specialized Divisions, 544
Engineers (Mining, Metallurgical and Petroleum), American Institute of (Iron and Steel Institute and the Metallurgical Society of the) : Special Meeting in the United States and Canada, 651
Engineers (Radio), British Institution of : Grant of a Charter to the, 651
Engineers (Scientists and), engaged in research and development in Colleges and Universities in the United States for 1958 : Report on (United States National Science Foundation), 225
Engineers (Women), in the U.S.S.R., a Study of the Education, Employment and Prospects of Women Engineers in the U.S.S.R. (L. S. Souter and R. Winsdale), 30
England, S., and others : Taste Thresholds and Food Dislikes, 1328
England (Rural), Council for the Preservation of : Twenty-second National Conference (The Impact of Industrial Development on the Countryside), 959
England (South-East) : The Changing Flora of (J. E. Lousley), 133
  Invertebrate Life in (John Sankey), 132
England (South-West) : Age of the Orogeny and Granites in (T. R. Owen), 486 ; (N. E. Butcher), 486
England (Southern) : The Lower Tertiary Floras of (Marjorie Elizabeth Jane Chandler), 1, Palaeocene Floras, London Clay Flora (Supplement), *review* by Prof. John Walton, 209
  Tropical Floras in the Tertiary in, *review* by Prof. John Walton, 209
England and Wales, National Foundation for Educational Research in : *Technical Education Abstracts from British Sources*, Vol. 1, No. 1, September–December 1960, 21
English Electric Valve Co., Ltd. : Travelling Wave Tubes—their Characteristics and Operation, 1348
English Family : Thalassaemia in an, and in a German Family (Dr. F. Vella and S. A. Ibrahim), 822

English Place-Name Society : Vols. 30, 31 and 32, The Place-Names of the West Riding of Yorkshire (Prof. A. H. Smith), Part 1, Lower and Upper Strafforth and Staincross Wapentakes ; Part 2, Osgoldcross and Agbrigg Wapentakes ; Part 3, Morley Wapentake, *review* by Prof. H. C. Darby, 208
English Rural Life, Museum of : Annual Report for 1960 (University of Reading), 544
English–Russian Geological Dictionary (T. A. Sofiano), (edited by I. D. Borneman, et *al*.), *review* by Dr. S. I. Tomkeieff, 9
Engravings (Rock), of the Sydney–Hawkesbury District (F. D. McCarthy), 854
Engström, Prof. Arne : X-Ray Microscopy, *review*, 1332
Enhanced Plant-Growth with Two Systemic Insecticides (M. M. H. Wallace), 513
Enhancement by Chemical Agents : Induction of Tumours by Polyoma Virus—(K. E. K. Rowson, F. J. C. Roe, J. K. Ball and Dr. M. H. Salaman), 893
Enhancement of Inhibition of Intestinal Absorption by Cetrimide–Phloridzin Combination (J. A. Nissim), 37
Enlargement of Salivary Gland in Mice Treated with *Iso*propylnoradrenaline (Dr. K. Brown-Grant), 1076
Enomoto, E., and others : Toxicology of *Penicillium islandicum*, 864
Enquiries (Scientific), in Parliament : Recent, 857
Ensinck, John (Rich, Dr. Clayton, and) : Effect of Sodium Fluoride on Calcium Metabolism of Human Beings, 184
Entamoeba histolytica : Induced Increase in Resistance of, to Fumagillin (Dr. Mitsuru Nakamura), 413
Enteropathy (Exudative) : Calcium Metabolism in Man with Calcium-45—Malabsorption Syndrome and (Dr. G. Milhaud and Dr. P. Vesin), 872
Enterprise : The Human Side of (D. McGregor), 1332
Entomological Society (Royal) : Symposium on Insect Polymorphism, 1145
Environment on the Bed of the Sea : Life and (Prof. C. M. Yonge), 986
Environmental Conditions (Controlled) : Flower Initiation in Kent Wild White Clover (*Trifolium repens* L.), under (R. J. Haggar), 1120
Environmental Factors and Bryophytes (Kathryn Benson-Evans), 255
Enzyme (New), connected with Protein Synthesis: Evidence for a (Hans G. Boman and Ingvar Svensson), 674
Enzyme (Receptor-destroying), and Proteases (Influenza Viruses,) : Action of, on Blood Group Agglutinogen M^g (Prof. Georg F. Springer and Dr. Karlheinz Stalder), 187
Enzyme Catalysis : Dextransucrase *V* and the Role of Metal Ions in (W. Brock Neely and Julie Hallmark), 385
Enzyme Injections : Effect of, on Mitosis in Regenerating Liver (Dr. Gaston de Lamirande), 400
Enzyme Synthesis (Induced), in Aqueous Suspensions of Starved Stationary Phase *Aerobacter aerogenes* (R. E. Strange), 1272
Enzyme System (Rat Lens) : Catalysis of Incorporation of ^{14}C-Uridine into Rat Lens Ribonucleic Acid by (Dr. Anima Devi and Dr. N. K. Sarkar), 1094
Enzyme or 'Heat Catalysed' Hydrolysis : Amino-Acid Liberation from Proteins by (S. M. Hardy), 1061
Enzymes of the Urea Cycle in Rat Liver : Effect of Adrenalectomy on the Activity of (Patricia McLean), 1302
Enzymic Activity of the Central Nervous System (Neurochemical Commission of the World Federation of Neurology International Symposium on), 1349
Enzymic Binding of Hydroxylamine by Fumaric Acid (Prof. Nathan Grossowicz and the late Yehudith Lichtenstein), 412
Enzymic Breakdown of Stigmatic Cuticula of Flowers (W. Heinen and H. F. Linskens), 1416
Enzymic Conversion of Tetrahydrofolic Acid to Folinic Acid by Plant Protein (E. H. Byers, J. Stewart and T. J. Bond), 179
Enzymic Degradation of Deoxyribonucleic Acid into Sub-Units : Kinetics of the (Dr. Giorgio Bernardi and Prof. Charles Sadron), 809
Enzymic (Non-) Hydroxylation of Polycyclic Aromatic Hydrocarbons (F. Dewhurst and G. Calcutt), 808
Eoliths and Prehistorical Tools, 23
Eosinophiles (Circulating), in Man : Effects of Corticotrophin, Hydrocortisone and Methopyrapone on the (Dr. N. J. Kothari and Dr. J. C. Saunders), 1105
Ephrussi, Prof. B. : Elected a foreign associate of the United States National Academy of Sciences, 18 ; work of, 18
Epidemiology : Stochastic Population Models in Ecology and (Prof. M. S. Bartlett), *review*, 843
Epidermoid Carcinoma Cells (Human), in Culture : Biochemical Aspects of Resistance to 6-Mercaptopurine in (R. W. Brockman, George G. Kelley, Patricia Stutts and Virginia Copeland), 469
Epinephrine Action : Potentiation of (Prof. R. Imaizumi, Y. Hashimoto and M. Oka), 186
Epitaxial Growth of Crystals on One-Degree Orientated Substrates (Dr. A. Goswami), 160
Epithelium (Intestinal) : Localization of the 'Sugar Pump' in the (Dr. T. Z. Csáky and G. W. Fernald), 709
Epps, F. J. : South-Eastern Union of Scientific Societies Annual Congress, 132
Epstein, H. T., and others : Some Properties of the Deoxyribonucleic Acid of Phage Alpha, 1097
Epstein, Dr. William L., and others : An Antigen in Lesions of Molluscum Contagiosum, 509
Equator : Height of Night-time *F* Layer Irregularities at the (Dr. G. S. Kent and Dr. J. R. Koster), 1083
Equatorial Undercurrent (Eastward), in the Atlantic : Evidence of an, from Measurements of Current Shear (Arthur D. Voorhis), 157
Equatorial (Trans-) Backscatter Observations of Magnetically Controlled Ionization (J. A. Thomas), 792
Equipment Exhibition (Instrument Symposium and Research) : Eleventh Annual (Washington Sections of the American Association of Clinical Chemists, the American Chemical Society, the Instrument Society of America, the Society of American Bacteriologists, the Society of Applied Spectroscopy and the Society for Experimental Biology and Medicine), 959
Eradication (Malaria) : Effect of Population Movements on (R. Mansell Prothero), 863
Eradication (Simultaneous), of Three Ectoparasitic Species from a Colony of Laboratory Mice (Dr. Nigel Bateman), 721
Ergonomics Research Society and the British Occupational Hygiene Society : Discussion on the Design and Use of Respirators, 655
Ergun, Sabri, and others : Electron Microscopic Observations of Structure in Ultra-Thin Sections of Vitrinite in Coal, 1361
Ernsting, J., and Gabb, J. E. : Design and Use of Respirators (Communication between Persons wearing Breathing Equipment), 655
Erythematosis (Disseminated Lupus) : Antileucoyte Cytoplasm, Anti-Leucocyte Nuclei and Anti-Platelets in (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Erythrocyte (Human) : Sialic Acids and the Electrokinetic Charge of the (G. M. W. Cook, Dr. D. H. Heard and Dr. G. V. F. Seaman), 44
Erythrocyte Ghosts of Mediated Sugar Transport : Persistence in (Paul G. LeFevre), 970
Erythrocyte Membrane : Dimer Formation and the Transfer of Glycerol across the (Dr. W. D. Stein), 352
Erythrocytes : A Positive Feedback Mechanism of Adenosine Triphosphate Synthesis in (Makoto Nakao, Takashi Motegi, Toshiko Nakao, Saburo Yamazoe and Haruhisa Yoshikawa), 283
Erythrocytes among Armenians in Iran : Virtual Absence of Glutathione Instability of the (Dr. J. E. Bowman and Dr. D. G. Walker), 221
Erythrocytes (Rabbit) : Recovery and *in vivo* Survival of (Dr. G. F. Doebbler, R. G. Buchheit and A. P. Rinfret), 1405
Erythrocytes (Sheep) : Antibody Response in Rabbits following Injection of, into Lateral Ventricle of Brain (Prof. B. D. Janković, Dr. M. Draškoci and Dr. K. Isaković), 288
Erythropoiesis : Effect of Bilateral Section of the Splanchnic Nerve on (Fumimaro Takaku, Kunitake Hirashima and Prof. Shigeo Okinaka), 500
Erythropoietin : Bio-Assay of, in Mice made Polycythaemic by Exposure to Air at a Reduced Pressure (Dr. P. Mary Cotes and Dr. D. R. Bangham), 1065
Escherichia coli : Influence of Salts on the Uptake of Streptomycin by (Paul H. Plotz, Donald T. Dubin and Bernard D. Davis), 1324
  Radiation Sensitivity of, to β-Rays in Dry and Wet Condition (S. B. Bhattacharjee and N. N. Das Gupta), 1015
Eschscholzia californica Cham., *Argemone mexicana* L. and *A. ochroleuca* Sweet : Intra-ovarian Pollination in (Prof. P. Maheshwari and Kusum Kanta), 304
Escobar del Rey, Dr. F., and others : Increase of the Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at Early Stages of Propylthiouracil Treatment in the Rat, 1171
Essential Oil from Fresh *Cannabis sativa* and its Use in Identification (L. Martin, Dr. D. Morison Smith and C. G. Farmilo), 774
Esserman, N. A. : Retirement as director of the National Standards Laboratory of the Australian Commonwealth Scientific and Industrial Research Organization, 542 ; work of, 542
Esters (Amino-Acid) : Effect of, on the Growth of Fibroblast Cultures (Dr. I. Fehér, A. Döklen and V. Selmeci), 494
Esters (Fatty Acid), of Riboflavin : Hydrolysis of, by Pancreatic Lipase (Prof. Kunio Yagi, Yoshiko Yamamoto and Jun Okuda), 174
Esters (Some New), of Chromone-2-Carboxylic Acid (Juan X. De Vries and Kempis Vidal-Beretervide), 171
Esters (Sterol) : Isolation of, from Human Faeces (Francis Aylward and Pamela Wills), 1397
Estimating Ionospheric Absorption Heights from Recovery Times : A Method of (Syed Ziauddin), 1084
Estimation of Protein in Cellular Material (Prof. David Racusen and D. B. Johnstone), 492
Estimation of Specific Proteins in Serum and other Mixtures (Dr. D. A. Darcy), 1163
Estimation of Time of Generation of Living Cells (R. J. M. Fry, S. Lesher and H. I. Kohn), 290
Estimation (Quantitative), of Peak Areas in Gas–Liquid Chromatography (Dr. K. K. Carroll), 377
Estimation (Quantitative), of Phosphatases (Phosphatases in *Schistosoma mansoni*), (Dr. D. L. H. Robinson), 473
Estuary (Tropical West African) : Seasonal Fluctuations and Distribution of Nitrite in a (J. C. D. Watts), 929
Estudios Ecologicos para el Aprovechamiento de la Tierra en Nicaragua (Dr. B. W. Taylor), 1259
Ethane : Reaction of, with a Clean Rhodium Film (Dr. Richard W. Roberts), 170
Ethanol (Carbon Dioxide and), as Factors controlling the Growth of Etiolated Oat Seedlings (Dr. C. L. Mer), 260
Ether (Ethylene Glycol Monomethyl), a New Fixative for Histological Work (Prof. Walter B. Shelley and Ralph Florence), 719
Ether (Trimethylsilyl) Derivatives of Alcohols : Peak-Shift Technique in Gas–Liquid Chromatography— (Stanley H. Langer and Peter Pantages), 141
3-O-Ethers of D-Glucosamine : A Convenient Synthesis of Muramic Acid and other (Dr. Roy Gigg and Patricia M. Carroll), 495
Ethers (Glycol Monoalkyl) : Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and (A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas), 378
Ethological Method for Investigating the Properties of Nervous Systems : Response to Series of Subliminal Stimuli—an (A. J. Pontin), 512
Ethoxide (Polymeric Titanium), and Titanium Oxide Ethoxides : Structures of (Prof. D. C. Bradley and A. H. Westlake), 273 ; (R. L. Martin and G. Winter), 274
Ethoxides (Titanium Oxide) : Structures of Polymeric Titantium Ethoxide and (Prof. D. C. Bradley, and A. H. Westlake), 273 ; (R. L. Martin and G. Winter), 274
Ethylene Evolution and Sub-Cellular Particles (Prof. Stanley P. Burg and Ellen A. Burg), 967
Ethylene Glycol Monomethyl Ether, a New Fixative for Histological Work (Prof. Walter B. Shelley and Ralph Florence), 719
Ethylene Oxide : Effect of, on the Tapping of *Hevea brasiliensis* (D. H. Taysum), 1319
Ethylenes (*cis* and *trans* Dichloro- and Dibromo-) : Ionization Potentials of (Dr. J. Momigny), 1089
Etiolated Oat Seedlings : Carbon Dioxide and Ethanol as Factors controlling the Growth of (Dr. C. L. Mer), 260
Etiolated Pea Plants : Correlative Inhibition of Formation of Chlorophyll in the Leaves of (Jiří Úlehla), 613
Eto, Morifusa, and others : Biological Activity of a Tri-*o*-Cresyl Phosphate Metabolite, 1396

Ettlinger, Dr. G. : Learning in Two Sense-Modalities, 308
Ettlinger, Dr. G., and others : Cross-modal Transfer in Man, 1225
Eucollagen (Dr. A. Courts), 1097
Europe (Central) : Some Bacterial Plant Diseases of, *review* by Dr. W. J. Dowson, 4
Europe (Unified) : The Non-Ferrous Metals Industry in a (Baron Snoy et d'Oppuers), 546
Europe (Western and Central) : Introduction of the Potato into (V. S. Lekhnovitch), 518
European Birds for Caging : Trade in, 124
European Conference on Broadcasting (International Telecommunication Union), 449
European Economic Co-operation, Organization for : Report on the School Curriculum in Mathematics (edited by Howard Fehr), 335
European Economic Co-operation, Organization for (European Nuclear Energy Agency) : New Trends in Atomic Research and their International Significance (Dr. L. Kowarski), 853
European Malacological Congress : First (Conchological Society of Great, Britain and Ireland and the Malacological Society of London), 126
European Nematologists, Society of : Sixth International Symposium on Nematology, 1048
European Nuclear Energy Agency of the Organization for European Economic Co-operation : New Trends in Atomic Research and their International Significance (Dr. L. Kowarski), 853
European Organic Geochemistry Group : Proposal to form a, 336
European Organization for Nuclear Research and the Max Planck Institute, Heidelberg : Conference on Fission and Spallation Phenomena and their Application to Cosmic Rays, 24
European Parasites of Man, *review* by Dr. G. Lapage, 107
European Standards for Drinking Water (United Nations, World Health Organization), 1261
European Symposium on Food Technology, 959
European Symposium on Vitamin B_{12} and Intrinsic Factor, 1154
Eutrichocheles modestus (Herbst) in the Near-shore Waters of Porto Novo, South India : Occurrence of (K. Balasubrahmanyan and Dr. J. Jacob), 830
Eutrophic Lakes (Fresh-water), of India : Hydrogen Ion Concentration, Plankton and Fish in (Dr. S. M. Das), 511
Evaluation of Quantal Neutralization Tests (Prof. S. Fazekas de St. Groth), 891
Evans, E., and others : Significance of the Anti-Sporulation Effect of Copper Fungicides in the Control of Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach, 476
Evans, Dr. J. H. : A Phytoplankton Multi-Sampler and its Use in Lake Victoria, 53
Evaporated Carbon Films of Large Area for Adsorption Studies by Electron Bombardment (A. E. Bell, J. Pritchard and Prof. K. W. Sykes), 487
Evaporation Resistance (Specific) : Compressibility and, of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers (A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas), 378
Evaporative Loss of Water from the Skin of the Ox : Increase of, in response to Local Heating of the Hypothalamus (Dr. D. L. Ingram, J. A. McLean and Dr. G. C. Whittow), 81
Evelyn, John : Fumifugium, or the Smoake of London Dissipated, reprint (National Society for Clean Air), 752
Evernden, Dr. J. F., and others : Age of Bed I, Olduvai Gorge, Tanganyika, 478
Evidence that the L-Asparaginase Activity of Guinea Pig Serum is responsible for its Antilymphoma Effects (J. D. Broome), 1114
Evidence for a Cholinergic Mechanism in Sympathetic Transmission : Lack of (Dr. J. E. Gardiner and Dr. J. W. Thompson), 86
Evidence of an Eastward Equatorial Undercurrent in the Atlantic from Measurements of Current Shear (Arthur D. Voorhis), 157
Evidence of a 'Hunger Motivational' System in the Lateral Hypothalamus of the Rat (Dr. P. J. Morgane), 672
Evidence for a New Enzyme connected with Protein Synthesis (Hans G. Boman and Ingvar Svensson), 674
Evidence of Thyroid Protease Action : Thyroglobulin— (Dr. A. D. Litonjua), 357
Evidence (Experimental), for the Mechanism of Photosynthesis (Dr. H. T. Witt, A. Müller and B. Rumberg), 194
Evidence (Infra-Red Spectroscopic), of Four-fold Co-ordination of Titanium in Barium Orthotitanate (P. Tarte), 1002
Evolution : Issues in, *review* by Dr. P. G. Fothergill, 425
Evolution after Darwin (edited by Sol Tax and Charles Callender), Vol. 3, Issues in Evolution, *review* by Dr. P. G. Fothergill, 425
Evolution of the Sweet Potato (*Ipomoea batatas* (L.) Lam.), (Dr. D. E. Yen), 93
Evolution (Ethylene), and Sub-Cellular Particles (Prof. Stanley P. Burg and Ellen A. Burg), 967
l'Évolution des Vertébrés : Théories de (Erik Jarvik), traduction du Prof. J. P. Lehman, *review* by Dr. E. Trewavas, 945
Ewe : Removal of Milk Constituents from the Mammary Gland of the (A. K. Lascelles), 1404
Ewer, Dr. Rosalie F. : Awarded a Royal Society and Nuffield Foundation Commonwealth Bursary, 126
Ewing, Maurice, and others : The Floors of the Oceans, I, The North Atlantic—Text to accompany the Physiographic Diagram of the North Atlantic (Geological Society of America, Special Paper 65), *review* by Dr. G. E. R. Deacon, 531
Exacting Strains (Nutritionally), of *Staphylococcus pyogenes* : Metabolic and Genetic Investigations of (Janice B. Edgar and Dr. B. A. D. Stocker), 1121
Examination by Light Microscopy : A Method of Replicating Dry or Moist Surfaces for (Joan Sampson), 932
Examination (Immunoelectrophoretic), of Agammaglobulinaemia (Fred Rosen, Charles A. Janeway and Prof. David Gitlin), 1109
Examination (Polarographic), of Sialic Acids (Barbara Robert, Max E. Rafelson and Ladislas Robert), 596
Examination (Water Treatment and), Society for : Autumn Meeting, 856
Examining the Rate, and Primary Products, of Reactive Bimolecular Collisions : A Molecular Beam Reactor for (C. W. Nutt and A. J. Biddlestone), 798
Example of Mechanical Co-ordination of Cilia (Dr. M. A. Sleigh), 931
Excess of Charge : An Unsteady Universe with (Ll. G. Chambers), 1082
Excess (Charge) : The Steady Universe with (Ll. G. Chambers), 262
Excess Supply of Heavy Metals to Barley during Germination : Effect of, with special reference to Catalase and Peroxidase (Dr. S. C. Agarwala, A. Kumar and C. P. Sharma), 726
Exchangers (Modified Cellulose Ion), and their Use in Ribonucleic Acid Fractionation (Dr. L. Bosch, G. van der Wende and Dr. H. Bloemendal), 349
Exchanges of Sodium Ions in the Central Nervous System of an Insect (*Periplaneta americana* L.), (J. E. Treherne), 1223
Excitable Tissues : Outflux of Inorganic and Organic Phosphate during Membrane Depolarization of (Dr. L. G. Abood, Prof. K. Koketsu and I. Koyama), 395
Excitation (π-Electronic), and Ionization Energies of Condensed Ring Aromatic Hydrocarbons (Dr. J. B. Birks and M. A. Slifkin), 761
Exclusion (Ion) : Separation Methods based on Molecular Sieving and (J. Porath and E. B. Lindner), 69
Exclusive Iodination of the *A* Chain : An Unreactive Tyrosine Residue in Insulin and the (Dr. P. H. Springell), 1372
Excreta (Internal Organs, Fluids and), of Rabbits : Effect of Polyvinyl Alcohol on the Distribution of Iodine-131 in the (H. F. Nowak and J. Kucharski), 665, *erratum*, 1045
Excretion of Inulin by Malpighian Tubules (Dr. J. A. Ramsay and Dr. J. A. Riegel), 1115
Excretion (Aldosterone), and Potassium Retention in Subjects living at High Altitude (P. J. Ayres, Rona C. Hurter, Dr. E. S. Williams and J. Rundo), 78
Excretion (Decreased Urinary), of 2-Aminoethanol by Irradiated Human Beings (Dr. Ralph R. Cavalieri, M. T. Van Metre and W. I. Sivertsen), 1303
Excretion (Hydroxyproline) : Influence of Scurvy and Lathyrism (Odoratism) on (Dr. G. R. Martin, S. E. Mergenhagen and D. J. Prockop), 1008
Excretion (Urinary), of Histamine : Relationship between the Decrease in, and the Initiation of the Labour Process (Dr. Halina Wiskont-Buczkowska), 290
Excretory Ducts in the Genus *Notopterus* (Lacepede), (Madhu Sudan Sharma), 1118
Exhaled Air of Diabetic Patients : Rapid Infra-Red Determination of Acetone in the Blood and the (Dr. Richard D. Stewart, Edward A. Boettner and Bruce P. Stubbs), 1008
Exhibition : International Instruments and Measurement, 1352
Exhibition (Research Equipment) : Eleventh Annual Instrument Symposium and (Washington Sections of the American Association of Clinical Chemists, the American Chemical Society, the Instrument Society of America, the Society of American Bacteriologists, the Society of Applied Spectroscopy and the Society for Experimental Biology and Medicine), 959
Exhibitions : Code of Practice for the Display of Sources of Ionizing Radiations at, 1249
Existence (Atomic) : A Concept of (Prof. W. H. Watson), 658
Exosphere (Terrestrial), at Heights of 1,000–1,700 km. (Max Römer), 238
Exothermic Compositions : Solid Propellant and (Dr. James Taylor), *review* by Dr. W. B. Littler, 3
Expansion Coefficient (Specific Heat and), of Liquid Helium-3 under Pressure below 0·1° K. (Dr. D. F. Brewer and J. R. G. Keyston), 1261
Expansion Cracks in Mortar : The Widening of, by Salt Solutions (J. A. Roberts and H. E. Vivian), 125, *erratum*, 1145
Expected Rate of Senescence and Age-dependent Mortality in Fish (Dr. Alex Comfort), 822
Expedition : North Borneo, 1961 (Royal Society of London in co-operation with the Government of North Borneo), 26
Expenditure (Capital), of Colleges and Universities in the United States for research and development in the Natural and Social Sciences in 1958 (United States National Science Foundation), 545
Expermental Agriculture and Animal Husbandry (*Australian Journal of*), No. 1, (Australian Institute of Agricultural Science), 544
Experimental Animals : Demonstration of Anti-Depressant or Stimulant Properties of Imipramine in (Dr. D. R. Maxwell and Helen T. Palmer), 84
Experimental Apparatus for Contact Microradiography at 200-500 V. (Dr. A. Recourt and G. H. F. de Vries), 1185
Experimental Biology and Medicine, Society for (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
Experimental Evidence for the Mechanism of Photosynthesis (Dr. H. T. Witt, A. Müller and B. Rumberg), 194
Experimental Hepatitis in Mice : Blockage of Transformation of Tryptophan to Nicotinic Acid by (M. Piazza, F. Tancredi and Prof. E. Quagliariello), 601
Experimental Medical Research : Feldberg Foundation for German–English Scientific Exchange for, 1143
Experimental Method in Biology from the Time of Antonio Vallisneri to the Present (University of Padua Symposium on), 546
Experimental Morphology, Journal of Embryology and (Editorial Board of the) : International Conference on Embryology, 24
Experimental Silicosis : Immunological Observations in (J. H. Jones and Prof. A. G. Heppleston), 1212
Experimental Transmission of *Borrelia anserina* (Sakharoff 1891) by *Aedes aegypti* (J. A. Roberts), 1225
Experimental Tumor Research : Progress in (edited by F. Homburger), Vol. I, *review* by Prof. F. Bergel, 1231
Experimental Uraemia (Acute) : Elimination of Bovine Serum Albumin in (Dr. G. Gowland, F. G. Smiddy, Dr. R. G. Burwell and F. M. Parsons), 1210
Experimentally Induced Extrahepatic Cholestasis : Immunological Study of (Dr. Roy G. Shorter and R. G. Lack), 610
Experiments on the Compression of Plasma in Cusp Geometry (Dr. T. K. Allen and Dr. R. J. Bickerton), 794
Experiments (Animal), in the Control of Animal Diseases (Research Defence Society Pamphlet on), 226
Experiments (Free-choice) : Comparison between Water and Alcohol Consumption in Six Animal Species in (Dr. Alpo Arvola and Dr. Olof Forsander), 819
Experiments (Voltage-Clamp), with *Nitella* (G. P. Findlay), 812
Exploded Wires : Electrically (Thermal Radiation Laboratory of the Geophysics Research Directorate of the United States Air Force Cambridge Research Laboratories Conference on), 1250
Exploding Wire Plasma : Magnetic Mirror Confinement of (E. J. Seykora), 995
Exploration and Introduction : Plant (Food and Agriculture Organization of the United Nations Technical Meeting on), 1240

Explosions (Nuclear) : Carbon-14 from, as a Short-term Dating System—Use to determine the Origin of Heartwood (Dr. A. T. Wilson), 714
Explosive Crystals : Slow Decomposition of, and their Damage by Fission Fragments (Dr. F. P. Bowden and H. M. Montagu-Pollock), 556
Explosives Technology, *review* by Dr. W. B. Littler, 3
Export : Public Investment and (Sir Walter Benton Jones), 334
Exposure to Air at a Reduced Pressure : Bio-Assay of Erythropoietin in Mice made Polycythaemic by (Dr. P. Mary Cotes and Dr. D. R. Bangham), 1065
Exposure to Cold : Changes in Blood Serotonin during (Dr. Paul Gordon), 183
Exposure (Chronic), to Radiostrontium : Assessment of, by Urinary Assay (Dr. C. L. Comar and Jay Georgi), 390
Expression (Theoretical), for Solubility Product (Prof. B. N. Bhar), 1004 ; (Prof. K. Krishnamurti), 1004
Extensive Air Showers in the Atmosphere : Photography of Cerenkov Light from (D. A. Hill and N. A. Porter), 690
External Conditions : Effect of, on the Phase of Radio Signals (D. E. Watt-Carter and R. L. Corke), 1286
External Sources : Long-Term Effects of Ionizing Radiations from—Report of the Subcommittee on, of the Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 849), 556
Extinction Coefficient (Average), for Re-absorption of Fluorescence Radiation through Uranin Solution (Dr. K. K. Rohatgi and G. S. Singhal), 799
Extracellular Diphtheria Proteins from Culture Filtrates of Different Iron Concentrations : Electrophoretic and Immuno-electrophoretic Patterns of (Bertold J. Radola), 382
Extraction of Heat-Labile Alkaloids from Plants (Dr. A. R. Mattocks), 1281
Extraction of Lipids from the Precursor of the Stylet-Sheath Material of a Pentatomid (P. W. Miles), 911
Extraction (Southern Italian) : Haemoglobin *P* in a Family of (Prof. E. Silvestroni, I. Bianco and C. Brancati), 292
Extractives : Wood (Plant Phenolics Group Meeting on), 229
Extracts (*Grewia populifolia*) : Effects of, on the Development of Chick Embryos (S. K. Brahma and N. Kundu), 506
Extragalactic Radio Sources : Distribution of Brightness in (A. T. Moffet and P. Maltby), 453
Extrahepatic Cholestasis : Immunological Study of Experimentally Induced (Dr. Roy G. Shorter and R. G. Lack), 610
Extra-Mural Libraries (University) in Great Britain : Aims, Organization and Methods of (E. P. Pritchard), 649
Exudative Enteropathy : Calcium Metabolism in Man with Calcium-45—Malabsorption Syndrome and (Dr. G. Milhaud and Dr. P. Vesin), 872
Eye of the Opossum, *Didelphis virginiana* : Cholesterol in the Tapetum Lucidum of the (Antoinette Pirie), 708
Eye (Bee's) : Analysis of Polarized Light by the (Prof. Donald Kennedy and Dr. Edward R. Baylor), 34
Eye Vergence : Barbiturates and (Gerald Westheimer and Cyril Rashbass), 833
Eyes of the Greenland Shark : Copepod *Ommatokoita elongata* (Grant) in the—a Possible Cause of Mutual Dependence (Bjørn Berland), 829
Eyles, Dr. D. E., and others : Isolation of *Plasmodium knowlesi* from Philippine Macaques, 1117
Eyles, J. M. : The Major Achievements of Science, *review*, 110
Eyles, Dr. V. A. : The Life and Scientific Work of Sir James Hall (Bruce-Preller Lecture of the Royal Society of Edinburgh), 345
Eyquem, Dr. A. : Blood Groups in Infra-human Species (Blood Group Investigations—Chimpanzees, Horses, Mules, Donkeys, Sheep, Pigs and Cattle), 655
Eyquem, Dr. A., and others : Serum Factor *Gm*^{a} among the Iranians, 717
Ezra-Cohn, H. E., and Cook, Prof. S. F. : Blood-Typing Compact Human Bone Tissue, 1267

F LAYER Irregularities (Night-time), at the Equator : Height of (Dr. G. S. Kent and Dr. J. R. Koster), 1083
F Region of the Ionosphere : Group and Phase Velocities of a Travelling Disturbance in the (Dr. L. H. Heisler and Dr. J. D. Whitehead), 157
Fabric of the Heavens (Dr. Stephen Toulmin and June Goodfield), *review* by Dr. M. A. Hoskin, 840
Face-centred Cubic Molybdenum Monocarbide : A New Phase formed by High-Pressure Treatment— (E. V. Clougherty, K. H. Lothrop and J. A. Kafalas), 1194
Fact Sheets on U.S. Nuclear Power Projects, third edition, 958
Factor (Intrinsic) : Vitamin B$_{12}$ and (European Symposium on), 1154
Factor (Rigidity), of Gelatin Gels (Dr. Audrey Todd), 567
Factories : Colouring in (Factory Buildings Studies No. 8, Department of Scientific and Industrial Research), 336
Factories : Colouring in (Factory Buildings Studies No. 8, Department of Scientific and Industrial Research), 336
Factors influencing the Composition of the Cellulose-decomposing Microflora on Soil Crumb Plates (A. Keynan, Y. Henis and P. Keller), 307
Factors controlling the Growth of Etiolated Oat Seedlings : Carbon Dioxide and Ethanol as (Dr. C. L. Mer), 260
Factors (Environmental), and Bryophytes (Kathryn Benson-Evans), 255
Factors (Serum) : Influence of, on the Species-specificity of Tissue Thromboplastin (Christine Hawkey and J. L. Stafford), 920
Factory (Chemical) : Quality Measurement in the (A. J. Clark and C. W. Munday), (Department of Scientific and Industrial Research), 752
Factory Buildings Studies No. 8 : Colouring in Factories (Department of Scientific and Industrial Research), 336
Facts and Figures about Viewing and Listening (British Broadcasting Corporation), 1051
Facts and Theories of Shape Discrimination (Dr. P. C. Dodwell), 578 ; (Dr. N. S. Sutherland), 581
Faeces (Human) : Isolation of Sterol Esters from (Francis Aylward and Pamela Wills), 1397
Fahmy, Dr. Myrtle J. (Fahmy, Dr. O. G., and) : Induction of Mutations by Deoxyribonucleic Acid in *Drosophila melanogaster*, 776
Fahmy, Dr. O. G., and Fahmy, Dr. Myrtle J. : Induction of Mutations by Deoxyribonucleic Acid in *Drosophila melanogaster*, 776
Failure of a Dog to Discriminate between Strontium-90 and Calcium given Orally (Dr. Betsy J. Stover, M. Goldman and A. C. Andersen), 713
Failure of Nucleotides to Diffuse Freely into the Embryo of *Pteridium aquilinum* (P. R. Bell), 91
Falconer, Dr. D. S., and Bloom, Dr. Joyce L. : Inheritance of Susceptibility to Induced Pulmonary Tumours in Mice, 1070
Falls (Meteorite), throughout the Ages : The Frequency of (Prof. Herbert Dingle), 482 ; (Prof. Hans Pettersson), 482
Family of Southern Italian Extraction : Haemoglobin *P* in a (Prof. E. Silvestroni, I. Bianco and C. Brancati), 292
Family (English Family and in a German) : Thalassaemia in an (Dr. F. Vella and S. A. Ibrahim), 822
Family (A New), of Potent Carcinogens—Benzopyridocarbazoles (Prof. A. Lacassagne, Prof. N. P. Buu-Hoï, F. Zajdela, F. Périn and P. Jacquignon), 1005
Family (Puerto Rican) : The Diego Factor in a—a case of Anti-Diego in Monozygotic Twins (Dr. Hubert J. Van Peenen, Dr. John Scudder, James A. Jack and Erika Awer), 188
Family (Sardinian) : Association of Haemoglobin *N* and Microcythaemia in a (Prof. E. Silvestroni and I. Bianco), 1208
Far Infra-Red : A Proposal for a Tunable Source of Radiation for the, using Beats between Optical Masers (Dr. D. C. Lainé), 795
Far-Red Irradiation (Red and) : Effect of, on the Germination Process in Seeds of *Pinus sylvestris* L. (Bengt Nyman), 1219
Far Ultra-Violet : Irradiated Lithium Fluoride as an Optical Filter in the (J. L. Weeks, S. Gordon and G. M. A. C. Meaburn), 1186
Faraday Society : Informal Discussion on Graphite, 27
  General Discussion on the Structure and Properties of Ionic Melts, 338, 443
Farm Buildings : Bibliography of Research Publications on, Section 5, Buildings for the Drying and Storage of Grain (Agricultural Research Council), 440
Farm Buildings in Britain : Survey of (Agricultural Research Council), 440
Farmer, J. B., and Petch, B. : Electrolyte Retention produced by the Anti-aldosterone *SC* 5233, 1308
Farmilo, C. G., and others : Essential Oil from Fresh *Cannabis sativa* and its Use in Identification, 774
5,10,15 ^{14}C-Farnesol Pyrophosphate : Incorporation of, into *Phycomyces carotenoids* (H. Yamamoto, H. Yokoyama, K. Simpson, T. O. M. Nakayama and C. O. Chichester), 1299
Farradane, J. : The National Reference Library of Science and Invention, 734
Fast-Neutron Spectroscopy (B. V. Rybakov and V. A. Sidorov), (edited by N. A. Vlasov), translated from the Russian, *review* by Dr. P. A. Egelstaff, 8
Farrington, Prof. B. : Science and the Classics, 1337
Fat : Metabolic Production of Sucrose from (Prof. H. Beevers), 433
  Role of the Lung in Metabolism of, during Ontogeny of the Rat (Dr. Z. Vacek, P. Hahn and O. Koldovsky), 85
Fatigue in a Fluid-filled Pulsating Elastic Tube (Group-Capt. John Erskine Malcolm), 1012
Fatty Acid Esters of Riboflavin : Hydrolysis of, by Pancreatic Lipase (Prof. Kunio Yagi, Yoshiko Yamamoto and Jun Okuda), 174
Fatty Acids (Free) : Stimulation of Lipogenesis in Thyroid Tissue *in vitro* by (Prof. Norbert Freinkel, Dr. Augusto D. Litonjua and Edward C. Saef), 804
Fatty Acids (Polyenoic) : Biosynthesis of, in Human Whole Blood (Dr. F. Leupold and G. Kremer), 805
Fault (Great Glen) : Continuation of the, beyond the Moray Firth (Dr. Derek Flinn), 589 ; (Dr. Robert M. Appleby), 1190
Fauna ('Lusitanian') : Origin of the British Insular Races of Small Mammals and of the (G. B. Corbet), 1037
Favelukes, Dr. G., and others : Control of Specificity in Haemoglobin Synthesis, 1365
Fazekas de St. Groth, Prof. S. : Evaluation of Quantal Neutralization Tests, 891
Fearnhead, R. W. : Title of reader in dental anatomy in the University of London (at the London Hospital Medical College) conferred on, 229
Feasible Directions : Methods of—a Study in Linear and Non-Linear Programming (Dr. G. Zoutendijk), *review* by Dr. M. V. Wilkes, 9.
Feather Keratin : Structure of (the late Prof. W. T. Astbury and E. Beighton), 171
Featherstone, M. S., and Cunningham, Catherine (Alistair Heron and Sheila M. Chown, assisted by) : Ageing and the Semi-Skilled—a Survey in Manufacturing Industry on Merseyside (Medical Research Council, Memorandum No. 40), 1249
Fecundity and Ageing (Blood-Feeding,), in *Aedes aegypti* var. *Queenslandensis* (Dr. M. M. J. Lavoipierre), 575
Federal Technical College, Zurich : Appointment of Dr. W. F. Berg as professor of the Photographic Institute, 1043
Federation of Malaya Institute for Medical Research : Studies from the, No. 29, Malaysian Parasites, 35-49 (edited by W. W. Macdonald), *review* by P. F. Mattingly, 948
Feedback Inhibition controlling the Biosynthesis of Isoleucine : Release from the (Masaya Hayashibe and Teijiro Uemura), 1417
Feedback Mechanism (A Positive), of Adenosine Triphosphate Synthesis in Erythrocytes (Makoto Nakao, Takashi Motegi, Toshiko Nakao, Saburo Yamazoe and Haruhisa Yoshikawa), 283
Feeding (Blood-), Fecundity and Ageing in *Aedes aegypti* var. *Queenslandensis* (Dr. M. M. J. Lavoipierre), 575
Feeding (Thyroid) : Augmentation of Toxicity of Monoamine Oxidase Inhibitor by (Richard N. Carrier and Dr. Paul V. Buday), 1107
Feeding Conditions (Normal) : Soluble Material in the Gastro-intestinal Tract of Rats under (Dr. Anne S. Cole), 502
Fehér, Dr. I., and others : Effect of Amino-Acid Esters on the Growth of Fibroblast Cultures, 494
Fehr, Howard (edited by) : Report on the School Curriculum in Mathematics (Organization for European Economic Co-operation), 335
Feibelman, Walter A. : The Alpha Virginis (Spica) Nebula, 1083
Feinberg, Dr. H. : Relationship between Velocity of Cardiac Contraction and Size of the Heart—a Hypothesis, 1373
Feinberg, Dr. J. G. : Pinnal Anaphylaxis, an Additional Anaphylactic Site, 712
Feinendegen, Dr. L. E., and others : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture, 1398
Feldberg Foundation for German–English Scientific Exchange for Experimental Medical Research, 1143

Fellowship : The Selby, 24
Fellowships : IBM Research (International Business Machines, United Kingdom, Ltd.), 442
  Industrial Research (J. Langham Thompson, Ltd.), 545
  Science Study (Unesco), 1250
  Wellcome Research (Animal Health Trust Awards), 1250
Fellowships in Chemistry (Ramsay Memorial Trust Awards for 1961-62), 337
Fellowships for Plankton Research (Lamont Geological Observatory), 754
Fellowships (Harkness) for 1962 of the Commonwealth Fund, New York, 1045
Fellowships (Eleanor Roosevelt), for Research on Cancer (International Union against Cancer), 125
Fellowships (Post-Doctoral), in Protein Chemistry (Division of Protein Chemistry of the Australian Commonwealth Scientific and Industrial Research Organization), 336
Fellowships (Research) : Carlsberg–Wellcome (Awards for 1960-61), 754
Fellowships and Grants in the field of Radiology and Nuclear Medicine for 1962-63 (James Picker Foundation), 228
Female Rat Flea, *Xenopsylla cheopis* Roths. : An Abnormal Form of (M. I. D. Sharma and G. C. Joshi), 727
Female Rats (Suckled and Unsuckled) : Induction of Cancer in (Prof. Giuliano Briziarelli), 921
Feng, Dr. P. C., and others : High Concentration of (–)-Noradrenaline in *Portulaca oleracea* L., 1108
Fermentation of a Synthetic Medium lacking Amino-acids : Formation of Phenethyl Alcohol and Tyrosol during (R. Stevens), 913
Fern Embryos in Sterile Culture : Growth of (Prof. Augustus E. DeMaggio and Prof. Ralph H. Wetmore), 94
Fernald, G. W. (Csáky, Dr. T. Z., and) : Localization of the 'Sugar Pump' in the Intestinal Epithelium, 709
Fernando, S. T. : Parasite Biology (Developmental Stages of *Mecistocirrus digitatus*), 135
Ferns : Antimicrobial Substances from (Prof. Jasper C. Maruzzella), 518
Ferri, A., and Ling, T. : Space-Flight and Re-entry Trajectories (Dynamics of Terminal Re-entry—Effect of Varying Lift on Re-entry Trajectories), 960
Ferric Dimethyldithiocarbamate : Effect of, on the Rooting of Cuttings of *Hevea brasiliensis* (G. H. Tinley), 1217
Ferrites (Manganese and Nickel) : Magnetic Relaxation in Magnetite and in its Solid Solutions with (A. Bragiński and Th. Merceron), 898
Ferro-Alloy Industry in India (National Metallurgical Laboratory, Jamshedpur, Symposium on), 1144
Ferrobacillus ferrooxidans : Morphological Variation in, related to the Rate of Iron Oxidation (M. P. Silverman and M. H. Rogoff), 1221
Ferrous Solutions : Radiolytic Oxidation of, with Standardized Internal Sources of Polonium-210 (J. Steyn and D. van As), 903
Fertility in the Domestic Fowl : Effect of Varying Doses of Undiluted Semen on (G. C. Taneja and R. S. Gowe), 828
Feulgen-Stained Somatic Cell Nucleus as a Homogeneously Absorbing Object (Dr. Simon Iversen), 150
Fever (Rheumatic) : Amino-acid Composition of Serum Mucoprotein in (Prof. D. O'Brien, C. H. M. Walker and D. Walker), 922
Few : The Chosen—an Examination of some Aspects of University Selection in Britain (W. D. Furneaux), *review* by Dr. W. H. King, 947
Fibre (Active Muscle) : Ionic Currents in Membrane of (Dr. H. Jenerick), 1074
Fibre Optics : A Hypodermic Probe using (David D. Capellaro, Narinder S. Kapany and Dr. Charles Long), 927
Fibres (Insect Muscle) : Spontaneous Miniature Potentials from (P. N. R. Usherwood), 814
Fibres (Muscle Target), a Newly Recognized Sign of Denervation (Dr. W. K. Engel), 389
Fibres (Taste), in the Carp : Function of (Jihei Konishi and Prof. Yngve Zotterman), 286
Fibrin : Conversion of, to Desmofibrin (K. Buluk, T. Januszko and J. Olbromski), 1093
Fibrinolysins : Demonstration by Paper Electrophoresis of an Auto-Immunological Precipitin possibly due to (Marion I. Cooper and J. R. Sykes), 1212
Fibrinolysis (Spontaneous Plasma) : Determination of, by a Practical Procedure (Dr. H. Yatzidis), 604
Fibroblast Cultures : Effect of Amino-Acid Esters on the Growth of (Dr. I. Fehér, A. Döklen and V. Selmeci), 494
Fibrous Silica (Dr. Wolfgang Haller), 662
Field Museum of Natural History, Chicago : Botanical Series, Vol. 13, Part 5, No. 1, Flora of Peru (J. Francis Macbride), *review* by N. Y. Sandwith, 214
Fielder, Dr. Gilbert : Structure of the Moon's Surface, *review* by D. H. P. Jones, 424
Figgis, B. N., and others : Inorganic Polymers (Dialkyl Tin Oxides), 1047
Figures (Facts and), about Viewing and Listening (British Broadcasting Corporation), 1051
Filamentous Saprophyte from Wookey Hole Caves : Further Observations on a (Dr. A. J. Willis), 724
Film (Clean Rhodium): Reaction of Ethane with a (Dr. Richard W. Roberts), 170
Films (Evaporated Carbon), of Large Area for Adsorption Studies by Electron Bombardment (A. E. Bell, J. Pritchard and Prof. K. W. Sykes), 487
Films (Nickel-Iron) : Variation of Anisotropy Axis Dispersion with Thickness of (H. Clow), 996
Filter (Optical), in the Far Ultra-Violet : Irradiated Lithium Fluoride as an (J. L. Weeks, S. Gordon and G. M. A. C. Meaburn), 1186
Filter Pads (Asbestos), for Virus Filtration : A Method of treating (N. St. G. Hyslop), 305
Filter Paper Chromatograms : A Spray Method for the Simultaneous Detection of Iodide and Iodinated Amino-Acids on (Dr. A. Van Zyl and M. Bhaga), 275
Filter Theory : Perceptual Defence and (Neville Moray), 940
Filtrate (Culture), of *Helminthosporium sativum* : Production of Symptoms of Barley Leaf-Spot Disease by (S. K. Gayed), 725
Filtrates (Culture), of Different Iron Concentrations : Electrophoretic and Immuno-electrophoretic Patterns of Extracellular Diphtheria Proteins from (Bertold J. Radola), 382
Filtration' ('Gel), of Organic Compounds (B. Cortis-Jones), 272
Filtration (Gel), of Sea-Water—Separation of Free and Bound Forms of Vitamin B_{12} (Dr. K. W. Daisley), 868
Filtration (Virus) : A Method of Treating Asbestos Filter Pads for (N. St. G. Hyslop), 305
Financing Government Research in Great Britain, 205
Finches (Weaver-), *Quelea quelea* : Melanism in Captive (Dr. B. Lofts), 993
Findlay, G. P. : Voltage-Clamp Experiments with *Nitella*, 812
Fine, B. C. : Pattern of Pyrethrin-resistance in Houseflies, 884
Fine, Dr. J. M., and others : Electrophoresis in a Thin Layer of Starch-Gel followed by a Plastifying Treatment, 72
Fine Measuring Instruments (Fourteenth International Instructional Course by Carl Zeiss, Jena, at the Imperial College of Science and Technology, London), 1045
Fine Structure (Genetic), of the *ad*$_7$ Locus of *Schizosaccharomyces pombe* : Distribution of X-Ray- and Nitrous Acid-induced Mutations in the (Dr. Herbert Gutz), 1127
Finger, G. C., and Starr, L. D. : 2,6-Difluoropyridine, 595
Finlay, Ian R. : Appointed director of the Royal Scottish Museum, Edinburgh, 648 ; work of, 648
Finlayson, Dr. L. H. : A New Approach to Biology Teaching, 554
Firsoff, V. A. : Surface of the Moon, *review* by D. H. P. Jones, 424
Firsov, G. G., and others : A Soviet View of British Libraries (translated by A. Thompson), (Library Association, Pamphlet No. 22), 752
First Anthocyanins appearing during the Ripening of Blueberries (Dr. Heikki Suomalainen and A. J. A. Keränen), 498
First Motion of a Refracted *P*-Wave : Possible Method for Increasing the Signal-Noise Ratio in the Detection of the (F. A. Key, J. K. Wright, E. W. Carpenter and B. M. H. Stott), 1382
First Pamphlet on Clear Air, 752
First Science Museum : Africa's (Murray Schoonraad), 848
Fischer, Dr. R., and others : Taste Thresholds and Food Dislikes, 1328
Fish : Expected Rate of Senescence and Age-dependent Mortality in (Dr. Alex Comfort), 822
Fish in Nutrition (Food and Agriculture Organization of the United Nations International Conference on), 1045
Fish (Hydrogen Ion Concentration, Plankton and), in Fresh-water Eutrophic Lakes of India (Dr. S. M. Das), 511
Fish (Marine Teleost), *Pollachius virens* : Pharmacological Characterization of the Oxytocic Peptides in the Pituitary of a (Prof. H. Heller, B. T. Pickering, J. Maetz and F. Morel), 670
Fish Muscle : Cholinergic Transmission in (K. Krnjević), 1403
Fisher, Sir Ronald A. : Elected a Member of the Biology Section of the Leopoldina German Academy of Sciences, 122
Fisheries (Salmon and Freshwater), Committee on : Report of the, 962
Fishes (Air-breathing) : A Hydrostatic Function of the Accessory Respiratory Organs in (Dr. S. Z. Qasim and R. Hasan), 396
Fishing in Madagascar (Dr. Rudolph Kreuzer), 238
Fishing (Salmon and Freshwater), in Great Britain (Dr. J. W. Jones), 962
Fishing Research Vessel (Food and Agriculture Organization of the United Nations Forum on the), 229
Fission Fragments : Damage in Stainless Steel due to (P. R. B. Higgins and A. C. Roberts), 1158
  Slow Decomposition of Explosive Crystals and their Damage by (Dr. F. P. Bowden and H. M. Montagu-Pollock), 556
Fission and Spallation Phenomena and their Application to Cosmic Rays (European Organization for Nuclear Research and the Max Planck Institute, Heidelberg, Conference on), 24
Fitt, E., and others : Isolation and Properties of Deoxyribonucleic Acid from Mammalian Sperm, 1375
Fitzwilliam Museum, Cambridge : Annual Report for 1960, 1249
Fixation of Yeast Protoplasts for Electron Microscopy (Dr. P. F. Elbers), 1022
Fixation (Dark-) of Carbon Dioxide in Leaves of *Bryophyllum fedtschenkoi* : An Endogenous Rhythm in the Rate of (Diana M. Warren and Malcolm B. Wilkins), 686
Fixation (Symbiotic Nitrogen) : Root Temperature and (A. H. Gibson), 1080
Fixative (New), for Histological Work : Ethylene Glycol Monomethyl Ether, a (Prof. Walter B. Shelley and Ralph Florence), 719
Fixatives (Formalin) : Use of Veronal Buffers in (Dr. S. J. Holt and Dr. R. Marian Hicks), 832
Flagellum (Flimmer-) of the Sponge (Björn A. Afzelius), 1318
Flagle, Charles D., and others : Operations Research and Systems Engineering, *review* by Prof. L. S. Goddard, 105
Flames (Diborane–Hydrazine) : Formation of Boron Nitride in (W. G. Berg and W. E. Wilson), 380
Flash-Lamp : Photo-Polarography with a (Dr. H. Berg and H. Schweiss), 1270
Flavonoid Content of Resistant and Susceptible Strains of Cotton : Leaf Curl Virus and the (Dr. Esam M. Moustafa), 415
Flea (Female Rat), *Xenopsylla cheopis* Roths. : An Abnormal Form of (M. I. D. Sharma and G. C. Joshi), 727
Fleas (Siphonaptera) : Double and Single Spermathecae in (Hon. Miriam Rothschild), 1322
Fleming, Dr. Launcelot : The Humanity of Man, 1229
Fleming Memorial Fund for Medical Research : Grants from the, 959
Fleš, D., and others : Inhibition of Chemi-Luminescence of Luminol by means of Oximes, 177
Flies (Dragon-) : Spiracle Control in (Dr. P. L. Miller), 621
Flies (House-) : Pattern of Pyrethrin-resistance in (B. C. Fine), 884
Flight (Space-) and Re-entry Trajectories (International Academy of Astronautics Symposium on), 960
Flimmer-flagellum of the Sponge (Björn A. Afzelius), 1318
Flinn, Dr. Derek : Continuation of the Great Glen Fault beyond the Moray Firth, 589
Floor : The Ocean, *review* by Dr. G. E. R. Deacon, 531
Floors of the Oceans, I, The North Atlantic—Text to accompany the Physiographic Diagram of the North Atlantic (Bruce C. Heezen, Marie Tharp and Maurice Ewing), (Geological Society of America, Special Paper 65), *review* by Dr. G. E. R. Deacon, 531
Flora (The Changing), of South-East England (J. E. Lousley), 133
Flora of Peru (J. Francis Macbride), *review* by N. Y. Sandwith, 214
Flora Europaea : Second Symposium on (Dr. V. H. Heywood), 446
Floral Initiation in Cocksfoot (*Dactylis glomerata* L.) : Effect of Spacing and Nitrogen-level on (D. M. Calder and J. P. Cooper), 195

Floras (Lower Tertiary), of Southern England (Marjorie Elizabeth Jane Chandler), I, Palaeocene Floras, London Clay Flora (Supplement), *review* by Prof. John Walton, 209
Floras (Tropical), in the Tertiary in Southern England, *review* by Prof. John Walton, 209
Florence, Ralph (Shelley, Prof. Walter B., and) : Ethylene Glycol Monomethyl Ether, a New Fixative for Histological Work, 719
Florey, Sir Howard : Elected an Honorary Fellow of the Royal College of Surgeons of England, 126
Florkin, Prof. Marcel : Elected a Member of the Biochemistry Section of the Leopoldina German Academy of Sciences, 122
Flotation : Power Requirements and Scale-up in (Prof. Nathaniel Arbiter and Prof. Colin C. Harris), 268
Flow of Dry Granules through Apertures : Minimum Energy Theorem for (R. L. Brown), 458
Flow (Rheopectic) : A Clear Instance of (J. B. Yannas and R. N. Gonzalez), 1384
Flow Calorimeter (Continuous) : Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of High Surface Area using a (G. H. Bell and A. J. Groszek), 1184
Flower Initiation in the Carnation in Response to Photoperiod (Dr. G. P. Harris and Juliet E. Griffin), 614
Flower Initiation in Kent Wild White Clover (*Trifolium repens* L.), under Controlled Environmental Conditions (R. J. Haggar), 1120
Flowering and Internode-Length in *Pisum sativum* : Effects of Gibberellic Acid and Allogibberic Acid on (I. C. Murfet and H. N. Barber), 514
Flowers : Enzymic Breakdown of Stigmatic Cuticula of (W. Heinen and H. F. Linskens), 1416
Fluctuations (Seasonal), and Distribution of Nitrite in a Tropical West African Estuary (J. C. D. Watts), 929
Fluid Mechanics at Glasgow, 853
Fluid–Fluid Interfaces in Steady Motion (Prof. Walter Rose), 242
Fluid-filled Pulsating Elastic Tube : Fatigue in a (Group-Capt. John Erskine Malcolm), 1012
Fluids and Excreta (Internal Organs,), of Rabbits : Effect of Polyvinyl Alcohol on the Distribution of Iodine-131 in the (H. F. Nowak and J. Kucharski), 665, *erratum*, 1045
Fluorescein-labelled Conjugates : Simplified Procedure for Removing Nonspecific Staining Components from (G. D. Johnson), 70
Fluorescence Radiation : Average Extinction Coefficient for Re-absorption of, through Uranin Solution (Dr. K. K. Rohatgi and G. S. Singhal), 799
Fluorescence and Ultra-violet Absorption Spectra of 3,4-Benzopyrene : Effect of Ozone on the (Z. Morlin and K. M. Sáringer), 907
Fluorescent Material : Use of a Different Type of, as an Optical Maser (Jordan J. Markham and Dickron Mergerian), 586
Fluoride : Differential Inhibition of Human Serum Cholinesterase with—Recognition of Two New Phenotypes (Prof. H. Harris and Mary Whittaker), 496
Fluoride-compensated Substitution of Bivalent Cations in the Magnetoplumbite Structure (Prof. E. Banks and M. Robbins), 1387
Fluoride (Irradiated Lithium), as an Optical Filter in the Far Ultra-Violet (J. L. Weeks, S. Gordon and G. M. A. C. Meaburn), 1186
Fluoride (Sodium) : Effect of, on Calcium Metabolism of Human Beings (Dr. Clayton Rich and John Ensinck), 184
Fluorine Chemistry, *review* by Dr. Richard Dresdner, 319
  Advances in (edited by Prof. M. Stacey, Prof. J. C. Tatlow and Dr. A. G. Sharp), Vol. I, *review* by Dr. Richard Dresdner, 319
p-Fluorophenylalanine : Effect of, on the Multiplication of Foot-and-Mouth Disease Virus (F. Brown, D. N. Planterose and Doreen L. Stewart), 414
  Influence of, on the Production of Viral Ribonucleic Acid and on the Utilizability of Viral Protein during Multiplication of Fowl Plague Virus (Dr. Christoph Scholtissek and Rudolf Rott), 1023
5-Fluorouracil : Effect of, on Hepatic Induced Increase of Tryptophan-Pyrrolase (Sancia Gaetani and M. Antonietta Spadoni), 1296
Fluorverbindungen (Organischen) : Chemie der (Milos Hudlicky), *review* by Dr. Richard Dresdner, 319
'Flux' : Neutron (K. L. Stretch), 60 ; (Dr. W. R. Harper), 695
Fly (Blow-), *Phormia regina* Meigen : Response of the Labellar Hairs of the, to Protein (D. I. Wallis), 917
Fly (Damsel-) Pigments : Isolation and Fractionation of some (C. B. Coulson and P. Mary Lord), 1300
Fodor, Prof. G., and others : Chemical Character of the Pigments of a New Nitrogen-fixing Micro-organism, 1413
Foetal Haemoglobins : Abnormal (Prof. E. Silvestroni and I. Bianco), 397
Foetal and Neonatal Physiology (*British Medical Bulletin*, Vol. 17, No. 2), (British Council), 344
Fogh, J. E. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Radiation affects Cell Division much more than Cell Growth), 340
Foldes, L. P. : Appointed to the University of London readership in economics at the London School of Economics and Political Science, 651
Folinic Acid : Enzymic Conversion of Tetrahydrofolic Acid to, by Plant Protein (E. H. Byers, J. Stewart and T. J. Bond), 179
Folk-life Study, Society for : Formation of a, 544
Folkers, C. S. (Soltys, M. A., and) : Immunity to Protozoa (Immunity in Trypanosomiasis), 552
Folkes, B. F., and others : An Introduction to the Biology of Micro-organisms, *review* by Prof. B. C. J. G. Knight, 1032
Follicle (Skin) Population : Composition of the, in Seven Rajasthan Sheep Breeds (Sunanda Narayan), 1115
Follicles (Testis), in Miridae : The Number of (Dennis Leston), 93
Fomes annosus : Inoculation of Pine Stumps against Infection by (Dr. J. Rishbeth), 826
Fonds National de la Recherche Scientifique : Trente-troisième Rapport Annuel, 441
Food Dislikes : Taste Thresholds and (Dr. R. Fischer, F. Griffin, S. England and S. M. Garn), 1328
Food Science (Australian Commonwealth Scientific and Industrial Research Organization Conference on), 337, 546
Food Technology : European Symposium on, 959
Food and Agriculture Organization (United Nations) : Forum in Tokyo on the Fishing Research Vessel, 229
  Meetings on Nutrition in Latin America, 959
  Appointment of Dr. Fabian Recalde as a nutrition officer, 1045
  International Conference on Fish in Nutrition, 1045
  Technical Meeting on Plant Exploration and Introduction, 1240
Food and Agriculture Organization (United Nations), and others : Conference on the Use of Radioisotopes in Animal Biology and the Medical Sciences, 959
Food and Drug Directorate : Research in the (Dr. Ross A. Chapman), 335
Foods (Honeybee Larval) : Chromatographic Investigation of the Methly Alcohol/Chloroform-soluble Constituents of (N. G. Patel, M. H. Haydak and R. Lovell), 362
Foodstuffs : Freeze-drying of (Borough Polytechnic, London, Symposium on), 651
Foot-and-Mouth Disease Virus : Effect of *p*-Fluorophenylalanine on the Multiplication of (F. Brown, D. N. Planterose and Doreen L. Stewart), 414
Forces (Interparticle) : Role of, in Development of Upper Plastic Limit of Clays (Dr. Raymond Yong and E. T. Selig), 938
Ford, the late L. T. : Collection of British Microlepidoptera belonging to, acquired by the British Museum (Natural History), 124
Foreign Languages (Science, Librarianship and) : Science Information Personnel—the New Profession of Information combining (Leonard Cohen and Kenneth Craven), 452
Forest Ecosystem : Proceedings of the Symposium on (Montreal, 1959), (International Society for Tropical Ecology), 755
Forest Insect Pests and Diseases in Canada (Prof. M. L. Anderson), 1260
Forest Products Research 1960—Report of the Products Research Steering Committee with the Report of the Director of Forest Products Research (Department of Scientific and Industrial Research), 760
Forestry, Canada Department of (Forest Entomology and Pathology Branch) : Annual Report of the Forest Insect and Disease Survey (1960), 1260
Forestry, Director of (New Zealand) : Report for 1959-60, 451
Forestry Commission : Forty-fifth Annual Report for 1959-60, 656
Forestry (State), in Great Britain : Four Decades of (Prof. M. L. Anderson), 656
Forestry (State), in New Zealand : Four Decades of, 451
Form (An Abnormal), of Female Rat Flea, *Xenopsylla cheopis* Roths. (M. I. D. Sharma and G. C. Joshi), 727
Formaldehyde : Mechanism of Mutagenesis induced by—the Essential Role of the 6-Amino-Group of Adenylic Acid (or Adenosine) in the Mediation of the Mutagenic Activity of Formaldehyde (Dr. Thomas Alderson), 251
Formalin Fixatives : Use of Veronal Buffers in (Dr. S. J. Holt and Dr. R. Marian Hicks), 832
Formation of Boron Nitride in Diborane–Hydrazine Flames (W. G. Berg and W. E. Wilson), 380
Formation of Calcium Chloraluminate by Interaction of Portland Cement with Sea-Water and Chloride Solutions (Dr. L. Heller and Dr. M. Ben-Yair), 488
Formation of Chalcopyrite by Reaction between Chalcocite and Pyrrhotite in Cold Solution (W. M. B. Roberts), 560
Formation of Chlorophyll in the Leaves of Etiolated Pea Plants : Correlative Inhibition of (Jiří Ulehla), 613
Formation of *G.P.* Zones (Clusters rich in Solute) : Resistivity Changes due to (H. Herman and Prof. J. B. Cohen), 63
Formation of 5-Hydroxytryptamine in an Aqueous Solution of 5-Hydroxytryptophan treated with X-Rays (M. Jović and Z. Supek), 383
Formation of Phenethyl Alcohol and Tyrosol during Fermentation of a Synthetic Medium lacking Amino-acids (R. Stevens), 913
Formation (Dimer), and Glucose Transfer across the Membrane of the Red Blood Cell (Dr. W. D. Stein), 1277
Formation (Dimer), and the Transfer of Glycerol across the Erythrocyte Membrane (Dr. W. D. Stein), 352
Formation (Lanthionine), in Keratin (Dr. C. Earland and D. J. Raven), 384
Formation (Radiation-induced), and Reactions of the Formyl Radical Ion (John Holian, Dr. George Scholes and Prof. Joseph J. Weiss), 1386
Formation (Supposed), of Hydrogen Peroxide during the Autoxidation of Haemoglobin : Reactions of Haemoproteins with Hydrogen Peroxide and the (Prof. D. Keilin), 769
Formation (Ternary Compound), in the System $CaO–Al_2O_3—MgO$ (J. H. Welch), 559
Formation and Structure of Paper (Technical Section of the British Paper and Board Makers' Association Conference on), 126
Formation and Utilization of Poly-β-Hydroxybutyric Acid by Knallgas Bacteria (*Hydrogenomonas*), (Prof. H. G. Schlegel, G. Gottschalk and R. von Bartha), 463
Forms (α- and β-), of Selected Sugars : Relative Sweetness of (Rose Marie Pangborn and Sandra C. Gee), 810
Forms (Culture), of *Trypanosoma rhodesiense* : Cyclical Infection of *Glossina mortisans* with (the late Prof. R. M. Gordon and J. K. Miller), 1317
Forms (Free and Bound), of Vitamin B_{12} : Gel Filtration of Sea-Water—Separation of (Dr. K. W. Daisley), 868
Formyl Radical Ion : Radiation-induced Formation and Reactions of the (John Holian, Dr. George Scholes and Prof. Joseph J. Weiss), 1386
Forsander, Dr. Olof (Arvola, Dr. Alpo, and) : Comparison between Water and Alcohol Consumption in Six Animal Species in Free-choice Experiments, 819
Forstwirtschaft (Land- und), Biologische Bundesanstalt für : Proceedings of the Fourth Conference on Potato Virus Diseases, Braunschweig, 1960 (edited by J. Brandes, R. Bartels, V. Völk and C. Wetter), 657
Forsyth, D. C. T. (Cooper, G. A., and) : *Pneumodermopsis paucidens* in the North Sea, 1412
Forthcoming International Scientific and Technical Conferences (Department of Scientific and Industrial Research, List No. 29), 959
Fossil Chlorins in a Triassic Sediment (Max Blumer and Gilbert Omenn), 161
Fothergill, Dr. P. G. : Issues in Evolution, *review*, 425
Foundations of Modern Analysis (Prof. J. Dieudonné), *review* by Prof. L. S. Goddard, 6
Four Decades of State Forestry in Great Britain (Prof. M. L. Anderson), 656
Four Decades of State Forestry in New Zealand, 451
Four-fold Co-ordination of Titanium in Barium Orthotitanate : Infra-Red Spectroscopic Evidence of (P. Tarte), 1002
Fowl (Domestic) : Effect of Varying Doses of Undiluted Semen on Fertility in the (G. C. Taneja and R. S. Gowe), 828
Fowl Plague Virus : Influence of *p*-Fluorophenylalanine on the Production of Viral Ribonucleic Acid and on the Utilizability of Viral Protein during Multiplication of (Dr. Christoph Scholtissek and Rudolf Rott), 1023

Fraction (Alkaloidal), from the Bark of the Jamaican Shade Tree *Pithecolobium samath* Benth. : Pharmacology of the (B. E. Leonard and Dr. H. S. A. Sherratt), 287
Fractionation of some Damselfly Pigments : Isolation and (C. B. Coulson and P. Mary Lord), 1300
Fractionation (Chromatographic), of Hog Thyroglobulin (Prof. Nobuo Ui, Osamu Tarutani, Yoichi Kondo and Hiroko Tamura), 1199
Fractionation (Ribonucleic Acid) : Modified Cellulose Ion Exchangers and their Use in (Dr. L. Bosch, G. van der Wende and Dr. H. Bloemendal), 349
Fractionation (Sub-) of Human Serum Leucine Aminopeptidase (Prof. Clyde A. Dubbs, Charlotte Vivonia and Jean M. Hilburn), 1203
Fractions (Cell), from Myocardial Slices : Effect of Adrenalectomy and Corticosteroids on Distribution of Radioactivity in Protein of (Dr. Edward I. Weinshelbaum and Dr. Ira G. Wool), 1401
Fractions (Collagen), in the Skin of New-born and Adult Rats (Nestor Gonzalez Cadavid, Berta Denduchis and Roberto E. Mancini), 1297
Fractions (Serum Protein), of the Newly Hatched Chick : Comparison of the, with those of Adult Birds using Starch-gel Electrophoresis (A. Amin), 708
Fractions (Sub-Cellular), of the Brain of the Rat during 'Metrazol' Convulsions : Changes in the Pentose-Nucleic Acid Content of (Prof. G. P. Talwar, B. Sadasivudu and V. S. Chitre), 1007
Fracture and Fusion of Coccid Chromosomes (Prof. W. Spencer Brown), 1419
Fraeijs de Veubeke, B. : Space-Flight and Re-entry Trajectories (Orbital Transfer and Rendezvous), 960
Fraenkel, Prof. Abraham : Abstract Set Theory, second edition, *review* by Dr. R. G. Cooke (June 17, 1047), *erratum*, 126
Fragaria (Strawberry) : Identity of L-Ascorbic Acid formed from D-Glucose by the (Dr. Frank A. Loewus and Stanley Kelly), 1059
Fragments (Fission) : Damage in Stainless Steel due to (P. R. B. Higgins and A. C. Roberts), 1158
  Slow Decomposition of Explosive Crystals and their Damage by (Dr. F. P. Bowden and H. M. Montagu-Pollock), 556
France : Megalithic Tombs of, *review* by Prof. C. F. C. Hawkes, 637.
  The Prehistoric Chamber Tombs of (Dr. Glyn Daniel), *review* by Prof. C. F. C. Hawkes, 637
Francis, Sir Frank : At the sixty-seventh Annual Conference of the Museums Association (The Problem of Display), 553
Francki, Dr. R. I. B., and Matthews, Dr. R. E. F. : Stimulation of Turnip Yellow Mosaic Virus Protein Production by 2-Thiouracil, 1078
Frank, Prof. F. C. : X-Ray Diffraction Spikes of Diamond, 1188
Frank, O., and others : Vitamin-Levels in Blood and Serum, 78
Franklin, Prof. Kenneth J. : William Harvey—Englishman, 1578-1657, *review*, 740
Franks, Dr. D. : Blood Groups in Infra-human Species (Horse Blood Groups), 654
Franks, F. (Eagland, D., and) : *p*H of Soap Solutions, 1003
Fraser, Dr. Donald : Awarded the second Borden Award of the Nutrition Society of Canada, 335
Fraser, I. D., and Raper, Dr. A. B. : Solubility of Denatured Haemoglobin Variants in Acid Buffers, 355
Frazer, Dr. J. F. D. : At the Annual Congress of the South-Eastern Union of Scientific Societies (Problems of Managing Nature Reserves), 133
Free Fatty Acids : Stimulation of Lipogenesis in Thyroid Tissue *in vitro* by (Prof. Norbert Freinkel, Dr. Augusto D. Litonjua and Edward C. Saef), 804
Free Radicals : Isoelectronic Principle, Thermochemistry and Reactivity of related (Dr. Peter Gray and J. C. J. Thynne), 1357
  Spatial Distribution of, as studied by Electron Spin Resonance (Robert C. Smith and S. J. Wyard), 897
Free-choice Experiments : Comparison between Water and Alcohol Consumption in Six Animal Species in (Dr. Alpo Arvola and Dr. Olof Forsander), 819
Free and Bound Forms of Vitamin B_{12} : Gel Filtration of Sea-Water—Separation of (Dr. K. W. Daisley), 868
Freeze-dried Anti-haemophilic Globulin : Stability of (F. Nour-Eldin), 503
Freeze-drying of Foodstuffs (Borough Polytechnic, London, Symposium on), 651
Freezing (Intracellular), and Frost Resistance in Egg-Cells of the Sea Urchin (E. Asahina)—Intracellular Freezing as a Cause of Frost-killing in relatively Rapidly Frozen Cell Suspensions, 1263 ; A Mechanism to Prevent the Seeding of Intracellular Ice in Freezing Living Cells, 1264
Freinkel, Prof. Norbert, and others : Stimulation of Lipogenesis in Thyroid Tissue *in vitro* by Free Fatty Acids, 804
Fremlin, Dr. J. H. : The Dust Cloud about the Earth, 31
Frenkel, Ayala, and Hirsch, Dr. Willy : Spontaneous Development of *L* Forms of Streptococci requiring Secretions of other Bacteria or Sulphydryl Compounds for Normal Growth, 728
Frequencies (N–H Stretching), of Primary Amides : A New Relation between the (Prof. P. G. Puranik and K. Venkata Ramiah), 796
Frequency : Damping without Influence on (A. Bloch), 1086
Frequency of the 'Alpha' Rhythm, Reaction Time and Age (Dr. Walter W. Surwillo), 823
Frequency of Meteorite Falls throughout the Ages (Prof. Herbert Dingle), 482 ; (Prof. Hans Pettersson), 482
Frequency (Discrete) Solar Noise Bursts : Very-Low-Frequency Modulation of (Dr. Jules Aarons, Santimay Basu, William Kidd and Richard Allen), 56
Frequency (Very-Low-) Modulation of Discrete Frequency Solar Noise Bursts (Dr. Jules Aarons, Santimay Basu, William Kidd and Richard Allen), 56
Fresh *Cannabis sativa* : Essential Oil from, and its Use in Identification (L. Martin, Dr. D. Morison Smith and C. G. Farmilo), 774
Freshwater Eutrophic Lakes of India : Hydrogen Ion Concentration, Plankton and Fish in (Dr. S. M. Das), 511
Freshwater Fisheries (Salmon and), Committee on : Report of the, 962
Freshwater Fishing (Salmon and), in Great Britain (Dr. J. W. Jones), 962
Friction : Effective Contact Stresses and (Dr. D. H. Trollope), 376
Friedell, G. H., and others : Effect of Alpha-Amylase on the Ehrlich Mouse Ascites Tumour, 1113
Friedrich, W. : Vitamin B_{12} and Intrinsic Factor (Specific Effects of Vitamin B_{12} Deficiency), 1155
Friend, J. (Redfearn, Dr. E. R., and) : Oxidation–Reduction Reactions of Plastoquinone in Isolated Chloroplasts, 806
Friend, M. T. (Birch, H. F., and) : Resistance of Humus to Decomposition, 731
Friends of the Lake District : Newsletter for June 1961, 855
Frings, Hubert, and Frings, Mable : Sound Production and Sound Reception by Insects, *review* by Prof. V. B. Wigglesworth, 428
Frings, Mable, and Frings, Hubert : Sound Production and Sound Reception by Insects, *review* by Prof. V. B. Wigglesworth, 428
Frith, Dr. Ronald : Terrestrial Accretion from the Solar Wind, 1182
Frog Muscles at Low Temperature : Inhibition by Potassium of the Mechanical Responses of (H. Lorković), 1011
Frontali, Dr. N. : Activity of Glutamic Acid Decarboxylase in Insect Nerve Tissue, 178
Frost Resistance : Intracellular Freezing and, in Egg-Cells of the Sea Urchin (E. Asahina)—Intracellular Freezing as a Cause of Frost-killing in relatively Rapidly Frozen Cell Suspensions, 1263 ; A Mechanism to Prevent the Seeding of Intracellular Ice in Freezing Living Cells, 1264
Fructose and Lactic Acid Metabolism in the Perfused Liver of Premature Lambs (W. H. H. Andrews, H. G. Britton and Dr. D. A. Nixon), 1307
Fruit (An Early Cretaceous (Hanterivian) Angiosperm), from California (M. E. J. Chandler and D. I. Axelrod), 442
Fruit (Larger), in the Raspberry : Mutation for (D. L. Jennings), 302
Fruits (Developing) : Stimulants of Cell Division in (D. S. Letham and E. G. Bollard), 1119
Fry, R. J. M., and others : Estimation of Time of Generation of Living Cells, 290
Fuchs, Prof. Walter : Elected a Member of the Agriculture Section of the Leopoldina German Academy of Sciences, 122
Fucus vesiculosus (Seaweed), (Gibberellin-like Substances in Plants), (Margaret Radley), 685
Fuel, Institute of : Election of Dr. James Burns as president, 23
  Election of Sir Harold Hartley to honorary membership, 23
Fujimoto, Kentaroh, and others : Isolation of Nigerose and Kojibiose from Dextrans, 278
Fujino, Dr. Masahiro, and others : Controlling Mechanism of the Mechanical Activity of Muscle and that of Blood Coagulation, 80
Fulton, J. D. : Immunity of Protozoa (Toxoplasmosis), 552
Fumagillin : Induced Increase in Resistance of *Entamoeba histolytica* to (Dr. Mitsuru Nakamura), 413
Fumaric Acid : Enzymic Binding of Hydroxylamine by (Prof. Nathan Grossowicz and the late Yehudith Lichtenstein), 412
Fumifugium, or the Smoake of London Dissipated (John Evelyn), reprint (National Society for Clean Air), 752
Funaki, Dr. S. : Spontaneous Spike-Discharges of Vascular Smooth Muscle, 1102
Function of Muscle : The Structure and (edited by Dr. G. H. Bourne), Vol. 2, Biochemistry and Physiology ; Vol. 3, Pharmacology and Disease, *review* by Dr. D. H. Sproull, 948
Function of Taste Fibres in the Carp (Jihei Konishi and Prof. Yngve Zotterman), 286
Function (A Hydrostatic), of the Accessory Respiratory Organs in Air-breathing Fishes (Dr. S. Z. Qasim and R. Hasan), 396
Function (Psychological) : Cerebral Dominance and its relation to (Prof. O. L. Zangwill), *review* by Sir Russell Brain, 840
Function (Thyroid), in Active, Sleeping and Torpid Hummingbirds (Prof. C. J. Shellabarger, R. C. Lasiewski and G. E. Hyncik), 1318
Function (Transfer), of a Biological Photoreceptor (Dr. Lawrence Stark and Dr. Howard T. Hermann), 1173
Functions of the Mammalian Carotid Rete Mirabile (Dr. C. H. Barnett and C. D. Marsden), 88
Functions (Visual Gnostic), and Emotional Behaviour : Changes in, following Unilateral Temporal Pole Damage in the 'Split-Brain' Monkey (Dr. J. L. deC. Downer), 50
Fungi : Dictionary of the (Ainsworth and Bisby's), fifth edition by Dr. G. C. Ainsworth, *review* by Prof. John Colhoun, 109
Fungi Pathogenic to Man : Use of Desertomycin Medium in Routine Selective Isolation of (J. Uri and Zs. Herpay), 1015
Fungi (Nematode-trapping), in the Soil : Agar Disk Method for the Direct Observation of (Roderic C. Cooke), 1411
Fungicides (Copper) : Significance of the Anti-Sporulation Effect of, in the Control of Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476
Fungitoxic Compounds : Transformation in Plants of Sodium Dimethyldithiocarbamate into other (H. M. Dekhuijzen), 198
Furneaux, W. D. : The Chosen Few—an Examination of some Aspects of University Selection in Britain, *review* by Dr. W. H. King, 947
Further Education : New Routes to—Papers presented at the B.A.C.I.E. Spring Conference, London, 1961 (British Association for Commercial and Industrial Education), 739
Furunculosis in Lough Neagh Pollan *Coregonus pollan*, Thompson (K. U. Vickers and R. McClean), 930
Fusion of Coccid Chromosomes : Fracture and (Prof. W. Spencer Brown), 1419
Future of Britain : Science and the (Labour Party), 837
Future of Nuclear Physics in Australia (Prof. E. W. Titterton), 639
Füzi, Dr. M., and Csóka, R. : Leptospirosis of White Laboratory Rats, 1123
Fyfe, Prof. W. S. (Clark, R. H., and) : Ultrabasic Liquids, 158

GABB, J. E. (Ernsting, J., and) : Design and Use of Respirators (Communication between Persons wearing Breathing Equipment), 655
Gabbe, E. E. : Vitamin B_{12} and Intrinsic Factor (Freedom of B_{12} Coenzyme from Cyanide), 1154
Gabor, Prof. D. : The Electron Microscope, *review*, 316
Gaddis, A. M., and others : Girard *T* Reagent for Carbonyls, 1391
Gaddum, Dr. J. H. : Remington's Practice of Pharmacy, *review*, 8
Gadea, E. : Nematology (Free-living Nematodes of Freshwater and Moss Biotopes in Spain), 1048
Gaetani, Sancia, and Spodani, M. Antonietta : Effect of 5-Fluorouracil on Hepatic Induced Increase of Tryptophan-Pyrrolase, 1296
Gaillard, Blanche D. E. : Separation of Linear from Branched Polysaccharides by Precipitation as Iodine Complexes, 1295
Gair, R., *et al.* : Nematology (Differences between Varieties of Spring Barley in their effect on Rate of Increase of *Heterodera avenae*), 1048
Galactic Radio Emission : Spectrum of (Dr. F. Graham Smith), 1381

Galactolipids : Lipids of the Common Pea (*Pisum sativum* L.) with special reference to the Occurrence of (S. Adhikari, Dr. F. B. Shorland and R. O. Weenink), 1301
L-Galactono-γ-lactone : Conversion of, into L-Ascorbic Acid by Plants (Dr. G. A. D. Jackson, R. B. Wood and M. V. Prosser), 282
Galileo Galilei, and others : The Controversy on the Comets of 1618 (translated by Stillman Drake and Dr. C. D. O'Malley), *review* by Colin A. Ronan, 314
Gallagher, C. H., and others : Electron Transport System and Phosphoproteins of Mitochondria, 1309
Galleries : Museums and (Dr. F. S. Wallis), 862
Galleries (Museums and), Standing Committee on : Sixth Report (1959-60), 862
Gallium : Thermoelectric Power of Single Crystals of (P. Horner), 58
Galloway, Dr. Ian A. : *Obituary* of Prof. Camille Guérin, 851
Galpin, Dr. J. V., and others : Bacteria of the Intestine (Studies on the Bacterial Flora in the Intestinal Tract of Pigs, 2, Streptococci : Selective Enumeration and Differentiation of the Dominant Groups), 1152
Galton : From, to Crowther (Prof. George Seth), 989
γ-Amino-*n*-butyric Acids (Glutamic and) : Analogues of, having Potent Actions on Mammalian Neurones (Dr. J. C. Watkins and Dr. D. R. Curtis), 1010
γ-Globulin (Mouse) : An Iso-Antigen (*γBA*) of, present in Inbred Strains (A. Kelus and J. K. Moor-Jankowski), 1405
γ-Glutamyl Transpeptidase : Histochemical Demonstration of (Prof. Z. Albert, Dr. Marian Orlowski and Dr. A. Szewczuk), 767
γ-Irradiated Lead Silicate Glass : Absorption Spectra of (R. S. Barker, T. K. Harrison, D. A. Richardson and R. Rimmer), 374
γ-Ray Counting Method : Measurement of the Thorium Content of Natural Materials by a (Prof. J. H. J. Poole and F. N. Byrne), 62
Ganglia (Cockroach) : An Acid Mucopolysaccharide in (Doreen E. Ashhurst), 1224
Gangolly, S. R., and Pandalai, K. M. : Possibilities of Vegetative Propagation in the Coconut (*Cocos nucifera* L.), 1218
Garattini, Prof. S., and others : Effect of Serotonin on Absorption and Distribution of Calcium-45 in the Rat, 185
Garay, A. S. (Sági, F., and) : Effect of Gibberellic Acid on Growth and Indoleacetic Acid Oxidase Activity of *Lupinus albus* L. exposed to Different Photoperiods, 824
García, Esperanza, and others : New *Rh* Phenotype, *Dcce*^{t}*elf*, found in a Chibcha Indian Tribe, 503
García García, M. D., and others : Increase of the Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at Early Stages of Propylthiouracil Treatment in the Rat, 1171
Gardiner, Dr. J. E., and Thompson, Dr. J. W. : Lack of Evidence for a Cholinergic Mechanism in Sympathetic Transmission, 86
Gardner, Prof. Eldon J. : Principles of Genetics, *review* by Dr. Kenneth S. Dodds, 636
Gargoylism : Inclusions of Mucopolysaccharide in the Lymphocytes of Patients with (Ursula Mittwoch), 1315
Garn, S. M., and others : Taste Thresholds and Food Dislikes, 1328
Garnham, Prof. P. C. C. : Immunity to Protozoa, 551
  Techniques in Tropical Pathology, *review*, 1236
Garratt, —. : Hazards of the Animal House (Hazards associated with the Use of Radioactive Substances in Animal Experiments), 339
Garrett, W. G. (Walker, G. F., and) : Complexes of Vermiculite with Amino-Acids, 1389
Garrison, Dr. W. M. : Effects of Ionizing Radiation on Organic and Biological Solids (Chemical Changes on Irradiation), 1151
Garrod, D. J. (Elder, H. Y., and) : A Natural Hybrid of *Tilapia nigra* and *Tilapia leucosticta* from Lake Naivasha, Kenya Colony, 722
Garrod, Prof. L. P. : Title of professor emeritus of bacteriology (at St. Bartholomew's Hospital Medical College) conferred on, 546
Garvan, Antony N. B., and Welsh, Peter C.: The Victorian American—Lithographs from the Harry T. Peters 'American on Stone' Collection (Smithsonian Institution, Publication 4466), 226
Garvey, Dr. Justine S. : Separation and *in vitro* Culture of Cells from Liver Tissue, 972
Gas : Ignition of, by Sparks produced by Breaking Wires at High Speed (Dr. R. L. Gordon, Dr. H. Lord and D. W. Widgington), 1085
Gas from Coal in Britain : Production of, 225
Gas (Nitrogen) : Distribution of Isotopic Nitrogen in, during Denitrification (R. D. Hauck and D. R. Bouldin), 871
Gas Chromatography : Detection by Ionization of Atmospheric Gases during Analysis by (Dr. Arthur Karmen, Laura Giuffrida and Robert L. Bowman), 906
  Determination of Hydrogen in Gaseous Mixtures by (P. J. Kipping), 270
  Integration with Ionization Detectors in (P. M. E. M. van der Grinten and Dr. A. Dijkstra), 1195
  Role of Pressure Gradients in obtaining Minimum Time in (Prof. J. Calvin Giggings), 1291
Gas Chromatography Abstracts, 1958 (edited by C. E. H. Knapman, assisted by C. G. Scott), *review* by A. Goldup, 1135
Gas Chromatography Abstracts, 1959 (edited by C. E. H. Knapman, assisted by C. G. Scott), *review* by A. Goldup, 1135
Gas Discharge Devices : Physics of (Electronics Group of the Institute of Physics and the Physical Society Conference on), 546
Gas-cooled Reactor at the Windscale Factory of the United Kingdom Atomic Energy Authority : Progress on the Advanced, 853
Gas–Liquid Chromatography : Peak-Shift Technique in—Trimethylsilyl Ether Derivatives of Alcohols (Stanley H. Langer and Peter Pantages), 141
  Quantitative Estimation of Peak Areas in (Dr. K. K. Carroll), 377
Gas–Liquid Chromatography (Pyrolysis and), on the Microgram Scale (C. E. Roland Jones and A. F. Moyles), 663
Gas-Phase Reactions of the Halogens and their Compounds (Dr. J. H. Thomas and Dr. E. Whittle), 129
Gaseous Mixtures : Determination of Hydrogen in, by Gas-Chromatography (P. J. Kipping), 270
Gaseous Oxygen : Permeation of, through Vitreous Silica (Dr. Francis J. Norton), 701
Gases : Kirkendall Effect in (Prof. E. A. Mason, L. Miller and P. C. Carman), 375
Gases (Atmospheric) : Detection by Ionization of, during Analysis by Gas Chromatography (Dr. Arthur Karmen, Laura Giuffrida and Robert L. Bowman), 906
Gasparic, Ing. : Paper Chromatography, 760
Gasparski, Dr. J. : Blood Groups in Infra-human Species (Red Cell Antigens of the European Bison identified by Cattle Blood Typing Reagents), 655
Gass, Dr. I. G. : Appointed lecturer in the Department of Geology in the University of Leeds, 650
Gass, Saul I. : Linear Programming, *review* by Dr. M. V. Wilkes, 9
Gasterosteus aculeatus L. (Stickleback) : Significance of the Regeneration of Lateral Plates of the (Tadéusz Penczak), 621
Gastro-intestinal Tract of Rats under Normal Feeding Conditions : Soluble Material in the (Dr. Anne S. Cole), 502
Gates, Dr. David M. : Appointed editor of Section D, Radio Propagation, of the *Journal of Research* of the United States National Bureau of Standards, 857
Gates, G. E. : Taxonomy of the Earthworm in India, 227
Gayed, S. K. : Production of Symptoms of Barley Leaf-Spot Disease by Culture Filtrate of *Helminthosporium sativum*, 725
Gazey, B. K. : Appointed Research Fellow in the Department of Electrical Engineering, University of Birmingham, 337
Gazzard, D., and others : Action of Carbon Dioxide on 6-Aminopenicillanic Acid, 910
Gear, J. R., and Spenser, Prof. I. D. : Biosynthesis of Hydrastine, 1393
Gebbie, H. A., and others : Molecular Emission Spectroscopy from 2μ to 12μ by a Michelson Interferometer, 264
Gee, Prof. G. : Inorganic Polymers, 1046
Gee, Sandra C. (Pangborn, Rose Marie, and) : Relative Sweetness of α- and β-Forms of Selected Sugars, 810
Geill, Torben, and others : Serum Lipids in relation to the Intake of Alcohol and Sodium Chloride, 1095
'Gel Filtration' of Organic Compounds (B. Cortis-Jones), 272
Gel Filtration of Sea-Water—Separation of Free and Bound Forms of Vitamin B_{12} (Dr. K. W. Daisley), 868
Gel (Starch-) : Electrophoresis in a Thin Layer of, followed by a Plastifying Treatment (J. Groulade, Dr. J. M. Fine and C. Ollivier), 72
Gel (Starch-) Electrophoresis : Comparison of the Serum Protein Fractions of the Newly Hatched Chick with those of Adult Birds using (A. Amin), 708
Gelatin Gels : Rigidity Factor of (Dr. Audrey Todd), 567
Gelatine and Glue Research Association (British) : Twenty-first Meeting of the Research Panel (Dr. D. A. Sutton), 857
Gelfand, Dr. M., and Carr. W. R. : Diabetes Mellitus in Africans in Rhodesia, 227
Gels (Co-precipitated) : Tetragonal Mullite-like Phase from (J. Ossaka), 1000
Gels (Gelatin) : Rigidity Factor of (Dr. Audrey Todd), 567
Genera of Polypodiaceae in Eastern India : Cytology of some (G. Panigrahi and S. N. Patnaik), 1207
General Medical Sciences : Highlights of Research Progress in, 1960 (United States Department of Health, Education and Welfare, Public Health Service Publication No. 815), 658
General Register Office (Ministry of Health and) : Report on Hospital In-Patient Enquiry for the Year 1959, Part I, Preliminary Tables, 345
General Relativity : A New Approach to, *review* by Dr. G. J. Whitrow, 424
Generating Liver : Influence of Plasma Proteins in the Control of Mitosis-rates in (Dr. J. T. Hemingway), 706
Generation of Living Cells : Estimation of Time of (R. J. M. Fry, S. Lesher and H. I. Kohn), 290
Generation of Radicals in Pairs and the Kinetics of Radical Chain Reactions in Solution (Dr. P. E. M. Allen and C. R. Patrick), 1194
Genetic Control of Protein Structure : Haemoglobin F and the (Prof. V. M. Ingram), 608 ; (Dr. Wolf W. Zuelzer and Abner R. Robinson), 609
Genetic Fine Structure of the *ad*$_{7}$ Locus of *Schizosaccharomyces pombe* : Distribution of X-Ray- and Nitrous Acid-induced Mutations in the (Dr. Herbert Gutz), 1127
Genetic Investigations (Metabolic and), of Nutritionally Exacting Strains of *Staphylococcus pyogenes* (Janice B. Edgar and Dr. B. A. D. Stocker), 1121
Genetic Research (Prof. Arne Müntzing), *review* by Dr. Kenneth S. Dodds, 636
Genetica (Prof. Enrique Sanchez-Monge y Parellada), *review* by Prof. S. C. Harland, 213
Genetics : Chemical, *review* by Dr. Charlotte Auerbach, 841
  Principles of (Prof. Eldon J. Gardner), *review* by Dr. Kenneth S. Dodds, 636
  Reviews of, *review* by Dr. Kenneth S. Dodds, 636
Genetics of a Difference in the Male Genitalia of East and West African Stocks of *Papilio dardanus* (Lep.), (J. R. G. Turner, C. A. Clarke and P. M. Sheppard), 935
Genetics (Bacterial) : Papers on (selected by Edward A. Adelberg), *review* by L. B. Quesnel, 320
Genetics (Biometrical) : Discriminant Analysis in (Dr. S. K. Jain), 1420
Genetics (Chemical) : An Outline of (Prof. Bernard S. Strauss), *review* by Dr. Charlotte Auerbach, 841
Genetics (Human) : Principles of (Curt Stern), second edition, *review* by Dr. G. Ainsworth Harrison, 110
Genitalia (Male), of East and West African Stocks of *Papilio dardanus* (Lep.) : Genetics of a Difference in the (J. R. G. Turner, C. A. Clarke and P. M. Sheppard), 935
Geochemical Society : Proposal to form a European Section of the Organic Geochemistry Group of the, 336
Geographical Society (Royal) : Grant of £200 for three years from the Gulbenkian Foundation, in aid of the expeditions, 754
Geography : Technology and (Prof. S. H. Beaver), 986
Geological Dictionary : English–Russian (T. A. Sofiano), (edited by I. D. Borneman, *et al*.), *review* by Dr. S. I. Tomkeieff, 9
  Russian–English (T. A. Sofiano), (edited by A. P. Lebedev and V. E. Khain), *review* by Dr. S. I. Tomkeieff, 9
Geological Observatory (Lamont) : Fellowships for Plankton Research, 754
Geological Society of America : Special Paper 65, The Floors of the Oceans, I, The North Atlantic—Text to accompany the Physiographic Diagram of the North Atlantic (Bruce C. Heezen, Marie Tharp and Maurice Ewing), *review* by Dr. G. E. R. Deacon, 531
Geological Society of India : *Journal* of the, Vol. I, 1348
Geological Studies, Centre for (Lisbon) : Grant from the Gulbenkian Foundation, 754

Geological Survey of Great Britain—England and Wales : Memoir on the Geology of the Country around Monmouth and Chepstow (Dr. F. B. A. Welch and Dr. F. M. Trotter), 227
Geology of the Country around Monmouth and Chepstow (Memoirs of the Geological Survey of Great Britain—England and Wales), (Dr. F. B. A. Welch and Dr. F. M. Trotter), 227
Geomagnetic Disturbances in the Auroral Zone : Electric Currents associated with (Dr. F. J. Scrase), 963
Geometrical Illusions : Centrally Produced (S. Papert), 733
Geometrical Models for Complementation Mapping of Allelic Series : Limitations of (Prof. Elof Axel Carlson), 788
Geometry (Cusp) : Experiments on the Compression of Plasma in (Dr. T. K. Allen and Dr. R. J. Bickerton), 794
Geometry (Molecular), of a 1:1 Crystalline Complex between 1,3,7,9-Tetramethyluric Acid and Pyrene (F. De Santis, E. Giglio, Dr. A. M. Liquori and A. Ripamonti), 900
Georgi, Jay (Comar, Dr. C. L., and) : Assessment of Chronic Exposure to Radio-strontium by Urinary Assay, 390
Geotropism in Plant Roots : Dependence on Light of (J. V. Lake and G. Slack), 300
Gerald, P. S., and others : Haptoglobin Patterns in Cord Blood Serums, 717
Germ Cells (Immature), of *Drosophila* : Chromosome Rearrangements induced by X-Rays in (Seymour Abrahamson), 523
German Family : Thalassaemia in an English Family and in a (Dr. F. Vella and S. A. Ibrahim), 822
Germination : Effect of Excess Supply of Heavy Metals to Barley during, with special reference to Catalase and Peroxidase (Dr. S. C. Agarwala, A. Kumar and C. P. Sharma), 726
Germination Process in Seeds of *Pinus sylvestris* L. : Effect of Red and Far-red Irradiation on the (Bengt Nyman), 1219
Gerrard, W. : Inorganic Polymers (BN Polymers), 1047
Gerritsen, F. (Brunn, P., and) : Stability of Coastal Inlets, *review* by W. J. Reid, 426
Gey, G. O. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (A Technique for Studying Population Dynamics of Lymphoblasts in Culture), 341
Gharpurey, M. K., and others : Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers, 378
Ghosts (Erythrocyte), of Mediated Sugar Transport : Persistence in (Paul G. LeFevre), 970
Giant Cell Axons in *Aplysia depilans* : The Path of the (Dr. G. M. Hughes and L. Tauc), 404
Giant Cells (Periodic Acid–Schiff Positive), in the Mouse Thymus Cortex (D. Metcalf and M. Ishidate), 305
Giant Nerve Cell under Abnormal Conditions : Electrical Activity of a (Dr. Yutaka Oomura, Sachio Ozaki and Takashi Maéno), 1265
Gibberellic Acid : Effect of, on Growth and Indoleacetic Acid Oxidase Activity of *Lupinus albus* L. exposed to Different Photoperiods (F. Sági and A. S. Garay), 824
  Effect of Spraying with, on Yield and Composition (Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid), (R. Stevens, J. B. Roberts and I. H. Williams), 360
  Treatment of Hops (*Humulus lupulus* L.) with—Effect of Spraying with Gibberellic Acid on Yield and Composition (R. Stevens, J. B. Roberts and I. H. Williams), 360 ; Effect on Growth and Development (I. H. Williams), 361
Gibberellic Acid and Allogibberic Acid : Effects of, on Flowering and Internode-Length in *Pisum sativum* (I. C. Murfet and H. N. Barber), 514
Gibberellin-like Substances in Plants (Margaret Radley)—Leguminous Root Nodules, 684 ; Seaweed (*Fucus vesiculosus*), 685
Gibson, A. H. : Root Temperature and Symbiotic Nitrogen Fixation, 1080
Giddings, Prof. J. Calvin : Role of Pressure Gradients in obtaining Minimum Time in Gas Chromatography, 1291
Giebisch, Dr. Gerhard (Windhager, Dr. Erich E., and) : Comparison of Short-Circuit Current and Net Water Movement in Single Perfused Proximal Tubules of Rat Kidneys, 1205
Gigg, Dr. Roy, and Carroll, Patricia M. : A Convenient Synthesis of Muramic Acid and other 3-O-Ethers of D-Glucosamine, 495
Giglio, E., and others : Molecular Geometry of a 1:1 Crystalline Complex between 1,3,7,9-Tetramethyluric Acid and Pyrene, 900
Gilchrist, R. C. J. H. (Wight, W., and) : The Concept of Kind of Tea, 14
Gill, Prof. W. D. : Appointed to the chair of oil technology at the Imperial College of Science and Technology, London, 651
Gillman, J. (Dijkstra, J., and) : Chromatographic Separation of Biologically Active Components from Commercial Trypan Blue, 803
Gilmour, Dr. D. G. : Blood Groups of Animals other than Man, 653
  Blood Groups in Infra-human Species (Blood Groups of Chickens), 654
Ginsburg, Michael, and others : Incorporation of Histamine into Liver Protein *in vivo*, 1204
Girard *T* Reagent for Carbonyls (A. M. Gaddis, Rex Ellis and G. T. Currie), 1391
Gitlin, Prof. David, and others : Immuno-electrophoretic Examination of Agammaglobulinaemia, 1109
Giuffrida, Laura, and others : Detection by Ionization of Atmospheric Gases during Analysis by Gas Chromatography, 906
Giustina, G., and others : Intermediate Pool between Amino-Acids and Globin in Duck Blood *in vitro*, 40
Glacial Acetic Acid : Use of, for Killing Parasitic Nematodes for Collection Purposes (Bjørn Berland), 1320
Gland (Mammary), of the Cow and Rat : Leucine Aminopeptidase in the (Prof. S. Talanti and Prof. Väinö K. Hopsu), 86
Gland (Mammary), of the Ewe : Removal of Milk Constituents from the (A. K. Lascelles), 1404
Gland (Salivary), of Mice Treated with *Iso*propylnoradrenaline : Enlargement of (Dr. K. Brown-Grant), 1076
Glands (Rectal), in Woodlice (Dr. Minati Gupta), 406
Glass, G. B. J. : Vitamin B$_{12}$ and Intrinsic Factor (Hydroxocobalamin in Body Tissues), 1155
Glass in the Electronics Industry, 1143
Glass (γ-Irradiated Lead Silicate) : Absorption Spectra of (R. S. Barker, T. K. Harrison, D. A. Richardson and R. Rimmer), 374
Glasshouse Crops Research Institute : Appointment of Dr. F. T. Last as head of the Department of Mycology and Bacteriology, 1250
Globin in Duck Blood *in vitro* : Intermediate Pool between Amino-Acids and (L. Bianco, G. Giustina and E. Lazzarini), 40
Globulin (Anti-haemophilic) : Stability of Freeze-dried (F. Nour-Eldin), 503
Globulin (Coating), causing a Positive Antiglobulin Consumption Test : Nature of the (D. Nelken), 1110
γ-Globulin (Mouse) : An Iso-Antigen (*γBA*) of, present in Inbred Strains (A. Kelus and J. K. Moor-Jankowski), 1405
Globulins (Myeloma) : Comparison of Reduced Alkylated Derivatives of some, and Bence-Jones Proteins (Dr. M. D. Poulik and Dr. G. M. Edelman), 1274
Glossary of Petroleum Terms (edited by George Sell), third edition (Institute of Petroleum), 22
Glossina mortisans : Cyclical Infection of, with Culture Forms of *Trypanosoma rhodesiense* (the late Prof. R. M. Gordon and J. K. Miller), 1317
Glover, J. (Gwynne, M. D., and) : Light Rainfall and Plant Survival—Measurement of Stem Flow Run-off, 1321
Glow Lamp (Neon), 1911-61 : The Development of the (Prof. H. E. Watson), 1040
Gluckman, Prof. M. : African Jurisprudence (Presidential Address to Section N (Sociology) of the British Association for the Advancement of Science), 991
D-Glucosaccharo-1,4-Lactone : Inhibition of β-Glucuronidase by Oral Administration of (S. Akamatsu, Dr. A. Kiyomoto, S. Harigaya and S. Ohshima), 1298
D-Glucosamine : A Convenient Synthesis of Muramic Acid and other 3-O-Ethers of (Dr. Roy Gigg and Patricia M. Carroll), 495
D-Glucosamine Hydrochloride Isomers (Mannosyl)—*N*-Mannosyl D-Glucosamine Hydrochloride (Dr. S. A. Barker, Dr. K. Murray and Prof. M. Stacey), 142 ; 6-*O*-Mannosyl D-Glucosamine Hydrochloride (Dr. S. A. Barker, Dr. K. Murray, Prof. M. Stacey and D. B. E. Stroud), 143
Glucose : Effect of Thyroxine and 2,4-Dinitrophenol on the Rate of Utilization of (Dr. L. Macho), 604
D-Glucose : Identity of L-Ascorbic Acid formed from, by the Strawberry (*Fragaria*), (Dr. Frank A. Loewus and Stanley Kelly), 1059
Glucose in Serum : Simultaneous Determination of Dextran and (A. Rédei and S. Nagy), 173
Glucose Transfer across the Membrane of the Red Blood Cell : Dimer Formation and (Dr. W. D. Stein), 1277
Glucose (Blood), and Liver Glycogen in Rabbits : Effect of Bee Venom on Concentration of (Prof. A. H. Mohamed and K. Zaki), 605
Glucose (1-(Indole-3-acetyl)-β-D), a New Compound in the Metabolism of Indole-3-acetic Acid in Plants (Dr. M. H. Zenk), 493
Glucose-6-phosphatase Activity in Liver and Kidney at Birth : Changes in (M. J. R. Dawkins), 72
β-Glucuronidase : Inhibition of, by Oral Administration of D-Glucosaccharo-1,4-Lactone (S. Akamatsu, Dr. A. Kiyomoto, S. Harigaya and S. Ohshima), 1298
Glueckauf, Dr. E., and Watts, R. E. : Non-Uniformity of Cross-linking in Ion-Exchange Polymers, 904
Glutamic Acid Decarboxylase in Insect Nerve Tissue : Activity of (Dr. N. Frontali), 178
Glutamic and γ-Amino-*n*-butyric Acids having Potent Actions on Mammalian Neurones : Analogues of (Dr. J. C. Watkins and Dr. D. R. Curtis), 1010
Glutamic-Oxalacetic Transaminase (Rat Liver) : Intracellular Distribution Pattern of (Dr. Herbert J. Eichel and Josef Bukovsky), 243
γ-Glutamyl Transpeptidase : Histochemical Demonstration of (Prof. Z. Albert, Dr. Marian Orlowski and Dr. A. Szewczuk), 767
Glutathione Instability of the Erythrocytes among Armenians in Iran : Virtual Absence of (Dr. J. E. Bowman and Dr. D. G. Walker), 221
Glyceraldehyde Phosphate Dehydrogenase (Triphosphopyridine Nucleotide-linked), in a Non-Photosynthetic Plant Tissue (C. R. Benedict and Dr. H. Beevers), 71
Glycerol : Dimer Formation and the Transfer of, across the Erythrocyte Membrane (Dr. W. D. Stein), 352
α-Glycerophosphate Dehydrogenase (Mitochondrial), in the Nervous System : Histochemical and Homogenization Studies of (R. Hess and Dr. A. G. E. Pearse), 718
α-Glycerophosphate Dehydrogenase Activity (Mitochondrial), in Skeletal Muscle : Direct Relationship of Phosphorylase and (Dr. A. G. E. Pearse), 504
[2-^{14}C]-Glycine : Breakdown of, during Paper Chromatography (A. K. Huggins and V. Moses), 668
Glycogen (Liver) : Effect of Bee Venom on Concentration of Blood Glucose and, in Rabbits (Prof. A. H. Mohamed and K. Zaki), 605
Glycol Monoalkyl Ethers : Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and (A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas), 378
Glycol Monomethyl Ether (Ethylene), a New Fixative for Histological Work (Prof. Walter B. Shelley and Ralph Florence), 719
Glycolysis and Respiration of Tumours (Dr. Alan C. Aisenberg), *review* by Prof. F. Dickens, 1335
Glycosidases (Intracellular), of Dextran-producing Bacteria (Dr. R. W. Bailey and Prof. E. J. Bourne), 277
Gm^{a} (Serum Factor), among the Iranians (Luba Podliachouk, Dr. A. Eyquem, R. Choaripour and M. Eftekhari), 717
Gnostic Functions (Visual), and Emotional Behaviour : Changes in, following Unilateral Temporal Pole Damage in the 'Split-Brain' Monkey (Dr. J. L. deC. Downer), 50
Goals for Science in the United States, 312
Goddard, Prof. L. S. : Modern Style in Mathematical Analysis, *review*, 6
  Operations Research and Systems Engineering, *review*, 105
  Mathematics and Queueing, *review*, 211
  Combinatorial Analysis, *review*, 321
Goffart, H. : Nematology (Effect of *Ditylenchus dipsaci* in Sugar Beet), 1048
Gogte, V. N., and others : Anticancer Properties of Thio-diglycollic Acid, 388
Gold, Eleanor, and others : Effect of Administration of Lysergic Acid Diethylamide on Serotonin-Levels in the Body, 499
Gold Medal and Prize of the Beilby Memorial Fund awarded to Dr. Constantin Edeleanu and to Prof. Jack Nutting, 224
Goldberg, Prof. Edward D., and others: Iron-55 from Nuclear Detonations, 162
Goldfeder, A. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Electron Microscopic and Biochemical Studies of Tumour Mitochondria), 341
Goldie, L., and Green, J. M. : Spike and Wave Discharges and Alterations of Conscious Awareness, 200

Goldman, M., and others : Failure of a Dog to Discriminate between Strontium-90 and Calcium given Orally, 713
Goldsmith, P., and Brown, F. : World-Wide Circulation of Air within the Stratosphere, 1033
Goldstein, D. J. : Mechanism of Differential Staining of Nucleic Acids, 407
Goldup, A. : Gas Chromatography Abstracts, 1958, *review*, 1135
  Gas Chromatography Abstracts, 1959, *review*, 1135
Goma, L. K. H. : Maxillary Index in *Anopheles gambiae* Giles, 405
Gonzalez, R. N. (Yannas, J. B., and) : A Clear Instance of Rheopectic Flow, 1384
Gooberman, Dr. G. : Ultrasonic Absorption of Polymer Solutions, 693
Good, Dr. N. E. : Photophosphorylation and the Hill Reaction, 678
Goodfield, June (Toulmin, Dr. Stephen, and) : The Fabric of the Heavens, *review* by Dr. M. A. Hoskin, 840
Goodman, Dr. J. F. : Appointed head of the Basic Research Department of Thomas Hedley and Co., Newcastle-upon-Tyne, 229
Goodman, Dr. J. W.: Blood Groups in Infra-human Species (Experiments in which Blood or Bone Marrow, or both, were Injected into Irradiated Mice), 654
Goodman, Prof. Lionel (Krishna, V. G., and) : Polarization of Triplet→Singlet Emission Spectra, 800
Goodman, Prof. Morris, and Poulik, Emily : Serum Transferrins in the Genus *Macaca*—Species Distribution of Nineteen Phenotypes, 1407
Goodman, N. S., and others : Regulation of Pyrogallol Oxidations by Alcohols and Sugars, 180
Goodwin, Prof. T. W., and Horton, Dr. A. A. : Biosynthesis of Riboflavin in Cell-free Systems, 772
Goodwin, Prof. T. W., and Passorn, Dr. P. N. A. : Purine and Pyrimidine Metabolism in *Rhodospirillum rubrum*, 276
Gordon, Dr. G. : Assessment of Pain (Electrophysiological Approach to Central Mechanisms of Somatic Sensation), 1353
Gordon, Hugh McL. : Thiabendazole, a Highly Effective Anthelmintic for Sheep, 1409
Gordon, Dr. M. : Appointed to the University of London readership in physical chemistry at the Imperial College of Science and Technology, 337
Gordon, Dr. Paul: Changes in Blood Serotonin during Exposure to Cold, 183
Gordon, Dr. R. L., and others : Ignition of Gas by Sparks produced by Breaking Wires at High Speed, 1085
Gordon, Prof. R. M. : *Obituary* by Prof. T. H. Davey, 852
Gordon, the late Prof. R. M., and Miller, J. K. : Cyclical Infection of *Glossina morsitans* with Culture Forms of *Trypanosoma rhodesiense*, 1317
Gordon, S., and others : Irradiated Lithium Fluoride as an Optical Filter in the Far Ultra-Violet, 1186
Gorer, Dr. P. A. : *Obituary* by J. R. Batchelor, 18
Goswami, Dr. A. : Epitaxial Growth of Crystals on One-Degree Orientated Substrates, 160
Gottschalk, G., and others : Formation and Utilization of Poly-β-Hydroxybutyric Acid by Knallgas Bacteria (*Hydrogenomonas*), 463
Gough, John S. : Interviewing in Twenty-six Steps, 228
Gould, S. : Flora Europaea (Problems of Abstracting and Indexing European Taxonomic Literature), 448
Goulden, Dr. J. D. S. : Quantitative Analysis of Milk and other Emulsions by Infra-Red Absorption, 905
Gove, H. E., and others : Production of Neon in Stars, 1381
Government of India, and others : To set up and operate an Aeronautical Research Laboratory and three Civil Aviation Training Centres, 1043
Government of Morocco, and others : To set up and operate an Aeronautical Research Laboratory and three Civil Aviation Training Centres, 1043
Government of North Borneo (Royal Society of London in co-operation with the) : North Borneo Expedition 1961, 26
Government of Thailand, and others : To set up and operate an Aeronautical Research Laboratory and three Civil Aviation Training Centres, 1043
Government of the United Arab Republic, and others : To set up and operate an Aeronautical Research Laboratory and three Civil Aviation Training Centres, 1043
Government Research in Great Britain : Financing, 205
Government Responsibility in Scientific Research and Development, 837
Gowe, R. S. (Taneja, G. C., and) : Effect of Varying Doses of Undiluted Semen on Fertility in the Domestic Fowl, 828
Gowland, Dr. G., and others : Elimination of Bovine Serum Albumin in Acute Experimental Uraemia, 1210
G.P. Zones (Clusters rich in Solute) : Resistivity Changes due to Formation of (H. Herman and Prof. J. B. Cohen), 63
Graczyk, Dr. Cz. : At the Second International Measurement Conference (A New Method of Measuring Rate of Flow of Coal Dust), 1351
Graddon, Dr. D. P. : An Introduction to Co-ordination Chemistry, *review* by Prof. C. A. Coulson, 1233
Gradients (Pressure) : Role of, in obtaining Minimum Time in Gas Chromatography (Prof. J. Calvin Giddings), 1291
Graduates : Employment of—a Durham University Survey, 649
Grafted Skin (Orthotopically), : Regeneration of Elements of Donor Origin in, following the Homograft Response (Dr. J. K. Dineen and Dr. Aleksander Szenberg), 153
Graham, Prof. Kenneth : Air-Swallowing, a Mechanism in Photic Reversal of the Beetle *Trypodendron*, 519
Grain : Buildings for the Drying and Storage of (Bibliography of Research Publications on Farm Buildings, Section 5), (Agricultural Research Council), 440
Grainger, Dr. John : Land-Use Survey—Nicaragua, 1259
Grammar Schools : Biology for (Science Masters Association and the Association of Women Science Teachers), 555
  Chemistry for (Science Masters Association and the Association of Women Science Teachers), 555
  Physics for (Science Masters Association and the Association of Women Science Teachers), 555
Granites in South-West England : Age of the Orogeny and (T. R. Owen), 486 ; (N. E. Butcher), 486
Grant, C. H. B. (Mackworth-Praed, C. W., and) : Birds of Eastern and North-Eastern Africa, second edition, *review* by Sir Landsborough Thomson, 9
Grant, Dr. Julius : A Laboratory Handbook of Pulp and Paper Manufacture, second edition, *review* by Dr. F. Lyth Hudson, 320
  Pulp and Paper, *review*, 1135
Grants from the Fleming Memorial Fund for Medical Research, 959
Grants for Research in Chemistry (Chemical Society), 651
Grants for Research in Marine Biology or Oceanography (Challenger Society), 24
Grants and Awards for 1962 (Lalor Foundation), 1348
Grants (American), for Nuclear Equipment in Argentina, Brazil and Israel, 442
Grants (Fellowships and), in the field of Radiology and Nuclear Medicine for 1962-63 (James Picker Foundation), 228
Grants (Research), in Radiological Health (United States Public Health Service), 442
Granules in Neurones of the Brain of the Leech : Neurosecretion and (Irvine R. Hagadorn and Richard S. Nishioka), 1013
Granules (Dry), through Apertures : Minimum Energy Theorem for Flow of (R. L. Brown), 458
Granville, W. C. : At the Maxwell Colour Centenary Conference (Colour Appearance), 11
Graphite (Faraday Society Informal Discussion on), 27
  Atomic Structure of, and Graphitization (Dr. G. E. Bacon), 27
Graphitization : Atomic Structure of Graphite and (Dr. G. E. Bacon), 27
Gräsbeck, R. : Vitamin B_{12} and Intrinsic Factor (Efforts to Purify and Isolate Intrinsic Factor), 1155 ; (Radioactive Vitamin B_{12}), 1156
Grass (Rye)-dominant Pasture grazed by Cows : Growth of, and Properties of New Zealand Butterfat (F. H. McDowall, W. A. McGillivray and J. C. Hawke), 303
Grassi, Horatio, and others : The Controversy on the Comets of 1618 (translated by Stillman Drake and Dr. C. D. O'Malley), *review* by Colin A. Ronan, 314
Gravity Survey on the Aira Caldera, Kyusyu, Japan (Izumi Yokoyama), 966
Gray, Prof. J. A. B. : Assessment of Pain (Peripheral Pain Mechanisms), 1353
Gray, Dr. L. H. : Effects of Ionizing Radiation on Organic and Biological Solids, 1151
Gray, Dr. Peter, and Thynne, J. C. J. : Isoelectronic Principle, Thermochemistry and Reactivity of related Free Radicals, 1357
Great Age for Science : A (Dr. Warren Weaver), 312
Great Britain : Aims, Organization and Methods of University Extra-Mural Libraries in (E. P. Pritchard), 649
  Conservation of Water in, 421
  Financing Government Research in, 205
  Four Decades of State Forestry in (Prof. M. L. Anderson), 656
  Salmon and Freshwater Fishing in (Dr. J. W. Jones), 962
  See also under Britain
Great Glen Fault : Continuation of the, beyond the Moray Firth (Dr. Derek Flinn), 589 ; (Dr. Robert M. Appleby), 1190
Green, A. F., and others : Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine, 1312
Green (A.T.) Book : The—Dedicated to Arnold Trevor Green by his colleagues of the British Ceramic Research Association, *review* by Prof. R. W. Douglas, 207
Green, D. H. (Miller, J. A., and) : Preliminary Age-Determinations in the Lizard Area, 159
Green, Prof. H. N., and others : A Relation between the Reactivity of Human Red Cells to Antiglobulin Tests and their Surface Structure, 923
Green, Dr. J. : A Biology of Crustacea, *review* by Dr. J. A. Allen, 111
  The Physiology of Crustacea, *review*, 428
Green, J. M. (Goldie, L., and) : Spike and Wave Discharges and Alterations of Conscious Awareness, 200
Green, R. : Appearance of the *T*-Phase, 997
Greenaway, Frank : Dalton's Irish Rival, *review*, 533
Greenbaum, Dr. A. L., and Leal, R. S. : Effect of Pituitary Growth Hormone on Cholesterogenesis, 600
Greenblatt, Dr. Robert B. (Mahesh, Dr. Virendra B., and) : Physiology and Pathogenesis of the Stein–Leventhal Syndrome—Urinary Androsterone, Etiocholanalone, Pregnanetriol and Δ^5-Pregnenetriol on Ovarian Origin in a Patient with Stein–Leventhal Syndrome, 888 ; Role of FSH in the Pathogenesis of the Stein–Leventhal Syndrome, 889
Greenland, Dr. D. J., and others : Role of Polysaccharides in Stabilization of Natural Soil Aggregates, 1283
Greenland Shark : Copepod *Ommatokoita elongata* (Grant) in the Eye of the—a Possible Cause of Mutual Dependence (Bjørn Berland), 829
Greenshields, R. N. (Cooper, R. A., and) : Sucrases in *Phaseolus vulgaris*, 601
Greenstadt, Eugene W. : Magnetic Storms in Interplanetary Space as observed by *Pioneer V*, 329
Greenstein, Dr. Jesse P., and Winitz, Dr. Milton : Chemistry of the Amino Acids, *review* by Sir Hans Krebs, 1132
Gregory, Prof. K. F., and Shyu, Wei-jen : Apparent Cytoplasmic Inheritance of Tyrosinase Competence in *Streptomyces scabies*, 465
Gregory, R. L., and others : Measuring Visual Constancy for Stationary or Moving Objects, 416
Greig, Prof. J. : The British Electrical and Allied Industries Research Association, 450
Gresham, D. C. : At the Maxwell Colour Centenary Conference (Colour Printing), 11
Grewia populifolia Extracts : Effects of, on the Development of Chick Embryos (S. K. Brahma and N. Kundu), 506
Grey Speck Syndrome of Oats : Manganese and Magnesium in the (P. B. Vose and Dr. D. J. Griffiths), 299
Griffenhagen, George B. : Drug Supplies during the American Revolution (United States National Museum, Bulletin No. 225), 761
Griffin, F., and others : Taste Thresholds and Food Dislikes, 1328
Griffin, Juliet E. (Harris, Dr. G. P., and) : Flower Initiation in the Carnation in Response to Photoperiod, 614
Griffith, Dr. G. W. : Awarded a grant from the Lady Tata Memorial Trust, for research on leukaemia and allied diseases, 228
Griffith, Prof. J. S. : The Theory of Transition-Metal Ions, *review* by Prof. C. A. Coulson, 1233
Griffith, Dr. R. L., and others : Electron Spin Resonance Absorptions induced by Light in Adsorbed Layers of Cyanine Dyes on Silver Bromide Microcrystals, 902
Griffiths, Dr. D. J. (Vose, P. B., and) : Manganese and Magnesium in the Grey Speck Syndrome of Oats, 299
Grigor, B. A. : Awarded a New Zealand fellowship in chemistry for 1961-62 by the Ramsay Memorial Trust, 337
Grime, J. Philip : Measurement of Leaf Colour, 614

Griswold, Dr. Ernest, and others : Inorganic Chemistry, *review* by Dr. M. F. Lappert, 1133
Grivsky, E. M., and others : Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine, 1312
Groen, Prof. J. J. : At the Third International Congress of Dietetics (Influence of Nutrition and Ways of Life on Blood Cholesterol and the Prevalence of Atherosclerosis and Coronary Heart Disease among Trappist and Benedictine Monks), 1343
Groote Eylandt Aborigines : Classification of Kin, Age Structure and Marriage amongst the (Dr. Frederick G. G. Rose), *review* by Dr. R. Needham, 6
Grosberg, Dr. P. : Appointed to the research chair of textile engineering in the Department of Textile Industries in the University of Leeds, 650, 1346 ; work of, 1346
Gross β-Activity in the Thames River (Conn.), (D. M. Skauen, T. Hatfield, D. Hanlon and J. S. Rankin, jun.), 1017
Grossi, E., and others : Effect of Serotonin on Absorption and Distribution of Calcium-45 in the Rat, 185
Grossowicz, Prof. Nathan, and Lichtenstein, the late Yehudith : Enzymic Binding of Hydroxylamine by Fumaric Acid, 412
Groszek, A. J. (Bell, G. H., and) : Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of High Surface Area using a Continuous Flow Calorimeter, 1184
Groulade, J., and others : Electrophoresis in a Thin Layer of Starch-Gel followed by a Plastifying Treatment, 72
Ground-Level : Seasonal Variations of Caesium-137 in Air at (K. G. Vohra, V. S. Bhatnagar and C. Rangarajan), 747
Ground-Level Air : Comparison of Beryllium-7 and Caesium-137 Radioactivity in (Dr. P. F. Gustafson, M. A. Kerrigan and S. S. Brar), 454
Group Velocity : Wave Propagation and (Dr. Léon Brillouin), *review* by Prof. M. H. L. Pryce, 104
Group and Phase Velocities of a Travelling Disturbance in the *F* Region of the Ionosphere (Dr. L. H. Heisler and Dr. J. D. Whitehead), 157
Grove, Jean M. : *Obituary* of W. V. Lewis, 332
Grover, Prof. Elliot B., and Hamby, Prof. D. S. : Handbook of Textile Testing and Quality Control, *review* by W. J. Onions, 106
Growing Old in a Mechanized World (F. Le Gros Clark), *review* by Prof. L. S. Hearnshaw, 532
Growth : Histidine Decarboxylase and (D. Mackay, Joan D. Reid and Dr. D. M. Shepherd), 1311
    Increase in Elastin Content of the Human Aorta during (Dr. V. Scarselli), 710
Growth of Etiolated Oat Seedlings : Carbon Dioxide and Ethanol as Factors controlling the (Dr. C. L. Mer), 260
Growth of Fern Embryos in Sterile Culture (Prof. Augustus E. DeMaggio and Prof. Ralph H. Wetmore), 94
Growth of Fibroblast Cultures : Effect of Amino-Acid Esters on the (Dr. I. Fehér, A. Döklen and V. Selmeci), 494
Growth of Mouse Carcinoma : Effect of Irradiated Normal Tissues on the (Dr. Arthur Compton and Prof. Charles A. Pannett), 1101
Growth of Ryegrass-dominant Pasture grazed by Cows, and Properties of New Zealand Butterfat (F. H. McDowall, W. A. McGillivray and J. C. Hawke), 303
Growth (Enhanced Plant-), with Two Systemic Insecticides (M. M. H. Wallace), 513
Growth (Epitaxial), of Crystals on One-Degree Orientated Substrates (Dr. A. Goswami), 160
Growth (Normal) : Spontaneous Development of *L* Forms of Streptococci requiring Secretions of other Bacteria or Sulphydryl Compounds for (Ayala Frenkel and Dr. Willy Hirsch), 728
Growth (Plant), in Irradiated Soil (G. D. Bowen and A. D. Rovira), 936
Growth and Development : Effect on (Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid), (I. H. Williams), 361
Growth and Indoleacetic Acid Oxidase Activity of *Lupinus albus* L. exposed to Different Photoperiods : Effect of Gibberellic Acid on (F. Sági and A. S. Garay), 824
Growth and Photosynthesis in Submerged Plants : Inhibition of, with Tetramethylthiuram Disulphide and Sodium Dimethyldithiocarbamate and Reversal of this Inhibition of Photosynthesis (P. E. Björn Lindahl), 51
Growth Hormone (Pituitary) : Effect of, on Cholesterogenesis (Dr. A. L. Greenbaum and R. S. Leal), 600
Growth-Rate of the Suckling of various Species and the Percentage of Total Calories from Protein in the Milk : Correlation between (Dr. F. W. Bernhart), 358
Growth Stimulation by High Indole-3-Acetic Acid Concentrations (T. A. Truelsen), 1410
Gruenberg, Dr. K. W. : Title of reader in pure mathematics (at Queen Mary College, London) conferred on, 546, 651
Grune, Dr. : Paper Chromatography (Value of New Types of Paper which have Alumina or Silica Gel incorporated into the Cellulose Matrix at the Manufacturing Stage), 759
Guanethidine : Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and (A. L. A. Boura, F. C. Copp, A. F. Green, H. F. Hodson, G. K. Ruffell, M. F. Sim, E. Walton and E. M. Grivsky), 1312
Guérin, Prof. Camille : *Obituary* by Dr. Ian A. Galloway, 851
Guest, J. R. : Vitamin B_{12} and Intrinsic Factor (Relationships between Vitamin B_{12} and Folic Acid), 1156
Guggenheim (Daniel and Florence) International Astronautics Award of the International Academy of Astronautics awarded to Sir Bernard Lovell, 856
Guide (Retrieval), to Thermophysical Properties Research Literature, Vol. I (edited by Y. S. Touloukian), Book 1, Part A, Guide to Substance Classification and Numerical Codes ; Book 1, Part B, Dictionary of Synonyms and Trade Names ; Book 1, Part C, Dictionary of Substances ; Book 2, Classified Search Index ; Book 3, Master Bibliography, Author Index, *review* by Prof. A. R. Ubbelohde, 109
Guiducci, Mario, and others : The Controversy on the Comets of 1618 (translated by Stillman Drake and Dr. C. D. O'Malley), *review* by Colin A. Ronan, 314
Guinea Pig Lymphocytes : Chemistry of a Mucopolysaccharide produced by (Helen Muir and A. H. E. Marshall), 706
Guinea Pig Serum : Evidence that the L-Asparaginase Activity of, is responsible for its Antilymphoma Effects (J. D. Broome), 1114
Guinea Pigs : Passive Transfer of Contact Sensitivity to Picryl Chloride in, with Subcellular Material (Dr. J. L. Turk), 915
Guinness Son and Co., Ltd. (Arthur) : Guinness Awards for Science Teachers in Training, 338
Gulbenkian Foundation : Conference at the University of Birmingham on the Biology Syllabus for Sixth Forms, 554
    Grants made during the first half of 1961, 754
    Report for 1955-1959, 855
Gupta, Dr. Minati : Rectal Glands in Woodlice, 406
Gustafson, Dr. P. F., and others : Comparison of Beryllium-7 and Caesium-137 Radioactivity in Ground-Level Air, 454
Gut (Isolated) : Dependence on Substrate of the Electrical Potential across the (Dr. T. W. Clarkson, A. C. Cross and S. Toole), 501
Guthrie Lecture of the Institute of Physics and the Physical Society (Dr. D. Shoenberg), 126
Gutmann, Dr. F., and Netschey, A. : Semiconductivity of Chlorpromazine, 1390
Gutz, Dr. Herbert : Distribution of X-Ray- and Nitrous Acid-induced Mutations in the Genetic Fine Structure of the ad_7 Locus of *Schizosaccharomyces pombe*, 1125
Gwynne, M. D., and Glover, J. : Light Rainfall and Plant Survival—Measurement of Stem Flow Run-off, 1321
Gysels, H. : Nematology (Post-embryonic Growth in *Panagrellus silusiae*), 1048

HAAS, A. : At the Second International Measurement Congress (Calibration of Contact Type Surface Thermometers), 1351
Haber, Dr. C. P. : Inorganic Polymers (Phosphonitrilic Compounds), 1047
Hacking, K. : Some Aspects of Optical Lens Performance, Part I, Secondary Flare in Lenses (B.B.C. Engineering Monograph No. 36), 1355
Haddon, E. E. : Appointed director of the Chemical Defence Experimental Establishment, Porton, 751 ; work of, 751
Haddow, Prof. A. : The Chester Beatty Research Institute (of the Institute of Cancer Research, Royal Cancer Hospital), 430
Hador, Dr. S. Ben : Biorheology and Microcirculation (Effect of Adrenaline on the Small Blood Vessels of the Conjunctiva and Nailfold), 758 ; (Observations and Experiments on Vasomotion in Human Capillaries), 758
Haemagglutinins from *Clerodendrum viscosum* Vent (Lieut.-Gen. G. W. G. Bird), 292
Haematites (Natural and Artificial) : Hydrogen Reduction Characteristics of (John Henderson), 268
Haematology : Lectures on (edited by F. G. J. Hayhoe), *review* by Dr. M. E. Nutt, 7
    Some Aspects of, *review* by Dr. M. E. Nutt, 7
Haematopoietic System (Human) : Effects of Ionizing Radiation on the—Report of the Subcommittee on Haematologic Effects, Committee on Pathologic Effects of Atomic Radiation (United States Academy of Sciences—National Research Council, Publication No. 875), 556
Haemoglobin : Reactions of Haemoproteins with Hydrogen Peroxide and the Supposed Formation of Hydrogen Peroxide during the Autoxidation of (Prof. D. Keilin), 769
Haemoglobin 'Barts' and the Sickling Phenomenon (Dr. Lie-Injo Luan Eng), 1314
Haemoglobin *F* and the Genetic Control of Protein Structure (Prof. V. M. Ingram), 608 ; (Dr. Wolf W. Zuelzer and Abner R. Robinson), 609
Haemoglobin *N* and Microcythaemia in a Sardinian Family : Association of (Prof. E. Silvestroni and I. Bianco), 1208
Haemoglobin *P* in a Family of Southern Italian Extraction (Prof. E. Silvestroni, I. Bianco and C. Brancati), 292
Haemoglobin Synthesis : Control of Specificity in (Dr. J. Bishop, Dr. G. Favelukes, Prof. R. Schweet and Dr. E. Russell), 1365
Haemoglobin Variants (Denatured), in Acid Buffers : Solubility of (I. D. Fraser and Dr. A. B. Raper), 355
Haemoglobins : Abnormal Foetal (Prof. E. Silvestroni and I. Bianco), 397
Haemoglobins (Abnormal Human) : Thalassaemia and the (Dr. Harvey A. Itano and Prof. Linus Pauling), 398
Haemophilic (Anti-) Globulin : Stability of Freeze-dried (F. Nour-Eldin), 503
Haemoproteins : Reactions of, with Hydrogen Peroxide and the Supposed Formation of Hydrogen Peroxide during the Autoxidation of Haemoglobin (Prof. D. Keilin), 769
Haemorrhage (Thrombocytopaenic) : Treatment of, with Œstriol Succinate (Dr. Hubert Poliwoda), 400
Haenel, Dr. H. : Bacteria of the Intestine (Micro-ecological Rules in the Composition of the Intestinal Flora of Man), 1152
Hafnium Oxides (Zirconium and) : Reduction of (F. K. McTaggart), 1192
Hagadorn, Irvine R., and Nishioka, Richard S. : Neurosecretion and Granules in Neurones of the Brain of the Leech, 1013
Haggar, R. J. : Flower Initiation in Kent Wild White Clover (*Trifolium repens* L.), under Controlled Environmental Conditions, 1120
Hague, Prof. Bernard : *Obituary* by Dr. J. Lamb, 750
Hague, John L. : Appointed chief of the Standard Reference Materials Section of the United States National Bureau of Standards, 334 ; work of, 334
Hague, N. G. M. (Call, F., and) : Nematology (Diffusion of Ethylene Dibromide through Soil), 1049
Hahn, P., and others : Role of the Lung in Metabolism of Fat during Ontogeny of the Rat, 85
Hail Samples : Radar Reflectivities of (R. H. Douglas), 266
Haine, M. E. : The Electron Microscope, *review* by Prof. D. Gabor, 316
Hair in Yellow *A^{vy}/a* Mice : 5-Methyltryptophan and Darkening of the (Dr. Vivianne T. Nachmias), 934
Hairs (Labellar), of the Blowfly, *Phormia regina* Meigen : Response of the, to Protein (D. I. Wallis), 917
Hais, Dr. I. M. : Paper Chromatography, 760
Hajnal, J. : Title of reader in demography in the University of London (at the London School of Economics and Political Science) conferred on, 337
Hall, D. M., and Jones, R. L. : Physiological Significance of Surface Wax on Leaves, 95
Hall, Dr. E. T. : Grant of £1,500 from the Paul Instrument Fund, for the design and manufacture of a recording instrument to give a direct reading of wave-length, 225

Hall, Sir James : The Life and Scientific Work of (Dr. V. A. Eyles), 345
Hall, J. M. : Stress Effects on Thermoremanent Magnetization, 586
Hall, Jr., Marshall (edited by Richard Bellman and) : Combinatorial Analysis—Proceedings of the American Mathematical Society Symposia in Applied Mathematics, Vol. 10), *review* by Prof. L. S. Goddard, 321
Hall, V. C. : At the Maxwell Colour Centenary Conference (Colour Printing—Automatic or Semi-automatic Colour Correction), 11
Haller, Dr. Wolfgang : Fibrous Silica, 662
Halliday, Dr. June W. (Inglis, Dr. James A., and) : Thromboplastic Activity of Red Cells, 821
Hallmark, Julie (Neely, W. Brock, and) : Dextransucrase *V* and the Role of Metal Ions in Enzyme Catalysis, 385
Halmos, M. (Beck, Dr. M. T., and) : Stability Constant of Copper (II) Complexes of Reduction Products of 2,2'-Dipyridyl, 1090
Halogens and their Compounds : Gas-Phase Reactions of the (Dr. J. H. Thomas and Dr. E. Whittle), 129
Halsbury, Earl of : Elected president of the Machine Tool Industry Research Association, 546
Halsey, G. (Burkin, A. R., and) : Chemisorption at Solid–Liquid Interfaces, 348
Hambler, Dr. D. J. : A Poikilohydrous, Poikilochlorophyllous Angiosperm from Africa, 1415
Hamby, Prof. D. S. (Grover, Prof. Elliot B., and) : Handbook of Textile Testing and Quality Control, *review* by W. J. Onions, 106
Hamilton, William C. (Rouayheb, George M., and) : Deterioration of Solid-coated Capillary Columns on Standing, 801
Hamilton, W. J. : Ovum Humanum, *review*, 110
Hanaki, Akira (Ishidate, Prof. Morizo, and) : Oxidative Demethylation and Carcinogenicity of 4-N-Dimethylaminoazobenzene Derivatives, 1198
Hancock Medal of the Institution of the Rubber Industry awarded to M. M. Heywood, 755
Handbook : Biochemists' (edited by Dr. Cyril Long), *review* by Dr. D. Recaldin, 635
Handbook (A Laboratory), of Pulp and Paper Manufacture (Dr. Julius Grant), second edition, *review* by Dr. F. Lyth Hudson, 320
Handbooks (New), for Lunar Studies, *review* by D. H. P. Jones, 424
Handedness and Cerebral Dominance : Speech, *review* by Sir Russell Brain, 840
Handling (Safe), of Radioactive Substances, 1257
Hanging Mercury Drop (Rotating) : Use of the, in Analysis—Anodic Stripping using a Rotating Mercury Drop (Dr. J. J. Engelsman and A. M. J. M. Claassens), 240 ; Trace Analysis with a Rotating Hanging Mercury Drop (Dr. M. van Swaay and R. S. Deelder), 241
Hankinson, Dr. J. : Assessment of Pain (Relief of Pain by destruction of Intracerebral Structures under the guidance of Stereoscopic Radiography), 1353
Hanlon, D., and others : Gross Beta-Activity in the Thames River (Conn.), 1017
Hanstrom, Bertil, and others (edited by) : South African Animal Life—Results of the Lund University Expedition in 1950-1951, Vol. 7, *review* by Prof. O. Lowenstein, 1135
Happold, Prof. F. C. : The Training of Biochemists, 956
Haptoglobin : Action of Neuraminidase on (Prof. Max E. Rafelson, jun., Lise Cloarec, Jean Moretti and Max F. Jayle), 279
Haptoglobin Patterns in Cord Blood Serums (A. R. Rausen, P. S. Gerald and Dr. L. K. Diamond), 717
Haptoglobinuria in Nephrotic Syndromes (A. Marnay), 74
Harbison, Prof. F., and Myers, Prof. C. A. : Management in the Industrial World, 1332
Harbottle, Dr. Garman (Veljković, Slobodanka, and) : Recoil-Atom Reactions at Low Temperature, 1287
Harden, Dr. D. B. : Presidential Address to the Museums Association, 553
Hardening of the Cuticle in the Silkworm, *Bombyx mori* : Role of Lipid in the (Shigemi Kawase), 279
Harders, Dr. H. : Biorheology and Microcirculation (Technical Difficulties involved in Direct Microscopic Observations of Microcirculation *in vivo*), 757
Harding, D. : Limnological Trends in Lake Kariba, 119
Hardisty, Dr. M. W. : Sex Composition of Lamprey Populations, 1116
  Oocyte Numbers as a Diagnostic Character for the Identification of Ammocoete Species, 1215
Hardy, Dr. J. D. : Assessment of Pain (Pain Threshold in relation to Measurement of the Intensity of Human Pain and the Effects of Analgesics), 1354
Hardy, S. M. : Amino-Acid Liberation from Proteins by Enzyme or 'Heat Catalysed' Hydrolysis, 1061
Harigaya, S., and others : Inhibition of β-Glucuronidase by Oral Administration of D-Glucosaccharo-1,4-Lactone, 1298
Harkness Fellowships for 1962 of the Commonwealth Fund, New York, 1045
Harland, Prof. S. C. : Genetica, *review*, 213
Harper, Dr. W. R. : Neutron Terminology, 60
  Neutron 'Flux', 695
Harrington, H. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Effects of Radiation on Cell Division), 341
Harris, Prof. Colin C. (Arbiter, Prof. Nathaniel, and) : Power Requirements and Scale-up in Flotation, 268
Harris, Dr. G. P., and Griffin, Juliet E. : Flower Initiation in the Carnation in Response to Photoperiod, 614
Harris, Prof. G. W. : At the Scientific Publications Council Meeting on the Publication of Scientific Books (Contractual Relationships), 132
  Appointed to the chair of anatomy in the University of Oxford, 957 ; work of, 957
Harris, Prof. H., and Whittaker, Mary : Differential Inhibition of Human Serum Cholinesterase with Fluoride— Recognition of Two New Phenotypes, 496
Harris, J. M., and West, Dr. G. B. : Anaphylactoid Reaction in Rats, 399
Harris, M., and Morrison, Dr. G. A. : Multiple Inhibitions of Cell Metabolism by a Single Substance, 1276
Harrison, Dr. B. D. : Plant Viruses, *review*, 111
Harrison, Dr. E. R. : Terrestrial Accretion from the Solar Wind, 1182
Harrison, Dr. G. Ainsworth : Principles of Human Genetics, *review*, 110
Harrison, M. B. (Mai, W. F., and) : Nematology (*Heterodera rostochiensis* is held in check on Long Island by Nematicidal Treatment, Cultural Methods and Legal Regulation), 1049
Harrison, Prof. R. J. : Elected to the Fullerian professorship of physiology at the Royal Institution, 122 ; work of, 122
Harrison, T. K., and others : Absorption Spectra of γ-Irradiated Lead Silicate Glass, 374
Hart, B. L. J. : Appointed principal of the Computer Application Training Centre of International Computers and Tabulators, Ltd., 24
Hartley, Sir Harold : Elected to honorary membership of the Institute of Fuel, 23
Hartley, William : Plant Exploration and Introduction (Phytogeographical Basis of Pasture Plant Introduction), 1240
Harvey, Dr. A. : Welsh College of Advanced Technology, 127
Harvey, J. J. (Salaman, Dr. M. H., and) : Leukaemia in the Progeny of Mice inoculated with a Leukaemogenic Virus, 509
Harvey, William—Englishman, 1578-1657 (Prof. Kenneth J. Franklin), *review*, 740
Harwell : Visit of Russian Scientists to, 957
Hasan, R. (Qasim, Dr. S. Z., and) : A Hydrostatic Function of the Accessory Respiratory Organs in Air-breathing Fishes, 396
Hashimoto, Y., and others : Potentiation of Epinephrine Action, 186
Haslett (Caroline) Memorial Trust : Women Engineers in the U.S.S.R., a Study of the Education, Employment and Prospects of Women Engineers in the U.S.S.R. (L. S. Souter and R. Winsdale), 30
Hatfield, T., and others : Gross Beta-Activity in the Thames River (Conn.), 1017
Hauck, R. D., and Bouldin, D. R. : Distribution of Isotopic Nitrogen in Nitrogen Gas during Denitrification, 871
Hawke, J. C., and others : Growth of Ryegrass-dominant Pasture grazed by Cows, and Properties of New Zealand Butterfat, 303
Hawker, Lilian E., and others : An Introduction to the Biology of Micro-organisms, *review* by Prof. B. C. J. G. Knight, 1032
Hawkes, Prof. C. F. C. : Megalithic Tombs of France, *review*, 637
Hawkes, Dr. I. : Appointed lecturer in the Postgraduate School of Mining, University of Sheffield, 337
Hawkes, Prof. J. G. : Plant Exploration and Introduction (Greater International Collaboration in Collecting Expeditions and the Subsequent Distribution of the Material), 1240
Hawkesbury District (Sydney–) : Rock Engravings of the (F. D. McCarthy), 854
Hawkey, Christine, and Stafford, J. L. : Influence of Serum Factors on the Species-specificity of Tissue Thromboplastin, 920
Hawkins, T. H. : Scientific Attitude to Management, *review*, 209
Hay, J. B., and others : Teichoic Acids and the Structure of Bacterial Walls, 570
Hayashibe, Masaya, and Uemura, Teijiro : Release from the Feedback Inhibition controlling the Biosynthesis of Isoleucine, 1417
Haydak, M. H., and others : Chromatographic Investigation of the Methyl Alcohol/Chloroform-soluble Constituents of Honeybee Larval Foods, 362
Hayes, T. J., and others : Determination of Uranium as Pyrophosphate, 1291
Hayhoe, F. G. J. (edited by) : Lectures on Haematology, *review* by Dr. M. E. Nutt, 7
Hayman, R. W. : Mammals of Northern Rhodesia, *review*, 8
Haynes, Prof. L. J., and others : High Concentration of (–)-Noradrenaline in *Portulaca oleracea* L., 1108
Hayward, Dr. A. F. : Awarded a Carlsberg–Wellcome Research Fellowship, 754
Hazard, Dr. C. : Lunar Occultation of a Radio Source, 58
Hazard, Dr. Elia Ch. and others : Biochemical Changes in Lethal Staphylococcal Infections in Mice, 783
Hazards of the Animal House (Laboratory Animals Centre Discussion on), 339
Hazards (Health), from Radioactive Materials : Introductory Manual on the Control of (Medical Research Council, Memorandum No. 39), new edition, 1257
Head, Richard B. : Steroids as Ice Nucleators, 1058
Heading Time in a Bulk-Hybrid Population of Barley : Stabilizing Selection for (Dr. S. K. Jain), 1123
Heald, Dr. P. J. : Phosphorus Metabolism of Brain, *review* by Dr. R. V. Coxon, 318
Health, Ministry of : Abstracts of Efficiency in the Hospital Service, 441
Health, Ministry of, and General Register Office : Report on Hospital In-Patient Enquiry for the Year 1959, Part I, Preliminary Tables, 345
Health, United States National Institutes of, and others : Conference on Transfer of Calcium and Strontium across Biological Membranes, 1250
Health in Yugoslavia (United Nations, World Health Organization, *WHO Chronicle*, Vol. 15, No. 7), 1260
Health, Education and Welfare, United States Department of : Public Health Service Publication No. 815, Highlights of Research Progress in General Medical Sciences (1960), 658
  Public Health Service Publication No. 854, Research Highlights in Ageing, 1145
Health (Mental) : Teaching of Psychiatry and (United Nations, World Health Organization, Public Health Papers No. 9), 1248
Health (Mental), World Federation for : Grant from the Gulbenkian Foundation, 754
Health (Mental) Statistics, 855
Health (Radiological) : Research Grants in (United States Public Health Service), 442
Health Education : Teacher Preparation for (United Nations, World Health Organization, Technical Report Series No. 193—Report of a joint WHO/Unesco Expert Committee), 441
Health Hazards from Radioactive Materials : Introductory Manual on the Control of (Medical Research Council, Memorandum No. 39), new edition, 1257
Health Service (Public), United States : Research Grants in Radiological Health, 442
Health Services (Public) : The Planning of (United Nations, World Health Organization, Technical Report Series No. 215—Fourth Report of the Expert Committee on Public Health Administration), 441
Healy, M. J. R. : Rapid Statistical Calculations, *review*, 1236
Heard, Dr. D. H., and others : Sialic Acids and the Electrokinetic Charge of the Human Erythrocyte, 44
Hearnshaw, Prof. L. S. : The Problem of Retirement, *review*, 532
Heart : Relationship between Velocity of Cardiac Contraction and Size of the—a Hypothesis (Dr. H. Feinberg), 1373

Heart of the Spinal Dog : Stimulation of the, by Phenoxybenzamine (B. G. Benfey and D. R. Varma), 919
Heart of the Turtle : Cycochrome c in the (Prof. Gunnar Biörck and Dr. Sven Paléus), 712
Heartwood : Carbon-14 from Nuclear Explosions as a Short-term Dating System—Use to determine the Origin of (Dr. A. T. Wilson), 714
Heat (Specific) and Expansion Coefficient of Liquid Helium-3 under Pressure below 0·1° K. (Dr. D. F. Brewer and J. R. G. Keyston), 1261
Heat Capacity and Resistivity Anomalies in Palladium Hydride (Paul Mitacek, jun., and Prof. J. G. Aston), 271
'Heat Catalysed' Hydrolysis : Amino-Acid Liberation from Proteins by Enzyme or (S. M. Hardy), 1061
Heat-Labile Alkaloids from Plants : Extraction of (Dr. A. R. Mattocks), 1281
Heat Shocks (Radiant) : Mutagenic Effect of, on Phanerogamous Plants (Dr. Bror Pettersson), 1167
Heath, Dr. I. D. : Staining of Sulphated Mucopolysaccharides, 1370
Heath, Prof. O. V. S. (Mansfield, Dr. T. A., and) : Photoperiodic Effects on Stomatal Behaviour in *Xanthium pennsylvanicum*, 974
Heating (Local), of the Hypothalamus : Increase of Evaporative Loss of Water from the Skin of the Ox in response to (Dr. D. L. Ingram, J. A. McLean and Dr. G. C. Whittow), 81
Heats of Adsorption of Organic Vapours on Adsorbents of High Surface Area using a Continuous Flow Calorimeter : Measurements of (G. H. Bell and A. J. Groszek), 1184
Heavens : The Fabric of the (Dr. Stephen Toulmin and June Goodfield), *review* by Dr. M. A. Hoskin, 840
Heavy Metals : Effect of Excess Supply of, to Barley during Germination, with special reference to Catalase and Peroxidase (Dr. S. C. Agarwala, A. Kumar and C. P. Sharma), 726
Heavy Water : Effects of, on Poliovirus Multiplication—Results and Speculations on Mechanism (Dr. David Kritchevsky, Dr. Richard I. Carp and Dr. Hilary Koprowski), 250
Hebrew University : Seminar at the Medical School on Biorheology and Microcirculation, 757
Hedley, A. B. : Appointed to the Central Electricity Authority research fellowship in fuel technology in the University of Sheffield, 229
Hedley and Co., Thomas (Newcastle-upon-Tyne) : Appointment of Dr. J. F. Goodman as head of the Basic Research Department, 229
Heezen, Bruce C., and others : The Floors of the Oceans, I, The North Atlantic—Text to accompany the Physiographic Diagram of the North Atlantic (Geological Society of America, Special Paper 65), *review* by Dr. G. E. R. Deacon, 531
Heftmann, Erich, and Mosettig, Erich : Biochemistry of Steroids, *review* by Dr. D. T. Elmore, 111
Height of Night-time *F* Layer Irregularities at the Equator (Dr. G. S. Kent and Dr. J. R. Koster), 1083
Heights of 1,000–1,700 km. : Terrestrial Exosphere at (Max Römer), 238
Heights (Ionospheric Absorption) : A Method of Estimating, from Recovery Times (Syed Ziauddin), 1084
Heinen, W., and Linskens, H. F. : Enzymic Breakdown of Stigmatic Cuticula of Flowers, 1416
Heinrich, H. C. : Vitamin B_{12} and Intrinsic Factor (Freedom of B_{12} Coenzyme from Cyanide), 1154 ; (Specific Effects of Vitamin B_{12} Deficiency), 1155
Heisenberg, Prof. W. K. : Elected a foreign associate of the United States National Academy of Sciences, 18 ; work of, 19
Heisler, Dr. L. H., and Whitehead, Dr. J. D. : Group and Phase Velocities of a Travelling Disturbance in the *F* Region of the Ionosphere, 157
Heitzmann, R. J. : Appointed Research Fellow in the Department of Chemistry, University of Birmingham, 337
Hela, Prof. Ilmo : Appointed chief scientist in charge of the programme of research on the effects of radioactivity in the sea, to be conducted at Monaco, 122 ; work of, 122
Helback, Hans : Ecological Aspects of Irrigation in Ancient Mesopotamia, 227
Helgason, Dr. Bjarni : Soil Temperatures in Iceland, 939
Helium (Solid) : Bond-Lengths in (Dr. A. F. Schuch), 591
Helium-3 (Liquid), under Pressure below 0·1° K. : Specific Heat and Expansion Coefficient of (Dr. D. F. Brewer and J. R. G. Keyston), 1261
Heller, Prof. H., and others : Pharmacological Characterization of the Oxytocic Peptides in the Pituitary of a Marine Teleost Fish (*Pollachius virens*), 670
Heller, Dr. L., and Ben-Yair, Dr. M. : Formation of Calcium Chloraluminate by Interaction of Portland Cement with Sea-Water and Chloride Solutions, 488
Helminthology, Journal of : Supplement in honour of R. T. Leiper on the occasion of his 80th Birthday, 1961, *review* by Dr. G. Lapage, 843
Helminthosporium sativum : Production of Symptoms of Barley Leaf-Spot Disease by Culture Filtrate of (S. K. Gayed), 725
Helminths (Soil-transmitted) : Control of (Prof. Paul C. Beaver), 1249
Helson, Prof. H. : At the Maxwell Colour Centenary Conference (Colour Appearance), 11
Hemihydrate (Calcium Sulphate) : Reactivity of (M. J. Ridge, H. Surkevicius and Angela A. Milne), 704
Hemileia vastatrix B. et Br. (Coffee Rust) : Spore Liberation and Dispersal of (R. W. Rayner), 725
Hemingway, Dr. J. T. : Influence of Plasma Proteins in the Control of Mitosis-rates in Generating Liver, 706
Henderson, Dr. B. : Appointed Research Fellow in the Department of Physical Metallurgy, University of Birmingham, 337
Henderson, John : Hydrogen Reduction Characteristics of Natural and Artificial Haematites, 268
Henderson Trust Lectures : No. 19, Cerebral Dominance and its relation to Psychological Function (Prof. O. L. Zangwill), *review* by Sir Russell Brain, 840
Henis, Y., and others : Factors influencing the Composition of the Cellulose-decomposing Microflora on Soil Crumb Plates, 307
Henle (Loop of) : Stimulation by Aldosterone of Sodium Transport in the (Dr. J. Crabbé), 817
Henn, T. R. : Science and Poetry, 534
Henry, S. Mark (Block, Richard J., and) : Metabolism of the Sulphur Amino-Acids and of Sulphate in *Blattella germanica*, 392
Hensel, Prof. Herbert : Awarded a Prize by the Feldberg Foundation for German–English Scientific Exchange for Experimental Medical Research, 1143
  The Electrophysiology of Thermoreceptors, 1143
  Assessment of Pain (Film of Afferent Impulses in Human Single Cutaneous Nerve Fibres), 1353
Heparin : Release of, from the Mast Cells of the Rat (G. T. Archer), 90
  Stimulation by, of Parenchymal Liver Cell Proliferation by Normal Adult Rats (Dr. M. Zimmerman and Evemarie Celozzi), 1014
Heparin-neutralizing Properties (Oncogenic and) : Surfen, a Quinoline with (DeWitt T. Hunter, jun., and Joseph M. Hill), 1378
Hepatic Cells (Normal Rat) : Nucleolar Localization of Succinic Dehydrogenase in (P. De, R. Chatterjee and Dr. S. Mitra), 925
Hepatic Induced Increase of Tryptophan-Pyrrolase : Effect of 5-Fluorouracil on (Sancia Gaetani and M. Antonietta Spadoni), 1296
Hepatitis (Experimental), in Mice : Blockage of Transformation of Tryptophan to Nicotinic Acid by (M. Piazza, F. Tancredi and Prof. E. Quagliariello), 601
Hepatoma No. 5123 : Energy Pathways of (Dr. Alan C. Aisenberg and Harold P. Morris), 1314
Hepatoma (Ascitic) : Comparison of Pyridine Nucleotides in the Liver and in an (M. Wintzerith, N. Klein, L. Mandel and Prof. P. Mandel), 467
Heppleston, Prof. A. G. (Jones, J. H., and) : Immunological Observations in Experimental Silicosis, 1212
Herbert, J. D., and others : A New 'Wedge' Type Instability observed in a 'Theta' Pinch, 1054
Herbert, W. J. : Awarded a Research Training Scholarship by the Animal Health Trust, 1250
Herdan, Dr. Gustav : Type-Token Mathematics, a Textbook of Mathematical Linguistics, *review* by Dr. G. H. Toulmin, 211
Herman, H., and Cohen, Prof. J. B. : Resistivity Changes due to Formation of G.P. Zones (Clusters rich in Solute), 63
Herman, L., and Herman, R. : New Afterglow Emission associated with Metastable Nitrogen Atoms, 346
Herman, R. (Herman, L., and) : New Afterglow Emission associated with Metastable Nitrogen Atoms, 346
Hermann, Dr. Howard T. (Stark, Dr. Lawrence, and) : Transfer Function of a Biological Photoreceptor, 1173
Hermiston, S. T. : Design and Use of Respirators (Apparatus for Protecting People who handled Radioactive Substances), 656
Heron, Alistair, and Chown, Sheila M., assisted by M. S. Featherstone and Catherine Cunningham : Ageing and the Semi-skilled—a Survey in Manufacturing Industry on Merseyside (Medical Research Council, Memorandum No. 40), 1249
Herpay, Zs. (Uri, J., and) : Use of Desertomycin Medium in Routine Selective Isolation of Fungi Pathogenic to Man, 1015
Herzberg, F. : The Motivation of Work, 1332
Hess, R., and Pearse, Dr. A. G. E. : Histochemical and Homogenization Studies of Mitochondrial α-Glycerophosphate Dehydrogenase in the Nervous System, 718
Hess, Prof. Seymour L. : Introduction to Theoretical Meteorology, *review* by Dr. Robert P. Pearce, 314
Heterocyclic Chemistry, *review* by Dr. G. L. Buchanan, 425
Heterocyclic Compounds (edited by Dr. E. H. Rodd), (Chemistry of Carbon Compounds, Vol. 4, Part C), *review* by Dr. James D. Loudon, 214
  An Introduction to the Chemistry of (R. M. Acheson), *review*, 320
Heterocyclic Systems with Bridgehead Nitrogen Atoms (William L. Mosby), Part 1, *review* by Dr. G. L. Buchanan, 425
Heterodera rostochiensis Woll. : Assessing the Damage caused by (E. B. Brown), 937
Heterogeneous Lactic Dehydrogenases in Malignant Effusions : Diagnostic Significance of (R. Richterich, K. Zuppinger and E. Rossi), 507
Hevea brasiliensis : Effect of Ethylene Oxide on the Tapping of (D. H. Taysum), 1319
  Effect of Ferric Dimethyldithiocarbamate on the Rooting of Cuttings of (G. H. Tinley), 1217
Hewish, Dr. A. : Elected to a Fellowship in Churchill College, Cambridge, on appointment as College lecturer in physics, 856
Hewitt, Dr. H. B. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Response of Mouse Leukaemic Cells—using an *in vivo* Bio-Assay—to Radiation and its Variation with Different Degrees of Oxygenation), 341
  Transplantation of Murine Leukaemia by Unconventional Routes, 1213
Hewitt, Dr. H. B., and Wilson, Dr. C. W. : Awarded the David Anderson Berry Prize for 1961 of the Royal Society of Edinburgh, 229
Hexachlorobenzene Intoxication : Nervous and Biochemical Disturbances following (Dr. F. De Matteis, B. E. Prior and Prof. C. Rimington), 363
Hexosamine : Partial Acid-Hydrolysis of a Mucopolysaccharide without Appreciable *N*-Deacetylation of (Dr. T. J. Painter and Prof. W. T. J. Morgan), 39
Hexosamine and Sialic Acid Contents in Cells (Atsushi Kimura, Yasushi Nagai, Dr. Koh-Iti Turumi, Yoshiko Kawashima and Haruo Sato), 596
Heyneman, Dr. Donald : A Natural Population of Anomalous Branched Tapeworms, *Hymenolepis nana* (Cestoda : Hymenolepididae), in a Colony of *DBA/1* Mice, 297
Heywood, J. : Research by Sixth-Form Schoolboys, 860
Heywood, M. M. : Awarded the Hancock Medal of the Institution of the Rubber Industry, 755
Heywood, Dr. V. H. : Flora Europaea, 446
Hickman, B. S., and others : Scattering of Long Wave-length Neutrons by Irradiated Beryllium Oxide, 1385
Hicks, Dr. R. Marian (Holt, Dr. S. J., and) : Use of Veronal Buffers in Formalin Fixatives, 832
Higgins, P. R. B., and Roberts, A. C. : Damage in Stainless Steel due to Fission Fragments, 1158
Higgins, William : The Life and Work of, Chemist (1763-1825), (Prof. T. S. Wheeler and Prof. J. R. Partington), *review* by Frank Greenaway, 533
Higgs, J. W. Y. : At the sixth-seventh Annual Conference of the Museums Association (Display of Folk Material), 553
High Altitude : Aldosterone Excretion and Potassium Retention in Subjects living at (P. J. Ayres, Rona C. Hurter, Dr. E. S. Williams and J. Rundo), 78
High Concentration of (–)-Noradrenaline in *Portulaca oleracea* L. (Dr. P. C. Feng, Prof. L. J. Haynes and Dr. K. E. Magnus), 1108
High Energy Nuclear Physics (Institute of Physics and the Physical Society Symposium on), 1349
High Indole-3-Acetic Acid Concentrations : Growth Stimulation by (T. A. Truelsen), 1410

High Polymers : Elimination of the Absorption Correction in the Viscometry of Dilute Solutions of (H. van Oene and Prof. L. H. Cragg), 1160
  Viscosity–Temperature Relationships for Dilute Solutions of (Dr. W. R. Moore), 1292
High Polymers (Semiconducting) : Photochemical Production of (Prof. Gerald Oster, Dr. Gisela K. Oster and Dr. Marian Kryszewski), 164
High-Pressure Treatment : A New Phase formed by—Face-centred Cubic Molybdenum Monocarbide (E. V. Clougherty, K. H. Lothrop and J. A. Kafalas), 1194
High Pressures : Melting and Polymorphism of Indium Antimonide at (A. Jayaraman, R. C. Newton and Dr. G. C. Kennedy), 1288
High-purity Zinc : Determination of Indium in (Naïm Košaric and the late G. Leliaert), 703
High Speed : Ignition of Gas by Sparks produced by Breaking Wires at (Dr. R. L. Gordon, Dr. H. Lord and D. W. Widginton), 1085
High Surface Area : Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of, using a Continuous Flow Calorimeter (G. H. Bell and A. J. Groszek), 1184
High Temperature Compounds : Sintered (Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals Symposium on), 1250
Higher Plants : Some Characteristics of Mitochondria Extracted from (Dr. Walter D. Bonner, jun., and Dinor O. Voss), 682
Highlights of Research Progress in General Medical Sciences, 1960 (United States Department of Health, Education and Welfare, Public Health Service Publication No. 815), 658
Highlights (Research), in Ageing (United States Department of Health, Education and Welfare, Public Health Service Publication No. 854), 1145
Highly Effective Anthelmintic for Sheep : Thiabendazole, a (Hugh McL. Gordon), 1409
Highnam, Dr. K. C. : Induced Changes in the Amounts of Material in the Neurosecretory System of the Desert Locust, 199
Hilburn, Jean M., and others : Sub-fractionation of Human Serum Leucine Aminopeptidase, 1203
Hildemann, Dr. W. H. : Blood Groups in Infra-human Species (Genetic Specificities of Amphibians and Reptiles), 654
Hill, Sir Austin Bradford : Title of professor emeritus of medical statistics (at the London School of Hygiene and Tropical Medicine) conferred on, 546
Hill, D. A., and Porter, N. A. : Photography of Cerenkov Light from Extensive Air Showers in the Atmosphere, 690
Hill, Prof. D. : Assessment of Pain (Central Mechanisms of Pain), 1353
Hill, Joseph M. (Hunter, jun., DeWitt T., and) : Surfen, a Quinoline with Oncogenic and Heparin-neutralizing Properties, 1378
Hill, Dr. June : Foetal and Neonatal Physiology (Reaction of the New-born Animal to Environmental Temperatures), 345
Hill Reaction : Photophosphorylation and the (Mary Stiller and Prof. Birgit Vennesland), 677 ; (Dr. N. E. Good), 678 ; (Dr. André T. Jagendorf), 679
Hiller, H. : Space-Flight and Re-entry Trajectories (A Generalized Study of Two-dimensional Trajectories in the Region of the Earth and Moon), 960
Hilton, W. F. : Space-Flight and Re-entry Trajectories (Dynamics of Terminal Re-entry—Re-entry Paths which avoid the Zones of Radiation), 960
Himalaya : Science in the (Dr. L. G. C. E. Pugh), 429
Hirashima, Kunitake, and others : Effect of Bilateral Section of the Splanchnic Nerve on Erythropoiesis, 500
Hirsch, Dr. P. B. : Grant of £3,900 from the Paul Instrument Fund, for the construction of an electron optical instrument, 225
Hirsch, Dr. Willy (Frenkel, Ayala, and) : Spontaneous Development of *L* Forms of Streptococci requiring Secretions of other Bacteria or Sulphydryl Compounds for Normal Growth, 728
Histamine : Incorporation of, into Liver Protein *in vivo* (Isabel Wajda, Michael Ginsburg and Prof. Heinrich Waelsch), 1204
  Relationship between the Decrease in Urinary Excretion of, and the Initiation of the Labour Process (Dr. Halina Wiskont-Buczkowska), 290
Histamine Metabolism : Anabolic Agents and (Dr. K. S. Kim), 1368
Histidine Decarboxylase and Growth (D. Mackay, Joan D. Reid and Dr. D. M. Shepherd), 1311
Histochemical Demonstration of Gamma-Glutamyl Transpeptidase (Prof. Z. Albert, Dr. Marian Orlowski and Dr. A. Szewczuk), 767
Histochemical and Homogenization Studies of Mitochondrial α-Glycerophosphate Dehydrogenase in the Nervous System (R. Hess and Dr. A. G. E. Pearse), 718
Histological Work : Ethylene Glycol Monomethyl Ether, a New Fixative for (Prof. Walter B. Shelley and Ralph Florence), 719
Histone (Calf Thymus) : Effect of, on Rats bearing Transplantable Acute Myeloid Leukaemia (Dr. Lubomir Hnilica and Dr. Viktor Holoubek), 922
Histones isolated from Different Tissues : Influence of Polyvalent Ions on (I. P. S. Agrell and E. G. Christensson), 284
History of the American Philosophical Society (E. G. Conklin), 335
History of Medicine and Pharmacy : Second Annual Report and List of Members (1960-61) of the Faculty of the (Worshipful Society of Apothecaries of London), 21
History of the Sheffield Smelting Company, Ltd., 1760-1960 : Two Hundred Precious Metal Years—a (Ronald E. Wilson), *review* by Prof. R. W. K. Honeycombe, 108
History (Quaternary) : Two Unexplored Records of (A. H. Stride and R. Bowers), 1000
History (A Social), of Engineering (Prof. W. H. G. Armytage), *review* by Prof. M. Postan, 946
Hnilica, Dr. Lubomir, and Holoubek, Dr. Viktor : Effect of Calf Thymus Histone on Rats bearing Transplantable Acute Myeloid Leukaemia, 922
Hobbs, Dr. Betty : Bacteria of the Intestine (The Public Health Significance of *Salmonella* Carriers in Livestock and Birds), 1153
Hobbs, Dr. Betty, and others : Bacteria of the Intestine (Studies on the Growth, Sporulation and Carriage of *Clostridium welchii* with special reference to Food Poisoning Strains), 1153
Hodges, H. W. M.: At the sixty-seventh Annual Conference of the Museums Association (Problems of Conservation), 554
Hodgkin, Dr. Dorothy, and Lenhert, Dr. P. G. : Vitamin B_{12} and Intrinsic Factor (Vitamin B_{12} Coenzyme), 1154
Hodgman, —.: Hazards of the Animal House (Hazards encountered in dealing with Dogs and Cats), 339
Hodgson, G. W., and Peake, Eric : Metal Chlorin Complexes in Recent Sediments as Initial Precursors to Petroleum Porphyrin Pigments, 766
Hodson, H. F., and others : Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine, 1312
Hog Thyroglobulin : Chromatographic Fractionation of (Prof. Nobuo Ui, Osamu Tarutani, Yoichi Kondo and Hiroko Tamura), 1199
Högbom, Dr. J. A. (Christiansen, Prof. W. N., and) : Cross-Antenna of the Proposed Benelux Radio Telescope, 215
Holeysovsky, Dr. V., and others : Paper Chromatography (Film showing the Technique of Paper Chromatography of Amino-Acids), 759
Holian, John, and others : Radiation-induced Formation and Reactions of the Formyl Radical Ion, 1386
Holistic Approach to Selenology (Brian Warner), 586
Hollingsworth, D. F. : Tradition, Science and Practice in Dietetics, 1343
Holmes, Dr. J. L. : Appointed a lecturer in chemistry at the University o Edinburgh, 443
Holoubek, Dr. Viktor (Hnilica, Dr. Lubomir, and) : Effect of Calf Thymus Histone on Rats bearing Transplantable Acute Myeloid Leukaemia, 922
Holt, Dr. S. J., and Hicks, Dr. R. Marian : Use of Veronal Buffers in Formalin Fixatives, 832
Holttum, Dr. R. E. : Plant Taxonomy as a Scientific Discipline (Presidential Address to Section K (Botany) of the British Association for the Advancement of Science), 990
Homburger, F. (edited by) : Progress in Experimental Tumor Research, Vol. I, *review* by Prof. F. Bergel, 1231
Homogeneously Absorbing Object : The Feulgen-Stained Somatic Cell Nucleus as a (Dr. Simon Iversen), 150
Homogenization Studies (Histochemical and), of Mitochondrial α-Glycerophosphate Dehydrogenase in the Nervous System (R. Hess and Dr. A. G. E. Pearse), 718
Homograft Response : Regeneration of Elements of Donor Origin in Orthotopically Grafted Skin following the (Dr. J. K. Dineen and Dr. Aleksander Szenberg), 153
Homotypic Long Bones : Number and Arrangement of Osteons in Corresponding Regions of (Dr. G. Marotti), 1400
Honeybee Larval Foods : Chromatographic Investigation of the Methyl Alcohol/Chloroform-soluble Constituents of (N. G. Patel, M. H. Haydak and R. Lovell), 362
Honeycombe, Prof. R. W. K. : From Buttons to Bullion, *review*, 108
Hönl, Prof. Helmut : Elected a Member of the Physics Section of the Leopoldina German Academy of Sciences, 122
Hope, A. B. : The Action Potential in Cells of *Chara*, 811
Hope (New), for African Wildlife, 958
Hopes of To-morrow, 1030
Hopkins, Dr. C. A., and Williams, M. O. : Parasite Biology (Application of New Laboratory Techniques), 135
Hopkins, Sir Frederick Gowland : Memoir to mark the Centenary of the birth of (Prof. Ernest Baldwin), (Van den Berghs, Ltd.), 439, *erratum*, 546
Hops (*Humulus lupulus* L.) : Treatment of, with Gibberellic Acid—Effect of Spraying with Gibberellic Acid on Yield and Composition (R. Stevens, J. B. Roberts and I. H. Williams), 360 ; Effect on Growth and Development (I. H. Williams), 361
Hopsu, Prof. Väinö K. (Talanti, Prof. S., and) : Leucine Aminopeptidase in the Mammary Gland of the Cow and Rat, 86
Hormone : An Immunological Study of a Human Pituitary Interstitial Cell-stimulating (N. R. Moudgal and Prof. Choh Hao Li), 192
Hormone (Brain), of the Silkworm, *Bombyx mori* (Mamori Ichikawa and Hironori Ishizaki), 933
Hormone (Pituitary Growth) : Effect of on Cholesterogenesis (Dr. A. L. Greenbaum and R. S. Leal), 600
Horn af Rantzien, Dr. H. : *Obituary* by Dr. C. Edelstam, 121
Hornby Libraries : Brown, Picton and (Liverpool Libraries, Museums and Arts Committee), 21
Horner, P. : Thermoelectric Power of Single Crystals of Gallium, 58
Horner, R. G. : At the Maxwell Colour Centenary Conference (Problems of Colour Reproduction), 11
Horticultural Society (Royal) : Bowles Memorial Travel Scholarship, 1349
Horticultural Society (Royal), and others : Third World Orchid Conference, London, 1960—Proceedings of the, *review* by V. S. Summerhayes, 213
Horticulture (Agriculture and), in Northern Ireland : Aids to, 959
Horton, Dr. A. A. (Goodwin, Prof. T. W., and) : Biosynthesis of Riboflavin in Cell-free Systems, 772
Horton-Smith, C. : Immunity to Protozoa (Local Immunity in Coccidiosis), 553
Hoskin, Dr. M. A. : Background to Astronomy, *review*, 840
Hospital In-Patient Enquiry : Report on, for the Year 1959, Part I, Preliminary Tables (Ministry of Health and General Register Office), 345
Hospital Physicists' Association : Douglas Lea Memorial Lecture (Dr. E. L. Powers), 1045
Hospital Service : Abstracts of Efficiency in the (Ministry of Health), 441
Hospitals : Sterilizing Materials for (Brian Watkin), 1044
Hot Spring Areas with Acid–Sulphate–Chloride Waters (Dr. A. J. Ellis and S. H. Wilson), 696
Hotz, Dr. G. : Effects of Ionizing Radiation on Organic and Biological Solids (Role of Oxygen in Radiation Damage), 1150
Houbolt, J. C. : Space-Flight and Re-entry Trajectories (Techniques of Orbital Rendezous), 960
Houde, Dr. R. W. : Assessment of Pain (Pathological and Experimental Pain in terms of the 'Ridit' Unit), 1354
Houghton-Evans, Dr. W. : Appointed lecturer in architectural engineering at the University of Leeds, 650
Hounam, R. F. : Design and Use of Respirators (Apparatus for Protecting People who handled Radioactive Substances), 656
House of Commons : Debate on Shipping and Shipbuilding, 543
  Debate on Apprenticeship and Training, 737
  Debate on Science, 837
  Adjournment Debate on the Research Work of the Nature Conservancy, 962
  Debate on the Social Sciences, 1354

House of Lords : Committee stage of the Rating and Valuation Bill, 123
Houseflies : Pattern of Pyrethrin-resistance in (B. C. Fine), 884
House-Mouse : Pulmonary Tumours and Group VII in the (Dr. Joyce L. Bloom), 1124 ; (Dr. J. A. H. Tatchell), 1125
Housing and Local Government, Ministry of : Pollution of Water by Tipped Refuse—Report of the Technical Committee on the Experimental Disposal of House Refuse in Wet and Dry Pits, 864
How, M. J. : Awarded a general fellowship in chemistry for 1961-62 by the Ramsay Memorial Trust, 337
How Old are the Angiosperms? : (D. I. Axelrod), 442 ; (—. Scott, —. Barghoorn, and —. Leopold,), 442
Howard, F. T. (edited by) : Sector-Focused Cyclotrons—Proceedings of an Informal Conference, Sea Island, Georgia, 1959 (United States National Academy of Sciences—National Research Council, Nuclear Science Series, Report No. 26, Publication No. 656), *review* by M. Snowden, 532
Howard, Dr. J. G. : Resistance to Infection with *Salmonella paratyphi C* in Mice parasitized with a relatively Avirulent Strain of *Salmonella typhymurium*, 87
Hsu, K. C., and others : Antibodies to Trophoblasts during the Post-Partum Period, 510
Hubbard, Dr. J. I., and Schmidt, Robert F. : Stimulation of Motor Nerve Terminals, 1103
Hudlicky, Milos : Chemie der Organischen Fluorverbindungen, *review* by Dr. Richard Dresdner, 319
Hudson, Dr. F. Lyth : A Laboratory Handbook of Pulp and Paper Manufacture, *review*, 320
Hudson, Dr. P. S. : Plant Exploration and Introduction (Report of the British Commonwealth Potato Collection), 1240 ; (Gene Resources of the World), 1241
Huggins, A. K., and Moses, V. : Breakdown of [2^{14}-C]-Glycine during Paper Chromatography, 668
Huggins, William H., and others : Operations Research and Systems Engineering, *review* by Prof. L. S. Goddard, 105
Hughes, Dr. Agnes M. : Title of reader in biology in the University of London (at the Royal Free Hospital School of Medicine) conferred on, 229
Hughes, Dr. E. O. : Appointed scientific attaché to the Canadian Embassy in Washington, 542 ; work of, 542
Hughes, Dr. G. M., and Tauc, L. : The Path of the Giant Cell Axons in *Aplysia depilans*, 404
Hughes, W. L. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Effects of Radiation on Cell Division), 341
Huis in't Veld, L. G., and others : Neutral 17-Ketosteroids in Porcine Urine, 175
Hulka, J. F., and others : Antibodies to Trophoblasts during the Post-Partum Period, 510
Hulme, Dr. A. C. (Jones, Dr. J. D., and) : Preparation of Mitochondria from the Peel of Apples, 370
Human Anatomy : Atlas of (edited by Prof. Ferenc Kiss and Prof. János Szentagothai), Vol. 1, Osteology, Syndesmology, Myology ; Vol. 2, Splanchnology, Endocrine Glands, Heart ; Vol. 3, Nervous System, Vascular System, Lymphatic System, Sense Organs, *review* by Prof. J. M. Yoffey, 1232
  Illustrations of, *review* by Prof. J. M. Yoffey, 1232
Human Aorta during Growth : Increase in Elastin Content of the (Dr. V. Scarselli), 710
Human Aortic Tissue : Degree of Polymerization of Haluronic Acid isolated from (Dr. Sv. Bertelsen and K. Marcker), 386
Human Beings : Effect of Sodium Fluoride on Calcium Metabolism of (Dr. Clayton Rich and John Ensinck), 184
Human Beings (Irradiated) : Decreased Urinary Excretion of 2-Aminoethanol by (Dr. Ralph R. Cavalieri, M. T. Van Metre and W. I. Sivertsen), 1303
Human Blood-Group A Substance : Two Serologically Active Trisaccharides isolated from (I. A. F. Lister Cheese and Prof. W. T. J. Morgan), 149
Human Body : Determination of Caesium-137/Potassium Ratios in Diet and in the, by Urine Analysis (A. Morgan and Gloria M. Arkell), 1100
Human Bone Tissue : Blood-Typing Compact (H. E. Ezra-Cohn and Prof. S. F. Cook), 1267
Human Cancer Cells (HeLa) in Culture : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Human Chromosome Counts in relation to Age : Distribution of (Patricia A. Jacobs, W. M. Court Brown and Dr. Richard Doll), 1178
Human Epidermoid Carcinoma Cells in Culture : Biochemical Aspects of Resistance to 6-Mercaptopurine in (R. W. Brockman, George G. Kelley, Patricia Stutts and Virginia Copeland), 469
Human Erythrocyte : Sialic Acids and the Electrokinetic Charge of the (G. M. W. Cook, Dr. D. H. Heard and Dr. G. V. F. Seaman), 44
Human Faeces : Isolation of Sterol Esters from (Francis Aylward and Pamela Wills), 1397
Human Genetics : Principles of (Curt Stern), second edition, *review* by Dr. G. Ainsworth Harrison, 110
Human Haematopoietic System : Effects of Ionizing Radiation on the—Report of the Subcommittee on Haematologic Effects, Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 875), 556
Human Haemoglobins (Abnormal) : Thalassaemia and the (Dr. Harvey A. Itano and Prof. Linus Pauling), 398
Human Pituitary Interstitial Cell-stimulating Hormone : An Immunological Study of a (N. R. Moudgal and Prof. Choh Hao Li), 192
Human Red Cells : Common Carrier System for Sugar Transport in (Dr. L. Lacko and Dr. M. Burger), 881
  A Relation between the Reactivity of, to Antiglobulin Tests and their Surface Structure (Judith E. Davis, Prof. H. N. Green and Pamela W. Tymms), 923
Human Serum Cholinesterase with Fluoride : Differential Inhibition of—Recognition of Two New Phenotypes (Prof. H. Harris and Mary Whittaker), 496
Human Serum Leucine Aminopeptidase : Sub-fractionation of (Prof. Clyde A. Dubbs, Charlotte Vivonia and Jean M. Hilburn), 1203
Human Side of Enterprise (D. McGregor), 1332
Human Urine (Normal) : Quantitative Determination of Metadrenaline and Normetadrenaline in (Dr. Kaoru Yoshinaga, Dr. Chuichi Itoh, Dr. Nozomu Ishida, Dr. Tatsuo Sato and Dr. Yasuo Wada), 599
Human Vision : Limits of (Dr. R. A. Weale), 471
Human Whole Blood : Biosynthesis of Polyenoic Fatty Acids in (Dr. F. Leupold and G. Kremer), 805
Human and Mouse Tumour Cells : Selective Uptake of Specifically Bound Cobalt-58 Vitamin B_{12} by (Dr. Bernard A. Cooper and Dr. William Paranchych), 393
Humanity of Man (Sir Wilfrid Le Gros Clark), 977 ; (Dr. Launcelot Fleming), 1229
Humanum : Ovum (Prof. Landrum B. Shettles), *review* by W. J. Hamilton, 110
Hume, Capt. C. W. : Assessment of Pain, 1354
Hummingbirds (Active, Sleeping and Torpid) : Thyroid Function in (Prof. C. J. Shellabarger, R. C. Lasiewski and G. E. Hyncik), 1318
Humphreys, O. W. : *Obituary* of J. Walter Ryde, 17
Humulus lupulus L. (Hops) : Treatment of, with Gibberellic Acid—Effect of Spraying with Gibberellic Acid on Yield and Composition (R. Stevens, J. B. Roberts and I. H. Williams), 360 ; Effect on Growth and Development (I. H. Williams), 361
Humus : Isolation of Anthraquinone from (Kyoichi Kumada, Akio Suzuki and Kazuyuki Aizawa), 415
  Resistance of, to Decomposition (H. F. Birch and M. T. Friend), 731
Hunger : A Strategy for the War on (Sir Joseph Hutchinson and Prof. A. H. Bunting), 539
'Hunger Motivational' System : Evidence of a, in the Lateral Hypothalamus of the Rat (Dr. P. J. Morgane), 672
Hunt, Dr. J. N. : Appointed reader in physical oceanography at the Imperial College of Science and Technology, London, 443, 1349
Hunt, Dr. R. W. G. : At the Maxwell Colour Centenary Conference (Problems of Colour Reproduction), 11
Hunter, jun., Dewitt T., and Hill, Joseph M. : Surfen, a Quinoline with Oncogenic and Heparin-neutralizing Properties, 1378
Hunter, G. : Studies in Management, 1332
Hurter, Rona C., and others : Aldosterone Excretion and Potassium Retention in Subjects living at High Altitude, 78
Hutchinson, Sir Joseph, and Bunting, Prof. A. H. : A Strategy for the War on Hunger, 539
Hutner, S. H., and others : Vitamin-Levels in Blood and Serum, 78
Hutson, Dr. V. C. L. : Appointed lecturer in applied mathematics at the University of Sheffield, 377
Hyaluronic Acid isolated from Human Aortic Tissue : Degree of Polymerization of (Dr. Sv. Bertelsen and K. Marcker), 386
Hyaluronidase : Urinary Inhibitors of (P. J. Knudsen and J. Koefoed), 1306
Hyashi, Dr. K. : Effects of Ionizing Radiation on Organic and Biological Solids (Reactions in the Solid State), 1150
Hybrid (Bulk-) Population of Barley : Stabilizing Selection for Heading Time in a (Dr. S. K. Jain), 1123
Hybrid (A Natural), of *Tilapia nigra* and *Tilapia leucosticta* from Lake Naivasha, Kenya Colony (H. Y. Elder and D. J. Garrod), 722
Hybridization : Effect of, on the Primary Polydipsic Trait of an Inbred Strain of Mice (Dr. Emanuel Silverstein), 523
Hyderabad Regional Research Laboratory : Annual Report (1959-60), 757
Hydra : Sexual Differentiation in, in relation to Population Density (Helen D. Park, Christyna Mecca and Anne Ortmeyer), 92
Hydrastine : Biosynthesis of (J. R. Gear and Prof. I. D. Spenser), 1393
Hydrated Salts (Molten) : Molar Sound Velocity in (P. R. K. L. Padmini and Prof. B. Ramachandra Rao), 694
Hydrazine (Diborane–) Flames : Formation of Boron Nitride in (W. G. Berg and W. E. Wilson), 380
Hydrazoic Acid (Nitric Acid–) : A New Inorganic Reaction— (Dr. J. Mašek), 166
Hydrazones (α-Keto-Acid) : Application of the Nickel Reaction and Thermal Isomerization of, for their Chromatographic Analysis (A. M. Tokushige and Dean E. S. Cook), 797
Hydride (Palladium) : Heat Capacity and Resistivity Anomalies in (Paul Mitacek, jun., and Prof. J. G. Aston), 271
Hydrocarbons (Condensed Ring Aromatic) : π-Electronic Excitation and Ionization Energies of (Dr. J. B. Birks and M. A. Slifkin), 761
Hydrocarbons (Polycyclic Aromatic) : Non-Enzymic Hydroxylation of (F. Dewhurst and G. Calcutt), 808
Hydrochloride Isomers : Mannosyl D-Glucosamine—*N*-Mannosyl D-Glucosamine Hydrochloride (Dr. S. A. Barker, Dr. K. Murray and Prof. M. Stacey), 142 ; 6-*O*-Mannosyl D-Glucosamine Hydrochloride (Dr. S. A. Barker, Dr. K. Murray, Prof. M. Stacey and D. B. E. Stroud), 143
Hydrocortisone and Methopyrapone (Corticotrophin) : Effects of, on the Circulating Eosinophiles in Man (Dr. N. J. Kothari and Dr. J. C. Saunders), 1105
Hydrocortisone, the Sulphydryl Group, and the Catechol Amines : Interaction of (Dr. Sidney Roston), 490
Hydrogen : Selectivity of Palladium Catalysts for the Oxidation of Carbon Monoxide in the Presence of (P. Mars and Th. van de Mond), 1003
Hydrogen in Gaseous Mixtures : Determination of, by Gas-Chromatography (P. J. Kipping), 270
Hydrogen Cyanide : Mechanism of Synthesis of Adenine from, under Possible Primitive Earth Conditions (Prof. J. Oró), 1193
Hydrogen Ion Concentration, Plankton and Fish in Fresh-water Eutrophic Lakes of India (Dr. S. M. Das), 511
Hydrogen Peroxide : Cell Transplants of Avian Lymphomatosis treated with (Dr. V. L. Sanger), 1406
  Reactions of Haemoproteins with, and the Supposed Formation of Hydrogen Peroxide during the Autoxidation of Haemoglobin (Prof. D. Keilin), 769
Hydrogen Reduction Characteristics of Natural and Artificial Haematites (John Henderson), 268
Hydrogen Transfer (Electron and) : Coupling of Phosphorylation to, by a Chemi-Osmotic Type of Mechanism (Dr. Peter Mitchell), 144
Hydrogenolysis of 2,4-Dinitrophenylhydrazones of Aromatic Keto-Acids : Side-Reactions during (Dr. John H. Menkes), 285
Hydrogenomonas (Knallgas Bacteria) : Formation and Utilization of Poly-β-Hydroxybutyric Acid by (Prof. H. G. Schlegel, G. Gottschalk and R. von Bartha), 463
Hydrolysates (Protein) : A Simplified Method for Separating Amino-Acid Mixtures from, on One Ion-Exchange Column during Preparation (J. Liebster, K. Kopoldová and M. Dobiášová), 1198

Hydrolysis of Fatty Acid Esters of Riboflavin by Pancreatic Lipase (Prof. Kunio Yagi, Yoshiko Yamamoto and Jun Okuda), 174
Hydrolysis (Alkaline), of *M*- and *N*-Blood Group Substances : Products of Mild Acid and (Dr. Elzbieta Romanowska), 1408
Hydrolysis ('Heat Catalysed') : Amino-Acid Liberation from Proteins by Enzyme or (S. M. Hardy), 1061
Hydrolysis (Partial Acid-), of a Mucopolysaccharide without Appreciable *N*-Deacetylation of Hexosamine (Dr. T. J. Painter and Prof. W. T. J. Morgan), 39
Hydromechanics Research Association (British) : Open Days (S. T. Bonnington), 652
Hydrostatic Function of the Accessory Respiratory Organs in Air-breathing Fishes (Dr. S. Z. Qasim and R. Hasan), 396
Hydrostatic Pressure : Mechanism of Sensitivity to, in the Prawn, *Palaemonetes varians* Leach (Peter S. B. Digby), 366
Hydroxylamine : Enzymic Binding of, by Fumaric Acid (Prof. Nathan Grossowicz and the late Yehudith Lichtenstein), 412
Hydroxylation (Non-Enzymic), of Polycyclic Aromatic Hydrocarbons (F. Dewhurst and G. Calcutt), 808
Hydroxyproline Excretion : Influence of Scurvy and Lathyrism (Odoratism) on (Dr. G. R. Martin, S. E. Mergenhagen and D. J. Prockop), 1008
5-Hydroxytryptamine : Formation of, in an Aqueous Solution of 5-Hydroxytryptophan treated with X-Rays (M. Jović and Z. Supek), 383
5-Hydroxytryptophan treated with X-Rays : Formation of 5-Hydroxytryptamine in an Aqueous Solution of (M, Jović and Z. Supek), 383
Hylander, Dr. N. : Flora Europaea (Report of the Nomenclature Subcommittee), 449
Hymenolepis nana (Cestoda : Hymenolepididae) : A Natural Population of Anomalous Branched Tapeworms, in a Colony of *DBA*/1 Mice (Dr. Donald Heyneman), 297
Hyncik, G. E., and others : Thyroid Function in Active, Sleeping and Torpid Hummingbirds, 1318
Hypodermic Probe using Fibre Optics (David F. Capellaro, Narinder S. Kapany and Dr. Charles Long), 927
Hypomagnesaemia in Lactating Cows given Artificial Rations Low in Magnesium : Rapid Development of (J. A. F. Rook), 1019
Hypoprothrombinaemia (Coumarin-induced) : Lack of Effect of Coenzyme Q_{10} on (Prof. Murray Weiner, Dr. Theodore Chenkin and Dr. Peter G. Dayton), 711
Hypothalamus : Action of the (John Erskine Malcolm), 606
  Increase of Evaporative Loss of Water from the Skin of the Ox in response to Local Heating of the (Dr. D. L. Ingram, J. A. McLean and Dr. G. C. Whittow), 81
Hypothalamus (Lateral), of the Rat : Evidence of a 'Hunger Motivational' System in the (Dr. P. J. Morgane), 672
Hypothesis (Chelation), of Auxin Action : Improbability of the (Dr. D. D. Perrin), 253
Hyslop, N. St. G. : A Method of Treating Asbestos Filter Pads for Virus Filtration, 305

IBALL, Dr. J. (Cole, Dr. A. J., and) : Digital Computers, their Use and Control, 554
IBM Co. : New Data-processing Centre, 23
IBM Research Fellowships (International Business Machines, United Kingdom, Ltd.), 442
Ibrahim, S. A. (Vella, Dr. F., and) : Thalassaemia in an English and in a German Family, 822
Ice Nucleators : Steroids as (Richard B. Head), 1058
Iceland : Soil Temperatures in (Dr. Bjarni Helgason), 939
Ichikawa, Mamori, and Ishizaki, Hironori : Brain Hormone of the Silkworm, *Bombyx mori*, 933
ICSU Review, Vol. 3, No. 1, 865
Identical Amino-Acid Sequences : Immunochemical Distinction between Insulins with (Dr. Solomon A. Berson and Dr. Rosalyn S. Yalow), 1392
Identification : Essential Oil from Fresh *Cannabis sativa* and its Use in (L. Martin, D. Morison Smith and C. G. Farmilo), 774
Identification of Ammocoete Species : Oocyte Numbers as a Diagnostic Character for the (Dr. M. W. Hardisty), 1215
Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of *Penicillium chrysogenum* (A. Ballio, Prof. E. B. Chain, F. Dentice di Accadia, M. Mauri, K. Rauer, M. J. Schlesinger and Sondra Schlesinger), 909
Identity of L-Ascorbic Acid formed from D-Glucose by the Strawberry (*Fragaria*), (Dr. Frank A. Loewus and Stanley Kelly), 1059
Identity (Microstructural and Chemical), of the Bear Creek and Narraburra Iron Meteorites (Dr. H. J. Axon), 1287
Iggo, Dr. A. : Assessment of Pain (Conduction in Single *C* Fibres of Impulses initiated by Noxious Stimuli in the Viscera, Muscles and Skin), 1353
Ignition of Gas by Sparks produced by Breaking Wires at High Speed (Dr. R. L. Gordon, Dr. H. Lord and D. W. Widginton), 1085
Illingworth, J. R. : The National Physical Laboratory—Open Days, 112
Illinois Institute of Technology, Chicago : International Symposium on Photoelasticity, 755
Illusions (Geometrical) : Centrally Produced (S. Papert), 733
Illustrations of Human Anatomy, *review* by Prof. J. M. Yoffey, 1232
Imaizumi, Prof. R., and others : Potentiation of Epinephrine Action, 186
Imidazole : Electronic Properties of (S. Aftergut and G. P. Brown), 379
Imipramine : Demonstration of Anti-Depressant or Stimulant Properties of, in Experimental Animals (Dr. D. R. Maxwell and Helen T. Palmer), 84
Immature Germ Cells of *Drosophila* : Chromosome Rearrangements induced by X-Rays in (Seymour Abrahamson), 523
Immunity to Protozoa (British Society for Immunology Symposium on), 551
Immunity (Non-Specific), in Pneumococcal Infection of Mice (Dr. G. N. Cooper and Dr. A. E. Stuart), 295
Immunochemical Distinction between Insulins with Identical Amino-Acid Sequences (Dr. Solomon A. Berson and Dr. Rosalyn S. Yalow), 1392
Immunoelectrophoretic Examination of Agammaglobulinaemia (Fred Rosen, Charles A. Janeway and Prof. David Gitlin), 1109
Immuno-electrophoretic Patterns (Electrophoretic and), of Extracellular Diphtheria Proteins from Culture Filtrates of Different Iron Concentrations (Bertold J. Radola), 382
Immunofluorescence of Pleuropneumonia-like Organisms isolated from Tissue Cell Cultures (Major W. F. Malizia, M. F. Barile and D. B. Riggs), 190
Immunological Observations in Experimental Silicosis (J. H. Jones and Prof. A. G. Heppleston), 1212
Immunological Study of Experimentally Induced Extrahepatic Cholestasis (Dr. Roy G. Shorter and R. G. Lack), 610
Immunological Study of a Human Pituitary Interstitial Cell-stimulating Hormone (N. R. Moudgal and Prof. Choh Hao Li), 192
Immunological (Auto-) Precipitin possibly due to Fibrinolysins : Demonstration by Paper Electrophoresis of an (Marion I. Cooper and J. R. Sykes), 1212
Immunology, British Society for : Symposium on Immunity to Protozoa, 551
Impact of Industrial Development on the Countryside (Council for the Preservation of Rural England Conference on), 959
Imperial Chemical Industries, Ltd. : New Research Laboratories for the Paints Division of (J. W. Dorling), 25
Imperial College of Science and Technology, London : Appointment of Dr. J. S. Webb to the University of London chair of applied geochemistry, 229
  Advanced Study Institute on Underwater Acoustics, 229
  Appointment of Dr. M. Gordon to the University of London readership in physical chemistry, 337
  Appointment of Dr. C. B. Alcock as reader in chemical metallurgy, 443, 1349
  Appointment of Dr. J. N. Hunt as reader in physical oceanography, 443, 1349
  Appointment of Dr. J. A. Kitchener as reader in the science of mineral processing, 443, 1349
  Title of professor emeritus of chemical engineering conferred on Prof. D. M. Newitt, 546
  Appointment of Prof. W. D. Gill to the chair of oil technology, 651
  Fourteenth International Instruction Course by Carl Zeiss, Jena, on Fine Measuring Instruments, 1045
  Title of professor of electrical engineering in the University of London conferred on Dr. J. H. Westcott, 1349
  Appointment of Dr. J. R. Butler as reader in pure geochemistry, 1349
Implant (Sarcoma), and Staphylococcus Infection : Development in Mice of Prolonged Non-specific Resistance to, following Repository Injection of Lipopolysaccharide (Samuel J. Prigal), 1111
Implant Material in the Rat : Biological Incorporation of (James Calnan), 1017
Implants (Zinc), in Rat Bladders : Incorporation of Phosphorus-32 into Urinary Concretions forming on (Dr. Richard F. Keeler and Dr. Stuart A. Lovelace), 715
Importance of Magnesium in the Control of Plasma Calcium in the Calf (Dr. R. H. Smith), 181
Importance of Nitrogen in Crop Production (Prof. N. R. Dhar), 544
Impregnated (Cathodically), and Cooled Austenite : Transformation of (E. G. Ramachandran and C. Dasarathy), 998
Improbability of the Chelation Hypothesis of Auxin Action (Dr. D. D. Perrin), 253
Improving Biological Communications in the United States, 23
In-Patient Enquiry (Hospital) : Report on, for the Year 1959, Part I, Preliminary Tables (Ministry of Health and General Register Office), 345
Inactivating Efficiency of Radiation : Comparative Analysis of, on Different Organisms (Dr. M. Terzi), 461
Inactivation and Reactivation of Chymotrypsin (T. Therattil Antony), 178
Inbred Mouse Strains : Social Dominance in (Prof. G. Lindzey, H. Winston and M. Manosevitz), 474
Inbred Strain of Mice : Effect of Hybridization on the Primary Polydipsic Trait of an (Dr. Emanuel Silverstein), 523
Inbred Strains : An Iso-Antigen ($\gamma B A$) of Mouse γ-Globulin present in (A. Kelus and J. K. Moor-Jankowski), 1405
Inclusion of Parental Somatic Cells in Sponge Larvae (Frederick E. Warburton), 1317
Inclusions of Mucopolysaccharide in the Lymphocytes of Patients with Gargoylism (Ursula Mittwoch), 1315
Incompressible Aerodynamics (edited by Bryan Thwaites), *review* by Prof. Leon Trilling, 315
Incorporation of 5,10,15 ^{14}C-Farnesol Pyrophosphate into *Phycomyces carotenoids* (H. Yamamoto, H. Yokoyama, K. Simpson, T. O. M. Nakayama and C. O. Chichester), 1299
Incorporation of ^{14}C-Uridine into Rat Lens Ribonucleic Acid by Rat Lens Enzyme Systems : Catalysis of (Dr. Anima Devi and Dr. N. K. Sarkar), 1094
Incorporation of Deoxyribonucleic Acid by Mammalian Cells *in vitro* (Prof. E. R. M. Kay), 387
Incorporation of Histamine into Liver Protein *in vivo* (Isabel Wajda, Michael Ginsburg and Prof. Heinrich Waelsch), 1204
Incorporation of Phosphorus-32 into Urinary Concretions forming on Zinc Implants in Rat Bladders (Dr. Richard F. Keeler and Dr. Stuart A. Lovelace), 715
Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture : Effect of Ribonuclease and Deoxyribonuclease on (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Incorporation (Biological), of Implant Material in the Rat (James Calnan), 1017
Increase in Elastin Content of the Human Aorta during Growth (Dr. V. Scarselli), 710
Increase of Evaporative Loss of Water from the Skin of the Ox in response to Local Heating of the Hypothalamus (Dr. D. L. Ingram, J. A. McLean and Dr. G. C. Whittow), 81
Increase of Oxygen Consumption induced by Potassium in Muscle : Inhibition by Physostigmine of the (Dr. Ivan Novotny and František Vyskočil), 916
Increase of the Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at Early Stages of Propylthiouracil Treatment in the Rat (Dr. E. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171
Increase in Specific Bactericidal Antibodies after Administration of Endotoxin (J. Gabriel Michael, James L. Whitby and Maurice Landy), 296
Increase (Hepatic Induced), of Tryptophan-Pyrrolase : Effect of 5-Fluorouracil on (Sancia Gaetani and M. Antonietta Spadoni), 1296

Increase (Induced), in Resistance of *Entamoeba histolytica* to Fumagillin (Dr. Mitsuru Nakamura), 413
Increasing the Signal-Noise Ratio in the Detection of the First Motion of a Refracted *P*-Wave : Possible Method for (F. A. Key, J. K. Wright, E. W. Carpenter and B. M. H. Stott), 1382
Index (Maxillary), in *Anopheles gambiae* Giles (L. K. H. Goma), 405
Index Biologicus : Publication of (Biological and Medical Abstracts, Ltd.), 544
India : Ferro-Alloy Industry in (National Metallurgical Laboratory, Jamshedpur, Symposium on), 1144
  Hydrogen Ion Concentration, Plankton and Fish in Fresh-water Eutrophic Lakes of (Dr. S. M. Das), 511
  Occurrence of *Salmonellae* in Domestic Animals and Poultry in (V. K. Sharma and Prof. C. M. Singh), 622
  Taxonomy of the Earthworm in (G. E. Gates), 227
India, Geological Society of : *Journal* of the, Vol. 1, 1348
India, Government of, and others : To set up and operate an Aeronautical Research Laboratory and three Civil Aviation Training Centres, 1043
India (Eastern) : Cytology of some Genera of Polypodiaceae in (G. Panigrahi and S. N. Patnaik), 1207
India (South) : Occurrence of *Eutrichocheles modestus* (Herbst) in the Near-shore Waters of Porto Novo (K. Balasubrahmanyan and Dr. J. Jacob), 830
Indian Central Oilseeds Committee : Castorseed and its Products, 753
Indian Science Congress : Presidential Address, Importance of Nitrogen in Crop Production (Prof. N. R. Dhar), 544
Indian Society of Theoretical and Applied Mechanics, and Unesco : Symposium on Non-Linear Physical Problems, 855
Indian Tribe (Chibcha) : New *Rh* Phenotype, *Dccefetf*, found in a (Dr. Miguel Layrisse, Zulay Layrisse, Esperanza García, Dr. Johannes Wilbert and Joaquín Parra R.), 503
Indies (West) : Research on Volcanoes in the, 225
Indigotin Content of Woad (C. Berkeley), 1414
Indium in High-purity Zinc : Determination of (Naïm Koŝaric and the late G. Leliaert), 703
Indium Antimonide at High Pressures : Melting and Polymorphism of (A. Jayaraman, R. C. Newton and Dr. G. C. Kennedy), 1288
Individual (Inter-) Variability in Pigmentation (André Leguebe), 1326
Indole-acetic Acid Oxidase Activity of *Lupinis albus* L. exposed to Different Photoperiods : Effect of Gibberellic Acid on Growth and (F. Sági and A. S. Garay), 824
Indole-3-acetic Acid : Effects of Kinetin and, on Multiplication Rates of *Paramecium* (Mary Annunciata McManus and Kathleen Sullivan), 619
Indole-3-acetic Acid in Plants : 1-(Indole-3-acetyl)-β-D-Glucose, a New Compound in the Metabolism of (Dr. M. H. Zenk), 493
Indole-3-acetic Concentrations : Growth Stimulation by High (T. A. Truelsen), 1410
1-(Indole-3-acetyl)-β-D-Glucose, a New Compound in the Metabolism of Indole-3-acetic Acid in Plants (Dr. M. H. Zenk), 493
Indole–Iodine : Spin Resonance and Electrical Conductivity of (B. Smaller, Dr. I. Isenberg and S. L. Baird, jun.), 168
Induced Changes in the Amounts of Material in the Neurosecretory System of the Desert Locust (Dr. K. C. Highnam), 199
Induced Enzyme Synthesis in Aqueous Suspensions of Starved Stationary Phase *Aerobacter aerogenes* (R. E. Strange), 1272
Induced Increase in Resistance of *Entamoeba histolytica* to Fumagillin (Dr. Mitsuru Nakamura), 413
Induced Increase (Hepatic), of Tryptophan-Pyrrolase : Effect of 5-Fluorouracil on (Sancia Gaetani and M. Antonietta Spadoni), 1296
Induced Pulmonary Tumours in Mice : Inheritance of Susceptibility to (Dr. D. S. Falconer and Dr. Joyce L. Bloom), 1070
Induction of Cancer in Suckled and Unsuckled Female Rats (Prof. Giuliano Briziarelli), 921
Induction of Mutations by Deoxyribonucleic Acid in *Drosophila melanogaster* (Dr. O. G. Fahmy and Dr. Myrtle J. Fahmy), 776
Induction of Tumours by Polyoma Virus—Enhancement by Chemical Agents (K. E. K. Rowson, F. J. C. Roe, J. K. Ball and Dr. M. H. Salaman), 893
Industrial Development : The Impact of, on the Countryside (Council for the Preservation of Rural England Conference on), 959
Industrial Education (Commercial and), British Association for : Interviewing in Twenty-six Steps (John S. Gough), 228
  New Routes to Further Education—Papers presented at the B.A.C.I.E. Spring Conference, London (1961), 739
Industrial Relations in the Welfare State, 1129
Industrial Research in Britain, 1252
Industrial Research Associations in the Government Scheme : Research for Industry 1960—a Report on Work done by (Department of Scientific and Industrial Research), 1252
Industrial Research Fellowships (J. Langham Thompson, Ltd.), 545
Industrial World : Management in the (Prof. F. Harbison and Prof. C. A. Myers), 1332
l'Industrie Minérale, Société de : Sixth International Mineral Processing Congress, 545
Industry : Radioactive Tracers in Chemistry and (Pascaline Daudel), translated by Dr. Ulli Eisner, *review* by Prof. J. R. Partington, 637
Industry 1960 : Research for—a Report on Work done by Industrial Research Associations in the Government Scheme (Department of Scientific and Industrial Research), 1252
Industry in Britain : The Structure of (Prof. G. C. Allen), 1332
Industry (Chemical), Society of : Formation of the Industrial Water and Effluents Group, 853
Industry (Chemical), Society of (Food Group) : Symposium on Recent Advances in Processing Cereals, 755
Industry (Electrical) : Magnetic Materials in the (P. R. Bardell), second edition, *review* by A. C. Lynch, 428
Industry (Electrical Power) : Corrosion Problems in the (Battersea College of Technology Summer School on), 651
Industry (Electronics) : Glass in the, 1143
Industry (Ferro-Alloy), in India (National Metallurgical Laboratory, Jamshedpur, Symposium on), 1144
Industry (Manufacturing), on Merseyside : Ageing and the Semi-Skilled—a Survey in (Alistair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine Cunningham), 1249
Industry (Non-Ferrous Metals), in a Unified Europe (Baron Snoy et d'Oppuers), 546
Industry (Nuclear Energy) : Corrosion in the (Société de Chimie Industrielle Symposium on), 651
Industry (Rubber), Institution of the : Colwyn Medal awarded to Dr. L. R. G. Treloar, 755
  Hancock Medal awarded to M. M. Heywood, 755
Infection by *Fomes annosus* : Inoculation of Pine Stumps against (Dr. J. Rishbeth), 826
Infection with *Salmonella paratyphi C* : Resistance to, in Mice parasitized with a relatively Avirulent Strain of *Salmonella typhimurium* (Dr. J. G. Howard), 87
Infection (Cyclical), of *Glossina mortisans* with Culture Forms of *Trypanosoma rhodesiense* (the late Prof. R. M. Gordon and J. K. Miller), 1317
Infection (Non-Lethal Pathological), of Roots (F. J. Newhook), 615
Infection (Pneumococcal), of Mice : Non-Specific Immunity in (Dr. G. N. Cooper and Dr. A. E. Stuart), 295
Infection (Staphylococcus) : Development in Mice of Prolonged Non-specific Resistance to Sarcoma Implant and, following Repository Injection of Lipopolysaccharide (Samuel J. Prigal), 1111
Infection (Viral), in the Chick Embryo : Mechanism of Recovery from (Dr. Samuel Baron and Alick Isaacs), 97
Infections (Lethal Staphylococcal), in Mice : Biochemical Changes in (George W. Counts, Dr. Ian M. Smith, Dr. Joseph I. Routh, Dr. Elia Ch. Hazard and J. F. McTavish), 783
Infectious Ribonucleic Acid from Poliovirus : Chromatographic Analysis of (C. Cocito, A. Prinzie and P. De Somer), 573
Infectious Ribonucleic Acids derived from Mouse Brains infected with Two Kinds of Arbor Virus Group *B* (Prof. Masahiro Nakamura), 624
Infeld, L.: Theory of the Red Shift and the Nature of Planck's Constant, 1184
Infinitely Many Variates : An Introduction to (Prof. Enders A. Robinson), *review* by Prof. D. V. Lindley, 214
Influence of Electrical Field on Pupation and Oviposition in *Nepytia phantasmaria* Stkr. (Lepidoptera : Geometridae), (Dr. D. K. Edwards), 976
Influence of *p*-Fluorophenylalanine on the Production of Viral Ribonucleic Acid and on the Utilizability of Viral Protein during Multiplication of Fowl Plague Virus (Dr. Christoph Scholtissek and Rudolf Rott), 1023
Influence on Frequency : Damping without (A. Bloch), 1086
Influence of Moisture Changes on Deformation of Wood under Stress (L. D. Armstrong and G. N. Christensen), 869
Influence of Plasma Proteins in the Control of Mitosis-rates in Generating Liver (Dr. J. T. Hemingway), 706
Influence of Polyvalent Ions on Histones isolated from Different Tissues (I. P. S. Agrell and E. G. Christensson), 284
Influence of Salts on the Uptake of Streptomycin by *Escherichia coli* (Paul H. Plotz, Donald T. Dubin and Bernard D. Davis), 1324
Influence of Scurvy and Lathyrism (Odoratism) on Hydroxyproline Excretion (Dr. G. R. Martin, S. E. Mergenhagen and D. J. Prockop), 1008
Influence of Serum Factors on the Species-specificity of Tissue Thromboplastin (Christine Hawkey and J. L. Stafford), 920
Influence (Thymus), in Leukaemogenesis : Analysis of the (Dr. J. F. A. P. Miller), 248
Influenza Viruses, Receptor-destroying Enzyme and Proteases : Action of, on Blood Group Agglutinogen M^g (Prof. Georg F. Springer and Dr. Karlheinz Stalder), 187
Information combining Science, Librarianship and Foreign Languages : Science Information Personnel—the New Profession of (Leonard Cohen and Kenneth Craven), 452
Information (Biological) : Storage and Retrieval of (Dr. G. C. Ainsworth), 12
Information (Published) : The Retrieval of, *review* by Prof. A. R. Ubbelohde, 109
Information (Scientific), Institute for (Philadelphia) : Services, Publications and Techniques, 1045
Infra-Human Species : Blood Groups in (New York Academy of Sciences Conference on), 653
Infra-Red (Far) : A Proposal for a Tunable Source of Radiation for the, using Beats between Optical Masers (Dr. D. C. Lainé), 795
Infra-Red Absorption : Quantitative Analysis of Milk and other Emulsions by (Dr. J. D. S. Goulden), 905
Infra-Red Arts, *review* by Dr. E. S. Waight, 315
Infra-Red Determination (Rapid), of Acetone in the Blood and the Exhaled Air of Diabetic Patients (Dr. Richard D. Stewart, Edward A. Boettner and Bruce T. Stubbs), 1008
Infra-Red Methods (G. K. T. Conn and D. G. Avery), *review* by Dr. E. S. Waight, 315
Infra-Red Spectra of Water-soluble Carbonates (Herman A. Szymanski and Richard Povinelli), 64
Infra-Red Spectroscopic Evidence of Four-fold Co-ordination of Titanium in Barium Orthotitanate (P. Tarte), 1002
Ingle, Ltd., W. L. : Grant of £1,000 during two years to the Procter Department of Food and Leather Science in the University of Leeds, 650
Inglis, Helene S., and others : Biosynthesis of the Pyrethrins, 281
Inglis, Dr. James A., and Halliday, Dr. June W. : Thromboplastic Activity of Red Cells, 821
Ingold, Sir Christopher : Title of professor emeritus of chemistry (at University College, London) conferred on, 546
Ingram, Dr. D. G. (Saison, Dr. R., and) : Blood Groups in Infra-human Species (Blood Groups of Pigs), 654
Ingram, Prof. D. J. E. : From Radar to Spectroscopy—New Regions of the Spectrum, 1146
  Effects of Ionizing Radiation on Organic and Biological Solids (Use of Electron-Spin Resonance in the Study of Proteins), 1151
Ingram, Dr. D. L., and others : Increase of Evaporative Loss of Water from the Skin of the Ox in response to Local Heating of the Hypothalamus, 81
Ingram, Prof. V. M. : Haemoglobin *F* and the Genetic Control of Protein Structure, 608
Inhaled Radioactive Particles : Effects of—Report of the Subcommittee on Inhalation Hazards, Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 848), 556
Inheritance of Susceptibility to Induced Pulmonary Tumours in Mice (Dr. D. S. Falconer and Dr. Joyce L. Bloom), 1070
Inheritance (Apparent Cytoplasmic), of Tyrosinase Competence in *Streptomyces scabies* (Prof. K. F. Gregory and Wei-jen Shyu), 465
Inhibition of Chemi-Luminescence of Luminol by means of Oximes (Prof. K. Weber, J. Matković and D. Fleš), 177
Inhibition of β-Glucuronidase by Oral Administration of D-Glucosaccharo-1,4-Lactone (S. Akamatsu, Dr. A. Kiyomoto, S. Harigaya and S. Ohshima), 1298

Inhibition of Growth and Photosynthesis in Submerged Plants with Tetramethylthiuram Disulphide and Sodium Dimethyldithiocarbamate and Reversal of this Inhibition of Photosynthesis (P. E. Björn Lindahl), 51
Inhibition of Intestinal Absorption by Cetrimide–Phloridzin Combination : Enhancement of (J. A. Nissim), 37
Inhibition by Oxygen of Biosynthesis and Activity of Nitrate-Reductase in *Aerobacter aerogenes* (Francis Pichinoty and Liliana D'Ornano), 879
Inhibition of Phosphorylation by Atractyloside (Dr. A. Bruni and A. R. Contessa), 818
Inhibition of Photosynthesis : Inhibition of Growth and Photosynthesis in Submerged Plants with Tetramethylthiuram Disulphide and Sodium Dimethyldithiocarbamate and Reversal of this (P. E. Björn Lindahl),51
Inhibition by Physostigmine of the Increase of Oxygen Consumption induced by Potassium in Muscle (Dr. Ivan Novotny and František Vyskočil), 916
Inhibition by Potassium of the Mechanical Responses of Frog Muscles at Low Temperature (H. Lorković), 1011
Inhibition of Ultrasonic Decolorization of Rhodamine B in Presence of Organic Substances (Prof. Satya Prakash and Sheo Prakash), 1292
Inhibition (Correlative), of Formation of Chlorophyll in the Leaves of Etiolated Pea Plants (Jiří Úlehla), 613
Inhibition (Differential), of Human Serum Cholinesterase with Fluoride—Recognition of Two New Phenotypes (Prof H. Harris and Mary Whittaker), 496
Inhibition (Feedback), controlling the Biosynthesis of Isoleucine : Release from the (Masaya Hayashibe and Teijiro Uemura), 1417
Inhibition (Oxygen), of Respiration in *Azotobacter* (M. J. Dilworth and C. A. Parker), 520
Inhibition (Specific), of Anti-D Antibody by Colominic Acid (Prof. William C. Boyd and Egli Reeves), 511
Inhibitions (Multiple), of Cell Metabolism by a Single Substance (M. Harris and Dr. G. A. Morrison), 1276
Inhibitor of Liver Alcohol Dehydrogenase in Preparations of Reduced Diphosphopyridine Nucleotide (Dr. Keith Dalziel), 1098
Inhibitor (Monoamine Oxidase) : Augmentation of Toxicity of, by Thyroid Feeding (Richard N. Carrier and Dr. Paul V. Buday), 1107
Inhibitors (Urinary), of Hyaluronidase (P. J. Knudsen and J. Koefoed), 1306
Initial Precursors to Petroleum Porphyrin Pigments : Metal Chlorin Complexes in Recent Sediments as (G. W. Hodgson and Eric Peake), 766
Initial Vibrations of a Supported Beam (Dr. R. V. Sharman and D. White), 692
Initiation of the Labour Process : Relationship between the Decrease in Urinary Excretion of Histamine and the (Dr. Halina Wiskont-Buczkowska), 290
Initiation (Floral), in Cocksfoot (*Dactylis glomerata* L.) : Effect of Spacing and Nitrogen-level on (D. M. Calder and J. P. Cooper), 195
Initiation (Flower), in the Carnation in Response to Photoperiod (Dr. G. P. Harris and Juliet E. Griffin), 614
Initiation (Flower), in Kent Wild White Clover (*Trifolium repens* L.), under Controlled Environmental Conditions (R. J. Haggar),1120
Injection of Sheep Erythrocytes into Lateral Ventricle of Brain : Antibody Response in Rabbits following (Prof. B. D. Janković, Dr. M. Draškoci and Dr. K. Isaković), 288
Injection (Repository), of Lipopolysaccharide : Development in Mice of Prolonged Non-specific Resistance to Sarcoma Implant and Staphylococcus Infection following (Samuel L. Prigal), 1111
Injections of some Intermediary Metabolites : Effects of, on the 'Insulin Response' of Normal Rabbits (Dr. H. D. Brahmachari and G. Raghupathy Sarma), 491, *erratum*, 755
Injections (Enzyme) : Effect of, on Mitosis in Regenerating Liver (Dr. Gaston de Lamirande), 400
Injector (*Nimrod*) : Successful Operation of the (National Institute for Research in Nuclear Science, Harwell), 1043
Inlets : Coastal, *review* by W. J. Reid, 426
Inlets (Coastal) : Stability of (P. Brunn and F. Gerritsen), *review* by W. J. Reid, 426
Inoculation of Pine Stumps against Infection by *Fomes annosus* (Dr. J. Rishbeth), 826
Inorganic Analysis : The Use of Organic Reagents in (A. I. Busev and N. G. Polianskii), translated from the Russian by J. T. Greaves, *review* by Dr. D. T. Lewis, 1336
Inorganic Chemistry (Dr. Jacob Kleinberg, Dr. William J. Argersinger, Jr., and Dr. Ernest Griswold), *review* by Dr. M. F. Lappert, 1133
  Modern Aspects of (Prof. H. J. Emeléus and Dr. J. S. Anderson), third edition, *review* by Prof. J. R. Partington, 637
Inorganic Compounds : Co-ordination of the Thiocyanate Group in (A. Turco and C. Pecile), 66
Inorganic Polymers : International Symposium on, at the University of Nottingham, 1046
Inorganic Reaction : A New—Nitric Acid–Hydrazoic Acid (Dr. J. Mašek), 166
Inorganic and Organic Phosphate : Outflux of, during Membrane Depolarization of Excitable Tissues (Dr. L. G. Abood, Prof. K. Koketsu and I. Koyama), 395
Insect (*Periplaneta americana* L.) : Exchanges of Sodium Ions in the Central Nervous System of an (J. E. Treherne), 1223
Insect Muscle Fibres : Spontaneous Miniature Potentials from (P. N. R. Usherwood), 814
Insect Nerve Tissue : Activity of Glutamic Acid Decarboxylase in (Dr. N. Frontali), 178
Insect Pests and Diseases (Forest), in Canada (Prof. M. L. Anderson), 1260
Insect Polymorphism (Royal Entomological Society Symposium on), 1145
Insecticides : Effects of, on Birds, 440
Insecticides (Systemic) : Enhanced Plant-Growth with Two (M. M. H. Wallace), 513
Insects : Sound Production and Sound reception by (Mable Frings and Hubert Frings), *review* by Prof. V. B. Wigglesworth, 428
Instability (Glutathione), of the Erythrocytes among Armenians in Iran : Virtual Absence of (Dr. J. E. Bowman and Dr. D. G. Walker), 211
Instability (A New 'Wedge' Type), observed in a 'Theta' Pinch (J. K. Wright, R. D. Medford, J. D. Herbert and R. C. Pottinger), 1054
Instance (A Clear), of Rheopectic Flow (J. B. Yannas and R. N. Gonzalez), 1384
Instituto de Fomento Nacional y Organizacion de las Naciones Unidas para la Agricultura y Alimentacion : Estudios Ecologicos, Vol. 1, Estudios Ecologicos para el Aprovechamiento de la Tierra en Nicaragua (Dr. B. W. Taylor), 1259
Instrument for Measuring Television Signal-to-Noise Ratio (S. M. Edwardson), 1355
Instrument Society of America (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
Instrument Symposium and Research Equipment Exhibition : Eleventh Annual (Washington Sections of the American Association of Clinical Chemists, the American Chemical Society, the Instrument Society of America, the Society of American Bacteriologists, the Society of Applied Spectroscopy and the Society for Experimental Biology and Medicine), 959
Instruments : Fine Measuring (Fourteenth International Instructional Course by Carl Zeiss, Jena, at the Imperial College of Science and Technology, London), 1045
Instruments and Measurement Exhibition : International, 1352
Insular Races (British), of Small Mammals : Origin of the, and of the 'Lusitanian' Fauna (G. B. Corbet), 1037
Insulin : An Unreactive Tyrosine Residue in, and the Exclusive Iodination of the *A* Chain (Dr. P. H. Springell), 1372
'Insulin Response' of Normal Rabbits: Effects of Injections of some Intermediary Metabolites on the (Dr. H. D. Brahmachari and G. Raghupathy Sarma), 491, *erratum*, 755
Insulins with Identical Amino-Acid Sequences : Immunochemical Distinction between (Dr. Solomon A. Berson and Dr. Rosalyn S. Yalow), 1392
Insulins (Cod and Bovine): A Comparison of (Dr. S. Wilson and Dr. G. H. Dixon), 876
Intake of Alcohol and Sodium Chloride : Serum Lipids in relation to the (Erling Lund, Torben Geill, Prof. Henrik Dam and Gunhild Kristensen), 1095
Integrated (Time-) Measurements of Submarine Irradiance (Ronald Currie and Laurence Draper), 661
Integration with Ionization Detectors in Gas Chromatography (P. M. E. M. van der Grinten and Dr. A. Dijkstra), 1195
Integration and Behaviour : Sense Organs, (edited by Talbot H. Waterman), (The Physiology of Crustacea, Vol. 2), *review* by Dr. J. Green, 428
Intensity (Light) : Effects of, on Reproduction in S.48 Timothy (*Phleum pratense* L.), (Dr. G. J. A. Ryle), 196
Inter-Individual Variability in Pigmentation (André Leguebe), 1326
Inter-Molecular Energy Transfer in Photosensitized Oxidation-Reduction : Paths of (N. Takeyama), 1359
Inter-Society Color Council (U.S.A.), (Colour Group (Great Britain)), and the : Maxwell Colour Centenary Conference, 10
Interaction of Hydrocortisone, the Sulphydryl Group, and the Catechol Amines (Dr. Sidney Roston), 490
Interaction of Lipids and Trypsin in Dimethyl Sulphoxide–Water Systems (Prof. A. Lukton, H. Donohue and Prof. F. A. Bettelheim), 565
Interaction of 14-MeV. Neutrons with a Silicon Semiconductor Nuclear Particle Detector (Dr. W. M. Deuchars and G. P. Lawrence), 995
Interaction of Portland Cement with Sea-Water and Chloride Solutions : Formation of Calcium Chloraluminate by (Dr. L. Heller and Dr. M. Ben-Yair), 488
Interfaces (Cell) : Characteristics of (American Society for Cell Biology Symposium on), 1045
Interfaces (Fluid–Fluid), in Steady Motion (Prof. Walter Rose), 242
Interfaces (Solid–Liquid) : Chemisorption at (A. R. Burkin and G. Halsey), 348
Interferometer (Michelson) : Molecular Emission Spectroscopy from 2μ to 12μ by a (H. A. Gebbie, G. Roland and L. Delbouille), 264
Interferon (Dr. A. Isaacs), 755
Interior of the Earth (Dr. R. Stoneley), 983
Intermediary Metabolites : Effects of Injections of some, on the 'Insulin Response' of Normal Rabbits (Dr. H. D. Brahmachari and G. Raghupathy Sarma), 491, *erratum*, 755
Intermediate Pool between Amino-Acids and Globin in Duck Blood *in vitro* (L. Bianco, G. Giustina and E. Lazzarini), 40
Internal Organs Fluids and Excreta of Rabbits : Effect of Polyvinyl Alcohol on the Distribution of Iodine-131 in the (H. F. Nowak and J. Kucharski), 665, *erratum*, 1045
Internal Sources (Standardized), of Polonium-210 : Radiolytic Oxidation of Ferrous Solutions with (J. Steyn and D. van As), 903
Internally Deposited Radionuclides : Removal of, by Triethylenetetraaminehexaacetic Acid (Prof. A. Catsch and D. Schindewolf-Jordan),715
International Academy of Astronautics : Daniel and Florence Guggenheim International Astronautics Award to be given to Sir Bernard Lovell, 856
  Symposium on Space-Flight and Re-entry Trajectories, 960
International Atomic Energy Agency : Appointment of Dr. Arno Sigvard Eklund as director-general, 338
  Grants from the United States for Nuclear Equipment in Argentina, Brazil and Israel, 442
  International Conference on Plasma Physics and Controlled Nuclear Fission Research, 443
  Purpose, Origin, Structure, Growth and Achievements, 1143
  Courses on Practical Applications of Nuclear Energy, 1143
International Atomic Energy Agency, and others : Conference on the Use of Radioisotopes in Animal Biology and the Medical Sciences, 959
International Botanical Congress : Symposium on Forest Ecosystem (Montreal, 1959)—Proceedings of the, 755
International Business Machines, United Kingdom, Ltd. : IBM Research Fellowships, 442
  Endowed Research Time, 442
International Centre for the Study of the Preservation and the Restoration of Cultural Property, Rome : Works and Publications, 3, Climatology and Conservation in Museums, 1050
International Civil Aviation Organization, and others : To set up and operate an Aeronautical Research Laboratory and three Civil Aviation Training Centres, 1043
International Commission on Radiological Units and Measurements : Report of the (United States National Bureau of Standards, Handbook No. 78), 28
International Committee of Dietitians (British Dietetic Association and an) : Third International Congress of Dietetics, 1343
International Computers and Tabulators, Ltd. : Appointment of B. L. J. Hart as principal of the Computer Application Training Centre, 24

INDEX

I

International Conference on Elementary Particles (CERN), 126
International Conference on Embryology (Editorial Board of the *Journal of Embryology and Experimental Morphology*), 24
International Conference on Magnetism and Crystallography (Science Council, the Physical Society and the Crystallographic Society of Japan), 24
International Conference on Neurosecretion : Third (University of Bristol), 856
International Conference on Pigment Cell Biology (New York Academy of Sciences), 1045
International Conference on Plasma Physics and Controlled Nuclear Fission Research (International Atomic Energy Agency), 443
International Conference on Timber Engineering (University of Southampton), 338
International Congress of Aviation and Cosmonautical Medicine (C.E.R.M.A.), 338
International Congress of Dietetics : Third (British Dietetic Association and an International Committee of Dietitians), 1343
International Convention on Starch (Dr. E. Dux), 236
International Co-operation : The Urgency of, 943
International Council for Bird Preservation : Seventh Conference of the European Continental Section (Phyllis Barclay-Smith), 550
International Council for Bird Preservation (British Section) : Annual Report for 1960, 124
International Council of Scientific Unions : Year Book for 1961, 123
  Ninth General Assembly, 752
  ICSU Review, Vol. 3, No. 1, 865
International Instructional Course (Fourteenth), by Carl Zeiss, Jena, at the Imperial College of Science and Technology, London, on Fine Measuring Instruments, 1045
International Instruments and Measurement Exhibition, 1352
International Measurement Conference : Second (J. A. Place), 1350
International Meeting of the Institute of Management Sciences : Proceedings of the Sixth (Paris, 1959)—Management Sciences, Models and Techniques (edited by Prof. C. West and Prof. Michel Verhulst), *review* by T. H. Hawkins, 209
International Meeting on Psychophysiology, Neuropharmcology and Biochemistry of Audiogenic Seizure (Centre National de la Recherche Scientifique), 959
International Mineral Processing Congress : Sixth (Société de l'Industrie Minérale), 545
International Nematology Symposium : Sixth (Society of European Nematologists), 24, 1048
International Scientific and Technical Conferences : Forthcoming (Department of Scientific and Industrial Research, List No. 29), 959
International Significance : New Trends in Atomic Research and their (Dr. L. Kowarski), 853
International Society for Tropical Ecology : Proceedings of the Symposium on Forest Ecosystem (Montreal, 1959), 755
International Symposium on Enzymic Activity of the Central Nervous System (Neurochemical Commission of the World Federation of Neurology), 1349
International Symposium on Inorganic Polymers at the University of Nottingham, 1046
International Symposium on Photoelasticity (Illinois Institute of Technology, Chicago), 755
International Symposium on Tissue Transplantation (University of Chile, and Societies of Santiago and Valparaiso), 24
International Telecommunication Union : European Conference on Broadcasting, 449
International Union against Cancer : Eleanor Roosevelt Fellowships for Research on Cancer, 125
  Grant from the Gulbenkian Foundation, 754
International Union for the Conservation of Nature and the Commission for Technical Co-operation in Africa : Conference on the Conservation of Nature and Natural Resources in Modern African States, 958
International Youth Federation for the Study and Conservation of Nature : Course in Conservation, by the Verein Naturpark in the Lüneburger Heide, Germany, 1045
Internode-Length in *Pisum sativum* : Effects of Gibberellic Acid and Allogibberic Acid on Flowering and (I. C. Murfet and H. N. Barber), 514
Interparticle Forces : Role of, in Development of Upper Plastic Limit of Clays (Dr. Raymond Yong and E. T. Selig), 938
Interplanetary Space : Magnetic Storms in, as observed by *Pioneer V* (Eugene W. Greenstadt), 329
Interpretation of Cosmic Noise Measurements at 3·8 Mc./s from Satellite 1960 η1 (J. H. Chapman and A. R. Molozzi), 480
Interstitial Cell-stimulating Hormone (Human Pituitary) : An Immunological Study of a (N. R. Moudgal and Prof. Choh Hao Li), 192
Interstitial Species (Littoral), from Anglesey representing Three Families New to Britain (P. J. S. Boaden), 512
Interviewing in Twenty-six Steps (John S. Gough), 228
Intestinal Absorption : Enhancement of Inhibition of, by Cetrimide–Phloridzin Combination (J. A. Nissim), 37
Intestinal Epithelium : Localization of the 'Sugar Pump' in the (Dr. T. Z. Csáky and G. W. Fernald), 709
Intestine : Bacteria of the (Society for Applied Bacteriology Symposium on), 1151
Intoxication (Hexachlorobenzene) : Nervous and Biochemical Disturbances following (Dr. F. De Matteis, B. E. Prior and Prof. C. Rimington), 363
Intracellular Distribution Pattern of Rat Liver Glutamic-Oxalacetic Transaminase (Dr. Herbert J. Eichel and Josef Bukovsky), 243
Intracellular Freezing and Frost Resistance in Egg-Cells of the Sea Urchin (E. Asahina)— Intracellular Freezing as a Cause of Frost-killing in relatively Rapidly Frozen Cell Suspensions, 1263 ; A Mechanism to Prevent the Seeding of Intracellular Ice in Freezing Living Cells, 1264
Intracellular Glycosidases of Dextran-producing Bacteria (Dr. R. W. Bailey and Prof. E. J. Bourne), 277
Intra-ovarian Pollination in *Eschscholzia californica* Cham., *Argemone mexicana* L. and *A. ochroleuca* Sweet (Prof. P. Maheshwari and Kusum Kanta), 304
Intrinsic Factor : Vitamin B_{12} and (European Symposium on), 1154
Introduction : Plant Exploration and (Food and Agriculture Organization of the United Nations Technical Meeting on), 1240
Introduction to the Biology of Micro-organisms (Lilian E. Hawker, A. H. Linton, B. F. Folkes and M. J. Carlile), *review* by Prof. B. C. J. G. Knight, 1032
Introduction to the Chemistry of Heterocyclic Compounds (R. M. Acheson), *review*, 320
Introduction to Co-ordination Chemistry (Dr. D. P. Graddon), *review* by Prof. C. A. Coulson, 1233
Introduction to Infinitely Many Variates (Prof. Enders A. Robinson), *review* by Prof. D. V. Lindley, 214
Introduction of the Potato into Western and Central Europe (V. S. Lekhnovitch), 518
Introduction to Theoretical Meteorology (Prof. Seymour L. Hess), *review* by Dr. Robert P. Pearce, 314
Introduction to Transition-Metal Chemistry—Ligand-Field Theory (Dr. Leslie E. Orgel), *review* by Prof. C. A. Coulson, 1233
Introductory Manual on the Control of Health Hazards from Radioactive Materials (Medical Research Council, Memorandum No. 39), new edition, 1257
Inulin : Excretion of, by Malpighian Tubules (Dr. J. A. Ramsay and Dr. J. A. Riegel), 1115
Invariant Casein Substrate for Rennet Standardization, derivable from Diverse Sources of Cows' Milk (Dr. G. W. Scott Blair and J. C. Oosthuizen), 697
Invention (Science and) National Reference Library of, 1 ; (Leslie Wilson), 733 ; (J. Farradane), 734
  Aslib Report of the Discussion Meeting on, 649
Inversion of a Laplace Transform arising from a Problem in Applied Chemical Kinetics (T. Wood), 589
Invertebrate Life in South-East England (John Sankey), 132
Investigating the Properties of Nervous Systems : Response to Series of Subliminal Stimuli—an Ethological Method for (A. J. Pontin), 512
Investigation (Chromatographic), of the Methyl Alcohol/Chloroform-soluble Constituents of Honeybee Larval Foods (N. G. Patel, M. H. Haydak and R. Lovell), 362
Investigations (Metabolic and Genetic), of Nutritionally Exacting Strains of *Staphylococcus pyogenes* (Janice B. Edgar and Dr. B. A. D. Stocker), 1121
Investment (Public), and Export (Sir Walter Benton Jones), 334
Iodide and Iodinated Amino-Acids : A Spray Method for the Simultaneous Detection of, on Filter Paper Chromatograms (Dr. A. Van Zyl and M. Bhaga), 275
Iodinated Amino-Acids : A Spray Method for the Simultaneous Detection of Iodide and, on Filter Paper Chromatograms (Dr. A. Van Zyl and M. Bhaga), 275
Iodinated Wools : Supercontraction of (O. A. Swanepoel), 908
Iodination (Exclusive), of the *A* Chain: An Unreactive Tyrosine Residue in Insulin and the (Dr. P. H. Springell), 1372
Iodine-131 in the Internal Organs, Fluids and Excreta of Rabbits : Effect of Polyvinyl Alcohol on the Distribution of (H. F. Nowak and J. Kucharski), 665, *erratum*, 1045
Iodine-131 (Thyroidal) : Increase of the Rate of Release of, and of Circulating Thyrotrophic Activity at Early Stages of Propylthiouracil Treatment in the Rat (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171
Iodine (Indole–) : Spin Resonance and Electrical Conductivity of (B. Smaller, Dr. I. Isenberg and S. L. Baird, jun.), 168
Iodine Complexes : Separation of Linear from Branched Polysaccharides by Precipitation as (Blanche D. E. Gaillard), 1295
Ion (Formyl Radical) : Radiation-induced Formation and Reactions of the (John Holian, Dr. George Scholes and Prof. Joseph J. Weiss), 1386
Ion-Exchange Column (One), during Preparation : A Simplified Method for Separating Amino-Acid Mixtures from Protein Hydrolysates on (J. Liebster, K. Kopoldová and M. Dobiášová), 1198
Ion-Exchange Polymers : Non-Uniformity of Cross-linking in (Dr. E. Glueckauf and R. E. Watts), 904
Ion Exchangers (Modified Cellulose), and their Use in Ribonucleic Acid Fractionation (Dr. L. Bosch, G. van der Wende and Dr. H. Bloemendal), 349
Ion Exclusion : Separation Methods based on Molecular Sieving and (J. Porath and E. B. Lindner), 69
Ion Pump : Electrical Properties of Orientated Lipid on a Biological Membrane—an Electrostatic Diffusion Barrier and (Dr. J. W. L. Beament), 217
Ionic Charge ('Effective'), in Crystals (Dr. W. Cochran), 60
Ionic Currents in Membrane of Active Muscle Fibre (Dr. H. Jenerick), 1074
Ionic Melts : The Structure and Properties of (Faraday Society General Discussion on), 338, 443
Ionization of Atmospheric Gases : Detection by, during Analysis by Gas Chromatography (Dr. Arthur Karmen, Laura Giuffrida and Robert L. Bowman), 906
Ionization (Magnetically Controlled) : Trans-Equatorial Backscatter Observations of (J. A. Thomas), 792
Ionization Detectors in Gas Chromatography : Integration with (P. M. E. M. van der Grinten and Dr. A. Dijkstra), 1195
Ionization Energies of Condensed Ring Aromatic Hydrocarbons : π-Electronic Excitation and (Dr. J. B. Birks and M. A. Slifkin), 761
Ionization Potentials of *cis* and *trans* Dichloro- and Dibromo-ethylenes (Dr. J. Momigny), 1089
Ionizing Radiation : Effects of, on the Human Haematopoietic System—Report of the Subcommittee on Haematologic Effects, Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 875), 556
  Effects of, on Organic and Biological Solids (Société de Chimie Physique and the British Biophysical Society Discussion on), 1150
Ionizing Radiations at Exhibitions : Code of Practice for the Display of Sources of, 1249
Ionizing Radiations from External Sources : Long-Term Effects of—Report of the Subcommittee on, of the Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 849), 556
Ionosphere : Connexion between Micropulsations and the (C. M. Lock and P. J. Stevens), 584
  Group and Phase Velocities of a Travelling Disturbance in the *F* Region of the (Dr. L. H. Heisler and Dr. J. D. Whitehead), 157
Ionospheric Absorption Heights : A Method of Estimating, from Recovery Times (Syed Ziauddin), 1084
Ionospheric Irregularities : Scintillations and the Latitude Distribution of (D. G. Singleton), 482

Ions (Divalent Metal), in the Binding of Tetracycline to Macromolecules: Mediation of (Dr. Kurt W. Kohn), 1156
Ions (Metal), in Enzyme Catalysis: Dextransucrase *V* and the Role of (W. Brock Neely and Julie Hallmark), 385
Ions (Polyvalent): Influence of, on Histones isolated from Different Tissues (I. P. S. Agrell and E. G. Christiensson), 284
Ions (Sodium): Exchanges of, in the Central Nervous System of an Insect (*Periplaneta americana* L.), (J. E. Treherne), 1223
Ions (Transition-Metal): The Theory of (Prof. J. S. Griffith), *review* by Prof. C. A. Coulson, 1233
Ipomoea batatas (L.) Lam. (Sweet Potato): Evolution of the (Dr. D. E. Yen), 93
Iran: Virtual Absence of Glutathione Instability of the Erythrocytes among Armenians in (Dr. J. E. Bowman and Dr. D. G. Walker), 221
Iranians: Serum Factor Gm^a among the (Luba Podliachouk, Dr. A. Eyquem, R. Choaripour and M. Eftekhari), 717
Ireland (Northern): Aids to Agriculture and Horticulture in, 959
Irish Rival: Dalton's, *review* by Frank Greenaway, 533
Iron-55 from Nuclear Detonations (Rama, Minoru Koide and Prof. Edward D. Goldberg), 162
Iron Concentrations (Different): Electrophoretic and Immuno-electrophoretic Patterns of Extracellular Diphtheria Proteins from Culture Filtrates of (Bertold J. Radola), 382
Iron Meteorites (Bear Creek and Narraburra): Microstructural and Chemical Identity of the (Dr. H. J. Axon), 1287
Iron Oxidation: Morphological Variation in *Ferrobacillus ferrooxidans* related to the Rate of (M. P. Silverman and M. H. Rogoff), 1221
Iron (Nickel-) Films: Variation of Anisotropy Axis Dispersion with Thickness of (H. Clow), 996
Iron and Steel Institute and the Institute of Metals (Powder Metallurgy Joint Group): Winter Meeting—Symposium on Sintered High Temperature Compounds, 1250
Iron and Steel Institute and the Metallurgical Society of the American Institute of Mining, Metallurgical and Petroleum Engineers: Special Meeting in the United States and Canada, 651
Iron and Steel Research Association (British): Open Days at the Sheffield Station, 548
  Computer Applications Section of the Operational Research Department renamed the Systems Evaluation Section, 1249
Irradiance (Submarine): Time-integrated Measurements of (Ronald Currie and Laurance Draper), 661
Irradiated Beryllium Oxide: Scattering of Long Wave-length Neutrons by (T. M. Sabine, A. W. Pryor and B. S. Hickman), 1385
Irradiated Human Beings: Decreased Urinary Excretion of 2-Aminoethanol by (Dr. Ralph R. Cavalieri, M. T. Van Metre and W. I. Sivertsen), 1303
Irradiated Lithium Fluoride as an Optical Filter in the Far Ultra-Violet (J. L. Weeks, S. Gordon and G. M. A. C. Meaburn), 1186
Irradiated Normal Tissues: Effect of, on the Growth of Mouse Carcinoma (Dr. Arthur Compton and Prof. Charles A. Pannett), 1101
Irradiated Soil: Plant Growth in (G. D. Bowen and A. D. Rovira), 936
γ-Irradiated Lead Silicate Glass: Absorption Spectra of (R. S. Barker, T. K. Harrison, D. A. Richardson and R. Rimmer), 374
Irradiation (Circumscribing), of Surface Structures of Cylindrical Animal and Plant Cells with Polonium-210 α-Particles (R. M. Bergström, R. F. Blåfield anf M. W. Brenner), 1165
Irradiation (Red and Far-Red): Effect of, on the Germination Process in Seeds of *Pinus sylvestris* L. (Bengt Nyman), 1219
Irradiation (Ultra-Violet): Effect of, on the Electrical Conductivity of Cathodes coated with Barium Oxide (Dr. C. H. B. Mee), 1383
Irradiation Decomposition of Carbon Dioxide (T. B. Copestake and N. S. Corney), 1192
Irregularities (Ionospheric): Scintillations and the Latitude Distribution of (D. G. Singleton), 482
Irregularities (Night-time *F* Layer), at the Equator: Height of (Dr. G. S. Kent and Dr. J. R. Koster), 1083
Irrigation in Ancient Mesopotamia: Ecological Aspects of (Hans Helback), 227
Irwin, Dr. J. W.: Biorheology and Microcirculation (Film showing the Pulmonary Microcirculation in the Living Rabbit), 758; (Microcirculation of the Inner Ear), 758
Irwin, Dr. M. R.: Blood Groups in Infra-human Species (Role of Individual Red Cell Antigens in Speciation), 654
Isaacs, Dr. A.: Interferon, 755
Isaacs, Alick (Baron, Dr. Samuel, and): Mechanism of Recovery from Viral Infection in the Chick Embryo, 97
Isaković, Dr. K., and others: Antibody Response in Rabbits following Injection of Sheep Erythrocytes into Lateral Ventricle of Brain, 288
Isenberg, Dr. I., and others: Spin Resonance and Electrical Conductivity of Indole–Iodine, 168
Ishibashi, Takaaki, and others: Synaptic Transmission in Neurosecretory Cells, 183
Ishida, Dr. Nozomu, and others: Quantitative Determination of Metadrenaline and Normetadrenaline in Normal Human Urine, 599
Ishidate, M. (Metcalf, D., and): Periodic Acid–Schiff Positive Giant Cells in the Mouse Thymus Cortex, 305
Ishidate, Prof. Morizo, and Hanaki, Akira: Oxidative Demethylation and Carcinogenicity of 4-N-Dimethylaminoazobenzene Derivatives, 1198
Ishizaki, Hironori (Ichikawa, Mamori, and): Brain Hormone of the Silkworm, *Bombyx mori*, 933
Iso-Antigen (γB^A) of Mouse γ-Globulin present in Inbred Strains (A. Kelus and J. K. Moor-Jankowski), 1405
Isoelectronic Principle, Thermochemistry and Reactivity of related Free Radicals (Dr. Peter Gray and J. C. J. Thynne), 1357
Isolated Chloroplasts: Oxidation-Reduction Reactions of Plastoquinone in (Dr. E. R. Redfearn and J. Friend), 806
Isolated Gut: Dependence on Substrate of the Electrical Potential across the (Dr. T. W. Clarkson, A. C. Cross and S. Toole), 501
Isolated Plant Protoplasts: Properties of (E. C. Cocking), 780
Isolated or Overlapping Normal Probability Curves: Calculation of the Areas of (Dr. D. Morison Smith and John C. Bartlet), 688
Isolating Bacteria capable of Producing 6-Aminopenicillanic Acid from Benzylpenicillin: A Method for (Prof. Yukio Kameda, Yukio Kimura, Etsuko Toyoura and Tomoo Omori), 1122
Isolation of Anthraquinone from Humus (Kyoichi Kumada, Akio Suzuki and Kazuyuki Aizawa), 415
Isolation of a New Antioxidant from Oats (Dr. D. G. H. Daniels and H. F. Martin), 1302
Isolation of Nigerose and Kojibiose from Dextrans (Kazuo Matsuda, Hiroshi Watanabe, Kentaroh Fujimoto and Prof. Kiyoshi Aso), 278
Isolation of *Plasmodium knowlesi* from Philippine Macaques (F. L. Lambrecht, Dr. F. L. Dunn and Dr. D. E. Eyles), 1117
Isolation of Proteins: The Separation and (edited by Dr. P. Alexander and R. J. Block), (A Laboratory Manual of Analytical Methods of Protein Chemistry, including Polypeptides, Vol. 1), *review* by Dr. D. T. Elmore, 320
Isolation of Sterol Esters from Human Faeces (Francis Aylward and Pamela Wills), 1397
Isolation (Routine Selective), of Fungi Pathogenic to Man: Use of Desertomycin Medium in (J. Uri and Zs. Herpay), 1015
Isolation and Fractionation of some Damselfly Pigments (C. B. Coulson and P. Mary Lord), 1300
Isolation and Properties of Deoxyribonucleic Acid from Mammalian Sperm (Dr. E. Borenfreund, E. Fitt and Dr. A. Bendich), 1375
Isoleucine: Release from the Feedback Inhibition controlling the Biosynthesis of (Masaya Hayashibe and Teijiro Uemura), 1417
Isomerization (Thermal), of Alpha-Keto-Acid Hydrazones: Application of the Nickel Reaction and, for their Chromatographic Analysis (A. M. Tokushige and Dean E. S. Cook), 797
Isomers: Mannosyl D-Glucosamine Hydrochloride—*N*-Mannosyl D-Glucosamine Hydrochloride (Dr. S. A. Barker, Dr. K. Murray and Prof. M. Stacey), 142; 6-*O*-Mannosyl D-Glucosamine Hydrochloride (Dr. S. A. Barker, Dr. K. Murray, Prof. M. Stacey and D. B. E. Stroud), 143
Isopods: Copper in (Dr. W. Wieser), 1020
*Iso*propylnoradrenaline: Enlargement of Salivary Gland in Mice Treated with (Dr. K. Brown-Grant), 1076
Isotactic Polypropylene: Crystallization of, using a Light Depolarization Technique (J. H. Magill), 1092
Isotopic Nitrogen: Distribution of, in Nitrogen Gas during Denitrification (R. D. Hauck and D. R. Bouldin), 871
Isotopic Norepinephrine: Effects of Drugs on Uptake of, by Cat Tissues (Hans J. Dengler, Herbert E. Spiegel and Elwood O. Titus), 816
Israel: Specimens of very early Pleistocene Age from certain Beds in (Dr. Desmond Clark), 23
Israel (Argentina, Brazil and): Grants from the United States to the International Atomic Energy Agency for Nuclear Equipment in, 442
Issaly, A., and others: Role of the Pentose Phosphate Pathway in *Pasteurella multocida*, 727
Issues in Evolution, *review* by Dr. P. G. Fothergill, 425
Italian (Southern) Extraction: Haemoglobin *P* in a Family of (Prof. E. Silvestroni, I. Bianco and C. Brancati), 292
Itano, Dr. Harvey A., and Pauling, Prof. Linus: Thalassaemia and the Abnormal Human Haemoglobins, 398
Ito, Dr. M., and others: Potential Changes produced by Application of Current Steps in Motonueurones, 1104
Ito, Dr. Toshio: Sterol Requirements of the Silkworm, *Bombyx mori*, 882
Itoh, Dr. Chuichi, and others: Quantitative Determination of Metadrenaline and Normetadrenaline in Normal Human Urine, 599
Iversen, Dr. Simon: The Feulgen-Stained Somatic Cell Nucleus as a Homogeneously Absorbing Object, 150
Ivri, Dr. M.: Biorheology and Microcirculation (Effect of Adrenaline on the Small Blood Vessels of the Conjunctiva and Nailfold), 758; (Observations and Experiments on Vasomotion in Human Capillaries), 758

Jack, James A., and others: The Diego Factor in a Puerto Rican Family—a Case of Anti-Diego in Monozygotic Twins, 188
Jackson, Dr. G. A. D., and others: Conversion of L-Galactono-γ-lactone into L-Ascorbic Acid by Plants, 282
Jacob, Dr. J.: Assessment of Pain (Testing of Analgesic Drugs on Animals), 1354
Jacob, Dr. J. (Balasubrahmanyan, K., and): Occurrence of *Eutrichocheles modestus* (Herbst) in the Near-shore Waters of Porto Novo, South India, 830
s'Jacob, J. J.: Nematology (*Ditylenchus radicicola* of Dutch strain damaging *Poa annua* but not Barley), 1048
Jacobs, Patricia A., and others: Distribution of Human Chromosome Counts in relation to Age, 1178
Jacquignon, P., and others: A New Family of Potent Carcinogens—Benzopyridocarbazoles, 1005
Jaenicke, L.: Vitamin B_{12} and Intrinsic Factor (Relationships between Vitamin B_{12} and Folic Acid), 1156
Jagendorf, Dr. André T.: Photophosphorylation and the Hill Reaction, 679
Jagnow, G.: Numbers and Types of Bacteria from the Rhizosphere of Pasture Plants—Possible Occurrence of *L*-Forms, 1220
Jain, S. C. (Krishnan, the late Sir K. S., and): Quenching of Cation Vacancies in Doped Crystals of Sodium Chloride, 162
Jain, Dr. S. K.: Stabilizing Selection for Heading Time in a Bulk-Hybrid Population of Barley, 1123
  Discriminant Analysis in Biometrical Genetics, 1420
Jamaican Shade Tree *Pithecolobium samath* Benth.: Pharmacology of the Alkaloidal Fraction from the Bark of the (B. E. Leonard and Dr. H. S. A. Sherratt), 287
James, B. L.: Parasite Biology (Parasites in relation to Natural Fauna), 135
James, Prof. W. O.: Respiratory Metabolism in Plants, *review*, 1031
Janeway, Charles A., and others: Immunoelectrophoretic Examination of Agammaglobulinaemia, 1109
Janković, Prof. B. D., and others: Antibody Response in Rabbits following Injection of Sheep Erythrocytes into Lateral Ventricle of Brain, 288
Januszko, T., and others: Conversion of Fibrin to Desmofibrin, 1093
Japanese Planetarium Equipment, 753
Japolsky, Dr. N. S.: Apparent Recession of Nebulae and the Nature of Radio Stars, 480
Jarvik, Erik: Théories de l'Évolution des Vertébrés (traduction du Prof. J. P. Lehman), *review* by Dr. E. Trewavas, 945
Jarvis, T. R., and others: Pulse-shortening in Electron Linear Accelerators, 483
Jayaraman, A., and others: Melting and Polymorphism of Indium Antimonide at High Pressures, 1288
Jayle, Max F., and others: Action of Neuraminidase on Haptoglobin, 279
Jenerick, Dr. H.: Ionic Currents in Membrane of Active Muscle Fibre, 1074

Jennings, D. L. : Mutation for Larger Fruit in the Raspberry, 302
Jensen, the late Dr. C. E., and others : Displacement Potentials and Bending of Rod-like Polyelectrolytes, 484
Jensen, E. J. : Alberta Sub-bituminous Coals as Drilling Fluid Thinners (Research Council of Alberta, Information Series No. 23), 1144
Jepson, Dr. J. B., and Smith, Dr. I. : Paper Chromatography (Film showing the Principles of Chromatography), 759
Jesty, L. C. : At the Maxwell Colour Centenary Conference (Problems of Colour Reproduction), 11
Jírovec, Prof. Otto : Parasitologie für Ärzte, *review* by Dr. G. Lapage, 107
Job Analysis (H. E. Roff and T. E. Watson), 336
Jobs : The Content of, 336
Johnson, Prof. A. W. : Vitamin B_{12} and Intrinsic Factor (Synthetic Approaches to the Corrin Ring System), 1154
Johnson, B. Connor : Vitamin B_{12} and Intrinsic Factor (Biological Function of Vitamin B_{12}), 1155
Johnson, G. D. : Simplified Procedure for Removing Non-specific Staining Components from Fluorescein-labelled Conjugates, 70
Johnson, Nora H. : Pollution from Refuse Tips, 864
  Drinking-Water Standards, 1261
Johnstone, D. B. (Racusen, Prof. David, and) : Estimation of Protein in Cellular Material, 492
Jones, A. W. : Elected president for 1961-62 of the Scientific Instrument Manufacturers' Association of Great Britain, 546
Jones, C. E. Roland, and Moyles, A. F. : Pyrolysis and Gas–Liquid Chromatography on the Microgram Scale, 663
Jones, D. de G., and Masterson, H. G. : Effect of Chloride Concentration on the Aqueous Corrosion of a Magnesium Alloy, 165
Jones, D. H. P. : New Handbooks for Lunar Studies, *review*, 424
Jones, G. R. N., and others : Tissue Dilution Artefact—a Re-interpretation of Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogenesis, 1203
Jones, Dr. J. D., and Hulme, Dr. A. C. : Preparation of Mitochondria from the Peel of Apples, 370
Jones, J. Graham : Design and Use of Respirators (Respirators in the Iron and Steel Industry), 656
Jones, J. H., and Heppleston, Prof. A. G. : Immunological Observations in Experimental Silicosis, 1212
Jones, Dr. J. P. : Appointed to the Westland readership in helicopter engineering in the University of Southampton, 443
Jones, Dr. J. W. : Salmon and Freshwater Fishing in Great Britain, 962
Jones, Dr. O. C., and Clarke, Dr. F. J. J. : A New Photometric Technique using a Variable Shutter Device, 1290
Jones, R. L. (Hall, D. M., and) : Physiological Significance of Surface Wax on Leaves, 95
Jones, S. Rees : Climatology and Conservation in Museums, 1050
Jones, Dr. Thomas O. : Appointed head of the Office of Antarctic Programs of the United States National Science Foundation, 125
Jones, Sir Walter Benton : Public Investment and Export, 334
Joseph, Dr. Norman R., and others: Distribution of Sodium and Potassium in certain Cells and Tissues, 1175
Joshi, G. C. (Sharma, M. I. D., and) : An Abnormal Form of Female Rat Flea, *Xenopsylla cheopis* Roths., 727
Journal of Embryology and Experimental Morphology (Editorial Board of the) : International Conference on Embryology, 24
Journal of Helminthology : Supplement in honour of R. T. Leiper on the occasion of his 80th Birthday, 1961, *review* by Dr. G. Lapage, 843
Journal of the Museums Association, No. 1, 226
Journalism (Selected), from the English Reviews by Stendahl, with Translations of other Critical Writings (edited by G. Strickland), 1031
Jović, M., and Supek, Z. : Formation of 5-Hydroxytryptamine in an Aqueous Solution of 5-Hydroxytryptophan treated with X-Rays, 383
Joyner, L. P. : Parasite Biology, 134
Judah, J. D., and others : Electron Transport System and Phosphoproteins of Mitochondria, 1309
Judd, Dr. D. B. : At the Maxwell Colour Centenary Conference (A Fresh Analysis of the Colour Mixture Curves obtained by Maxwell), 10
Juhlin, Lennart (Shelley, Prof. Walter B., and) : A New Test for Detecting Anaphylactic Sensitivity—the Basophil Reaction, 1056
Jung, Prof. C. G. : *Obituary* by Dr. Clifford Allen, 223
  The Structure and Dynamics of the Psyche (translated by R. F. C. Hull), *review* by Dr. C. Allen, 1235
Jungian Psychology, *review* by Dr. C. Allen, 1235
Jurand, A., and Tuft, Dr. Peter : A Morphogenetic Effect of Beta-Mercaptoethanol—Distribution of Water in the Chick Blastoderm, 1073
Jurisprudence : African (Prof. M. Gluckman), 991
Juvenile Mandible from Olduvai (Dr. L. S. B. Leakey), 417
Juvenile Phase of Apple Seedlings : Shortening the (A. I. Campbell), 517

KABATA, Dr. Z. : Parasite Biology (Marine Parasitology), 134
Kacker, K. P., and others : Basic Dyestuffs in Clay Mineralogy, 696
Kaempfe, L. : Nematology (Disappointing Results of Ultrasonic Vibrations against Encysted *Heterodera* Larvae), 1049
Kaesberg, Prof. Paul (Yamazaki, H., and) : Unusual Base Composition of the Ribonucleic Acid of Wild Cucumber Mosaic Virus, 96
Kafalas, J. A., and others : A New Phase formed by High-Pressure Treatment—Face-centred Cubic Molybdenum Monocarbide, 1194
Kakulas, B. A. : Myopathy affecting the Rottnest Quokka (*Setonix brachyurus*) reversed by α-Tocopherol, 402
Kallistratos, G. : Vitamin B_{12} amd Intrinsic Factor (Broad Bean (*Vicia faba*) Plants can take up Vitamin B_{12} from Nutrient Solutions), 1155
Kameda, Prof. Yukio, and others : A Method for Isolating Bacteria capable of Producing 6-Aminopenicillanic Acid from Benzylpenicillin, 1122
Kamlet, Mortimer J. (edited by) : Organic Electronic Spectral Data, Vol. I, 1946-1952, *review* by Dr. E. G. Kellett, 107
Kanta, Kusum (Maheshwari, Prof. P., and) : Intra-ovarian Pollination in *Eschscholzia californica* Cham., *Argemone mexicana* L. and *A. ochroleuca* Sweet, 304
Kantorowich, R. H. : Appointed professor of town and country planning in the University of Manchester, 443
Kapany, Narinder S., and others : A Hypodermic Probe using Fibre Optics, 927
Kapustinsky, Prof. A. F. : *Obituary* by Prof. S. I. Tomkeieff, 647
Kariba (Lake) : Limnological Trends in (D. Harding), 119
Karmen, Dr. Arthur, and others : Detection by Ionization of Atmospheric Gases during Analysis by Gas Chromatography, 906
Karroo Dolerites (Weathered) : Climate and (Dr. H. H. Weinert), 325
Kassanis, Dr. B. : Potato Virus Diseases, 657
Katchalsky, Prof. A. : Biorheology and Microcirculation (Differences in Osmotic Fragility under Normal and Gradual Conditions of Haemolysis), 758
Kato, Yutaka, and Nabatoff, Robert A. : Treatment of Varicose Veins, 335
Katz, Dr. Sidney : Low-Angle Light Scattering and the Molecular Weight of Deoxyribonucleic Acid, 280
Katznelson, Dr. H., and Sirois, Dr. J. C. : Auxin Production by Species of *Arthrobacter*, 1323
Kawase, Shigemi : Role of Lipid in the Hardening of the Cuticle in the Silkworm, *Bombyx mori*, 279
Kawashima, Yoshiko, and others : Hexosamine and Sialic Acid Contents in Cells, 596
Kay, Prof. E. R. M. : Incorporation of Deoxyribonucleic Acid by Mammalian Cells *in vitro*, 387
Kay, Prof. H., and Weiss, Dr. A. D. : Relationship between Simple and Serial Reaction Times, 790
Keele, Prof. C. A. : Assessment of Pain, 1353
Keele, Dr. K. D. : Assessment of Pain (History of Theories of Pain from Primitive Times), 1353
Keeler, Dr. Richard F., and Lovelace, Dr. Stuart A. : Incorporation of Phosphorus-32 into Urinary Concretions forming on Zinc Implants in Rat Bladders, 715
Keilin, Prof. D. : Reactions of Haemoproteins with Hydrogen Peroxide and the Supposed Formation of Hydrogen Peroxide during the Autoxidation of Haemoglobin, 769
Keller, P., and others : Factors influencing the Composition of the Cellulose-decomposing Microflora on Soil Crumb Plates, 307
Kellett, Dr. E. G. : A Catalogue of the Ultra-Violet, *review*, 107
Kelley, George G., and others : Biochemical Aspects of Resistance to 6-Mercaptopurine in Human Epidermoid Carcinoma Cells in Culture, 469
Kelly, Stanley (Loewus, Dr. Frank A., and) : Identity of L-Ascorbic Acid formed from D-Glucose by the Strawberry (*Fragaria*), 1059
Kelus, A., and Moor-Jankowski, J. K. : An Iso-Antigen ($\gamma B\Delta$) of Mouse γ-Globulin present in Inbred Strains, 1405
Kemeny, L. G. : Topology of Linear Neutron Kinetics, 263
Kemény, T. : At the Second International Measurement Conference (An Automatic Electronic Potentiometer), 1350
Kempf, W. (Tegge, G., and) : At the International Starch Conference (Effect of certain Cations on Starch), 237
Kendall, D. G. : Theory of Markov Processes, *review*, 321
Kendall, Prof. M. G. : Re-elected president of the Royal Statistical Society, 126
Kennaway and the Carcinogens (Prof. Antoine Lacassagne), 743
Kennedy, Prof. Donald, and Baylor, Dr. Edward R. : Analysis of Polarized Light by the Bee's Eye, 34
Kennedy, Dr. G. C., and others : Melting and Polymorphism of Indium Antimonide at High Pressures, 1288
Kent, Dr. G. S., and Koster, Dr. J. R. : Height of Night-time *F* Layer Irregularities at the Equator, 1083
Kent Wild White Clover (*Trifolium repens* L.) : Flower Initiation in, under Controlled Environmental Conditions (R. J. Haggar), 1120
Kenya Colony : A Natural Hybrid of *Tilapia nigra* and *Tilapia leucosticta* from Lake Naivasha (H. Y. Elder and D. J. Garrod), 722
Kepler, Johann, and others : The Controversy on the Comets of 1618 (translated by Stillman Drake and Dr. C. D. O'Malley), *review* by Colin A. Ronan, 314
Keränen, A. J. A. (Suomalainen, Dr. Keikki, and): The First Anthocyanins appearing during the Ripening of Blueberries, 498
Keratin : Lanthionine Formation in (Dr. C. Earland and D. J. Raven), 384
Keratin (Feather) : Structure of (the late Prof. W. T. Astbury and E. Beighton), 171
Keratin (Wool) : Crystallization in (Dr. Allan Skertchly), 138
Kerly, Dr. Margaret : The Training of Biochemists (University and Departmental Entrance Requirements), 955
Kermack, Dr. K. A. : Awarded a Royal Society and Nuffield Foundation Commonwealth Bursary, 126
Kerrigan, M. A., and others : Comparison of Beryllium-7 and Caesium-137 Radioactivity in Ground-Level Air, 454
Kershaw, Prof. W. E., and others : Techniques in Tropical Pathology, *review* by Prof. P. C. C. Garnham, 1236
α-Keto-Acid Hydrazones : Application of the Nickel Reaction and Thermal Isomerization of, for their Chromatographic Analysis (A. M. Tokushige and Dean E. S. Cook), 797
Keto-Acids (Aromatic) : Side-Reactions during Hydrogenolysis of 2,4-Dinitrophenylhydrazones of (Dr. John H. Menkes), 285
Keto-Acids and Amines in Rumen Liquid of Cattle (Dr. C. J. G. van der Horst), 73
17-Ketosteroids (Neutral), in Porcine Urine (L. G. Huis in't Veld, B. Louwerens and W. Reilingh), 175
Kettering Laboratory, Charles F. (Ohio) : Appointment of Dr. Leo P. Vernon as director of research, 229
Key, Dr. B. J. : Appointed lecturer in neuropharmacology at the University of Birmingham, 337
Key, F. A., and others : Possible Method for Increasing the Signal-Noise Ratio in the Detection of the First Motion of a Refracted *P*-Wave, 1382
Keynan, A., and others : Factors influencing the Composition of the Cellulose-decomposing Microflora on Soil Crumb Plates, 307
Keyston, J. R. G. (Brewer, Dr. D. F., and) : Specific Heat and Expansion Coefficient of Liquid Helium-3 under Pressure below 0·1° K., 1261
Khain, V. E. (edited by A. P. Lebedev and) : Russian–English Geological Dictionary (T. A. Sofiano), *review* by Dr. S. I. Tomkeieff, 9
Khintchine, A. Y. : Mathematical Methods in the Theory of Queueing (translated by D. M. Andrews and M. H. Quenouille), *review* by Prof. L. S. Goddard, 211
Khosla, Dr. A. N. : Shell Roof for 325 feet Auditorium Span at the University of Roorkee (Presidential Address at the fifth Congress on Theoretical and Applied Mechanics), 855
Kidd, D. E. : Appointed research associate in nuclear physics in the University of Birmingham, 337

Kidd, Dr. G. L. : Assessment of Pain (Possible Mechanisms of Phantom Pain), 1353
Kidd, William, and others : Very-Low-Frequency Modulation of Discrete Frequency Solar Noise Bursts, 56
Kidney (Liver and) : Changes in Glucose-6-phosphatase Activity in, at Birth (M. J. R. Dawkins), 72
Kidneys (Rat) : Comparison of Short-Circuit Current and Net Water Movement in Single Perfused Proximal Tubules of (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Kidron, I. : At the second International Measurement Conference (Measurement of Dynamic Flow by means of Hot-wire Anemometers of the Constant-current and Constant-temperature Type), 1351
Killing Parasitic Nematodes for Collection Purposes : Use of Glacial Acetic Acid for (Bjørn Berland), 1320
Kim, Dr. K. S. : Anabolic Agents and Histamine Metabolism, 1368
Kimber, G. : Basis of the Diploid-like Meiotic Behaviour of Polyploid Cotton, 98
Kimura, Atsushi, and others : Hexosamine and Sialic Acid Contents in Cells, 596
Kimura, Yukio, and others : A Method for Isolating Bacteria capable of Producing 6-Aminopenicillanic Acid from Benzylpenicillin, 1122
Kin, Age Structure and Marriage amongst the Groote Eylandt Aborigines : Classification of (Dr. Frederick G. G. Rose), *review* by Dr. R. Needham, 6
Kind of Tea : The Concept of (W. Wight and R. C. J. H. Gilchrist), 14
Kinds (Two), of Arbor Virus Group *B* : Infectious Ribonucleic Acids derived from Mouse Brains infected with (Prof. Masahiro Nakamura), 624
Kinetics of the Enzymic Degradation of Deoxyribonucleic Acid into Sub-Units (Dr. Giorgio Bernardi and Prof. Charles Sadron), 809
Kinetics of Radical Chain Reactions in Solution : Generation of Radicals in Pairs and the (Dr. P. E. M. Allen and C. R. Patrick), 1194
Kinetics (Applied Chemical) : Inversion of a Laplace Transform arising from a Problem in (T. Wood), 589
Kinetics (Crystallization), in Polyvinylchloride and Thermoelastic Relaxation induced by Ultrasonics (Dr. V. Nardi), 563
Kinetics (Linear Neutron) : Topology of (L. G. Kemeny), 263
Kinetin and Indole-3-acetic Acid : Effects of, on Multiplication Rates of *Paramecium* (Mary Annunciata McManus and Kathleen Sullivan), 619
King, Dr. A. S.: Appointed professor of veterinary anatomy in the University of Liverpool, 648 ; work of, 648
King, Dr. W. H. : University Selection, *review*, 947
King's College, London : Appointment of S. B. Watkins to the University of London readership in chemical engineering, 546, 651
  Title of professor of civil engineering conferred on J. K. T. L. Nash, 651
  Title of professor of powder technology conferred on Dr. H. E. Rose, 651
  Title of reader in engineering science conferred on Dr. J. M. Zarek, 651
  Grant of £10,000 from the Gulbenkian Foundation, to establish a Gulbenkian Fund, 855
King-Hele, D. G. : Space-Flight and Re-entry Trajectories, 960
  A Collection of Tables and Nomograms for the Processing of Observations made on Artificial Earth Satellites, *review*, 1135
  Satellite and Space Orbits, *review*, 1334
King-Hele, D. G., and Walker, D. M. C. : Density of the Upper Atmosphere determined by Satellite Orbits, and its Variation during 1957-61, 114
Kinship in Australia : Age and, *review* by Dr. R. Needham, 6
Kipping, P. J. : Determination of Hydrogen in Gaseous Mixtures by Gas-Chromatography, 270
Kirk, M. : Appointed lecturer in the Department of Social Studies in the University of Leeds, 650
Kirk, Raymond E., and Othmer, Donald F. (edited by) : Encyclopedia of Chemical Technology, second Supplement Volume edited by Anthony Standen, *review* by R. H. Villet, 1131
Kirkendall Effect in Gases (Prof. E. A. Mason, L. Miller and P. C. Carman), 375
Kirschner, Prof. Leonard B. : Thermodynamics and Osmoregulation, 815
Kiss, Prof. Ferenc, and Szentágothai, Prof. János (edited by) : Atlas of Human Anatomy, Vol. 1, Osteology, Syndesmology, Myology ; Vol. 2, Splanchnology, Endocrine Glands, Heart ; Vol. 3, Nervous System, Vascular System, Lymphatic System, Sense Organs, *review* by Prof. J. M. Yoffey, 1232
Kitchell, Dr. R. L. : Assessment of Pain (Testing of Analgesic Drugs on Animals), 1354
Kitchener, Dr. J. A. : Appointed reader in the science of mineral processing at the Imperial College of Science and Technology, London, 443, 1349
  A Much-studied Protozoon, *review*, 1133
Kiyasu, Prof. J., and others : Urea Synthesis in the Living Rat Brain, 288
Kiyomoto, Dr. A., and others : Inhibition of β-Glucuronidase by Oral Administration of D-Glucosaccharo-1,4-Lactone, 1298
Klein, M. Wintzerith N., and others : Comparison of Pyridine Nucleotides in the Liver and in an Ascitic Hepatoma, 467
Kleinberg, Dr. Jacob, and others : Inorganic Chemistry, *review* by Dr. M. F. Lappert, 1133
Klingsberg, Erwin (edited by) : Pyridine and its Derivatives, *review* by Dr. G. L. Buchanan, 425
Klyne, Prof. W. : Stereochemistry of Natural Phenolic Compounds (Determination of Absolute Stereochemistry), 235
Knallgas Bacteria (*Hydrogenomonas*) : Formation and Utilization of Poly-β-Hydroxybutyric Acid by (Prof. H. G. Schlegel, G. Gottschalk and R. von Bartha), 463
Knapman, C. E. H., (edited by, assisted by C. G. Scott) : Gas Chromatography Abstracts, 1958, *review* by A. Goldup, 1135
  Gas Chromatography Abstracts, 1959, *review* by A. Goldup, 1135
Knight, Prof. B. C. J. G. : The Biology of Micro-organisms, *review*, 1032
Knight, Dr. C. : Paper Chromatography (New Ion Exchange Papers in which the Active Exchange Group is built into the Cellulose Molecule), 759
Kniper, Gerard P. (edited by) : Orthographic Atlas of the Moon (compiled by D. W. G. Arthur and E. A. Whitaker), Edition 'A' showing the Standard Orthographic Coordinate Grid, Part 1, Central Area, *review* by D. H. P. Jones, 424
Knowlden, J. A., and others : Bacteria of the Intestine (Studies on the Growth, Sporulation and Carriage of *Clostridium welchii* with special reference to Food Poisoning Strains), 1153
Knox, Dr. Graham R.: Appointed lecturer in the Department of Chemistry, Royal College of Science and Technology, Glasgow, 443
Knox, Prof. R., and Smith, J. T. : Use of Cellulose Acetate Membranes for Detecting Penicillinase-producing Organisms, 926
Knudsen, P. J., and Koefoed, J. : Urinary Inhibitors of Hyaluronidase, 1306
Kobayahi, Y., and others : Toxicology of *Penicillium islandicum*, 864
Koch, Dr. R. : Effects of Ionizing Radiation on Organic and Biological Solids (Sulphur Compounds as Protective Agents), 1151
Kochloefl, Dr. K., and others : Paper Chromatography (Film showing the Technique of Paper Chromatography of Amino-Acids), 759
Koefoed, J. (Knudsen, P. J., and) : Urinary Inhibitors of Hyaluronidase, 1306
Koelensmid, Dr. Blanche : Bacteria of the Intestine (Faecal Bacteria in Foods cannot be regarded with the same significance as in Water), 1154
Koenigsberger, Dr. F. : Appointed to the newly created chair of machine tool engineering in the University of Manchester, 19 ; work of, 19
Kohll, E. A. : Elected to a Postgraduate Studentship (for a course in chemical engineering) at Churchill College, Cambridge, 856
Kohn, H. I., and others : Estimation of Time of Generation of Living Cells, 290
Kohn, Dr. Kurt W. : Mediation of Divalent Metal Ions in the Binding o Tetracycline to Macromolecules, 1156
Koide, Minoru, and others : Iron-55 from Nuclear Detonations, 162
Kojibiose : Isolation of Nigerose and, from Dextrans (Kazuo Matsuda, Hiroshi Watanabe, Kentaroh Fujimoto and Prof. Kiyoshi Aso), 278
Koketsu, Prof. K., and others : Outflux of Inorganic and Organic Phosphate during Membrane Depolarization of Excitable Tissues, 395
Koldovsky, O., and others : Role of the Lung in Metabolism of Fat during Ontogeny of the Rat, 85
Koll, Dr. W. : Assessment of Pain (Effects of Morphine and other Drugs on Nociceptive Spinal Reflexes), 1353
Koller, H. R. (Medam, E. C., and) : Report on Computer Systems for Searching Chemical Literature (United States National Bureau of Standards Research Information Centre and Advisory Service on Information Processing), 23
Kolnai, Dr. A. T. : Appointed Lloyd-Muirhead Fellow in social philosophy in the University of Birmingham, 337
Kolthoff, I. M., and others (edited by) : Organic Analysis, Vol. 4, *review* by Dr. D. T. Lewis, 741
Kompaneets, A. S. (Zeldovich, Ia. B., and) : Theory of Detonation, *review* by Dr. W. L. Murray, 1236
Kon, Dr. S. K. : Vitamin B_{12} and Intrinsic Factor, 1156
Kondo, Yoichi, and others : Chromatographic Fractionation of Hog Thyroglobulin, 1199
Konishi, Jihei, and Zotterman, Prof. Yngve : Function of Taste Fibres in the Carp, 286
Kooy, J. M. J. : Space-Flight and Re-entry Trajectories (Trajectories for Lunar and Interplanetary Missions—Astrodynamics and Planetary Research), 960
Kopelman, A., and others : Urea Synthesis in the Living Rat Brain, 288
Kopoldová, K., and others : A Simplified Method for Separating Amino-Acid Mixtures from Protein Hydrolysates on One Ion-Exchange Column during Preparation, 1198
Koprowski, Dr. Hilary, and others : Effects of Heavy Water on Poliovirus Multiplication—Results and Speculations on Mechanism, 250
Košaric, Naïm, and Leliaert, the late G. : Determination of Indium in High-purity Zinc, 703
Koster, Dr. J. R. (Kent, Dr. G. S., and) : Height of Night-time *F* Layer Irregularities at the Equator, 1083
Kothari, Dr. N. J., and Saunders, Dr. J. C. : Effects of Corticotrophin, Hydrocortisone and Methopyrapone on the Circulating Eosinophiles in Man, 1105
Kotnis, L. B., and others : Anticancer Properties of Thio-diglycollic Acid, 388
Kotzé, J. J. : Management of Pine Stands for Pulpwood, 855
Kováč, Dr. L. : Changes of Permeability of Yeast with Sodium Deoxycholate, 698
Kovalevsky, J. : Space-Flight and Re-entry Trajectories (Near Earth Satellites—Analytical Review of Orbital Perturbations), 960
Kowarski, Dr. L. : New Trends in Atomic Research and their International Significance, 853
Koyama, I., and others : Outflux of Inorganic and Organic Phosphate during Membrane Depolarization of Excitable Tissues, 395
Krabbendam, H. : Netherlands fellowship in chemistry renewed for 1961-62 by the Ramsay Memorial Trust, 337
Krassilnikov, Prof. N. A.: Diagnostik der Bakterien und Actinomyceten, *review* by Dr. K. Zinnemann, 1333
Krebs, Sir Hans : Amino-Acids, *review*, 1132
Kremer, G. (Leupold, Dr. F., and) : Biosynthesis of Polyenoic Fatty Acids in Human Whole Blood, 805
Kreuzer, Dr. Rudolph : Fishing in Madagascar, 238
Krishna, V. G., and Goodman, Prof. Lionel : Polarization of Triplet→Singlet Emission Spectra, 800
Krishnamurti, Prof. K. : Theoretical Expression for Solubility Product, 1004
Krishnan, Sir K. S. : *Obituary* by Dr. D. Shoenberg, 541
Krishnan, the late Sir K. S., and Jain, S. C. : Quenching of Cation Vacancies in Doped Crystals of Sodium Chloride, 162
Krishnan, Prof. P. S., and others : Diurnal Variation in Transaminase Activity in the Cactus Plant, 516
Kristensen, Gunhild, and others: Serum Lipids in relation to the Intake of Alcohol and Sodium Chloride, 1095
Kritchevsky, Dr. David, and others : Effects of Heavy Water on Poliovirus Multiplication—Results and Speculations on Mechanism, 250
Krnjević, K. : Cholinergic Transmission in Fish Muscle, 1403
Kryszewski, Dr. Marian, and others : Photochemical Production of Semiconducting High Polymers, 164
Kubelka, Vl., and Rybák, M. : Action of Tetracycline on the Level of Properdin, 820
Kucharski, J. (Nowak, H. F., and) : Effect of Polyvinyl Alcohol on the Distribution of Iodine-131 in the Internal Organs, Fluids and Excreta of Rabbits, 665, *erratum*, 1045
Kühnau, Prof. J. : Vitamin B_{12} and Intrinsic Factor (Importance of the newly revealed Structure of Vitamin B_{12} Coenzyme), 1154
Kuiper, K. : Nematology (Invasion of New Polder-Soils from the Zuider Zee Area by Plant Nematodes), 1048
Kulkarni, S. B., and others : Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers, 378

Kumada, Kyoichi, and others : Isolation of Anthraquinone from Humus, 415
Kumar, A., and others : Effect of Excess Supply of Heavy Metals on Barley during Germination, with special reference to Catalase and Peroxidase, 726
Kumta, U. S., and Tappel, A. L. : Radiation Damage to Proteins, 1304
Kundu, N. (Brahma, S. K., and) : Effects of *Grewia populifolia* Extracts on the Development of Chick Embryos, 506
Kuyama, Shimpei, and others : A Nemin Assay Procedure, 155
Kwashiorkor : Partition of Urine Nitrogen in (Dr. J. C. Edozien and E. J. Phillips), 47
Kyusyu, Japan : Gravity Survey on the Aira Caldera (Izumi Yokoyama), 966

LABELLAR Hairs of the Blowfly, *Phormia regina* Meigen : Response of the, to Protein (D. I. Wallis), 917
Labile (Heat-) Alkaloids from Plants : Extraction of (Dr. A. R. Mattocks), 1281
Laboratory (A Mobile), for U.H.V. and V.H.F. Television Surveys (E. W. Taylor and L. C. Munn), 1355
Laboratory (A New), for Research on Creep, 1049
Laboratory Animals Centre : Discussion on Hazards of the Animal House, 339
Laboratory Handbook of Pulp and Paper Manufacture (Dr. Julius Grant), second edition, *review* by Dr. F. Lyth Hudson, 320
Laboratory Manual of Analytical Methods of Protein Chemistry, including Polypeptides (edited by Dr. P. Alexander and R. J. Block), Vol. I, The Separation and Isolation of Proteins, *review* by Dr. D. T. Elmore, 320
Laboratory Mice : Simultaneous Eradication of Three Ectoparasitic Species from a Colony of (Dr. Nigel Bateman), 721
Laboratory Rats (White) : Leptospirosis of (Dr. M. Füzi and R. Csóka), 1123
Labour Party : Science and the Future of Britain, 837
Labour Process : Relationship between the Decrease in Urinary Excretion of Histamine and the Initiation of the (Dr. Halina Wiskont-Buczkowska), 290
Lacassagne, Prof. Antoine : Kennaway and the Carcinogens, 743
Lacassagne, Prof. Antoine, and others : A New Family of Potent Carcinogens—Benzopyridocarbazoles, 1005
Lack, Dr. C. H., and others : Action of Plasmin on Cartilage Matrix *in vivo*, 1402
Lack, R. G. (Shorter, Dr. Roy G., and) : Immulogical Study of Experimentally Induced Extrahepatic Cholestasis, 610
Lack of Effect of Coenzyme Q_{10} on Coumarin-induced Hypoprothrombinaemia (Prof. Murray Weiner, Dr. Theodore Chenkin and Dr. Peter G. Dayton), 711
Lack of Evidence for a Cholinergic Mechanism in Sympathetic Transmission (Dr. J. E. Gardiner and Dr. J. W. Thompson), 86
Lacko, Dr. L., and Burger, Dr. M. : Common Carrier System for Sugar Transport in Human Red Cells, 881
Lactating Cows given Artificial Rations Low in Magnesium: Rapid Development of Hypomagnesaemia in (J. A. F. Rook), 1019
Lactic Acid Metabolism (Fructose and), in the Perfused Liver of Premature Lambs (W. H. H. Andrews, H. G. Britton and Dr. D. A. Nixon), 1307
Lactic Dehydrogenases (Heterogeneous), in Malignant Effusions : Diagnostic Significance of (R. Richterich, K. Zuppinger and E. Rossi), 507
Lagerlof, Dr. B. : Awarded a grant from the Lady Tata Memorial Trust, for research on leukaemia and allied diseases, 228
Lainé, Dr. D. C. : A Proposal for a Tunable Source of Radiation for the Far Infra-Red using Beats between Optical Masers, 795
Lake, J. V., and Slack, G. : Dependence on Light of Geotropism in Plant Roots, 300
Lake in British Columbia containing Old Sea-Water (Dr. P. M. Williams, Prof. W. H. Matthews and Prof. G. L. Pickard), 830
Lake District, Friends of the : Newsletter for June 1961, 855
Lake Kariba : Limnological Trends in (D. Harding), 119
Lake Naivasha, Kenya Colony : A Natural Hybrid of *Tilapia nigra* and *Tilapia leucosticta* from (H. Y. Elder and D. J. Garrod), 722
Lake Victoria : A Phytoplankton Multi-Sampler and its Use in (Dr. J. H. Evans), 53
Lakes (Fresh-water Eutrophic), of India : Hydrogen Ion Concentration, Plankton and Fish in (Dr. S. M. Das), 511
Lalor Foundation : Grants and Awards for 1962, 1348
Lamb, H. H. : Melbourne Symposium on Antarctic Meteorology, *review*, 210
Lamb, Dr. John : Appointed to the James Watt chair of electrical engineering in the University of Glasgow, 224 ; work of, 224
Obituary of Prof. Bernard Hague, 750
Lambert, J. M. (Williams, Prof. W. T., and) : Nodal Analysis of Associated Populations, 202
Lambrecht, F. L., and others : Isolation of *Plasmodium knowlesi* from Philippine Macaques, 1117
Lambs (Premature) : Fructose and Lactic Acid Metabolism in the Perfused Liver of (W. H. H. Andrews, H. G. Britton and Dr. D. A. Nixon), 1307
Lamont Geological Observatory : Fellowships for Plankton Research, 754
Lamp (Flash-): Photo-Polarography with a (Dr. H. Berg and H. Schweiss), 1270
Lamp (Neon Glow), 1911-61 : The Development of the (Prof. H. E. Watson), 1040
Lamprey Populations : Sex Composition of (Dr. M. W. Hardisty), 1116
Land, Dr. Frank : The Language of Mathematics, *review* by W. J. Langford, 638
Land- und Forstwirtschaft, Biologische Bundesanstalt für : Proceedings of the Fourth Conference on Potato Virus Diseases, Braunschweig, 1960 (edited by J. Brandes, R. Bartels, V. Völk and C. Wetter), 657
Land and Man in the Western Weald (Prof. S. W. Wooldridge), 132
Land-Use Survey—Nicaragua (Dr. John Grainger), 1259
Landau, Dr. J. : Biorheology and Microcirculation (Effect of Adrenaline on the Small Blood Vessels of the Conjunctiva and Nailfold), 758 ; (Observations and Experiments on Vasomotion in Human Capillaries), 758
Landsberg, Prof. P. T. : Thermodynamics with Quantum Statistical Illustrations, *review* by Dr. W. E. Parry, 1236
Landy, Maurice, and others : Increase in Specific Bacterial Antibodies after Administration of Endotoxin, 296
Láng, L., and others : Chemical Character of the Pigments of a New Nitrogen-fixing Micro-organism, 1413
Langer, Stanley H., and Pantages, Peter : Peak-Shift Technique in Gas-Liquid Chromatography—Trimethylsilyl Ether Derivatives of Alcohols, 141
Langford, W. J. : Mathematical Delight, *review*, 638
Langham, W. L., and others : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Methods for Growing HeLa Cells Suspensions in the Presence of Tritiated Thymidine and Tritium Water), 341
Language : Mathematics and, *review* by Dr. G. H. Toulmin, 211
Language of Mathematics (Dr. Frank Land), *review* by W. J. Langford, 638
Language (Modern) Association of America : Science Information Personnel —the New Profession of Information combining Science, Librarianship and Foreign Languages (Leonard Cohen and Kenneth Craven), 452
Languages (Science, Librarianship and Foreign) : Science Information Personnel—the New Profession of Information combining (Leonard Cohen and Kenneth Craven), 452
Lanthionine Formation in Keratin (Dr. C. Earland and D. J. Raven), 384
Lanyon, Dr. W. E., and Tavolga, Dr. W. N. (edited by) : Animal Sounds and Communication, *review* by Dr. W. H. Thorpe, 741
Lapage, Dr. G. : European Parasites of Man, *review*, 107
Journal of Helminthology, Supplement in honour of R. T. Leiper on the occasion of his 80th Birthday, *review*, 843
Laplace Transform : Inversion of a, arising from a Problem in Applied Chemical Kinetics (T. Wood), 589
Lappert, Dr. M. F. : Inorganic Chemistry, *review*, 1133
Large Lymphocytes and the Simonsen Phenomenon (Dr. Aleksander Szenberg and N. L. Warner), 920
Larger Fruit in the Raspberry : Mutation for (D. L. Jennings), 302
Larvae (Sponge) : Inclusion of Parental Somatic Cells in (Frederick E. Warburton), 1317
Larval Foods (Honeybee) : Chromatographic Investigation of the Methyl Alcohol/Chloroform-soluble Constituents of (N. G. Patel, M. H. Haydak and R. Lovell), 362
Lascelles, A. K. : Removal of Milk Constituents from the Mammary Gland of the Ewe, 1404
Lasiewski, R. C., and others : Thyroid Function in Active, Sleeping and Torpid Hummingbirds, 1318
Lasnitzki, Dr. Ilse : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology, 340 ; (Irradiation can change the Pattern of Differentiation in Organized Tissues grown *in vitro*), 342
Lasnitzki, Dr. Ilse, and others : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Action of Radiation on Freshly Explanted Cultures of Organized Tissues), 341
Last, Dr. F. T. : Appointed head of the Department of Mycology and Bacteriology at the Glasshouse Crops Research Institute, 1250
Laszlo, Imre : Appointed to an I.C.I. Research Fellowship in the University of Edinburgh, 545
Lateral Hypothalamus of the Rat : Evidence of a 'Hunger Motivational' System in the (Dr. P. J. Morgane), 672
Lateral Plates of the Stickleback (*Gasterosteus aculeatus* L.) : Significance of the Regeneration of (Tadeusz Penczak), 621
Lateral Ventricle of Brain : Antibody Response in Rabbits following Injection of Sheep Erythrocytes into (Prof. B. D. Janković, Dr. M. Draškoci and Dr. K. Isaković), 288
Lathyrism (Odoratism) : Influence of Scurvy and, on Hydroxyproline Excretion (Dr. G. R. Martin, S. E. Mergenhagen and D. J. Prockop), 1008
Latin America : Nutrition in (Food and Agriculture Organization of the United Nations Meetings on), 959
Latitude Distribution of Ionospheric Irregularities : Scintillations and the (D. G. Singleton), 482
Latrodectus geometricus C. Koch (Spider) : Cycle and Rhythm in the Ovulation of the (A. Bouillon and R. Lekie), 620
Lattice Silicates (Layer) : Surface Symmetry and Cell Dimensions of (E. W. Radoslovich), 67
Laubengayer, Prof. A. W. : Inorganic Polymers (BN Polymers), 1047
Lavallee, W. H., and Rhode, R. A. : Nematology (Movements of Larvae of *Pratylenchus penetrans*), 1048
Lavenne, F., and Leyh, P. : Design and Use of Respirators (Performance of Two Closed-circuit Breathing Sets), 656
Lavoipierre, Dr. M. M. J. : Blood-Feeding, Fecundity and Ageing in *Aedes aegypti* var. *Queenslandensis*, 575
Law, C. N. : Recombination in the X-Chromosome of *Drosophila melanogaster*, 1180
Law (Medicine, Science and the) : Vol. I, No. 4, 753
Lawden, D. F. : Space-Flight and Re-entry Trajectories (Orbital Transfer and Rendezvous), 960
Lawrence, Dr. B. E. : Education in Uganda (Presidential Address to Section L (Education) of the British Association for the Advancement of Science), 990
Lawrence, G. P. (Deuchars, Dr. W. M., and) : Interaction of 14-MeV. Neutrons with a Silicon Semiconductor Nuclear Particle Detector, 995
Lawrence, M., and Vivian, H. E. : The Action of Calcium Chloride on Mortar and Concrete, 125, *erratum*, 1145
Laws, Dr. R. M. : Appointed senior investigator in the Nuffield Unit of Tropical Animal Ecology, Mweya, Uganda, 651
Layer (Thin), of Starch-Gel : Electrophoresis in a, followed by a Plastifying Treatment (J. Groulade, Dr. J. M. Fine and C. Ollivier), 72
Layer Lattice Silicates : Surface Symmetry and Cell Dimensions of (E. W. Radoslovich), 67
Layers (Adsorbed), of Cyanine Dyes on Silver Bromide Microcrystals : Electron Spin Resonance Absorptions induced by Light in (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Layrisee, Dr. Miguel, and others : New *Rh* Phenotype, $Dcce^{t}e^{t}f$, found in a Chibcha Indian Tribe, 503
Layrisee, Zulay, and others : New *Rh* Phenotype, $Dcce^{t}e^{t}f$, found in a Chibcha Indian Tribe, 503
Lazarus, Dr. S., and others : Biorheology and Microcirculation (Capillary Strength Tests in Scurvy and their Reactions to Vitamins C and P Therapy), 758
Lazenby, Dr. Alec : Weeds in Britain, *review*, 842

Lazzarini, E., and others : Intermediate Pool between Amino-Acids and Globin in Duck Blood *in vitro*, 40
LeFevre, Paul G. : Persistence in Erythrocyte Ghosts of Mediated Sugar Transport, 970
LeGalley, Donald P. (edited by) : Ballistic Missile and Space Technology—Proceedings of the Fifth Symposium held at Los Angeles, 1960, Vol. 1, Bioastronautics and Electronics, with Invited Addresses ; Vol. 2, Propulsion and Auxiliary Power Systems ; Vol. 3, Guidance, Navigation, Tracking and Space Physics ; Vol. 4, Re-entry and Vehicle Design, *review* by W. H. Stephens, 1131
Le Grand, Prof. Yves : At the Maxwell Colour Centenary Conference (Complexities of the Visual Process), 10
Le Play Society : Presidential Address to the concluding Conference (Sir John Russell), 1144
Lea (Douglas) Memorial Lecture of the Hospital Physicists' Association (Dr. E. L. Powers), 1045
Leach, Gerald Adrian : Appointed to the editorship of *Discovery*, 1043 ; work of, 1043
Leach, Dr. S. : Effects of Ionizing Radiation on Organic and Biological Solids (Reactions in the Solid State), 1150
Lead in Urine : Determination of, by Atomic Absorption Spectroscopy (J. B. Willis), 381
Lead Monoxide : Rotational Analysis of Absorption Bands of (Dr. R. F. Barrow, J. L. Deutsch and D. N. Travis), 374
Lead Silicate Glass (γ-Irradiated) : Absorption Spectra of (R. S. Barker, T. K. Harrison, D. A. Richardson and R. Rimmer), 374
Leaf Blight : Susceptibility to, caused by *Cochliobolus heterostrophus*, in Nigerian Varieties of Maize (C. L. M. van Eijnatten), 515
Leaf Colour : Measurement of (J. Philip Grime), 614
Leaf Curl Virus and the Flavonoid Content of Resistant and Susceptible Strains of Cotton (Dr. Esam M. Moustafa), 415
Leaf-Spot Disease (Barley) : Production of Symptoms of, by Culture Filtrate of *Helminthosporium sativum* (S. K. Gayed), 725
Leakey, Dr. L. S. B. : The Juvenile Mandible from Olduvai, 417
Leakey, Dr. L. S. B., and others : Age of Bed I, Olduvai Gorge, Tanganyika, 478
Leal, R. S. (Greenbaum, Dr. A. L., and) : Effect of Pituitary Growth Hormone on Cholesterogenesis, 600
Learning in Two Sense-Modalities (Dr. G. Ettlinger), 308
Learning (Paired-Associate), of Nonsense Syllables : Stimulus-Response Compatibility in the (A. D. Baddeley), 1327
Leaves : Physiological Significance of Surface Wax on (D. M. Hall and R. L. Jones), 95
Leaves of *Bryophyllum fedtschenkoi* : An Endogenous Rhythm in the Rate of Dark-Fixation of Carbon Dioxide in the (Diana M. Warren and Malcolm B. Wilkins), 686
Leaves of Etiolated Pea Plants : Correlative Inhibition of Formation of Chlorophyll in the (Jiří Úlehla), 613
Lebedev, A. P., and Khain, V. E. (edited by) : Russian–English Geological Dictionary (T. A. Sofiano), *review* by Dr. S. I. Tomkeieff, 9
Lectures on Haematology (edited by F. G. J. Hayhoe), *review* by Dr. M. E. Nutt, 7
Lederer, Prof. Edgar : Elected a Member of the Biochemistry Section of the Leopoldina German Academy of Sciences, 122
Lederer, G. : Appointed a lecturer in pure mathematics at the University of Sheffield, 229
Lederer, Dr. M. : Paper Chromatography (Separation of particular families of Compounds—Results of Australian Workers in the Inorganic Field), 759
Lee, D. L. : Parasite Biology (Application of New Laboratory Techniques), 135
Lee, J. A. (Marples, J. A. C., and) : Structure of δ-Plutonium, 1391
Lee, W. H. (Allam, D. S., and) : Ultrasonic Velocity in Aqueous Silver Nitrate Solution, 1388
Leech : Neurosecretion and Granules in Neurones of the Brain of the (Irvine R. Hagadorn and Richard S. Nishioka), 1013
Lees, J., and others : Synthesis and X-Ray Analysis of Diamond, 865
Lefebvre, Prof. A. H. : Appointed to the chair of aircraft propulsion at the College of Aeronautics, Cranfield, 1042 ; work of, 1042
Leguebe, André : Inter-Individual Variability in Pigmentation, 1326
Leguminous Root Nodules (Gibberellin-like Substances in Plants), (Margaret Radley), 684
Leiper, R. T. : *Journal of Helminthology*, Supplement in honour of, on the occasion of his 80th Birthday, 1961, *review* by Dr. G. Lapage, 843
Lekhnovitch, V. S. : Introduction of the Potato into Western and Central Europe, 518
Lekie, R. (Bouillon, A., and) : Cycle and Rhythm in the Ovulation of the Spider *Latrodectus geometricus* C. Koch, 620
Leliaert, the late G. (Košaric, Naîm, and) : Determination of Indium in High-purity Zinc, 703
Length (Internode-), in *Pisum sativum* : Effects of Gibberellic Acid and Allogibberic Acid on Flowering and (I. C. Murfet and H. N. Barber), 514
Lengths (Bond-), in Solid Helium (Dr. A. F. Schuch), 591
Lenhert, Dr. P. G. (Hodgkin, Dr. Dorothy, and) : Vitamin B$_{12}$ and Intrinsic Factor (Vitamin B$_{12}$ Coenzyme), 1154
Lens (Optical) Performance : Some Aspects of, Part 1, Secondary Flare in Lenses (K. Hacking) ; Part 2, The Design of Lens Hoods (W. N. Sproson), 1355
Lens (Rat) Enzyme System : Catalysis of Incorporation of ^{14}C-Uridine into Rat Ribonucleic Acid by (Dr. Anima Devi and Dr. N. K. Sarkar), 1094
Lens (Rat) Ribonucleic Acid : Catalysis of Incorporation of ^{14}C-Uridine into, by Rat Lens Enzyme System (Dr. Anima Devi and Dr. N. K. Sarkar), 1094
Leonard, B. E., and Sherratt, Dr. H. S. A. : Pharmacology of the Alkaloidal Fraction from the Bark of the Jamaican Shade Tree *Pithecolobium samath* Benth., 287
Leopold, —., and others : How Old are the Angiosperms?, 442
Leopoldina German Academy of Sciences : Election of new Members, 122
Leprosy : Rehabilitation in—Report of the Scientific Meeting on (United Nations, World Health Organization, Technical Report Series No. 221), 1248
Leptospirosis of White Laboratory Rats (Dr. M. Füzi and R. Csóka), 1123
Lesher, S., and others : Estimation of Time of Generation of Living Cells, 290
Lesion Number and Concentration (Tobacco Mosaic Virus (TMV)) : Relationship of, to the Rate of Lesion Production on Pinto Bean (John Piacitelli and Prof. Vincent Santilli), 624
Lesion Production on Pinto Bean : Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the (John Piacitelli and Prof. Vincent Santilli), 624
Lesions of Molluscum Contagiosum : An Antigen in (Dr. William L. Epstein, Irene P. Senecal and Dr. Alan M. Massing), 509
Leston, Dennis : The Number of Testis Follicles in Miridae, 93
Lethal Staphylococcal Infections in Mice : Biochemical Changes in (George W. Counts, Dr. Ian M. Smith, Dr. Joseph I. Routh, Dr. Elia Ch. Hazard and J. F. McTavish), 783
Lethal (Non-) Pathological Infection of Roots (F. J. Newhook), 615
Letham, D. S., and Bollard, E. G. : Stimulants of Cell Division in Developing Fruits, 1119
Letterer, Prof. Ernst : Elected a Member of the Pathology Section of the Leopoldina German Academy of Sciences, 122
Letts, H. J. R. : Design and Use of Respirators (Industrial Gas and Dust Masks), 656
Leuallen, E. Emerson, and others (edited by) : Remington's Practice of Pharmacy, twelfth edition, *review* by Dr. J. H. Gaddum, 8
Leucine Aminopeptidase in the Mammary Gland of the Cow and Rat (Prof. S. Talanti and Prof. Väinö K. Hopsu), 86
Leucine Aminopeptidase (Human Serum) : Sub-fractionation of (Prof. Clyde A. Dubbs, Charlotte Vivonia and Jean M. Hilburn), 1203
Leucobases (Thiazine) : Photo-Sensitivity of (D. W. Menzies), 505
Leucocyte (Anti-) Cytoplasm, Anti-Leucocyte Nuclei and Anti-Platelets in Disseminated Lupus Erythematosis (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Leucocyte (Anti-) Nuclei and Anti-Platelets (Anti-Leucocyte Cytoplasm,), in Disseminated Lupus Erythematosis (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Leukaemia in the *AkR* Mouse after X-Irradiation *in utero* (Donald Porteous), 291
Leukaemia in the Progeny of Mice inoculated with a Leukaemogenic Virus (Dr. M. H. Salaman and J. J. Harvey), 509
Leukaemia (Murine) : Transplantation of, by Unconventional Routes (Dr. Harold B. Hewitt), 1213
Leukaemia (Transplantable Acute Myeloid) : Effect of Calf Thymus Histone on Rats bearing (Dr. Lubomir Hnilica and Dr. Viktor Holoubek), 922
Leukaemia and Allied Diseases : Research on (Lady Tata Memorial Trust Awards for 1960-61), 228
Leukaemogenesis : Analysis of the Thymus Influence in (Dr. J. F. A. P. Miller), 248
Leukaemogenic Virus : Leukaemia in the Progeny of Mice inoculated with a (Dr. M. H. Salaman and J. J. Harvey), 509
Leupold, Dr. F., and Kremer, G. : Biosynthesis of Polyenoic Fatty Acids in Human Whole Blood, 805
Lev, Dr. M. : Bacteria of the Intestine (Germ-free Animals and their Use in Elucidating the Action of the Gut Flora on the Host), (read by Dr. Cuthbert), 1152
Level of Properdin : Action of Tetracycline on the (Vl. Kubelka and M. Rybák), 820
Levels of Succinic Dehydrogenase during Chemical Carcinogenesis : Tissue Dilution Artefact—a Re-interpretation of Variations in (G. R. N. Jones, L. Bitensky, J. Chayen and Prof. G. J. Cunningham), 1203
Levels (Atomic Energy), in Crystals (J. L. Prather), 1348
Levels (Vitamin-), in Blood and Serum (H. Baker, O. Frank, I. Pasher, Dr. H. Sobotka and S. H. Hutner), 78
Leversen, A. I. : Palaeogeologic Maps, *review* by Dr. C. Downie, 842
Levi, P. : Appointed Bernard Hickson Fellow in the Department of Botany in the University of Leeds, 650
Levine, Dr. S., and others : Discreteness-of-Charge Effect and the Stability Theory of Colloids, 699
Lewis, Dr. D. (edited by) : Digestive Physiology and Nutrition of the Ruminant—Proceedings of the University of Nottingham Seventh Easter School in Agricultural Science, *review* by Dr. I. W. McDonald, 1334
Lewis, Dr. D. T. : Analysis of Organic Compounds, *review*, 741
    The Use of Organic Reagents in Inorganic Analysis, *review*, 1336
Lewis, W. V. : *Obituary* by Jean M. Grove, 332
Lewison, G. R. G. : Elected to a Research Studentship in engineering at Churchill College, Cambridge, 856
Leyh, P. (Lavenne, F., and) : Design and Use of Respirators (Performance of Two Closed-circuit Breathing Sets), 656
Li, Prof. Choh Hao (Moudgal, N. R., and) : An Immunological Study of a Human Pituitary Interstitial Cell-stimulating Hormone, 192
Liberation (Amino-Acid), from Proteins by Enzyme or 'Heat Catalysed' Hydrolysis (S. M. Hardy), 1061
Liberation and Dispersal (Spore), of Coffee Rust *Hemileia vastatrix* B. et Br. (R. W. Rayner), 725
Librarianship and Foreign Languages (Science,) : Science Information Personnel—the New Profession of Information combining (Leonard Cohen and Kenneth Craven), 452
Libraries : Brown, Picton and Hornby (Liverpool Libraries, Museums and Arts Committee), 21
Libraries (British) : A Soviet View of (V. I. Shunkov, G. G. Firsov and N. I. Tyulina), translated by A. Thompson (Library Association, Pamphlet No. 22), 752
Libraries (University Extra-Mural), in Great Britain : Aims, Organization and Methods of (E. P. Pritchard), 649
Library of the United States Naval Observatory : Resources and Treasures of the (Marjorie S. Clopine), 22
Library (National Lending), for Science and Technology : *N.L.L. News*, No. 1, 1347
Library (National Reference), of Science and Invention : 1 ; (Leslie Wilson), 733 ; (J. Farradane), 734
    Aslib Report of the Discussion Meeting on the, 649
Library (South African Public) : Grey Bibliographies No. 7, A Bibliography of African Bibliographies, covering the Territories South of the Sahara, fourth edition, 959
Library Association : Annual Report of the Council for 1960, 21
    Special Subject List No. 34, Solar Energy Technology, 1954-1959 (T. Brimelow), 228
    Pamphlet No. 21, Aims, Organization and Methods of University Extra-Mural Libraries in Great Britain (E. P. Pritchard), 649
    Pamphlet No. 22, A Soviet View of British Libraries (V. I. Shunkov, G. G. Firsov and N. I. Tyulina), translated by A. Thompson, 752

Library Association (School) : Grant of £3,900 for three years from the Gulbenkian Foundation, 754
Lichtenstein, the late Yehudith (Grossowicz, Prof. Nathan, and) : Enzymic Binding of Hydroxylamine by Fumaric Acid, 412
Lidén, Kurt, and others : Radiocaesium and Potassium in Norwegians, 436
Lie-Injo, Dr. Luan Eng : Haemoglobin 'Barts' and the Sickling Phenomenon, 1314
Liebeck, H. : Appointed to a lectureship in mathematics at the University College of North Staffordshire, 229
Liebster, J., and others : A Simplified Method for Separating Amino-Acid Mixtures from Protein Hydrolysates on One Ion-Exchange Column during Preparation, 1198
Lienau, C. C. (edited by Quincy Wright and) : Statistics of Deadly Quarrels (Dr. Lewis F. Richardson), *review* by Sir Cyril Burt, 427
Liese, Prof. Walter : Elected a Member of the Hygiene Section of the Leopoldina German Academy of Sciences, 122
Life (Animal) : South African—Results of the Lund University Expedition in 1950-1951, Vol. 7 (edited by Bertil Hanstrom, Per Brinck and Gustaf Rudebeck), *review* by Prof. O. Lowenstein, 1135
Life (English Rural), Museum of : Annual Report for 1960 (University of Reading), 544
Life (Folk-) Study, Society for : Formation of a, 544
Life (Invertebrate), in South-East England (John Sankey), 132
Life (Wild-) : New Hope for African, 958
Life and Environment on the Bed of the Sea (Prof. C. M. Yonge), 986
Life and Scientific Work of Sir James Hall (Dr. V. A. Eyles), 345
Life and Work of William Higgins, Chemist (1763-1825), (Prof. T. S. Wheeler and Prof. J. R. Partington), *review* by Frank Greenaway, 533
Light : Dependence on, of Geotropism in Plant Roots (J. V. Lake and G. Slack), 300
Light in Adsorbed Layers of Cyanine Dyes on Silver Bromide Microcrystals : Electron Spin Resonance Absorptions induced by (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Light (Cerenkov), from Extensive Air Showers in the Atmosphere: Photography of (D. A. Hill and N. A. Porter), 690
Light (Polarized) : Analysis of, by the Bee's Eye (Prof. Donald Kennedy and Dr Edward R. Baylor), 34
Light Depolarization Technique : Crystallization of Isotactic Polypropylene using a (J. H. Magill), 1092
Light Intensity : Effects of, on Reproduction in S.48 Timothy (*Phleum pratense* L.), (Dr. G. J. A. Ryle), 196
Light Microscopy : A Method of Replicating Dry or Moist Surfaces for Examination by (Joan Sampson), 932
Light Rainfall and Plant Survival—Measurement of Stem Flow Run-off (M. D. Gwynne and J. Glover), 1321
Light Scattering (Low-Angle), and the Molecular Weight of Deoxyribonucleic Acid (Dr. Sidney Katz), 280
Limit (Upper Plastic), of Clays : Role of Interparticle Forces in Development of (Dr. Raymond Yong and E. T. Selig), 938
Limitations of Geometrical Models for Complementation Mapping of Allelic Series (Prof. Elof. Axel Carlson), 788
Limits of Human Vision (Dr. R. A. Weale), 471
Limnological Trends in Lake Kariba (D. Harding), 119
Lindahl, P. E. Björn : Inhibition of Growth and Photosynthesis in Submerged Plants with Tetramethylthiuram Disulphide and Sodium Dimethyldithiocarbamate and Reversal of this Inhibition of Photosynthesis, 51
Lindley, Prof. D. V. : An Introduction to Infinitely Many Varieties, *review*, 214
Lindner, E. B. (Porath, J., and) : Separation Methods based on Molecular Sieving and Ion Exclusion, 69
Lindstrom, G. R., and others : Role of Polysaccharides in Stabilization of Natural Soil Aggregates, 1283
Lindzey, Prof. G., and others : Social Dominance in Inbred Mouse Strains, 474
Linear Accelerators (Electron) : Pulse-shortening in (Dr. M. C. Crowley-Milling, T. R. Jarvis, C. W. Miller and G. Saxon), 483
Linear Neutron Kinetics : Topology of (L. G. Kemeny), 263
Linear Programming (Saul I. Gass), *review* by Dr. M. V. Wilkes, 9
Linear from Branched Polysaccharides : Separation of, by Precipitation as Iodine Complexes (Blanche D. E. Gaillard), 1295
Linear and Non-Linear Programming : Methods of Feasible Directions—a Study in (Dr. G. Zoutendijk), *review* by Dr. M. V. Wilkes, 9
Linear (Non-) Physical Problems (Indian Society of Theoretical and Applied Mechanics and Unesco Symposium on), 855
Ling, T. (Ferri, A., and) : Space-Flight and Re-entry Trajectories (Dynamics of Terminal Re-entry—Effect of Varying Lift on Re-entry Trajectories), 960
Linguistics (Mathematical) : Type-Token Mathematics, a Textbook of (Dr. Gustav Herdan), *review* by Dr. G. H. Toulmin, 211
Linnell, Prof. W. H. : Title of professor emeritus of pharmaceutical chemistry (at the University of London School of Pharmacy) conferred on, 546
Linskens, H. F. (Heinen, W., and) : Enzymic Breakdown of Stigmatic Cuticula of Flowers, 1416
Linton, A. H., and others : An Introduction to the Biology of Micro-organisms, *review* by Prof. B. C. J. G. Knight, 1032
Lipase (Pancreatic) : Hydrolysis of Fatty Acid Esters of Riboflavin by (Prof. Kunio Yagi, Yoshiko Yamamoto and Jun Okuda), 174
Lipid : Role of, in the Hardening of the Cuticle in the Silkworm, *Bombyx mori* (Shigemi Kawase), 279
Lipid (Orientated), on a Biological Membrane : Electrical Properties of—an Electrostatic Diffusion Barrier and Ion Pump (Dr. J. W. L. Beament), 217
Lipid and Lipidophilic Substances as Adjuvants : Effectiveness of (D. W. Dresser), 1169
Lipidophilic Substances (Lipid and), as Adjuvants : Effectiveness of (D. W. Dresser), 1169
Lipids of the Common Pea (*Pisum sativum* L.) with special reference to the Occurrence of Galactolipids (S. Adhikari, Dr. F. B. Shorland and R. O. Weenink), 1301
Lipids from the Precursor of the Stylet-Sheath Material of a Pentatomid : Extraction of (P. W. Miles), 911
Lipids and Trypsin : Interaction of, in Dimethyl Sulphoxide–Water Systems (Prof. A. Lukton, H. Donohue and Prof. F. A. Bettelheim), 565
Lipids (Serum), in relation to the Intake of Alcohol and Sodium Chloride (Erling Lund, Torben Geill, Prof. Henrik Dam and Gunhild Kristensen), 1095
Lipids (Serum), and Atherosclerosis in Rabbits : Effects of Carrageenin on (Dr. Katsumi Murata), 189
Lipogenesis in Thyroid Tissue *in vitro* : Stimulation of, by Free Fatty Acids (Prof. Norbert Freinkel, Dr. Augusto D. Litonjua and Edward C. Saef), 804
Lipopolysaccharide : Development in Mice of Prolonged Non-specific Resistance to Sarcoma Implant and Staphylococcus Infection following Repository Injection of (Samuel J. Prigal), 1111
Lipopolysaccharide (Bacterial) : Sensitivity of Mice to, following Alteration of Activity of the Reticulo-Endothelial System (Dr. G. N. Cooper and Dr. A. E. Stuart), 294
Liquid Helium-3 under Pressure below 0·1° K. : Specific Heat and Expansion Coefficient of (Dr. D. F. Brewer and J. R. G. Keyston), 1261
Liquid (Gas–) Chromatography : Peak-Shift Technique in—Trimethylsilyl Ether Derivatives of Alcohols (Stanley H. Langer and Peter Pantages), 141
Liquid (Gas–) Chromatography (Pyrolysis and), on the Microgram Scale (C. E. Roland Jones and A. F. Moyles), 663
Liquid/Liquid Coalescence : The Marangoni Effect and (G. D. M. MacKay and S. G. Mason), 488
Liquid (Rumen), of Cattle : Keto-Acids and Amines in (Dr. C. J. G. van der Horst), 73
Liquids : Ultrabasic (R. H. Clark and Prof. W. S. Fyfe), 158
Liquori, Dr. A. M., and others : Molecular Geometry of a 1:1 Crystalline Complex between 1,3,7,9-Tetramethyluric Acid and Pyrene. 900
Listening (Viewing and) : Facts and Figures about (British Broadcasting Corporation), 1051
Lister Institute of Preventive Medicine, London : Report of the Governing Body (1961), 1356
Liston, Prof. J. (Colwell, R. R., and) : Taxonomy of *Xanthomonas* and *Pseudomonas*, 617
Literature (Chemical) : Report on Computer Systems for Searching (E. C. Medam and H. R. Koller), (United States National Bureau of Standards Research Information Centre and Advisory Service on Information Processing), 23
Literature (Scientific) : The Use of (B. C. Vickery), 1347
Literature (Thermophysical Properties Research) : Retrieval Guide to, Vol. 1 (edited by Y. S. Touloukian), Book 1, Part A, Guide to Substance Classification and Numerical Codes ; Book 1, Part B, Dictionary of Synonyms and Trade Names ; Book 1, Part C, Dictionary of Substances ; Book 2, Classified Search Index ; Book 3, Master Bibliography, Author Index, *review* by Prof. A. R. Ubbelohde, 109
Litherland, A. E., and others : Production of Neon in Stars, 1381
Lithium Fluoride (Irradiated), as an Optical Filter in the Far Ultra-Violet (J. L. Weeks, S. Gordon and G. M. A. C. Meaburn), 1186
Litonjua, Dr. Augusto D. : Thyroglobulin—Effect of Acid, 356 ; Evidence of Thyroid Protease Action, 357
Litonjua, Dr. Augusto D., and others : Stimulation of Lipogenesis in Thyroid Tissue *in vitro* by Free Fatty Acids, 804
Little (Arthur D.) Research Institute : Annual Report for 1960 (June 17, 1068), *erratum*, 126
Little, Dr. K. : At the Twenty-first Meeting of the Research Panel of the British Gelatine and Glue Research Association (Structural Re-orientation of Gelatine), 860
Littler, Dr. W. B. : Explosives Technology, *review*, 3
Littoral Interstitial Species from Anglesey representing Three Families New to Britain (P. J. S. Boaden), 512
Liver : Comparison of Pyridine Nucleotides in the, and in an Ascitic Hepatoma (M. Wintzerith N. Klein, L. Mandel and Prof. P. Mandel), 467
Liver (Generating) : Influence of Plasma Proteins in the Control of Mitosis rates in (Dr. J. T. Hemingway), 706
Liver (Perfused), of Premature Lambs : Fructose and Lactic Acid Metabolism in the (W. H. H. Andrews, H. G. Britton and Dr. D. A. Nixon), 1307
Liver (Rat) : Effect of Adrenalectomy on the Activity of Enzymes of the Urea Cycle in (Patricia McLean), 1302
Liver (Rat) Glutamic-Oxalacetic Transaminase : Intracellular Distribution Pattern of (Dr. Herbert J. Eichel and Josef Bukovsky), 243
Liver (Regenerating) : Effect of Enzyme Injections on Mitosis in (Dr. Gaston de Lamirande), 400
Liver Alcohol Dehydrogenase : An Inhibitor of, in Preparations of Reduced Diphosphopyridine Nucleotide (Dr. Keith Dalziel), 1098
Liver Cell Proliferation (Parenchymal), in Normal Adult Rats : Stimulation by Heparin of (Dr. M. Zimmerman and Evemarie Celozzi), 1014
Liver Glycogen (Blood Glucose and), in Rabbits : Effect of Bee Venom on Concentration of (Prof. A. H. Mohamed and K. Zaki), 605
Liver Protein *in vivo* : Incorporation of Histamine into (Isabel Wajda, Michael Ginsburg and Prof. Heinrich Waelsch), 1204
Liver Tissue : Separation and *in vitro* Culture of Cells from (Dr. Justine S. Garvey), 972
Liver and Kidney : Changes in Glucose-6-phosphatase Activity in, at Birth (M. J. R. Dawkins), 72
Liverpool College of Technology : Postgraduate Courses in Nuclear Studies, 338
Liverpool Libraries, Museums and Arts Committee : Brown, Picton and Hornby Libraries, 21
Living Cells : Estimation of Time of Generation of (R. J. M. Fry, S. Lesher and H. I. Kohn), 290
Living Organisms : Atomic Radiations and (R. H. Mole), 556
Living Rat Brain : Urea Synthesis in the (R. K. Davies, A. J. Defalco, D. Shander, A. Kopelman and Prof. J. Kiyasu), 288
Livingstone, I. : Appointed lecturer in economic statistics at the University of Sheffield, 337
Lizard Area : Preliminary Age-Determinations in the (J. A. Miller and D. H. Green), 159
Ljunggren, Dr. Hans : Transfer of Virulence in *Rhizobium trifolii*, 623
Local Heating of the Hypothalamus : Increase of Evaporative Loss of Water from the Skin of the Ox in response to (Dr. D. L. Ingram, J. A. McLean and Dr. G. C. Whittow), 81
Localization of the 'Sugar Pump' in the Intestinal Epithelium (Dr. T. Z. Csáky and G. W. Fernald), 709
Localization in Tissues : Paper Electrophoresis of Proteins while Preserving the Pattern of their (Dr. Zh. A. Medvedev), 1021

Localization (Nucleolar), of Succinic Dehydrogenase in Normal Rat Hepatic Cells (P. De, R. Chatterjee and Dr. S. Mitra), 925
Location of Polygenes (Prof. J. M. Thoday), 368
Lock, C. M., and Stevens, P. J. : Connexion between Micropulsations and the Ionosphere, 584
Locus (*ad₇*) of *Schizosaccharomyces pombe* : Distribution of X-Ray- and Nitrous Acid-induced Mutations in the Genetic Fine Structure of the (Dr. Herbert Gutz), 1127
Locust (Desert) : Induced Changes in the Amounts of Material in the Neurosecretory System of the (Dr. K. C. Highnam), 199
Lodge, R., and others : Investigation into Blood Groups in a Paternity Case, 753
Loewus, Dr. Frank A., and Kelly, Stanley : Identity of L-Ascorbic Acid formed from D-Glucose by the Strawberry (*Fragaria*), 1059
Lofts, Dr. B. : Melanism in Captive Weaver-Finches (*Quelea quelea*), 993
London Hospital Medical College : Appointment of H. D. Ritchie to the University of London readership in surgery, 229
  Title of reader in dental anatomy in the University of London conferred on R. W. Fearnhead, 229
London School of Economics and Political Science : Title of reader in demography in the University of London conferred on J. Hajnal, 337
  Title of professor emeritus of geography conferred on Prof. R. O. Buchanan, 546
  Appointment of Dr. Brian Abel-Smith to the University of London readership in social administration, 651
  Appointment of L. P. Foldes to the University of London readership in economics, 651
  Appointment of Harry Townsend to the University of London readership in economics, 651
  Title of reader in economics conferred on J. Wiseman, 651
  Appointment of James Durbin to the University of London chair of statistics, 1247
London School of Hygiene and Tropical Medicine : Title of professor emeritus of medical statistics conferred on Sir Austin Bradford Hill, 546
London Transport : Retirement of A. T. Wilford as director of research, 751
  Appointment of H. E. Styles as director of research, 751
Long, Dr. Charles, and others : A Hypodermic Probe using Fibre Optics, 927
Long, Dr. Cyril (edited by) : Biochemists' Handbook, *review* by Dr. D. Recaldin, 635
Long, P. L. : Immunity to Protozoa (Local Immunity in Coccidiosis), 553
Long, P. L. (Rose, M. E., and) : Parasite Biology (Application of New Laboratory Techniques), 135
Long Ashton Research Station, Bristol (R. W. Marsh), 547
Long Bones (Homotypic) : Number of Arrangement of Osteons in Corresponding Regions of (Dr. G. Marotti), 1400
Long-Chain Alcohols and Glycol Monoalkyl Ethers : Compressibility and Specific Evaporation Resistance of the Monolayers of (A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas), 378
Long-Range Radio Communication by Satellite Microwave Dipoles—the West Ford Project, 1237
Long-Term Effects of Ionizing Radiations from External Sources—Report of the Subcommittee on, of the Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 849), 556
Long Wave-length Neutrons : Scattering of, by Irradiated Beryllium Oxide (T. M. Sabine, A. W. Pryor and B. S. Hickman), 1385
Lonsdale, Dame Kathleen : *Obituary* of Prof. W. T. Astbury, 332
Looney, W. B. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Some Limitations of Labelled Compounds in Radiobiological Studies), 341
Loop of Henle : Stimulation by Aldosterone of Sodium Transport in the (Dr. J. Crabbé), 817
Lord, Dr. H., and others : Ignition of Gas by Sparks produced by Breaking Wires at High Speed, 1085
Lord, P. Mary (Coulson, C. B., and) : Isolation and Fractionation of some Damselfly Pigments, 1300
Lords, House of : Committee stage of the Rating and Valuation Bill, 123
Lorković, H. : Inhibition by Potassium of the Mechanical Responses of Frog Muscles at Low Temperature, 1011
Loss (Evaporative) of Water from the Skin of the Ox : Increase of, in response to Local Heating of the Hypothalamus (Dr. D. L. Ingram, J. A. McLean and Dr. G. C. Whittow), 81
Lothrop, K. H., and others : A New Phase formed by High-Pressure Treatment—Face-centred Cubic Molybdenum Monocarbide, 1194
Loudon, Dr. James D. : Chemistry of Carbon Compounds, *review*, 214
Lough Neagh Pollan *Coregonus pollan*, Thompson : Furunculosis in (K. U. Vickers and R. McClean), 930
Loughman, Dr. B. C. : Effect of Boric Acid on the Phosphoglucomutase of Pea Seeds, 1399
Lousley, J. E. : At the Annual Congress of the South-Eastern Union of Scientific Societies (The Changing Flora of South-East England), 133
Louwerens, B., and others : Neutral 17-Ketosteroids in Porcine Urine, 175
Lovelace, Dr. Stuart A. (Keeler, Dr. Richard F., and) : Incorporation of Phosphorus-32 into Urinary Concretions forming on Zinc Implants in Rat Bladders, 715
Lovell, Sir Bernard : Appointed scientific director to the British Space Development Co., Ltd., 230
  Awarded the first Daniel and Florence Guggenheim International Astronautics Award, 856
Lovell, R., and others : Chromatographic Investigation of the Methyl Alcohol/Chloroform-soluble Constituents of Honeybee Larval Foods, 362
Low-Angle Light Scattering and the Molecular Weight of Deoxyribonucleic Acid (Dr. Sidney Katz), 280
Low Temperature : Inhibition by Potassium of the Mechanical Responses of Frog Muscles at (H. Lorković), 1011
  Recoil-Atom Reactions at (Slobodanka Veljković and Dr. Garman Harbottle), 1287
Lowenstein, Prof. O. : South African Animal Life, *review*, 1135
Lowenstein, Prof. O. E. : Biology Syllabus for Sixth Forms, 554
Lower Tertiary Floras of Southern England (Marjorie Elizabeth Jane Chandler), I, Palaeocene Floras, London Clay Flora (Supplement), *review* by Prof. John Walton, 209
Loynes, R. M. : Elected to a Junior Research Fellowship for research in mathematics at Churchill College, Cambridge, 856
Lucas, Ian A. McK. : Appointed to the chair of agriculture in University College of North Wales, Bangor, 439 ; work of, 439
Lüdi, Prof. W. : Flora Europaea, 447
Lukton, Prof. A., and others : Interaction of Lipids and Trypsin in Dimethyl Sulphoxide–Water Systems, 565
Luminescence (Chemi-) of Luminol : Inhibition of, by means of Oximes (Prof. K. Weber, J. Matković and D. Fleš), 177
Luminol : Inhibition of Chemi-Luminescence of, by means of Oximes (Prof. K. Weber, J. Matković and D. Fleš), 177
Lunar Occultation of a Radio Source (Dr. C. Hazard), 58
Lunar Studies : New Handbooks for, *review* by D. H. P. Jones, 424
Lund, Erling, and others : Serum Lipids in relation to the Intake of Alcohol and Sodium Chloride, 1095
Lung : Role of the, in Metabolism of Fat during Ontogeny of the Rat (Dr. Z. Vacek, P. Hahn and O. Koldovsky), 85
Lung-books in the Devonian Palaeocharinidae (Arachnida), (Dr. M. F. Claridge and A. G. Lyon), 1190
Lupinus albus L. exposed to Different Photoperiods : Effect of Gibberellic Acid on Growth and Indoleacetic Acid Oxidase Activity of (F. Sági and A. S. Garay), 824
Lupus Erythematosis (Disseminated) : Anti-Leucocyte Cytoplasm, Anti-Leucocyte Nuclei and Anti-Platelets in (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Lush, I. E. : Awarded a Wellcome Research Fellowship by the Animal Health Trust, 1250
'Lusitanian' Fauna : Origin of the British Insular Races of Small Mammals and of the (G. B. Corbet), 1037
Lymphocytes of Patients with Gargoylism : Inclusions of Mucopolysaccharide in the (Ursula Mittwoch), 1315
Lymphocytes (Guinea Pig) : Chemistry of a Mucopolysaccharide produced by (Helen Muir and A. H. E. Marshall), 706
Lymphocytes (Large), and the Simonsen Phenomenon (Dr. Aleksander Szenberg and N. L. Warner), 920
Lymphomatosis (Avian) : Cell Transplants of, treated with Hydrogen Peroxide (Dr. V. L. Sanger), 1406
Lynch, A. C. : Magnetic Materials in the Electrical Industry, *review*, 428
Lyon, A. G. (Claridge, Dr. M. F., and) : Lung-books in the Devonian Palaeocharinidae (Arachnida), 1190
Lysergic Acid Diethylamide : Effect of Administration of, on Serotonin-Levels in the Body (Dr. D. V. Siva Sankar, D. Barbara Sankar, Edward Phipps and Eleanor Gold), 499
Lysogeny : Absence of, in *Pasteurella pestis* (Dr. D. A. Smith), 522

MACACA (Genus) : Serum Transferrins in the—Species Distribution of Nineteen Phenotypes (Prof. Morris Goodman and Emily Poulik), 1407
Macaques (Philippine) : Isolation of *Plasmodium knowlesi* from (F. L. Lambrecht, Dr. F. L. Dunn and Dr. D. E. Eyles), 117
MacArthur, Dr. I. : *Obituary* of Prof. W. T. Astbury, 331
Macbride, J. Francis : Flora of Peru, *review* by N. Y. Sandwith, 214
Maccabaean Prize and Medal for 1962 (Worshipful Society of Apothecaries of London), 650
McCall, Richard C., and others : Radiocaesium and Potassium in Norwegians, 436
McCallien, Prof. W. J. (Bailey, Sir Edward, and) : Structure of the Northern Apennines, 1136
McCann, Dr. W. P. : Appointed Leverhulme research officer in the University College of North Staffordshire, 229
McCarthy, F. D. : Cave Art of the Conjola District, New South Wales, 854
  Rock Engravings of the Sydney–Hawkesbury District, 854
McCartney, J. T., and others : Electron Microscopic Observations of Structure in Ultra-Thin Sections of Vitrinite in Coal, 1361
McClean, R. (Vickers, K. U., and) : Furunculosis in Lough Neagh Pollan *Coregonus pollan*, Thompson, 930
McCoy, Dr. J. H. : Bacteria of the Intestine, 1153
McCrea, Prof. W. H. : Cosmology, *review*, 1336
McCulloch, N. G. : Appointed an honorary life governor of the Cotton, Silk and Man-made Fibres Research Association, 443
Macdonald, D. F. : The State and the Trade Unions, 1129
Macdonald, Gordon J. F. (Munk, Walter H., and) : The Rotation of the Earth, *review* by Prof. S. K. Runcorn, 313
McDonald, Dr. I. W. : Ruminant Digestion and Nutrition, *review*, 133
Macdonald, W. W. (edited by) : Malaysian Parasites, 35-49 (Studies from the Institute for Medical Research, Federation of Malaya, No. 29), *review* by P. F. Mattingly, 948
McDowall, F. H., and others : Growth of Ryegrass-dominant Pasture grazed by Cows, and Properties of New Zealand Butterfat, 303
Mace, Prof. C. A. : Title of professor emeritus of psychology (at Birkbeck College, London) conferred on, 546
Macek, Dr. K. : Paper Chromatography, 760
Macfarlane, Dr. G. G. : Appointed director of the Royal Radar Establishment, Malvern, 1145
McGillivray, W. A., and others : Growth of Ryegrass-dominant Pasture grazed by Cows, and Properties of New Zealand Butterfat, 303
McGivering, T., and others : Management in Britain, 1332
McGovern, V. J. : Mast Cells in Photosensitivity Reactions, 90
McGowan, Rt. Hon. the Lord : *Obituary* by R. Brightman, 1041
McGreavy, C. : Appointed I.C.I. Research Fellow in the University of Edinburgh, 337
McGregor, D. : The Human Side of Enterprise, 1332
McGregor, I. A. : Immunity to Protozoa (Use of Gamma-Globulin in the Cure of Malaria), 553
Machine Tool Engineering at Manchester, 19
Machine Tool Industry Research Association : Election of the Earl of Halsbury as president, 546
Macho, Dr. L. : Effect of Thyroxine and 2,4-Dinitrophenol on the Rate of Utilization of Glucose, 604
McIndoe Memorial Research Unit, Queen Victoria Hospital, East Grinstead : Appointment of Dr. Morten Simonsen as director of research, 337
McIntosh, J. F., and others : Biochemical Changes in Lethal Staphylococcal Infections in Mice, 783
Mackay, D., and others : Histidine Decarboxylase and Growth, 1311
MacKay, D. B. : Appointed chief officer of the Official Seed Testing Station of the National Institute of Agricultural Botany, 856

MacKay, G. D. M., and Mason, S. G. : The Marangoni Effect and Liquid/Liquid Coalescence, 488
McKeen, C. D. (Mountain, W. B., and) : Nematology (An Interaction between *Verticillium albo-atrum* and *Pratylenchus penetrans*), 1048
McKenzie, A. E. E. : The Major Achievements of Science, *review* by J. M. Eyles, 110
Mackworth-Praed, C. W., and Grant, C. H. B. : Birds of Eastern and North-Eastern Africa, second edition, *review* by Sir Landsborough Thomson, 9
McLean, J. A., and others : Increase of Evaporative Loss of Water from the Skin of the Ox in response to Local Heating of the Hypothalamus, 81
McLean, Patricia : Effect of Adrenalectomy on the Activity of Enzymes of the Urea Cycle in Rat Liver, 1302
McLean, Stuart : An Autoradiographic Effect, 690
Macleod, Dr. John : Arthropod Transmission of Micro-organisms, 885
McLoughlin, J. V. : Splitting of Serum Albumin during Electrophoresis on Paper, 1305
McManus, Mary Annunciata, and Sullivan, Kathleen : Effects of Kinetin and Indole-3-acetic Acid on Multiplication Rates of *Paramecium*, 619
McMichael, Dr. D. F. : Awarded a Royal Society and Nuffield Foundation Commonwealth Bursary, 126
Macnae, Dr. W. : Awarded a Royal Society and Nuffield Foundation Commonwealth Bursary, 126
McNaughton, G. S., and Woodward, R. N. : Barium-140 in New Zealand Rainwater, 1344
McNeil, —.: Hazards of the Animal House (Hazards associated with Farm Animals), 339
Macromolecules : Mediation of Divalent Metal Ions in the Binding of Tetracycline to (Dr. Kurt W. Kohn), 1156
McTaggart, F. K. : Reduction of Zirconium and Hafnium Oxides, 1192
Madagascar : Fishing in (Dr. Rudolph Kreuzer), 238
Maddocks, Dr. W. R. : *Obituary* of Prof. J. H. Andrew, 121
Madshus, Kjell, and others : Radiocaesium and Potassium in Norwegians, 436
Maegraith, Prof. B. G. : Immunity to Protozoa (Non-invasive Strains of Parasites), 552
Maegraith, Prof. B. G., and others : Techniques in Tropical Pathology, *review* by Prof. P. C. C. Garnham, 1236
Maéno, Takashi, and others : Electrical Activity of a Giant Nerve Cell under Abnormal Conditions, 1265
Maetz, J., and others : Pharmacological Characterization of the Oxytocic Peptides in the Pituitary of a Marine Teleost Fish (*Pollachius virens*), 670
Magat, Prof. M. : Effects of Ionizing Radiation on Organic and Biological Solids (Reactions in the Solid State), 1150
Magdalene College, Cambridge : Grant of £5,500 to the Pepys Library, for preparation and publication of a catalogue, 855
Maggs, F. A. P. : Design and Use of Respirators (Removal of Gases by Activated Charcoal), 655
Magill, J. H. : Crystallization of Isotactic Polypropylene using a Light Depolarization Technique, 1092
Magnesium : Importance of, in the Control of Plasma Calcium in the Calf (Dr. R. H. Smith), 181
  Rapid Development of Hypomagnesaemia in Lactating Cows given Artificial Rations Low in (J. A. F. Rook), 1019
Magnesium (Manganese and), in the Grey Speck Syndrome of Oats (P. B. Vose and Dr. D. J. Griffiths), 299
Magnesium Alloy : Effect of Chloride Concentration on the Aqueous Corrosion of a (D. de G. Jones and H. G. Masterson), 165
Magnetic Fields : Proposed Use of, in Electron Microscopic Radioautography (Lucien G. Caro), 1188
Magnetic Materials in the Electrical Industry (P. R. Bardell), second edition, *review* by A. C. Lynch, 428
Magnetic Mirror Confinement of Exploding Wire Plasma (E. J. Seykora), 995
Magnetic Quantities : Dimensions of Electrical and (Dr. A. E. Martin), 588
Magnetic Relaxation in Magnetite and in its Solid Solutions with Manganese and Nickel Ferrites (A. Bragiński and Th. Merceron), 898
Magnetic Storms in Interplanetary Space as observed by *Pioneer V* (Eugene W. Greenstadt), 329
Magnetically Controlled Ionization : Trans-Equatorial Backscatter Observations of (J. A. Thomas), 792
Magnetism and Crystallography (Science Council, the Physical Society and the Crystallographic Society of Japan International Conference on), 24
Magnetite : Magnetic Relaxation in, and in its Solid Solutions with Magganese and Nickel Ferrites (A. Bragiński and Th. Merceron), 898
Magnetization (Thermoremanent) : Stress Effects on (Dr. P. M. Stott and Dr. F. D. Stacey), 585 ; (J. M. Hall), 586
Magnetoplumbite Structure : Fluoride-compensated Substitution of Bivalent Cations in the (Porf. E. Banks and M. Robbins), 1387
Magnus, Dr. K. E., and others : High Concentration of (–)-Noradrenaline in *Portulaca oleracea* L., 1108
Mahesh, Dr. Virendra B., and Greenblatt, Dr. Robert B. : Physiology and Pathogenesis of the Stein–Leventhal Syndrome—Urinary Androsterone, Etiocholanolone, Pregnanetriol and Δ^5-Pregnenetriol of Ovarian Origin in a Patient with Stein–Leventhal Syndrome, 888 ; Role of FSH in the Pathogenesis of the Stein–Leventhal Syndrome, 889
Maheshwari, Prof. P., and Baldev, B. : Artificial Production of Buds from the Embryos of *Cuscuta reflexa*, 197
Maheshwari, Prof. P., and Kanta, Kusum : Intra-ovarian Pollination in *Eschscholzia californica* Cham., *Argemone mexicana* L. and *A. ochroleuca* Sweet, 304
Mahoney, M. Colette, and others : Chromosomal Elimination induced by 6-Aza-Uracil, 627
Mai, W. F., and Harrison, M. B. : Nematology (*Heterodera rostochiensis* is held in check on Long Island by Nematicidal Treatment, Cultural Methods and Legal Regulation), 1049
Maitland, B. J. G. : Mammals of the British Isles, *review*, 110
Maize : Susceptibility to Leaf Blight, caused by *Cochliobolus heterostrophus*, in Nigerian Varieties of (C. L. M. van Eijnatten), 515
Major Achievements of Science (A. E. E. McKenzie), *review* by J. M. Eyles, 110
Making : Astronomy in the, *review* by Colin A. Ronan, 314
Malabsorption Syndrome and Exudative Enteropathy : Calcium Metabolism in Man with Calcium-45— (Dr. G. Milhaud and Dr. P. Vesin), 872
Malacological Congress : First European (Conchological Society of Great Britain and Ireland and the Malacological Society of London), 126
Malacological Society of London (Conchological Society of Great Britain and Ireland and the) : First European Malacological Congress, 126
Malaria Eradication : Effect of Population Movements on (R. Mansell Prothero), 863
Malaya (Federation of) Institute for Medical Research : Studies from the, No. 29, Malaysian Parasites, 35-49 (edited by W. W. Macdonald), *review* by P. F. Mattingly, 948
Malaysian Parasites (edited by W. W. Macdonald), 35-49 (Studies from the Institute for Medical Research, Federation of Malaya, No. 29), *review* by P. F. Mattingly, 948
Malcolm, Group-Captain John Erskine : Action of the Hypothalamus, 606
  Fatigue in a Fluid-filled Pulsating Elastic Tube, 1012
Male Genitalia of East and West African Stocks of *Papilio dardanus* (Lep.) : Genetics of a Difference in the (J. R. G. Turner, C. A. Clarke and P. M. Sheppard), 935
Malignant Effusions : Diagnostic Significance of Heterogeneous Lactic Dehydrogenases in (R. Richterich, K. Zuppinger and E. Rossi), 507
Malizia, Major W. F., and others : Immunofluorescence of Pleuropneumonia-like Organisms isolated from Tissue Cell Cultures, 190
Malpighian Tubules : Excretion of Inulin by (Dr. J. A. Ramsay and Dr. J. A. Riegel), 1115
Maltby, P. : Central Component of the Radio Source Centaurus *A*, 793
Maltby, P. (Moffet, A. T., and) : Distribution of Brightness in Extragalactic Radio Sources, 453
Malyjev, A. : At the International Starch Conference (Use of the Dextrose–Sodium Chloride Double Salt to facilitate the Crystallization of Dextrose in Factories in the U.S.S.R.), 237
Mammal Society (Australian) : Election of Officers for 1961-62, 755
Mammalian Carotid Rete Mirabile : Functions of the (Dr. C. H. Barnett and C. D. Marsden), 88
Mammalian Cells *in vitro* : Incorporation of Deoxyribonucleic Acid by (Prof. E. R. M. Kay), 387
Mammalian Neurones : Analogues of Glutamic and γ-Amino-*n*-butyric Acids having Potent Actions on (Dr. J. C. Watkins and Dr. D. R. Curtis), 1010
Mammalian Sperm : Isolation and Properties of Deoxyribonucleic Acid from (Dr. E. Borenfreund, E. Fitt and Dr. A. Bendich), 1375
Mammalian Tissue Cell Surfaces : Sialic Acid as a Structural Component of some (Dr. Leonard Weiss), 1108
Mammals of the British Isles (Philip Street), *review* by B. J. G. Maitland, 110
Mammals of Northern Rhodesia (W. F. H. Ansell), *review* by R. W. Hayman, 8
Mammals (Small) : Origin of the British Insular Races of, and of the 'Lusitanian' Fauna (G. B. Corbet), 1037
Mammary Gland of the Cow and Rat : Leucine Aminopeptidase in the (Prof. S. Talanti and Prof. Väinö K. Hopsu), 86
Mammary Gland of the Ewe : Removal of Milk Constituents from the (A. K. Lascelles), 1404
Man : Blood Groups of Animals other than (Dr. D. G. Gilmour), 653
  Cross-modal Transfer in (M. Cole, S. L. Chorover and Dr. G. Ettlinger), 1225
  Effects of Corticotrophin, Hydrocortisone and Methopyrapone on the Circulating Eosinophiles in (Dr. N. J. Kothari and Dr. J. C. Saunders), 1105
  European Parasites of, *review* by Dr. G. Lapage, 107
  The Humanity of (Sir Wilfrid Le Gros Clark), 977 ; (Dr. Launcelot Fleming), 1229
  Use of Desertomycin Medium in Routine Selective Isolation of Fungi Pathogenic to (J. Uri and Zs. Herpay), 1015
Man with Calcium-45 : Calcium Metabolism in—Malabsorption Syndrome and Exudative Enteropathy (Dr. G. Milhaud and Dr. P. Vesin), 872
Man's Emergence : Dating (Dr. K. P. Oakley), 988
Man and Animals : Assessment of Pain in, 1353
Man (Land and), in the Western Weald (Prof. S. W. Wooldridge), 132
Management : Scientific Attitude to, *review* by T. H. Hawkins, 209
  Studies in (G. Hunter), 1332
Management in Britain (T. McGivering, D. Matthews and W. H. Scott), 1332
  Training for, 1331
Management in the Industrial World (Prof. F. Harbison and Prof. C. A. Myers), 1332
Management of Pine Stands for Pulpwood (J. J. Kotzé), 855
Management (Personnel), Institute of : Job Analysis (H. E. Roff and T. E. Watson), 336
Management Education, Foundation for : Grant of £16,000 a year for seven years to the University of Leeds, 228
Management Sciences, Institute of : Proceedings of the Sixth International Meeting (Paris, 1959)—Management Sciences, Models and Techniques (edited by Prof. C. West and Prof. Michel Verhulst), *review* by T. H. Hawkins, 209
Management Sciences, Models and Techniques—Proceedings of the Sixth International Meeting of the Institute of Management Sciences, Paris, 1959 (edited by Prof. C. West and Prof. Michel Verhulst), *review* by T. H. Hawkins, 209
Management Studies in Britain, 1251
Manager : The (L. F. Urwick), 1332
Managing Nature Reserves : Problems of (Dr. J. F. D. Frazer), 133
Manchester College of Science and Technology : Appointment of Ronald Beresford Dew as visiting professor of Industrial Administration, 651
Mancini, Roberto E., and others : Collagen Fractions in the Skin of New-born and Adult Rats, 1297
Mandel, L., and others : Comparison of Pyridine Nucleotides in the Liver and in an Ascitic Hepatoma, 467
Mandel, Prof. P., and others : Comparison of Pyridine Nucleotides in the Liver and in an Ascitic Hepatoma, 467
Mandible (The Juvenile), from Olduvai (Dr. L. S. B. Leakey), 417
Manganese and Magnesium in the Grey Speck Syndrome of Oats (P. B. Vose and Dr. D. J. Griffiths), 299
Manganese and Nickel Ferrites : Magnetic Relaxation in Magnetite and in its Solid Solutions with (A. Bragiński and Th. Merceron), 898
Mankiewicz, Dr. E. : Mycobacteriophages isolated from Persons with Tuberculous and Non-tuberculous Conditions, 1416
Mann, Dr. F. G. : Russian Work in Organo-Phosphorus Chemistry, *review*, 1232
Mann, Dr. H. H. : Classification of the Tea Plant, 409

Mann, Dr. K. J. : Biorheology and Microcirculation, 757
Mannosyl D-Glucosamine Hydrochloride Isomers—*N*-Mannosyl D-Glucosamine Hydrochloride (Dr. S. A. Barker, Dr. K. Murray and Prof. M. Stacey), 142 ; 6-*O*-Mannosyl D-Glucosamine Hydrochloride (Dr. S. A. Barker, Dr. K. Murray, Prof. M. Stacey and D. B. E. Stroud), 143
Manoach, Dr. M. (Weinman, Dr. J., and): Biorheology and Microcirculation (Photoelectric Techniques for Studying the Peripheral Circulation in Man), 758
Manosevitz, M., and others : Social Dominance in Inbred Mouse Strains, 474
Mansfield, Dr. T. A., and Heath, Prof. O. V. S. : Photoperiodic Effects on Stomatal Behaviour in *Xanthium pennsylvanicum*, 974
Manson, L. A. : Vitamin B_{12} and Intrinsic Factor (Biological Function of Vitamin B_{12}), 1155
Manson Bahr, P. E. C. : Immunity to Protozoa (Immunization of a Population in Kenya exposed to Epidemic Kala-azar with an Avirulent Ground Squirrel Strain of the Organism), 553
Manual of Bacteria and Actinomyces, *review* by Dr. K. Zinnemann, 1333
Manual (Introductory), on the Control of Health Hazards from Radioactive Materials (Medical Research Council, Memorandum No. 39), new edition, 1257
Manual (A Laboratory), of Analytical Methods of Protein Chemistry, including Polypeptides (edited by Dr. P. Alexander and R. J. Block), Vol. 1, The Separation and Isolation of Proteins, *review* by Dr. D. T. Elmore, 320
Manufacture (Pulp and Paper) : A Laboratory Handbook of (Dr. Julius Grant), second edition, *review* by Dr. F. Lyth Hudson, 320
Manufacturing Industry on Merseyside : Ageing and the Semi-Skilled—a Survey in (Alistair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine Cunningham), 1249
Map Publications by the Ordnance Survey of Great Britain : Recent, 958
Mapping (Complementation), of Allelic Series : Limitations of Geometrical Models for (Prof. Elof Axel Carlson), 788
Maps : Palaeogeologic (A. I. Leversen), *review* by Dr. C. Downie, 842
Marangoni Effect and Liquid/Liquid Coalescence (G. D. M. MacKay and S. G. Mason), 488
March, Dr. N. H. : Appointed to the newly created second chair of physics in the University of Sheffield, 337, 543 ; work of, 543
Marcker, K. (Bertelsen, Dr. Sv., and) : Degree of Polymerization of Hyaluronic Acid isolated from Human Aortic Tissue, 386
Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas (Prof. William Randolph Taylor), *review* by Prof. Lily Newton, 1234
Marine Biology or Oceanography : Research in (Challenger Society Grants for), 24
Marine Laboratory (Sandy Hook) : Fellowships for Plankton Research, 754
Marine Teleost Fish (*Pollachius virens*) : Pharmacological Characterization of the Oxytocic Peptides in the Pituitary of a (Prof. H. Heller, B. T. Pickering, J. Maetz and F. Morel), 670
Marini-Bettolo, Prof. : Paper Chromatography, 760
Markham, Jordan J., and Mergerian, Dickron : Use of a Different Type of Fluorescent Material as an Optical Maser, 586
Markham, Dr. Roy : Still More Nucleic Acids, *review*, 742
Markov Processes : Theory of (E. B. Dynkin), translated from the Russian by D. E. Brown ; edited by T. Köváry, *review* by D. G. Kendall, 321
Marley, Dr. E., and others : Tryptamine Receptors in the Central Nervous System, 1068
Marmur, Prof. J., and others : Some Properties of the Deoxyribonucleic Acid of Phage Alpha, 1097
Marnay, A. : Haptoglobinuria in Nephrotic Syndromes, 74
Marotti, Dr. G. : Number and Arrangement of Osteons in Corresponding Regions of Homotypic Long Bones, 1400
Marples, J. A. C., and Lee, J. A. : Structure of δ-Plutonium, 1391
Marriage amongst the Groote Eylandt Aborigines : Classification of Kin, Age Structure and (Dr. Frederick G. G. Rose), *review* by Dr. R. Needham, 6
Mars, P., and van de Mond, Th. : Selectivity of Palladium Catalysts for the Oxidation of Carbon Monoxide in the Presence of Hydrogen, 1003
Marsden, C. D. (Barnett, Dr. C. H., and) : Functions of the Mammalian Carotid Rete Mirabile, 88
Marsh, R. W. : Long Ashton Research Station, Bristol, 547
Marshall, A. H. E. (Muir, Helen, and) : Chemistry of a Mucopolysaccharide produced by Guinea Pig Lymphocytes, 706
Martelli, G. : Appointed a lecturer in the Department of Physics, University of Birmingham, 337
Martin, Dr. A. E. : Dimensions of Electrical and Magnetic Quantities, 588
Martin, Eric W., and others (edited by) : Remington's Practice of Pharmacy, twelfth edition, *review* by Dr. J. H. Gaddum, 8
Martin, Dr. G. R., and others : Influence of Scurvy and Lathyrism (Odoratism) on Hydroxyproline Excretion, 1008
Martin, H. F. (Daniels, Dr. D. G. H., and) : Isolation of a New Antioxidant from Oats, 1302
Martin. L., and others : Essential Oil from Fresh *Cannabis sativa* and its Use in Identification, 774
Martin, R. L., and Winter, G. : Structures of Polymeric Titanium Ethoxide and Titanium Oxide Ethoxides, 274
Maruzzella. Prof. Jasper C. : Antimicrobial Substances from Ferns, 518
Marx, Dr. R. : Effects of Ionizing Radiation on Organic and Biological Solids (Use of Electron-Spin Resonance to Identify and Measure the Free Radicals produced by Irradiation), 1150
Mašek, Dr. J. : A New Inorganic Reaction—Nutric Acid–Hydrazoic Acid, 166
Maser (Optical) : Use of a Different Type of Fluorescent Material as an (Jordan J. Markham and Dickron Mergerian), 586
Masers (Optical) : A Proposal for a Tunable Source of Radiation for the Far Infra-Red using Beats between (Dr. D. C. Lainé), 795
Mason, Prof. E. A., and others : Kirkendall Effect in Gases, 375
Mason, R. : Dispersal of Tropical Seeds by Ocean Currents, 408
Mason, Dr. R. : Effects of Ionizing Radiation on Organic and Biological Solids (Post-irradiation Thermo-luminescence), 1150
Mason, S. G. (MacKay, G. D. M., and) : The Marangoni Effect and Liquid/Liquid Coalescence, 488
Mass Spectrometry (Mass Spectrometry Group of the Institute of Petroleum and Committee *E*-14 of the American Society for Testing Materials Conference on), 24
Mass Spectrometry : Determination by, of the Structure of Proline Derivatives from Apples (Dr. K. Biemann, G. G. J. Deffner and Prof. F. C. Steward), 380
Massey, A. G., and Orgel, Dr. L. E. : A Reversible Photochromic Polymer, 1387
Massey, Prof. Sir Harrie : The New Age in Physics, *review* by Prof. W. E. Burcham, 423
Massing, Dr. Alan M., and others : An Antigen in Lesions of Molluscum Contagiosum, 509
Mast Cells : Mechanism of Action of Antigen–Antibody Complexes—their Effect on (Dr. Ivan Mota), 572
Mast Cells in Photosensitivity Reactions (V. J. McGovern), 90
Mast Cells of the Rat : Release of Heparin from the (G. T. Archer), 90
Masterson, H. G. (Jones, D. de G., and) : Effect of Chloride Concentration on the Aqueous Corrosion of a Magnesium Alloy, 165
Material in the Neurosecretory System of the Desert Locust : Induced Changes in the Amounts of (Dr. K. C. Highnam), 199
Material (Cellular) : Estimation of Protein in (Prof. David Racusen and D. B. Johnstone), 492
Material (Fluorescent) : Use of a Different Type of, as an Optical Maser (Jordan J. Markham and Dickron Mergerian), 586
Material (Implant), in the Rat : Biological Incorporation of (James Calnan), 1017
Material (Soluble), in the Gastro-intestinal Tract of Rats under Normal Feeding Conditions (Dr. Anne S. Cole), 502
Material (Stylet-Sheath), of a Pentatomid : Extraction of Lipids from the Precursor of the (P. W. Miles), 911
Material (Subcellular) : Passive Transfer of Contact Sensitivity to Picryl Chloride in Guinea Pigs, with (Dr. J. L. Turk), 915
Materials (Agricultural) : Strontium-90 in Milk and, in the United Kingdom, 1959-1960 (Agricultural Research Council, Report No. 4), 20
Materials (Magnetic), in the Electrical Industry (P. R. Bardell), second edition, *review* by A. C. Lynch, 428
Materials (Natural) : Measurement of the Thorium Content of, by a γ-Ray Counting Method (Prof. J. H. J. Poole and F. N. Byrne), 62
Materials (Radioactive) : Introductory Manual on the Control of Health Hazards from (Medical Research Council, Memorandum No. 39), new edition, 1257
Materials (Sterilizing), for Hospitals (Brian Watkin), 1044
Mathematical Analysis : Modern Style in, *review* by Prof. L. S. Goddard, 6
Mathematical Delight, *review* by W. J. Langford, 638
Mathematical Education in Britain : Crisis in, 103
Mathematical Linguistics : Type-Token Mathematics, a Textbook of (Dr. Gustav Herdan), *review* by Dr. G. H. Toulmin, 211
Mathematical Methods in the Theory of Queueing (A. Y. Khintchine), translated by D. M. Andrews and M. H. Quenouille, *review* by Prof. L. S. Goddard, 211
Mathematical Society (American) : Proceedings of Symposia in Applied Mathematics, Vol. 10, Combinatorial Analysis (edited by Richard Bellman and Marshall Hall, Jr.), *review* by Prof. L. S. Goddard, 321
Mathematical Society (Australian) : Second Summer Research Institute, 1145
Mathematical Studies in the Psychology of War, *review* by Sir Cyril Burt, 427
Mathematical Study of the Causes and Origins of War : Arms and Security, a (Dr. Lewis F. Richardson), edited by Nicolas Rashevsky and Ernesto Trucco, *review* by Sir Cyril Burt, 427
Mathematicians : The Atomic Problem—a Challenge to Physicists and (Lancelot Law Whyte), *review* by Prof. R. Eisenschitz, 214
Mathematics at Leeds, 122
Mathematics: The Language of (Dr. Frank Land), *review* by W. J. Langford, 638
    School Curriculum in (edited by Howard Fehr), (Organization for European Economic Co-operation Report on), 335
    Teaching of (Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom Conference of professors of mathematics from the Universities of Great Britain to consider the), 1145
    Type-Token—a Textbook of Mathematical Linguistics (Dr. Gustav Herdan), *review* by Dr. G. H. Toulmin, 211
Mathematics Teachers : Shortage of, in Britain, 127
Mathematics (Applied), at Belfast, 19
Mathematics (Applied), at Manchester, 647
Mathematics (Pure), at Aberystwyth, 333
Mathematics (School) : New Thinking in, 335
Mathematics and Language, *review* by Dr. G. H. Toulmin, 211
Mathematics and Queueing, *review* by Prof. L. S. Goddard, 211
Matheson, Prof. A. A. : Digital Computers, their Use and Control, 554
Matković, J., and others : Inhibition of Chemi-Luminescence of Luminol by means of Oximes, 177
Matrix (Cartilage), *in vivo* : Action of Plasmin on (Dr. C. H. Lock, A. J. Anderson and S. Y. Ali), 1402
Matson, B. A. : Awarded a Wellcome Research Fellowship by the Animal Health Trust, 1250
Matsuda, Kazuo, and others : Isolation of Nigerose and Kojibiose from Dextrans, 278
Matsuno, Takao, and Yamanouchi, Toshihiko : A New Triterpenoid Sapogenin of Animal Origin (Sea Cucumber), 75
Matsushima, Tatsuaki, and others : Controlling Mechanism of the Mechanical Activity of Muscle and that of Blood Coagulation, 80
Matthew, Sir Bryan : The Senses and the Sciences (Presidential Address to Section I (Physiology and Biochemistry) of the British Association for the Advancement of Science), 989
Matthews, D., and others : Management in Britain, 1332
Matthews, Dr. R. E. F. (Francki, Dr. R. I. B., and) : Stimulation of Turnip Yellow Mosaic Virus Protein Production by 2-Thiouracil, 1078
Matthews, Prof. W. H., and others : A Lake in British Columbia containing Old Sea-Water, 830
Matthias, Fernan Alonso : Awarded a Spanish fellowship in chemistry for 1961-62 by the Ramsay Memorial Trust, 337
Mattingly, P. F. : Ecto- and Endo-Parasites of South-East Asia, *review*, 948
Mattocks, Dr. A. R. : Extraction of Heat-Labile Alkaloids from Plants, 1281
Maturity (Sexual), of Mice : The Effect of various Diets upon (J. Szepsenwol), 1112
Mauri, M., and others : Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of *Penicillium chrysogenum*, 909
Maxillary Index in *Anopheles gambiae* Giles (L. K. H. Goma), 405
Maxwell, Dr. D. R., and Palmer, Helen T. : Demonstration of Anti-Depressant or Stimulant Properties of Imipramine in Experimental Animals, 84

Maxwell Colour Centenary (Prof. W. D. Wright), 10
Meaburn, G. M. A. C., and others : Irradiated Lithium Fluoride as an Optical Filter in the Far Ultra-Violet, 1186
Measurement Conference : Second International (J. A. Place), 1350
Measurement of Leaf Colour (J. Philip Grime), 614
Measurement of Stem Flow Run-off : Light Rainfall and Plant Survival—(M. D. Gwynne and J. Glover), 1321
Measurement of the Thorium Content of Natural Materials by a γ-Ray Counting Method (Prof. J. H. J. Poole and F. N. Byrne), 62
Measurement of Tissue Slice Respiration : Oxygen Electrode for (E. H. Strickland, F. D. Ziegler and Prof. A. Anthony), 969
Measurement (Quality), in the Chemical Factory (A. J. Clark and C. W. Munday), (Department of Scientific and Industrial Research), 752
Measurements of Current Shear : Evidence of an Eastward Equatorial Undercurrent in the Atlantic from (Arthur D. Voorhis), 157
Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of High Surface Area using a Continuous Flow Calorimeter (G. H. Bell and A. J. Groszek), 1184
Measurements (Age), on Antarctic Rocks (Queen Maud Land), (Sarah Deutsch, Prof. E. E. Picciotto and M. Reinharz), 1286
Measurements (Cosmic Noise), at 3·8 Mc./s. from Satellite 1960 $\eta 1$: Interpretation of (J. H. Chapman and A. R. Molozzi), 480
Measurements (Radiological Units and) : Report of the International Commission on (United States National Bureau of Standards, Handbook No. 78), 28
Measurements (Time-integrated), of Submarine Irradiance (Ronald Currie and Laurence Draper), 661
Measuring Instruments : Fine (Fourteenth International Instructional Course by Carl Zeiss, Jena, at the Imperial College of Science and Technology, London), 1045
Measuring Television Signal-to-Noise Ratio : An Instrument for (S. M. Edwardson), 1355
Measuring Visual Constancy for Stationary or Moving Objects (S. M. Anstis, C. D. Shopland and R. L. Gregory), 416
Mecca, Christyna, and others : Sexual Differentiation in *Hydra* in relation to Population Density, 92
Mechanical Activity of Muscle and that of Blood Coagulation : Controlling Mechanism of the (Dr. Mashiro Fujino, Saburo Yamaguchi, Masa Takauji, Kohki Uchida, Masahide Yasuda and Tatsuaki Matsushima), 80
Mechanical Co-ordination of Cilia : An Example of (Dr. M. A. Sleigh), 931
Mechanical Responses of Frog Muscles at Low Temperature : Inhibition by Potassium of the (H. Lorković), 1011
Mechanics (Fluid), at Glasgow, 853
Mechanics (Soil) : K. Terzaghi, Founder of the Science of, *review* by Kevin Nash, 210
    From Theory to Practice in—Selections from the Writings of Karl Terzaghi, *review* by Kevin Nash, 210
Mechanics (Theoretical and Applied) : Proceedings of the Fifth Congress on (Indian Society of Theoretical and Applied Mechanics), 855
Mechanics (Theoretical and Applied), Indian Society of, and Unesco : Symposium on Non-Linear Physical Problems, 855
Mechanism : Coupling of Phosphorylation to Electron and Hydrogen Transfer by a Chemi-Osmotic Type of (Dr. Peter Mitchell), 144
Mechanism of Action of Antigen–Antibody Complexes—their Effect on Mast Cells (Dr. Ivan Mota), 572
Mechanism of Differential Staining of Nucleic Acids (D. J. Goldstein), 407
Mechanism of Mutagenesis induced by Formaldehyde—the Essential Role of the 6-Amino Group of Adenylic Acid (or Adenosine) in the Mediation of the Mutagenic Activity of Formaldehyde (Dr. Thomas Alderson), 251
Mechanism in Photic Reversal of the Beetle *Trypodendron* : Air-Swallowing, a (Prof. Kenneth Graham), 519
Mechanism of Photosynthesis : Experimental Evidence for the (Dr. H. T. Witt, A. Müller and B. Rumberg), 194
Mechanism of Recovery from Viral Infection in the Chick Embryo (Dr. Samuel Baron and Alick Isaacs), 97
Mechanism of Sensitivity to Hydrostatic Pressure in the Prawn, *Palaemonetes varians* Leach (Peter S. B. Digby), 366
Mechanism of Synthesis of Adenine from Hydrogen Cyanide under Possible Primitive Earth Conditions (Prof. J. Oró), 1193
Mechanism (Cholinergic), in Sympathetic Transmission : Lack of Evidence for a (Dr. J. E. Gardiner and Dr. J. W. Thompson), 86
Mechanism (Controlling), of the Mechanical Activity of Muscle and that of Blood Coagulation (Dr. Masahiro Fujino, Saburo Yamaguchi, Masa Takauji, Kohki Uchida, Masahide Yasuda and Tatsuaki Matsushima), 80
Mechanism (A Positive Feedback), of Adenosine Triphosphate Synthesis in Erythrocytes (Makoto Nakao, Takashi Motegi, Toshiko Nakao, Saburo Yamazoe and Haruhisa Yoshikawa), 283
Mechanized World : Growing Old in a (F. Le Gros Clark), *review* by Prof. L. S. Hearnshaw, 532
Medam, E. C., and Koller, H. R. : Report on Computer Systems for Searching Chemical Literature (United States National Bureau of Standards Research Information Centre and Advisory Service on Information Processing), 23
Medford, R. D., and others : A New 'Wedge' Type Instability observed in a 'Theta' Pinch, 1054
Media (Anisotropic Elastic) : Surface Waves in (V. T. Buchwald and A. Davis), 899
Media (Culture), of *Penicillium chrysogenum* : Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from (A. Ballio, Prof. E. B. Chain, F. Dentice di Accadia, M. Mauri, K. Rauer, M. J. Schlesinger and Sondra Schlesinger), 909
Media (Multi-carbohydrate) : A Micro-method for Performing the Phenylalanine Test on Cultures from (D. J. Stewart), 521
Mediated Sugar Transport : Persistence in Erythrocyte Ghosts of (Paul G. LeFevre), 970
Mediation of Divalent Metal Ions in the Binding of Tetracycline to Macromolecules (Dr. Kurt W. Kohn), 1156
Medical Abstracts (Biological and), Ltd. : Publication of *Index Biologicus*, 544
Medical Bulletin (British) : Vol. 17, No. 2, Foetal and Neonatal Physiology (British Council), 344
Medical Research in East Africa, 226
Medical Research, East African Council for : Report for 1959-60, 226
Medical Research, Fleming Memorial Fund for : Grants from the, 959
Medical Research, Institute for (Federation of Malaya) : Studies from the, No. 29, Malaysian Parasites, 35-49 (edited by W. W. Macdonald), *review* by P. F. Mattingly, 948
Medical Research (Experimental) : Feldberg Foundation for German–English Scientific Exchange for, 1143
Medical Research Council : Appointment of Lord Shawcross as a member, 546
    Appointment of Prof. M. L. Rosenheim and Prof. Wilson Smith as members, 1142
    Establishment at the University of Durham of a Research Group on Demyelinating Diseases, 1143
    Feldberg Foundation for Experimental Medical Research, 1143
    Change of Address, 1143
    Memorandum No. 40, Ageing and the Semi-skilled—a Survey in Manufacturing Industry on Merseyside (Alistair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine Cunningham), 1249
    Memorandum No. 39, Introductory Manual on the Control of Health Hazards from Radioactive Materials, new edition, 1257
Medical Research Council (Statistical Research Unit) : Title of professor emeritus of medical statistics conferred on Sir Austin Bradford Hill, 546
Medical Sciences : The Use of Radioisotopes in Animal Biology and the (International Atomic Energy Agency, the Food and Agriculture Organization and the World Health Organization of the United Nations, Conference on), 959
Medical Sciences (General) : Highlights of Research Progress in, 1960 (United States Department of Health, Education and Welfare, Public Health Service Publication No. 815), 658
Medical Statistics at Belfast, 20
Medication : Skin (British Pharmaceutical Conference Symposium on), 755
Medicine : Aviation and Cosmonautical (C.E.R.M.A. International Congress on), 338
Medicine (Experimental Biology and), Society for (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
Medicine (Nuclear) : Fellowships and Grants in the field of Radiology and, 1962-63 (James Picker Foundation), 228
Medicine (Preventive), Lister Institute of : Report of the Governing Body (1961), 1356
Medicine and Pharmacy : Second Annual Report and List of Members (1960-61) of the Faculty of the History of (Worshipful Society of Apothecaries of London), 21
Medicine, Science and the Law : Vol. 1, No. 4, 753
Medium (Desertomycin) : Use of, in Routine Selective Isolation of Fungi Pathogenic to Man (J. Uri and Zs. Herpay), 1015
Medium (Synthetic), lacking Amino-acids : Formation of Phenethyl Alcohol and Tyrosol during Fermentation of a (R. Stevens), 913
Medvedev, Dr. Zh. A. : Paper Electrophoresis of Proteins while Preserving the Pattern of their Localization in Tissues, 1021
Mee, Dr. C. H. B. : Effect of Ultra-Violet Irradiation on the Electrical Conductivity of Cathodes coated with Barium Oxide, 1383
Megalithic Tombs of France, *review* by Prof. C. F. C. Hawkes, 637
Meiosis in the Oogonium and Antheridium of *Pythium debaryanum* Hesse (Dr. Eva Sansome), 827
Meiotic Behaviour (Diploid-like), of Polyploid Cotton : Basis of the (G. Kimber), 98
Melander, Stig E. J. : Oxytocinase Activity of Plasma of Pregnant Women, 176
Melanism in Captive Weaver-Finches (*Quelea quelea*), (Dr. B. Lofts), 993
Melbourne Symposium on Antarctic Meteorology, *review* by H. H. Lamb, 210
Meleka, A. H., and Barr, W. : An Autoradiographic Effect, 692
Mellon Institute : Appointment of Dr. P. C. Cross as president and chief executive officer, 439
    Annual Pittsburgh Diffraction Conference, 755
Melting and Polymorphism of Indium Antimonide at High Pressures (A. Jayaraman, R. C. Newton and Dr. G. C. Kennedy), 1288
Melts (Ionic) : The Structure and Properties of (Faraday Society General Discussion on), 338, 443
Membrane : Changes in the, during Recurrent Disinhibition of Spinal Motoneurones (Dr. Victor J. Wilson and Paul R. Burgess), 918
Membrane of Active Muscle Fibre : Ionic Currents in (Dr. H. Jenerick), 1074
Membrane of the Red Blood Cell : Dimer Formation and Glucose Transfer across the (Dr. W. D. Stein), 1277
Membrane (Biological) : Electrical Properties of Orientated Lipid on a—an Electrostatic Diffusion Barrier and Ion Pump (Dr. J. W. L. Beament), 217
Membrane (Erythrocyte) : Dimer Formation and the Transfer of Glycerol across the (Dr. W. D. Stein), 352
Membrane (Pit), of Wood Cells : Staining Reaction of the (R. K. Bamber), 409
Membrane Depolarization of Excitable Tissues : Outflux of Inorganic and Organic Phosphate during (Dr. L. G. Abood, Prof. K. Koketsu and I. Koyama), 395
Membranes (Biological) : Transfer of Calcium and Strontium across (United States Atomic Energy Commission, United States National Institutes of Health, United States National Science Foundation and the New York State Veterinary College Conference on), 1250
Membranes (Cellulose Acetate) : Use of, for Detecting Penicillinase-producing Organisms (Prof. R. Knox and J. T. Smith), 926
Membranes (Permselective) : Poisoning and Sign Reversal of (Dr. Francis de Körösy), 1363
Menkes, Dr. John H. : Side-Reactions during Hydrogenolysis of 2,4-Dinitrophenyl-hydrazones of Aromatic Keto-Acids, 285
Mental Health : Teaching of Psychiatry and (United Nations, World Health Organization, Public Health Papers No. 9), 1248
Mental Health, World Federation for : Grant from the Gulbenkian Foundation, 754
Mental Health Statistics, 855
Menzies, D. W. : Photo-Sensitivity of Thiazine Leucobases, 505
Mer, Dr. C. L. : Carbon Dioxide and Ethanol as Factors controlling the Growth of Etiolated Oat Seedlings, 260
β-Mercaptoethanol : A Morphogenetic Effect of—Distribution of Water in the Embryo (Dr. Peter Tuft), 1072 : Distribution of Water in the Chick Blastoderm (A. Jurand and Dr. Peter Tuft), 1073

6-Mercaptopurine : Biochemical Aspects of Resistance to, in Human Epidermoid Carcinoma Cells in Culture (R. W. Brockman, George G. Kelley, Patricia Stutts and Virginia Copeland), 469
Merceron, Th. (Bragiński, A., and) : Magnetic Relaxation in Magnetite and in its Solid Solutions with Manganese and Nickel Ferrites, 898
Mercury Drop (Rotating Hanging) : Use of the, in Analysis—Anodic Stripping using a Rotating Mercury Drop (Dr. J. J. Engelsman and A. M. J. M. Claassens), 240 ; Trace Analysis with a Rotating Hanging Mercury Drop (Dr. M. van Swaay and R. S. Deelder), 241
Mergenhagen, S. E., and others : Influence of Scurvy and Lathyrism (Odoratism) on Hydroxyproline Excretion, 1008
Mergerian, Dickron (Markham, Jordan J., and) : Use of a Different Type of Fluorescent Material as an Optical Maser, 586
Mering, Prof. J. : Graphite (Defaults Cristallins dans le Graphite), 27
Merriam, Sir Laurence : Elected president for 1961-62 of the Plastics Institute, 959
Merrick, M. J. : At the Twenty-first Meeting of the Research Panel of the British Gelatine and Glue Research Association (Glueing in the Furniture Industry), 860
Merseyside : Ageing and the Semi-Skilled—a Survey in Manufacturing Industry on (Alistair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine Cunningham), 1249
Mesopotamia (Ancient) : Ecological Aspects of Irrigation in (Hans Helback), 227
Metabolic Defects : Nutrition and (Nutrition Society Symposium on), 125
Metabolic Production of Sucrose from Fat (Prof. H. Beevers), 433
Metabolic Stability of Body Constituents (Dr. A. N. Davison and Dr. J. Dobbing), 844
Metabolic and Genetic Investigations of Nutritionally Exacting Strains of *Staphylococcus pyogenes* (Janice B. Edgar and Dr. B. A. D. Stocker), 1121
Metabolism : Tumour, *review* by Prof. F. Dickens, 1335
Metabolism of Fat during Ontogeny of the Rat : Role of the Lung in (Dr. Z. Vacek, P. Hahn and O. Koldovsky), 85
Metabolism of Indole-3-acetic Acid in Plants : 1-(Indole-3-acetyl)-β-D-Glucose, a New Compound in the (Dr. M. H. Zenk), 493
Metabolism of the Sulphur Amino-Acids and of Sulphate in *Blattella germanica* (Richard J. Block and S. Mark Henry), 392
Metabolism (Calcium), of Human Beings : Effect of Sodium Fluoride on (Dr. Clayton Rich and John Ensinck), 184
Metabolism (Calcium), in Man with Calcium-45—Malabsorption Syndrome and Exudative Enteropathy (Dr. G. Milhaud and Dr. P. Vesin), 872
Metabolism (Cell) : Multiple Inhibitions of, by a Single Substance (M. Harris and Dr. G. A. Morrison), 1276
Metabolism (Fructose and Lactic Acid), in the Perfused Liver of Premature Lambs (W. H. H. Andrews, H. G. Britton and Dr. D. A. Nixon), 1307
Metabolism (Histamine) : Anabolic Agents and (Dr. K. S. Kim), 1368
Metabolism (Phosphorus), of Brain (Dr. P. J. Heald), *review* by Dr. R. V. Coxon, 318
Metabolism (Purine and Pyrimidine), in *Rhodospirillum rubrum* (Prof. T. W. Goodwin and Dr. P. N. A. Passorn), 276
Metabolism (Respiratory), in Plants (Prof. Harry Beevers), *review* by Prof. W. O. James, 1031
Metabolite (Tri-*o*-Cresyl Phosphate) : Biological Activity of a (Prof. John E. Casida, Morifusa Eto and Ronald L. Baron), 1396
Metabolites of Vitamin B_{12} in Bile after Parenteral Administration of the Vitamin (Dr. John B. Stokes), 807
Metabolites (Intermediary) : Effects of Injections of some, on the 'Insulin Response' of Normal Rabbits (Dr. H. D. Brahmachari and G. Raghupathy Sarma), 491, *erratum*, 755
Metabolites (Urinary), of ^{14}C-Tetrachloroethylene in Mice (Dr. Sven Yllner), 820
Metadrenaline and Normetadrenaline in Normal Human Urine : Quantitative Determination of (Dr. Kaoru Yoshinaga, Dr. Chuichi Itoh, Dr. Nozomu Ishida, Dr. Tatsuo Sato and Dr. Yasuo Wada), 599
Metal Chlorin Complexes in Recent Sediments as Initial Precursors to Petroleum Porphyrin Pigments (G. W. Hodgson and Eric Peake), 766
Metal Ions in Enzyme Catalysis : Dextransucrase V and the Role of (W. Brock Neely and Julie Hallmark), 385
Metal Ions (Divalent), in the Binding of Tetracycline to Macromolecules : Mediation of (Dr. Kurt W. Kohn), 1156
Metal (Precious) Years : Two Hundred—a History of the Sheffield Smelting Company, Ltd., 1760-1960 (Ronald E. Wilson), *review* by Prof. R. W. K. Honeycombe, 108
Metallurgical Laboratory (National), Jamshedpur : Symposium on the Ferro-Alloy Industry in India, 1144
Metallurgical Society of the American Institute of Mining, Metallurgical and Petroleum Engineers (Iron and Steel Institute and the) : Special Meeting in the United States and Canada, 651
Metallurgical and Petroleum Engineers (Mining,) American Institute of (Iron and Steel Institute and the Metallurgical Society of the) : Special Meeting in the United States and Canada, 651
Metals : The de Haas-van Alphen Effect and the Electronic Structure of (Dr. D. Shoenberg), 126
Metals, Institute of : Autumn Meeting, 546
  Autumn Lecture, The Non-Ferrous Metals Industry in a Unified Europe (Baron Snoy et d'Oppuers), 546
Metals, Institute of (Iron and Steel Institute and the) (Powder Metallurgy Joint Group) : Winter Meeting—Symposium on Sintered High Temperature Compounds, 1250
Metals (Heavy) : Effect of Excess Supply of, to Barley during Germination, with special reference to Catalase and Peroxidase (Dr. S. C. Agarwala, A. Kumar and C. P. Sharma), 726
Metals (Non-Ferrous) Industry in a Unified Europe (Baron Snoy et d'Oppuers), 546
Metastable Nitrogen Atoms : New Afterglow Emission associated with (L. Herman and R. Herman), 346
Metcalf, D., and Ishidate, M. : Periodic Acid–Schiff Positive Giant Cells in the Mouse Thymus Cortex, 305
Meteorite Falls throughout the Ages : The Frequency of (Prof. Herbert Dingle), 482 ; (Prof. Hans Pettersson), 482
Meteorites : Organic Constituents of (Michael H. Briggs), 1137
Meteorites (Iron) : Microstructural and Chemical Identity of the Bear Creek and Narraburra (Dr. H. J. Axon), 1287
Meteorological Society (Royal) : Election of Officers for 1961-62, 443
Meteorology : Antarctic—Proceedings of the Symposium held in Melbourne, 1959 (Commonwealth of Australia, Bureau of Meteorology), *review* by H. H. Lamb, 210
  Theoretical, *review* by Dr. Robert P. Pearce, 314
Meteorology, Commonwealth of Australia Bureau of : Antarctic Meteorology—Proceedings of the Symposium held in Melbourne, 1959, *review* by H. H. Lamb, 210
Meteorology (Antarctic) : Melbourne Symposium on, *review* by H. H. Lamb, 210
Meteorology (Theoretical) : Introduction to (Prof. Seymour L. Hess), *review* by Dr. Robert P. Pearce, 314
Methaemoglobin in Chronic Copper Poisoning of Sheep (J. R. Todd and R. H. Thompson), 89
Method of Estimating Ionospheric Absorption Heights from Recovery Times (Syed Ziauddin), 1084
Method for Isolating Bacteria capable of Producing 6-Aminopenicillanic Acid from Benzylpenicillin (Prof. Yukio Kameda, Yukio Kimura, Etsuko Toyoura and Tomoo Omori), 1122
Method of Replicating Dry or Moist Surfaces for Examination by Light Microscopy (Joan Sampson), 932
Method of Treating Asbestos Filter Pads for Virus Filtration (N. St. G. Hyslop), 305
Method (Agar Disk), for the Direct Observation of Nematode-trapping Fungi in the Soil (Roderic C. Cooke), 1411
Method (Ethological), for Investigating the Properties of Nervous Systems : Response to Series of Subliminal Stimuli—an (A. J. Pontin), 512
Method (Experimental), in Biology from the Time of Antonio Vallisneri to the Present (University of Padua Symposium on), 546
Method (γ-Ray Counting) : Measurement of the Thorium Content of Natural Materials by a (Prof. J. H. J. Poole and F. N. Byrne), 62
Method (A Micro-), for Performing the Phenylalanine Test on Cultures from Multi-carbohydrate Media (D. J. Stewart), 521
Method (A New Two-Stage), for Cross-linking Proteins (Dr. Felix Borek), 1293
Method (Possible), for Increasing the Signal-Noise Ratio in the Detection of the First Motion of a Refracted *P*-Wave (F. A. Key, J. K. Wright, E. W. Carpenter and B. M. H. Scott), 1382
Method (A Simplified), for Separating Amino-Acid Mixtures from Protein Hydrolysates on One Ion-Exchange Column during Preparation (J. Liebster, K. Kopoldová and M. Dobiášová), 1198
Method (A Spray), for the Simultaneous Detection of Iodide and Iodinated Amino-Acids on Filter Paper Chromatograms (Dr. A. Van Zyl and M. Bhaga), 275
Methods : Infrared (G. K. T. Conn and D. G. Avery), *review* by Dr. E. S. Waight, 315
Methods of Feasible Directions, a Study in Linear and Non-Linear Programming (Dr. G. Zoutendijk), *review* by Dr. M. V. Wilkes, 9
Methods (Aims, Organization and), of University Extra-Mural Libraries in Great Britain (E. P. Pritchard), 649
Methods (Analytical), of Protein Chemistry (including Polypeptides) : A Laboratory Manual of (edited by Dr. P. Alexander and R. J. Block), Vol. 1, The Separation and Isolation of Proteins, *review* by Dr. D. T. Elmore, 320
Methods (Mathematical), in the Theory of Queueing (A. Y. Khintchine), translated by D. M. Andrews and M. H. Quenouille, *review* by Prof. L. S. Goddard, 211
Methods (Modern), of Traffic Control (Exhibition at the Science Museum, London, by the Westinghouse Brake and Signal Co., Ltd.), 1044
Methods (Physical), of Organic Chemistry (edited by Arnold Weissberger), third edition, Parts 3 and 4, *review* by Dr. W. A. Waters, 317
Methods (Separation), based on Molecular Sieving and Ion Exclusion (J. Porath and E. B. Lindner), 69
Methods (Simple, Quick), for Detection of 6-Amino-penicillanic Acid (J. Uri and F. Sztaricskai), 1223
Methopyrapone (Corticotrophin, Hydrocortisone and) : Effects of, on the Circulating Eosinophiles in Man (Dr. N. J. Kothari and Dr. J. C. Saunders), 1105
Methyl Alcohol/Chloroform-soluble Constituents of Honeybee Larval Foods : Chromatographic Investigation of the (N. G. Patel, M. H. Haydak and R. Lovell), 362
5-Methyltryptophan and Darkening of the Hair in Yellow A^{y}/a Mice (Dr. Vivianne T. Nachmias), 934
'Metrazol' Convulsions : Changes in the Pentose–Nucleic Acid Content of Sub-Cellular Fractions of the Brain of the Rat during (Prof. G. P. Talwar, B. Sadasivudu and V. S. Chitre), 1007
Meusel, Prof. H. : Flora Europaea (Problems of Abstracting and Indexing European Taxonomic Literature), 448
Mevalonic Acid in Carrots : Occurrence of (Prof. V. V. Modi and D. K. Patwa), 1202
Mice : Biochemical Changes in Lethal Staphylococcal Infections in (George W. Counts, Dr. Ian M. Smith, Dr. Joseph I. Routh, Dr. Elia Ch. Hazard and J. F. McTavish), 783
  Blockage of Transformation of Tryptophan to Nicotinic Acid by Experimental Hepatitis in (M. Piazza, F. Tancredi and Prof. E. Quagliariello), 601
  Development in, of Prolonged Non-specific Resistance to Sarcoma Implant and Staphylococcus Infection following Repository Injection of Lipopolysaccharide (Samuel J. Prigal), 1111
  The Effect of various Diets upon Sexual Maturity of (J. Szepsenwol), 1112
  Effect of Added Vitamins on the Phagocytic Activity of the Reticulo-Endothelial System of (J. Cordingley and Prof. T. Nicol), 82
  Effect of Hybridization on the Primary Polydipsic Trait of an Inbred Strain of (Dr. Emanuel Silverstein), 523
  Inheritance of Susceptibility to Induced Pulmonary Tumours in (Dr. D. S. Falconer and Dr. Joyce L. Bloom), 1070
  Non-Specific Immunity in Pneumococcal Infection of (Dr. G. N. Cooper and Dr. A. E. Stuart), 295
  Sensitivity of, to Bacterial Lipopolysaccharide following Alteration of Activity of the Reticulo-Endothelial System (Dr. G. N. Cooper and Dr. A. E. Stuart), 294
  Sex-Ratio in (Margaret J. Cook and Augustin Vlček), 89
  Urinary Metabolites of ^{14}C-Tetrachloroethylene in (Dr. Sven Yllner), 820
Mice (*DBA/1*) : A Natural Population of Anomalous Branched Tapeworms, *Hymenolepis nana* (Cestoda : Hymenolepididae), in a Colony of (Dr. Donald Heyneman), 297

Mice (Laboratory) : Simultaneous Eradication of Three Ectoparasitic Species from a Colony of (Dr. Nigel Bateman), 721
Mice (Yellow *A^vy^a*) : 5-Methyltryptophan and Darkening of the Hair in (Dr. Vivianne T. Nachmias), 934
Mice Inoculated with a Leukaemogenic Virus : Leukaemia in the Progeny of (Dr. M. H. Salaman and J. J. Harvey), 509
Mice Parasitized with a relatively Avirulent Strain of *Salmonella typhimurium* : Resistance to Infection with *Salmonella paratyphi* C in (Dr. J. G. Howard), 87
Mice made Polycythaemic by Exposure to Air at a Reduced Pressure : Bio-Assay of Erythropoietin in (Dr. P. Mary Cotes and Dr. D. R. Bangham), 1065
Mice Treated with *Iso*propylnoradrenaline : Enlargement of Salivary Gland in (Dr. K. Brown-Grant), 1076
Michael, J. Gabriel, and others : Increase in Specific Bactericidal Antibodies after Administration of Endotoxin, 296
Michalec, Dr. C. : Paper Chromatography (Paper Chromatography of Solvents, Reagents and Techniques useful in this field), 759
Michelson Interferometer : Molecular Emission Spectroscopy from 2μ to 12μ by a (H. A. Gebbie, G. Roland and L. Delbouille), 264
Microbial (Anti-) Agents and Chemotherapy (American Society for Microbiology Interscience Conference on), 651, 1250
Microbial (Anti-) Substances from Ferns (Prof. Jasper C. Maruzzella), 518
Microbiological Research Institute (Colonial), Trinidad : Closing of the, 20
Microbiology, American Society for : Interscience Conference on Antimicrobial Agents and Chemotherapy, 651, 1250
Microcirculation : Biorheology and (Medical School of the Hebrew University Seminar on), 757
Microcrystals (Silver Bromide) : Electron Spin Resonance Absorptions induced by Light in Adsorbed Layers of Cyanine Dyes on (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Microcythaemia in a Sardinian Family : Association of Haemoglobin *N* and (Prof. E. Silvestroni and I. Bianco), 1208
Microfibrils (Cellulose) : Constitution of (D. T. Dennis and Prof. R. D. Preston), 667
Microflora (Cellulose-decomposing), on Soil Crumb Plates : Factors influencing the Composition of the (A. Keynan, Y. Henis and P. Keller), 307
Microgram Scale : Pyrolysis and Gas–Liquid Chromatography on the (C. E. Roland Jones and A. F. Moyles), 663
Microlepidoptera (British), (Collection of), belonging to the late L. T. Ford acquired by the British Museum (Natural History), 124
Micro-method for Performing the Phenylalanine Test on Cultures from Multicarbohydrate Media (D. J. Stewart), 521
Micro-organism (New Nitrogen-fixing) : Chemical Character of the Pigments of a (Gy. Németh, F. Uresch, Prof. G. Fodor and L. Láng), 1413
Micro-organisms : Arthropod Transmission of (Dr. John Macleod), 885
  The Biology of, *review* by Prof. B. C. J. G. Knight, 1032
  An Introduction to the Biology of (Lilian E. Hawker, A. H. Linton, B. F. Folkes and M. J. Carlile), *review* by Prof. B. C. J. G. Knight, 1032
Micro-organisms which produce Antibiotics : Activation of Amino-Acids in (Dr. O. Ciferri, Dr. M. Di Girolamo and Dr. A. Bendicenti Di Girolamo), 411
Micropulsations and the Ionosphere : Connexion between (C. M. Lock and P. J. Stevens), 584
Microradiography (Contact), at 200-500 V. : An Experimental Apparatus for (Dr. A. Recourt and G. H. F. de Vries), 1185
Microscope : The Electron (M. E. Haine), *review* by Prof. D. Gabor, 316
Microscopic Observations (Electronic), of Structure in Ultra-Thin Sections of Vitrinite in Coal (J. T. McCartney, Sabri Ergun and R. E. Walline), 1361
Microscopic (Electron) Radioautography : Proposed Use of Magnetic Fields in (Lucien G. Caro), 1188
Microscopy : X-Ray (Dr. V. E. Cosslett and Dr. W. C. Nixon), *review* by Prof. Arne Engström, 1332
Microscopy (Electron) : Fixation of Yeast Protoplasts for (Dr. P. F. Elbers), 1022
Microscope (Electron) Observations of the Receptors in the Sensory Vesicle of the Ascidian Tadpole (Noël Dilly), 786
Microscopy (Light) : A Method of Replicating Dry or Moist Surfaces for Examination by (Joan Sampson), 932
Microstructural and Chemical Identity of the Bear Creek and Narraburra Iron Meteorites (Dr. H. J. Axon), 1287
Microwave Dipoles (Satellite) : Long-Range Radio Communication by—the West Ford Project, 1237
Migirdycian, Dr. E. : Effects of Ionizing Radiation on Organic and Biological Solids (Reactions in the Solid State), 1150
Mild Acid and Alkaline Hydrolysis of *M*- and *N*-Blood Group Substances : Products of (Dr. Elzbieta Romanowska), 1408
Miles, Dr. Charles P. : Peripheral Position of Sex Chromatin, 626
Miles, P. W. : Extraction of Lipids from the Precursor of the Stylet-Sheath Material of a Pentatomid, 911
Milhaud, Dr. G., and Vesin, Dr. P. : Calcium Metabolism in Man with Calcium-45—Malabsorption Syndrome and Exudative Enteropathy, 872
Milk : Correlation between Growth-Rate of the Suckling of various Species and the Percentage of Total Calories from Protein in the (Dr. F. W. Bernhart), 358
Milk (Cows') : An Invariant Casein Substrate for Rennet Standardization, derivable from Diverse Sources of (Dr. G. W. Scott Blair and J. C. Oosthuizen), 697
Milk Constituents : Removal of, from the Mammary Gland of the Ewe (A. K. Lascelles), 1404
Milk and Agricultural Materials : Strontium-90 in, in the United Kingdom, 1959-1960 (Agricultural Research Council, Report No. 4), 20
Milk and other Emulsions : Quantitative Analysis of, by Infra-Red Absorption (Dr. J. D. S. Goulden), 905
Miller, C. W., and others : Pulse-shortening in Electron Linear Accelerators, 483
Miller, J. A. : Elected to a Junior Research Fellowship for research in geophysics at Churchill College, Cambridge, 856
Miller, J. A., and Green, D. H. : Preliminary Age-Determinations in the Lizard Area, 159
Miller, Dr. J. F. A. P. : Analysis of the Thymus Influence in Leukaemogenesis, 248
Miller, J. K. (Gordon, the late Prof. R. M., and) : Cyclical Infection of *Glossina morsitans* with Culture Forms of *Trypanosoma rhodesiense*, 1317
Miller, L., and others : Kirkendall Effect in Gases, 375
Miller, Dr. P. L. : Spiracle Control in Dragonflies, 621
Mills, Lord : New Laboratory of the British Welding Research Association opened by, 1147
Milne, Angela A., and others : Reactivity of Calcium Sulphate Hemihydrate, 704
Milton, Dr. A. S. : Appointed I.C.I. Research Fellow in the University of Edinburgh, 337
Mineral Processing Congress : Sixth International (Société de l'Industrie Minérale), 545
Mineralogy (Clay) : Basic Dyestuffs in (Dr. V. S. Ramachandran, K. P. Kacker and N. K. Patwardhan), 696
Miniature Potentials (Spontaneous), from Insect Muscle Fibres (P. N. R. Usherwood), 814
Minimum Energy Theorem for Flow of Dry Granules through Apertures (R. L. Brown), 458
Minimum Time in Gas Chromatography : Role of Pressure Gradients in obtaining (Prof. J. Calvin Giddings), 1291
Mining, Metallurgical and Petroleum Engineers, American Institute of (Iron and Steel Institute and the Metallurgical Society of the) : Special Meeting in the United States and Canada, 651
Minolta Planetarium Projectors (Chiyoda Kogaku Seiko K.K.), 753
Miridae : The Number of Testis Follicles in (Dennis Leston), 93
Mirror (Magnetic) Confinement of Exploding Wire Plasma (E. J. Seykora), 995
Mirsky, Dr. L. : Appointed to a readership in pure mathematics at the University of Sheffield, 229
Mish, Dr. L. B., and others : A Sealing Compound for Use in Biological Work, 410
Missle (Ballistic), and Space Technology—Proceedings of the Fifth Symposium held at Los Angeles, 1960 (edited by Donald P. LeGalley), Vol. 1, Bioastronautics and Electronics, with Invited Addresses ; Vol. 2, Propulsion and Auxiliary Power Systems ; Vol. 3, Guidance, Navigation, Tracking and Space Physics ; Vol. 4, Re-entry and Vehicle Design, *review* by W. H. Stephens, 1131
Mitacek, jun., Paul, and Aston, Prof. J. G. : Heat Capacity and Resistivity Anomalies in Palladium Hydride, 271
Mitchell, Benjamin : Appointed to a lectureship in veterinary surgery at the University of Edinburgh, 545
Mitchell, Jr., John, and others (edited by) : Organic Analysis, Vol. 4, *review* by Dr. D. T. Lewis, 741
Mitchell, Dr. Peter : Coupling of Phosphorylation to Electron and Hydrogen Transfer by a Chemi-Osmotic Type of Mechanism, 144
Mitchison, Dr. N. A. : Appointed reader in zoology at the University of Edinburgh, 443
Mitochondria : Electron Transport System and Phosphoproteins of (K. Ahmed, J. D. Judah and C. H. Gallagher), 1309
Mitochondria from the Peel of Apples : Preparation of (Dr. J. D. Jones and Dr. A. C. Hulme), 370
Mitochondria Extracted from Higher Plants : Some Characteristics of (Dr. Walter D. Bonner, jun., and Dinor O. Voss), 682
Mitochondrial α-Glycerophosphate Dehydrogenase in the Nervous System : Histochemical and Homogenization Studies of (R. Hess and Dr. A. G. E. Pearse), 718
Mitochondrial α-Glycerophosphate Dehydrogenese Activity in Skeletal Muscle : Direct Relationship of Phosphorylase and (Dr. A. G. E. Pearse), 504
Mitosis in Regenerating Liver : Effect of Enzyme Injections on (Dr. Gaston de Lamirande), 400
Mitosis-rates in Generating Liver : Influence of Plasma Proteins in the Control of (Dr. J. T. Hemingway), 706
Mitra, Dr. S., and others : Nucleolar Localization of Succinic Dehydrogenase in Normal Rat Hepatic Cells, 925
Mittwoch, Ursula : Inclusions of Mucopolysaccharide in the Lymphocytes of Patients with Gargoylism, 1315
Mixtures : Estimation of Specific Proteins in Serum and other (Dr. D. A. Darcy), 1163
Mixtures (Amino-Acid), from Protein Hydrolysates : A Simplified Method for Separating, on One Ion-Exchange Column during Preparation (J. Liebster, K. Kopoldová and M. Dobiášová), 1198
Mixtures (Gaseous) : Determination of Hydrogen in, by Gas-Chromatography (P. J. Kipping), 270
Miyake, M., and others : Toxicology of *Penicillium islandicum*, 864
Mobile Laboratory for U.H.V. and V.H.F. Television Surveys (E. W. Taylor and L. C. Munn), 1355
Mocquot, Dr. G. (Dickinson, Dr. Anne, and) : Bacteria of the Intestine (Studies on the Bacterial Flora in the Alimentary Tract of Pigs, 1, Enterobacteriaceae and other Gram-negative Bacteria), 1152
Mocquot, Dr. G., and others : Bacteria of the Intestine (Studies on the Bacterial Flora in the Alimentary Tract of Pigs, 2, Streptococci : Selective Enumeration and Differentiation of the Dominant Groups), 1152
Modalities (Two Sense-) : Learning in (Dr. G. Ettlinger), 308
Models (Geometrical), for Complementation Mapping of Allelic Series : Limitations of (Prof. Elof Axel Carlson), 788
Models (Stochastic Population), in Ecology and Epidemiology (Prof. M. S. Bartlett), *review*, 843
Models and Techniques (Sciences,) : Management—Proceedings of the Sixth International Meeting of the Institute of Management Sciences, Paris, 1959 (edited by Prof. C. West and Prof. Michel Verhulst), *review* by T. H. Hawkins, 209
Modern African States : Conservation of Nature and Natural Resources in (International Union for the Conservation of Nature and the Commission for Technical Co-operation in Africa Conference on), 958
Modern Analysis : Foundations of (Prof. J. Dieudonné), *review* by Prof. L. S. Goddard, 6
Modern Aspects of Inorganic Chemistry (Prof. H. J. Emeléus and Dr. J. S. Anderson), third edition, *review* by Prof. J. R. Partington, 637
Modern Language Association of America : Science Information Personnel—the New Profession of Information combining Science, Librarianship and Foreign Languages (Leonard Cohen and Kenneth Craven), 452

Modern Methods of Traffic Control (Exhibition at the Science Museum, London, by the Westinghouse Brake and Signal Co., Ltd.), 1044
Modern Style in Mathematical Analysis, *review* by Prof. L. S. Goddard, 6
Modi, Prof. V. V., and Patwa, D. K.: Occurrence of Mevalonic Acid in Carrots, 1202
Modified Cellulose Ion Exchangers and their Use in Ribonucleic Acid Fractionation (Dr. L. Bosch, G. van der Wende and Dr. H. Bloemendal), 349
Modulation (Very-Low-Frequency), of Discrete Frequency Solar Noise Bursts (Dr. Jules Aarons, Santimay Basu, William Kidd and Richard Allen), 56
Moeckel, W. E.: Space-Flight and Re-entry Trajectories (Interplanetary Trajectories for Electrically Propelled Space Vehicles), 960
Moelwyn-Hughes, Dr. E. A.: At the Scientific Publications Council Meeting on the Publication of Scientific Books (Ownership of Copyright), 131; (Contractual Relationships), 132
Moffet, A. T., and Maltby, P.: Distribution of Brightness in Extragalactic Radio Sources, 453
Moggi, Dr. G.: Flora Europaea (Floristic Investigation in the Southern half of Italy and in Sicily), 447
Mohamed, Prof. A. H., and Zaki, K.: Effect of Bee Venom on Concentration of Blood Glucose and Liver Glycogen in Rabbits, 605
Moist Surfaces (Dry or): A Method of replicating, for Examination by Light Microscopy (Joan Sampson), 932
Moisture Changes: Influence of, on Deformation of Wood under Stress (L. D. Armstrong and G. N. Christensen), 869
Molar Sound Velocity in Molten Hydrated Salts (P. R. K. L. Padmini and Prof. B. Ramachandra Rao), 694
Mole, R. H.: Atomic Radiations and Living Organisms, 556
Mole's Snout: Nerve Endings in the (J. Armstrong and Dr. T. Andrew Quilliam), 1379
Molecular Beam Reactor for Examining the Rate, and Primary Products, of Reactive Bimolecular Collisions (C. W. Nutt and A. J. Biddlestone), 798
Molecular Emission Spectroscopy from 2μ to 12μ by a Michelson Interferometer (H. A. Gebbie, G. Roland and L. Delbouille), 264
Molecular Geometry of a 1:1 Crystalline Complex between 1,3,7,9-Tetramethyluric Acid and Pyrene (F. De Santis, E. Giglio, Dr. A. M. Liquori and A. Ripamonti), 900
Molecular Sieving and Ion Exclusion: Separation Methods based on (J. Porath and E. B. Lindner), 69
Molecular Structure (Crystal and), of Diphenyl (Dr. G. B. Robertson), 593
Molecular Weight of Deoxyribonucleic Acid: Low-Angle Light Scattering and the (Dr. Sidney Katz), 280
Molecular (Inter-) Energy Transfer in Photo-sensitized Oxidation-Reduction: Paths of (N. Takeyama), 1359
Molecule (Acceptor): Electron Affinity of the, in Charge-Transfer Complexes (Dr. M. E. Peover), 702
Mollin, D. L.: Vitamin B_{12} and Intrinsic Factor (Relationships between Vitamin B_{12} and Folid Acid), 1156
Molluscum Contagiosum: An Antigen in Lesions of (Dr. William L. Epstein, Irene P. Senecal and Dr. Alan M. Massing), 509
Molozzi, A. R. (Chapman, J. H., and): Interpretation of Cosmic Noise Measurements at 3·8 Mc./s. from Satellite 1960 $\eta 1$, 480
Molten Hydrated Salts: Molar Sound Velocity in (P. R. K. L. Padmini and Prof. B. Ramachandra Rao), 694
Molybdenum Monocarbide: A New Phase formed by High-Pressure Treatment—Face-centred Cubic (E. V. Clougherty, K. H. Lothrop and J. A. Kafalas), 1194
Momigny, Dr. J.: Ionization Potentials of *cis* and *trans* Dichloro- and Dibromo-ethylenes, 1089
Monkey ('Split-Brain'): Changes in Visual Gnostic Functions and Emotional Behaviour following Unilateral Temporal Pole Damage in the (Dr. J. L. deC. Downer), 50
Monmouth and Chepstow: Geology of the Country around (Memoirs of the Geological Survey of Great Britain—England and Wales), (Dr. F. B. A. Welch and Dr. F. M. Trotter), 227
Monoalkyl Ethers (Glycol): Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and (A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas), 378
Monoamine Oxidase Inhibitor: Augmentation of Toxicity of, by Thyroid Feeding (Richard N. Carrier and Dr. Paul V. Buday), 1107
Monocarbide (Molybdenum): A New Phase formed by High-Pressure Treatment—Face-centred Cubic (E. V. Clougherty, K. H. Lothrop and J. A. Kafalas), 1194
Monograph Prizes (American Academy of Arts and Sciences), 442
Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers: Compressibility and Specific Evaporation Resistance of the (A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas), 378
Monomethyl Ether (Ethylene Glycol), a New Fixative for Histological Work (Prof. Walter B. Shelley and Ralph Florence), 719
Monoxide (Carbon): Selectivity of Palladium Catalysts for the Oxidation of, in the Presence of Hydrogen (P. Mars and Th. van de Mond), 1003
Monoxide (Lead): Rotational Analysis of Absorption Bands of (Dr. R. F. Barrow, J. L. Deutsch and D. N. Travis), 374
Monozygotic Twins: The Diego Factor in a Puerto Rican Family—a Case of Anti-Diego in (Dr. Hubert J. Van Peenen, Dr. John Scudder, James A. Jack and Erika Awer), 188
Montagu-Pollock, H. M. (Bowden, Dr. F. P., and): Slow Decomposition of Explosive Crystals and their Damage by Fission Fragments, 556
Montefinale, Dr. Alberto (Papée, Dr. Henry M., and): Chemical Composition of Precipitable Water Vapour over the United States, 136
Months (Winter): Warm-water Species in the Plankton off Newfoundland during (V. Bainbridge), 1216
Moon: Surface of the (V. A. Firsoff), *review* by D. H. P. Jones, 424
  Orthographic Atlas of the (compiled by D. W. G. Arthur and E. A. Whitaker), edited by Gerard P. Kniper, Edition 'A' showing the Standard Orthographic Coordinate Grid, Part 1, Central Area, *review* by D. H. P. Jones, 424
Moon's Surface: Structure of the (Dr. Gilbert Fielder), *review* by D. H. P. Jones, 424
Mooney, E. F.: Inorganic Polymers (BN Polymers), 1047
Moor-Jankowski, J. K. (Kelus, A., and): An Iso-Antigen ($\gamma B4$) of Mouse γ-Globulin present in Inbred Strains, 1405
Moore, Dr. W. R.: Viscosity–Temperature Relationships for Dilute Solutions of High Polymers, 1292
Moray, Neville: Perceptual Defence and Filter Theory, 940
Moray Firth: Continuation of the Great Glen Fault beyond the (Dr. Derek Flinn), 589; (Dr. Robert M. Appleby), 1190
Morel, F., and others: Pharmacological Characterization of the Oxytocic Peptides in the Pituitary of a Marine Teleost Fish (*Pollachius virens*), 670
Moretti, Jean, and others: Action of Neuraminidase on Haptoglobin, 279
Morgan, A., and Arkell, Gloria M.: Determination of Caesium-137/Potassium Ratios in Diet and in the Human Body by Urine Analysis, 1100
Morgan, Prof. W. T. J. (Cheese, I. A. F. Lister, and): Two Serologically Active Trisaccharides isolated from Human Blood-Group *A* Substance, 149
Morgan, Prof. W. T. J. (Painter, Dr. T. J., and): Partial Acid-Hydrolysis of a Mucopolysaccharide without Appreciable *N*-Deacetylation of Hexosamine, 39
Morgane, Dr. P. J.: Evidence of a 'Hunger Motivational' System in the Lateral Hypothalamus of the Rat, 672
Morita, Hiromichi, and others: Synaptic Transmission in Neurosecretory Cells, 183
Morlin, Z., and Sáringer, K. M.: Effect of Ozone on the Fluorescence and Ultra-violet Absorption Spectra of 3,4-Benzopyrene, 907
Morocco, Government of, and others: To set up and operate an Aeronautical Research Laboratory and three Civil Aviation Training Centres, 1043
Morphine-like Drugs: Effects of, in Chicks (C. Schneider), 607
Morphogenetic Effect of Beta-Mercaptoethanol—Distribution of Water in the Embryo (Dr. Peter Tuft), 1072; Distribution of Water in the Chick Blastoderm (A. Jurand and Dr. Peter Tuft), 1073
Morphological Variation in *Ferrobacillus ferrooxidans* related to the Rate of Iron Oxidation (M. P. Silverman and M. H. Rogoff), 1221
Morphology (Experimental), Journal of Embryology and (Editorial Board of the): International Conference on Embryology, 24
Morreale de Escobar, G., and others: Increase of the Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at Early Stages of Propylthiouracil Treatment in the Rat, 1171
Morris, Harold P. (Aisenberg, Dr. Alan C., and): Energy Pathways of Hepatoma No. 5123, 1314
Morris, Prof. J. N.: At the Third International Congress of Dietetics (Large-Scale Individual Dietary Surveys on Middle-aged Men), 1343
Morrison, Dr. G. A.: Appointed lecturer in the Department of Organic Chemistry in the University of Leeds, 650
Morrison, Dr. G. A. (Harris, M., and): Multiple Inhibitions of Cell Metabolism by a Single Substance, 1276
Mortality (Age-dependent), in Fish: Expected Rate of Senescence and (Dr. Alex Comfort), 822
Mortar: The Widening of Expansion Cracks in, by Salt Solutions (J. A. Roberts and H. E. Vivian), 125, *erratum*, 1145
Mortar and Concrete: The Action of Calcium Chloride on (M. Lawrence and H. E. Vivian), 125, *erratum*, 1145
Morton, Prof. R. A.: The Training of Biochemists (Educational Aspects of a University Course should not be neglected in favour of 'Training'), 955
Morton, Prof. V. C.: Retirement from the chair of pure mathematics in the University College of Wales, Aberystwyth, 333; work of, 333
Mosaic Virus (Tobacco) (TMV): Chromatography of, on Chitin Columns (P. M. Townsley), 626
Mosaic Virus (Tobacco) (TMV) Lesion Number and Concentration: Relationship of, to the Rate of Lesion Production on Pinto Bean (John Piacitelli and Prof. Vincent Santilli), 624
Mosaic Virus (Turnip Yellow) Protein Production: Stimulation of, by 2-Thiouracil (Dr. R. I. B. Francki and Dr. R. E. F. Matthews), 1078
Mosaic Virus (Wild Cucumber): Unusual Base Composition of the Ribonucleic Acid of (H. Yamazaki and Prof. Paul Kaesberg), 96
Mosby, William L.: Heterocyclic Systems with Bridgehead Nitrogen Atoms, Part 1, *review* by Dr. G. L. Buchanan, 425
Moses (Robert) Niagara Power Project, 22
Moses, V. (Huggins, A. K., and): Breakdown of [2-^{14}C]-Glycine during Paper Chromatography, 668
Mosettig, Erich (Heftmann, Erich, and): Biochemistry of Steroids, *review* by Dr. D. T. Elmore, 111
Mossel, Dr. D. A. Q. (Buttiaux, Dr. R., and): Bacteria of the Intestine (The Significance of Organisms of Supposed Faecal Origin in Food and Water), 1153
Mota, Dr. Ivan: Mechanism of Action of Antigen–Antibody Complexes—their Effect on Mast Cells, 572
Motegi, Takashi, and others: A Positive Feedback Mechanism of Adenosine Triphosphate Synthesis in Erythrocytes, 283
Motion (First), of a Refracted *P*-Wave: Possible Method for Increasing the Signal-Noise Ratio in the Detection of the (F. A. Key, J. K. Wright, E. W. Carpenter and B. M. H. Stott), 1382
Motion (Steady): Fluid–Fluid Interfaces in (Prof. Walter Rose), 242
Motivation of Work (F. Herzberg), 1332
Motoneurones: Potential Changes produced by Application of Current Steps in (T. Araki, Dr. M. Ito and T. Oshima), 1104
Motoneurones (Spinal): Changes in the Membrane during Recurrent Disinhibition of (Dr. Victor J. Wilson and Paul R. Burgess), 918
Motor Nerve Terminals: Stimulation of (Dr. J. I. Hubbard and Robert F. Schmidt), 1103
Mott, Dr. Joan: Foetal and Neonatal Physiology (Ability of Young Mammals to withstand a Total Lack of Oxygen), 344
Motz, Dr. H.: Grant of £3,000 (in supplement of a previous grant) from the Paul Instrument Fund, for the construction of a linear accelerator working at 1·6 cm. (*J*-band), 225
Moudgal, N. R., and Li, Prof. Choh Hao: An Immunological Study of a Human Pituitary Interstitial Cell-stimulating Hormone, 192
Mountain, W. B., and McKeen, C. D.: Nematology (An Interaction between *Verticillium albo-atrum* and *Pratylenchus penetrans*), 1048
Mouriz García, J., and others: Increase of the Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at Early Stages of Propylthiouracil Treatment in the Rat, 1171
Mouse (*AkR*): Leukaemia in the, after X-Irradiation *in utero* (Donald Porteous), 291
Mouse (House-): Pulmonary Tumours and Group VII in the (Dr. Joyce L. Bloom), 1124; (Dr. J. A. H. Tatchell), 1125
Mouse Ascites Tumour (Ehrlich): Effect of Alpha-Amylase on the (Dr. F. Steckerl, A. Ofodile, R. R. Campbell and G. H. Friedell), 1113

Mouse Brains infected with Two Kinds of Arbor Virus Group *B* : Infectious Ribonucleic Acids derived from (Prof. Masahiro Nakamura), 624
Mouse Carcinoma : Effect of Irradiated Normal Tissues on the Growth of (Dr. Arthur Compton and Prof. Charles A. Pannett), 1101
Mouse γ-Globulin : An Iso-Antigen ($\gamma B A$) of, present in Inbred Strains (A. Kelus and J. K. Moor-Jankowski), 1405
Mouse Strains (Inbred) : Social Dominance in (Prof. G. Lindzey, H. Winston and M. Manosevitz), 474
Mouse Thymus Cortex : Periodic Acid–Schiff Positive Giant Cells in the (D. Metcalf and M. Ishidate), 305
Mouse Tumour Cells (Human and) : Selective Uptake of Specifically Bound Cobalt-58 Vitamin B_{12} by (Dr. Bernard A. Cooper and Dr. William Paranchych), 393
Moustafa, Dr. Esam M. : Leaf Curl Virus and the Flavonoid Content of Resistant and Susceptible Strains of Cotton, 415
Movement : The Phenomenological (Dr. Herbert Spiegelberg), *review* by F. I. G. Rawlins, 5
Movement (Net Water) : Comparison of Short-Circuit Current and, in Single Perfused Proximal Tubules of Rat Kidneys (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Movements (Population) : Effect of, on Malaria Eradication (R. Mansell Prothero), 863
Moving Objects (Stationary or) : Measuring Visual Constancy for (S. M. Anstis, C. D. Shopland and R. L. Gregory), 416
Moyles, A. F. (Jones, C. E. Roland, and) : Pyrolysis and Gas–Liquid Chromatography on the Microgram Scale, 663
Much-studied Protozoon : A, *review* by Dr. J. A. Kitching, 1133
Mucopolysaccharide : Inclusions of, in the Lymphocytes of Patients with Gargoylism (Ursula Mittwoch), 1315
  Partial Acid-Hydrolysis of a, without Appreciable *N*-Deacetylation of Hexosamine (Dr. T. J. Painter and Prof. W. T. J. Morgan), 39
Mucopolysaccharide produced by Guinea Pig Lymphocytes : Chemistry of a (Helen Muir and A. H. E. Marshall), 706
Mucopolysaccharide (An Acid), in Cockroach Ganglia (Doreen E. Ashhurst), 1224
Mucopolysaccharides (Sulphated) : Staining of (Dr. I. D. Heath), 1370
Mucoprotein (Serum), in Rheumatic Fever : Amino-acid Composition of (Prof. D. O'Brien, C. H. M. Walker and D. Walker), 922
Mud-Thinners : Sub-bituminous Coals as Oil-well Drilling, 1144
Muggleton, Dr. L. M. : Appointed to a lectureship in electrical engineering at the University of Edinburgh, 545
Muir, Helen, and Marshall, A. H. E. : Chemistry of a Mucopolysaccharide produced by Guinea Pig Lymphocytes, 706
Mukerji, S. K., and others : Diurnal Variation in Transaminase Activity in the Cactus Plant, 516
Müller, Dr. A. : Effects of Ionizing Radiation on Organic and Biological Solids (Role of Oxygen in Radiation Damage), 1150
Müller, Dr. A., and others : Experimental Evidence for the Mechanism of Photosynthesis, 194
Muller, Dr. H. H. : The Problem of whether Stains consist of Human or Animal Blood—Testing for Haemoglobin in the Stain, 753
Mullite-like Phase (Tetragonal), from Co-precipitated Gels (J. Ossaka), 1000
Multi-carbohydrate Media : A Micro-method for Performing the Phenylalanine Test on Cultures from (D. J. Stewart), 521
Multi-Sampler (A Phytoplankton), and its Use in Lake Victoria (Dr. J. H. Evans), 53
Multiple Inhibitions of Cell Metabolism by a Single Substance (M. Harris and Dr. G. A. Morrison), 1276
Multiplication of Foot-and-Mouth Disease Virus : Effect of *p*-Fluorophenylalanine on the (F. Brown, D. N. Planterose and Doreen L. Stewart), 414
Multiplication of Fowl Plague Virus : Influence of *p*-Fluorophenylalanine on the Production of Viral Ribonucleic Acid and on the Utilizability of Viral Protein during (Dr. Christoph Scholtissek and Rudolf Rott), 1023
Multiplication Rates of *Paramecium* : Effects of Kinetin and Indole-3-acetic Acid on (Mary Annunciata McManus and Kathleen Sullivan), 619
Multiplication (Poliovirus) : Effects of Heavy Water on—Results and Speculations on Mechanism (Dr. David Kritchevsky, Dr. Richard I. Carp and Dr. Hilary Koprowski), 250
Munday, C. W. (Clark, A. J., and) : Quality Measurement in the Chemical Factory (Department of Scientific and Industrial Research), 752
Munk, Walter H., and Macdonald, Gordon J. F. : The Rotation of the Earth, *review* by Prof. S. K. Runcorn, 313
Munn, L. C. (Taylor, E. W., and) : A Mobile Laboratory for U.H.V. and V.H.F. Television Surveys (B.B.C. Engineering Monographs No. 34), 1355
Munro, Dr. H. N., and others : Biorheology and Microcirculation (Capillary Strength Tests in Scurvy and their Reactions to Vitamins C and P Therapy), 758
Munro, T. R. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (A Technique which allows Selected Parts of Single Cells to be Irradiated with α-Particles), 341
Müntzing, Prof. Arne : Genetic Research, *review* by Dr. Kenneth S. Dodds, 636
Muramic Acid and other 3-O-Ethers of D-Glucosamine : A Convenient Synthesis of (Dr. Roy Gigg and Patricia M. Carroll), 495
Murata, Dr. Katsumi : Effects of Carrageenin on Serum Lipids and Atherosclerosis in Rabbits, 189
Murfet, I. C., and Barber, H. N. : Effects of Gibberellic Acid and Allogibberic Acid on Flowering and Internode-Length in *Pisum sativum*, 514
Murine Leukaemia : Transplantation of, by Unconventional Routes (Dr. Harold B. Hewitt), 1213
Murray, Dr. K., and others : Mannosyl D-Glucosamine Hydrochloride Isomers—*N*-Mannosyl D-Glucosamine Hydrochloride, 142
  Mannosyl D-Glucosamine Hydrochloride Isomers—6-*O*-Mannosyl D-Glucosamine Hydrochloride, 143
Murray, Dr. W. L. : Theory of Detonation, *review*, 1236
Muscle : Controlling Mechanism of the Mechanical Activity of, and that of Blood Coagulation (Dr. Masahuri Fujino, Saburo Yamaguchi, Masa Takauji. Kohki Uchida, Masahide Yasuda and Tatsuaki Matsushima), 80
  Inhibition by Physostigmine of the Increase of Oxygen Consumption induced by Potassium in (Dr. Ivan Novotny and František Vyskočil), 916
  The Physiology, Pharmacology and Pathology of, *review* by Dr. D. H. Sproull, 948
  The Structure and Function of (edited by Dr. G. H. Bourne), Vol. 2, Biochemistry and Physiology ; Vol. 3, Pharmacology and Disease, *review* by Dr. D. H. Sproull, 948
Muscle (Fish) : Cholinergic Transmission in (K. Krnjević), 1403
Muscle (Skeletal) : Direct Relationship of Phosphorylase and Mitochondrial α-Glycerophosphate Dehydrogenase Activity in (Dr. A. G. E. Pearse), 504
Muscle (Vascular Smooth) : Spontaneous Spike-Discharges of (Dr. S. Funaki), 1102
Muscle Fibre (Active) : Ionic Currents in Membrane of (Dr. H. Jenerick), 1074
Muscle Fibres (Insect) : Spontaneous Miniature Potentials from (P. N. R. Usherwood), 814
Muscle Target Fibres, a Newly Recognized Sign of Denervation (Dr. W. K. Engel), 389
Muscles (Frog), at Low Temperature: Inhibition by Potassium of the Mechanical Responses of (H. Lorković), 1011
Muscles ('Quick' and 'Catch' Adductor), in Brachiopods (Dr. M. J. S. Rudwick), 1021
Museum of Applied Science of Victoria, Melbourne : Report for 1959-60, 124
Museum of English Rural Life : Annual Report for 1960 (University of Reading), 544
Museum (Coryndon Memorial), Nairobi : Annual Report for 1959, 335
Museum (Fitzwilliam), Cambridge : Annual Report for 1960, 1249
Museum (Royal Ontario), Toronto : Appointment of Dr. W. E. Swinton as head of the Division of Life Sciences, 24
Museum (Royal Scottish), Edinburgh : Retirement of Dr. Douglas A. Allan from the directorship, 648
  Appointment of Ian R. Finlay as director, 648
  Appointment of Cyril Aldred as keeper of the Department of Art and Ethnography, 648
  Annual Report for 1960, 958
Museum (Science) : Africa's First (Murray Schoonraad), 848
Museum (South African) : Annual Report for 1959-60, 1044
Museum (United States National) : Bulletin No. 225, Drug Supplies during the American Revolution (George B. Griffenhagen), 761
Museum (Western Australian) : Annual Report for 1959-60, 335
Museums : Climatology and Conservation in (International Centre for the Study of the Preservation and the Restoration of Cultural Property: Works and Publications, 3), 1050
Museums and Galleries (Dr. F. S. Wallis), 862
Museums and Galleries, Standing Committee on : Sixth Report (1959-60), 862
Museums (National), of Southern Rhodesia : Annual Report for 1960, 650
Museums Association : Annual Report for 1960-61, 226
  Journal of the, No. 1, 226
  Sixty-seventh Annual Conference (Dr. F. S. Wallis), 553
  Election of Dr. Mary Woodall as president, 554
Mutagenesis induced by Formaldehyde : Mechanism of—the Essential Role of the 6-Amino Group of Adenylic Acid (or Adenosine) in the Mediation of the Mutagenic Activity of Formaldehyde (Dr. Thomas Alderson), 251
Mutagenic Action of the Alkylating Agents : Specificity of the (Prof. Bernard S. Strauss), 730
Mutagenic Effect of Radiant Heat Shocks on Phanerogamous Plants (Dr. Bror Pettersson), 1167
Mutation for Larger Fruit in the Raspberry (D. L. Jennings), 302
Mutations : Induction of, by Deoxyribonucleic Acid in *Drosophila melanogaster* (Dr. O. G. Fahmy and Dr. Myrtle J. Fahmy), 776
Mutations of *Tribolium confusum* Duv. : Sterile Crosses between (Prof. John Stanley), 934
Mutations (X-Ray- and Nitrous Acid-induced), in the Genetic Fine Structure of the ad_7 Locus of *Schizosaccharomyces pombe* : Distribution of (Dr. Herbert Gutz), 1127
Mutual Dependence : Copepod *Ommatokoita elongata* (Grant) in the Eyes of the Greenland Shark—a Possible Cause of (Bjørn Berland), 829
Mycobacteriophages isolated from Persons with Tuberculous and Non-tuberculous Conditions (Dr. E. Mankiewicz), 1416
Mycosphaerella musicola Leach : Significance of the Anti-Sporulation Effect of Copper Fungicides in the Control of Sigatoka Disease of Bananas caused by (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476
Myeloid Leukaemia (Transplantable Acute) : Effect of Calf Thymus Histone on Rats bearing (Dr. Lubomir Hnilica and Dr. Viktor Holoubek), 922
Myeloma Globulins : Comparison of Reduced Alkylated Derivatives of some, and Bence-Jones Proteins (Dr. M. D. Poulik and Dr. G. M. Edelman), 1274
Myers, Prof. C. A. (Harbison, Prof. F., and) : Management in the Industrial World, 1332
Myocardial Slices : Effect of Adrenalectomy and Corticosteroids on Distribution of Radioactivity in Protein of Cell Fractions from (Dr. Edward I. Weinshelbaum and Dr. Ira G. Wool), 1401
Myopathy affecting the Rottnest Quokka (*Setonix brachyurus*) reversed by α-Tocopherol (B. A. Kakulas), 402

NABATOFF, Robert A. (Kato, Yutaka, and) : Treatment of Varicose Veins, 335
Nachmias, Dr. Vivianne T. : 5-Methyltryptophan and Darkening of the Hair in Yellow A^{v}/a Mice, 934
Nagai, Yasushi, and others : Hexosamine and Sialic Acid Contents in Cells, 596
Nagy, S. (Rédei, A., and) : Simultaneous Determination of Dextran and Glucose in Serum, 173
Naivasha (Lake), Kenya Colony : A Natural Hybrid of *Tilapia nigra* and *Tilapia leucosticta* from (H. Y. Elder and D. J. Garrod), 722
Nakamura, Prof. Masahiro : Infectious Ribonucleic Acids derived from Mouse Brains infected with Two Kinds of Arbor Virus Group *B*, 624
Nakamura, Dr. Mitsuru : Induced Increase in Resistance of *Entamoeba histolytica* to Fumagillin, 413
Nakao, Makoto, and others : A Positive Feedback Mechanism of Adenosine Triphosphate Synthesis in Erythrocytes, 283
Nakao, Toshiko, and others : A Positive Feedback Mechanism of Adenosine Triphosphate Synthesis in Erythroyctes, 283

Nakayama, T. O. M., and others : Incorporation of 5,10,15 ^{14}C-Farnesol Pyrophosphate into *Phycomyces carotenoids*, 1299
Names (The Place-) of the West Riding of Yorkshire (Prof. A. H. Smith), Part 1, Lower and Upper Strafforth and Staincross Wapentakes ; Part 2, Osgoldcross and Agbrigg Wapentakes ; Part 3, Morley Wapentake, *review* by Prof. H. C. Darby, 208
Nanaimo, British Columbia : Swarming of the Polychaete *Odontosyllis phosphorea* Moore, var. *Nanaimoensis* Berkeley, near (E. Berkeley), 1321
Narayan, Sunanda : Composition of the Skin Follicle Population in Seven Rajasthan Sheep Breeds, 1115
Nardi, Dr. V. : Crystallization Kinetics in Polyvinylchloride and Thermoelastic Relaxation induced by Ultrasonics, 563
Narraburra Iron Meteorites (Bear Creek and) : Microstructural and Chemical Identity of the (Dr. H. J. Axon), 1287
Narurkar, M. V., and others : Anticancer Properties of Thio-diglycollic Acid, 388
Nash, J. K. T. L. : Title of professor of civil engineering (at King's College, London) conferred on, 651
Nash, Kevin : K. Terzaghi, Founder of the Science of Soil Mechanics, *review*, 210
Nathan, Dr. P. W. : Assessment of Pain (Traces of Pain remaining in the Nervous System, as in the case of Phantom Limbs), 1353
National Academy of Sciences (United States) : Election of foreign associates, 18
National Academy of Sciences (United States)—National Research Council : Grant of 56,000 dollars from the National Science Foundation, for a one-year study of Chemical Notation Systems, 23
  Report on Science in Space, 124
  Sector-Focused Cyclotrons—Proceedings of an Informal Conference, Sea Island, Georgia, 1959 (edited by F. T. Howard), (Nuclear Science Series, Report No. 26, Publication No. 656), *review* by M. Snowden, 532
  Publication No. 848, Effects of Inhaled Radioactive Particles, 556
  Publication No. 849, Long-Term Effects of Ionizing Radiations from External Sources, 556
  Publication No. 875, Effects of Ionizing Radiation on the Human Haematopoietic System, 556
National Bureau of Standards (United States) : Handbook No. 78, Report of the International Commission on Radiological Units and Measurements, 28
  Appointment of Martin T. Decker as chief of the Tropospheric Measurements Section, 334
  Appointment of John L. Hague as chief of the Standard Reference Materials Section, 334
  Appointment of Dr. John K. Taylor as chief of the Applied Analytical Research Section of the Analytical and Inorganic Chemistry Division, 334
  Appointment of Dr. David M. Gates as editor of Section D, Radio Propagation, of the *Journal of Research*, 856
  Appointment of Dr. Harry C. Allen, jun., as chief of the Analytical and Inorganic Chemistry Division, 1346
  Monograph No. 19, Atomic Energy Levels in Crystals (J. L. Prather), 1348
National Bureau of Standards, United States (Informations System Branch of the Office of Naval Research Information Centre) : Symposium on Optical Character Recognition, 1145
National Bureau of Standards (United States), (Research Information Centre and Advisory Service on Information Processing) : Report on Computer Systems for Searching Chemical Literature (E. C. Medam and H. R. Koller), 23
National Cancer Institute (United States), and others (sponsored by the) : Proceedings of a Conference on Biological Activities of Steroids in relation to Cancer (edited by Gregory Pincus and Erwin P. Vollmer), *review* by E. Reid, 1235
National Chemical Laboratory : Report for 1960 ; Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for 1960 (Department of Scientific and Industrial Research), 342
National Council for Technological Awards : Report for 1960-61, 754
National Foundation for Educational Research in England and Wales : *Technical Education Abstracts from British Sources*, Vol. 1, No. 1. September–December 1960, 21
National Foundation for Scientific Research, Belgium : Thirty-third Annual Report, 441
National Institute of Agricultural Botany : Appointment of D. B. MacKay as chief officer of the Official Seed Testing Station, 856
National Institute for Research in Dairying : Annual Report for 1960, 237
National Institute for Research in Nuclear Science, Harwell : Successful Operation of the *Nimrod* Injector, 1043
National Institutes of Health (United States), and others : Conference on Transfer of Calcium and Strontium across Biological Membranes, 1250
National Lending Library for Science and Technology : *N.L.L. News*, No. 1, 1347
National Reference Library of Science and Invention, 1 ; (Leslie Wilson), 733 ; (J. Farradane), 734
  Aslib Report of the Discussion Meeting on the, 649
National Metallurgical Laboratory, Jamshedpur : Symposium on the Ferro-Alloy Industry in India, 1144
National Museum (United States) : Bulletin No. 225, Drug Supplies during the American Revolution (George B. Griffenhagen), 761
National Museums of Southern Rhodesia : Annual Report for 1960, 650
National Organization of Scientific Research, 632
National Parks Commission : Appointment of Colonel J. F. Williams-Wynne as a member, 546
National Physical Laboratory : Open Days (J. R. Illingworth), 112
National Research Council of Canada : Problems of the North (Translation of *Problemy Severa*), No. 1, 1958 (translated December 1960), *review* by Dr. Terence Armstrong, 841
National Research Council (United States), (National Academy of Sciences) : Grant of 56,000 dollars from the National Science Foundation, for a one-year study of Chemical Notation Systems, 23
  Report on Science in Space, 124
  Sector-Focused Cyclotrons—Proceedings of an Informal Conference, Sea Island, Georgia, 1959 (edited by F. T. Howard), (Nuclear Science Series, Report No. 26, Publication No. 656), *review* by M. Snowden, 532
  Publication No. 848, Effects of Inhaled Radioactive Particles, 556
  Publication No. 849, Long-Term Effects of Ionizing Radiations from External Sources, 556
  Publication No. 875, Effects of Ionizing Radiation on the Human Haematopoietic System, 556
National Research Development Corporation : Report for 1960, 343
National Science Foundation (United States) : Grant of 56,000 dollars to the National Academy of Sciences—National Research Council, for a one-year study of Chemical Notation Systems, 23
  Establishment of an Office of Antarctic Programs, 125
  Appointment of Dr. Thomas O. Jones as head of the Office of Antarctic Programs, 125
  Reviews of Data on Research and Development, No. 27, Report on Scientists and Engineers engaged in research and development in Colleges and Universities in the United States for 1958, 225
  Reviews of Data on Research and Development, No. 28, Capital Expenditure of Colleges and Universities in the United States for research and development in the Natural and Social Sciences in 1958, 545
  Science Information Personnel—the New Profession of Information combining Science, Librarianship and Foreign Languages (Leonard Cohen and Kenneth Craven), 452
National Science Foundation (United States), and others : Conference on Transfer of Calcium and Strontium across Biological Membranes, 1250
National Society for Clean Air : Fumifugium, or the Smoake of London Dissipated (John Evelyn), reprint, 752
  Twenty-eighth Conference, 1250
National Spectra Index—Plans and Problems (P. S. Davison), 1250
National University (Australian), Canberra : Hon. D.Sc. degree awarded to Sir Rudolph Peters and to Dr. J. L. Pawsey, 443
National University of Ireland : Appointment of Dr. Eva M. Philbin to the chair of organic chemistry in University College, Dublin, 439
NATO : Advanced Study Institute Programme (1962), 442
Natural History, Field Museum of (Chicago) : Botanical Series, Vol. 13, Part 5, No. 1, Flora of Peru (J. Francis Macbride), *review* by N. Y. Sandwith, 214
Natural Hydrid of *Tilapia nigra* and *Tilapia leucosticta* from Lake Naivasha, Kenya Colony (H. Y. Elder and D. J. Garrod), 722
Natural Materials : Measurement of the Thorium Content of, by a γ-Ray Counting Method (Prof. J. H. J. Poole and F. N. Byrne), 62
Natural Phenolic Compounds : Stereochemistry of (Plant Phenolics Group Symposium on), 234
Natural Population of Anomalous Branched Tapeworms, *Hymenolepis nana* (Cestoda : Hymenolepididae), in a Colony of *DBA*/1 Mice (Dr. Donald Heyneman), 297
Natural Radioactivity in Tobacco and Tobacco Smoke (Dr. V. C. Runeckles), 322
Natural Resources (Nature and), in Modern African States : Conservation of (International Union for the Conservation of Nature and the Commission for Technical Co-operation in Africa Conference on), 958
Natural Rubber Producers' Research Association : Annual Report, 854
Natural Soil Aggregates : Role of Polysaccharides in Stabilization of (Dr. D. J. Greenland, G. R. Lindstrom and Dr. J. P. Quirk), 1283
Natural and Artificial Haematites : Hydrogen Reduction Characteristics of (John Henderson), 268
Nature, International Union for the Conservation of, and the Commission for Technical Co-operation in Africa : Conference on the Conservation of Nature and Natural Resources in Modern African States, 958
Nature, International Youth Federation for the Study and Conservation of : Course in Conservation, by the Verein Naturpark in the Lüneburger Heide, Germany, 1045
Nature Conservancy : Research Work of the (House of Commons Adjournment Debate on), 962
  Shapwick Heath Nature Reserve, 1248
  Wye and Crundale Downs Nature Reserve, 1248
Nature Reserve : Shapwick Heath (Nature Conservancy), 1248
  Wye and Crundale Downs (Nature Conservancy), 1248
Nature Reserves : Problems of Managing (Dr. J. F. D. Frazer), 133
Nature of the Coating Globulin causing a Positive Antiglobulin Consumption Test (D. Nelken), 1110
Nature of Planck's Constant: Theory of the Red Shift and the (L. Infeld), 1184
Nature of Radio Stars : Apparent Recession of Nebulae and the (Dr. N. S. Japolsky), 480
Nature and Natural Resources in Modern African States : Conservation of (International Union for the Conservation of Nature and the Commission for Technical Co-operation in Africa Conference on), 958
Naval Observatory (United States) : Reprint No. 19, Resources and Treasures of the Library (Marjorie S. Clopine), 22
Naval Research, United States Office of (Information Systems Branch) : Symposium on Redundancy Techniques for Computing Systems, 1349
Nayler, J. H. C., and others : Action of Carbon Dioxide on 6-Aminopenicillanic Acid, 910
  Some Novel Acid-Stable Penicillins, 1091
Neagh (Lough) Pollan *Coregonus pollan*, Thompson : Furunculosis in (K. U. Vickers and R. McClean), 930
Neal, Dr. P. B. N. : Appointed senior Research Fellow in mechanical engineering at the University of Sheffield, 229
Neale, D. M. : At the Scientific Publications Council Meeting on the Publication of Scientific Books (Ownership of Copyright), 131
Near-shore Waters of Porto Novo, South India : Occurrence of *Eutrichocheles modestus* (Herbst) in the (K. Balasubrahmanyan and Dr. J. Jacob), 830
Nebula : The Alpha Virginis (Spica), (Walter A. Feibelman), 1083
Nebulae : Apparent Recession of, and the Nature of Radio Stars (Dr. N. S. Japolsky), 480
Needham, Dr. A. E. : Adaptive Value of Regenerative Ability, 720
Needham, Dr. R. : Age and Kinship in Australia, *review*, 6
Needler, Dr. W. C., and others : Electron Spin Resonance Absorptions induced by Light in Adsorbed Layers of Cyanine Dyes on Silver Bromide Microcrystals, 902
Neely, W. Brock, and Hallmark, Julie : Dextransucrase *V* and the Role of Metal Ions in Enzyme Catalysis, 385
Nelken, D. : Nature of the Coating Globulin causing a Positive Antiglobulin Consumption Test, 1110

Nematode-trapping Fungi in the Soil : Agar Disk Method for the Direct Observation of (Roderic C. Cooke), 1411
Nematodes (Parasitic), for Collection Purposes : Use of Glacial Acetic Acid for killing (Bjørn Berland), 1320
Nematologists (European), Society of : Sixth International Symposium on Nematology, 24, 1048
Nematology (Society of European Nematologists Sixth International Symposium on), 24, 1048
Németh, Gy., and others : Chemical Character of the Pigments in a New Nitrogen-fixing Micro-organism, 1413
Nemin Assay Procedure (E. J. Winkler, Shimpei Kuyama and Prof. David Pramer), 155
Neon in Stars : Production of (H. E. Gove, A. E. Litherland and M. A. Clark), 1381
Neon Glow Lamp (1911-51) : The Development of the (Prof. H. E. Watson), 1040
Neonatal Physiology : Foetal and (*British Medical Bulletin*, Vol. 17, No. 2), (British Council), 344
Nephrotic Syndromes : Haptoglobinuria in (A. Marnay), 74
Nepytia phantasmaria Stkr. (Lepidoptera : Geometridae) : Influence of Electrical Field on Pupation and Oviposition in (Dr. D. K. Edwards), 976
Nerve (Splanchnic) : Effect of Bilateral Section of the, on Erythropoiesis (Fumimaro Takaku, Kunitake Hirashima and Prof. Shigeo Okinaka), 500
Nerve Cell (Giant), under Abnormal Conditions : Electrical Activity of a (Dr. Yutaka Oomura, Sachio Ozaki and Takashi Maéno), 1265
Nerve Endings in the Mole's Snout (J. Armstrong and Dr. T. Andrew Quilliam), 1379
Nerve Terminals (Motor) : Stimulation of (Dr. J. I. Hubbard and Robert F. Schmidt), 1103
Nerve Tissue (Insect) : Activity of Glutamic Acid Decarboxylase in (Dr. N. Frontali), 178
Nervous System : Chemistry and the, *review* by Dr. R. V. Coxon, 318
  Histochemical and Homogenization Studies of Mitochondrial α-Glycerophosphate Dehydrogenase in the (R. Hess and Dr. A. G. E. Pearse), 718
Nervous System (Central) : Enzymic Activity of the (Neurochemical Commission of the World Federation of Neurology International Symposium on), 1349
  Pharmacologically Active Substances present in the (Dr. Paul V. Buday), 245
  Tryptamine Receptors in the (Dr. J. R. Vane, Dr. H. O. J. Collier and Dr. S. J. Corne, and Dr. E. Marley and Dr. P. B. Bradley), 1068
Nervous System (Central), of an Insect (*Periplaneta americana* L.) : Exchanges of Sodium Ions in the (J. E. Treherne), 1223
Nervous Systems : Response to Series of Subliminal Stimuli—an Ethological Method for Investigating the Properties of (A. J. Pontin), 512
Nervous and Biochemical Disturbances following Hexachlorobenzene Intoxication (Dr. F. De Matteis, B. E. Prior and Prof. C. Rimington), 363
Net Water Movement : Comparison of Short-Circuit Current and, in Single Perfused Proximal Tubules of Rat Kidneys (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Netschey, A. (Gutmann, Dr. F., and) : Semi-conductivity of Chlorpromazine, 1390
Neuraminidase : Action of, on Haptoglobin (Prof. Max E. Rafelson, jun., Lise Cloarec, Jean Moretti and Max F. Jayle), 279
Neurochemistry of Nucleotides and Amino Acids—a Symposium of the Section on Neurochemistry, American Academy of Neurology (edited by Roscoe O. Brady and Donald B. Tower), *review* by Dr. R. V. Coxon, 318
Neurology, American Academy of (Section on Neurochemistry) : Symposium on the Neurochemistry of Nucleotides and Amino Acids (edited by Roscoe O. Brady and Donald B. Tower), *review* by Dr. R. V. Coxon, 318
Neurology, World Federation of (Neurochemical Commission) : International Symposium on Enzymic Activity of the Central Nervous System, 1349
Neurone : The Adrenergic (Sir Lindor Brown), 1143
Neurone-blocking Agents (Adrenergic), related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine (A. L. A. Boura, F. C. Copp, A. F. Green, H. F. Hodson, G. K. Ruffell, M. F. Sim, E. Walton and E. M. Grivsky), 1312
Neurones of the Brain of the Leech : Neurosecretion and Granules in (Irvine R. Hagadorn and Richard S. Nishioka), 1013
Neurones (Mammalian) : Analogues of Glutamic and γ-Amino-*n*-butyric Acids having Potent Actions on (Dr. J. C. Watkins and Dr. D. R. Curtis), 1010
Neurones (Spinal), of some Teleosts : Pigment Bodies of the (Prof. A. Stolk), 924
Neuropharmacology and Biochemistry (Psychophysiology,), of Audiogenic Seizure (Centre National de la Recherche Scientifique International Meeting on), 959
Neurosecretion (University of Bristol International Conference on), 856
Neurosecretion and Granules in Neurones of the Brain of the Leech (Irvine R. Hagadorn and Richard S. Nishioka), 1013
Neurosecretory Cells : Synaptic Transmission in (Hiromichi Morita, Takaaki Ishibashi and Satoru Yamashita), 183
Neurosecretory System of the Desert Locust : Induced Changes in the Amounts of Material in the (Dr. K. C. Highnam), 199
Neurospora : Chromosomes of, 658
Neutral 17-Ketosteroids in Porcine Urine (L. G. Huis in't Veld, B. Louwerens and W. Reilingh), 175
Neutralization Tests (Quantal) : Evaluation of (Prof. S. Fazekas de St. Groth), 891
Neutron Activation : Rapid Determination of Ytterbium in Aqueous Solutions and in Thulium Oxide by (Minoru Okada), 1090
Neutron Capture Therapy, 1258
Neutron 'Flux' (K. L. Stretch), 60 ; (Dr. W. R. Harper), 695
Neutron Kinetics (Linear) : Topology of (L. G. Kemeny), 263
Neutron Terminology (Dr. W. R. Harper), 60
Neutron (Fast-) Spectroscopy (B. V. Rybakov and V. A. Sidorov), (edited by N. A. Vlasov), translated from the Russian, *review* by Dr. P. A. Egelstaff, 8
Neutrons (Long Wave-length) : Scattering of, by Irradiated Beryllium Oxide (T. M. Sabine, A. W. Pryor and B. S. Hickman), 1385
Neutrons (14-MeV.) : Interaction of, with a Silicon Semiconductor Nuclear Particle Detector (Dr. W. M. Deuchars and G. P. Lawrence), 995
Neville, Prof. E. H. : *Obituary* by Prof. R. Rado, 1345
New to Britain : Littoral Interstitial Species from Anglesey representing Three Families (P. J. S. Boaden), 512
New Afterglow Emission associated with Metastable Nitrogen Atoms (L. Herman and R. Herman), 346
New Age in Physics (Prof. Sir Harrie Massey), *review* by Prof. W. E. Burcham, 423
New American Electricity Plant, 22
New Antioxidant from Oats : Isolation of a (Dr. D. G. H. Daniels and H. F. Martin), 1302
New Approach to Biology Teaching (Dr. L. H. Finlayson), 554
New Approach to General Relativity, *review* by Dr. G. J. Whitrow, 424
New Compound in the Metabolism of Indole-3-acetic Acid in Plants : 1-(Indole-3-acetyl)-β-D-Glucose, a (Dr. M. H. Zenk), 493
New Computing Centre (IBM Co.), 23
New Considerations on Survival Curves and Target Theory (Dr. E. L. Powers), 1045
New Data-processing Centre (IBM Co.), 23
New Delhi Council of Scientific and Industrial Research : Annual Technical Report (1959-60), 1254
New Enzyme connected with Protein Synthesis : Evidence for a (Hans G. Boman and Ingvar Svensson), 674
New Esters of Chromone-2-Carboxylic Acid : Some (Juan X. de Vries and Kempis Vidal-Beretervide), 171
New Family of Potent Carcinogens—Benzopyridocarbazoles (Prof. A. Lacassagne, Prof. N. P. Buu-Hoï, F. Zajdela, F. Périn and P. Jacquignon), 1005
New Fixative for Histological Work : Ethylene Glycol Monomethyl Ether, a (Prof. Walter B. Shelley and Ralph Florence), 719
New Handbooks for Lunar Studies, *review* by D. H. P. Jones, 424
New Hope for African Wildlife, 958
New Inorganic Reaction—Nitric Acid–Hydrazoic Acid (Dr. J. Mašek), 166
New Laboratory for Research on Creep, 1049
New National Nature Reserves, 1248
New Nitrogen-fixing Micro-organism : Chemical Character of the Pigments of a (Gy. Németh, F. Uresch, Prof. G. Fodor and L. Láng), 1413
New Phase formed by High-Pressure Treatment—Face-centred Cubic Molybdenum Monocarbide (E. V. Clougherty, K. H. Lothrop and J. A. Kafalas), 1194
New Phenotypes (Two) : Differential Inhibition of Human Serum Cholinesterase with Fluoride—Recognition of (Prof. H. Harris and Mary Whittaker), 496
New Photometric Technique using a Variable Shutter Device (Dr. O. C Jones and Dr. F. J. J. Clarke), 1290
New Profession of Information combining Science, Librarianship and Foreign Languages : Science Information Personnel—the (Leonard Cohen and Kenneth Craven), 452
New Regions of the Spectrum : From Radar to Spectroscopy— (Prof. D. J. E. Ingram), 1146
New Relation between the N–H Stretching Frequencies of Primary Amides (Prof. P. G. Puranik and K. Venkata Ramiah), 795
New *Rh* Phenotype, $Dcce^{l}e^{i}f$, found in a Chibcha Indian Tribe (Dr. Miguel Layrisse, Zulay Layrisse, Esperanza García, Dr. Johannes Wilbert and Joaquin Parra R.), 503
New Routes to Further Education—Papers presented at the B.A.C.I.E. Spring Conference, London, 1961 (British Association for Commercial and Industrial Education), 739
New Series of Anti-tumour Agents : N,N'-Alkylene-*bis* (Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds—a (A. S. Tomcufcik, S. D. Willson, A. W. Vogel and A. Sloboda), 611
New South Wales : Cave Art of the Conjola District (F. D. McCarthy), 854
New Technique for the Production of Synthetic Corundum (E. A. D. White), 901
New Test for Detecting Anaphylactic Sensitivity—the Basophil Reaction (Prof. Walter B. Shelley and Lennart Juhlin), 1056
New Thinking in School Mathematics, 335
New Trends in Atomic Research and their International Significance (Dr. L. Kowarski), 853
New Triterpenoid Sapogenin of Animal Origin (Sea Cucumber), (Takao Matsuno and Toshihiko Yamanouchi), 75
New Two-Stage Method for Cross-linking Proteins (Dr. Felix Borek), 1293
New Universal Buffer System (Dr. D. A. Ellis), 1099
New 'Wedge' Type Instability observed in a 'Theta' Pinch (J. K. Wright, R. D. Medford, J. D. Herbert and R. C. Pottinger), 1054
New York Academy of Sciences : Symposium on the Use of Animal Cell, Tissue and Organ Cultures in Radiobiology, 340
  Conference on Blood Groups in Infra-human Species, 653
  Fifth International Conference on Pigment Cell Biology, 1045
New York State Veterinary College, and others : Conference on Transfer of Calcium and Strontium across Biological Membranes, 1250
New Zealand : Four Decades of State Forestry in, 451
  Report of the Director of Forestry for 1959-60, 451
New Zealand Butterfat : Growth of Ryegrass-dominant Pasture grazed by Cows, and Properties of (F. H. McDowall, W. A. McGillivray and J. C. Hawke), 303
New Zealand Phytochemical Survey, 545
New Zealand Rainwater : Barium-140 in (G. S. McNaughton and R. N. Woodward), 1344
New-born and Adult Rats : Collagen Fractions in the Skin of (Nestor Gonzalez Cadavid, Berta Denduchis and Roberto E. Mancini), 1297
Newfoundland : Warm-water Species in the Plankton off, during Winter Months (V. Bainbridge), 1216
Newham, Dr. J. : Appointed I.C.I. Research Fellow in the University of Edinburgh, 337
Newhook, F. J. : Non-Lethal Pathological Infection of Roots, 615
Newitt, Prof. D. M. : Title of professor emeritus of chemical engineering (at the Imperial College of Science and Technology, London), conferred on, 546
Newly Hatched Chick : Comparison of the Serum Protein Fractions of the, with those of Adult Birds using Starch-gel Electrophoresis (A. Amin), 708
Newly Recognized Sign of Denervation : Muscle Target Fibres, a (Dr. W. K. Engel), 389

- Newman, R. P. : British Welding Research Association—New Laboratory, 1147
- Newnham, I. E. : Appointed chief of the Division of Mineral Chemistry of the Australian Commonwealth Scientific and Industrial Research Organization, 543 ; work of, 543
- Newton, Prof. Lily : Alcological Study on the Eastern Coasts of the Americas, *review*, 1234
- Newton, R. C., and others : Melting and Polymorphism of Indium Antimonide at High Pressures, 1288
- Niagara Power Plant : The Robert Moses, 22
- Nicaragua: Estudios Ecologicos para el Aprovechamiento de la Tierra en (Dr. B. W. Taylor), 1259
- Nickel Complexes (Chromium, Uranium, Tungsten and), of Thioglycolic Acid (V. M. Bhuchar), 489
- Nickel Ferrites (Manganese and) : Magnetic Relaxation in Magnetite and in its Solid Solutions with (A. Bragiński and Th. Merceron), 898
- Nickel-Iron Films : Variation of Anisotropy Axis Dispersion with Thickness of (H. Clow), 996
- Nickel Oxide : Porous (I. S. Solet), 64
- Nickel Reaction and Thermal Isomerization of Alpha-Keto-Acid Hydrazones : Application of the, for their Chromatographic Analysis (A. M. Tokushige and Dean E. S. Cook), 797
- Nickerson, Dorothy : At the Maxwell Colour Centenary Conference (Colour Appearance), 11
- Nicol, Prof. T. (Cordingley, J., and) : Effect of Added Vitamins on the Phagocytic Activity of the Reticulo-Endothelial System of Mice, 82
- Nicotinic Acid : Blockage of Transformation of Tryptophan to, by Experimental Hepatitis in Mice (M. Piazza, F. Tancredi and Prof. E. Quagliariello), 601
- Nigerian Varieties of Maize : Susceptibility to Leaf Blight, caused by *Cochliobolus heterostrophus*, in (C. L. M. van Eijnatten), 515
- Nigerose and Kojibiose : Isolation of, from Dextrans (Kazuo Matsuda, Hiroshi Watanabe, Kentaroh Fujimoto and Prof. Kiyoshi Aso), 278
- Night Sky in August, 338 ; in September, 755 ; in October, 1145
- Night-time F Layer Irregularities at the Equator : Height of (Dr. G. S. Kent and Dr. J. R. Koster), 1083
- *Nimrod* Injector : Successful Operation of the (National Institute for Research in Nuclear Science, Harwell), 1043
- Nishioka, Richard S. (Hagadorn, Irvine R., and) : Neurosecretion and Granules in Neurones of the Brain of the Leech, 1013
- Nissim, J. A. : Enhancement of Inhibition of Intestinal Absorption by Cetrimide–Phloridzin Combination, 37
- *Nitella* : Relative Volumes of Cytoplasm and Vacuole in (Dr. Paul Saltman and Dr. Tyge Christensen), 825
  - Voltage-Clamp Experiments with (G. P. Findlay), 812
- Nitrate-Reductase in *Aerobacter aerogenes* : Inhibition by Oxygen of Biosynthesis and Activity of (Francis Pichinoty and Liliane D'Ornano), 879
- Nitric Acid–Hydrazoic Acid : A New Inorganic Reaction— (Dr. J. Mašek), 166
- Nitride (Boron) : Formation of, in Diborane–Hydrazine Flames (W. G. Berg and W. E. Wilson), 380
- Nitrite in a Tropical West African Estuary : Seasonal Fluctuations and Distribution of (J. C. D. Watts), 929
- Nitrogen in Crop Production : Importance of (Prof. N. R. Dhar), 544
- Nitrogen (Isotopic) : Distribution of, in Nitrogen Gas during Denitrification (R. D. Hauck and D. R. Bouldin), 871
- Nitrogen (Urine), in Kwashiorkor : Partition of (Dr. J. C. Edozien and E. J. Phillips), 47
- Nitrogen Atoms (Bridgehead) : Heterocyclic Systems with (William L. Mosby), Part I, *review* by Dr. G. L. Buchanan, 425
- Nitrogen Atoms (Metastable) : New Afterglow Emission associated with (L. Herman and R. Herman), 346
- Nitrogen Fixation (Symbiotic) : Root Temperature and (A. H. Gibson), 1080
- Nitrogen Gas : Distribution of Isotopic Nitrogen in, during Denitrification (R. D. Hauck and D. R. Bouldin), 871
- Nitrogen Level : Effect of Spacing and, on Floral Initiation in Cocksfoot (*Dactylis glomerata* L.), (D. M. Calder and J. P. Cooper), 195
- Nitrogen Problem : The, 544
- Nitrogen-fixing Micro-organism (New) : Chemical Character of the Pigments of a (Gy. Németh, F. Uresch, Prof. G. Fodor and L. Láng), 1413
- Nitrous Acid-induced Mutations (X-Ray- and), in the Genetic Fine Structure of the ad_7 Locus of *Schizosaccharomyces pombe* : Distribution of (Dr. Herbert Gutz), 1127
- Nixon, Dr. D. A. (Alexander, Pauline, and) : Foetal and Neonatal Physiology (The Foetal Kidney), 344
- Nixon, Dr. D. A., and others : Fructose and Lactic Acid Metabolism in the Perfused Liver of Premature Lambs, 1307
- Nixon, Dr. W. C. (Cosslett, Dr. V. E., and) : X-Ray Microscopy, *review* by Prof. Arne Engström, 1332
- Nodal Analysis of Associated Populations (Prof. W. T. Williams and J. M. Lambert), 202
- Nodules : Leguminous Root (Gibberellin-like Substances in Plants), (Margaret Radley), 684
- Noise Bursts (Solar) : Very-Low-Frequency Modulation of Discrete Frequency (Dr. Jules Aarons, Santimay Basu, William Kidd and Richard Allen), 56
- Nolte, H. W. : Nematology (Temporary Benefit from 'Vapam' against *Pratylenchus* in Pine Nurseries), 1049
- Nomenclature of Silica (I. Bergman), 270
- Nomograms : A Collection of Tables and, for the Processing of Observations made on Artificial Earth Satellites (I. D. Zhongolovich and V. M. Amelin), *review* by D. G. King-Hele, 1135
- Non-Aquatic Reptiles : Swimming Ability of (R. R. Tercafs), 125
- Non-Enzymic Hydroxylation of Polycyclic Aromatic Hydrocarbons (F. Dewhurst and G. Calcutt), 808
- Non-Ferrous Metals Industry in a Unified Europe (Baron Snoy et d'Oppuers), 546
- Non-Lethal Pathological Infection of Roots (F. J. Newhook), 615
- Non-Linear Physical Problems (Indian Society of Theoretical and Applied Mechanics and Unesco Symposium on), 855
- Non-Linear Programming (Linear and) : Methods of Feasible Directions—a Study in (Dr. G. Zoutendijk), *review* by Dr. M. V. Wilkes, 9
- Non-Photosynthetic Plant Tissue : Triphosphopyridine Nucleotide-linked Glyceraldehyde Phosphate Dehydrogenase in a (C. R. Benedict and Dr. H. Beevers), 71
- Non-Random Occurrence of Amino-Acid Replacements (C. R. Woese), 1196
- Non-Randomness in Amino-Acid 'Alleles' (Richard V. Eck), 1284
- Non-Specific Immunity in Pneumococcal Infection of Mice (Dr. G. N. Cooper and Dr. A. E. Stuart), 295
- Non-Specific Resistance (Prolonged), to Sarcoma Implant and Staphylococcus Infection following Repository Injection of Lipopolysaccharide: Development in Mice of (Samuel J. Prigal), 1111
- Non-Specific Staining Components from Fluorescein-labelled Conjugates : Simplified Procedure for removing (G. D. Johnson), 70
- Non-Tuberculous Conditions (Tuberculous and) : Mycobacteriophages isolated from Persons with (Dr. E. Mankiewicz), 1416
- Non-Uniformity of Cross-linking in Ion-Exchange Polymers (Dr. E. Glueckauf and R. E. Watts), 904
- Nonsense Syllables : Stimulus-Response Compatibility in the Paired-Associate Learning of (A. D. Baddeley), 1327
- Nonweiler, Dr. T. R. F. : Appointed to the Mechan chair of aeronautics and fluid mechanics in the University of Glasgow, 853 ; work of, 853
  - Space-Flight and Re-entry Trajectories (Dynamics of Terminal Re-entry —Errors in Re-entry Guidance), 960
- (–)-Noradrenaline : High Concentration of, in *Portulaca oleracea* L. (Dr. P. C. Feng, Prof. L. J. Haynes and Dr. K. E. Magnus), 1108
- Nordhagen, Prof. R. : Flora Europaea, 447
- Norepinephrine (Isotopic) : Effects of Drugs on Uptake of, by Cat Tissues (Hans J. Dengler, Herbert E. Spiegel and Elwood O. Titus), 816
- Norfolk Broads : Status of the, 751
- Normal Adult Rats : Stimulation by Heparin of Parenchymal Liver Cell Proliferation in (Dr. M. Zimmerman and Evemarie Celozzi), 1014
- Normal Feeding Conditions : Soluble Material in the Gastro-intestinal Tract of Rats under (Dr. Anne S. Cole), 502
- Normal Growth : Spontaneous Development of *L* Forms of Streptococci requiring Secretions of other Bacteria or Sulphydryl Compounds for (Ayala Frenkel and Dr. Willy Hirsch), 728
- Normal Human Urine : Quantitative Determination of Metadrenaline and Normetadrenaline in (Dr. Kaoru Yoshinaga, Dr. Chuichi Itoh, Dr. Nozomu Ishida, Dr. Tatsuo Sato and Dr. Yasuo Wada), 599
- Normal Probability Curves (Isolated or Overlapping) : Calculation of the Areas of (Dr. D. Morison Smith and John C. Bartlet), 688
- Normal Rabbits : Effects of Injections of some Intermediary Metabolites on the 'Insulin Response' of (Dr. H. D. Brahmachari and G. Raghupathy Sarma), 491, *erratum*, 755
- Normal Rat Hepatic Cells : Nucleolar Localization of Succinic Dehydrogenase in (P. De, R. Chatterjee and Dr. S. Mitra), 925
- Normal Tissues (Irradiated) : Effect of, on the Growth of Mouse Carcinoma (Dr. Arthur Compton and Prof. Charles A. Pannett), 1101
- Norman, A. G. (edited by) : Advances in Agronomy, Vol. 12, *review* by G. V. Dyke, 213
- Normetadrenaline in Normal Human Urine : Quantitative Determination of Metadreanaline and (Dr. Kaoru Yoshinaga, Dr. Chuichi Itoh, Dr. Nozomu Ishida, Dr. Tatsuo Sato and Dr. Yasuo Wada), 599
- Norreys, J. J. (Brown, Allan, and) : Uranium Disilicide, 61
- Norrish, Prof. R. G. W. : Chemistry and the Spectroscope (Presidential Address to Section B (Chemistry) of the British Association for the Advancement of Science), 984
- North, Dr. A. C. T., and Rich, Prof. Alexander : X-Ray Diffraction Studies of Bacterial Viruses, 1242
- North : Problems of the (Translation of *Problemy Severa*), No. 1, 1958 (translated December 1960), *review* by Dr. Terence Armstrong, 841
- North Atlantic : The (The Floors of the Oceans, I, Text to accompany the Physiographic Diagram of the North Atlantic), (Bruce C. Heezen, Marie Tharp and Maurice Ewing), (Geological Society of America, Special Paper 65), *review* by Dr. G. E. R. Deacon, 531
- North Borneo, Government of (Royal Society of London in co-operation with the) : North Borneo Expedition 1961, 26
- North Borneo Expedition 1961 (Royal Society of London in co-operation with the Government of North Borneo), 26
- North Sea : *Pneumodermopsis paucidens* in the (G. A. Cooper and D. C. T. Forsyth), 1412
- North-Eastern Africa (Eastern and) : Birds of (C. W. Mackworth-Praed and C. H. B. Grant), second edition, *review* by Sir Landsborough Thomson, 9
- Northern Apennines : Structure of the (Sir Edward Bailey and Prof. W. J. McCallien), 1136
- Northern Ireland : Aids to Agriculture and Horticulture in, 959
- Northern Rhodesia : Blindness in Africans in (Dr. C. M. Phillips), 753
  - Mammals of (W. F. H. Ansell), *review* by R. W. Hayman, 8
- Norton, Dr. Francis J. : Permeation of Gaseous Oxygen through Vitreous Silica, 701
- Norwegians : Radiocaesium and Potassium in (Johan Baarli, Kjell Madshus, Kurt Lidén and Richard C. McCall), 436
- Norwich Meeting of the British Association for the Advancement of Science (D. D. Aldridge), 16
- Norwich and its Region (British Association for the Advancement of Science), 956
- *Notopterus* (Lacepede) : Excretory Ducts in the Genus (Madhu Sudan Sharma), 1118
- Nour-Eldin, F. : Stability of Freeze-dried Anti-haemophilic Globulin, 503
- Novel Acid-stable Penicillins : Some (F. P. Doyle, J. H. C. Nayler, H. Smith and E. R. Stove), 1091
- Novotny, Dr. Ivan, and Vyskočil, František : Inhibition by Physostigmine of the Increase of Oxygen Consumption induced by Potassium in Muscle, 916
- Nowak, H. F., and Kucharski, J. : Effect of Polyvinyl Alcohol on the Distribution of Iodine-131 in the Internal Organs, Fluids and Excreta of Rabbits, 665, *erratum*, 1045
- Nuclear Detonations : Iron-55 from (Rama, Minoru Koide and Prof. Edward D. Goldberg), 162
- Nuclear Energy : Practical Applications of (International Atomic Energy Agency Courses on), 1143
  - United Kingdom Courses in, 125
- Nuclear Energy Agency (European), of the Organization for European Economic Co-operation : New Trends in Atomic Research and their International Significance (Dr. L. Kowarski), 853

Nuclear Energy Industry : Corrosion in the (Société de Chimie Industrielle Symposium on), 651
Nuclear Energy Study Commission (Laboratory of Mass Spectrometry), Lisbon : Grant from the Gulbenkian Foundation, 754
Nuclear Equipment in Argentina, Brazil and Israel : Grants from the United States to the International Atomic Energy Agency for, 442
Nuclear Explosions : Carbon-14 from, as a Short-term Dating System—Use to determine the Origin of Heartwood (Dr. A. T. Wilson), 714
Nuclear Fission Research (Controlled) : Plasma Physics and (International Atomic Energy Agency International Conference on), 443
Nuclear Medicine : Fellowships and Grants in the field of Radiology and, 1962-63 (James Picker Foundation), 228
Nuclear Particle Detector (Silicon Semiconductor) : Interaction of 14-MeV. Neutrons with a (Dr. W. M. Deuchars and G. P. Lawrence), 995
Nuclear Physics : Atomic and (Prof. Robert S. Shankland), second edition, *review* by Dr. E. B. Paul, 8
  High Energy (Institute of Physics and the Physical Society Symposium on), 1349
Nuclear Physics in Australia : Future of (Prof. E. W. Titterton), 639
Nuclear Power Projects (United States) : Fact Sheets on, third edition, 958
Nuclear Reactor Calculations : Digital Computers and (Ward C. Sangren), *review* by Dr. M. V. Wilkes, 9
Nuclear Reactor Chemistry (Oak Ridge National Laboratory Second Conference on), 651
Nuclear Reactor Technology : Analytical Chemistry in (Oak Ridge National Laboratory Fifth Conference on), 651
Nuclear Research, European Organization for, and the Max Planck Institute, Heidelberg : Conference on Fission and Spallation Phenomena and their Application to Cosmic Rays, 24
Nuclear Science, National Institute for Research in (Harwell) : Successful Operation of the *Nimrod* Injector, 1043
Nuclear Studies (British Council), 125
Nuclear Studies (Liverpool College of Technology Postgraduate Courses in), 338
Nucleators (Ice) : Steroids as (Richard B. Head), 1058
Nuclei dividing Amitotically : Deoxyribonucleic Acid Content of (Prof. K. W. Cleland), 504
Nuclei (Anti-Leucoyte), and Anti-Platelets (Anti-Leucocyte Cytoplasm,), in Disseminated Lupus Erythematosis (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Nucleic Acid (Pentose–) Content of Sub-Cellular Fractions of the Brain of the Rat during 'Metrazol' Convulsions : Changes in the (Prof. G. P. Talwar, B. Sadasividu and V. S. Chitre), 1007
Nucleic Acids : The (edited by Erwin Chargaff and J. N. Davidson), Vol. 3, *review* by Dr. Roy Markham, 742
  Mechanism of Differential Staining of (D. J. Goldstein), 407
  Still More, *review* by Dr. Roy Markham, 742
Nucleolar Localization of Succinic Dehydrogenase in Normal Rat Hepatic Cells (P. De, R. Chatterjee and Dr. S. Mitra), 925
Nucleolus : Deoxyribonucleic Acid Structures in the (Dr. Elsa H. J. O'Donnell), 1325
Nucleosides (Tritiated Pyrimidine-) : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of, into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Nucleotide (Reduced Diphosphopyridine) : An Inhibitor of Liver Alcohol Dehydrogenase in Preparations of (Dr. Keith Dalziel), 1098
Nucleotide-linked (Triphosphopyridine) Glyceraldehyde Phosphate Dehydrogenase in a Non-Photosynthetic Plant Tissue (C. R. Benedict and Dr. H. Beevers), 71
Nucleotides : Failure of, to Diffuse Freely into the Embryo of *Pteridium aquilinum* (P. R. Bell), 91
Nucleotides (Pyridine), in the Liver and in an Ascitic Hepatoma : Comparison of (M. Wintzerith N. Klein, L. Mandel and Prof. P. Mandel), 467
Nucleotides and Amino Acids : The Neurochemistry of—a Symposium of the Section on Neurochemistry, American Academy of Neurology (edited by Roscoe O. Brady and Donald B. Tower), *review* by Dr. R. V. Coxon, 318
Nucleus (The Feulgen-Stained Somatic Cell), as a Homogeneously Absorbing Object (Dr. Simon Iversen), 150
Nuffield Foundation : Sixteenth Annual Report (1960-61), 1148
Nuffield Foundation (Royal Society and): Commonwealth Bursaries Scheme, Awards, 126
Nuffield Unit of Tropical Animal Ecology : Appointment of Dr. R. M. Laws as senior investigator, 651
Number of Testis Follicles in Miridae (Dennis Leston), 93
Number and Arrangement of Osteons in Corresponding Regions of Homotypic Long Bones (Dr. G. Marotti), 1400
Number and Concentration (Lesion) : Relationship of Tobacco Mosaic Virus (TMV), to the Rate of Lesion Production on Pinto Bean (John Piacitelli and Prof. Vincent Santilli), 624
Numbers (Oocyte), as a Diagnostic Character for the Identification of Ammocoete Species (Dr. M. W. Hardisty), 1215
Numbers and Types of Bacteria from the Rhizosphere of Pasture Plants—Possible Occurrence of *L*-Forms (G. Jagnow), 1220
Nutrition : Current Problems in (Nutrition Society of Canada Symposium on), 335
  Fish in (Food and Agriculture Organization of the United Nations International Conference on), 1045
  Ruminant Digestion and, *review* by Dr. I. W. McDonald, 1334
Nutrition in Latin America (Food and Agriculture Organization of the United Nations Meetings on), 959
Nutrition of the Ruminant : Digestive Physiology and—Proceedings of the University of Nottingham Seventh Easter School in Agricultural Science, 1960 (edited by Dr. D. Lewis), *review* by Dr. I. W. McDonald, 1334
Nutrition Society : Symposium on Nutrition and Metabolic Defects, 125
  Symposium on Agricultural Problems in Developing Countries, 1250
Nutrition Society (Scottish Group) : Symposium on Nutrition and Poultry, 856
Nutrition Society of Canada : Election of Officers, 335
  Borden Award awarded to Dr. Donald Fraser, 335
  Symposium on Current Problems in Nutrition, 335
Nutrition and Metabolic Defects (Nutrition Society Symposium on), 125
Nutrition and Poultry (Scottish Branch of the Nutrition Society Symposium on), 856
Nutritionally Exacting Strains of *Staphylococcus pyogenes* : Metabolic and Genetic Investigations of (Janice B. Edgar and Dr. B. A. D. Stocker) 1121
Nutt, C. W., and Biddlestone, A. J. : A Molecular Beam Reactor for Examining the Rate, and Primary Products, of Reactive Bimolecular Collisions, 798
Nutt, Dr. M. E. : Some Aspects of Haematology, *review*, 7
Nutting, Prof. Jack : Awarded a Gold Medal and Prize from the Beilby Memorial Fund, 224 ; work of, 224
Nyman, Bengt : Effect of Red and Far-red Irradiation on the Germination Process in Seeds of *Pinus sylvestris* L., 1219

OAK Ridge National Laboratory : Second Conference on Nuclear Reactor Chemistry, 651
  Fifth Conference on Analytical Chemistry in Nuclear Reactor Technology, 651
Oakley, Dr. K. P. : Dating Man's Emergence (Presidential Address to Section H (Anthropology) of the British Association for the Advancement of Science), 988
Oat Seedlings (Etiolated) : Carbon Dioxide and Ethanol as Factors controlling the Growth of (Dr. C. L. Mer), 260
Oats : Isolation of a New Antioxidant from (Dr. D. G. H. Daniels and H. F. Martin), 1302
  Manganese and Magnesium in the Grey Speck Syndrome of (P. B. Vose and Dr. D. J. Griffiths), 299
Objects (Stationary or Moving) : Measuring Visual Constancy for (S. M. Anstis, C. D. Shopland and R. L. Gregory), 416
O'Brien, Prof. D., and others : Amino-acid Composition of Serum Mucoprotein in Rheumatic Fever, 922
Observation (Direct), of Nematode-trapping Fungi in the Soil : Agar Disk Method for the (Roderic C. Cooke), 1411
Observations made on Artificial Earth Satellites : A Collection of Tables and Nomograms for the Processing of (I. D. Zhongolovich and V. M. Amelin), *review* by D. G. King-Hele, 1135
Observations (Electron Microscope), of the Receptors in the Sensory Vesicle of the Ascidian Tadpole (Noël Dilly), 786
Observations (Electronic Microscopic), of Structure in Ultra-Thin Sections of Vitrinite in Coal (J. T. McCartney, Sabri Ergun and R. E. Walline), 1361
Observations (Further), on a Filamentous Saprophyte from Wookey Hole Caves (Dr. A. J. Willis), 724
Observations (Immunological), in Experimental Silicosis (J. H. Jones and Prof. A. G. Heppleston), 1212
Observations (Trans-Equatorial Backscatter), of Magnetically Controlled Ionization (J. A. Thomas), 792
Observatory (Lamont Geological) : Fellowships for Plankton Research, 754
Observatory (Naval), United States : Reprint No. 19, Resources and Treasures of the Library (Marjorie S. Clopine), 22
Obtaining Minimum Time in Gas Chromatography : Role of Pressure Gradients in (Prof. J. Calvin Giddings), 1291
Occultation (Lunar), of a Radio Source (Dr. C. Hazard), 58
Occupational Hygiene Society, British (Ergonomics Research Society and the) : Discussion on the Design and Use of Respirators, 655
Occurrence of Ascarylose : Dideoxysugars of *Pasteurella pseudotuberculosis*-specific Polysaccharides, and the (Dr. D. A. L. Davies), 43
Occurrence of *Eutrichocheles modestus* (Herbst) in the Near-shore Waters of Porto Novo, South India (K. Balasubrahmanyan and Dr. J. Jacob), 830
Occurrence of Galactolipids : Lipids of the Common Pea (*Pisum sativum* L.) with special reference to the (S. Adhikari, Dr. F. B. Shorland and R. O. Weenink), 1301
Occurrence of Mevalonic Acid in Carrots (Prof. V. V. Modi and D. K. Patwa), 1202
Occurrence of an *Rh*-like Antigen in Red Cells of the Sheep (B. Urbaschek and Prof. K. Trautwein), 193
Occurrence of *Salmonellae* in Domestic Animals and Poultry in India (V. K. Sharma and Prof. C. M. Singh), 622
Occurrence (Non-Random), of Amino-Acid Replacements (C. R. Woese), 1196
Occurrence (Possible), of *L*-Forms : Numbers and Types of Bacteria from the Rhizosphere of Pasture Plants— (G. Jagnow), 1220
Ocean Currents : Dispersal of Tropical Seeds by (R. Mason), 408
Ocean Floor : The, *review* by Dr. G. E. R. Deacon, 531
Oceanography : Research in Marine Biology or (Challenger Society Grants for), 24
Oceans : The Floors of the, 1, The North Atlantic—Text to accompany the Physiographic Diagram of the North Atlantic (Bruce C. Heezen, Marie Tharp and Maurice Ewing), (Geological Society of America, Special Paper 65), *review* by Dr. G. E. R. Deacon, 531
O'Connor, Cecilia M. (edited by G. E. W. Wolstenholme and) : Ciba Foundation Symposium on Quinones in Electron Transport, *review* by A. I. Scott, 321
O'Donnell, Dr. Elsa H. J. : Deoxyribonucleic Acid Structures in the Nucleolus, 1325
Odontosyllis phosphorea Moore (Polychaete), var. *Nanaimoensis* Berkeley : Swarming of the, near Nanaimo, B.C. (E. Berkeley), 1321
Odoratism (Lathyrism) : Influence of Scurvy and, on Hydroxyproline Excretion (Dr. G. R. Martin, S. E. Mergenhagen and D. J. Prockop), 1008
Odour Receptors (M. H. Briggs and R. B. Duncan), 1310
Odqvist, Prof. F. K. G. : Engineering Theories of Creep (Presidential Address at the Symposium at the University of Roorkee on Non-Linear Physical Problems), 855
Œstriol Succinate : Treatment of Thrombocytopaenic Haemorrhage with (Dr. Hubert Poliwoda), 400
Ofodile, A., and others : Effect of Alpha-Amylase on the Ehrlich Mouse Ascites Tumour, 1113
Ogram, G. R. : Appointed a Research Fellow in the Department of Industrial Metallurgy, University of Birmingham, 337
Ohshima, S., and others : Inhibition of β-Glucoronidase by Oral Administration of D-Glucosaccharo-1,4-Lactone, 1298

Oil (Essential), from Fresh *Cannabis sativa* and its Use in Identification (L. Martin, Dr. D. Morison Smith and C. G. Farmilo), 774
Oil Consumption in the United Kingdom during 1960, 123
Oil and Sulphide Ore : Black Shale, (Prof. K. C. Dunham), 985
Oilseeds Committee (Indian Central) : Castorseed and its Products, 753
Oil-well Drilling Mud-Thinners : Sub-bituminous Coals as, 1144
Oka, M., and others : Potentiation of Epinephrine Action, 186
Okada, Minoru : Rapid Determination of Ytterbium in Aqueous Solutions and in Thulium Oxide by Neutron Activation, 1090
Okamura, Dr. S. : Effects of Ionizing Radiation on Organic and Biological Solids (Reactions in the Solid State), 1150
Okinaka, Prof. Shigeo, and others : Effect of Bilateral Section of the Splanchnic Nerve on Erythropoiesis, 500
Okuda, Jun, and others : Hydrolysis of Fatty Acid Esters on Riboflavin by Pancreatic Lipase, 174
Olbromski, J., and others : Conversion of Fibrin to Desmofibrin, 1093
Olcott, H. S., and others : Selenomethionine as an Antioxidant, 1201
Old (Growing), in a Mechanized World (F. Le Gros Clark), *review* by Prof. L. S. Hearnshaw, 532
Old Sea-Water : A Lake in British Columbia containing (Dr. P. M. Williams, Prof. W. H. Matthews and Prof. G. L. Pickard), 830
Old Smyrna : Archaeology of, *review* by Dr. Hugh Plommer, 212
Oldfield, Prof. R. C. : *Obituary* of Prof. A. W. P. Wolters, 646
Olduvai : The Juvenile Mandible from (Dr. L. S. B. Leakey), 417
Olduvai Gorge, Tanganyika : Age of Bed I (Dr. L. S. B. Leakey, Dr. J. F. Evernden and Dr. G. H. Curtis), 478
Oliver Memorial Fund : Award for Blood Transfusion work, 336
Ollerenshaw, C. B. : Parasite Biology (Importance of Temperature and Moisture-Levels on Different Stages in the Life-Cycle of *Fasciola hepatica*), 134
Ollis, Dr. W. D. : Stereochemistry of Natural Phenolic Compounds (Relative Configuration of the Rotenoids), 236
Ollivier, C., and others : Electrophoresis in a Thin Layer of Starch-Gel followed by a Plastifying Treatment, 72
Omenn, Gilbert (Blumer, Max, and) : Fossil Chlorins in a Triassic Sediment, 161
Ommatokoita elongata Grant (Copepod), in the Eyes of the Greenland Shark—a Possible Cause of Mutual Dependence (Bjørn Berland), 829
Omori, Tomoo, and others : A Method for Isolating Bacteria capable of Producing 6-Aminopenicillanic Acid from Benzylpenicillin, 1122
Oncogenic and Heparin-neutralizing Properties : Surfen, a Quinoline with (DeWitt, T. Hunter, jun., and Joseph M. Hill), 1378
Oncorhynchus keta (Chum Salmon) : Effect of Thyroxine plus Thiourea on the Early Development of the (Prof. M. A. Ali), 1214
One-Degree Orientated Substrates : Epitaxial Growth of Crystals on (Dr. A. Goswami), 160
One Ion-Exchange Column during Preparation : A Simplified Method for Separating Amino-Acid Mixtures from Protein Hydrolysates on (J. Liebster, K. Kopoldová and M. Dobiášová), 1198
Onions, W. J. : Testing Textiles, *review*, 106
Ontario Museum (Royal), Toronto : Appointment of Dr. W. E. Swinton as head of the Division of Life Sciences, 24
Ontario Research Foundation : Annual Report for 1960, 1254
Ontogeny of the Rat : Role of the Lung in Metabolism of Fat during (Dr. Z. Vacek, P. Hahn and O. Koldovsky), 85
Onuki, Y. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Effect of Radiation on the Chromosomes of Leucocytes cultured from the Human Buffy Coat), 340
Oocyte Numbers as a Diagnostic Character for the Identification of Ammocoete Species (Dr. M. W. Hardisty), 1215
Oogonium and Antheridium of *Pythium debaryanum* Hesse : Meiosis in the (Dr. Eva Sansome), 827
Oomura, Dr. Yutaka, and others : Electrical Activity of a Giant Nerve Cell under Abnormal Conditions, 1265
Oostenbrink, M. : Nematology (Nematode Populations in Meadows and Arable Land in the Netherlands), 1048
Oosthuizen, J. C. (Blair, Dr. G. W. Scott, and) : An Invariant Casein Substrate for Rennet Standardization, derivable from Diverse Sources of Cows' Milk, 697
Oota, Yukito, and others : Sub-Unit of Ribosomal Ribonucleic Acid from Yeast, 598
Operations Research and Systems Engineering (Charles D. Flagle, William H. Huggins and Robert H. Roy), *review* by Prof. L. S. Goddard, 105
Opossum, *Didelphis virginiana* : Cholesterol in the Tapetum Lucidum of the Eye of the (Antoinette Pirie), 708
Oppenheimer, Prof. H. R. : Plant Exploration and Introduction (The Origin of Cultivated Wheat), 1240
Optical Character Recognition (Information Systems Branch of the Office of Naval Research Information Centre of the United States National Bureau of Standards Symposium on), 1145
Optical Filter in the Far Ultra-Violet : Irradiated Lithium Fluoride as an (J. L. Weeks, S. Gordon and G. M. A. C. Meaburn), 1186
Optical Lens Performance : Some Aspects of, Part 1, Secondary Flare in Lenses (K. Hacking) ; Part 2, The Design of Lens Hoods (W. N. Sproson), 1355
Optical Maser : Use of a Different Type of Fluorescent Material as an (Jordan J. Markham and Dickron Mergerian), 586
Optical Masers : A Proposal for a Tunable Source of Radiation for the Far Infra-Red using Beats between (Dr. D. C. Lainé), 795
Optics (Fibre) : A Hypodermic Probe using (David F. Capellaro, Narinder S. Kapany and Dr. Charles Long), 927
Oral Administration of D-Glucosaccharo-1,4-Lactone : Inhibition of β-Glucuronidase by (S. Akamatsu, Dr. A. Kiyomoto, S. Harigaya and S. Ohshima), 1298
Orally : Failure of a Dog to Discriminate between Strontium-90 and Calcium given (Dr. Betsy J. Stover, M. Goldman and A. C. Andersen), 713
Orbits : Satellite and Space, *review* by D. G. King-Hele, 1334
Orbits (Satellite) : Density of the Upper Atmosphere determined by, and its Variation during 1957-61 (D. G. King-Hele and D. M. C. Walker), 114
Orchid Conference (sponsored by the American Orchid Society, the British Orchid Growers' Association and the Royal Horticultural Society, London, 1960) : Proceedings of the Third World, *review* by V. S. Summerhayes, 213
Orchid Growers' Association (British), and others : Third World Orchid Conference, London, 1960—Proceedings of the, *review* by V. S. Summerhayes, 213
Orchid Society (American), and others : Third World Orchid Conference, London, 1960—Proceedings of the, *review* by V. S. Summerhayes, 213
Ord-Smith, Dr. R. J. : Digital Computers, their Use and Control (Computer Applications), 554
Ordnance Survey of Great Britain : Recent Map Publications by the, 958
Ore (Sulphide) : Black Shale, Oil and (Prof. K. C. Dunham), 985
Organ Cultures (Animal Cell, Tissue and), in Radiobiology : Use of (New York Academy of Sciences Symposium on), 340
Organic Analysis (edited by John Mitchell, Jr., I. M. Kolthoff, E. S. Proskauer and A. Weissberger), Vol. 4, *review* by Dr. D. T. Lewis, 741
Organic Chemistry : Physical Methods of (edited by Arnold Weissberger), third edition, Parts 3 and 4, *review* by Dr. W. A. Waters, 317
Organic Compounds : Analysis of, *review* by Dr. D. T. Lewis, 741
  'Gel Filtration' of (B. Cortis-Jones), 272
  Radiation Chemistry of (Dr. A. J. Swallow), *review* by Dr. S. J. Thomson, 317
Organic Constituents of Meteorites (Michael H. Briggs), 1137
Organic Electronic Spectral Data : Vol. 1, 1946-1952 (edited by Mortimer J. Kamlet) ; Vol. 2, 1953-1955 (edited by Herbert E. Ungnade), *review* by Dr. E. G. Kellett, 107
Organic Geochemistry Group (European) : Proposal to form a, 336
Organic Phosphate (Inorganic and) : Outflux of, during Membrane Depolarization of Excitable Tissues (Dr. L. G. Abood, Prof. K. Koketsu and I. Koyama), 395
Organic Reagents in Inorganic Analysis : The Use of (A. I. Busev and N. G. Polianskii), translated from the Russian by J. T. Greaves, *review* by Dr. D. T. Lewis, 1336
Organic Substances : Inhibition of Ultrasonic Decolorization of Rhodamine *B* in Presence of (Prof. Satya Prakash and Sheo Prakash), 1292
Organic Vapours : Measurements of Heats of Adsorption of, on Adsorbents of High Surface Area using a Continuous Flow Calorimeter (G. H. Bell and A. J. Groszek), 1184
Organic and Biological Solids : Effects of Ionizing Radiation on (Société de Chimie Physique and the British Biophysical Society Discussion on), 1150
Organischen Fluorverbindungen : Chemie der (Milos Hudlicky), *review* by Dr. Richard Dresdner, 319
Organisms (Different) : Comparative Analysis of Inactivating Efficiency of Radiation on (Dr. M. Terzi), 461
Organisms (Living) : Atomic Radiations and (R. H. Mole), 556
Organisms (Penicillinase-producing) : Use of Cellulose Acetate Membranes for detecting (Prof. R. Knox and J. T. Smith), 926
Organisms (Pleuropneumonia-like), isolated from Tissue Cell Cultures : Immunofluorescence of (Major W. F. Malizia, N. F. Barile and D. B. Riggs), 190
Organization for European Economic Co-operation : Report on the School Curriculum in Mathematics (edited by Howard Fehr), 335
Organization for European Economic Co-operation (European Nuclear Energy Agency) : New Trends in Atomic Research and their International Significance (Dr. L. Kowarski), 853
Organization (National), of Scientific Research, 632
Organization and Methods (Aims,), of University Extra-Mural Libraries in Great Britain (E. P. Pritchard), 649
Organo-Metal Compounds, *review* by Dr. D. S. Payne, 316
Organo-Metallic Compounds (Prof. G. E. Coates), second edition, *review* by Dr. D. S. Payne, 316
Organo-Phosphorus Chemistry : Russian Work in, *review* by Dr. F. G. Mann, 1232
Organo-Phosphorus Compounds : Soviet Research on, 1949-1956 (in English translation), *review* by Dr. F. G. Mann, 1232
Organs (Accessory Respiratory), in Air-breathing Fishes : A Hydrostatic Function of the (Dr. S. Z. Qasim and R. Hasan), 396
Organs (Internal), Fluids and Excreta of Rabbits : Effect of Polyvinyl Alcohol on the Distribution of Iodine-131 in the (H. F. Nowak and J. Kucharski), 665, *erratum*, 1045
Organs (Sense), Integration and Behaviour (edited by Talbot H. Waterman), (The Physiology of Crustacea, Vol. 2), *review* by Dr. J. Green, 428
Orgel, Dr. L. E. : An Introduction to Transition-Metal Chemistry—Ligand-Field Theory, *review* by Prof. C. A. Coulson, 1233
Orgel, Dr. L. E. (Massey, A. G., and) : A Reversible Photochromic Polymer, 1387
Oriental and African Studies, School of : Grant of £15,000 from the Gulbenkian Foundation, 754
Orientated Lipid on a Biological Membrane : Electrical Properties of—an Electrostatic Diffusion Barrier and Ion Pump (Dr. J. W. L. Beament), 217
Orientated Substrates (One-Degree) : Epitaxial Growth of Crystals on (Dr. A. Goswami), 160
Origin of the British Insular Races of Small Mammals and of the 'Lusitanian' Fauna (G. B. Corbet), 1037
Origin of Heartwood : Carbon-14 from Nuclear Explosions as a Short-term, Dating System—Use to determine the (Dr. A. T. Wilson), 714
Origin (Animal), Sea Cucumber : A New Triterpenoid Sapogenin of (Takao Matsuno and Toshihiko Yamanouchi), 75
Origins of Petroleum (Sir Robert Robinson), 346
Origins of War : Arms and Security, a Mathematical Study of the Causes and (Dr. Lewis F. Richardson), edited by Nicolas Rashevsky and Ernesto Trucco, *review* by Sir Cyril Burt), 427
Orlowski, Dr. Marian, and others : Histochemical Demonstration of Gamma-Glutamyl Transpeptidase, 767
Ormerod, M. G. : Effects of Ionizing Radiation on Organic and Biological Solids (Sulphur Compounds as Protective Agents), 1151
Ormerod, W. E. : Immunity to Protozoa (Development of Immunity in *Trypanosoma lewisi* Infections), 551
Oró, Prof. J. : Mechanism of Synthesis of Adenine from Hydrogen Cyanide under Possible Primitive Earth Conditions, 1193
Orogeny and Granites in South-West England : Age of the (T. R. Owen), 486 ; (N. E. Butcher), 486
Orthographic Atlas of the Moon (compiled by D. W. G. Arthur and E. A. Whitaker), edited by Gerard P. Kniper, Edition 'A' showing the Standard Orthographic Coordinate Grid, Part 1, Central Area, *review* by D. H. P. Jones, 424

Orthotitanate (Barium) : Infra-Red Spectroscopic Evidence of Four-fold Co-ordination of Titanium in (P. Tarte), 1002
Orthotopically Grafted Skin : Regeneration of Elements of Donor Origin in, following the Homograft Response (Dr. J. K. Dineen and Dr. Aleksander Szenberg), 153
Ortmeyer, Anne, and others : Sexual Differentiation in *Hydra* in relation to Population Density, 92
Osawa, Syozo, and others : Sub-Unit of Ribosomal Ribonucleic Acid from Yeast, 598
Oscillations (Sustained), in a Catalytic Chemical System (R. A. Spangler and F. M. Snell), 457
Osgood, F. E. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Effect of Radiation on Normal and Malignant Human Haemic Cells *in vivo* and *in vitro*), 340
Oshima, T., and others : Potential Changes produced by Application of Current Steps in Motoneurones, 1104
Osmoregulation : Thermodynamics and (Prof. Leonard B. Kirschner), 815
Osmotic (Chemi-) Type of Mechanism : Coupling of Phosphorylation to Electron and Hydrogen Transfer by a (Dr. Peter Mitchell), 144
Osol, Arthur, and others (edited by) : Remington's Practice of Pharmacy, twelfth edition, *review* by Dr. J. H. Gaddum, 8
Ossaka, J. : Tetragonal Mullite-like Phase from Co-precipitated Gels, 1000
Osteons : Number and Arrangement of, in Corresponding Regions of Homotypic Long Bones (Dr. G. Marotti), 1400
Oster, Prof. Gerald, and others : Photochemical Production of Semiconducting High Polymers, 164
Oster, Dr. Gisela K., and others : Photochemical Production of Semiconducting High Polymers, 164
Otaka, Eiko, and others : Sub-Unit of Ribosomal Ribonucleic Acid from Yeast, 598
Oteifa, A. B. : Nematology (The Chief Plant Nematodes of Crops in Egypt), 1048
Oteifa, B. A., and Elgindi, D. M. : Nematology (Radiotracer Techniques using Phosphorus-32 applied to a study of Galling in Tomatoes by *Meloidogyne javanica* and its Effect on Uptake of Nutrient), 1049
Othmer, Donald F. (edited by Raymond E. Kirk and) : Encyclopedia of Chemical Technology, second Supplement Volume edited by Anthony Standen, *review* by R. F. Villet, 1131
Outflux of Inorganic and Organic Phosphate during Membrane Depolarization of Excitable Tissues (Dr. L. G. Abood, Prof. K. Koketsu and I. Koyama), 395
Outline of Chemical Genetics (Prof. Bernard S. Strauss), *review* by Dr. Charlotte Auerbach, 841
Outward Bound Trust : Grant of £12,000 from the Gulbenkian Foundation, 855
Overlapping Normal Probability Curves (Isolated or) : Calculation of the Areas of (Dr. D. Morison Smith and John C. Bartlet), 688
Overseas Countries : Technical Assistance to (June 17, 1039), *errata*, 24
Oviposition in *Nepytia phantasmaria* Stkr. (Lepidoptera : Geometridae) : Influence of Electrical Field on Pupation and (Dr. D. K. Edwards), 976
Ovulation of the Spider *Latrodectus geometricus* C. Koch : Cycle and Rhythm in the (A. Bouillon and R. Lekie), 620
Ovum Humanum (Prof. Landrum B. Shettles), *review* by W. J. Hamilton, 110
Owen, David : Appointed head of the Department of Operational Research and Cybernetics of the United Steel Companies, Ltd., 443
Owen, Dr. R. D. : Blood Groups in Infra-human Species (Usefulness of Rats as Laboratory Animals), 653
Owen, Dr. R. D., and Anderson, Dr. D. R. : Blood Groups in Infra-human Species (A Study of Blood Groups in the Rhesus Monkey), 653
Owen, T. R. : Age of the Orogeny and Granites in South-West England, 486
Ox : Increase of Evaporative Loss of Water from the Skin of the, in response to Local Heating of the Hypothalamus (Dr. D. L. Ingram, J. A. McLean and Dr. G. C. Whittow), 81
Oxfordshire : Coal in (Dr. C. J. Stubblefield and Dr. F. M. Trotter), 953
Oxidase Activity (Indoleacetic Acid), of *Lupinus albus* L. exposed to Different Photoperiods : Effect of Gibberellic Acid on Growth and (F. Sági and A. S. Garay), 824
Oxidase (Monoamine) Inhibitor : Augmentation of Toxicity of, by Thyroid Feeding (Richard N. Carrier and Dr. Paul V. Buday), 1107
Oxidation of Carbon Monoxide in the Presence of Hydrogen: Selectivity of Palladium Catalysts for the (P. Mars and Th. van de Mond), 1003
Oxidation (Aniline), initiated by Ultra-sonic Waves (Dr. Wiktor Wawrzyczek), 701
Oxidation (Iron) : Morphological Variation in *Ferrobacillus ferrooxidans* related to the Rate of (M. P. Silverman and M. H. Rogoff), 1221
Oxidation (Radiolytic), of Ferrous Solutions with Standardized Internal Sources of Polonium-210 (J. Steyn and D. van As), 903
Oxidation-Reduction (Photo-sensitized) : Paths of Inter-Molecular Energy Transfer in (N. Takeyama), 1359
Oxidation-Reduction Reactions of Plastoquinone in Isolated Chloroplasts (Dr. E. R. Redfearn and J. Friend), 806
Oxidations (Pyrogallol) : Regulation of, by Alcohols and Sugars (B. Z. Siegel, S. M. Siegel and N. S. Goodman), 180
Oxidative Demethylation and Carcinogenicity of 4-N-Dimethylaminoazobenzene Derivatives (Prof. Marizo Ishidate and Akira Hanaki), 1198
Oxide (Barium) : Effect of Ultra-Violet Irradiation on the Electrical Conductivity of Cathodes coated with (Dr. C. H. B. Mee), 1383
Oxide (Beryllium) : Scattering of Long Wave-length Neutrons by Irradiated (T. M. Sabine, A. W. Pryor and B. S. Hickman), 1395
Oxide (Deuterium): Ultra-violet Absorption Spectra of Proteins in (Dr. G. H. Beaven and Elizabeth Bailey), 914
Oxide (Ethylene): Effect of, on the Tapping of *Hevea brasiliensis* (D. H. Taysum), 1319
Oxide (Nickel) : Porous (I. S. Solet), 64
Oxide (Thulium) : Rapid Determination of Ytterbium in Aqueous Solutions and in, by Neutron Activation (Minoru Okada), 1090
Oxide (Titanium) Ethoxides : Structures of Polymeric Titanium Ethoxide and (Prof. D. C. Bradley and A. H. Westlake), 273 ; (R. L. Martin and G. Winter), 274
Oxide Spinels : Dependence on Temperature of the Distribution of Cations in (Ranajit K. Datta and Prof. Rustum Roy), 169
Oxides (Zirconium and Hafnium) : Reduction of (F. K. McTaggart), 1192
Oximes : Inhibition of Chemi-Luminescence of Luminol by means of (Prof. K. Weber, J. Matković and D. Fleš), 177
Oxygen : Inhibition by, of Biosynthesis and Activity of Nitrate-Reductase in *Aerobacter aerogenes* (Francis Pichinoty and Liliane D'Ornano), 879
Oxygen (Gaseous) : Permeation of, through Vitreous Silica (Dr. Francis J. Norton), 701
Oxygen Consumption induced by Potassium in Muscle : Inhibition by Physostigmine of the Increase of (Dr. Ivan Novotny and František Vyskočil), 916
Oxygen Electrode for Measurement of Tissue Slice Respiration (E. H. Strickland, F. D. Ziegler and Prof. A. Anthony), 969
Oxygen Inhibition of Respiration in *Azotobacter* (M. J. Dilworth and C. A. Parker), 520
Oxytocic Peptides in the Pituitary of a Marine Teleost Fish (*Pollachius virens*): Pharmacological Characterization of the (Prof. H. Heller, B. T. Pickering, J. Maetz and F. Morel), 670
Oxytocinase Activity of Plasma of Pregnant Women (Stig E. J. Melander), 176
Ozaki, Sachio, and others : Electrical Activity of a Giant Nerve Cell under Abnormal Conditions, 1265
Ozone : Effect of, on the Fluorescence and Ultra-violet Absorption Spectra of 3,4-Benzopyrene (Z. Morlin and K. M. Sáringer), 907

PACE-MAKER of Respiration : Active Cation Transport as a (Dr. R. Whittam), 603
Paddock, N. L. : Inorganic Polymers (Phosphonitrilic Compounds), 1047
Padmini, P. R. K. L., and Rao, Prof. B. Ramachandra : Molar Sound Velocity in Molten Hydrated Salts, 694
Pain : The Assessment of (Universities Federation for Animal Welfare Symposium on), 1353
Painter, R. B. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Effects of Radiation on Cell Division), 341
Painter, Dr. T. J., and Morgan, Prof. W. T. J. : Partial Acid-Hydrolysis of a Mucopolysaccharide with Appreciable *N*-Deacetylation of Hexosamine, 39
Paired-Associate Learning of Nonsense Syllables : Stimulus-Response Compatibility in the (A. D. Baddelley), 1327
Pairs : Generation of Radicals in, and the Kinetics of Radical Chain Reactions in Solution (Dr. P. E. M. Allen and C. R. Patrick), 1194
Palaemonetes varians Leach (Prawn) : Mechanism of Sensitivity to Hydrostatic Pressure in the (Peter S. B. Digby), 366
Palaeocharinidae (Devonian) (Arachnida) : Lung-books in the (Dr. M. F. Claridge and A. G. Lyon), 1190
Palaeogeologic Maps (A. I. Leversen), *review* by Dr. C. Downie, 842
Paléus, Dr. Sven (Biörck, Prof. Gunnar, and) : Cytochrome *c* in the Heart of the Turtle, 712
Palladium Catalysts for the Oxidation of Carbon Monoxide in the Presence of Hydrogen : Selectivity of (P. Mars and Th. van de Mond), 1003
Palladium Hydride : Heat Capacity and Resistivity Anomalies in (Paul Mitacek, jun., and Prof. J. G. Aston), 271
Palm, Dr. J. : Blood Groups in Infra-human Species (Blood Groups and Histocompatibility Factors of Rats), 654
Palmer, Helen T. (Maxwell, Dr. D. R., and) : Demonstration of Anti-Depressant or Stimulant Properties of Imipramine in Experimental Animals, 84
Pancreatic Lipase : Hydrolysis of Fatty Acid Esters of Riboflavin by (Prof. Kunio Yagi, Yoshiko Yamamoto and Jun Okuda), 174
Pandalai, K. M. (Gangolly, S. R., and) : Possibilities of Vegetative Propagation in the Coconut (*Cocos nucifera* L.), 1218
Pangborn, Rose Marie, and Gee, Sandra C. : Relative Sweetness of α- and β-Forms of Selected Sugars, 810
Panigrahi, G., and Patnaik, S. N. : Cytology of some Genera of Polypodiaceae in Eastern India, 1207
Pannett, Prof. Charles A. (Compton, Dr. Arthur, and) : Effect of Irradiated Normal Tissues on the Growth of Mouse Carcinoma, 1101
Pantages, Peter (Langer, Stanley H., and) : Peak-Shift Technique in Gas–Liquid Chromatography—Trimethylsilyl Ether Derivatives of Alcohols, 141
Pantek, Henry R. : Brain Waves and 'Blood Waves', 577
Paoletti, P., and others : Effect of Serotonin on Absorption and Distribution of Calcium-45 in the Rat, 185
Paoletti, R., and others : Effect of Serotonin on Absorption and Distribution of Calcium-45 in the Rat, 185
Papée, Dr. Henry M., and Montefinale, Dr. Alberto : Chemical Composition of Precipitable Water Vapour over the United States, 136
Paper : Colorimetric Detection of Penicillins and Cephalosporins on (Robert Thomas), 1161
  The Formation and Structure of (Technical Section of the British Paper and Board Makers' Association Conference on), 126
  Pulp and—Chemistry and Chemical Technology (James P. Casey), Vol. 2, Papermaking, second edition, *review* by Dr. Julius Grant, 1135
  Splitting of Serum Albumin during Electrophoresis on (J. V. McLoughlin), 1305
Paper (Diethyl-amino-ethyl (DEAE) Cellulose) : Chromatography of Proteins on (C. C. Curtain), 1269
Paper Chromatograms (Filter) : A Spray Method for the Simultaneous Detection of Iodide and Iodinated Amino-Acids on (Dr. A. Van Zyl and M. Bhaga), 275
Paper Chromatography (Czech Chemical Society Conference on), 759
  Breakdown of [2-^{14}C]-Glycine during (A. K. Huggins and V. Moses), 668
  RM and Solvent Composition in (Dr. Edward Soczewiński), 68
Paper Electrophoresis : Demonstration by, of an Auto-Immunological Precipitin possibly due to Fibrinolysins (Marion I. Cooper and J. R. Sykes), 1212
Paper Electrophoresis of Proteins while Preserving the Pattern of their Localization in Tissues (Dr. Zh. A. Medvedev), 1021
Paper Manufacture (Pulp and) : A Laboratory Handbook of (Dr. Julius Grant), second edition, *review* by Dr. F. Lyth Hudson, 320
Paper and Board Makers' Association, British (Technical Section) : Conference on the Formation and Structure of Paper, 126
Papert, S. : Centrally Produced Geometrical Illusions, 733
Papilio dardanus (Lep.) : Genetics of a Difference in the Male Genitalia of East and West African Stocks of (J. R. G. Turner, C. A. Clarke and P. M. Sheppard), 935
Paramecium : Effects of Kinetin and Indole-3-acetic Acid on Multiplication Rates of (Mary Annunciata McManus and Kathleen Sullivan), 619

Paranchych, Dr. William (Cooper, Dr. Bernard A., and) : Selective Uptake of Specifically Bound Cobalt-58 Vitamin B_{12} by Human and Mouse Tumour Cells, 393
Parasite Biology (Parasitology Group of the Institute of Biology Meeting on), 134
Parasites : Malaysian (edited by W. W. Macdonald), 35-49 (Studies from the Institute for Medical Research, Federation of Malaya, No. 29), *review* by P. F. Mattingly, 948
Parasites (Ecto- and Endo), of South-East Asia, *review* by P. F. Mattingly, 948
Parasites (European), of Man, *review* by Dr. G. Lapage, 107
Parasitic Nematodes for Collection Purposes : Use of Glacial Acetic Acid for killing (Bjørn Berland), 1320
Parasitologie für Ärzte (Prof. Otto Jírovec), *review* by Dr. G. Lapage, 107
Parenchymal Liver Cell Proliferation in Normal Adult Rats : Stimulation by Heparin of (Dr. M. Zimmerman and Evemarie Celozzi), 1014
Parental Somatic Cells : Inclusion of, in Sponge Larvae (Frederick E. Warburton), 1317
Parenteral Administration of the Vitamin : Metabolites of Vitamin B_{12} in Bile after (Dr. John B. Stokes), 807
Park, Helen D., and others : Sexual Differentiation in *Hydra* in relation to Population Density, 92
Parker, C. A. (Dilworth, M. J., and) : Oxygen Inhibition of Respiration in *Azotobacter*, 520
Parkes, Dr. A. S. : Biological Control of Conception (Oliver Bird Lecture), 1256
Parks (National) Commission : Appointment of Colonel J. F. Williams-Wynne as a member, 546
Parkyn, Prof. D. G. : Analysis of Satellite Data, 896
Parliament : Recent Scientific Enquiries in, 857
Parr, Geoffrey : *Obituary* by T. H. Bridgewater, 332
Parra R., Joaquín, and others : New *Rh* Phenotype, *Dcce^{l}e^{i}f*, found in a Chibcha Indian Tribe, 503
Parry, Dr. W. E. : Thermodynamics with Quantum Statistical Illustrations, *review*, 1236
Parsons, F. M., and others : Elimination of Bovine Serum Albumin in Acute Experimental Uraemia, 1210
Parthenogenesis (Pathological), in a Viviparous Toothcarp (Prof. A. Stolk), 507
Partial Acid-Hydrolysis of a Mucopolysaccharide without Appreciable *N*-Deacetylation of Hexosamine (Dr. T. J. Painter and Prof. W. T. J. Morgan), 39
Particles : Elementary (CERN International Conference on), 126
  Wettability and the Capture, Carriage and Deposition of, by Raindrops (R. R. Davies), 616
Particles (Inhaled Radioactive) : Effects of—Report of the Subcommittee on Inhalation Hazards, Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 848), 556
Particles (Sub-Cellular) : Ethylene Evolution and (Prof. Stanley P. Burg and Ellen A. Burg), 967
α-Particles (Polonium-210) : Circumscribing Irradiation of Surface Structures of Cylindrical Animal and Plant Cells with (R. M. Bergström, R. F. Blåfield and M. W. Brenner), 1165
Partington, Prof. J. R. : Progress in Chemistry, *review*, 637
Partington, Prof. J. R. (Wheeler, Prof. T. S., and) : The Life and Work of William Higgins, Chemist (1763-1825), *review* by Frank Greenaway, 533
Partition of Urine Nitrogen in Kwashiorkor (Dr. J. C. Edozien and E. J. Phillips), 47
Pasher, I., and others : Vitamin-Levels in Blood and Serum, 78
Pasirouk, K. : At the International Starch Conference (New Potato Washing Machine in use in Russian Starch Factories), 237
Passive Transfer of Contact Sensitivity to Picryl Chloride in Guinea Pigs, with Subcellular Material (Dr. J. L. Turk), 915
Passorn, Dr. P. N. A. (Goodwin, Prof. T. W., and) : Purine and Pyrimidine Metabolism in *Rhodospirillum rubrum*, 276
Pasteurella multocida : Role of the Pentose Phosphate Pathway in (Inda M. de Issaly, A. Issaly and Prof. A. O. M. Stoppani), 727
Pasteurella pestis : Absence of Lysogeny in (Dr. D. A. Smith), 522
Pasteurella pseudotuberculosis-specific Polysaccharides : Dideoxysugars of, and the Occurrence of Ascarylose (Dr. D. A. L. Davies), 43
Pasture (Ryegrass-dominant), grazed by Cows : Growth of, and Properties of New Zealand Butterfat (F. H. McDowall, W. A. McGillivray and J. C. Hawke), 303
Pasture Plants : Numbers and Types of Bacteria from the Rhizosphere of—Possible Occurrence of *L*-Forms (G. Jagnow), 1220
Patel, N. G., and others : Chromatographic Investigation of the Methyl Alcohol/Chloroform-soluble Constituents of Honeybee Larval Foods, 362
Path of the Giant Cell Axons in *Aplysia depilans* (Dr. G. M. Hughes and L. Tauc), 404
Pathogenesis (Physiology and), of the Stein–Leventhal Syndrome (Dr. Virendra B. Mahesh and Dr. Robert B. Greenblatt)—Urinary Androsterone, Etiocholanolone, Pregnanetriol and Δ^5-Pregnenetriol of Ovarian Origin in a Patient with Stein–Leventhal Syndrome, 888 ; Role of FSH in the Pathogenesis of the Stein–Leventhal Syndrome, 889
Pathogenic to Man : Use of Desertomycin Medium in Routine Selective Isolation of Fungi (J. Uri and Zs. Herpay), 1015
Pathogens: Bacterial Plant (C. Stapp), translated by A. Schoenfeld, *review* by Dr. W. J. Dowson, 4
Pathological Infection (Non-Lethal), of Roots (F. J. Newhook), 615
Pathological Parthenogenesis in a Viviparous Toothcarp (Prof. A. Stolk), 507
Pathology of Muscle : The Physiology, Pharmacology and, *review* by Dr. D. H. Sproull, 948
Pathology (Tropical) : Techniques in (Prof. B. G. Maegraith, Prof. W. E. Kershaw and D. Dagnall), *review* by Prof. P. C. C. Garnham, 1236
Paths of Inter-Molecular Energy Transfer in Photo-sensitized Oxidation-Reduction (N. Takeyama), 1359
Pathway (Pentose Phosphate) : Role of the, in *Pasteurella multocida* (Inda M. de Issaly, A. Issaly and Prof. A. O. M. Stoppani), 727
Pathways (Energy), of Hepatoma No. 5123 (Dr. Alan C. Aisenberg and Harold P. Morris), 1314
Patients with Gargoylism : Inclusions of Mucopolysaccharide in the Lymphocytes of (Ursula Mittwoch), 1315
Patients (Diabetic) : Rapid Infra-Red Determination of Acetone in the Blood and the Exhaled Air of (Dr. Richard D. Stewart, Edward A. Boettner and Bruce T. Stubbs), 1008
Patnaik, S. N. (Panigrahi, G., and) : Cytology of some Genera of Polypodiaceae in Eastern India, 1207
Patrick, C. R. (Allen, Dr. P. E. M., and) : Generation of Radicals in Pairs and the Kinetics of Radical Chain Reactions in Solution, 1194
Pattern of their Localization in Tissues : Paper Electrophoresis of Proteins while Preserving the (Dr. Zh. A. Medvedev), 1021
Pattern of Pyrethrin-resistance in Houseflies (B. C. Fine), 884
Pattern (Intracellular Distribution), of Rat Liver Glutamic-Oxalacetic Transaminase (Dr. Herbert J. Eichel and Josef Bukovsky), 243
Patterns (Electrophoretic and Immunoelectrophoretic), of Extracellular Diphtheria Proteins from Culture Filtrates of Different Iron Concentrations (Bertold J. Radola), 382
Patterns (Haptoglobin), in Cord Blood Serums (A. R. Rausen, P. S. Gerald and Dr. L. K. Diamond), 717
Patwa, D. K. (Modi, Prof. V. V., and) : Occurrence of Mevalonic Acid in Carrots, 1202
Patwardhan, N. K., and others : Basic Dyestuffs in Clay Mineralogy, 696
Paul, Dr. E. B. : Atomic and Nuclear Physics, *review*, 8
Paul Instrument Fund : Grant of £1,550 to Dr. E. T. Hall, for the design and manufacture of a recording instrument to give a direct reading of wave-length, 225
  Grant of £3,900 to Dr. P. B. Hirsch, for the construction of an electron optical instrument, 225
  Grant of £3,000 (in supplement of a previous grant) to Dr. H. Motz, for the construction of a linear accelerator working at 1·6 cm. (*J*-band), 225
  Grant of £2,284 to B. M. Turner, for the further development of micro-scale chromatography and electrophoresis techniques, 225
Pauling, Prof. Linus (Itano, Dr. Harvey A., and) : Thalassaemia and the Abnormal Human Haemoglobins, 398
Pawsey, Dr. J. L. : Awarded the degree of Hon.D.Sc. of the Australian National University, Canberra, 443
Payne, Dr. D. S. : Organo-Metal Compounds, *review*, 316
Pea (Common), *Pisum sativum* L. : Lipids of the, with special reference to the Occurrence of Galactolipids (S. Adhikari, Dr. F. B. Shorland and R. O. Weenink), 1301
Pea Plants (Etiolated) : Correlative Inhibition of Formation of Chlorophyll in the Leaves of (Jiří Úlehla), 613
Pea Seeds : Effect of Boric Acid on the Phosphoglucomutase of (Dr. B. C. Loughman), 1399
Peacock, A. C. : Design and Use of Respirators (Manufacture and Testing of Respirators), 656
Peacock, J. D.: Uranium in British Surface and Underground Waters, 1189
Peacock, W. J. : Sub-Chromatid Structure and Chromosome Duplication in *Vicia faba*, 832
Peak Areas in Gas–Liquid Chromatography : Quantitative Estimation of (Dr. K. K. Carroll), 377
Peak-Shift Technique in Gas–Liquid Chromatography—Trimethylsilyl Ether Derivatives of Alcohols (Stanley H. Langer and Peter Pantages), 141
Peake, Eric (Hodgson, G. W., and) : Metal Chlorin Complexes in Recent Sediments as Initial Precursors to Petroleum Porphyrin Pigments, 766
Pearce, Dr. Robert P. : Theoretical Meteorology, *review*, 314
Pearse, Dr. A. G. E. : Direct Relationship of Phosphorylase and Mitochondrial α-Glycerophosphate Dehydrogenase Activity in Skeletal Muscle, 504
Pearse, Dr. A. G. E. (Hess, R., and) : Histochemical and Homogenization Studies of Mitochondrial α-Glycerophosphate Dehydrogenase in the Nervous System, 718
Pecile, C. (Turco, A., and) : Co-ordination of the Thiocyanate Group in Inorganic Compounds, 66
Pedler, Dr. A. E. : Appointed a senior Research Fellow in the Department of Chemistry, University of Birmingham, 337
Peel of Apples : Preparation of Mitochondria from the (Dr. J. D. Jones and Dr. A. C. Hulme), 370
Pelser, Johannes : Appointment as technical managing director of the Reactor Centrum Nederland, 1042 ; work of, 1042
Penczak, Tadeusz : Significance of the Regeneration of Lateral Plates of the Stickleback (*Gasterosteus aculeatus* L.), 621
Penicillinase-producing Organisms : Use of Cellulose Acetate Membranes for detecting (Prof. R. Knox and J. T. Smith), 926
Penicillins (Acid-stable) : Some Novel (F. P. Doyle, J. H. C. Nayler, H. Smith and E. R. Stove), 1091
Penicillins and Cephalosporins : Colorimetric Detection of, on Paper (Robert Thomas), 1161
Penicillium chrysogenum : Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of (A. Ballio, Prof. E. B. Chain, F. Dentice di Accadia, M. Mauri, K. Rauer, M. J. Schlesinger and Sondra Schlesinger), 909
Penicillium islandicum : Toxicology of (K. Uraguchi, T. Tatsuno, M. Tsukioka, Y. Sakai, F. Sakai, Y. Kobayahi, M. Saito, M. Enomoto and M. Miyake), 864
Penman, Dr. H. L. : Elected president for 1961-62 of the Royal Meteorological Society, 443
Pennington, Dr. W. B. : Appointed to the chair of pure mathematics in the University College of Wales, Aberystwyth, 333 ; work of, 333
Pentatomid : Extraction of Lipids from the Precursor of the Stylet-Sheath Material of a (P. W. Miles), 911
Pentose–Nucleic Acid Content of Sub-Cellular Fractions of the Brain of the Rat during 'Metrazol' Convulsions : Changes in the (Prof. G. P. Talwar, B. Sadasivudu and V. S. Chitre), 1007
Pentose Phosphate Pathway : Role of the, in *Pasteurella multocida* (Inda M. de Issaly, A. Issaly and Prof. A. O. M. Stoppani), 727
Peover, Dr. M. E. : Electron Affinity of the Acceptor Molecule in Charge-Transfer Complexes, 702
Pepper, Dr. D. C. : Appointed to the newly established chair of physical chemistry in Trinity College, Dublin, 333 ; work of, 333
Peptides (Oxytocic), in the Pituitary of a Marine Teleost Fish (*Pollachius virens*) : Pharmacological Characterization of the (Prof. H. Heller, B. T. Pickering, J. Maetz and F. Morel), 670
Peptides (Small) : Chemical Aspects of the Structure of (Dr. Dorothy Wrinch), *review* by Prof. H. N. Rydon, 105
Percentage of Total Calories from Protein in the Milk : Correlation between Growth-Rate of the Suckling of various Species and the (Dr. F. W. Bernhart), 358
Perceptual Defence and Filter Theory (Neville Moray), 940

Performing the Phenylalanine Test on Cultures from Multi-carbohydrate Media : A Micro-method for (D. J. Stewart), 521
Perfused Liver of Premature Lambs : Fructose and Lactic Acid Metabolism in the (W. H. H. Andrews, H. G. Britton and Dr. D. A. Nixon), 1307
Perfused Proximal Tubules (Single), of Rat Kidneys : Comparison of Short-Circuit Current and Net Water Movement in (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Periconia circinata : A Selective Toxin produced by (R. P. Scheffer and R. B. Pringle), 912
Périn, F., and others : A New Family of Potent Carcinogens—Benzopyridocarbazoles, 1005
Periodic Acid–Schiff Positive Giant Cells in the Mouse Thymus Cortex (D. Metcalf and M. Ishidate), 305
Peripheral Position of Sex Chromatin (Dr. Charles P. Miles), 626
Peripheral Vision : Effect of Attention in (Arthur Thomson), 732 ; (B. Babington Smith), 732
Periplaneta americana L. (Insect) : Exchanges of Sodium Ions in the Central Nervous System of an (J. E. Treherne), 1223
Perlman, D. : Vitamin B$_{12}$ and Intrinsic Factor (B$_{12}$ Coenzymes), 1154
Perls, T. A. : At the Second International Measurement Conference (Use of Pyroelectric Transducers for Heat-transfer Measurements), 1351
Permeability of Yeast : Changes of, with Sodium Deoxycholate (Dr. L. Kováč), 698
Permeation of Gaseous Oxygen through Vitreous Silica (Dr. Francis J. Norton), 701
Permselective Membranes : Poisoning and Sign Reversal of (Dr. Francis de Körösy), 1363
Peroxidase (Catalase and) : Effect of Excess Supply of Heavy Metals to Barley during Germination, with special reference to (Dr. S. C. Agarwala, A. Kumar and C. P. Sharma), 726
Peroxide (Hydrogen) : Cell Transplants of Avian Lymphomatosis treated with (Dr. V. L. Sanger), 1406
    Reactions of Haemoproteins with, and the Supposed Formation of Hydrogen Peroxide during the Autoxidation of Haemoglobin (Prof. D. Keilin), 769
Perren, Dr. E. A. : Design and Use of Respirators, 655
    Retirement as director of the Chemical Defence Experimental Establishment, Porton, 750 ; work of, 750
Perrin, Dr. D. D. : Improbability of the Chelation Hypothesis of Auxin Action, 253
Perrin, Dawn R., and Bottomley, W. : Pisatin, an Antifungal Substance from *Pisum sativum* L., 76
Perrin de Brichambaut, G. : Plant Exploration and Introduction (Comparative Study of Agro-Climates in the Winter-Rainfall Regions), 1240
Perry, Jerome J. : Prodigiosin in an Actinomycete, 77
Perry, Prof. S. V. : The Training of Biochemists (Growth and Development of Biochemistry in Britain), 955
Persistence of Erythrocyte Ghosts of Mediated Sugar Transport (Paul G. LeFevre), 970
Personnel (Science Information)—the New Profession of Information combining Science, Librarianship and Foreign Languages (Leonard Cohen and Kenneth Craven), 452
Personnel Management, Institute of : Job Analysis (H. E. Roff and T. E. Watson), 336
Persons with Tuberculous and Non-tuberculous Conditions : Mycobacteriophages isolated from (Dr. E. Mankiewicz), 1416
'Perspex' : Polarization induced by Radiation in (Dr. V. J. Vanhuyse, G. J. Vanpraet and J. F. Van Landùyt), 595
Peru : Flora of (J. Francis Macbride), *review* by N. Y. Sandwith, 214
Pests and Diseases (Forest Insect), in Canada (Prof. M. L. Anderson), 1260
Petch, B. (Farmer, J. B., and) : Electrolyte Retention produced by the Antialdosterone *SC* 5233, 1308
Peters, Prof. B. G. : Society of European Nematologists, 1048
Peters (Harry T.) 'American on Stone' Collection : The Victorian American —Lithographs from the (Antony N. B. Garvan and Peter C. Welsh), 226
Peters, Sir Rudolph : Awarded the degree of Hon.D.Sc. of the Australian National University, Canberra, 443
Petersen, D. F., and others : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Methods for Growing HeLa Cell Suspensions in the Presence of Tritiated Thymidine and Tritium Water), 341
Petroleum : Origins of (Sir Robert Robinson), 346
Petroleum, Institute of : Glossary of Petroleum Terms (edited by George Sell), third edition, 22
Petroleum, Institute of (Mass Spectrometry Panel), and the American Society for Testing Materials (Committee *E*-14) : Conference on Mass Spectrometry, 24
Petroleum Engineers (Mining, Metallurgical and), American Institute of (Iron and Steel Institute and the Metallurgical Society of the) : Special Meeting in the United States and Canada, 651
Petroleum Information Bureau : U.K. Petroleum Industry Statistics relating to Consumption and Refinery Production 1959 and 1960, 123
Petroleum Porphyrin Pigments : Metal Chlorin Complexes in Recent Sediments as Initial Precursors to (G. W. Hodgson and Eric Peake), 766
Petroleum Terms : Glossary of (edited by George Sell), third edition (Institute of Petroleum), 22
Pettersson, Dr. Bror : Mutagenic Effect of Radiant Heat Shocks on Phanerogamous Plants, 1167
Pettersson, Prof. Hans : The Frequency of Meteorite Falls throughout the Ages, 482
Pfau, A. : Vitamin B$_{12}$ and Intrinsic Factor (Broad Bean (*Vicia faba*) Plants can take up Vitamin B$_{12}$ from Nutrient Solutions), 1155
Pfizer Group : Laboratories for Research into Virus Diseases, 20
*p*H of Soap Solutions (D. Eagland and F. Franks), 1003
Phage Alpha : Some Properties of the Deoxyribonucleic Acid of (S. Cordes, H. T. Epstein and Prof. J. Marmur), 1097
Phagocytic Activity of the Reticulo-Endothelial System of Mice : Effect of Added Vitamins on the (J. Cordingley and Prof. T. Nicol), 82
Phanerogamous Plants : Mutagenic Effect of Radiant Heat Shocks on (Dr. Bror Pettersson), 1167
Pharmaceutical Conference (British) : Symposium on Skin Medication, 755
Pharmacological Characterization of the Oxytocic Peptides in the Pituitary of a Marine Teleost Fish (*Pollachius virens*), (Prof. H. Heller, B. T. Pickering, J. Maetz and F. Morel), 670
Pharmacologically Active Substances present in the Central Nervous System (Dr. Paul V. Buday), 245
Pharmacology of the Alkaloidal Fraction from the Bark of the Jamaican Shade Tree *Pithecolobium samath* Benth. (B. E. Leonard and Dr. H. S. A. Sherratt), 287
Pharmacology and Pathology of Muscle : The Physiology, *review* by Dr. D. H. Sproull, 948
Pharmacy : Remington's Practice of (edited by Eric W. Martin, E. Fullerton Cook, E. Emerson Leuallen, Arthur Osol, Linwood F. Tice and Clarance T. Van Meter), twelfth edition, *review* by Dr. J. H. Gaddum, 8
Pharmacy (Medicine and) : Second Annual Report and List of Members (1960-61) of the Faculty of the History of (Worshipful Society of Apothecaries of London), 21
Phase of Radio Signals : Effect of External Conditions on the (D. E. Watt-Carter and R. L. Corke), 1286
Phase (Juvenile), of Apple Seedlings : Shortening the (A. I. Campbell), 517
Phase (A New), formed by High-Pressure Treatment—Face-centred Cubic Molybdenum Monocarbide (E. V. Clougherty, K. H. Lothrop and J. A. Kafalas), 1194
Phase (Stationary) *Aerobacter aerogenes* : Induced Enzyme Synthesis in Aqueous Suspensions of Starved (R. E. Strange), 1272
Phase (*T*-) : Appearance of the (R. Green), 997
Phase (Tetragonal Mullite-like), from Co-precipitated Gels (J. Ossaka), 1000
Phase Velocities (Group and), of a Travelling Disturbance in the *F* Region of the Ionosphere (Dr. L. H. Heisler and Dr. J. D. Whitehead), 157
Phaseolus vulgaris : Sucrases in (R. A. Cooper and R. N. Greenshields), 601
Phenethyl Alcohol and Tyrosol : Formation of, during Fermentation of a Synthetic Medium lacking Amino-acids (R. Stevens), 913
Phenolic Compounds (Natural) : Stereochemistry of (Plant Phenolics Group Symposium on), 234
Phenomena (Spallation) : Fission and, and their Application to Cosmic Rays (European Organization for Nuclear Research and the Max Planck Institute, Heidelberg, Conference on), 24
Phenomena (Thermo-electric), in Electrodialysis Systems (Dr. Lloyd H. Shaffer), 591
Phenomenological Movement : The (Dr. Herbert Spiegelberg), *review* by F. I. G. Rawlins, 5
Phenomenology : An Appraisal of, *review* by F. I. G. Rawlins, 5
Phenomenon (Sickling) : Haemoglobin 'Barts' and the (Dr. Lie-Injo Luan Eng), 1314
Phenomenon (Simonsen) : Large Lymphocytes and the (Dr. Aleksander Szenberg and N. L. Warner), 920
Phenotype (New *Rh*), $Dcce^{f}e^{f}$, found in a Chibcha Indian Tribe (Dr. Miguel Layrisse, Zulay Layrisse, Esperanza García, Dr. Johannes Wilbert and Joaquín Parra R.), 503
Phenotypes : Differential Inhibition of Human Serum Cholinesterase with Fluoride—Recognition of Two New (Prof. H. Harris and Mary Whittaker), 496
    Serum Transferrins in the Genus *Macaca*—Species Distribution of Nineteen (Prof. Morris Goodman and Emily Poulik), 1407
Phenoxybenzamine : Stimulation of the Heart of the Spinal Dog by (B. G. Benfey and D. R. Varma), 919
Phenylalanine Test on Cultures from Multi-carbohydrate Media : A Micro-method for Performing the (D. J. Stewart), 521
Philbin, Dr. Eva M. : Appointed to the chair of organic chemistry in University College, Dublin, 439 ; work of, 439
Philippine Macaques : Isolation of *Plasmodium knowlesi* from (F. L. Lambrecht, Dr. F. L. Dunn and Dr. D. E. Eyles), 1117
Philips Technical Review, Vol. 22, Nos. 9 and 10, devoted to the material Glass, 1143
Phillips, A. P., and Burrows, R. B. : Pyridine Derivatives with Anthelmintic Activity, 707
Phillips, Dr. C. M. : Blindness in Africans in Northern Rhodesia, 753
Phillips, Dr. D. C. : Effects of Ionizing Radiation on Organic and Biological Solids (Chemical Changes on Irradiation), 1151
Phillips, E. J. (Edozien, Dr. J. C., and) : Partition of Urine Nitrogen in Kwashiorkor, 47
Phillips, Dr. J. N. : Awarded a Royal Society and Nuffield Foundation Commonwealth Bursary, 126
Phillips, Prof. J. N. : Appointed reader in morbid anatomy at the Postgraduate Medical School of London, 1349
Philosophical Society (American) : Year Book 1960, 335
    History of the American Philosophical Society (E. G. Conklin), 335
Philosophical Society (Yorkshire) : Report for 1960, 442
Philosophy of Science, British Society for the : Sixth Annual Conference, 338
Phipps, Edward, and others : Effect of Administration of Lysergic Acid Diethylamide on Serotonin-Levels in the Body, 499
Phleum pratense L. (S.48 Timothy) : Effects of Light Intensity on Reproduction in (Dr. G. J. A. Ryle), 196
Phloridzin (Cetrimide–) Combination : Enhancement of Inhibition of Intestinal Absorption by (J. A. Nissim), 37
Phormia regina Meigen (Blowfly) : Response of the Labellar Hairs of the, to Protein (D. I. Wallis), 917
Phosphatase (Alkaline) : Distribution of (Phosphatases in *Schistosoma mansoni*), (Dr. D. L. H. Robinson), 473
Phosphatases : Quantitative Estimation of (Phosphatases in *Schistosoma mansoni*), (Dr. D. L. H. Robinson), 473
Phosphatases in *Schistosoma mansoni* (Dr. D. L. H. Robinson)—Distribution of Alkaline Phosphatase, 473 ; Quantitative Estimation of Phosphatases, 473
Phosphate (Inorganic and Organic) : Outflux of, during Membrane Depolarization of Excitable Tissues (Dr. L. G. Abood, Prof. K. Koketsu and I. Koyama), 395
Phosphate (Pentose) Pathway : Role of the, in *Pasteurella multocida* (Inda M. de Issaly, A. Issaly and Prof. A. O. M. Stoppani), 727
Phosphate Metabolite (Tri-*o*-Cresyl) : Biological Activity of a (Prof. John E. Casida, Morifusa Eto and Ronald L. Baron), 1396
Phosphate-dependent Degradation of Urea (R. C. Valentine and Prof. R. S. Wolfe), 925
Phosphoglucomutase of Pea Seeds : Effect of Boric Acid on the (Dr. B. C. Loughman), 1399
Phosphoproteins of Mitochondria : Electron Transport System and (K. Ahmed, J. D. Judah and C. H. Gallagher), 1309
Phosphorus-32 : Incorporation of, into Urinary Concretions forming on Zinc Implants in Rat Bladders (Dr. Richard F. Keeler and Dr. Stuart A. Lovelace), 715

Phosphorus Metabolism of Brain (Dr. P. J. Heald), *review* by Dr. R. V. Coxon, 318
Phosphorylase and Mitochondrial α-Glycerophosphate Dehydrogenase Activity in Skeletal Muscle : Direct Relationship of (Dr. A. G. E. Pearse), 504
Phosphorylation : Coupling of, to Electron and Hydrogen Transfer by a Chemi-Osmotic Type of Mechanism (Dr. Peter Mitchell), 144
  Inhibition of, by Atractyloside (Dr. A. Bruni and A. R. Contessa), 818
Photic Reversal of the Beetle *Trypodendron* : Air-Swallowing, a Mechanism in (Prof. Kenneth Graham), 519
Photochemical Production of Semiconducting High Polymers (Prof. Gerald Oster, Dr. Gisela K. Oster and Dr. Marian Kryszewski), 164
Photochromic Polymer : A Reversible (A. G. Massey and Dr. L. E. Orgel), 1387
Photoelasticity (Illinois Institute of Technology, Chicago, International Symposium on), 755
Photography at the Federal Technical College, Zurich, 1043
Photography of Cerenkov Light from Extensive Air Showers in the Atmosphere (D. A. Hill and N. A. Porter), 690
Photometric Technique (A New), using a Variable Shutter Device (Dr. O. C. Jones and Dr. F. J. J. Clarke), 1290
Photoperiod : Flower Initiation in the Carnation in Response to (Dr. G. P. Harris and Juliet E. Griffin), 614
Photoperiodic Effects on Stomatal Behaviour in *Xanthium pennsylvanicum* (Dr. T. A. Mansfield and Prof. O. V. S. Heath), 974
Photoperiods (Different) : Effect of Gibberellic Acid on Growth and Indoleacetic Acid Oxidase Activity of *Lupinus albus* L. exposed to (F. Sági and A. S. Garay), 824
Photophosphorylation and the Hill Reaction (Mary Stiller and Prof. Birgit Vennesland), 677 ; (Dr. N. E. Good), 678 ; (Dr. André T. Jagendorf), 679
Photo-Polarography with a Flash-Lamp (Dr. H. Berg and H. Schweiss), 1270
Photoreceptor (Biological) : Transfer Function of a (Dr. Lawrence Stark and Dr. Howard T. Hermann), 1173
Photo-Sensicivity of Thiazine Leucobases (D. W. Menzies), 505
Photosensitivity Reactions : Mast Cells in (V. J. McGovern), 90
Photo-Sensitized Oxidation-Reduction : Paths of Inter-Molecular Energy Transfer in (N. Takeyama), 1359
Photosynthesis : Experimental Evidence for the Mechanism of (Dr. H. T. Witt, A. Müller and B. Rumberg), 194
  Inhibition of Growth and Photosynthesis in Submerged Plants with Tetramethylthiuram Disulphide and Sodium Dimethyldithiocarbamate and Reversal of this Inhibition of (P. E. Björn Lindahl), 51
Photosynthesis in Submerged Plants : Inhibition of Growth and, with Tetramethylthiuram Disulphide and Sodium Dimethyldithiocarbamate and Reversal of this Inhibition of Photosynthesis (P. E. Björn Lindahl), 51
Photosynthetic (Non-) Plant Tissue : Triphosphopyridine Nucleotide-linked Glyceraldehyde Phosphate Dehydrogenase in a (C. R. Benedict and Dr. H. Beevers), 71
Phycomycetes isolated from Subcutaneous Phycomycosis in Uganda : Two (Dr. A. M. M. Wilson), 612
Phycomyces carotenoids : Incorporation of 5,10,15 ^{14}C-Farnesol Pyrophosphate into (H. Yamamoto, H. Yokoyama, K. Simpson, T. O. M. Nakayama and C. O. Chichester), 1299
Phycomycosis (Subcutaneous), in Uganda : Two Phycomycetes isolated from (Dr. A. M. M. Wilson), 612
Phylogeny : Vertebrate, *review* by Dr. E. Trewavas, 945
Physical Chemistry in Trinity College, Dublin, 333
Physical Methods of Organic Chemistry (edited by Arnold Weissberger), third edition, Parts 3 and 4, *review* by Dr. W. A. Waters, 317
Physical Problems : Non-Linear (Indian Society of Theoretical and Applied Mechanics and Unesco Symposium on), 855
Physical Society (Institute of Physics and the) : Guthrie Lecture (Dr. D. Shoenberg, The de Haas-van Alphen Effect and the Electronic Structure of Metals), 126
  Election of Officers, 228
  Residential Symposium on Some Aspects of the Physics of Space Research, 229
  Symposium on High Energy Nuclear Physics, 1349
Physical Society (Electronics Group of the Institute of Physics and the) : Conference on the Physics of Gas Discharge Devices, 546
Physical Society (Institute of Physics and the), and the British Radio Spectroscopy Group : Joint Conference on Radio Spectroscopy of Solids, 126
Physical Society (Non-Destructive Testing Group of the Institute of Physics and the), (British Society of Rheology and the) : Joint Conference on Rheological Techniques, 24
Physical Society, and others : International Conference on Magnetism and Crystallography, 24
Physicists' (Hospital) Association : Douglas Lea Memorial Lecture (Dr. E. L. Powers), 1045
Physicists and Mathematicians : The Atomic Problem—a Challenge to (Lancelot Law Whyte), *review* by Prof. R. Eisenschitz, 214
Physics at Sheffield, 543
Physics : Achievements in, *review* by Prof. W. E. Burcham, 423
  Atomic and Nuclear (Prof. Robert S. Shankland), second edition, *review* by Dr. E. B. Paul, 8
  The New Age in (Prof. Sir Harrie Massey), *review* by Prof. W. E. Burcham, 423
Physics of Gas Discharge Devices (Electronics Group of the Institute of Physics and the Physical Society Conference on), 546
Physics for Grammar Schools (Science Masters' Association and the Association of Women Science Teachers), 555
Physics of Space Research : Some Aspects of the (Institute of Physics and the Physical Society Residential Symposium on), 229
Physics, Institute of, and the Physical Society : Guthrie Lecture (Dr. D. Shoenberg, The de Haas-van Alphen Effect and the Electronic Structure of Metals), 126
  Election of Officers, 228
  Residential Symposium on Some Aspects of the Physics of Space Research, 229
  Symposium on High Energy Nuclear Physics, 1349
Physics, Institute of, and the Physical Society (Electronics Group) : Conference on the Physics of Gas Discharge Devices, 546
Physics, Institute of, and the Physical Society and the British Radio Spectroscopy Group : Joint Conference on Radio Spectroscopy of Solids, 126
Physics, Institute of, and the Physical Society (Non-Destructive Testing Group), (British Society of Rheology and the) : Joint Conference on Rheological Techniques, 24
Physics (Nuclear) : High Energy (Institute of Physics and the Physical Society Symposium on), 1349
Physics (Nuclear), in Australia : Future of (Prof. E. W. Titterton), 639
Physics (Plasma), and Controlled Nuclear Fission Research (International Atomic Energy Agency International Conference on), 443
Physiological Significance of Surface Wax on Leaves (D. M. Hall and R. L. Jones), 95
Physiology : Foetal and Neonatal (*British Medical Bulletin*, Vol. 17, No. 2), (British Council), 344
Physiology of Crustacea (edited by Talbot H. Waterman), Vol. 2, Sense Organs, Integration and Behaviour, *review* by Dr. J. Green, 428
Physiology (Digestive), and Nutrition of the Ruminant—Proceedings of the University of Nottingham Seventh Easter School in Agricultural Science, 1960 (edited by Dr. D. Lewis), *review* by Dr. I. W. McDonald, 1334
Physiology and Pathogenesis of the Stein–Leventhal Syndrome (Dr. Virendra A. Mahesh and Dr. Robert B. Greenblatt)—Urinary Androsterone, Etiocholanolone, Pregnanetriol and Δ^5-Pregnenetriol of Ovarian Origin in a Patient with Stein–Leventhal Syndrome, 888 ; Role of FSH in the Pathogenesis of the Stein–Leventhal Syndrome, 889
Physiology, Pharmacology and Pathology of Muscle, *review* by Dr. D. H. Sproull, 948
Physostigmine : Inhibition by, of the Increase of Oxygen Consumption induced by Potassium in Muscle (Dr. Ivan Novotny and František Vyskočil), 916
Phytochemical Survey : New Zealand, 545
Phytoplankton Multi-Sampler and its Use in Lake Victoria (Dr. J. H. Evans), 53
π-Electronic Excitation and Ionization Energies of Condensed Ring Aromatic Hydrocarbons (Dr. J. B. Birks and M. A. Slifkin), 761
Piacitelli, John, and Santilli, Prof. Vincent : Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the Rate of Lesion Production on Pinto Bean, 624
Piazza, A., and others : Blockage of Transformation of Tryptophan to Nicotinic Acid by Experimental Hepatitis in Mice, 601
Picciotto, Prof. E. E., and others : Age Measurements on Antarctic Rocks (Queen Maud Land), 1286
Pichi-Sermolli, Prof. R. E. G. : Flora Europaea (Regional Check-lists for *Flora Europaea*), 448
Pichinoty, Francis, and D'Ornano, Liliane : Inhibition by Oxygen of Biosynthesis and Activity of Nitrate-Reductase in *Aerobacter aerogenes*, 879
Pickard, Prof. G. L., and others : A Lake in British Columbia containing Old Sea-Water, 830
Picker (James) Foundation : Fellowships and Grants in the field of radiology and nuclear medicine for 1962-63, 228
Pickering, B. T., and others : Pharmacological Characterization of the Oxytocic Peptides in the Pituitary of a Marine Teleost Fish (*Pollachius virens*), 670
Pickering, Sir George : What is Basic Science ?, *review*, 530
Pickles, Dr. V. R. : Appointed to a readership in physiology in the University of Sheffield, 229
Picryl Chloride in Guinea Pigs : Passive Transfer of Contact Sensitivity to, with Subcellular Material (Dr. J. L. Turk), 915
Picton and Hornby Libraries : Brown, (Liverpool Libraries, Museums and Arts Committee), 21
Pierce, A. E. : Immunity to Protozoa (Local Immunity in Coccidiosis), 553
Pigment Bodies of the Spinal Neurones of some Teleosts (Prof. A. Stolk), 924
Pigment Cell Biology (New York Academy of Sciences fifth International Conference on), 1045
Pigmentation : Inter-Individual Variability in (André Leguebe), 1326
Pigments of a New Nitrogen-fixing Micro-organism : Chemical Character of the (Gy. Németh, F. Uresch, Prof. G. Fodor and L. Láng), 1413
Pigments (Damselfly) : Isolation and Fractionation of some (C. B. Coulson and P. Mary Lord), 1300
Pigments (Petroleum Porphyrin) : Metal Chlorin Complexes in Recent Sediments as Initial Precursors to (G. W. Hodgson and Eric Peake), 766
Pignatti, Dr. S. : Flora Europaea (Taxonomy and Relationship of the Genus *Limonium* in Italy and Spain), 447
Pillow, W. A. E. : Digital Computers, their Use and Control (Description of a Transportation Problem and its Solution), 554
Pinch ('Theta') : A New 'Wedge' Type Instability observed in a (J. K. Wright, R. D. Medford, J. D. Herbert and R. C. Pottinger), 1054
Pincus, Gregory, and Vollmer, Erwin P. (edited by) : Biological Activities of Steroids in relation to Cancer—Proceedings of a Conference sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, United States Department of Health, Education and Welfare, *review* by E. Reid, 1235
Pinder, Dr. A. R. : The Chemistry of the Terpenes, *review* by Prof. B. C. L. Weedon, 1234
Pine Stands for Pulpwood : Management of (J. J. Kotzé), 855
Pine Stumps : Inoculation of, against Infection by *Fomes annosus* (Dr. J. Rishbeth), 826
Pinnal Anaphylaxis, an Additional Anaphylactic Site (Dr. J. G. Feinberg), 712
Pinto Bean : Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the Rate of Lesion Production on (John Piacitelli and Prof. Vincent Santilli), 624
Pinus sylvestris L. : Effect of Red and Far-red Irradiation on the Germination Process in Seeds of (Bengt Nyman), 1219
Pioneer V : Magnetic Storms in Interplanetary Space as observed by (Eugene W. Greenstadt), 329
Piperidine in the Brain : Distribution of, and its Possible Significance in Behaviour (Dr. L. G. Abood, F. Rinaldi and Virginia Eagleton), 201
Pirie, Antoinette : Cholesterol in the Tapetum Lucidum of the Eye of the Opossum, *Didelphis virginiana*, 708
Pisatin, an Antifungal Substance from *Pisum sativum* L. (Dawn R. Perrin and W. Bottomley), 76

Pisum sativum : Effects of Gibberellic Acid and Allogibberic Acid on Flowering and Internode-Length in (I. C. Murfet and H. N. Barber), 514
Pisum sativum L. : Pisatin, an Antifungal Substance from (Dawn R. Perrin and W. Bottomley), 76
Pisum sativum L. (Common Pea) : Lipids of the, with special reference to the Occurrence of Galactolipids (S. Adhikari, Dr. F. B. Shorland and R. O. Weenink), 1301
Pit Membrane of Wood Cells : Staining Reaction of the (R. K. Bamber), 409
Pitcher, R. S. : Nematology (Importance of Nematodes as Vectors of Virus Pathogens), 1048
Pithecolobium samath Benth. (Jamaican Shade Tree) : Pharmacology of the Alkaloidal Fraction from the Bark of the (B. E. Leonard and Dr. H. S. A. Sherratt), 287
Pittsburgh Diffraction Conference : Annual (Mellon Institute, Pittsburgh), 755
Pituitary of a Marine Teleost Fish (*Pollachius virens*) : Pharmacological Characterization of the Oxytocic Peptides in the (Prof. H. Heller, B. T. Pickering, J. Maetz and F. Morel), 670
Pituitary Growth Hormone : Effect of, on Cholesterogenesis (Dr. A. L. Greenbaum and R. S. Leal), 600
Pituitary Interstitial Cell-stimulating Hormone (Human) : An Immunological Study of a (N. R. Moudgal and Prof. Choh Hao Li), 192
Place, J. A. : The International Measurement Conference, 1350
Place-Name Society (English) : Vols. 30, 31 and 32, The Place-Names of the West Riding of Yorkshire (Prof. A. H. Smith), Part 1, Lower and Upper Strafforth and Staincross Wapentakes ; Part 2, Osgoldcross and Agbrigg Wapentakes ; Part 3, Morley Wapentake, *review* by Prof. H. C. Darby, 208
Place-Names of the West Riding of Yorkshire (Prof. A. H. Smith), Part 1, Lower and Upper Strafforth and Staincross Wapentakes ; Part 2, Osgoldcross and Agbrigg Wapentakes ; Part 3, Morley Wapentake, *review* by Prof. H. C. Darby, 208
Planck (Max) Institute, Heidelberg (European Organization for Nuclear Research and the) : Conference on Fission and Spallation Phenomena and their Application to Cosmic Rays, 24
Planck's Constant : Theory of the Red Shift and the Nature of (L. Infeld), 1184
Planetarium Equipment : Japanese, 753
Planetarium Projectors : Minolta (Chiyoda Kogaku Seiko K. K.), 753
Plankton off Newfoundland during Winter Months : Warm-water Species in the (V. Bainbridge), 1216
Plankton Research : Fellowships for (Lamont Geological Observatory), 754
Plankton and Fish (Hydrogen Ion Concentration,), in Fresh-water Eutrophic Lakes of India (Dr. S. M. Das), 511
Planning of Public Health Services (United Nations, World Health Organization, Technical Report Series No. 215—Fourth Report of the Expert Committee on Public Health Administration), 441
Plant (Cactus) : Diurnal Variation in Transaminase Activity in the (S. K. Mukerji, G. G. Sanwal and Prof. P. S. Krishnan), 516
Plant (Tea) : Classification of the (Dr. H. H. Mann), 409
Plant Breeding : Principles of (Prof. R. W. Allard), *review* by Dr. Kenneth S. Dodds, 636
Plant Cells (Cylindrical Animal and) : Circumscribing Irradiation of Surface Structures of, with Polonium-210 α-Particles (R. M. Bergström, R. F. Blåfield and M. W. Brenner), 1165
Plant Diseases (Bacterial), of Central Europe : Some, *review* by Dr. W. J. Dowson, 4
Plant Exploration and Introduction (Food and Agriculture Organization of the United Nations Technical Meeting on), 1240
Plant Growth in Irradiated Soil (G. D. Bowen and A. D. Rovira), 936
Plant-Growth (Enhanced), with Two Systemic Insecticides (M. M. H. Wallace), 513
Plant Pathogens : Bacterial (C. Stapp), translated by A. Schoenfeld, *review* by Dr. W. J. Dowson, 4
Plant Phenolics Group : Meeting on Wood Extractives, 229
  Symposium on the Stereochemistry of Natural Phenolic Compounds, 234
Plant Protein : Enzymic Conversion of Tetrahydrogolic Acid to Folinic Acid by (E. H. Byers, J. Stewart and T. J. Bond), 179
Plant Protoplasts : Properties of Isolated (C. G. Cocking), 780
Plant Roots : Dependence on Light of Geotropism in (J. V. Lake and G. Slack), 300
Plant Survival : Light Rainfall and—Measurement of Stem Flow Run-off (M. D. Gwynne and J. Glover), 1321
Plant Taxonomy as a Scientific Discipline (Dr. R. E. Holttum), 990
Plant Tissue (Non-Photosynthetic) : Triphosphopyridine Nucleotide-linked Glyceraldehyde Phosphate Dehydrogenase in a (C. R. Benedict and Dr. H. Beevers), 71
Plant Viruses (Dr. Kenneth M. Smith), third edition, *review* by Dr. B. D, Harrison, 111
Planterose, D. N., and others : Effect of *p*-Fluorophenylalanine on the Multiplication of Foot-and-Mouth Disease Virus, 414
Plants : Conversion of L-Galactono-γ-lactone into L-Ascorbic Acid by (Dr. G. A. D. Jackson, R. B. Wood and M. V. Prosser), 282
  Extraction of Heat-Labile Alkaloids from (Dr. A. R. Mattocks), 1281
  Gibberellin-like Substances in (Margaret Radley)—Leguminous Root Nodules, 684 ; Seaweed (*Fucus vesiculosus*), 685
  1-(Indole-3-acetyl)-β-D-Glucose, a New Compound in the Metabolism of Indole-3-acetic Acid in (Dr. M. H. Zenk), 493
  Respiratory Metabolism in (Prof. Harry Beevers), *review* by Prof. W. O. James, 1031
  Transformation in, of Sodium Dimethyldithiocarbamate into other Fungitoxic Compounds (H. M. Dekhuijzen), 198
Plants (Etiolated Pea) : Correlative Inhibition of Formation of Chlorophyll in the Leaves of (Jiří Úlehla), 613
Plants (Higher) : Some Characteristics of Mitochondria Extracted from (Dr. Walter D. Bonner, jun., and Dinor O. Voss), 682
Plants (Pasture) : Numbers and Types of Bacteria from the Rhizosphere of —Possible Occurrence of *L*-Forms (G. Jagnow), 1220
Plants (Phanerogamous) : Mutagenic Effect of Radiant Heat Shocks on (Dr. Bror Pettersson), 1167
Plants (Submerged) : Inhibition of Growth and Photosynthesis in, with Tetramethylthiuram Disulphide and Sodium Dimethyldithiocarbamate and Reversal of this Inhibition of Photosynthesis (P. E. Björn Lindahl), 51
Plasma in Cusp Geometry : Experiments on the Compression of (Dr. T. K. Allen and Dr. R. J. Bickerton), 794
Plasma of Pregnant Women : Oxytocinase Activity of (Stig E. J. Melander), 176
Plasma (Exploding Wire) : Magnetic Mirror Confinement of (E. J. Seykora), 995
Plasma Calcium in the Calf : Importance of Magnesium in the Control of (Dr. R. H. Smith), 181
Plasma Fibrinolysis (Spontaneous) : Determination of, by a Practical Procedure (Dr. H. Yatzidis), 604
Plasma Physics and Controlled Nuclear Fission Research (International Atomic Energy Agency International Conference on), 443
Plasma Proteins : Influence of, in the Control of Mitosis-rates in Generating Liver (Dr. J. T. Hemingway), 706
Plasmin : Action of, on Cartilage Matrix *in vivo* (Dr. C. H. Lack, A. J. Anderson and S. Y. Ali), 1402
Plasmodium knowlesi : Isolation of, from Philippine Macaques (F. L. Lambrecht, Dr. F. L. Dunn and Dr. D. E. Eyles), 1117
Plastic Limit (Upper), of Clays : Role of Interparticle Forces in Development of (Dr. Raymond Yong and E. T. Selig), 938
Plastics (Plastics Institute Lectures on), 650
Plastics Institute : Election of Officers for 1961-62, 959
  Lectures on Plastics, 650
  Appointment of L. M. Read as chairman of the Council, 1145
  Appointment of members of the Building Committee, 1145
Plastifying Treatment : Electrophoresis in a Thin Layer of Starch-Gel followed by a (J. Groulade, Dr. J. M. Fine and C. Ollivier), 72
Plastoquinone in Isolated Chloroplasts : Oxidation-Reduction Reactions of (Dr. E. R. Redfearn and J. Friend), 806
Platelets (Anti-), (Anti-Leucocyte Cytoplasm, Anti-Leucocyte Nuclei, and), in Disseminated Lupus Erythematosis (Prof. J. Dausset, J. Colombani and M. Colombani), 1209
Plates (Lateral), of the Stickleback (*Gasterosteus aculeatus* L. : Significance of the Regeneration of (Tadeusz Penczak), 621
Platinum (Zero-valent) : Complex Compounds of (Dr. J. Chatt and G. A. Rowe), 1191
Platt, Prof. B. S. : At the Third International Congress of Dietetics (Nutrition of the Mother and her Child), 1344
Platzman, Prof. R. L. : Effects of Ionizing Radiation on Organic and Biological Solids (Present State of the Theory of Primary Processes), 1150
Pleistocene Age : Specimens of very early, from certain beds in Israel (Dr. Desmond Clark), 23
Pleuropneumonia-like Organisms isolated from Tissue Cell Cultures : Immunofluorescence of (Major W. F. Malizia, M. F. Barile and D. B. Riggs), 190
Plommer, Dr. Hugh : Archaeology of Old Smyrna, *review*, 212
Plotz, Paul H., and others : Influence of Salts on the Uptake of Streptomycin by *Escherichia coli*, 1324
δ-Plutonium : Structure of (J. A. C. Marples and J. A. Lee), 1391
Pneumococcal Infection of Mice : Non-Specific Immunity in (Dr. G. N. Cooper and Dr. A. E. Stuart), 295
Pneumodermopsis paucidens in the North Sea (G. A. Cooper and D. C. T. Forsyth), 1412
Podliachouk, Luba, and others : Serum Factor *Gma* among the Iranians, 717
Poetry : Science and (T. R. Henn), 534
Poggi, M., and others : Effect of Serotonin on Absorption and Distribution of Calcium-45 in the Rat, 185
Poikilohydrous, Poikilochlorophyllous Angiosperm from Africa (Dr. D. J. Hambler), 1415
Point of View : Science and Education—the Teachers', 555
Poisoning (Chronic Copper), of Sheep : Methaemoglobin in (J. R. Todd and R. H. Thompson), 89
Poisoning and Sign Reversal of Permselective Membranes (Dr. Francis de Körösy), 1363
Polar Research : Soviet, *review* by Dr. Terence Armstrong, 841
Polarization induced by Radiation in 'Perspex' (Dr. V. J. Vanhuyse, G. J. Vanpraet and J. F. Van Landuyt), 595
Polarization of Triplet→Singlet Emission Spectra (V. G. Krishna and Prof. Lionel Goodman), 800
Polarized Light : Analysis of, by the Bee's Eye (Prof. Donald Kennedy and Dr. Edward R. Baylor), 34
Polarographic Examination of Sialic Acids (Barbara Robert, Max E. Rafelson and Ladislas Robert), 596
Polarography (Photo-), with a Flash-Lamp (Dr. H. Berg and H. Schweiss), 1270
Polianskii, N. G. (Busev, A. I., and) : The Use of Organic Reagents in Inorganic Analysis (translated from the Russian by J. T. Greaves), *review* by Dr. D. T. Lewis, 1336
Poliovirus : Chromatographic Analysis of Infectious Ribonucleic Acid from (C. Cocito, A. Prinzie and P. De Somer), 573
Poliovirus Multiplication : Effects of Heavy Water on—Results and Speculations on Mechanism (Dr. David Kritchevsky, Dr. Richard I. Carp and Dr. Hilary Koprowski), 250
Polish Textile Research, 1045
Politechnika Lodzka : *Zeszyty Naukowe Politechniki Lodzkiej* (Journal of the), Textile Section, Vols. 6 and 7, 1045
Politics and Social Service : Science, (Earl of Cranbrook), 992
Poliwoda, Dr. Hubert : Treatment of Thrombocytopaenic Haemorrhage with Œstriol Succinate, 400
Pollachius virens (Marine Teleost Fish) : Pharmacological Characterization of the Oxytocic Peptides in the Pituitary of a (Prof. H. Heller, B. T. Pickering, J. Maetz and F. Morel), 670
Pollan (Lough Neagh), *Coregonus pollan*, Thompson : Furunculosis in (K. U. Vickers and R. McClean), 930
Pollard, Dr. F. H. : Paper Chromatography (Separation of particular families of Compounds—Present Work and Advances in the Inorganic Field), 759
Pollination (Intra-ovarian), in *Eschscholzia californica* Cham., *Argemone mexicana* L. and *A. ochroleuca* Sweet (Prof. P. Maheshwari and Kusum Kanta), 304
Pollution from Refuse Tips (Nora H. Johnson), 864
Pollution of Water by Tipped Refuse—Report of the Technical Committee on the Experimental Disposal of House Refuse in Wet and Dry Pits (Ministry of Housing and Local Government), 864
Polonium-210 : Radiolytic Oxidation of Ferrous Solutions with Standardized Internal Sources of (J. Steyn and D. van As), 903

Polonium-210 α-Particles : Circumscribing Irradiation of Surface Structures of Cylindrical Animal and Plant Cells with (R. M. Bergström, F. R. Blåfield and M. W. Brenner), 1165
Poly-β-Hydroxybutyric Acid : Formation and Utilization of, by Knallgas Bacteria (*Hydrogenomonas*), (Prof. H. G. Schlegel, G. Gottschalk and R. von Bartha), 463
Polyamino Carboxylic Acids : Effect of, on Catalase (Dr. Joan Bond and J. G. Cooper), 385
Polychaete *Odontosyllis phosphorea* Moore, var. *Nanaimoensis* Berkeley : Swarming of the, near Nanaimo, B.C. (E. Berkeley), 1321
Polycrystalline Boron : Electrical Effects observed in (C. H. Carmichael and M. Dore), 485
Polycyclic Aromatic Hydrocarbons : Non-Enzymic Hydroxylation of (F. Dewhurst and G. Calcutt), 808
Polycythaemic : Bio-Assay of Erythropoietin in Mice made, by Exposure to Air at a Reduced Pressure (Dr. P. Mary Cotes and Dr. D. R. Bangham), 1065
Polydipsic Trait (Primary), of an Inbred Strain of Mice : Effect of Hybridization on the (Dr. Emanuel Silverstein), 523
Polyelectrolytes (Rod-like) : Displacement Potentials and Bending of (Prof. J. A. Christiansen, the late Dr. C. E. Jensen and Dr. Th. Vilstrup), 484
Polyenoic Fatty Acids : Biosynthesis of, in Human Whole Blood (Dr. F. Leupold and G. Kremer), 805
Polygenes : Location of (Prof. J. M. Thoday), 368
Polymer: A Reversible Photochromic (A. G. Massey and Dr. L. E. Orgel), 1387
Polymer Solutions : Ultrasonic Absorption of (Dr. G. Gooberman), 693
Polymeric Titanium Ethoxide and Titanium Oxide Ethoxides : Structures of (Prof. D. C. Bradley and A. H. Westlake), 273 ; (R. L. Martin and G. Winter), 274
Polymerization of Hyaluronic Acid isolated from Human Aortic Tissue : Degree of (Dr. Sv. Bertelsen and K. Marcker), 386
Polymers : Differential Thermogravimetry versus Differential Thermometry in Studying the Pyrolysis of (Hugh C. Anderson), 1088
    Recent Developments in the Chemistry and Technology of (Bradford Institute of Technology Course on), 1349
Polymers (High) : Elimination of the Absorption Correction in the Viscometry of Dilute Solutions of (H. van Oene and Prof. L. H. Cragg), 1160
    Viscosity–Temperature Relationships for Dilute Solutions of (Dr. W. R. Moore), 1292
Polymers (Inorganic) : International Symposium on, at the University of Nottingham, 1046
Polymers (Ion-Exchange) : Non-Uniformity of Cross-linking in (Dr. E. Glueckauf and R. E. Watts), 904
Polymers (Semiconducting High) : Photochemical Production of (Prof. Gerald Oster, Dr. Gisela K. Oster and Dr. Marian Kryszewski), 164
Polymorphism : Insect (Royal Entomological Society Symposium on), 1145
Polymorphism (Melting and), of Indium Antimonide at High Pressures (A. Jayaraman, R. C. Newton and Dr. G. C. Kennedy), 1288
Polyoma Virus : Induction of Tumours by—Enhancement by Chemical Agents (K. E. K. Rowson, F. J. C. Roe, J. K. Ball and Dr. M. H. Salaman), 893
Polypeptides : A Laboratory Manual of Analytical Methods of Protein Chemistry, including (edited by Dr. P. Alexander and R. J. Block), Vol. 1, The Separation and Isolation of Proteins, *review* by Dr. D. T. Elmore, 320
Polyploid Cotton : Basis of the Diploid-like Meiotic Behaviour of (G. Kimber), 98
Polypodiaceae in Eastern India : Cytology of some Genera of (G. Panigrahi and S. N. Patnaik), 1207
Polypropylene (Isotactic) : Crystallization of, using a Light Depolarization Technique (J. H. Magill), 1092
Polysaccharides : Role of, in Stabilization of Natural Soil Aggregates (Dr. D. J. Greenland, G. R. Lindstrom and Dr. J. P. Quirk), 1283
Polysaccharides (Linear from Branched) : Separation of, by Precipitation as Iodine Complexes (Blanche D. E. Gaillard), 1295
Polysaccharides (*Pasteurellapseudotuberlosis*-specific) : Dideoxysugars of, and the Occurrence of Ascarylose (Dr. D. A. L. Davies), 43
Polyvalent Ions : Influence of, on Histones isolated from Different Tissues (I. P. S. Agrell and E. G. Christensson), 284
Polyvinyl Alcohol : Effect of, on the Distribution of Iodine-131 in the Internal Organs, Fluids and Excreta of Rabbits (H. F. Nowak and J. Kucharski), 665, *erratum*, 1045
Polyvinylchloride : Crystallization Kinetics in, and Thermoelastic Relaxation induced by Ultrasonics (Dr. V. Nardi), 563
Pomerat, C. M. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Effect of Radiation on the Chromosomes of Leucocytes cultured from the Human Buffy Coat), 340
Pontin, A. J. : Response to Series of Subliminal Stimuli—an Ethological Method for Investigating the Properties of Nervous Systems, 512
Pool (Intermediate), between Amino-Acids and Globin in Duck Blood *in vitro* (L. Bianco, G. Giustina and E. Lazzarini), 40
Poole, Prof. J. H. J., and Byrne, F. N. : Measurement of the Thorium Content of Natural Materials by a γ-Ray Counting Method, 62
Population (Bulk-Hybrid), of Barley : Stabilizing Selection for Heading Time in a (Dr. S. K. Jain), 1123
Population (A Natural), of Anomalous Branched Tapeworms, *Hymenolepis nana* (Cestoda : Hymenolepididae), in a Colony of *DBA*/1 Mice (Dr. Donald Heyneman), 297
Population (Skin Follicle) : Composition of the, in Seven Rajasthan Sheep Breeds (Sunanda Narayan), 1115
Population Density : Sexual Differentiation in *Hydra* in relation to (Helen D. Park, Christyna Mecca and Anne Ortmeyer), 92
Population Models (Stochastic), in Ecology and Epidemiology (Prof. M. S. Bartlett), *review*, 843
Population Movements : Effect of, on Malaria Eradication (R. Mansell Prothero), 863
Population Statistics in the United Kingdom, 1347
Population Trends : World, 1346
Populations (Associated) : Nodal Analysis of (Prof. W. T. Williams and J. M. Lambert), 202
Populations (Lamprey) : Sex Composition of (Dr. M. W. Hardisty), 1116
Porath, J., and Lindner, E. B. : Separation Methods based on Molecular Sieving and Ion Exclusion, 69
Porcine Urine : Neutral 17-Kerosteroids in (L. G. Huis in't Veld, B. Louwerens and W. Reilingh), 175
Porous Nickel Oxide (I. S. Solet), 64
Porphyrin Pigments (Petroleum) : Metal Chlorin Complexes in Recent Sediments as Initial Precursors to (G. W. Hodgson and Eric Peake), 766
Porteous, Donald : Leukaemia in the *AkR* Mouse after X-Irradiation *in utero*, 291
Porter, —.: Hazards of the Animal House (Traumatic Hazards not only from the Animals themselves but also from Damaged or Incorrectly Designed Equipment), 339
Porter, N. A. (Hill, D. A., and) : Photography of Cerenkov Light from Extensive Air Showers in the Atmosphere, 690
Portland Cement (Interaction of), with Sea-Water and Chloride Solutions : Formation of Calcium Chloraluminate by (Dr. L. Heller and Dr. M. Ben-Yair), 488
Porto Novo, South India : Occurrence of *Eutrichocheles modestus* (Herbst) in the Near-shore Waters of (K. Balasubrahmanyan and Dr. J. Jacob), 830
Portulaca oleracea L. : High Concentration of (–)-Noradrenaline in (Dr. P. C. Feng, Prof. L. J. Haynes and Dr. K. E. Magnus), 1108
Position (Peripheral), of Sex Chromatin (Dr. Charles P. Miles), 626
Positive Antiglobulin Consumption Test : Nature of the Coating Globulin causing a (D. Nelken), 1110
Positive Feedback Mechanism of Adenosine Triphosphate Synthesis in Erythrocytes (Makoto Nakao, Takashi Motegi, Toshiko Nakao, Saburo Yamazoe and Haruhisa Yoshikawa), 283
Possibilities of Vegetative Propagation in the Coconut (*Cocos nucifera* L.), (S. R. Gangolly and K. M. Pandalai), 1218
Possible Cause of Mutual Dependence : Copepod *Ommatokoita elongata* (Grant) in the Eyes of the Greenland Shark—a (Bjørn Berland), 829
Possible Method for Increasing the Signal-Noise Ratio in the Detection of the First Motion of a Refracted *P*-Wave (F. A. Key, J. K. Wright, E. W. Carpenter and B. M. H. Stott), 1382
Possible Occurrence of *L*-Forms : Numbers and Types of Bacteria from the Rhizosphere of Pasture Plants— (G. Jagnow), 1220
Possible Primitive Earth Conditions : Mechanism of Synthesis of Adenine from Hydrogen Cyanide under (Prof. J. Oró), 1193
Possible Significance in Behaviour : Distribution of Piperidine in the Brain and its (Dr. L. G. Abood, F. Rinaldi and Virginia Eagleton), 201
Post-Doctoral Fellowships in Protein Chemistry (Division of Protein Chemistry of the Australian Commonwealth Scientific and Industrial Research Organization), 336
Postgraduate Courses in Nuclear Studies (Liverpool College of Technology), 338
Postgraduate Medical School of London : Appointment of Prof. J. N. P. Davies to the University of London readership in morbid anatomy, 229
    Appointment of Prof. J. N. Phillips as reader in morbid anatomy, 1349
Post-Partum Period : Antibodies to Trophoblasts during the (J. F. Hulka, K. C. Hsu and S. M. Beiser), 510
Postan, Prof. M. : Social History of Engineering, *review*, 946
Potassium: Inhibition by, of the Mechanical Responses of Frog Muscles at Low Temperature (H. Lorković), 1011
    Radiocaesium and, in Norwegians (Johan Baarli, Kjell Madshus, Kurt Lidén and Richard C. McCall), 436
Potassium in Muscle : Inhibition by Physostigmine of the Increase of Oxygen Consumption induced by (Dr. Ivan Novotny and František Vyskočil), 916
Potassium Retention : Aldosterone Excretion and, in Subjects living at High Altitude (P. J. Ayres, Rona C. Hurter, Dr. E. S. Williams and J. Rundo), 78
Potassium (Sodium and), in certain Cells and Tissues : Distribution of (Dr. Norman R. Joseph, Dr. Milton B. Engel and Dr. Hubert R. Catchpole), 1175
Potato : Introduction of the, into Western and Central Europe (V. S. Lekhnovitch), 518
Potato (Sweet), *Ipomoea batatas* (L.) Lam. : Evolution of the (Dr. D. E Yen), 93
Potato Crop : Tuber Bulking in the (R. W. Radley, M. A. Taha and P. M Bremner), 782
Potato Virus Diseases : Proceedings of the Fourth Conference on (Braunschweig, 1960), edited by J. Brandes, R. Bartels, V. Völk and C. Wetter (Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig), 657
Potent Actions on Mammalian Neurones : Analogues of Glutamic and γ-Amino-*n*-butyric Acids having (Dr. J. C. Watkins and Dr. D. R. Curtis), 1010
Potent Carcinogens : A New Family of—Benzopyridocarbazoles (Prof. A. Lacassagne, Prof. N. P. Buu-Hoï, F. Zajdela, F. Périn and P. Jacquignon), 1005
Potential (The Action), in Cells of *Chara* (A. B. Hope), 811
Potential (Electrical), across the Isolated Gut : Dependence on Substrate of the (Dr. T. W. Clarkson, A. C. Cross and S. Toole), 501
Potential Changes produced by Application of Current Steps in Motoneurones (T. Araki, Dr. M. Ito and T. Oshima), 1104
Potentials (Displacement), and Bending of Rod-like Polyelectrolytes (Prof. J. A. Christiansen, the late Dr. C. E. Jensen and Dr. Th. Vilstrup), 484
Potentials (Ionization), of *cis* and *trans* Dichloro- and Dibromo-ethylenes (Dr. J. Momigny), 1089
Potentials (Spontaneous Miniature), from Insect Muscle Fibres (P. N. R. Usherwood), 814
Potentiation of Epinephrine Action (Prof. R. Imaizumi, Y. Hashimoto and M. Oka), 186
Pottinger, R. C., and others : A New 'Wedge' Type Instability observed in a 'Theta' Pinch, 1054
Poulik, Emily (Goodman, Prof. Morris, and) : Serum Transferrins in the Genus *Macaca*—Species Distribution of Nineteen Phenotypes, 1407
Poulik, Dr. M. D., and Edelman, Dr. G. M. : Comparison of Reduced Alkylated Derivatives of some Myeloma Globulins and Bence-Jones Proteins, 1274
Poulsen, Dr. E. : Awarded a Carlsberg–Wellcome Research Fellowship, 754
Poultry : Nutrition and (Scottish Group of the Nutrition Society Symposium on), 856
Poultry (Domestic Animals and), in India : Occurrence of *Salmonellae* in (V. K. Sharma and Prof. C. M. Singh), 622

Povinelli, Richard (Szymanski, Herman A., and) : Infra-Red Spectra of Water-soluble Carbonates, 64
Power (Thermoelectric), of Single Crystals of Gallium (P. Horner), 58
Power Plant : The Robert Moses Niagara, 22
Power Requirements and Scale-up in Flotation (Prof. Nathaniel Arbiter and Prof. Colin C. Harris), 268
Powers, Dr. E. L. : New Considerations on Survival Curves and Target Theory (Douglas Lea Memorial Lecture of the Hospital Physicists' Association), 1045
  Effects of Ionizing Radiation on Organic and Biological Solids (Role of Oxygen in Radiation Damage), 1150
Practical Applications of Nuclear Energy (International Atomic Energy Agency Courses on), 1143
Practical Procedure : Determination of Spontaneous Plasma Fibrinolysis by a (Dr. H. Yatzidis), 604
Practice : Code of, for the Display of Sources of Ionizing Radiations at Exhibitions, 1249
  From Theory to, in Soil Mechanics—Selections from the Writings of Karl Terzaghi, *review* by Kevin Nash, 210
Practice (Tradition, Science and), in Dietetics (D. F. Hollingsworth), 1343
Practice of Pharmacy (Remington's), (edited by Eric W. Martin, E. Fullerton Cook, E. Emerson Leuallen, Arthur Osol, Linwood F. Tice and Clarance T. Van Meter), twelfth edition, *review* by Dr. J. H. Gaddum, 8
Prakash, Prof. Satya, and Prakash, Sheo: Inhibition of Ultrasonic Decolorization of Rhodamine *B* in Presence of Organic Substances, 1292
Prakash, Sheo (Prakash, Prof. Satya, and) : Inhibition of Ultrasonic Decolorization of Rhodamine *B* in Presence of Organic Substances, 1292
Pramer, Prof. David, and others : A Nemin Assay Procedure, 155
Prather, J. L. : Atomic Energy Levels in Crystals (United States National Bureau of Standards, Monograph No. 19), 1348
Prawn, *Palaemonetes varians* Leach : Mechanism of Sensitivity to Hydrostatic Pressure in the (Peter S. B. Digby), 366
Precious Metal Years : Two Hundred—a History of the Sheffield Smelting Company, Ltd., 1760-1960 (Ronald E. Wilson), *review* by Prof. R. W. K. Honeycombe, 108
Precipitable Water Vapour over the United States : Chemical Composition of (Dr. Henry M. Papée and Dr. Alberto Montefinale), 136
Precipitation of Electrolytes : Electrical Effects during Dissolution and (R. P. Rastogi, B. P. Batra and R. K. Dass), 764
Precipitation as Iodine Complexes : Separation of Linear from Branched Polysaccharides by (Blanche D. E. Gaillard), 1295
Precipitin (Auto-Immunological), possibly due to Fibrinolysins : Demonstration by Paper Electrophoresis of an (Marion I. Cooper and J. R. Sykes), 1212
Precorticotrophin in Blood (Dr. P. R. Dasgupta), 1106
Precursor of the Stylet-Sheath Material of a Pentatomid : Extraction of Lipids from the (P. W. Miles), 911
Precursors (Initial), to Petroleum Porphyrin Pigments : Metal Chlorin Complexes in Recent Sediments as (G. W. Hodgson and Eric Peake), 766
Pregnant Women : Oxytocinase Activity of Plasma of (Stig E. J. Melander), 176
Prehistoric Chamber Tombs of France (Dr. Glyn Daniel), *review* by Prof. C. F. C. Hawkes, 637
Prehistorical Tools : Eoliths and, 23
Preliminary Age-Determinations in the Lizard Area (J. A. Miller and D. H. Green), 159
Prelog, Prof. V. : Elected a foreign associate of the United States National Academy of Sciences, 18 ; work of, 19
Premature Lambs : Fructose and Lactic Acid Metabolism in the Perfused Liver of (W. H. H. Andrews, H. G. Britton and Dr. D. A. Nixon), 1307
Preparation : A Simplified Method for Separating Amino-Acid Mixtures from Protein Hydrolysates on One Ion-Exchange Column during (J. Liebster, K. Kopoldová and M. Dobiášová), 1198
Preparation of Mitochondria from the Peel of Apples (Dr. J. D. Jones and Dr. A. C. Hulme), 370
Preparation (Teacher), for Health Education (United Nations, World Health Organization, Technical Report Series No. 193—Report of a joint WHO/Unesco Expert Committee), 441
Preparations of Reduced Diphosphopyridine Nucleotide : An Inhibitor of Liver Alcohol Dehydrogenase in (Dr. Keith Dalziel), 1098
Preparations (Cell-free Spleen), Incubated *in vitro* : Rise of Antibody Titre in (Dr. Dieter H. Sussdorf), 770
Presence of Hydrogen : Selectivity of Palladium Catalysts for the Oxidation of Carbon Monoxide in the (P. Mars and Th. van de Mond), 1003
Present : Experimental Method in Biology from the Time of Antonio Vallisneri to the (University of Padua Symposium on), 546
Preservation of Rural England, Council for the : Twenty-second National Conference (The Impact of Industrial Development on the Countryside), 959
Preservation and the Restoration of Cultural Property, International Centre for the Study of the (Rome) : Works and Publications, 3, Climatology and Conservation in Museums, 1050
Preservation (Bird), International Council for (British Section) : Annual Report for 1960, 124
Preserving the Pattern of their Localization in Tissues : Paper Electrophoresis of Proteins while (Dr. Zh. A. Medvedev), 1021
Pressure below 0·1° K. : Specific Heat and Expansion Coefficient of Liquid Helium-3 under (Dr. D. F. Brewer and J. R. G. Keyston), 1261
Pressure (High-) Treatment : A New Phase formed by—Face-centred Cubic Molybdenum Monocarbide (E. V. Clougherty, K. H. Lothrop and J. A. Kafalas), 1194
Pressure (Hydrostatic) : Mechanism of Sensitivity to, in the Prawn, *Palaemonetes varians* Leach (Peter S. B. Digby), 366
Pressure (Reduced) : Bio-Assay of Erythropoietin in Mice made Polycythaemic by Exposure to Air at a (Dr. P. Mary Cotes and Dr. D. R. Bangham), 1065
Pressure Differences in a Direct-Current Capillary Discharge (D. S. Smith), 265
Pressure Gradients : Role of, in obtaining Minimum Time in Gas Chromatography (Prof. J. Calvin Giddings), 1291
Pressures (High) : Melting and Polymorphism of Indium Antimonide at (A. Jayaraman, R. C. Newton and Dr. G. C. Kennedy), 1288
Preston, Prof. R. D. (Dennis, D. T., and) : Constitution of Cellulose Microfibrils, 667
Prevention of Ammonia Toxicity by Amino-acids concerned in the Biosynthesis of Urea (Dr. Francesco Salvatore and Dr. Vincenzo Bocchini), 705
Preventive Medicine, Lister Institute of (London): Report of the Governing Body (1961), 1356
Prigal, Samuel J. : Development in Mice of Prolonged Non-specific Resistance to Sarcoma Implant and Staphylococcus Infection following Repository Injection of Lipopolysaccharide, 1111
Primary Amides : A New Relation between the N–H Stretching Frequencies of (Prof. P. G. Puranik and K. Venkata Ramiah), 796
Primary Polydipsic Trait of an Inbred Strain of Mice : Effect of Hybridization on the (Dr. Emanuel Silverstein), 523
Primary Products, of Reactive Bimolecular Collisions : A Molecular Beam Reactor for Examining the Rate, and (C. W. Nutt and A. J. Biddlestone), 798
Primitive Earth Conditions : Mechanism of Synthesis of Adenine from Hydrogen Cyanide under Possible (Prof. J. Oró), 1193
Principals of the Universities of the United Kingdom, Committee of Vice-Chancellors and : Conference of professors of mathematics from the Universities of Great Britain, to consider the Teaching of Mathematics, 1145
Principle (Isoelectronic), Thermochemistry and Reactivity of related Free Radicals (Dr. Peter Gray and J. C. J. Thynne), 1357
Principles of Genetics (Prof. Eldon J. Gardner), *review* by Dr. Kenneth S. Dodds, 636
Principles of Human Genetics (Curt Stern), second edition, *review* by Dr. G. Ainsworth Harrison, 110
Principles of Plant Breeding (Prof. R. W. Allard), *review* by Dr. Kenneth S. Dodds, 636
Principles of Radiation Protection (Harwell Reactor School of the Atomic Energy Research Establishment Course on), 856
Principles (Energy), in Applied Statics (T. M. Charlton), *review* by Prof. R. C. Coates, 1134
Pringle, R. B. (Scheffer, R. P., and) : A Selective Toxin produced by *Periconia circinata*, 912
Prinzie, A., and others : Chromatographic Analysis of Infectious Ribonucleic Acid from Poliovirus, 573
Prior, B. E., and others : Nervous and Biochemical Disturbances following Hexachlorobenzene Intoxication, 363
Pritchard, E. B., and others : Space-Flight and Re-entry Trajectories (Dynamics of Terminal Re-entry—Methods of Control for Re-entry from Escape Speeds), 960
Pritchard, E. P. : Aims, Organization and Methods of University Extra-Mural Libraries in Great Britain (Library Association, Pamphlet No. 21), 649
Pritchard, J., and others : Evaporated Carbon Films of Large Area for Adsorption Studies by Electron Bombardment, 487
Prizes : Monograph (American Academy of Arts and Sciences), 442
Probability Curves (Isolated or Overlapping Normal) : Calculation of the Areas of (Dr. D. Morison Smith and John C. Bartlet), 688
Probe (A Hypodermic), using Fibre Optics (David F. Capellaro, Narinder S. Kapany and Dr. Charles Long), 927
Problem : The Atomic—a Challenge to Physicists and Mathematicians (Lancelot Law Whyte), *review* by Prof. R. Eisenschitz, 214
  A Combinatorial Assignment (Prof. C. Radhakrishna Rao), 100
  The Nitrogen, 544
Problem in Applied Chemical Kinetics : Inversion of a Laplace Transform arising from a (T. Wood), 589
Problem of Retirement, *review* by Prof. L. S. Hearnshaw, 532
Problem (Cancer) : A Clinical Prospect of the (Prof. D. W. Smithers), *review*, 1336
Problem (World) : Public Health as a, 441
Problems : Non-Linear Physical (Indian Society of Theoretical and Applied Mechanics and Unesco Symposium on), 855
Problems of Ageing : Research into, 1145
Problems of Managing Nature Reserves (Dr. J. F. D. Frazer), 133
Problems of the North (Translation of *Problemy Severa*), No. 1, 1958 (translated December 1960), *review* by Dr. Terence Armstrong, 841
Problems of Psychology, Nos. 1 and 2 (translation from the Russian *Voprosy Psikhologii*), 958
Problems (Agricultural), in Developing Countries (Nutrition Society Symposium on), 1250
Problems (Corrosion), in the Electrical Power Industry (Battersea College of Technology Summer School on Corrosion), 651
Problems (Current), in Nutrition (Nutrition Society of Canada Symposium on), 335
Procedure : A Nemin Assay (E. J. Winkler, Shimpei Kuyama and Prof. David Pramer), 155
Procedure (Practical) : Determination of Spontaneous Plasma Fibrinolysis by a (Dr. H. Yatzidis), 604
Procedure (Simplified), for Removing Non-specific Staining Components from Fluorescein-labelled Conjugates (G. D. Johnson), 70
Process (Germination), in Seeds of *Pinus sylvestris* L. : Effect of Red and Far-red Irradiation on the (Bengt Nyman), 1219
Process (Labour) : Relationship between the Decrease in Urinary Excretion of Histamine and the Initiation of the (Dr. Halina Wiskont-Buczkowska), 290
Processes (Markov) : Theory of (E. B. Dynkin), translated from the Russian by D. E. Brown, edited by T. Köváry, *review* by D. G. Kendall, 321
Processing Cereals : Recent Advances in (Food Group of the Society of Chemical Industry Symposium on), 755
Processing of Observations made on Artificial Earth Satellites : A Collection of Tables and Nomograms for the (I. D. Zhongolovich and V. M. Amelin), *review* by D. G. King-Hele, 1135
Processing (Chemical) : Technology of, *review* by R. H. Villet, 1131
Prochazka, Ing. : Paper Chromatography, 760
Prockop, D. J., and others : Influence of Scurvy and Lathyrism (Odoratism) on Hydroxyproline Excretion, 1008
Prodigiosin in an Actinomycete (Jerome J. Perry), 77
Produced (Centrally) Geometrical Illusions (S. Papert), 733
Producing 6-Aminopenicillanic Acid from Benzylpenicillin : A Method for Isolating Bacteria capable of (Prof. Yukio Kameda, Yukio Kimura, Etsuko Toyoura and Tomoo Omori), 1122
Product (Solubility) : Theoretical Expression for (Prof. B. N. Bhar), 1004; (Prof. K. Krishnamurti), 1004
Production of Gas from Coal in Britain, 225

Production of Neon in Stars (H. E. Gove, A. E. Litherland and M. A. Clark), 1381
Production of Symptoms of Barley Leaf-Spot Disease by Culture Filtrate of *Helminthosporium sativum* (S. K. Gayed), 725
Production of Synthetic Corundum : A New Technique for the (E. Q. D. White), 901
Production of Viral Ribonucleic Acid : Influence of *p*-Fluorophenylalanine on the, and on the Utilizability of Viral Protein during Multiplication of Fowl Plague Virus (Dr. Christoph Scholtissek and Rudolf Rott), 1023
Production (Artificial), of Buds from the Embryos of *Cuscuta reflexa* (Prof. P. Maheshwari and B. Baldev), 197
Production (Auxin), by Species of *Arthrobacter* (Dr. H. Katznelson and Dr. J. C. Sirois), 1323
Production (Crop) : Importance of Nitrogen in (Prof. N. R. Dhar), 544
Production (Lesion), on Pinto Bean : Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the Rate of (John Piacitelli and Prof. Vincent Santilli), 624
Production (Metabolic), of Sucrose from Fat (Prof. H. Beevers), 433
Production (Photochemical), of Semiconducting High Polymers (Prof. Gerald Oster, Dr. Gisela K. Oster and Dr. Marian Kryszewski), 164
Production (Sound), and Sound Reception by Insects (Mable Frings and Hubert Frings), *review* by Prof. V. B. Wigglesworth, 428
Production (Turnip Yellow Mosaic Virus Protein) : Stimulation of, by 2-Thiouracil (Dr. R. I. B. Francki and Dr. R. E. F. Matthews), 1078
Products : Castorseed and its (Council for Scientific and Industrial Research, India, Seminar on), 753
Products of Mild Acid and Alkaline Hydrolysis of *M*- and *N*-Blood Group Substances (Dr. Elzbieta Romanowska), 1408
Products (Primary), of Reactive Bimolecular Collisions : A Molecular Beam Reactor for Examining the Rate, and (C. W. Nutt and A. J. Biddlestone), 798
Products (Reduction), of 2,2'-Dipyridyl : Stability Constant of Copper (II) Complexes of (Dr. M. T. Beck and M. Halmos), 1090
Profession (New), of Information combining Science, Librarianship and Foreign Languages : Science Information Personnel—the (Leonard Cohen and Kenneth Craven), 452
Progeny of Mice inoculated with a Leukaemogenic Virus : Leukaenia in the (Dr. M. H. Salaman and J. J. Harvey), 509
Programming : Linear (Saul I. Gass), *review* by Dr. M. V. Wilkes, 9
Programming (Linear and Non-Linear) : Methods of Feasible Directions—a Study in (Dr. G. Zoutendijk), *review* by Dr. M. V. Wilkes, 9
Progress on the Advanced Gas-cooled Reactor at the Windscale Factory of the United Kingdom Atomic Energy Authority, 853
Progress in Chemistry, *review* by Prof. J. R. Partington, 637
Progress of Chemistry : Annual Reports on the, for 1959 (edited by Dr. R. S. Cahn), Vol. 61, *review* by Prof. J. R. Partington, 637
Progress in Experimental Tumor Research (edited by F. Homburger), Vol. I, *review* by Prof. F. Bergel, 1231
Progress Reports and Tumour Research, *review* by Prof. F. Bergel, 1231
Progress (Research), in General Medical Sciences, 1960 : Highlights of (United States Department of Health, Education and Welfare, Public Health Service Publication No. 815), 658
Proliferation (Parenchymal Liver Cell), in Normal Adult Rats : Stimulation by Heparin of (Dr. M. Zimmerman and Evemarie Celozzi), 1014
Proline Derivatives from Apples : Determination by Mass Spectrometry of the Structure of (Dr. K. Biemann, G. G. J. Deffner and Prof. F. C. Steward), 380
Prolonged Non-specific Resistance to Sarcoma Implant and Staphylococcus Infection following Repository Injection of Lipopolysaccharide : Development in Mice of (Samuel J. Prigal), 1111
Promotion of Science in Australian Schools, 543
Propagation (Vegetative), in the Coconut (*Cocos nucifera* L.) : Possibilities of (S. R. Gangolly and K. M. Pandalai), 1218
Propagation (Wave), and Group Velocity (Dr. Léon Brillouin), *review* by Prof. M. H. L. Pryce, 104
Propellant (Solid), and Exothermic Compositions (Dr. James Taylor), *review* by Dr. W. B. Littler, 3
Properdin : Action of Tetracycline on the Level of (Vl. Kubelka and M. Rybák), 820
Properties of Deoxyribonucleic Acid from Mammalian Sperm : Isolation and (Dr. E. Borenfruend, E. Fitt and Dr. A. Bendich), 1375
Properties of the Deoxyribonucleic Acid of Phage Alpha : Some (S. Cordes, H. T. Epstein and Prof. J. Marmur), 1097
Properties of Ionic Melts : The Structure and (Faraday Society General Discussion on), 338, 443
Properties of Isolated Plant Protoplasts (E. C. Cocking), 780
Properties of Nervous Systems : Response to Series of Subliminal Stimuli—an Ethological Method for Investigating the (A. J. Pontin), 512
Properties of New Zealand Butterfat : Growth of Ryegrass-dominant Pasture grazed by Cows, and (F. H. McDowall, W. A. McGillivray and J. C. Hawke), 303
Properties (Acaricidal), of Di-(*p*-chlorophenyl)-trifluoromethylcarbinol (K. R. S. Ascher and R. Cwilich), 1322
Properties (Anticancer), of Thio-diglycollic Acid (M. B. Sahasrabudhe, M. V. Narurkar, L. B. Kotnis, B. D. Tilak, L. C. Shah and V. N. Gogte), 388
Properties (Anti-Depressant or Stimulant), of Imipramine : Demonstration of, in Experimental Animals (Dr. D. R. Maxwell and Helen T. Palmer), 84
Properties (Electrical), or Orientated Lipid on a Biological Membrane—an Electrostatic Diffusion Barrier and Ion Pump (Dr. J. W. L. Beament), 217
Properties (Electronic), of Imidazole (S. Aftergut and G. P. Brown), 379
Properties (Oncogenic and Heparin-neutralizing) : Surfen, a Quinoline with (DeWitt T. Hunter, jun., and Joseph M. Hill), 1378
Properties (Thermophysical) Research Literature : Retrieval Guide to, Vol. I (edited by Y. S. Touloukian), Book 1, Part A, Guide to Substance Classification and Numerical Codes ; Book 1, Part B, Dictionary of Synonyms and Trade Names ; Book 1, Part C, Dictionary of Substances ; Book 2, Classified Search Index ; Book 3, Master Bibliography, Author Index, *review* by Prof. A. R. Ubbelohde, 109
Property (Cultural), International Centre for the Study of the Preservation and the Restoration of (Rome) : Works and Publications, 3, Climatology and Conservation in Museums, 1050
Proposal for a Tunable Source of Radiation for the Far Infra-Red using Beats between Optical Masers (Dr. D. C. Lainé), 795
Proposed Use of Magnetic Fields in Electron Microscopic Radioautography (Lucian G. Caro), 1188
Propulsion (Aircraft), at the College of Aeronautics, Cranfield, 1042
Propylthiouracil Treatment in the Rat : Increase of the Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at Early Stages of (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171
Proskauer, E. S., and others (edited by) : Organic Analysis, Vol. 4, *review* by Dr. D. T. Lewis, 741
Prospect (A Clinical), of the Cancer Problem (Prof. D. W. Smithers), *review*, 1336
Prosser, M. V., and others : Conversion of L-Galactono-γ-lactone into L-Ascorbic Acid by Plants, 282
Protease Action (Thyroid) : Thyroglobulin—Evidence of (Dr. A. D. Litonjua), 357
Proteases (Influenza Viruses, Receptor-destroying Enzyme and) : Action of, on Blood Group Agglutinogen M^g (Prof. Georg F. Springer and Dr. Karlheinz Stalder), 187
Protection (Radiation) : Principles of (Harwell Reactor School of the Atomic Energy Research Establishment Course on), 856
Protein : Response of the Labellar Hairs of the Blowfly, *Phormia regina* Meigen, to (D. I. Wallis), 917
Protein of Cell Fractions from Myocardial Slices : Effect of Adrenalectomy and Corticosteroids on Distribution of Radioactivity in (Dr. Edward I. Weinshelbaum and Dr. Ira G. Wool), 1401
Protein in Cellular Material : Estimation of (Prof. David Racusen and D. B. Johnstone), 492
Protein in the Milk : Correlation between Growth-Rate of the Suckling of various Species and the Percentage of Total Calories from (Dr. F. W. Bernhart), 358
Protein Chemistry : Post-Doctoral Fellowships in (Division of Protein Chemistry of the Australian Commonwealth Scientific and Industrial Research Organization), 336
Protein Chemistry (including Polypeptides) : A Laboratory Manual of Analytical Methods of (edited by Dr. P. Alexander and R. J. Block), Vol. I, The Separation and Isolation of Proteins, *review* by Dr. D. T. Elmore, 320
Protein Composition (Soybean) : Ultracentrifugal Differences in (W. J. Wolf, G. E. Babcock and A. K. Smith), 1395
Protein Hydrolysates : A Simplified Method for Separating Amino-Acid Mixtures from, on One Ion-Exchange Column during Preparation (J. Liebster, K. Kopoldová and M. Dobiášová), 1198
Protein Production (Turnip Yellow Mosaic Virus) : Stimulation of, by 2-Thiouracil (Dr. R. I. B. Francki and Dr. R. E. F. Matthews), 1078
Protein Structure : Haemoglobin *F* and the Genetic Control of (Prof. V. M. Ingram), 608 ; (Dr. Wolf W. Zuelzer and Abner R. Robinson), 609
Protein Synthesis : Evidence for a New Enzyme connected with (Hans G. Boman and Ingvar Svensson), 674
Protein (Liver), *in vivo* : Incorporation of Histamine into (Isabel Wajda, Michael Ginsburg and Prof. Heinrich Waelsch), 1204
Protein (Plant) : Enzymic Conversion of Tetrahydrofolic Acid to Folinic Acid by (E. H. Byers, J. Stewart and T. J. Bond), 179
Protein (Serum) Fractions of the Newly Hatched Chick : Comparison of the, with those of Adult Birds using Starch-gel Electrophoresis (A. Amin), 708
Protein (Viral), during Multiplication of Fowl Plague Virus : Influence of *p*-Fluorophenylalanine on the Production of Viral Ribonucleic Acid and on the Utilizability of (Dr. Christoph Scholtissek and Rudolf Rott), 1023
Proteins : Amino-Acid Liberation from, by Enzyme or 'Heat Catalysed' Hydrolysis (S. M. Hardy), 1061
  Chromatography of, on Diethyl-amino-ethyl (DEAE) Cellulose Paper (C. C. Curtain), 1269
  A New Two-Stage Method for Cross-linking (Dr. Felix Borek), 1293
  Paper Electrophoresis of, while Preserving the Pattern of their Localization in Tissues (Dr. Zh. A. Medvedev), 1021
  Radiation Damage to (U. S. Kumta and A. L. Tappel), 1304
  The Separation and Isolation of (edited by Dr. P. Alexander and R. J. Block), (A Laboratory Manual of Analytical Methods of Protein Chemistry, including Polypeptides, Vol. I), *review* by Dr. D. T. Elmore, 320
Proteins in Deuterium Oxide : Ultra-violet Absorption Spectra of (Dr. G. H. Beaven and Elizabeth Bailey), 914
Proteins (Bence-Jones) : Comparison of Reduced Alkylated Derivatives of some Myeloma Globulins and (Dr. M. D. Poulik and Dr. G. M. Edelman), 1274
Proteins (Extracellular Diphtheria), from Culture Filtrates of Different Iron Concentrations : Electrophoretic and Immuno-electrophoretic Patterns of (Bertold J. Radola), 382
Proteins (Phospho-) of Mitochondria : Electron Transport System and (K. Ahmed, J. D. Judah and C. H. Gallagher), 1309
Proteins (Plasma) : Influence of, in the Control of Mitosis-rates in Generating Liver (Dr. J. T. Hemingway), 706
Proteins (Specific), in Serum and others Mixtures : Estimation of (Dr. D. A. Darcy), 1163
Prothero, R. Mansell : Effect of Population Movements on Malaria Eradication, 863
Protoplasts (Plant) : Properties of Isolated (C. G. Cocking), 780
Protoplasts (Yeast) : Fixation of, for Electron Microscopy (Dr. P. F. Elbers), 1022
Protozoa : Immunity to (British Society for Immunology Symposium on), 551
Protozoon : A Much-studied, *review* by Dr. J. A. Kitching, 1133
Proximal Tubules (Single Perfused), of Rat Kidneys : Comparison of Short-Circuit Current and Net Water Movement in (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Pryce, Prof. M. H. L. : Wave Propagation and Group Velocity, *review*, 104
Pryor, A. W., and others : Scattering of Long Wave-Length Neutrons by Irradiated Beryllium Oxide, 1385
Pseudomonas (*Xanthomonas* and) : Taxonomy of (R. R. Colwell and Prof. J. Liston), 617
Psyche : The Structure and Dynamics of the (C. G. Jung), translated by R. F. C. Hull, *review* by Dr. C. Allen, 1235

Psychiatry and Mental Health : Teaching of (United Nations, World Health Organization, Public Health Papers No. 9), 1248
Psychological Function : Cerebral Dominance and its relation to (Prof. O. L. Zangwill), *review* by Sir Russell Brain, 840
Psychology : Jungian, *review* by Dr. C. Allen, 1235
Psychology : Problems of, Nos. 1 and 2, (translations from the Russian *Voprosy Psikhologii*), 958
Psychology of War: Mathematical Studies in the, *review* by Sir Cyril Burt, 427
Psychophysiology, Neuropharmacology and Biochemistry of Audiogenic Seizure (Centre National de la Recherche Scientifique International Meeting on), 959
Pteridium aquilinum : Failure of Nucleotides to Diffuse Freely into the Embryo of (P. R. Bell), 91
Public Exhibitions : Sources of Ionizing Radiation at, 1249
Public Health Service (United States) : Research Grants in Radiological Health, 442
Public Health Services : The Planning of (United Nations, World Health Organization, Technical Report Series No. 215—Fourth Report of the Expert Committee on Public Health Administration), 441
Public Investment and Export (Sir Walter Benton Jones), 334
Public Library (South African) : Grey Bibliographies No. 7, A Bibliography of African Bibliographies, covering the Territories South of the Sahara, fourth edition, 959
Publication : Purpose in, 527 ; *erratum*, 1250
Publication of Scientific Books (Scientific Publications Council Meeting on), 131
Publications (Research), on Farm Buildings : Bibliography of, Section 5, Buildings for the Drying and Storage of Grain (Agricultural Research Council), 440
Published Information: The Retrieval of, *review* by Prof. A. R. Ubbelohde, 109
Publisher : Author and (Dr. D. Richter), 131
Puerto Rican Family : The Diego Factor in a—a Case of Anti-Diego in Monozygotic Twins (Dr. Hubert J. Van Peenen, Dr. John Scudder, James A. Jack and Erika Awer), 188
Pugh, H. Ll. D., and others : Synthesis and X-Ray Analysis of Diamond, 865
Pugh, Dr. L. G. C. E. : Science in the Himalaya, 429
Pulmonary Tumours (Induced), in Mice : Inheritance of Susceptibility to (Dr. D. S. Falconer and Dr. Joyce L. Bloom), 1070
Pulmonary Tumours and Group VII in the House-Mouse (Dr. Joyce L. Bloom), 1124 ; (Dr. J. A. H. Tatchell), 1125
Pulp and Paper—Chemistry and Chemical Technology (James P. Casey), Vol. 2, Papermaking, second edition, *review* by Dr. Julius Grant, 1135
Pulp and Paper Manufacture : A Laboratory Handbook of (Dr. Julius Grant), second edition, *review* by Dr. F. Lyth Hudson, 320
Pulpwood : Management of Pine Stands for (J. J. Kotzé), 855
Pulsating Elastic Tube : Fatigue in a Fluid-filled (Group-Capt. John Erskine Malcolm), 1012
Pulse-shortening in Electron Linear Accelerators (Dr. M. C. Crowley-Milling, T. R. Jarvis, C. W. Miller and G. Saxon), 483
Pump (Ion): Electrical Properties of Orientated Lipid on a Biological Membrane—an Electrostatic Diffusion Barrier and (Dr. J. W. L. Beament), 217
Pupation and Oviposition in *Nepytia phantasmaria* Stkr. (Lepidoptera : Geometridae): Influence of Electrical Field on (Dr. D. K. Edwards), 976
Puranik, Prof. P. G., and Ramiah, K. Venkata : A New Relation between the N–H Stretching Frequencies of Primary Amides, 796
Pure Mathematics at Aberystwyth, 333
Purine and Pyrimidine Metabolism in *Rhodospirillum rubrum* (Prof. T. W. Goodwin and Dr. P. N. A. Passorn), 276
Purpose in Publication, 527 ; *erratum*, 1250
Purposes (Collection) : Use of Glacial Acetic Acid for Killing Parasitic Nematodes for (Bjørn Berland), 1320
Purseglove, Prof. J. W. : Plant Exploration and Introduction (Not all Cultivated Plants have necessarily arisen in the Eight Independent Centres of Origin recognised by Vavilov), 1240
Pyrene : Molecular Geometry of a 1:1 Crystalline Complex between 1,3,7,9-Tetramethyluric Acid and (F. De Santis, E. Giglio, Dr. A. M. Liquori and A. Ripamonti), 900
Pyrene Dimers (Isadore B. Berlman), 594
Pyrethrin-resistance in Houseflies : Pattern of (B. C. Fine), 884
Pyrethrins : Biosynthesis of the (Dr. Mary P. Crowley, Helene S. Inglis, M. Snarey and Dr. E. M. Thain), 281
Pyridine and its Derivatives (edited by Erwin Klingsberg), *review* by Dr. G. L. Buchanan, 425
Pyridine Derivatives with Anthelmintic Activity (A. P. Phillips and R. B. Burrows), 707
Pyridine Nucleotides in the Liver and in an Ascitic Hepatoma : Comparison of (M. Wintzerith N. Klein, L. Mandel and Prof. P. Mandel), 467
Pyrimidine Metabolism (Purine and), in *Rhodospirillum rubrum* (Prof. T. W. Goodwin and Dr. P. N. A. Passorn), 276
Pyrimidine-Nucleosides (Tritiated) : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of, into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Pyrogallol Oxidations : Regulation of, by Alcohols and Sugars (B. Z. Sigel, S. M. Siegel and N. S. Goodman), 180
Pyrolysis of Polymers : Differential Thermogravimetry versus Differential Thermometry in Studying the (Hugh C. Anderson), 1088
Pyrolysis and Gas–Liquid Chromatography on the Microgram Scale (C. E. Roland Jones and A. F. Moyles), 663
Pyrophosphate : Determination of Uranium as (Dr. F. M. Brewer), 1290 ; (J. S. Wright, T. J. Hayes and J. A. Ryan), 1291
Pyrophosphate (5,10,15 ^{14}C-Farnesol) : Incorporation of, into *Phycomyces carotenoids* (H. Yamamoto, H. Yokoyama, K. Simpson, T. O. M. Nakayama and C. O. Chichester), 1299
Pyrrhotite : Formation of Chalcopyrite by Reaction between Chalcocite and, in Cold Solution (W. M. B. Roberts), 560
Pyrrolase (Tryptophan-) : Effect of 5-Fluorouracil on Hepatic Induced Increase of (Sancia Gaetani and M. Antonietta Spadoni), 1296
Pythium debaryanum Hesse : Meiosis in Oogonium and Antheridium of (Dr. Eva Sansome), 827

QASIM, Dr. S. Z., and Hasan, R. : A Hydrostatic Function of the Accessory Respiratory Organs in Air-breathing Fishes, 396
Quagliariello, Prof. E., and others : Blockage of Transformation of Tryptophan to Nicotinic Acid by Experimental Hepatitis in Mice, 601
Quality Control : Handbook of Textile Testing and (Prof. Elliot B. Grover and Prof. D. S. Hamby), *review* by W. J. Onions, 106
Quality Control (Statistical), and Acceptance Sampling (Birmingham College of Advanced Technology Course on), 337
Quality Measurement in the Chemical Factory (A. J. Clark and C. W. Munday), (Department of Scientific and Industrial Research), 752
Quantal Neutralization Tests : Evaluation of (Prof. S. Fazekas de St. Groth), 891
Quantitative Analysis of Milk and other Emulsions by Infra-Red Absorption (Dr. J. D. S. Goulden), 905
Quantitative Determination of Metadrenaline and Normetadrenaline in Normal Human Urine (Dr. Kaoru Yoshinaga, Dr. Chuichi Itoh, Dr. Nozomu Ishida, Dr. Tatsuo Sato and Dr. Yasuo Wada), 599
Quantitative Estimation of Peak Areas in Gas–Liquid Chromatography (Dr. K. K. Carroll), 377
Quantitative Estimation of Phosphatases (Phosphatases in *Schistosoma mansoni*), (Dr. D. L. H. Robinson), 473
Quantities (Electrical and Magnetic) : Dimensions of (Dr. A. E. Martin), 588
Quantum Statistical Illustrations : Thermodynamics with (Prof. P. T. Landsberg), *review* by Dr. W. E. Parry, 1236
Quarrels (Deadly) : Statistics of (Dr. Lewis F. Richardson), edited by Quincy Wright and C. C. Lienau, *review* by Sir Cyril Burt, 427
Quaternary History : Two Unexplored Records of (A. H. Stride and R. Bowers), 1000
Queen Mary College, London : Appointment of Dr. F. G. A. Stone to the University of London readership in inorganic chemistry, 229
    Title of reader in pure mathematics conferred on Dr. K. W. Gruenberg, 546, 651
Queen Victoria Hospital, East Grinstead : Appointment of Dr. Morten Simonsen as director of research at the McIndoe Memorial Research Unit, 338
Queen's College (Mathematics Department), St. Andrews, in collaboration with Standard Telephones and Cables, Ltd. : Symposium on Digital Computers, their Use and Control, 554
Queen's University, Belfast : Appointment of Dr. A. Dalgarno to a chair of quantum mechanics, 19
    Appointment of Dr. E. A. Cheeseman to a personal chair of medical statistics, 20
Quelea quelea (Weaver-Finches) : Melanism in Captive (Dr. B. Lofts), 993
Quenching of Cation Vacancies in Doped Crystals of Sodium Chloride (the late Sir K. S. Krishnan and S. C. Jain), 162
Quenouille, M. H. : Rapid Statistical Calculations, *review* by M. J. R. Healy, 1236
Quesnel, L. B. : Papers on Bacterial Genetics, *review*, 320
    Papers on Bacterial Viruses, *review*, 320
Queueing : Mathematical Methods in the Theory of (A. Y. Khintchine), translated by D. M. Andrews and M. H. Quenouille, *review* by Prof. L. S. Goddard, 211
    Mathematics and, *review* by Prof. L. S. Goddard, 211
Quick Methods (Simple,), for Detection of 6-Amino-penicillanic Acid (J. Uri and F. Sztaricskai), 1223
'Quick' and 'Catch' Adductor Muscles in Brachiopods (Dr. M. J. S. Rudwick), 1021
Quilliam, J. P. : Appointed to the chair of pharmacology at St. Bartholomew's Hospital Medical College, London, 1349
Quilliam, Dr. T. Andrew (Armstrong, J., and) : Nerve Endings in the Mole's Snout, 1379
Quinoline with Oncogenic and Heparin-neutralizing Properties : Surfen, a (DeWitt, T. Hunter, jun., and Joseph M. Hill), 1378
Quinones in Electron Transport (Ciba Foundation Symposium on), (edited by G. E. W. Wolstenholme and Cecilia M. O'Connor), *review* by A. I. Scott, 321
Quirk, Dr. J. P., and others : Role of Polysaccharides in Stabilization of Natural Soil Aggregates, 1283
Quokka (Rottnest), *Setonix brachyurus* : Myopathy affecting the, reversed by α-Tocopherol (B. A. Kakulas), 402

RABBIT Erythrocytes : Recovery and *in vivo* Survival of (Dr. G. F. Doebbler, R. G. Buchheit and A. P. Rinfret), 1405
Rabbits : Antibody Response in, following Injection of Sheep Erythrocytes into Lateral Ventricle of Brain (Prof. B. D. Janković, Dr. M. Draškoci and Dr. K. Isaković), 288
    Effect of Bee Venom on Concentration of Blood Glucose and Liver Glycogen in (Prof. A. H. Mohamed and K. Zaki), 605
    Effect of Polyvinyl Alcohol on the Distribution of Iodine-131 in the Internal Organs, Fluids and Excreta of (H. F. Nowak and J. Kucharski), 665, *erratum*, 1045
    Effects of Carrageenin on Serum Lipids and Atherosclerosis in (Dr. Katsumi Murata), 189
Rabbits (Normal) : Effects of Injections of some Intermediary Metabolites on the 'Insulin Response' of (Dr. H. D. Brahmachari and G. Raghupathy Sarma), 491, *erratum*, 755
Rabinovitch, S. G. : At the Second International Measurement Conference (Electrical Measuring Instruments based on the Photo-compensating Amplifier), 1351
Races (British Insular), of Small Mammals : Origin of the, and of the 'Lusitanian' Fauna (G. B. Corbet), 1037
Racinskij, Prof. V. V. : Paper Chromatography (Chromatographic Separation in terms of the General Theory of Sorption Dynamics), 759
Racoveanu, Prof. N. : At the Second International Measurement Conference (A Novel Bi-parametric Displacement Transducer), 1351
Racusen, Prof. David, and Johnstone, D. B. : Estimation of Protein in Cellular Material, 492
Radar to Spectroscopy : From—New Regions of the Spectrum (Prof. D. J. E. Ingram), 1146
Radar Establishment (Royal), Malvern : Appointment of Dr. G. G. Macfarlane as director, 1145
Radar Reflectivities of Hail Samples (R. H. Douglas), 266
Radiant Heat Shocks : Mutagenic Effect of, on Phanerogamous Plants (Dr. Bror Pettersson), 1167
Radiation : Comparative Analysis of Inactivating Efficiency of, on Different Organisms (Dr. M. Terzi), 461
    Polarization induced by, in 'Perspex' (Dr. V. J. Vanhuyse, G. J. Vanpraet and J. F. Van Landuyt), 595

Radiation for the Far Infra-Red : A Proposal for a Tunable Source of, using Beats between Optical Masers (Dr. D. C. Lainé), 795
Radiation Incorporated, and others (sponsored by) : A Symposium on Space Trajectories (edited by the Technical Staff, Research Division, Radiation Incorporated), *review* by D. G. King-Hele, 1334
Radiation-induced Formation and Reactions of the Formyl Radical Ion (John Holian, Dr. George Scholes and Prof. Joseph J. Weiss), 1386
Radiation (Fluorescence) : Average Extinction Coefficient for Re-absorption of, through Uranin Solution (Dr. K. K. Rohatgi and G. S. Singhal), 799
Radiation (Ionizing) : Effects of, on the Human Haematopoietic System—Report of the Subcommittee on Haematologic Effects, Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 875), 556
  Effects of, on Organic and Biological Solids (Société de Chimie Physique and the British Biophysical Society Discussion on), 1150
Radiation Chemistry of Organic Compounds (Dr. A. J. Swallow), *review* by Dr. S. J. Thomson, 317
Radiation Damage to Proteins (U. S. Kumta and A. L. Tappel), 1304
Radiation Protection : Principles of (Harwell Reactor School of the Atomic Energy Research Establishment Course on), 856
Radiation Sensitivity of *Escherichia coli* to β-Rays in Dry and Wet Condition (S. B. Bhattacharjee and N. N. Das Gupta), 1015
Radiations (Atomic), and Living Organisms (R. H. Mole), 556
Radiations (Ionizing), at Exhibitions : Code of Practice for the Display of Sources of, 1249
Radiations (Ionizing), from External Sources : Long-Term Effects of—Report of the Subcommittee on, of the Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 849), 556
Radical Chain Reactions in Solution : Generation of Radicals in Pairs and the Kinetics of (Dr. P. E. M. Allen and C. R. Patrick), 1194
Radical Ion (Formyl) : Radiation-induced Formation and Reactions of the (John Holian, Dr. George Scholes and Prof. Joseph J. Weiss), 1386
Radicals in Pairs : Generation of, and the Kinetics of Radical Chain Reactions in Solution (Dr. P. E. M. Allen and C. R. Patrick), 1194
Radicals (Free) : Isoelectronic Principle, Thermochemistry and Reactivity of related (Dr. Peter Gray and J. C. J. Thynne), 1357
  Spatial Distribution of, as studied by Electron Spin Resonance (Robert C. Smith and S. J. Wyard), 897
Radio Communication (Long-Range), by Satellite Microwave Dipoles—the West Ford Project, 1237
Radio Emission (Galactic) : Spectrum of (Dr. F. Graham Smith), 1381
Radio Engineers, British Institution of : Grant of a Charter to the, 651
Radio Research 1960—the Report of the Radio Research Board and the Report of the Director of Radio Research (Department of Scientific and Industrial Research), 1255
Radio Research Board : Radio Research 1960—the Report of the, and the Report of the Director of Radio Research (Department of Scientific and Industrial Research), 1255
Radio Research Station, Slough : Open Days, 231
Radio Signals : Effect of External Conditions on the Phase of (D. E. Watt-Carter and R. L. Corke), 1286
Radio Source : Lunar Occultation of a (Dr. C. Hazard), 58
Radio Source Centaurus *A* : Central Component of the (P. Maltby), 793
Radio Sources (Extragalactic) : Distribution of Brightness in (A. T. Moffet and P. Maltby), 453
Radio Spectroscopy of Solids (Institute of Physics and the Physical Society and the British Radio Spectroscopy Group Joint Conference on), 126
Radio Spectroscopy Group, British (Institutute of Physics and the Physical Society and the): Joint Conference on Radio Spectroscopy of Solids, 126
Radio Stars : Apparent Recession of Nebulae and the Nature of (Dr. N. S. Japolsky), 480
Radio Telescope (Benelux) : Cross-Antenna of the Proposed (Prof. W. N. Christiansen and Dr. J. A. Högbom), 215
Radioactive Materials : Introductory Manual on the Control of Health Hazards from (Medical Research Council, Memorandum No. 39), new edition, 1257
Radioactive Particles (Inhaled) : Effects of—Report of the Subcommittee on Inhalation Hazards, Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 848), 556
Radioactive Substances : Safe Handling of, 1257
Radioactive Tracers in Chemistry and Industry (Pascaline Daudel), translated by Dr. Ulli Eisner, *review* by Prof. J. R. Partington, 637
Radioactivity in Protein of Cell Fractions from Myocardial Slices : Effect of Adrenalectomy and Corticosteroids on Distribution of (Dr. Edward I. Weinshelbaum and Dr. Ira G. Wool), 1401
Radioactivity in the Sea : Research on the Effects of, 122
Radioactivity (Beryllium-7 and Caesium-137), in Ground-Level Air : Comparison of (Dr. P. F. Gustafson, M. A. Kerrigan and S. S. Brar), 454
Radioactivity (Natural), in Tobacco and Tobacco Smoke (Dr. V. C. Runeckles), 322
Radioautography (Electron Microscopic) : Proposed Use of Magnetic Fields in (Lucien G. Caro), 1188
Radiobiology : Use of Animal Cell, Tissue and Organ Cultures in (New York Academy of Sciences Symposium on), 340
Radiocaesium and Potassium in Norwegians (Johan Baarli, Kjell Madshus, Kurt Lidén and Richard C. McCall), 436
Radioisotopes : The Use of, in Animal Biology and the Medical Sciences (International Atomic Energy Agency, the Food and Agriculture Organization and the World Health Organization of the United Nations, Conference on), 959
  Use of, in Biological Work in Burma (Dr. Maung Mya Thaung), 875
Radiological Health : Research Grants in (United States Public Health Service), 442
Radiological Units and Measurements : Report of the International Commission on (United States National Bureau of Standards, Handbook No. 78), 28
Radiology and Nuclear Medicine : Fellowships and Grants in the field of, 1962-63 (James Picker Foundation), 228
Radiolytic Oxidation of Ferrous Solutions with Standardized Internal Sources of Polonium-210 (J. Steyn and D. van As), 903
Radionuclides (Internally Deposited) : Removal of, by Triethylenetetramine-hexaacetic Acid (Prof. A. Catsch and D. Schindewolf-Jordan), 715
Radiostrontium : Assessment of Chronic Exposure to, by Urinary Assay (Dr. C. L. Comar and Jay Georgi), 390
Radley, Sir Gordon : World Communications (Presidential Address to Section G (Engineering) of the British Association for the Advancement of Science), 987
Radley, Margaret : Gibberellin-like Substances in Plants—Leguminous Root Nodules, 684 ; Seaweed (*Fucus vesiculosus*), 685
Radley, R. W., and others : Tuber Bulking in the Potato Crop, 782
Rado, Prof. R. : *Obituary* of Prof. E. H. Neville, 1345
Radola, Bertold J. : Electrophoretic and Immuno-electrophoretic Patterns of Extracellular Diphtheria Proteins from Culture Filtrates of Different Iron Concentrations, 382
Radoslovich, E. W. : Surface Symmetry and Cell Dimensions of Layer Lattice Silicates, 67
Radouco-Thomas, Dr. C. : Assessment of Pain (Testing of Analgesic Drugs on Animals), 1354
Rafelson, jun., Prof. Max E., and others: Action of Neuraminidase on Haptoglobin, 279
Rafelson, Max E., and others : Polarographic Examination of Sialic Acids, 596
Ragge, David R. : Cockroach Ecology, *review*, 106
Raibaud, Dr. P., and others : Bacteria of the Intestine (Studies on the Bacterial Flora in the Alimentary Tract of Pigs, 2, Streptococci : Selective Enumeration and Differentiation of the Dominant Groups), 1152
Railways in Rural Areas, 855
Raindrops : Wettability and the Capture, Carriage and Deposition of Particles by (R. R. Davies), 616
Rainfall (Light), and Plant Survival—Measurement of Stem Flow Run-off (M. D. Gwynne and J. Glover), 1321
Rainwater (New Zealand) : Barium-140 in (G. S. McNaughton and R. N. Woodward), 1344
Rajasthan Sheep Breeds : Composition of the Skin Follicle Population in seven (Sunanda Narayan), 1115
Rajewsky, Prof. B. : Effects of Ionizing Radiation on Organic and Biological Solids (Radicals produced on Irradiation of Keratin), 1151
Rama, and others : Iron-55 from Nuclear Detonations, 162
Ramachandran, E. G., and Dasarathy, C. : Transformation of Cathodically Impregnated and Cooled Austenite, 998
Ramachandran, Dr. V. S., and others : Basic Dyestuffs in Clay Mineralogy, 696
Ramiak, K. Venkata (Puranik, Prof. P. G., and): A New Relation between the N–H Stretching Frequencies of Primary Amides, 796
Ramsay, Dr. J. A., and Riegel, Dr. J. A. : Excretion of Inulin by Malpighian Tubules, 1115
Ramsay Memorial Trust : Awards of new Fellowships in Chemistry for 1961-62, 337
Randomness (Non-), in Amino-Acid 'Alleles' (Richard V. Eck), 1284
Rang, H. P. : Given the first award of the J. H. Burn fellowship (for 1961-62) in the Department of Pharmacology, University of Oxford, 229
Rangappa, Dr. K. S. : Cryoscopic Effect of Separation and Concentration of Solutes, 1087
Rangarajan, C., and others : Seasonal Variations of Caesium-137 in Air at Ground-Level, 747
Rankin, jun., J. S., and others : Gross Beta-Activity in the Thames River (Conn.), 1017
Rao, Prof. B. Ramachandra (Padmini, P. R. K. L., and): Molar Sound Velocity in Molten Hydrated Salts, 694
Rao, Prof. C. Radhakrishna : A Combinatorial Assignment Problem, 100
Rao, M. G. Seshagiri, and Rao, Prof. R. Ramachandra : Ultrasonic Velocity in Aqueous Solutions of some Electrolytes, 164
Rao, Prof. R. Ramachandra (Rao, M. G. Seshagiri, and) : Ultrasonic Velocity in Aqueous Solutions of some Electrolytes, 164
Raper, Dr. A. B. (Fraser, I. D., and) : Solubility of Denatured Haemoglobin Variants in Acid Buffers, 355
Rapid Determination of Ytterbium in Aqueous Solutions and in Thulium Oxide by Neutron Activation (Minoru Okada), 1090
Rapid Development of Hypomagnesaemia in Lactating Cows given Artificial Rations Low in Magnesium (J. A. F. Rook), 1019
Rapid Infra-Red Determination of Acetone in the Blood and the Exhaled Air of Diabetic Patients (Dr. Richard D. Stewart, Edward A. Boettner and Bruce T. Stubbs), 1008
Rapid Statistical Calculations (M. H. Quenouille), *review* by M. J. R. Healy, 1236
Raschke, K. : Nematology (Reduction of Hatching from *Heterodera* Cysts after Treatment with 'Trapex' due to Death of Larvae), 1049
Rashbass, Cyril (Westheimer, Gerald, and): Barbiturates and Eye Vergence, 833
Rashevsky, Nicolas, and Trucco, Ernesto (edited by) : Arms and Security, a Mathematical Study of the Causes and Origins of War (Dr. Lewis F. Richardson), *review* by Sir Cyril Burt, 427
Rasmusen, Dr. B. A. : Blood Groups in Infra-human Species (Blood Group Systems of Sheep), 655
Raspberry : Mutation for Larger Fruit in the (D. L. Jennings), 302
Rastogi, R. P., and others : Electrical Effects during Dissolution and Precipitation of Electrolytes, 764
Rat : Biological Incorporation of Implant Material in the (James Calnan), 1017
  Effect of Serotonin on Absorption and Distribution of Calcium-45 in the (Prof. S. Garattini, E. Grossi, P. Paoletti, R. Paoletti and M. Poggi), 185
  Evidence of a 'Hunger Motivational' System in the Lateral Hypothalamus of the (Dr. P. J. Morgane), 672
  Increase of the Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at Early Stages of Propylthiouracil Treatment in the (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171
  Leucine Aminopeptidase in the Mammary Gland of the Cow and (Prof. S. Talanti and Prof. Väinö K. Hopsu), 86
  Release of Heparin from the Mast Cells of the (G. T. Archer), 90
  Role of the Lung in Metabolism of Fat during Ontogeny of the (Dr. Z. Vacek, P. Hahn and O. Koldovsky), 85
Rat during 'Metrazol' Convulsions : Changes in the Pentose-Nucleic Acid Content of Sub-Cellular Fractions of the Brain of the (Prof. G. P. Talwar, B. Sadasividu and V. S. Chitre), 1007
Rat Bladders : Incorporation of Phosphorus-32 into Urinary Concretions forming on Zinc Implants in (Dr. Richard F. Keeler and Dr. Stuart A. Lovelace), 715

Rat Brain (Living) : Urea Synthesis in the (R. K. Davies, A. J. Defalco, D. Shander, A. Kopelman and Prof. J. Kiyasu), 288

Rat Flea (Female), *Xenopsylla cheopis* Roths. : An Abnormal Form of (M. I. D. Sharma and G. C. Joshi), 727

Rat Hepatic Cells (Normal) : Nucleolar Localization of Succinic Dehydrogenase in (P. De, R. Chatterjee and Dr. S. Mitra), 925

Rat Kidneys : Comparison of Short-Circuit Current and Net Water Movement in Single Perfused Proximal Tubules of (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205

Rat Lens Enzyme System : Catalysis of Incorporation of ^{14}C-Uridine into Rat Lens Ribonucleic Acid by (Dr. Anima Devi and Dr. N. K. Sarkar), 1094

Rat Lens Ribonucleic Acid : Catalysis of Incorporation of ^{14}C-Uridine into, by Rat Lens Enzyme System (Dr. Anima Devi and Dr. N. K. Sarkar), 1094

Rat Liver : Effect of Adrenalectomy on the Activity of Enzymes of the Urea Cycle in (Patricia McLean), 1302

Rat Liver Glutamic-Oxalacetic Transaminase : Intracellular Distribution Pattern of (Dr. Herbert J. Eichel and Josef Bukovsky), 243

Rate of Dark-Fixation of Carbon Dioxide in Leaves of *Bryophyllum fedtschenkoi* : An Endogenous Rhythm in the (Diana M. Warren and Malcolm B. Wilkins), 686

Rate of Iron Oxidation : Morphological Variation in *Ferrobacillus ferrooxidans* related to the (M. P. Silverman and M. H. Rogoff), 1221

Rate of Lesion Production on Pinto Bean : Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the (John Piacitelli and Prof. Vincent Santilli), 624

Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at Early Stages of Propylthiouracil Treatment in the Rat : Increase of the (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171

Rate of Utilization of Glucose : Effect of Thyroxine and 2,4-Dinitrophenol on the (Dr. L. Macho), 604

Rate, and Primary Products, of Reactive Bimolecular Collisions : A Molecular Beam Reactor for Examining the (C. W. Nutt and A. J. Biddlestone), 798

Rate (Expected), of Senescence and Age-dependent Mortality in Fish (Dr. Alex Comfort), 822

Rating and Valuation Bill : Scientific Societies and the, 123

Ratio (Sex-), in Mice (Margaret J. Cook and Augustin Vlček), 89

Ratio (Signal-Noise) : Possible Method for Increasing the, in the Detection of the First Motion of a Refracted *P*-Wave (F. A. Key, J. K. Wright, E. W. Carpenter and B. M. H. Stott), 1382

Ratio (Television Signal-to-Noise) : An Instrument for measuring (S. M. Edwardson), 1355

Rations (Artificial), Low in Magnesium : Rapid Development of Hypomagnesaemia in Lactating Cows given (J. A. F. Rook), 1019

Ratios (Caesium-137/Potassium) : Determination of, in Diet and in the Human Body by Urine Analysis (A. Morgan and Gloria M. Arkell), 1100

Rats : Anaphylactoid Reaction in (J. M. Harris and Dr. G. B. West), 399

Rats under Normal Feeding Conditions : Soluble Material in the Gastro-intestinal Tract of (Dr. Anne S. Cole), 502

Rats bearing Transplantable Acute Myeloid Leukaemia : Effect of Calf Thymus Histone on (Dr. Lubomir Hnilica and Dr. Viktor Holoubek), 922

Rats (New-born and Adult) : Collagen Fractions in the Skin of (Nestor Gonzalez Cadavid, Berta Denduchis and Roberto E. Mancini), 1297

Rats (Normal Adult) : Stimulation by Heparin of Parenchymal Liver Cell Proliferation in (Dr. M. Zimmerman and Evemarie Celozzi), 1014

Rats (Suckled and Unsuckled Female) : Induction of Cancer in (Prof. Giuliano Briziarelli), 921

Rats (White Laboratory) : Leptospirosis of (Dr. M. Füzi and R. Csóka), 1123

Rauer, K., and others : Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of *Penicillium chrysogenum*, 909

Rausen, A. R., and others : Haptoglobin Patterns in Cord Blood Serums, 717

Raven, D. J. (Earland, Dr. C., and) : Lanthionine Formation in Keratin, 384

Rawlins, F. I. G. : An Appraisal of Phenomenology, *review*, 5

γ-Ray Counting Method : Measurement of the Thorium Content of Natural Materials by a (Prof. J. H. J. Poole and F. N. Byrne), 62

Rayner, R. W. : Spore Liberation and Dispersal of Coffee Rust *Hemileia vastatrix* B. et Br., 725

β-Rays in Dry and Wet Condition : Radiation Sensitivity of *Escherichia coli* to (S. B. Bhattacharjee and N. N. Das Gupta), 1015

Rays (Cosmic) : Fission and Spallation Phenomena and their Application to (European Organization for Nuclear Research and the Max Planck Institute, Heidelberg, Conference on), 24

Re-absorption of Fluorescence Radiation through Uranin Solution : Average Extinction Coefficient for (Dr. K. K. Rohatgi and G. S. Singhal), 799

Re-entry Trajectories : Space-Flight and (International Academy of Astronautics Symposium on), 960

Re-interpretation of Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogenesis : Tissue Dilution Artefact—a (G. R. N. Jones, L. Bitensky, J. Chayen and Prof. G. J. Cunningham), 1203

Reaction : A New Inorganic—Nitric Acid–Hydrazoic Acid (Dr. J. Mašek), 166

Reaction between Chalcocite and Pyrrhotite in Cold Solution : Formation of Chalcopyrite by (W. M. B. Roberts), 560

Reaction of Ethane with a Clean Rhodium Film (Dr. Richard W. Roberts), 170

Reaction (Anaphylactoid), in Rats (J. M. Harris and Dr. G. B. West), 399

Reaction (Basophil) : A New Test for Detecting Anaphylactic Sensitivity—the (Prof. Walter B. Shelley and Lennart Juhlin), 1056

Reaction (Hill) : Photophosphorylation and the (Mary Stiller and Prof. Birgit Vennesland), 677 ; (Dr. N. E. Good), 678 ; (Dr. André T. Jagendorf), 679

Reaction (Nickel), and Thermal Isomerization of Alpha-Keto-Acid Hydrazones : Application of the, for their Chromatographic Analysis (A. M. Tokushige and Dean E. S. Cook), 797

Reaction (Staining), of the Pit Membrane of Wood Cells (R. K. Bamber), 409

Reaction Time and Age ('Alpha' Rhythm) : Frequency of the (Dr. Walter W. Surwillo), 823

Reaction Times (Simple and Serial) : Relationship between (Prof. H. Kay and Dr. A. D. Weiss), 790

Reactions of the Formyl Radical Ion : Radiation-induced Formation and (John Holian, Dr. George Scholes and Prof. Joseph J. Weiss), 1386

Reactions of Haemoproteins with Hydrogen Peroxide and the Supposed Formation of Hydrogen Peroxide during the Autoxidation of Haemoglobin (Prof. D. Keilin), 769

Reactions of Salt Solutions and Calcium Chloride on Mortar and Concrete, 125, *erratum*, 1145

Reactions (Gas-Phase), of the Halogens and their Compounds (Dr. J. H. Thomas and Dr. E. Whittle), 129

Reactions (Oxidation-Reduction), of Plastoquinone in Isolated Chloroplasts (Dr. E. R. Redfearn and J. Friend), 806

Reactions (Photosensitivity) : Mast Cells in (V. J. McGovern), 90

Reactions (Radical Chain), in Solution : Generation of Radicals in Pairs and the Kinetics of (Dr. P. E. M. Allen and C. R. Patrick), 1194

Reactions (Recoil-Atom), at Low Temperature (Slobodanka Veljković and Dr. Garman Harbottle), 1287

Reactions (Side-), during Hydrogenolysis of 2,4-Dinitrophenylhydrazones of Aromatic Keto-Acids (Dr. John H. Menkes), 285

Reactivation of Chymotrypsin : Inactivation and (T. Therattil Antony), 178

Reactive Bimolecular Collisions : A Molecular Beam Reactor for Examining the Rate, and Primary Products, of (C. W. Nutt and A. J. Biddlestone), 798

Reactivity of Calcium Sulphate Hemihydrate (M. J. Ridge, H. Surkevicius and Angela A. Milne), 704

Reactivity of related Free Radicals : Isoelectronic Principle, Thermochemistry and (Dr. Peter Gray and J. C. J. Thynne), 1357

Reactivity of Human Red Cells to Antiglobulin Tests : A Relation between the, and their Surface Structure (Judith E. Davis, Prof. H. N. Green and Pamela W. Tymms), 923

Reactor Centrum Nederland : Appointment of Johannes Pelser as technical managing director, 1042

Reactor (Gas-cooled), at the Windscale Factory of the United Kingdom Atomic Energy Authority : Progress on the Advanced, 853

Reactor (A Molecular Beam), for Examining the Rate, and Primary Products, of Reactive Bimolecular Collisions (C. W. Nutt and A. J. Biddlestone), 798

Reactor (Nuclear) Calculations : Digital Computers and (Ward C. Sangren), *review* by Dr. M. V. Wilkes, 9

Reactor (Nuclear) Chemistry (Oak Ridge National Laboratory Second Conference on), 651

Reactor (Nuclear) Technology : Analytical Chemistry in (Oak Ridge National Laboratory Fifth Conference on), 651

Read, Prof. C. : Parasite Biology (The Host–Parasite Interface—Molecular Basis of Parasitism), 134

Read, L. M. : Appointed chairman of the Council of the Plastics Institute, 1145

Reagent (Girard *T*), for Carbonyls (A. M. Gaddis, Rex Ellis and G. T. Currie), 1391

Reagents (Organic), in Inorganic Analysis : The Use of (A. I. Busev and N. G. Polianskii), translated from the Russian by J. T. Greaves, *review* by Dr. D. T. Lewis, 1336

Rearrangements (Chromosome), induced by X-Rays in Immature Germ Cells of *Drosophila* (Seymour Abrahamson), 523

Recalde, Dr. Fabian : Appointed a nutrition officer on the staff of the Food and Agriculture Organization of the United Nations, 1045

Recaldin, Dr. D. : A Reference Book for Biochemists, *review*, 635

Reception (Sound), by Insects : Sound Production and (Mable Frings and Hubert Frings), *review* by Prof. V. B. Wigglesworth, 428

Receptor-destroying Enzyme and Proteases (Influenza Viruses,) : Action of, on Blood Group Agglutinogen M^g (Prof. Georg F. Springer and Dr. Karlheinz Stalder), 187

Receptors : Odour (M. H. Briggs and R. B. Duncan), 1310

Receptors in the Sensory Vesicle of the Ascidian Tadpole : Electron Microscope Observations of the (Noël Dilly), 786

Receptors (Tryptamine), in the Central Nervous System (Dr. J. R. Vane, Dr. H. O. J. Collier and Dr. S. J. Corne, and Dr. E. Marley and Dr. P. B. Bradley), 1068

Recession (Apparent), of Nebulae and the Nature of Radio Stars (Dr. N. S. Japolsky), 480

Recherche Scientifique, Centre National de la : International Meeting on Psychophysiology, Neuropharmacology and Biochemistry of Audiogenic Seizure, 959

Recherche Scientifique, Fonds National de la : Trente-troisième Rapport Annuel, 441

Reckel, Dr. R. P. (Scheinberg, Dr. S. L., and) : Blood Groups in Infra-human Species (Red Cell Agglutinogen of Chickens identified by certain Plant Seed Extracts), 654

Recognition : Optical Character (Information Systems Branch of the Office of Naval Research Information Centre of the United States National Bureau of Standards Symposium on), 1145

Recognized Sign (Newly), of Denervation : Muscle Target Fibres, a (Dr. W. K. Engel), 389

Recoil-Atom Reactions at Low Temperature (Slobodanka Veljković and Dr. Garman Harbottle), 1287

Recombination in the *X*-Chromosome of *Drosophila melanogaster* (C. N. Law), 1180

Records (Two Unexplored), of Quaternary History (A. H. Stride and R. Bowers), 1000

Recourt, Dr. A., and de Vries, G. H. F. : An Experimental Apparatus for Contact Microradiography at 200-500 V., 1185

Recovery from Viral Infection in the Chick Embryo : Mechanism of (Dr. Samuel Baron and Alick Isaacs), 97

Recovery and *in vivo* Survival of Rabbit Erythrocytes (Dr. G. F. Doebbler, R. G. Buchheit and A. P. Rinfret), 1405

Recovery Times : A Method of Estimating Ionospheric Absorption Heights from (Syed Ziauddin), 1084

Rectal Glands in Woodlice (Dr. Minati Gupta), 406

Recurrent Disinhibition of Spinal Motoneurones: Changes in the Membrane during (Dr. Victor J. Wilson and Paul R. Burgess), 918

Red Blood Cell : Dimer Formation and Glucose Transfer across the Membrane of the (Dr. W. D. Stein), 1277

Red Cells : Thromboplastic Activity of (Dr. James A. Inglis and Dr. June W. Halliday), 821

Red Cells of the Sheep : Occurrence of an *Rh*-like Antigen in (B. Urbaschek and Prof. K. Trautwein), 193

Red Cells (Human) : Common Carrier System for Sugar Transport in (Dr. L. Lacko and Dr. M. Burger), 881
  A Relation between the Reactivity of, to Antiglobulin Tests and their Surface Structure (Judith E. Davis, Prof. H. N. Green and Pamela W. Tymms), 923
Red Deer Commission : Report for 1959-1960, 29
Red Shift : Theory of the, and the Nature of Planck's Constant (L. Infeld), 1184
Red and Far-red Irradiation : Effect of, on the Germination Process in Seeds of *Pinus sylvestris* L. (Bengt Nyman), 1219
Rédei, A., and Nagy, S. : Simultaneous Determination of Dextran and Glucose in Serum, 173
Redfearn, Dr. E. R., and Friend, J. : Oxidation-Reduction Reactions of Plastoquinone in Isolated Chloroplasts, 806
Redhardt, Dr. A. : Effects of Ionizing Radiation on Organic and Biological Solids (Radicals produced on Irradiation of Keratin), 1151
Reduced Alkylated Derivatives of some Myeloma Globulins : Comparison of, and Bence-Jones Proteins (Dr. M. D. Poulik and Dr. G. M. Edelman), 1274
Reduced Diphosphopyridine Nucleotide : An Inhibitor of Liver Alcohol Dehydrogenase in Preparations of (Dr. Keith Dalziel), 1098
Reduced Pressure : Bio-Assay of Erythropoietin in Mice made Polycythaemic by Exposure to Air at a (Dr. P. Mary Cotes and Dr. D. R. Bangham), 1065
Reductase (Nitrate-) in *Aerobacter aerogenes* : Inhibition by Oxygen of Biosynthesis and Activity of (Francis Pichinoty and Liliane D'Ornano), 879
Reduction of Zirconium and Hafnium Oxides (F. K. McTaggart), 1192
Reduction (Oxidation-) : Paths of Inter-Molecular Energy Transfer in Photosensitized (N. Takeyama), 1359
Reduction (Oxidation-) Reactions of Plastoquinone in Isolated Chloroplasts (Dr. E. R. Redfearn and J. Friend), 806
Reduction Products of 2,2'-Dipyridyl : Stability Constant of Copper (II) Complexes of (Dr. M. T. Beck and M. Halmos), 1090
Redundancy Techniques for Computing Systems (Information Systems Branch of the United States Office of Naval Research Symposium on), 1349
Rees, Dr. : Hazards of the Animal House (Precautions against Tuberculous Infection in the Animal House and Laboratory), 339
Reeves, Egli (Boyd, Prof. William C., and) : Specific Inhibition of Anti-D Antibody by Colominic Acid, 511
Reference Book : Civil Engineering (edited by J. Comrie), *review* by T. M. Charlton, 1032
Reference Book for Biochemists, *review* by Dr. D. Recaldin, 635
Reflectivities (Radar), of Hail Samples (R. H. Douglas), 266
Refracted *P*-Wave : Possible Method for Increasing the Signal-Noise Ratio in the Detection of the First Motion of a (F. A. Key, J. K. Wright, E. W. Carpenter and B. M. H. Stott), 1382
Refuse (Tipped) : Pollution of Water by—Report of the Technical Committee on the Experimental Disposal of House Refuse in Wet and Dry Pits (Ministry of Housing and Local Government), 864
Refuse Tips : Pollution from (Nora H. Johnson), 864
Regenerating Liver : Effect of Enzyme Injections on Mitosis in (Dr. Gaston de Lamirande), 400
Regeneration of Elements of Donor Origin in Orthotopically Grafted Skin following the Homograft Response (Dr. J. K. Dineen and Dr. Aleksander Szenberg), 153
Regeneration of Lateral Plates of the Stickleback (*Gasterosteus aculeatus* L.) : Significance of the (Tadeusz Penczak), 621
Regenerative Ability : Adaptive Value of (Dr. A. E. Needham), 720
Regional Advisory Council for Technological Education, London and Home Counties : Regional Development Plan for Courses for the Diploma in Technology, 1044
Regional Development Plan for Courses for the Diploma in Technology (Regional Advisory Council for Technological Education, London and Home Counties), 1044
Regional Research Laboratory, Hyderabad : Annual Report (1959-60), 757
Regions (Corresponding), of Homotypic Long Bones : Number and Arrangement of Osteons in (Dr. G. Marotti), 1400
Regions (New), of the Spectrum : From Radar to Spectroscopy— (Prof. D. J. E. Ingram), 1146
Registrar General : Statistical Review on Mental Health for 1957 and 1958, Supplement, 855
  Statistical Return for the First Quarter of 1961, 1347
Regulation of Pyrogallol Oxidations by Alcohols and Sugars (B. Z. Siegel, S. M. Siegel and N. S. Goodman), 180
Rehabilitation in Leprosy—Report of the Scientific Meeting on (United Nations, World Health Organization, Technical Report Series No. 221), 1248
Reid, E. : Steroids and Cancer, *review*, 1235
Reid, Joan D., and others : Histidine Decarboxylase and Growth, 1311
Reid, W. J. : Coastal Inlets, *review*, 426
Reilingh, W., and others : Neutral 17-Ketosteroids in Porcine Urine, 175
Reiner, Prof. M. : Biorheology and Microcirculation (Function of Models in Biorheology), 758
Reinfelds, Juris : Appointed to an I.C.I. Research Fellowship in the University of Edinburgh, 545
Reinharz, M., and others : Age Measurements on Antarctic Rocks (Queen Maud Land), 1286
Relation between the Reactivity of Human Red Cells to Antiglobulin Tests and their Surface Structure (Judith E. Davis, Prof. H. N. Green and Pamela W. Tymms), 923
Relation (A New), between the N–H Stretching Frequencies of Primary Amides (Prof. P. G. Puranik and K. Venkata Ramiah), 796
Relations (Industrial), in the Welfare State, 1129
Relationship between the Decrease in Urinary Excretion of Histamine and the Initiation of the Labour Process (Dr. Halina Wiskont-Buczkowska), 290
Relationship between Simple and Serial Reaction Times (Prof. H. Kay and Dr. A. D. Weiss), 790
Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the Rate of Lesion Production on Pinto Bean (John Piacitelli and Prof. Vincent Santilli), 624
Relationship between Velocity of Cardiac Contraction and Size of the Heart—a Hypothesis (Dr. H. Feinberg), 1373
Relationship (Direct), of Phosphorylase and Mitochondrial α-Glycerophosphate Dehydrogenase Activity in Skeletal Muscle (Dr. A. G. E. Pearse), 504
Relationships (Viscosity–Temperature), for Dilute Solutions of High Polymers (Dr. W. R. Moore), 1292
Relative Sweetness of α- and β-Forms of Selected Sugars (Rose Marie Pangborn and Sandra C. Gee), 810
Relative Volumes of Cytoplasm and Vacuole in *Nitella* (Dr. Paul Saltman and Dr. Tyge Christensen), 825
Relativity, the General Theory (J. L. Synge), *review* by Dr. G. J. Whitrow, 424
Relativity (General) : A New Approach to, *review* by Dr. G. J. Whitrow, 424
Relaxation (Magnetic), in Magnetite and in its Solid Solutions with Manganese and Nickel Ferrites (A. Bragiński and Th. Merceron), 898
Relaxation (Thermoelastic), induced by Ultrasonics : Crystallization Kinetics in Polyvinylchloride and (Dr. V. Nardi), 563
Release from the Feedback Inhibition controlling the Biosynthesis of Isoleucine (Masaya Hayashibe and Teijiro Uemura), 1417
Release of Heparin from the Mast Cells of the Rat (G. T. Archer), 90
Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at Early Stages of Propylthiouracil Treatment in the Rat : Increase of the Rate of (Dr. F. Escobar del Rey, G. Morreale de García, M. D. García García and J. Mouriz García), 1171
Remington's Practice of Pharmacy (edited by Eric W. Martin, E. Fullerton Cook, E. Emerson Leuallen, Arthur Osol, Linwood F. Tice and Clarence T. Van Meter), twelfth edition, *review* by Dr. J. H. Gaddum, 8
Removal of Internally Deposited Radionuclides by Triethylenetetraaminehexaacetic Acid (Prof. A. Catsch and D. Schindewolf-Jordan), 715
Removal of Milk Constituents from the Mammary Gland of the Ewe (A. K. Lascelles), 1404
Removing Non-specific Staining Components from Fluorescein-labelled Conjugates : Simplified Procedure for (G. D. Johnson), 70
Rennet Standardization : An Invariant Casein Substrate for, derivable from Diverse Sources of Cows' Milk (Dr. G. W. Scott Blair and J. C. Oosthuizen), 697
Replacements (Amino-Acid) : Non-Random Occurrence of (C. R. Woese), 1196
Replicating Dry or Moist Surfaces for Examination by Light Microscopy : A Method of (Joan Sampson), 932
Reports (Technical), of the United States Armed Forces, 1347
Repository Injection of Lipopolysaccharide : Development in Mice of Prolonged Non-specific Resistance to Sarcoma Implant and Staphylococcus Infection following (Samuel J. Prigal), 1111
Reproduction in *Rubus* : Biology of (Dr. G. J. Dowrick), 680
Reproduction in S.48 Timothy (*Phleum pratense* L.) : Effects of Light Intensity on (Dr. G. J. A. Ryle), 196
Reptiles (Non-aquatic) : Swimming Ability of (R. R. Tercafs), 125
Requirements (Power), and Scale-up in Flotation (Prof. Nathaniel Arbiter and Prof. Colin C. Harris), 268
Requirements (Sterol), of the Silkworm, *Bombyx mori* (Dr. Toshio Ito), 882
Research : Forest Products (1960)—Report of the Products Research Steering Committee with the Report of the Director of Forest Products Research (Department of Scientific and Industrial Research), 760
  Genetic (Prof. Arne Müntzing), *review* by Dr. Kenneth S. Dodds, 636
  Radio, 1960—the Report of the Radio Research Board and the Report of the Director of Radio Research (Department of Scientific and Industrial Research), 1255
Research by Sixth-Form Schoolboys (J. Heywood), 860
Research on Cancer : Eleanor Roosevelt Fellowships for (International Union against Cancer), 125
Research in Chemistry : Grants for (Chemical Society), 651
Research on Creep : A New Laboratory for, 1049
Research in Dairying, National Institute for : Annual Report for 1960, 237
Research on the Effects of Radioactivity in the Sea, 122
Research in the Food and Drug Directorate (Dr. Ross A. Chapman), 335
Research for Industry 1960—a Report on Work done by Industrial Research Associations in the Government Scheme (Department of Scientific and Industrial Research), 1252
Research on Leukaemia and Allied Diseases (Lady Tata Memorial Trust Awards for 1960-61), 228
Research in Marine Biology or Oceanography (Challenger Society Grants for), 24
Research in Nuclear Science, National Institute for (Harwell) : Successful Operation of the *Nimrod* Injector, 1043
Research into Problems of Ageing, 1145
Research into Virus Diseases (Pfizer Group Laboratories for), 20
Research on Volcanoes in the West Indies, 225
Research (Aeronautical), in Asia and Africa, 1043
Research (Atomic) : New Trends in, and their International Significance (Dr. L. Kowarski), 853
Research (Controlled Nuclear Fission) : Plasma Physics and (International Atomic Energy Agency International Conference on), 443
Research (Educational), in England and Wales, National Foundation for : *Technical Education Abstracts from British Sources*, Vol. 1, No. 1, September–December 1960, 21
Research (Experimental Medical) : Feldberg Foundation for German–English Scientific Exchange for, 1143
Research (Experimental Tumor) : Progress in (edited by F. Homburger), Vol. 1, *review* by Prof. F. Bergel, 1231
Research (Government), in Great Britain : Financing, 205
Research (Industrial), in Britain, 1252
Research (Medical), in East Africa, 226
Research (Medical), East African Council for : Report for 1959-60, 226
Research (Medical), Fleming Memorial Fund for : Grants from the, 959
Research (Medical), Institute for (Federation of Malaya) : Studies from the, No. 29, Malaysian Parasites, 35-49 (edited by W. W. Macdonald), *review* by P. F. Mattingly, 948
Research (Naval), United States Office of (Information Systems Branch) : Symposium on Redundancy Techniques for Computing Systems, 1349
Research (Nuclear), European Organization for, and the Max Planck Institute, Heidelberg : Conference on Fission and Spallation Phenomena and their Application to Cosmic Rays, 24
Research (Operations), and Systems Engineering (Charles D. Flagle, William H. Huggins and Robert H. Roy), *review* by Prof. L. S. Goddard, 105

Research (Plankton) : Fellowships for (Lamont Geological Observatory), 754
Research (Polar) : Soviet, *review* by Dr. Terence Armstrong, 841
Research (Scientific) : National Organization of, 632
Research (Scientific), in British Universities, 1960-61 (Department of Scientific and Industrial Research), 228
Research (Scientific), in the U.S.S.R., 123
Research (Scientific), National Foundation for (Belgium) : Thirty-third Annual Report, 441
Research (Shipbuilding), in Britain, 543
Research (Soviet), on Organo-Phosphorus Compounds, 1949-1956 (in English translation), *review* by Dr. F. G. Mann, 1232
Research (Space) : Some Aspects of the Physics of (Institute of Physics and the Physical Society Residential Symposium on), 229
  Further Advancements in, 631
Research (Textile) : Polish, 1045
Research (Tumour) : Progress Reports and, *review* by Prof. F. Bergel, 1231
Research (*Vision*), Vol. 1, Nos. 1-2, 650
Research Awards (Animal Health Trust), 1250
Research Council of Alberta : Information Series No. 23, Alberta Sub-bituminous Coals as Drilling Fluid Thinners (E. J. Jensen), 1144
Research Defence Society : Pamphlet on Animal Experiments in the Control of Animal Diseases, 226
Research Development Corporation (National) : Report for 1960, 343
Research Equipment Exhibition (Instrument Symposium and) : Eleventh Annual (Washington Sections of the American Association of Clinical Chemists, the American Chemical Society, the Instrument Society of America, the Society of American Bacteriologists, the Society of Applied Spectroscopy and the Society for Experimental Biology and Medicine), 959
Research Fellowships : Carlsberg–Wellcome (Awards for 1960-61), 754
  IBM (International Business Machines, United Kingdom, Ltd.), 442
  Industrial (J. Langham Thompson, Ltd.), 545
  Wellcome (Animal Health Trust Awards), 1250
Research Foundation (Ontario) : Annual Report for 1960, 1254
Research Grants in Radiological Health (United States Public Health Service), 442
Research Highlights in Ageing (United States Department of Health, Education and Welfare, Public Health Service Publication No. 854), 1145
Research Laboratory (Regional), Hyderabad : Annual Report (1959-60), 757
Research Literature (Thermophysical Properties) : Retrieval Guide to, Vol. 1 (edited by Y. S. Touloukian), Book 1, Part A, Guide to Substance Classification and Numerical Codes ; Book 1, Part B, Dictionary of Synonyms and Trade Names ; Book 1, Part C, Dictionary of Substances ; Book 2, Classified Search Index ; Book 3, Master Bibliography, Author Index, *review* by Prof. A. R. Ubbelohde, 109
Research Medal of the Royal Society of Victoria awarded to Dr. George Baker, 1247
Research Progress in General Medical Sciences, 1960 : Highlights of (United States Department of Health, Education and Welfare, Public Health Service Publication No. 815), 658
Research Publication on Farm Buildings : Bibliography of, Section 5, Buildings for the Drying and Storage of Grain (Agricultural Research Council), 440
Research Time : Endowed (International Business Machines, United Kingdom, Ltd.), 442
Research Training Scholarships (Animal Health Trust Awards), 1250
Research Vessel : The Fishing (Food and Agriculture Organization of the United Nations Forum on), 229
Research Work of the Nature Conservancy (House of Commons Adjournment Debate on), 962
Research and Development : Reviews of Data on, No. 27, Report on Scientists and Engineers engaged in research and development in Colleges and Universities in the United States for 1958 (United States National Science Foundation), 225
  Reviews of Data on, No. 28, Capital Expenditure of Colleges and Universities in the United States for research and development in the Natural and Social Sciences in 1958 (United States National Science Foundation), 545
Research and Development in Scotland, 440
Research and Development (Atomic Energy), in Britain, 857
Research and Development (Scientific) : Government Responsibility in, 837
Residue (An Unreactive Tyrosine), in Insulin and the Exclusive Iodination of the *A* Chain (Dr. P. H. Springell), 1372
Resistance of *Entamoeba histolytica* to Fumagillin : Induced Increase in (Dr. Mitsuru Nakamura), 413
Resistance of Humus to Decomposition (H. F. Birch and M. T. Friend), 731
Resistance to Infection with *Salmonella paratyphi C* in Mice parasitized with a relatively Avirulent Strain of *Salmonella typhimurium* (Dr. J. G. Howard), 87
Resistance to 6-Mercaptopurine in Human Epidermoid Carcinoma Cells in Culture : Biochemical Aspects of (R. W. Brockman, George G. Kelley, Patricia Stutts and Virginia Copeland), 469
Resistance (Frost) : Intracellular Freezing and, in Egg-Cells of the Sea Urchin (E. Asahina)—Intracellular Freezing as a Cause of Frost-killing in relatively Rapidly Frozen Cell Suspensions, 1263 ; A Mechanism to Prevent the Seeding of Intracellular Ice in Freezing Living Cells, 1264
Resistance (Prolonged Non-specific), to Sarcoma Implant and Staphylococcus Infection following Repository Injection of Lipopolysaccharide : Development in Mice of (Samuel J. Prigal), 1111
Resistance (Specific Evaporation) : Compressibility and, of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers (A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas), 378
Resistant and Susceptible Strains of Cotton : Leaf Curl Virus and the Flavonoid Content of (Dr. Esam M. Moustafa), 415
Resistivity Anomalies in Palladium Hydride : Heat Capacity and (Paul Mitacek, jun., and Prof. J. G. Aston), 271
Resistivity Changes due to Formation of *G.P.* Zones (Clusters rich in Solute), (H. Herman and Prof. J. B. Cohen), 63
Resonance : Electron Spin (Chemical Society Symposium on), 755
Resonance (Electron Spin) : Spatial Distribution of Free Radicals as studied by (Robert C. Smith and S. J. Wyard), 897
Resonance (Electron Spin), of Adenosine Triphosphate (D. B. Russell and S. J. Wyard), 65
Resonance (Spin), and Electrical Conductivity of Indole–Iodine (B. Smaller, Dr. I. Isenberg and S. L. Baird, jun.), 168
Resonance Absorptions (Electron Spin), induced by Light in Adsorbed Layers of Cyanine Dyes on Silver Bromide Microcrystals (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Resources (Water), of the United States (Prof. W. G. V. Balchin), 444
Resources (Water), of Wales : Report on the (Welsh Advisory Water Committee), 135
Respiration : Active Cation Transport as a Pace-maker of (Dr. R. Whittam), 603
Respiration in *Azotobacter* : Oxygen Inhibition of (M. J. Dilworth and C. A. Parker), 520
Respiration of Tumours : The Glycolysis and (Dr. Alan C. Aisenberg), *review* by Prof. F. Dickens, 1335
Respiration (Tissue Slice) : Oxygen Electrode for Measurement of (E. H. Strickland, F. D. Ziegler and Prof. A. Anthony), 969
Respirators : The Design and Use of (Ergonomics Research Society and the British Occupational Hygiene Society Discussion on), 655
Respiratory Metabolism in Plants (Prof. Harry Beevers), *review* by Prof. W. O. James, 1031
Respiratory Organs (Accessory), in Air-breathing Fishes : A Hydrostatic Function of the (Dr. S. Z. Qasim and R. Hasan), 396
Response of the Labellar Hairs of the Blowfly, *Phormia regina* Meigen, to Protein (D. I. Wallis), 917
Response to Photoperiod : Flower Initiation in the Carnation in (Dr. G. P. Harris and Juliet E. Griffin), 614
Response to Series of Subliminal Stimuli—an Ethological Method for Investigating the Properties of Nervous Systems (A. J. Pontin), 512
Response (Antibody), in Rabbits following Injection of Sheep Erythrocytes into Lateral Ventricle of Brain (Prof. B. D. Janković, Dr. M. Draškoci and Dr. K. Isaković), 288
Response (Homograft) : Regeneration of Elements of Donor Origin in Orthotopically Grafted Skin following the (Dr. J. K. Dineen and Dr. Aleksander Szenberg), 153
Response' ('Insulin), of Normal Rabbits : Effects of Injections of some Intermediary Metabolites on the (Dr. H. D. Brahmachari and G. Raghupathy Sarma), 491, *erratum*, 755
Response (Stimulus-) Compatibility in the Paired-Associate Learning of Nonsense Syllables (A. D. Baddeley), 1327
Responses (Mechanical), of Frog Muscles at Low Temperature : Inhibition by Potassium of the (H. Lorković), 1011
Responsibility (Government), in Scientific Research and Development, 837
Restoration of Cultural Property, International Centre for the Study of the Preservation and the (Rome) : Works and Publications, 3, Climatology and Conservation in Museums, 1050
Rete Mirabile (Mammalian Carotid) : Functions of the (Dr. C. H. Barnett and C. D. Marsden), 88
Retention (Electrolyte), produced by the Antialdosterone *SC* 5233 (J. B. Farmer and B. Petch), 1308
Retention (Potassium) : Aldosterone Excretion and, in Subjects living at High Altitude (P. J. Ayres, Rona C. Hurter, Dr. E. S. Williams and J. Rundo), 78
Reticulo-Endothelial System : Sensitivity of Mice to Bacterial Lipopolysaccharide following Alteration of Activity of the (Dr. G. N. Cooper and Dr. A. E. Stuart), 294
Reticulo-Endothelial System of Mice : Effect of Added Vitamins on the Phagocytic Activity of the (J. Cordingley and Prof. T. Nicol), 82
Retirement : The Problem of, *review* by Prof. L. S. Hearnshaw, 532
Retrieval of Biological Information : Storage and (Dr. G. C. Ainsworth), 12
Retrieval of Published Information, *review* by Prof. A. R. Ubbelohde, 109
Retrieval Guide to Thermophysical Properties Research Literature, Vol. 1 (edited by Y. S. Touloukian), Book 1, Part A, Guide to Substance Classification and Numerical Codes ; Book 1, Part B, Dictionary of Synonyms and Trade Names ; Book 1, Part C, Dictionary of Substances ; Book 2, Classified Search Index ; Book 3, Master Bibliography, Author Index, *review* by Prof. A. R. Ubbelohde, 109
Reversal of this Inhibition of Photosynthesis : Inhibition of Growth and Photosynthesis in Submerged Plants with Tetramethylthiuram Disulphide and Sodium Dimethyldithiocarbamate and (P. E. Björn Lindahl), 51
Reversal (Photic), of the Beetle *Trypodendron* : Air-Swallowing, a Mechanism in (Prof. Kenneth Graham), 519
Reversal (Sign), of Permselective Membranes : Poisoning and (Dr. Francis de Körösy), 1363
Reversible Photochromic Polymer (A. G. Massey and Dr. L. E. Orgel), 1387
Reviews of Data on Research and Development : No. 27, Report on Scientists and Engineers engaged in research and development in Colleges and Universities in the United States for 1958 (United States National Science Foundation), 225
  No. 28, Capital Expenditure of Colleges and Universities in the United States for research and development in the Natural and Social Sciences in 1958 (United States National Science Foundation), 545
Reviews of Genetics, *review* by Dr. Kenneth S. Dodds, 636
Revolution in Agriculture (Sir William Slater), 991
Revolution (American) : Drug Supplies during the (George B. Griffenhagen), 761
Rh Phenotype, *Dcceʹeʹf*, (New), found in a Chibcha Indian Tribe (Dr. Miguel Layrisse, Zulau Layrisse, Esperanza García, Dr. Johannes Wilbert and Joaquín Parra R.), 503
Rh-like Antigen in Red Cells of the Sheep : Occurrence of an (B. Urbaschek and Prof. K. Trautwein), 193
Rheological Techniques (British Society of Rheology and the Non-Destructive Testing Group of the Institute of Physics and the Physical Society Joint Conference on), 24
Rheology, British Society of, and the Non-Destructive Testing Group of the Institute of Physics and the Physical Society : Joint Conference on Rheological Techniques, 24
Rheopectic Flow : A Clear Instance of (J. B. Yannas and R. N. Gonzalez), 1384
Rheumatic Fever : Amino-acid Composition of Serum Mucoprotein in (Prof. D. O'Brien, C. H. M. Walker and D. Walker), 922
Rhizobium trifolii : Transfer of Virulence in (Dr. Hans Ljunggren), 623
Rhizosphere of Pasture Plants : Numbers and Types of Bacteria from the—Possible Occurrence of *L*-Forms (G. Jagnow), 1220
Rhodamine *B* : Inhibition of Ultrasonic Decolorization of, in Presence of Organic Substances (Prof. Satya Prakash and Sheo Prakash), 1292

Rhode, R. A. (Lavallee, W. H., and) : Nematology (Movements of Larvae of *Pratylenchus penetrans*), 1048
Rhodes, W. L. : At the Maxwell Colour Centenary Conference (Colour Printing), 11
Rhodesia : Diabetes Mellitus in Africans in (Dr. M. Gelfand and W. R. Carr), 227
Rhodesia (Northern) : Blindness in Africans in (Dr. C. M. Phillips), 753
  Mammals of (W. F. H. Ansell), *review* by R. W. Hayman, 8
Rhodesia (Southern), National Museums of : Annual Report for 1960, 650
Rhodium Film (Clean) : Reaction of Ethane with a (Dr. Richard W. Roberts), 170
Rhodospirillum rubrum : Purine and Pyrimidine Metabolism in (Prof. T. W. Goodwin and Dr. P. N. A. Passorn), 276
Rhythm ('Alpha'), Reaction Time and Age : Frequency of the (Dr. Walter W. Surwillo), 823
Rhythm (Cycle and), in the Ovulation of the Spider *Latrodectus geometricus* C. Koch (A. Bouillon and R. Lekie), 620
Rhythm (Diurnal), in Cave Crayfish (Prof. Frank A. Brown), 929
Rhythm (An Endogenous), in the Rate of Dark-Fixation of Carbon Dioxide in Leaves of *Bryophyllum fedtschenkoi* (Diana M. Warren and Malcolm B. Wilkins), 686
Riboflavin : Hydrolysis of Fatty Acid Esters of, by Pancreatic Lipase (Prof. Kunio Yagi Yoshiko Yamamoto and Jun Okuda), 174
Riboflavin in Cell-free Systems : Biosynthesis of (Prof. T. W. Goodwin and Dr. A. A. Horton), 772
Ribonuclease and Deoxyribonuclease : Effect of, on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Ribonucleic Acid of Wild Cucumber Mosaic Virus : Unusual Base Composition of the (H. Yamazaki and Prof. Paul Kaesberg), 96
Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Ribonucleic Acid (Infectious), from Poliovirus : Chromatographic Analysis of (C. Cocito, A. Prinzie and P. De Somer), 573
Ribonucleic Acid (Rat Lens) : Catalysis of Incorporation of ^{14}C-Uridine into, by Rat Lens Enzyme System (Dr. Anima Devi and Dr. N. K. Sarkar), 1094
Ribonucleic Acid (Ribosomal), from Yeast : Sub-Unit of (Eiko Otaka, Yukito Oota and Syozo Osawa), 598
Ribonucleic Acid (Viral) : Influence of *p*-Fluorophenylalanine on the Production of, and on the Utilizability of Viral Protein during Multiplication of Fowl Plague Virus (Dr. Christoph Scholtissek and Rudolf Rott), 1023
Ribonucleic Acid Fractionation : Modified Cellulose Ion Exchangers and their Use in (Dr. L. Bosch, G. van der Wende and Dr. H. Bloemendal), 349
Ribonucleic Acids (Infectious), derived from Mouse Brains infected with Two Kinds of Arbor Virus Group B (Prof. Masahiro Nakamura), 624
Ribosomal Ribonucleic Acid from Yeast : Sub-Unit of (Eiko Otaka, Yukito Oota and Syozo Osawa), 598
Rich, Prof. Alexander (North, Dr. A. C. T., and) : X-Ray Diffraction Studies of Bacterial Viruses, 1242
Rich, Dr. Clayton, and Ensinck, John : Effect of Sodium Fluoride on Calcium Metabolism of Human Beings, 184
Richards, Prof. E. J. : A Take-Over Bid in Acoustical Training, 748
Richardson, D. A., and others : Absorption Spectra of γ-Irradiated Lead Silicate Glass, 374
Richardson, Dr. Lewis F. : Arms and Security, a Mathematical Study of the Causes and Origins of War (edited by Nicolas Rashevsky and Ernesto Trucco), *review* by Sir Cyril Burt, 427
  Statistics of Deadly Quarrels (edited by Quincy Wright and C. C. Lienau), *review* by Sir Cyril Burt, 427
Richman, S. (Slobodkin, Prof. L. B., and): Calories/gm. in Species of Animals, 299
Richter, Dr. D. : Author and Publisher, 131
  At the Scientific Publications Council Meeting on the Publication of Scientific Books (Contractural Relationships), 132
Richterich, R., and others : Diagnostic Significance of Heterogeneous Lactic Dehydrogenases in Malignant Effusions, 507
Riddiford, Dr. L. : Appointed senior lecturer in the Department of Physics, University of Birmingham, 337
Ridge, M. J., and others : Reactivity of Calcium Sulphate Hemihydrate, 704
Ridges, A. P. : Immunity to Protozoa (Local Immunity to Occidiosis), 553
Ridgway, Dr. G. F. : Blood Groups in Infra-human Species (Extremely Difficult Work involved in Analysing Blood Groups of Salmon and Trout, primarily using Isoimmunizations), 654
Riding (West) of Yorkshire : The Place-Names of the (Prof. A. H. Smith), Part 1, Lower and Upper Strafforth and Staincross Wapentakes ; Part 2, Osgoldcross and Agbrigg Wapentakes ; Part 3, Morley Wapentake, *review* by Prof. H. C. Darby, 208
Riedel, P. : Vitamin B_{12} and Intrinsic Factor (Specific Effects of Vitamin B_{12} Deficiency), 1155
Riegel, Dr. J. A. (Ramsay, Dr. J. A., and) : Excretion of Inulin by Malpighian Tubules, 1115
Riek, R. : Immunity to Protozoa (Immunity in Piroplasmosis), 552
Riggs, D. B., and others : Immunofluorescence of Pleuropneumonia-like Organisms isolated from Tissue Cell Cultures, 190
Right Australopithecine Upper Arm Bone : An Australopithecine Scoop made from a (Prof. Raymond A. Dart), 372
Rigidity Factor of Gelatin Gels (Dr. Audrey Todd), 567
Rimington, Prof. C., and others : Nervous and Biochemical Disturbances following Hexachlorobenzene Intoxication, 363
Rimmer, D. : Appointed lecturer in the Department of Economics, University of Birmingham, 337
Rimmer, R., and others : Absorption Spectra of γ-Irradiated Lead Silicate Glass, 374
Rinaldi, F., and others : Distribution of Piperidine in the Brain and its Possible Significance in Behaviour, 201
Rinfret, A. P., and others : Recovery and *in vivo* Survival of Rabbit Erythrocytes, 1405
Ringold, Dr. Howard J., and others : A Biochemical Effect of Deuterium, 1294
Ripamonti, A., and others : Molecular Geometry of a 1:1 Crystalline Complex between 1,3,7,9-Tetramethyluric Acid and Pyrene, 900
Ripening of Blueberries : The First Anthocyanins appearing during the (Dr. Heikki Suomalainen and A. J. A. Keränen), 498
Rise of Antibody Titre in Cell-free Spleen Preparations Incubated *in vitro* (Dr. Dieter H. Sussdorf), 770
Rishbeth, Dr. J. : Inoculation of Pine Stumps against Infection by *Fomes annosus*, 826
Ritchie, H. D. : Appointed to the University of London readership in surgery at the London Hospital Medical School, 229
Ritter, M. : Nematology (Distribution of species of *Meloidogyne* in France), 1048
Rival : Dalton's Irish, *review* by Frank Greenaway, 533
River (Thames), Conn. : Gross β-Activity in the (D. M. Skauen, T. Hatfield, D. Hanlon and J. S. Rankin, jun.), 1017
Rixon, A. E. : At the sixty-seventh Annual Conference of the Museums Association (Problems of Conservation), 554
R_M and Solvent Composition in Paper Chromatography (Dr. Edward Soczewiński), 68
Road Research Laboratory : Open Days, 644
Robbins, M. (Banks, Prof. E., and) : Fluoride-compensated Substitution o Bivalent Cations in the Magnetoplumbite Structure, 1387
Robert, Barbara, and others : Polarographic Examination of Sialic Acids, 596
Robert, Ladislas, and others : Polarographic Examination of Sialic Acids, 596
Roberts, A. C. (Higgins, P. R. B., and) : Damage in Stainless Steel due to Fission Fragments, 1158
Roberts, Prof. E. J. : Retirement from the chair of agriculture in University College of North Wales, Bangor, 439 ; work of, 439
Roberts, J. A. : Experimental Transmission of *Borrelia anserina* (Sakharoff 1891) by *Aedes aegypti*, 1225
Roberts, J. A., and Vivian, H. E. : The Widening of Expansion Cracks in Mortar by Salt Solutions, 125, *erratum*, 1145
Roberts, J. B., and others : Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid—Effect of Spraying with Gibberellic Acid on Yield and Composition, 360
Roberts, Dr. Richard W. : Reaction of Ethane with a Clean Rhodium Film, 170
Roberts, W. M. B. : Formation of Chalcopyrite by Reaction between Chalcocite and Pyrrhotite in Cold Solution, 560
Robertson, Dr. G. B. : Crystal and Molecular Structure of Diphenyl, 593
Robertson, Muriel : Immunity to Protozoa (Parasites localized in the Skin or Mucous Membranes), 553
Robins, Dr. A. B. : Effects of Ionizing Radiation on Organic and Biological Solids, 1150
Robinson, Abner R. (Zuelzer, Dr. Wolf W., and) : Haemoglobin *F* and the Genetic Control of Protein Structure, 609
Robinson, Dr. D. L. H. : Phosphatases in *Schistosoma mansoni*—Distribution of Alkaline Phosphatase, 473 ; Quantitative Estimation of Phosphatases, 473
Robinson, Prof. Enders A. : An Introduction to Infinitely Many Variates, *review* by Prof. D. V. Lindley, 214
Robinson, Dr. F. A. : Appointed chairman of the board of directors of the Crookes Laboratories, 546
Robinson, Sir Robert : Origins of Petroleum, 346
Robson, Dr. J. S. : Appointed reader in therapeutics at the University of Edinburgh, 443
Rock Art : Australian, 854
Rock Engravings of the Sydney–Hawkesbury District (F. D. McCarthy), 854
Rocks (Antarctic) (Queen Maud Land) : Age Measurements on (Sarah Deutsch, Prof. E. E. Picciotto and M. Reinharz), 1286
Rod-like Polyelectrolytes : Displacement Potentials and Bending of (Prof. J. A. Christiansen, the late Dr. C. E. Jensen and Dr. Ty. Vilstrup), 484
Rodd, Dr. E. H. (edited by) : Chemistry of Carbon Compounds, Vol. 4, Part C, Heterocyclic Compounds, *review* by Dr. James D. Loudon, 214
Rodley, G. A. : New Zealand fellowship in chemistry renewed for 1961-62 by the Ramsay Memorial Trust, 337
Rodriguez, Dr. Maria Covadonga : Spanish fellowship in chemistry renewed for 1961-62 by the Ramsay Memorial Trust, 337
Roe, F. J. C., and others : Induction of Tumours by Polyoma Virus—Enhancement by Chemical Agents, 893
Roff, H. E., and Watson, T. E. : Job Analysis (Institute of Personnel Management), 336
Rogoff, M. H. (Silverman, M. P., and) : Morphological Variation in *Ferrobacillus ferrooxidans* related to the Rate of Iron Oxidation, 1221
Roguska-Wasilewska, L. : Nematology (*Heterodera schachtii* inhibited the Respiration of Rape far more than did *Eucephalobus elongatus*), 1048
Rohatgi, Dr. K. K., and Singhal, G. S. : Average Extinction Coefficient for Re-absorption of Fluorescence Radiation through Uranin Solution, 799
Roland, G., and others : Molecular Emission Spectroscopy from 2μ to 12μ by a Michelson Interferometer, 264
Role of Interparticle Forces in Development of Upper Plastic Limit of Clays (Dr. Raymond Yong and E. T. Selig), 938
Role of Lipid in the Hardening of the Cuticle in the Silkworm, *Bombyx mori* (Shigemi Kawase), 279
Role of the Lung in Metabolism of Fat during Ontogeny of the Rat (Dr. Z. Vacek, P. Hahn and O. Koldovsky), 85
Role of Metal Ions in Enzyme Catalysis : Dextransucrase *V* and the (W. Brock Neely and Julie Hallmark), 385
Role of the Pentose Phosphate Pathway in *Pasteurella multocida* (Inda M. de Issaly, A. Issaly and Prof. A. O. M. Stoppani), 727
Role of Polysaccharides in Stabilization of Natural Soil Aggregates (Dr. D. J. Greenland, G. R. Lindstrom and Dr. J. P. Quirk), 1283
Role of Pressure Gradients in obtaining Minimum Time in Gas Chromatography (Prof. J. Calvin Giddings), 1291
Romanowska, Dr. Elzbieta : Products of Mild Acid and Alkaline Hydrolysis of *M*- and *N*-Blood Group Substances, 1408
Römer, Max : Terrestrial Exosphere at Heights of 1,000–1,700 km., 238
Ronan, Colin A. : Astronomy in the Making, *review*, 314
Rook, J. A. F. : Rapid Development of Hypomagnesaemia in Lactating Cows given Artificial Rations Low in Magnesium, 1019
Roosevelt (Eleanor) Fellowships for Research on Cancer (International Union against Cancer), 125
Root Nodules : Leguminous (Gibberellin-like Substances in Plants), (Margaret Radley), 684

Root Temperature and Symbiotic Nitrogen Fixation (A. H. Gibson), 1080
Rooting of Cuttings of *Hevea brasiliensis* : Effect of Ferric Dimethyldithiocarbamate on the (G. H. Tinley), 1217
Roots : Non-Lethal Pathological Infection of (F. J. Newhook), 615
Roots (Plant) : Dependence on Light of Geotropism in (J. V. Lake and G. Slack), 300
Rose, Dr. Frederick G. G. : Classification of Kin, Age Structure and Marriage amongst the Groote Eylandt Aborigines, *review* by Dr. R. Needham, 6
Rose, Dr. H. E. : Title of professor of powder technology (at King's College, London), conferred on, 651
Rose, M. E. : Immunity to Protozoa (Local Immunity in Coccidiosis), 553
Rose, M. E., and Long, P. L.: Parasite Biology (Application of New Laboratory Techniques), 135
Rose, Prof. Walter : Fluid–Fluid Interfaces in Steady Motion, 242
Rosen, Fred, and others : Immunoelectrophoretic Examination of Agammaglobulinaemia, 1109
Rosenblum, Dr. : Vitamin B_{12} and Intrinsic Factor, 1156
Rosenheim, Prof. M. L. : Appointed a member of the Medical Research Council, 1142
Ross, Dr. H. : Plant Exploration and Introduction (The Potato Gene Centre and its Importance with respect to the Origin and Breeding of the Cultivated Potato), 1240
Ross, J. W. : Appointed junior Research Fellow in physics at the University of Sheffield, 337
Rossi, E., and others : Diagnostic Significance of Heterogeneous Lactic Dehydrogenases in Malignant Effusions, 507
Roston, Dr. Sidney : Interaction of Hydrocortisone, the Sulphydryl Group, and the Catechol Amines, 490
Rotating Hanging Mercury Drop : Use of the, in Analysis—Anodic Stripping using a Rotating Mercury Drop (Dr. J. J. Engelsman and A. M. J. M. Claassens), 240 ; Trace Analysis with a Rotating Hanging Mercury Drop (Dr. M. van Swaay and R. S. Deelder), 241
Rotation of the Earth (Walter H. Munk and Gordon J. F. Macdonald), *review* by Prof. S. K. Runcorn, 313
Rotational Analysis of Absorption Bands of Lead Monoxide (Dr. R. F. Barrow, J. L. Deutsch and D. N. Travis), 374
Roth, Dr. Louis M., and Willis, Dr. Edwin R. : The Biotic Associations of Cockroaches, *review* by David R. Ragge, 106
Rothschild, Hon. Miriam : Double and Single Spermathecae in Fleas (Siphonaptera), 1322
Rott, Rudolf (Scholtissek, Dr. Christoph, and) : Influence of *p*-Fluorophenylalanine on the Production of Viral Ribonucleic Acid and on the Utilizability of Viral Protein during Multiplication of Fowl Plague Virus, 1023
Rottnest Quokka (*Setonix brachyurus*) : Myopathy affecting the, reversed by α-Tocopherol (B. A. Kakulas), 402
Rouayheb, George M., and Hamilton, William C. : Deterioration of Solid-coated Capillary Columns on Standing, 801
Rounds, D. E. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Protective Effect of Yeast RNA), 341
Routes (New), to Further Education—Papers presented at the B.A.C.I.E. Spring Conference, London, 1961 (British Association for Commercial and Industrial Education), 739
Routes (Unconventional) : Transplantation of Murine Leukaemia by (Dr. Harold B. Hewitt), 1213
Routh, Dr. Joseph I., and others : Biochemical Changes in Lethal Staphylococcal Infections in Mice, 783
Routine Selective Isolation of Fungi Pathogenic to Man : Use of Desertomycin Medium in (J. Uri and Zs. Herpay), 1015
Rovira, A. D. (Bowen, G. D., and) : Plant Growth in Irradiated Soil, 936
Rowe, G. A. (Chatt, Dr. J., and) : Complex Compounds of Zero-valent Platinum, 1191
Rowlands, R. P. : Design and Use of Respirators (Apparatus for Protecting People who handled Radioactive Substances), 656
Rowles, Dr. S. L. : Awarded a Carlsberg–Wellcome Research Fellowship, 754
Rowson, K. E. K., and others : Induction of Tumours by Polyoma Virus—Enhancement by Chemical Agents, 893
Roy, Prof. Rustum (Datta, Ranajit K., and) : Dependence on Temperature of the Distribution of Cations in Oxide Spinels, 169
Roy, Robert H., and others : Operations Research and Systems Engineering, *review* by Prof. L. S. Goddard, 105
Royal Aircraft Establishment, Farnborough : Open Days (Peter Sanderson), 232
Royal Cancer Hospital : The Chester Beatty Research Institute of the Institute of Cancer Research (Prof. A. Haddow), 430
Royal College of Science and Technology, Glasgow : Appointment of Dr. David W. A. Sharp as senior lecturer in the Department of Chemistry, 443
  Appointment of Dr. Graham R. Knox as lecturer in the Department of Chemistry, 443
  Endowment of £800 made by Mrs. Hilda M. Beilby, to be known as the Kelvin Prizes, 1349
Royal College of Surgeons of England : Election of Sir Howard Florey and Dr. Owen H. Wangensteen as Honorary Fellows, 126
Royal Entomological Society : Symposium on Insect Polymorphism, 1145
Royal Free Hospital School of Medicine, London : Title of reader in biology in the University of London conferred on Dr. Agnes M. Hughes, 229
  Appointment of J. C. G. Coleridge as reader in physiology, 1349
Royal Geographical Society : Grant of £200 for three years from the Gulbenkian Foundation, in aid of the expeditions, 754
Royal Holloway College, London : Title of reader in organic chemistry in the University of London conferred on Dr. T. G. Bonner, 229
Royal Horticultural Society : Bowles Memorial Travel Scholarship, 1349
Royal Horticultural Society, and others : Third World Orchid Conference, London, 1960—Proceedings of the, *review* by V. S. Summerhayes, 213
Royal Institution : Election of Prof. R. J. Harrison to be Fullerian professor of physiology, 122
Royal Meteorological Society : Election of Officers for 1961-62, 443
Royal Ontario Museum, Toronto : Appointment of Dr. W. E. Swinton as head of the Division of Life Sciences, 24
Royal Radar Establishment, Malvern : Appointment of Dr. G. G. Macfarlane as director, 1145
Royal Scottish Museum, Edinburgh : Retirement of Dr. Douglas A. Allan from the directorship, 648
  Appointment of Ian R. Finlay as director, 648
  Appointment of Cyril Aldred as keeper of the Department of Art and Ethnography, 648
  Annual Report for 1960, 958
Royal Society of Edinburgh : David Anderson Berry Prize for 1961 awarded to (*a*) Dr. H. B. Hewitt and Dr. C. W. Wilson, and (*b*) to Dr. D. H. Sussdorf, 229
  Bruce-Preller Lecture (Dr. V. A. Eyles, The Life and Scientific Work of Sir James Hall), 345
Royal Society of London, in co-operation with the Government of North Borneo : North Borneo Expedition 1961, 26
Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme : Awards, 126
Royal Society of South Australia : Verco Medal presented to Dr. R. L. Specht, 1247
Royal Society of Victoria : First award of the Research Medal made to Dr. George Baker, 1247
Royal Statistical Society : Election of Officers, 126
Rubber (Natural) Producers' Research Association : Annual Report, 854
Rubber Industry, Institution of the : Colwyn Medal awarded to Dr. L. R. G. Treloar, 755
  Hancock Medal awarded to M. M. Heywood, 755
Rubus : Biology of Reproduction in (Dr. G. J. Dowrick), 680
Rudebeck, Gustaf, and others (edited by) : South African Animal Life—Results of the Lund University Expedition in 1950-1951, Vol. 7, *review* by Prof. O. Lowenstein, 1135
Rudorf, Prof. W. : Plant Exploration and Introduction (Plant Exploration Stations), 1241
Rudwick, Dr. M. J. S. : 'Quick' and 'Catch' Adductor Muscles in Brachiopods, 1021
Ruffell, G. K., and others : Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine, 1312
Rumberg, B., and others : Experimental Evidence for the Mechanism of Photosynthesis, 194
Rumen Liquid of Cattle : Keto-Acids and Amines in (Dr. C. J. G. van der Horst), 73
Ruminant : Digestive Physiology and Nutrition of the—Proceedings of the University of Nottingham Seventh Easter School in Agricultural Science, 1960 (edited by Dr. D. Lewis), *review* by Dr. I. W. McDonald, 1334
Ruminant Digestion and Nutrition, *review* by Dr. I. W. McDonald, 1334
Runcorn, Prof. S. K. : Rotation of the Earth, *review*, 313
Rundo, J., and others: Aldosterone Excretion and Potassium Retention in Subjects living at High Altitude, 78
Runeckles, Dr. V. C. : Natural Radioactivity in Tobacco and Tobacco Smoke, 322
Runge, H. : At the International Starch Conference (Automation in the Starch-producing Industry), 237
Rural Areas : Railways in, 855
Rural England, Council for the Preservation of : Twenty-second National Conference (The Impact of Industrial Development on the Countryside), 959
Rural Life (English), Museum of : Annual Report for 1960 (University of Reading), 544
Rushton, Dr. W. A. H. : At the Maxwell Colour Centenary Conference (Complexities of the Visual Process), 10
Russell, D. B., and Wyard, S. J. : Electron Spin Resonance of Adenosine Triphosphate, 65
Russell, Dr. E., and others : Control of Specificity in Haemoglobin Synthesis, 1365
Russell, Sir John : Presidential Address to the concluding Conference of the Le Play Society, 1144
Russian–English Geological Dictionary (T. A. Sofiano), (edited by A. P. Lebedev and V. E. Khain), *review* by Dr. S. I. Tomkeieff, 9
Russian Scientists : Visit of, to Harwell, 957
Russian Work in Organo-Phosphorus Chemistry, *review* by Dr. F. G. Mann, 1232
Rust (Coffee), *Hemileia vastatrix* B. et Br. : Spore Liberation and Dispersal of (R. W. Rayner), 725
Ryan, J. A., and others : Determination of Uranium as Pyrophosphate, 1291
Rybák, M. (Kubelka, Vl. and) : Action of Tetracycline on the Level of Properdin, 820
Rybakov, B. V., and Sidorov, V. A. : Fast-Neutron Spectroscopy (edited by N. A. Vlasov), translated from the Russian, *review* by Dr. P. A. Egelstaff, 8
Ryde, J. Walter : *Obituary* by O. W. Humphreys, 17
Rydon, Prof. H. N. : The Cyclol Revived, *review*, 105
Ryegrass-dominant Pasture grazed by Cows : Growth of, and Properties of New Zealand Butterfat (F. H. McDowall, W. A. McGillivray and J. C. Hawke), 303
Ryle, Dr. G. J. A. : Effects of Light Intensity on Reproduction in S.48 Timothy (*Phleum pratense* L.), 196

SAAMELI, Dr. K. (Berde, Dr. B., and) : Effect of Bradykinin on Uterine Activity, 83
Sabine, T. M., and others : Scattering of Long Wave-length Neutrons by Irradiated Beryllium Oxide, 1385
Sadasivudu, B., and others : Changes in the Pentose–Nucleic Acid Content of Sub-Cellular Fractions of the Brain of the Rat during 'Metrazol' Convulsions, 1007
Sadron, Prof. Charles (Bernardi, Dr. Giorgio, and) : Kinetics of the Enzymic Degradation of Deoxyribonucleic Acid into Sub-Units, 809
Saef, Edward C., and others : Stimulation of Lipogenesis in Thyroid Tissue *in vitro* by Free Fatty Acids, 804
Safe Handling of Radioactive Substances, 1257
Sági, F., and Garay, A. S. : Effect of Gibberellic Acid on Growth and Indoleacetic Acid Oxidase Activity of *Lupinus albus* L. exposed to Different Photoperiods, 824
Sahasrabudhe, M. B., and others : Anti-cancer Properties of Thio-diglycollic Acid, 388
Saison, Dr. R., and Ingram, Dr. D. G. : Blood Groups in Infra-human Species (Blood Groups of Pigs), 654
Saito, M., and others : Toxicology of *Penicillium islandicum*, 86

Sakai, F., and others : Toxicology of *Penicillium islandicum*, 864
Sakai, Y., and others : Toxicology of *Penicillium islandicum*, 864
Salaman, Dr. M. H., and Harvey, J. J. : Leukaemia in the Progeny of Mice inoculated with a Leukaemogenic Virus, 509
Salaman, Dr. M. H., and others : Induction of Tumours by Polyoma Virus—Enhancement by Chemical Agents, 893
Salisbury, Sir Edward : Weeds and Aliens, *review* by Dr. Alec Lazenby, 842
Salivary Gland in Mice Treated with *Iso*propylnoradrenaline : Enlargement of (Dr. K. Brown-Grant), 1076
Salmon, Dr. G. A. : Appointed J. W. Wootton Research Fellow in the Department of Chemistry in the University of Leeds, 650
Salmon (Chum), *Oncorhynchus keta* : Effect of Thyroxine plus Thiourea on the Early Development of the (Prof. M. A. Ali), 1214
Salmon and Freshwater Fisheries, Committee on : Report of the, 962
Salmon and Freshwater Fishing in Great Britain (Dr. J. W. Jones), 962
Salmonella paratyphi C : Resistance to Infection with, in Mice parasitized with a relatively Avirulent Strain of *Salmonella typhimurium* (Dr. J. G. Howard), 87
Salmonella typhimurium : Resistance to Infection with *Salmonella paratyphi C* in Mice parasitized with a relatively Avirulent Strain of (Dr. J. G. Howard), 87
Salmonellae : Occurrence of, in Domestic Animals and Poultry in India (V. K. Sharma and Prof. C. M. Singh), 622
Salt Solutions : The Widening of Expansion Cracks in Mortar by (J. A. Roberts and H. E. Vivian), 125, *erratum*, 1145
Saltman, Dr. Paul, and Christensen, Dr. Tyge : Relative Volumes of Cytoplasm and Vacuole in *Nitella*, 825
Salts : Influence of, on the Uptake of Streptomycin by *Escherichia coli* (Paul H. Plotz, Donald T. Dubin and Bernard D. Davis), 1324
Salts (Molten Hydrated) : Molar Sound Velocity in (P. R. K. L. Padmini and Prof. B. Ramachandra Rao), 694
Salvatore, Dr. Francesco, and Bocchini, Dr. Vincenzo : Prevention of Ammonia Toxicity by Amino-acids concerned in the Biosynthesis of Urea, 705
Sammons, the late Irene M., and Chessin, Prof. M. : Cactus Virus in the United States, 517
Sampler (A Phytoplankton Multi-), and its Use in Lake Victoria (Dr. J. H. Evans), 53
Samples (Hail) : Radar Reflectivities of (R. H. Douglas), 266
Sampling (Acceptance) : Statistical Quality Control and (Birmingham College of Advanced Technology Course on), 337
Sampson, Joan : A Method of Replicating Dry or Moist Surfaces for Examination by Light Microscopy, 932
Samuel, Dr. Eric : Appointed reader in pharmacology at the University of Edinburgh, 443
Sanchez-Monge y Parellada, Prof. Enrique : Genetica, *review* by Prof. S. C. Harland, 213
Sanders, P. F., and others : Use of Aninal Cell, Tissue and Organ Cultures in Radiobiology (Methods for Growing HeLa Cell Suspensions in the Presence of Tritiated Thymidine and Tritium Water), 341
Sanderson, Peter : The Royal Aircraft Establishment, Farnborough, 232
Sandstedt, R. M. (Schuster, M. L., and) : Nematology (Tissue-culture Experiments with *Meloidogyne*), 1048
Sandwith, N. Y. : Flora of Peru, *review*, 214
  Die Cactaceae, *review*, 1336
Sandy Hook Marine Laboratory : Fellowships for Plankton Research, 754
Sanger, Dr. V. L. : Cell Transplants of Avian Lymphomatosis treated with Hydrogen Peroxide, 1406
Sangren, Ward C. : Digital Computers and Nuclear Reactor Calculations, *review* by Dr. M. V. Wilkes, 9
Sankar, D. Barbara, and others : Effect of Administration of Lysergic Acid Diethylamide on Serotonin-Levels in the Body, 499
Sankey, John : At the Annual Congress of the South-Eastern Union of Scientific Societies (Invertebrate Life in South-East England), 132
Sansome, Dr. Eva : Meiosis in the Oogonium and Antheridium of *Pythium debaryanum* Hesse, 827
Santa Maria Hospital, Lisbon : Grant from the Gulbenkian Foundation, in aid of science and medicine, 754
Santilli, Prof. Vincent (Piacitelli, John, and) : Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the Rate of Lesion Production on Pinto Bean, 624
Sanwal, G. G., and others : Diurnal Variation in Transaminase Activity in the Cactus Plant, 516
Sapogenin (A New Triterpenoid), of Animal Origin (Sea Cucumber), (Takao Matsuno and Toshihiko Yamanouchi), 75
Saprophyte (Filamentous), from Wookey Hole Caves : Further Observations on a (Dr. A. J. Willis), 724
Sarcoma Implant and Staphylococcus Infection : Development in Mice of Prolonged Non-specific Resistance to, following Repository Injection of Lipopolysaccharide (Samuel J. Prigal), 1111
Sardinian Family : Association of Haemoglobin *N* and Microcythaemia in a (Prof. E. Silvestroni and I. Bianco), 1208
Sáringer, K. M. (Morlin, Z., and) : Effect of Ozone on the Fluorescence and Ultra-violet Absorption Spectra of 3,4-Benzopyrene, 907
Sarkar, Dr. N. K. (Devi, Dr. Anima, and) : Catalysis of Incorporation of ^{14}C-Uridine into Rat Lens Ribonucleic Acid by Rat Lens Enzyme System, 1094
Sarma, G. Raghupathy (Brahmachari, Dr. H. D., and) : Effects of Injections of some Intermediary Metabolites on the 'Insulin Response' of Normal Rabbits, 491, *erratum*, 755
Satellite 1960 $\eta 1$: Interpretation of Cosmic Noise Measurements at 3·8 Mc./s. from (J. H. Chapman and A. R. Molozzi), 480
Satellite Data : Analysis of (Prof. D. G. Parkyn), 896
Satellite Microwave Dipoles : Long-Range Radio Communication by—the West Ford Project, 1237
Satellite Orbits : Density of the Upper Atmosphere determined by, and its Variation during 1957-61 (D. G. King-Hele and D. M. C. Walker), 114
Satellite and Space Orbits, *review* by D. G. King-Hele, 1334
Satellites (Artificial Earth) : A Collection of Tables and Nomograms for the Processing of Observations made on (I. D. Zhongolovich and V. M. Amelin), *review* by D. G. King-Hele, 1135
Sato, Haruo, and others : Hexosamine and Sialic Acid Contents in Cells, 596
Sato, Dr. Tatsuo, and others : Quantitative Determination of Metadrenaline and Normetadrenaline in Normal Human Urine, 599
Saunders, Dr. J. C. (Kothari, Dr. N. J., and) : Effects of Corticotrophin, Hydrocortisone and Methopyrapone on the Circulating Eosinophiles in Man, 1105
Savary, A. : Nematology (*Ditylenchus dipsaci* increasingly causing Collar-rot of Beet in Switzerland), 1048
Saxon, G., and others : Pulse-shortening in Electron Linear Accelerators, 483
Scale (Microgram) : Pyrolysis and Gas–Liquid Chromatography on the (C. E. Roland Jones and A. F. Moyles), 663
Scale-up in Flotation : Power Requirements and (Prof. Nathaniel Arbiter and Prof. Colin C. Harris), 268
Scarselli, Dr. V. : Increase in Elastin Content of the Human Aorta during Growth, 710
Scattering of Long Wave-length Neutrons by Irradiated Beryllium Oxide (T. M. Sabine, A. W. Pryor and B. S. Hickman), 1385
Scattering (Low-Angle Light), and the Molecular Weight of Deoxyribonucleic Acid (Dr. Sidney Katz), 280
Scharf, Prof. Joachim Hermann : Elected a Member of the Anatomy Section of the Leopoldina German Academy of Sciences, 122
Scheffer, R. P., and Pringle, R. B. : A Selective Toxin produced by *Periconia circinata*, 912
Scheinberg, Dr. S. L., and Reckel, Dr. R. P. : Blood Groups in Infra-human Species (Red Cell Agglutinogen of Chickens identified by certain Plant Seed Extracts), 654
Schelling, Prof. Thomas C. : The Strategy of Conflict, *review* by Sir Cyril Burt, 427
Scherrer, Prof. J. : Assessment of Pain (Possible Mechanisms of Phantom Pain), 1353
Schilling, Dr. R. F. : Vitamin B$_{12}$ and Intrinsic Factor (Hydroxocobalamin in Body Tissues), 1155
Schindewolf-Jordan, D. (Catsch, Prof. A., and) : Removal of Internally Deposited Radionuclides by Triethylenetetraamine-hexaacetic Acid, 715
Schistosoma mansoni : Phosphatases in (Dr. D. L. H. Robinson)—Distribution of Alkaline Phosphatase, 473 ; Quantitative Estimation of Phosphatases, 473
Schizosaccharomyces pombe : Distribution of X-Ray- and Nitrous Acid-induced Mutations in the Genetic Fine Structure of the *ad*$_7$ Locus of (Dr. Herbert Gutz), 1127
Schlegel, Prof. H. G., and others : Formation and Utilization of Poly-β-Hydroxybutyric Acid by Knallgas Bacteria (*Hydrogenomonas*), 463
Schleich, A. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Radioprotective Properties of various Compounds), 341
Schlesinger, M. J., and others : Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of *Penicillium chrysogenum*, 909
Schlesinger, Sondra, and others : Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of *Penicillium chrysogenum*, 909
Schmidt, Robert F. (Hubbard, Dr. J. I., and) : Stimulation of Motor Nerve Terminals, 1103
Schmitz-Dumont, Prof. O. : Inorganic Polymers (Co-ordination Polymers), 1047
Schneider, C.: Effects of Morphone-like Drugs in Chicks, 607
Scholarship (Travel) : Bowles Memorial (Royal Horticultural Society), 1349
Scholarships : Research Training (Animal Health Trust Awards), 1250
Scholes, Dr. George, and others : Radiation-induced Formation and Reactions of the Formyl Radical Ion, 1386
Scholtissek, Dr. Christoph, and Rott, Rudolf : Influence of *p*-Fluorophenylalanine on the Production of Viral Ribonucleic Acid and on the Utilizability of Viral Protein during Multiplication of Fowl Plague Virus, 1023
School Curriculum in Mathematics (edited by Howard Fehr), (Organization for European Economic Co-operation Report on), 335
School Library Association : Grant of £3,900 for three years from the Gulbenkian Foundation, 754
School (Summer) on Corrosion—Corrosion Problems in the Electrical Power Industry (Battersea College of Technology), 651
School Mathematics : New Thinking in, 335
Schoolboys (Sixth-Form) : Research by (J. Heywood), 860
Schools (Australian) : Promotion of Science in, 543
Schools (Grammar) : Biology for (Science Masters' Association and the Association of Women Science Teachers), 555
  Chemistry for (Science Masters' Association and the Association of Women Science Teachers), 555
  Physics for (Science Masters' Association and the Association of Women Science Teachers), 555
Schoonraad, Murray : Africa's First Science Museum, 848
Schrek, R. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Immediate and Delayed Effects of Radiation on Human and Animal Lymphocytes), 340
Schubart, H. : At the sixty-seventh Annual Conference of the Museums Association (The Training of Picture Restorers), 553
Schuch, Dr. A. F. : Bond-Lengths in Solid Helium, 591
Schuster, M. L., and Sandstedt, R. M. : Nematology (Tissue-culture Experiments with *Meloidogyne*), 1048
Schütte, K. : Space-Flight and Re-entry Trajectories (Near Earth Satellites—Changes in the Orbit of *Echo* 1), 960
Schweet, Prof. R., and others : Control of Specificity in Haemoglobin Synthesis, 1365
Schweiss, H. (Berg, Dr. H., and) : Photo-Polarography with a Flash-Lamp, 1270
Science : Food (Australian Commonwealth Scientific and Industrial Research Organization Conference on), 337, 546
  A Great Age for (Dr. Warren Weaver), 312
  The Major Achievements of (A. E. E. McKenzie), *review* by J. M. Eyles, 110
Science in Australian Schools : Promotion of, 543
Science in the Himalaya (Dr. L. G. C. E. Pugh), 429
Science for Scientists and Technologists, 311
Science of Soil Mechanics : K. Terzaghi, Founder of the, *review* by Kevin Nash, 210
Science in Space (United States National Academy of Sciences—National Research Council), 124
Science in the United States : Goals for, 312

Science, British Society for the Philosophy of : Sixth Annual Conference, 338
Science Council, and others : International Conference on Magnetism and Crystallography, 24
Science Information Personnel—the New Profession of Information combining Science, Librarianship and Foreign Languages (Leonard Cohen and Kenneth Craven), 452
Science Masters' Association : Grant of £4,500 from the Gulbenkian Foundation, 754
Science Masters' Association and the Association of Women Science Teachers : Science and Education, a Policy Statement, 555
  Chemistry for Grammar Schools, 555
  Biology for Grammar Schools, 555
  Physics for Grammar Schools, 555
Science Museum : Africa's First (Murray Schoonraad), 848
Science Museum, London : Exhibition by the Westinghouse Brake and Signal Co., Ltd., of Modern Methods of Traffic Control, 1044
Science Museum Library, London : Books on the Chemical and Allied Industries, 22
Science Study Fellowships (Unesco), 1250
Science Teachers in Training (Arthur Guinness Son and Co., Ltd., Awards for), 338
Science Teachers (Women), Association of (Science Masters' Association and the) : Science and Education, a Policy Statement, 555
  Chemistry for Grammar Schools, 555
  Biology for Grammar Schools, 555
  Physics for Grammar Schools, 555
Science (Agricultural), Australian Institute of : *Australian Journal of Experimental Agriculture and Animal Husbandry*, No. 1, 544
Science (Agricultural), Easter School in : Proceedings of the University of Nottingham Seventh (1960)—Digestive Physiology and Nutrition of the Ruminant (edited by Dr. D. Lewis), *review* by Dr. I. W. McDonald, 1334
Science (Basic) : Disease and the Advancement of (edited by Dr. Henry K. Beecher), *review* by Sir George Pickering, 530
  What is ?, *review* by Sir George Pickering, 530
Science (Nuclear), National Institute for Research in (Harwell) : Successful Operation of the *Nimrod* Injector, 1043
Science and the Classics (Prof. B. Farrington), 1337
Science and Education, a Policy Statement (Science Masters' Association and the Association of Women Science Teachers), 555
Science and the Future of Britain (Labour Party), 837
Science and Invention : National Reference Library of, 1 ; (Leslie Wilson), 733 ; (J. Farradane), 734
  Aslib Report of the Discussion Meeting on, 649
Science and the Law (*Medicine*,) : Vol. 1, No. 4, 753
Science, Librarianship and Foreign Languages : Science Information Personnel—the New Profession of Information combining (Leonard Cohen and Kenneth Craven), 452
Science and Poetry (T. R. Henn), 534
Science, Politics and Social Service (Earl of Cranbrook), 992
Science and Practice (Tradition,), in Dietetics (D. F. Hollingsworth), 1343
Science and Technology, National Lending Library for : *N.L.L. News*, No. 1, 1347
Science and Technology, College of (Manchester) : Appointment of Ronald Beresford Dew as visiting professor of industrial administration, 651
Science and Technology, Royal College of (Glasgow) : Appointment of Dr. David W. A. Sharp as senior lecturer in the Department of Chemistry, 443
  Appointment of Dr. Graham R. Knox as lecturer in the Department of Chemistry, 443
  Endowment of £800 made by Mrs. Hilda M. Beilby, to be known as the Kelvin Prizes, 1349
Science and Television, 20
Sciences : The Senses and the (Sir Bryan Matthew), 989
  The Social (House of Commons Debate on), 1354
Sciences, New York Academy of : Symposium on the Use of Animal Cell, Tissue and Organ Cultures in Radiobiology, 340
  Conference on Blood Groups in Infra-human Species, 653
  Fifth International Conference on Pigment Cell Biology, 1045
Sciences, United States National Academy of: Election of foreign associates, 18
Sciences, U.S.S.R. Academy of : Mathematical Tables, Vol. 12, A Collection of Tables and Nomograms for the Processing of Observations made on Artificial Earth Satellites (I. D. Zhongolovich and V. M. Amelin), *review* by D. G. King-Hele, 1135
Sciences (Arts and), American Academy of : Monograph Prizes, 442
Sciences (General Medical) : Highlights of Research Progress in, 1960 (United States Department of Health, Education and Welfare, Public Health Service Publication No. 815), 658
Sciences (Management), Institute of: Proceedings of the Sixth International Meeting (Paris, 1959)—Management Sciences, Models and Techniques (edited by Prof. C. West and Prof. Michel Verhulst), *review* by T. H. Hawkins, 209
Sciences (Management), Models and Techniques—Proceedings of the Sixth International Meeting of the Institute of Management Sciences, Paris 1959 (edited by Prof. C. West and Prof. Michel Verhulst), *review* by T. H. Hawkins, 209
Sciences (Medical), The Use of Radioisotopes in Animal Biology and the (International Atomic Energy Agency, the Food and Agriculture Organization and the World Health Organization of the United Nations, Conference on), 959
Scientific Attitude to Management, *review* by T. H. Hawkins, 209
Scientific Books : Publication of (Scientific Publications Council Meeting on), 131
Scientific Discipline : Plant Taxonomy as a (Dr. R. E. Holttum), 990
Scientific Enquiries in Parliament : Recent, 857
Scientific Information, Institute for (Philadelphia) : Services, Publications and Techniques, 1045
Scientific Instrument Manufacturers' Association of Great Britain : Election of Officers, 546
Scientific Literature : The Use of (B. C. Vickery), 1347
Scientific Publications Council : Meeting on the Publication of Scientific Books, 131
Scientific Research : National Organization of, 632
Scientific Research in British Universities, 1960-61 (Department of Scientific and Industrial Research), 228
Scientific Research in the U.S.S.R., 123
Scientific Research, National Foundation for (Belgium) : Thirty-third Annual Report, 441
Scientific Research and Development : Government Responsibility in, 837
Scientific Societies, South-Eastern Union of : Annual Congress (F. J. Epps), 132
Scientific Societies and the Rating and Valuation Bill, 123
Scientific Teachers : Shortage of, in the United Kingdom, 123
Scientific Unions, International Council of : Year Book for 1961, 123
  Ninth General Assembly, 752
  ICSU Review, Vol. 3, No. 1, 865
Scientific Work of Sir James Hall : The Life and (Dr. V. A. Eyles), 345
Scientific and Industrial Research, Council for (India) : Seminar at Hyderabad on Castorseed and its Products, 753
  Annual Technical Report (1959-60), 1254
Scientific and Industrial Research, Department of : The National Physical Laboratory—Open Days (J. R. Illingworth), 112
  Memoirs of the Geological Survey of Great Britain—England and Wales, Geology of the Country around Monmouth and Chepstow (Dr. F. B. A. Welch and Dr. F. M. Trotter), 227
  Scientific Research in British Universities (1960-61), 228
  Report of the Research Council for 1960, 230
  Radio Research Station, Slough—Open Days, 231
  Factory Buildings Studies No. 8, Colouring in Factories, 336
  Report of the National Chemical Laboratory 1960 ; Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for 1960, 342
  Open Days at the Road Research Laboratory, 644
  Grant of £13,362 during two years to the Department of Geology in the University of Leeds, for an investigation into African rocks, 650
  Grant of £2,897 during two years, for a research project in the Department of Biochemistry in the University of Leeds, 650
  Quality Measurement in the Chemical Factory (A. J. Clark and C. W. Munday), 752
  Forest Products Research 1960—Report of the Products Research Steering Committee with the Report of the Director of Forest Products Research, 760
  List No. 29, Forthcoming International Scientific and Technical Conferences, 959
  Open Days at the Building Research Station, 1053
  Change of address, 1145
  Research for Industry 1960—a Report on Work done by Industrial Research Associations in the Government Scheme, 1252
  Radio Research 1960—the Report of the Radio Research Board and the Report of the Director of Radio Research, 1255
  N.L.L. News, No. 1, of the National Lending Library for Science and Technology, 1347
Scientific and Industrial Research Organization (Australian Commonwealth): Overall management of the Chemical Research Laboratories, 23
  Post-Doctoral Fellowships in Protein Chemistry, 336
  Conference on Food Science, 337
  Retirement of N. A. Esserman as director of the National Standards Laboratory, 542
  Appointment of I. E. Newnham as chief of the Division of Mineral Chemistry, 543
  New laboratories for the Division of Food Preservation, 546
  Division of Food Preservation Conference on Food Science, 546
Scientific and Technical Books : Recent, Supp. iv, July 28 ; Supp. iv, August 26 ; ccccxciii, September 30
Scientific and Technical Conferences : Forthcoming International (Department of Scientific and Industrial Research, List No. 29), 959
Scientifique (Recherche), Fonds National de la : Trente-troisième Rapport Annuel, 441
Scientists (Russian) : Visit of, to Harwell, 957
Scientists and Engineers engaged in research and development in Colleges and Universities in the United States for 1958 : Report on (United States National Science Foundation), 225
Scientists and Technologists : Science for, 311
Scintillations and the Latitude Distribution of Ionospheric Irregularities (D. G. Singleton), 482
Scoop (An Australopithecine), made from a Right Australopithecine Upper Arm Bone (Prof. Raymond A. Dart), 372
Scotland : Research and Development in, 440
Scott, A. I. : Ciba Foundation Symposium on Quinones in Electron Transport, *review*, 321
Scott, C. G. (edited by C. E. H. Knapman, assisted by) : Gas Chromatography Abstracts, 1958, *review* by A. Goldup, 1135
  Gas Chromatography Abstracts, 1959, *review* by A. Goldup, 1135
Scott, W. H., and others : Management in Britain, 1332
Scott, —. and others : How Old are the Angiosperms ?, 442
Scottish Museum (Royal), Edinburgh : Retirement of Dr. Douglas A. Allan from the directorship, 648
  Appointment of Ian R. Finlay as director, 648
  Appointment of Cyril Aldred as keeper of the Department of Art and Ethnography, 648
  Annual Report for 1960, 958
Scrase, Dr. F. J. : Electric Currents associated with Geomagnetic Disturbances in the Auroral Zone, 963
Scudder, Dr. John, and others : The Diego Factor in a Puerto Rican Family—a Case of Anti-Diego in Monozygotic Twins, 188
Scurvy and Lathyrism (Odoratism) : Influence of, on Hydroxyproline Excretion (Dr. G. R. Martin, S. E. Mergenhagen and D. J. Prockop), 1008
Sea : Life and Environment on the Bed of the (Prof. C. M. Yonge), 986
  Research on the Effects of Radioactivity in the, 122
  Speciation in the (Systematics Association and the Challenger Society Symposium on), 229
Sea (North) : *Pneumodermopsis paucidens* in the (G. A. Cooper and D. C. T. Forsyth), 1412
Sea Cucumber : A New Triterpenoid Sapogenin of Animal Origin (Takao Maysuno and Toshihiko Yamanouchi), 75
Sea Urchin : Intracellular Freezing and Frost Resistance in Egg-Cells of the (E. Asahina)—Intracellular Freezing as a Cause of Frost-killing in relatively Rapidly Frozen Cell Suspensions, 1263 ; A Mechanism to Prevent the Seeding of Intracellular Ice in Freezing Living Cells. 1264

Sea-Water : Gel Filtration of—Separation of Free and Bound Forms of Vitamin B_{12} (Dr. K. W. Daisley), 868
Sea-Water (Old) : A Lake in British Columbia containing (Dr. P. M. Williams, Prof. W. H. Matthews and Prof. G. L. Pickard), 830
Sea-Water and Chloride Solutions : Formation of Calcium Chloraluminate by Interaction of Portland Cement with (Dr. L. Heller and Dr. M. Ben-Yair), 488
Sea-weed (*Fucus vesiculosus*), (Gibberellin-like Substances in Plants), (Margaret Radley), 685
Sealing Compound for Use in Biological Work (G. Stotzky, W. Culbreth and Dr. L. B. Mish), 410
Seaman, Dr. G. V. F., and others : Sialic Acids and the Electrokinetic Charge of the Human Erythrocyte, 44
Searching *Chemical Literature* : Report on Computer Systems for (E. C. Medam and H. R. Koller), (United States National Bureau of Standards Research Information Centre and Advisory Service on Information Processing), 23
Seasonal Fluctuations and Distribution of Nitrite in a Tropical West African Estuary (J. C. D. Watts), 929
Seasonal Variations of *Caesium-137 in Air at Ground-Level* (K. G. Vohra, V. S. Bhatnagar and C. Rangarajan), 747
Secretions of other Bacteria or Sulphydryl Compounds for Normal Growth : Spontaneous Development of *L* Forms of Streptococci requiring (Ayala Frenkel and Dr. Willy Hirsch), 728
Section (Bilateral), of the Splanchnic Nerve : Effect of, on Erythropoiesis (Fumimaro Takaku, Kunitake Hirashima and Prof. Shigeo Okinaka), 500
Sections (Ultra-Thin), of Vitrinite in Coal : Electron Microscopic Observations of Structure in (J. T. McCartney, Sabri Ergun and R. E. Walline), 1361
Sector-Focused Cyclotrons—Proceedings of an Informal Conference, Sea Island, Georgia, 1959 (edited by F. T. Howard), (United States National Academy of Sciences—National Research Council, Nuclear Science Series, Report No. 26, Publication No. 656), *review* by M. Snowden, 532
Security : Arms and—a Mathematical Study of the Causes and Origins of War (*Dr. Lewis F. Richardson*), edited by Nicolas Rashevsky and Ernesto Trucco, *review* by Sir Cyril Burt, 427
Sediment (Triassic) : Fossil Chlorins in a (Max Blumer and Gilbert Omenn), 161
Sediments : Metal Chlorin Complexes in Recent, as Initial Precursors to Petroleum Porphyrin Pigments (G. W. Hodgson and Eric Peake), 766
Seed (Castor-) and its Products (*Council for Scientific and Industrial Research*, India, Seminar on), 753
Seedlings (Apple) : Shortening the Juvenile Phase of (A. I. Campbell), 517
Seedlings (Axenic), of *Drosera* (Richard M. Burger), 724
Seedlings (Etiolated Oat) : Carbon Dioxide and Ethanol as Factors controlling the Growth of (Dr. C. L. Mer), 260
Seeds of *Pinus sylvestris* L. : Effect of Red and Far-red Irradiation on the Germination Process in (Bengt Nyman), 1219
Seeds (Pea) : Effect of Boric Acid on the Phosphoglucomutase of (Dr. B. C. Loughman), 1399
Seeds (Tropical) : Dispersal of, by Ocean Currents (R. Mason), 408
Segré, Dr. G., and Silberberg, Dr. A. : Biorheology and Microcirculation (The Radial Distribution of Rigid Macroscopic Spheres in a Poiseuille Field of Flow), 758
Sehnal, L. : Space-Flight and Re-entry Trajectories (Near Earth Satellites—Luni-solar Perturbations on a 24-hr. Satellite), 960
Seizure (Audiogenic) : Psychophysiology, Neuropharmacology and Biochemistry of (*Centre National de la Recherche Scientifique International Meeting on*), 959
Selby Fellowship, 24
Selected Journalism from the English Reviews by Stendahl, with Translations of other Critical Writings (edited by G. Strickland), 1031
Selected Sugars : Relative Sweetness of α- and β-Forms of (Rose Marie Pangborn and Sandra C. Gee), 810
Selection : University, *review* by Dr. W. H. King, 947
Selection (Stabilizing), for Heading Time in a Bulk-Hybrid Population of Barley (Dr. S. K. Jain), 1123
Selective Isolation (Routine), of Fungi Pathogenic to Man : Use of Desertomycin Medium in (J. Uri and Zs. Herpay), 1015
Selective Toxin produced by *Periconia circinata* (R. P. Scheffer and R. B. Pringle), 912
Selective Uptake of Specifically Bound Cobalt-58 Vitamin B_{12} by Human and Mouse Tumour Cells (Dr. Bernard A. Cooper and Dr. William Paranchych), 393
Selectivity of Palladium Catalysts for the Oxidation of Carbon Monoxide in the Presence of Hydrogen (P. Mars and Th. van de Mond), 1003
Selenology : Holistic Approach to (Brian Warner), 586
Selenomethionine as an Antioxidant (H. S. Olcott, W. Duane Brown and J. Van der Veen), 1201
Seliber, B. A. : At the Second International Measurement Conference (*Electrical Measuring Instruments based on the Photo-compensating Amplifier*), 1351
Selig, E. T. (Yong, Dr. Raymond, and) : Role of Interparticle Forces in Development of Upper Plastic Limit of Clays, 938
Sell, George : Glossary of Petroleum Terms, third edition (Institute of Petroleum), 22
Selmeci, V., and others : Effect of Amino-Acid Esters on the Growth of Fibroblast Cultures, 494
Semen (Undiluated) : Effect of Varying Doses of, on Fertility in the Domestic Fowl (G. C. Taneja and R. S. Gowe), 828
Semiconducting High Polymers : Photochemical Production of (Prof. Gerald Oster, Dr. Gisela K. Oster and Dr. Marian Kryszewski), 164
Semiconductivity of Chlorpromazine (Dr. F. Gutmann and A. Netschey), 1390
Semiconductor Nuclear Particle Detector (Silicon) : Interaction of 14-MeV. Neutrons with a (Dr. W. M. Deuchars and G. P. Lawrence), 995
Semi-Skilled : Ageing and the—a Survey in Manufacturing Industry on Merseyside (Alistair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine Cunningham), 1249
Senecal, Irene P., and others : An Antigen in Lesions of Molluscum Contagiosum, 509
Senescence and Age-dependent Mortality in Fish : Expected Rate of (Dr. Alex Comfort), 822
Senneck, C. R. : Design and Use of Respirators (Application of Respiratory Devices in Mine Rescue), 656
Sense-Modalities (Two) : *Learning in* (Dr. G. Ettlinger), 308
Sense Organs, Integration and Behaviour (edited by Talbot H. Waterman), (The Physiology of Crustacea, Vol. 2), *review* by Dr. J. Green, 428
Senses and the Sciences (Sir Bryan Matthew), 989
Sensitivity to Hydrostatic Pressure in the Prawn, *Palaemonetes varians* Leach : Mechanism of (Peter S. B. Digby), 366
Sensitivity of Mice to Bacterial Lipopolysaccharide following Alteration of Activity of the Reticulo-Endothelial System (Dr. G. N. Cooper and Dr. A. E. Stuart), 294
Sensitivity (Anaphylactic) : A New Test for detecting—the Basophil Reaction (Prof. Walter B. Shelley and Lennart Juhlin), 1056
Sensitivity (Contact), to Picryl Chloride in Guinea Pigs : Passive Transfer of, with Subcellular Material (Dr. J. L. Turk), 915
Sensitivity (Radiation), of *Escherichia coli* to β-Rays in Dry and Wet Condition (S. B. Bhattacharjee and N. N. Das Gupta), 1015
Sensory Vesicle of the Ascidian Tadpole : Electron Microscope Observations of the Receptors in the (Noël Dilly), 786
Separating Amino-Acid Mixtures from Protein Hydrolysates on One Ion-Exchange Column during Preparation : A Simplified Method for (J. Liebster, K. Kopoldová and M. Dobiášová), 1198
Separation of Free and Bound Forms of Vitamin B_{12} : Gel Filtration of Sea-Water— (Dr. K. W. Daisley), 868
Separation of Linear from Branched Polysaccharides by Precipitation as Iodine Complexes (Blanche *D. E. Gaillard*), 1295
Separation Methods based on Molecular Sieving and Ion Exclusion (J. Porath and E. B. Lindner), 69
Separation (Chromatographic), of Biologically Active Components from Commercial Trypan Blue (J. Dijkstra and J. Gillman), 803
Separation and Concentration of Solutes : Cryoscopic Effect of (Dr. K. S. Rangappa), 1087
Separation and *in vitro* Culture of Cells from Liver Tissue (Dr. Justine S. Garvey), 972
Separation and Isolation of Proteins (edited by Dr. P. Alexander and R. J. Block), (A Laboratory Manual of Analytical Methods of Protein Chemistry, including Polypeptides, Vol. 1), *review* by Dr. D. T. Elmore, 320
Sequences (Amino-Acid) : Immunochemical Distinction between Insulins with Identical (Dr. Solomon A. Berson and Dr. Rosalyn S. Yalow), 1392
Sera (Blood) : Emulsifying Capacity of (Prof. B. Breyer), 174
Sergent, Edmond : Immunity to Protozoa (Premunition), 551
Serial Reaction Times (Simple and) : Relationship between (Prof. H. Kay and Dr. A. D. Weiss), 790
Series of Subliminal Stimuli : Response to—an Ethological Method for investigating the Properties of Nervous Systems (A. J. Pontin), 512
Series (New), of Anti-tumour Agents : N,N′-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds—a (A. S. Tomcufcik, S. D. Willson, A. W. Vogel and A. Sloboda), 611
Serologically Active Trisaccharides (Two), isolated from Human Blood-Group *A* Substance (I. A. F. Lister Cheese and Prof. W. T. J. Morgan), 149
Serotonin : Effect of, on Absorption and Distribution of Calcium-45 in the Rat (Prof. S. Garattini, E. Grossi, P. Paoletti, R. Paoletti and M. Poggi), 185
Serotonin (Blood) : Changes in, during Exposure to Cold (Dr. Paul Gordon), 183
Serotonin-Levels in the Body : Effect of Administration of Lysergic Acid Diethylamide on (Dr. D. V. Siva Sankar, D. Barbara Sankar, Edward Phipps and Eleanor Gold), 499
Serum : Simultaneous Determination of Dextran and Glucose in (A. Rédei and S. Nagy), 173
  Vitamin-Levels in Blood and (H. Baker, O. Frank, I. Pasher, Dr. H. Sobotka and S. H. Hutner), 78
Serum and other Mixtures : Estimation of Specific Proteins in (Dr. D. A. Darcy), 1163
Serum (Guinea Pig): Evidence that the L-Asparaginase Activity of, is responsible for its Antilymphoma Effects (J. D. Broome), 1114
Serum Albumin : Splitting of, during Electrophoresis on Paper (J. V. McLoughlin), 1305
Serum Albumin (Bovine) : Elimination of, in Acute Experimental Uraemia (Dr. G. Gowland, F. G. Smiddy, Dr. R. G. Burwell and F. M. Parsons), 1210
Serum Cholinesterase (Human), with Fluoride : Differential Inhibition of—Recognition of Two New Phenotypes (Prof. H. Harris and Mary Whittaker), 496
Serum Factor Gm^a among the Iranians (Luba Podliachouk, Dr. A. Eyquem, R. Choaripour and M. Eftekhari), 717
Serum Factors : Influence of, on the Species-specificity of Tissue Thromboplastin (Christine Hawkey and J. L. Stafford), 920
Serum Leucine Aminopeptidase (Human) : Sub-fractionation of (Prof. Clyde A. Dubbs, Charlotte Vivonia and Jean M. Hilburn), 1203
Serum Lipids in relation to the Intake of Alcohol and Sodium Chloride (Erling Lund, Torben Geill, Prof. Henrik Dam and Gunhild Kristensen), 1095
Serum Lipids and Atherosclerosis in Rabbits : Effects of Carrageenin on (Dr. Katsumi Murata), 189
Serum Mucoprotein in Rheumatic Fever : Amino-acid Composition of (Prof. D. O'Brien, C. H. M. Walker and D. Walker), 922
Serum Protein Fractions of the Newly Hatched Chick : Comparison of the, with those of Adult Birds using Starch-gel Electrophoresis (A. Amin), 708
Serum Transferrins in the Genus *Macaca*—Species Distribution of Nineteen Phenotypes (Prof. Morris Goodman and Emily Poulik), 1407
Serums (Cord Blood) : Haptoglobin Patterns in (A. R. Rausen, P. S. Gerald and Dr. L. K. Diamond), 717
Service : The World University (Hilda M. Browne), 1140
Service (Hospital): Abstracts of Efficiency in the (Ministry of Health), 441
Service (Social) : Science, Politics and (Earl of Cranbrook), 992
Services (Public Health) : The Planning of (United Nations, World Health Organization, Technical Report Series No. 215—Fourth Report of the Expert Committee on Public Health Administration), 441
Serville, Yvonne : At the Third International Congress of Dietetics (*The Means, indirect and direct, of Conveying the Finds of Research on the Science of Nutrition to the Consumer*), 1344

Seshadri, Prof. T. R. : Elected a Member of the Chemistry Section of the Leopoldina German Academy of Sciences, 122
Set Theory : Abstract (Prof. Abraham Fraenkel), second edition, *review* by Dr. R. G. Cooke (June 17, 1047), *erratum*, 126
Seth, Prof. George : From Galton to Crowther (Presidential Address to Section J (Psychology) of the British Association for the Advancement of Science), 989
Setinek, Dr. K., and others : Paper Chromatography (Film showing the Technique of Paper Chromatography of Amino-acids), 759
Setonix brachyurus (Rottnest Quokka) : Myopathy affecting the, reversed by α-Tocopherol (B. A. Kakulas), 402
Setser, Dr. D. W. : Awarded a United States fellowship in chemistry for 1961-62 by the Ramsay Memorial Trust, 337
Sex Chromatin : Peripheral Position of (Dr. Charles P. Miles), 626
Sex Composition of Lamprey Populations (Dr. M. W. Hardisty), 1116
Sex-Ratio in Mice (Margaret J. Cook and Augustin Vlček), 89
Sexual Differentiation in *Hydra* in relation to Population Density (Helen D. Park, Christyna Mecca and Anne Ortmeyer), 92
Sexual Maturity of Mice : The Effect of various Diets upon (J. Szepsenwol), 1112
Seykora, E. J. : Magnetic Mirror Confinement of Exploding Wire Plasma, 995
Shaffer, Dr. Lloyd H. : Thermo-electric Phenomena in Electrodialysis Systems, 591
Shah, L. C., and others : Anticancer Properties of Thio-diglycollic Acid, 388
Shale (Black), Oil and Sulphide Ore (Prof. K. C. Dunham), 985
Shander, D., and others : Urea Synthesis in the Living Rat Brain, 288
Shankland, Prof. Robert S. : Atomic and Nuclear Physics, second edition, *review* by Dr. E. B. Paul, 8
Shape Discrimination : Facts and Theories of (Dr. P. C. Dodwell), 578 ; (Dr. N. S. Sutherland), 581
Shapwick Heath Nature Reserve (Nature Conservancy), 1248
Shark (Greenland) : Copepod *Ommatokoita elongata* (Grant) in the Eyes of the—a Possible Cause of Mutual Dependence (Bjørn Berland), 829
Sharma, C. P., and others : Effect of Excess Supply of Heavy Metals to Barley during Germination, with special reference to Catalase and Peroxidase, 726
Sharma, M. I. D., and Joshi, G. C. : An Abnormal Form of Female Rat Flea, *Xenopsylla cheopis* Roths., 727
Sharma, Madhu Sudan : Excretory Ducts in the Genus *Notopterus* (Lacepede), 1118
Sharma, V. K., and Singh, Prof. C. M. : Occurrence of *Salmonellae* in Domestic Animals and Poultry in India, 622
Sharman, Dr. G. B. : Elected president for 1961-62 of the Australian Mammal Society, 755
Sharman, Dr. R. V., and White, D. : Initial Vibrations of a Supported Beam, 692
Sharp, Dr. A. G., and others (edited by) : Advances in Fluorine Chemistry, Vol. 1, *review* by Dr. Richard Dresdner, 319
Sharp, Dr. David W. A. : Appointed senior lecturer in the Department of Chemistry, Royal College of Science and Technology, Glasgow, 443
Shatsky, Dr. N. S. : *Obituary* by Prof. S. I. Tomkeieff, 542
Shaw, Dr. B. L. : Appointed lecturer in inorganic and structural chemistry at the University of Leeds, 228
Shaw, Dr. D. H. : Blood Groups in Infra-human Species (Role of Individual Red Cell Antigens in Speciation), 654
Shawcross, Lord : Appointed a member of the Medical Research Council, 546
Shear (Current) : Evidence of an Eastward Equatorial Undercurrent in the Atlantic from Measurements of (Arthur D. Voorhis), 157
Sheep : Methaemoglobin in Chronic Copper Poisoning of (J. R. Todd and R. H. Thompson), 89
  Occurrence of an *Rh*-like Antigen in Red Cells of the (B. Urbaschek and Prof. K. Trautwein), 193
  Thiabendazole, a Highly Effective Anthelmintic for (Hugh McL. Gordon), 1409
Sheep Breeds (Rajasthan) : Composition of the Skin Follicle Population in seven (Sunanda Narayan), 115
Sheep Erythrocytes : Antibody Response in Rabbits following Injection of, into Lateral Ventricle of Brain (Prof. B. D. Janković, Dr. M. Draškoci and Dr. K. Isaković), 288
Sheffield Smelting Company, Ltd., 1760-1960 : Two Hundred Precious Metal Years—a History of the (Ronald E. Wilson), *review* by Prof. R. W. K. Honeycombe, 108
Shellabarger, Prof. C. J., and others : Thyroid Function in Active, Sleeping and Torpid Hummingbirds, 1318
Shelley, Dr. Heather J. : Foetal and Neonatal Physiology (Reserves of Glycogen and their Changes at Birth and in Anoxia), 344
Shelley, Prof. Walter B., and Florence, Ralph : Ethylene Glycol Monomethyl Ether, a New Fixative for Histological Work, 719
Shelley, Prof. Walter B., and Juhlin, Lennart : A New Test for Detecting Anaphylactic Sensitivity—the Basophil Reaction, 1056
Shephard, R. J. : Design and Use of Respirators (Ergonomics of the Respirator), 655
Shepherd, A. M. : Nematology (*Heterodera gottingiana* Larvae), 1048
Shepherd, Dr. D. M., and others : Histidine Decarboxylase and Growth, 1311
Sheppard, P. M., and others : Genetics of a Difference in the Male Genitalia of East and West African Stocks of *Papilio dardanus* (Lep.), 935
Sherratt, Dr. H. S. A. (Leonard, B. E., and) : Pharmacology of the Alkaloidal Fraction from the Bark of the Jamaican Shade Tree *Pithecolobium samath* Benth., 287
Shettles, Prof. Landrum B. : Ovum Humanum, *review* by W. J. Hamilton, 110
Shipbuilding Research in Britain, 543
Shirling, F. C. S. : Design and Use of Respirators (Respirators in the Fire Service), 656
Shocks (Radiant Heat) : Mutagenic Effect of, on Phanerogamous Plants (Dr. Bror Pettersson), 1167
Shoenberg, Dr. D. : The de Haas-van Alphen Effect and the Electronic Structure of Metals (Guthrie Lecture of the Institute of Physics and the Physical Society), 126
  Obituary of Sir K. S. Krishnan, 541
Shoenberg, Isaac—Silver Jubilee of British Television, 751
Shopland, C. D., and others : Measuring Visual Constancy for Stationary or Moving Objects, 416
Shorland, Dr. F. B., and others : Lipids of the Common Pea (*Pisum sativum* L.) with special reference to the Occurrence of Galactolipids, 1301
Short-Circuit Current and Net Water Movement : Comparison of, in Single Perfused Proximal Tubules of Rat Kidneys (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Short-term Dating System : Carbon-14 from Nuclear Explosions as a—Use to determine the Origin of Heartwood (Dr. A. T. Wilson), 714
Shortage of Mathematics Teachers in Britain, 127
Shortage of Scientific Teachers in the United Kingdom, 123
Shortening the Juvenile Phase of Apple Seedlings (A. I. Campbell), 517
Shortening (Pulse-) in Electron Linear Accelerators (Dr. M. C. Crowley-Milling, T. R. Jarvis, C. W. Miller and G. Saxon), 483
Shorter, Dr. Roy G., and Lack, R. G. : Immunological Study of Experimentally Induced Extrahepatic Cholestasis, 610
Showers (Extensive Air), in the Atmosphere : Photography of Cerenkov Light from (D. A. Hill and N. A. Porter), 690
Shunkov, V. I., and others : A Soviet View of British Libraries (translated by A. Thompson), (Library Association, Pamphlet No. 22), 752
Shutter Device (Variable) : A New Photometric Technique using a (Dr. O. C. Jones and Dr. F. J. J. Clarke), 1290
Shyu, Wei-jen (Gregory, Prof. K. F., and) : Apparent Cytoplasmic Inheritance of Tyrosinase Competence in *Streptomyces scabies*, 465
Sialic Acid as a Structural Component of some Mammalian Tissue Cell Surfaces (Dr. Leonard Weiss), 1108
Sialic Acid Contents (Hexosamine and), in Cells (Atsushi Kimura, Yasishi Nagai, Dr. Koh-Iti Turumi, Yoshiko Kawashima and Haruo Sato), 596
Sialic Acids : Polarographic Examination of (Barbara Robert, Max E. Rafelson and Ladislas Robert), 596
Sialic Acids and the Electrokinetic Charge of the Human Erythrocyte (G. M. W. Cook, Dr. D. H. Heard and Dr. G. V. F. Seaman), 44
Sickling Phenomenon : Haemoglobin 'Barts' and the (Dr. Lie-Injo Luan Eng), 1314
Side-Reactions during Hydrogenolysis of 2,4-Dinitrophenylhydrazones of Aromatic Keto-Acids (Dr. John H. Menkes), 285
Sidorov, V. A. (Rybakov, B. V., and) : Fast-Neutron Spectroscopy (edited by N. A. Vlasov), translated from the Russian, *review* by Dr. P. A. Egelstaff, 8
Siegel, B. Z., and others : Regulation of Pyrogallol Oxidations by Alcohols and Sugars, 180
Siegel, S. M., and others : Regulation of Pyrogallol Oxidations by Alcohols and Sugars, 180
Sieving (Molecular), and Ion Exclusion : Separation Methods based on (J. Porath and E. B. Lindner), 69
Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach : Significance of the Anti-Sporulation Effect of Copper Fungicides in the Control of (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476
Sign (Newly Recognized), of Denervation : Muscle Target Fibres, a (Dr. W. K. Engel), 389
Sign Reversal of Permselective Membranes : Poisoning and (Dr. Francis de Körösy), 1363
Signal-Noise Ratio : Possible Method for Increasing the, in the Detection of the First Motion of a Refracted *P*-Wave (F. A. Key, J. K. Wright, E. W. Carpenter and B. M. H. Stott), 1382
Signal-to-Noise Ratio (Television) : An Instrument for measuring (S. M. Edwardson), 1355
Signals (Radio) : Effect of External Conditions on the Phase of (D. E. Watt-Carter and R. L. Corke), 1286
Significance of the Anti-Sporulation Effect of Copper Fungicides in the Control of Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476
Significance of the Regeneration of Lateral Plates of the Stickleback (*Gasterosteus aculeatus* L.), (Tadeusz Penczak), 621
Significance (Diagnostic), of Heterogeneous Lactic Dehydrogenases in Malignant Effusions (R. Richterich, K. Zuppinger and E. Rossi), 507
Significance (International) : New Trends in Atomic Research and their (Dr. L. Kowarski), 853
Significance (Physiological), of Surface Wax on Leaves (D. M. Hall and R. L. Jones), 95
Significance (Possible), in Behaviour : Distribution of Piperidine in the Brain and its (Dr. L. G. Abood, F. Rinaldi and Virginia Eagleton), 201
Silberberg, Dr. A. (Segré, Dr. G., and) : Biorheology and Microcirculation (The Radial Distribution of Rigid Macroscopic Spheres in a Poiseuille Field of Flow), 758
Silica : Fibrous (Dr. Wolfgang Haller), 662
  Nomenclature of (I. Bergman), 270
Silica (Vitreous) : Permeation of Gaseous Oxygen through (Dr. Francis J. Norton), 701
Silicate (Lead) Glass : Absorption Spectra of γ-Irradiated (R. S. Barker, T. K. Harrison, D. A. Richardson and R. Rimmer), 374
Silicates (Layer Lattice) : Surface Symmetry and Cell Dimensions of (E. W. Radoslovich), 67
Silicon Semiconductor Nuclear Particle Detector : Interaction of 14-MeV. Neutrons with a (Dr. W. M. Deuchars and G. P. Lawrence), 995
Silicosis (Experimental) : Immunological Observations in (J. H. Jones and Prof. A. G. Heppleston), 1212
Silkworm, *Bombyx mori* : Brain Hormone of the (Mamori Ichikawa and Hironori Ishizaki), 933
  Role of Lipid in the Hardening of the Cuticle in the (Shigemi Kawase), 279
  Sterol Requirements of the (Dr. Toshio Ito), 882
Silver Bromide Microcrystals : Electron Spin Resonance Absorptions induced by Light in Adsorbed Layers of Cyanine Dyes on (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Silver Jubilee of British Television : Isaac Shoenberg—, 751
Silver Nitrate Solution (Aqueous) : Ultrasonic Velocity in (D. S. Allam and W. H. Lee), 1388
Silverman, M. P., and Rogoff, M. H. : Morphological Variation in *Ferrobacillus ferrooxidans* related to the Rate of Iron Oxidation, 1221
Silverstein, Dr. Emanuel : Effect of Hybridization on the Primary Polydipsic Trait of an Inbred Strain of Mice, 523
Silvestroni, Prof. E., and Bianco, I. : Abnormal Foetal Haemoglobins, 397
  Association of Haemoglobin *N* and Microcythaemia in a Sardinian Family, 1208
Silvestroni, Prof. E., and others : Haemoglobin *P* in a Family of Southern Italian Extraction, 292

Sim, M. F., and others : Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine, 1312
Simonsen, Dr. Morten : Appointed director of research at the McIndoe Memorial Research Unit, Queen Victoria Hospital, East Grinstead, 337
Simonsen Phenomenon : Large Lymphocytes and the (Dr. Aleksander Szenberg and N. L. Warner), 920
Simple, Quick Methods for Detection of 6-Amino-penicillanic Acid (J. Uri and F. Sztaricskai), 1223
Simple and Serial Reaction Times : Relationship between (Prof. H. Kay and Dr. A. D. Weiss), 790
Simplified Method for Separating Amino-Acid Mixtures from Protein Hydrolysates on One Ion-Exchange Column during Preparation (J. Liebster, K. Kopoldová and M. Dobiášová), 1198
Simplified Procedure for Removing Non-specific Staining Components from Fluorescein-labelled Conjugates (G. D. Johnson), 70
Simpson, K., and others : Incorporation of 5,10,15 ^{14}C-Farnesol Pyrophosphate into *Phycomyces carotenoids*, 1299
Simultaneous Detection of Iodide and Iodinated Amino-Acids on Filter Paper Chromatograms : A Spray Method for the (Dr. A. Van Zyl and M. Bhaga), 275
Simultaneous Determination of Dextran and Glucose in Serum (A. Rédei and S. Nagy), 173
Simultaneous Eradication of Three Ectoparasitic Species from a Colony of Laboratory Mice (Dr. Nigel Bateman), 721
Singh, Prof. C. M. (Sharma, V. K., and) : Occurrence of *Salmonellae* in Domestic Animals and Poultry in India, 622
Singhal, G. S. (Rohatgi, Dr. K. K., and) : Average Extinction Coefficient for Re-absorption of Fluorescence Radiation through Uranin Solution, 799
Single Crystals of Gallium : Thermoelectric Power of (P. Horner), 58
Single Perfused Proximal Tubules of Rat Kidneys : Comparison of Short-Circuit Current and Net Water Movement in (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Single Spermathecae (Double and), in Fleas (Siphonaptera), (Hon. Miriam Rothschild), 1322
Single Substance : Multiple Inhibitions of Cell Metabolism by a (M. Harris and Dr. G. A. Morrison), 1276
Singleton, D. G. : Scintillations and the Latitude Distribution of Ionospheric Irregularities, 482
Sintered High Temperature Compounds (Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals Symposium on), 1250
Sirois, Dr. J. C. (Katznelson, Dr. H., and) : Auxin Production by Species of *Arthrobacter*, 1323
Siva Sankar, Dr. D. V., and others : Effect of Administration of Lysergic Acid Diethylamide on Serotonin-Levels in the Body, 499
Sivertsen, W. I., and others : Decreased Urinary Excretion of 2-Aminoethanol by Irradiated Human Beings, 1303
Six Animal Species in Free-choice Experiments : Comparison between Water and Alcohol Consumption in (Dr. Alpo Arvola and Dr. Olof Forsander), 819
Sixth-Form Schoolboys : Research by (J. Heywood), 860
Sixth Forms : Biology Syllabus for (Gulbenkian Foundation Conference on, at the University of Birmingham), 554
Size of the Heart : Relationship between Velocity of Cardiac Contraction and—a Hypothesis (Dr. H. Feinberg), 1373
Skauen, D. M., and others : Gross Beta-Activity in the Thames River (Conn.), 1017
Skeletal Muscle : Direct Relationship of Phosphorylase and Mitochondrial α-Glycerophosphate Dehydrogenase Activity in (Dr. A. G. E. Pearse), 504
Skertchly, Dr. Allan : Crystallization in Wool Keratin, 138
Skilled (Semi-) : Ageing and the—a Survey in Manufacturing Industry on Merseyside (Alistair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine Cunningham), 1249
Skin of New-born and Adult Rats : Collagen Fractions in the (Nestor Gonzalez Cadavid, Berta Denduchis and Roberto E. Mancini), 1297
Skin Follicle Population : Composition of the, in Seven Rajasthan Sheep Breeds (Sunanda Narayan), 1115
Skin Medication (British Pharmaceutical Conference Symposium on), 755
Skin of the Ox : Increase of Evaporative Loss of Water from the, in response to Local Heating of the Hypothalamus (Dr. D. L. Ingram, J. A. McLean and Dr. G. C. Whittow), 81
Skin (Orthotopically Grafted) : Regeneration of Elements of Donor Origin in, following the Homograft Response (Dr. J. K. Dineen and Dr. Aleksander Szenberg), 153
Skull : The Whaley (D. R. Brothwell), 854
Sky : The Night, in August, 338 ; in September, 755 ; in October, 1145
Slack, G. (Lake, J. V., and) : Dependence on Light of Geotropism in Plant Roots, 300
Slater, Sir William : The Revolution in Agriculture (Presidential Address to Section M (Agriculture) of the British Association for the Advancement of Science), 991
Sleeping and Torpid Hummingbirds (Active,) : Thyroid Function in (Prof. C. J. Shellabarger, R. C. Lasiewski and G. E. Hyncik), 1318
Sleigh, Dr. M. A. : An Example of Mechanical Co-ordination of Cilia, 931
Slifkin, M. A. (Birks, Dr. J. B., and) : π-Electronic Excitation and Ionization Energies of Condensed Ring Aromatic Hydrocarbons, 761
Sloan Foundation (Alfred P.), New York : A Great Age for Science (Dr. Warren Weaver), 312
Sloboda, A., and others : N,N'-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds—a New Series of Anti-tumour Agents, 611
Slobodkin, Prof. L. B., and Richman, S. : Calories/gm. in Species of Animals, 299
Slow Decomposition of Explosive Crystals and their Damage by Fission Fragments (Dr. F. P. Bowden and H. M. Montagu-Pollock), 556
Small Mammals : Origin of the British Insular Races of, and of the 'Lusitanian' Fauna (G. B. Corbet), 1037
Small Peptides : Chemical Aspects of the Structure of (Dr. Dorothy Wrinch), *review* by Prof. H. N. Rydon, 105
Smaller, Dr. B. : Effects of Ionizing Radiation on Organic and Biological Solids (Sulphur Compounds as Protective Agents), 1151
Smaller, Dr. B., and others : Spin Resonance and Electrical Conductivity of Indole–Iodine, 168
Smiddy, F. G., and others : Elimination of Bovine Serum Albumin in Acute Experimental Uraemia, 1210
Smith, A. D. M. : Vitamin B_{12} and Intrinsic Factor (B_{12} Deficiency characterized by Low Serum B_{12}), 1155
Smith, Prof. A. H. : The Place-Names of the West Riding of Yorkshire, Part 1, Lower and Upper Strafforth and Staincross Wapentakes ; Part 2, Osgoldcross and Agbrigg Wapentakes ; Part 3, Morley Wapentake, *review* by Prof. H. C. Darby, 208
Smith, A. K., and others : Ultracentrifugal Differences in Soybean Protein Composition, 1395
Smith, B. Babington : Effect of Attention in Peripheral Vision, 732
Smith, Dr. D. A. : Absence of Lysogeny in *Pasteurella pestis*, 522
Smith, Dr. D. Morison, and others : Essential Oil from Fresh *Cannabis sativa* and its Use in Identification, 774
Smith, Dr. D. Morison, and Bartlet, John C. : Calculation of the Areas of Isolated or Overlapping Normal Probability Curves, 688
Smith, D. S. : Pressure Differences in a Direct-Current Capillary Discharge, 265
Smith, Dr. E. Lester : Vitamin B_{12} and Intrinsic Factor (Specific Effects of Vitamin B_{12} Deficiency), 1155
Smith, Dr. F. Graham : Spectrum of Galactic Radio Emission, 1381
Smith, H., and others : Some Novel Acid-stable Penicillins, 1091
Smith, Dr. H. Williams : Bacteria of the Intestine (The Bacterial Flora of the Faeces of Animals with particular reference to its Development in the Young), 1151
Smith, Dr. I. (Jepson, Dr. J. B., and) : Paper Chromatography (Film showing the Principles of Chromatography), 759
Smith, Dr. I., and Weiss, J. B. : Paper Chromatography, 759
Smith, Dr. Ian M., and others : Biochemical Changes in Lethal Staphyloccal Infections in Mice, 783
Smith, Dr. J. A. B. : The National Institute for Research in Dairying, 237
Smith, J. T. (Knox, Prof. R., and) : Use of Cellulose Acetate Membranes for Detecting Penicillinase-producing Organisms, 926
Smith, Dr. K. F. : Appointed professor of experimental physics at the University of Sussex, 1349
Smith, Dr. Kenneth M. : Plant Viruses, third edition, *review* by Dr. B. D. Harrison, 111
Smith, M. J. A. : Appointed Mullard Research Fellow in physics in the University College of North Staffordshire, 229
Smith, Dr. Robert : Assessment of Pain (Quantitative Relation between Stimulus and Sensation), 1353
Smith, Robert C., and Wyard, S. J. : Spatial Distribution of Free Radicals as studied by Electron Spin Resonance, 897
Smith, Dr. R. H. : Importance of Magnesium in the Control of Plasma Calcium in the Calf, 181
Smith, Prof. Wilson : Appointed a member of the Medical Research Council, 1142
Smith-Rose, Dr. R. L. : *Obituary* of Dr. Lee de Forest, 749
Smithers, Prof. D. W. : A Clinical Prospect of the Cancer Problem, *review*, 1336
Smithers, S. R. : Parasite Biology (Application of New Laboratory Techniques), 135
Smithsonian Institution : Miscellaneous Collections, Vol. 141, The Biotic Associations of Cockroaches (Dr. Louis M. Roth and Dr. Edwin R. Willis), *review* by David R. Ragge, 106
  Publication 4466, The Victorian American—Lithographs from the Harry T. Peters 'American on Stone' Collection (Antony N. B. Garvan and Peter C. Welsh), 226
  Bulletin No. 225 of the United States National Museum, Drug Supplies during the American Revolution (George B. Griffenhagen), 761
Smoake of London Dissipated : Fumifugium, or the (John Evelyn), reprint (National Society for Clean Air), 752
Smoke (Cigarette) Condensate—Preparation and Routine Laboratory Estimation (H. R. Bentley and J. G. Burgan), second edition, 336
Smoke (Tobacco) : Natural Radioactivity in Tobacco and (Dr. V. C. Runeckles), 322
Smoking—its Influence on the Individual and its Role in Social Medicine (Dr. C. Van Proosdy), *review* by Dr. W. Carruthers, 108
Smoking : Effects of, *review* by Dr. W. Carruthers, 108
Smooth Muscle (Vascular) : Spontaneous Spike-Discharges of (Dr. S. Funaki), 1102
Smyrna (Old) : Archaeology of, *review* by Dr. Hugh Plommer, 212
Smyth, D. McCormack : Elected to a Research Studentship in education at Churchill College, Cambridge, 856
Snarey, M., and others : Biosynthesis of the Pyrethrins, 281
Snell, F. M. (Spangler, R. A., and) : Sustained Oscillations in a Catalytic Chemical System, 457
Snout (Mole's) : Nerve Endings in the (J. Armstrong and Dr. T. Andrew Quilliam), 1379
Snowden, M. : Sector-Focused Cyclotrons, *review*, 532
Snoy et d'Oppuers, Baron : The Non-Ferrous Metals Industry in a Unified Europe (Autumn Lecture of the Institute of Metals), 546
Soap Solutions : *p*H of (D. Eagland and F. Franks), 1003
Sobotka, Dr. H., and others : Vitamin-Levels in Blood and Serum, 78
Social Dominance in Inbred Mouse Strains (Prof. G. Lindzey, H. Winston and M. Manosevitz), 474
Social History of Engineering (Prof. W. H. G. Armytage), *review* by Prof. M. Postan, 946
Social Sciences (House of Commons Debate on), 1354
Social Service : Science, Politics and (Earl of Cranbrook), 992
Social Studies in Britain, 1354
Société de Chimie Industrielle : Symposium on Corrosion in the Nuclear Energy Industry, 651
Société de Chimie Physique and the British Biophysical Society : Discussion on the Effects of Ionizing Radiation on Organic and Biological Solids, 1150
Société de l'Industrie Minérale : Sixth International Mineral Processing Congress, 545
Société Royale de Zoologie d'Anvers : Bulletin No. 20, Swimming Ability of Non-aquatic Reptiles (R. R. Tercafs), 125
Societies (Scientific), and the Rating and Valuation Bill, 123
Socolovsky, Dr. V. : At the Third International Congress of Dietetics (Diet and Atherosclerosis), 1344

Soczewiński, Dr. Edward : R_M and Solvent Composition in Paper Chromatography, 68
Soddy (Frederick) Trust : Report, 854
Sodium Chloride : Quenching of Cation Vacancies in Doped Crystals of (the late Sir K. S. Krishnan and S. C. Jain), 162
  Serum Lipids in relation to the Intake of Alcohol and (Erling Lund, Torben Geill, Prof. Henrik Dam and Gunhild Kristensen), 1095
Sodium Deoxycholate : Changes of Permeability of Yeast with (Dr. L. Kováč), 698
Sodium Dimethyldithiocarbamate : Inhibition of Growth and Photosynthesis in Submerged Plants with Tetramethylthiuram Disulphide and, and Reversal of this Inhibition of Photosynthesis (P. E. Björn Lindahl), 51
  Transformation in Plants of, into other Fungitoxic Compounds (H. M. Dekhuijzen), 198
Sodium Fluoride : Effect of, on Calcium Metabolism of Human Beings (Dr. Clayton Rich and John Ensinck), 184
Sodium Ions : Exchanges of, in the Central Nervous System of an Insect (*Periplaneta americana* L.), (J. E. Treherne), 1223
Sodium Transport in the Loop of Henle : Stimulation by Aldosterone of (Dr. J. Crabbé), 817
Sodium and Potassium in certain Cells and Tissues : Distribution of (Dr. Norman R. Joseph, Dr. Milton B. Engel and Dr. Hubert R. Catchpole), 1175
Soehring, Dr. K. : Assessment of Pain (Testing of Analgesic Drugs on Animals), 1354
Sofiano, T. A. : English–Russian Geological Dictionary (edited by I. D. Borneman, *et al.*), *review* by Dr. S. I. Tomkeieff, 9
  Russian–English Geological Dictionary (edited by A. P. Lebedev and V. E. Khain), *review* by Dr. S. I. Tomkeieff, 9
Soil : Agar Disk Method for the Direct Observation of Nematode-trapping Fungi in the (Roderic C. Cooke), 1411
Soil (Irradiated) : Plant Growth in (G. D. Bowen and A. D. Rovira), 936
Soil Aggregates (Natural) : Role of Polysaccharides in Stabilization of (Dr. D. J. Greenland, G. R. Lindstrom and Dr. J. P. Quirk), 1283
Soil Crumb Plates : Factors influencing the Composition of the Cellulose-decomposing Microflora on (A. Keynan, Y. Henis and P. Keller), 307
Soil Mechanics : K. Terzaghi, Founder of the Science of, *review* by Kevin Nash, 210
  From Theory to Practice in—Selections from the Writings of Karl Terzaghi, *review* by Kevin Nash, 210
Soil Temperatures in Iceland (Dr. Bjarni Helgason), 939
Soil-transmitted Helminths : Control of (Prof. Paul C. Beaver), 1249
Sol, H. H. : Nematology (Importance of Nematodes as Vectors of Virus Pathogens), 1048
Solar Energy : Applications of, 228
Solar Energy Technology, 1954-1959 (T. Brimelow), 228
Solar Noise Bursts : Very-Low-Frequency Modulation of Discrete Frequency (Dr. Jules Aarons, Santimay Basu, William Kidd and Richard Allen), 56
Solar Wind : Terrestrial Accretion from the (Dr. Ronald Frith), 1182 ; (Dr. E. R. Harrison), 1182 ; (C. M. de Turville), 1183
Solet, I. S. : Porous Nickel Oxide, 64
Solid Helium : Bond-Lengths in (Dr. A. F. Schuch), 591
Solid Propellant and Exothermic Compositions (Dr. James Taylor), *review* by Dr. W. B. Littler, 3
Solid Solutions with Manganese and Nickel Ferrites : Magnetic Relaxation in Magnetite and in its (A. Bragiński and Th. Merceron), 898
Solid-coated Capillary Columns : Deterioration of, on Standing (George M. Rouayheb and William C. Hamilton), 801
Solid–Liquid Interfaces : Chemisorption at (A. R. Burkin and G. Halsey), 348
Solids : Radio Spectroscopy of (Institute of Physics and the Physical Society and the British Radio Spectroscopy Group Conference on), 126
Solids (Organic and Biological) : Effects of Ionizing Radiation on (Société de Chimie Physique and the British Biophysical Society Discussion on), 1150
Soltys, M. A., and Folkers, C. S. : Immunity to Protozoa (Immunity in Trypanosomiasis), 552
Solubility of Denatured Haemoglobin Variants in Acid Buffers (I. D. Fraser and Dr. A. B. Raper), 355
Solubility Product : Theoretical Expression for (Prof. B. N. Bhar), 1004 ; (Prof. K. Krishnamurti), 1004
Soluble Material in the Gastro-intestinal Tract of Rats under Normal Feeding Conditions (Dr. Anne S. Cole), 502
Solute : Resistivity Changes due to Formation of *G.P.* Zones (Clusters rich in) (H. Herman and Prof. J. B. Cohen), 63
Solutes : Cryoscopic Effect of Separation and Concentration of (Dr. K. S. Rangappa), 1087
Solution : Generation of Radicals in Pairs and the Kinetics of Radical Chain Reactions in (Dr. P. E. M. Allen and C. R. Patrick), 1194
Solution (Aqueous), of 5-Hydroxytryptophan treated with X-Rays : Formation of 5-Hydroxytryptamine in an (M. Jović and Z. Supek), 383
Solution (Aqueous Silver Nitrate) : Ultrasonic Velocity in (D. S. Allam and W. H. Lee), 1388
Solution (Cold) : Formation of Chalcopyrite by Reaction between Chalcocite and Pyrrhotite in (W. M. B. Roberts), 560
Solution (Uranin) : Average Extinction Coefficient for Re-absorption of Fluorescence Radiation through (Dr. K. K. Rohatgi and G. S. Singhal), 799
Solutions (Aqueous) : Ultrasonic Velocity in, of some Electrolytes (M. G. Seshagiri Rao and Prof. R. Ramachandra Rao), 164
Solutions (Aqueous), and in Thulium Oxide : Rapid Determination of Ytterbium in, by Neutron Activation (Minoru Okada), 1090
Solutions (Chloride) : Formation of Calcium Chloraluminate by Interaction of Portland Cement with Sea-Water and (Dr. L. Heller and Dr. M. Ben-Yair), 488
Solutions (Dilute), of High Polymers : Elimination of the Absorption Correction in the Viscometry of (H. van Oene and Prof. L. H. Cragg), 1160
  Viscosity–Temperature Relationships for (Dr. W. R. Moore), 1292
Solutions (Ferrous) : Radiolytic Oxidation of, with Standardized Internal Sources of Polonium-210 (J. Steyn and D. van As), 903
Solutions (Polymer) : Ultrasonic Absorption of (Dr. G. Gooberman), 693
Solutions (Salt) : The Widening of Expansion Cracks in Mortar by (J. A. Roberts and H. E. Vivian), 125, *erratum*, 1145
Solutions (Soap) : *p*H of (D. Eagland and F. Franks), 1003
Solutions (Solid), with Manganese and Nickel Ferrites : Magnetic Relaxation in Magnetite and in its (A. Bragiński and Th. Merceron), 898
Solvent Composition in Paper Chromatography : R_M and (Dr. Edward Soczewiński), 68
Somatic Cell Nucleus (The Feulgen-Stained), as a Homogeneously Absorbing Object (Dr. Simon Iversen), 150
Somatic Cells (Parental) : Inclusion of, in Sponge Larvae (Frederick E. Warburton), 1317
Sommerville, Dr. : Hazards of the Animal House (Natural Infections of Laboratory Rats and Mice which are Transmissible to Man), 339
Sound Production and Sound Reception by Insects (Mable Frings and Hubert Frings), *review* by Prof. V. B. Wigglesworth, 428
Sound Reception by Insects : Sound Production and (Mable Frings and Hubert Frings), *review* by Prof. V. B. Wigglesworth, 428
Sound Velocity (Molar), in Molten Hydrated Salts (P. R. K. L. Padmini and Prof. B. Ramachandra Rao), 694
Sounds used by Animals, *review* by Dr. W. H. Thorpe, 741
Sounds (Animal), and Communication (edited by Dr. W. E. Lanyon and Dr. W. N. Tavolga), *review* by Dr. W. H. Thorpe, 741
Source (Tunable), of Radiation for the Far Infra-Red using Beats between Optical Masers : A Proposal for a (Dr. D. C. Lainé), 795
Sources of Ionizing Radiations at Exhibitions : Code of Practice for the Display of, 1249
Sources (Diverse), of Cows' Milk : An Invariant Casein Substrate for Rennet Standardization, derivable from (Dr. G. W. Scott Blair and J. C. Oosthuizen), 697
Sources (Standardized Internal), of Polonium-210 : Radiolytic Oxidation of Ferrous Solutions with (J. Steyn and D. van As), 903
Souter, L. S., and Winsdale, R. : Women Engineers in the U.S.S.R., a Study of the Education, Employment and Prospects of Women Engineers in the U.S.S.R. (Caroline Haslett Memorial Trust), 30
South African Animal Life—Results of the Lund University Expedition in 1950-1951, Vol. 7 (edited by Bertil Hanstrom, Per Brinck and Gustaf Rudebeck), *review* by Prof. O. Lowenstein, 1135
South African Museum : Annual Report for 1959-60, 1044
South African Public Library : Grey Bibliographies No. 7, A Bibliography of African Bibliographies, covering Territories South of the Sahara, fourth edition, 959
South Australia, Royal Society of : Verco Medal presented to Dr. R. L. Specht, 1247
South India : Occurrence of *Eutrichocheles modestus* (Herbst) in the Near-shore Waters of Porto Novo (K. Balasubrahmanyan and Dr. J. Jacob), 830
South-East Asia : Ecto- and Endo-Parasites of, *review* by P. F. Mattingly, 948
South-East England : The Changing Flora of (J. E. Lousley), 133
  Invertebrate Life in (John Sankey), 132
South-Eastern Union of Scientific Societies : Annual Congress (F. J. Epps), 132
South-West England : Age of the Orogeny and Granites in (T. R. Owen), 486 ; (N. E. Butcher), 486
Southern England : The Lower Tertiary Floras of (Marjorie Elizabeth Jane Chandler), 1, Palaeocene Floras, London Clay Flora (Supplement), *review* by Prof. John Walton, 209
  Tropical Floras in the Tertiary in, *review* by Prof. John Walton, 209
Southern Italian Extraction : Haemoglobin *P* in a Family of (Prof. E. Silvestroni, I. Bianco and C. Brancati), 292
Southern Rhodesia (National Museums of) : Annual Report for 1960, 650
Soviet Journal of Atomic Energy : Supplement No. 6, Fast-Neutron Spectroscopy (B. V. Rybakov and V. A. Sidorov), (edited by N. A. Vlasov), translated from the Russian, *review* by Dr. P. A. Egelstaff, 8
Soviet Polar Research, *review* by Dr. Terence Armstrong, 841
Soviet Research on Organo-Phosphorus Compounds, 1949-1956 (in English translation), *review* by Dr. F. G. Mann, 1232
Soviet View of British Libraries (V. I. Shunkov, G. G. Firsov and N. I. Tyulina), translated by A. Thompson (Library Association, Pamphlet No. 22), 752
Soybean Protein Composition : Ultracentrifugal Differences in (W. J. Wolf, G. E. Babcock and A. K. Smith), 1395
Space : Science in (United States National Academy of Sciences—National Research Council), 124
Space (Interplanetary) : Magnetic Storms in, as observed by *Pioneer V* (Eugene W. Greenstadt), 329
Space Development Co., Ltd. (British) : Aims of the, 230
  Appointment of Sir Bernard Lovell as scientific director, 230
Space-Flight and Re-entry Trajectories (International Academy of Astronautics Symposium on), 960
Space Orbits : Satellite and, *review* by D. G. King-Hele, 1334
Space Research : Some Aspects of the Physics of (Institute of Physics and the Physical Society Residential Symposium on), 229
  Further Advancements in, 631
Space Technology : Ballistic Missile and—Proceedings of the Fifth Symposium held at Los Angeles, 1960 (edited by Donald P. LeGalley), Vol. 1, Bioastronautics and Electronics, with Invited Addresses ; Vol. 2, Propulsion and Auxiliary Power Systems ; Vol. 3, Guidance, Navigation, Tracking and Space Physics ; Vol. 4, Re-entry and Vehicle Design, *review* by W. H. Stephens, 1131
Space Trajectories—a Symposium sponsored by the American Astronautical Society, the Advanced Research Projects Agency and Radiation Incorporated (edited by the Technical Staff, Research Division, Radiation Incorporated), *review* by D. G. King-Hele, 1334
Spacing and Nitrogen-level : Effect of, on Floral Initiation in Cocksfoot (*Dactylis glomerata* L.), (D. M. Calder and J. P. Cooper), 195
Spadoni, M. Antonietta (Gaetani, Sancia, and) : Effect of 5-Fluorouracil on Hepatic Induced Increase of Tryptophan-Pyrrolase, 1296
Spallation Phenomena : Fission and, and their Application to Cosmic Rays (European Organization for Nuclear Research and the Max Planck Institute, Heidelberg, Conference on), 24
Spangler, R. A., and Snell, F. M. : Sustained Oscillations in a Catalytic Chemical System, 457
Sparks produced by Breaking Wires at High Speed : Ignition of Gas by (Dr. R. L. Gordon, Dr. H. Lord and D. W. Widginton), 1085
Spatial Distribution of Free Radicals as studied by Electron Spin Resonance (Robert C. Smith and S. J. Wyard), 897
Specht, Dr. R. L. : Presented with the Verco Medal of the Royal Society of South Australia, 1247 ; work of, 1247

Speciation in the Sea (Systematics Association and the Challenger Society Symposium on), 229
Species : Correlation between Growth-Rate of the Suckling of various, and the Percentage of Total Calories from Protein in the Milk (Dr. F. W. Bernhart), 358
Species of Animals : Calories/gm. in (Prof. L. B. Slobodkin and S. Richman), 299
Species of *Arthrobacter* : Auxin Production by (Dr. H. Katznelson and Dr. J. C. Sirois), 1323
Species (Ammocoete) : Oocyte Numbers as a Diagnostic Character for the Identification of (Dr. M. W. Hardisty), 1215
Species (Infra-human) : Blood Groups in (New York Academy of Sciences Conference on), 653
Species (Littoral Interstitial), from Anglesey representing Three Families New to Britain (P. J. S. Boaden), 512
Species (Three Ectoparasitic) : Simultaneous Eradication of, from a Colony of Laboratory Mice (Dr. Nigel Bateman), 721
Species (Warm-water), in the Plankton off Newfoundland during Winter Months (V. Bainbridge), 1216
Species Distribution of Nineteen Phenotypes : Serum Transferrins in the Genus *Macaca*— (Prof. Morris Goodman and Emily Poulik), 1407
Species-Specificity of Tissue Thromboplastin : Influence of Serum Factors on the (Christine Hawkey and J. L. Stafford), 920
Specific Bactericidal Antibodies : Increase in, after Administration of Endotoxin (J. Gabriel Michael, James L. Whitby and Maurice Landy), 296
Specific Evaporation Resistance : Compressibility and, of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers (A. V. Deo, S. B. Kulkarni, M. K. Gharpurey and A. B. Biswas), 378
Specific Heat and Expansion Coefficient of Liquid Helium-3 under Pressure below 0·1° K. (Dr. D. F. Brewer and J. R. G. Keyston), 1261
Specific Inhibition of Anti-*D* Antibody by Colominic Acid (Prof. William C. Boyd and Egli Reeves), 511
Specific Proteins in Serum and other Mixtures : Estimation of (Dr. D. A. Darcy), 1163
Specific (Non-) Immunity in Pneumococcal Infection of Mice (Dr. G. N. Cooper and Dr. A. E. Stuart), 295
Specifically Bound Cobalt-58 Vitamin B_{12} : Selective Uptake of, by Human and Mouse Tumour Cells (Dr. Bernard A. Cooper and Dr. William Paranchych), 393
Specificity in Haemoglobin Synthesis : Control of (Dr. J. Bishop, Dr. G. Favelukes, Prof. R. Schweet and Dr. E. Russell), 1365
Specificity of the Mutagenic Action of the Alkylating Agents (Prof. Bernard S. Strauss), 730
Spectra Index : A National—Plans and Problems (P. S. Davison), 1250
Spectra (Absorption), of γ-Irradiated Lead Silicate Glass (R. S. Barker, T. K. Harrison, D. A. Richardson and R. Rimmer), 374
Spectra (Infra-Red), of Water-soluble Carbonates (Herman A. Szymanski and Richard Povinelli), 64
Spectra (Triplet→Singlet Emission) : Polarization of (V. G. Krishna and Prof. Lionel Goodman), 800
Spectra (Ultra-violet Absorption), of 3,4-Benzopyrene : Effect of Ozone on the Fluorescence and (Z. Morlin and K. M. Sáringer), 907
Spectra (Ultra-violet Absorption), of Proteins in Deuterium Oxide (Dr. G. H. Beaven and Elizabeth Bailey), 914
Spectral Data : Organic Electronic, Vol. 1, 1946-1952 (edited by Mortimer J. Kamlet) ; Vol. 2, 1953-1955 (edited by Herbert E. Ungnade), *review* by Dr. E. G. Kellett, 107
Spectrometry : Mass (Mass Spectrometry Group of the Institute of Petroleum and Committee *E*-14 of the American Society for Testing Materials Conference on), 24
Spectrometry (Mass) : Determination by, of the Structure of Proline Derivatives from Apples (Dr. K. Biemann, G. G. J. Deffner and Prof. F. C. Steward), 380
Spectroscope : Chemistry and the (Prof. R. G. W. Norrish), 984
Spectroscopic Evidence (Infra-Red), of Four-fold Co-ordination of Titanium in Barium Orthotitanate (P. Tarte), 1002
Spectroscopy : From Radar to—New Regions of the Spectrum (Prof. D. J. E. Ingram), 1146
  Fast-Neutron (B. V. Rybakov and V. A. Sidorov), (edited by N. A. Vlasov), translated from the Russian, *review* by Dr. P. A. Egelstaff, 8
Spectroscopy (Applied), Society of (Washington Section), and others : Eleventh Annual Instrument Symposium and Research Equipment Exhibition, 959
Spectroscopy (Atomic Absorption) : Determination of Lead in Urine by (J. B. Willis), 381
Spectroscopy (Molecular Emission), from 2μ to 12μ by a Michelson Interferometer (H. A. Gebbie, G. Roland and L. Delbouille), 264
Spectroscopy (Radio), of Solids (Institute of Physics and the Physical Society and the British Radio Spectroscopy Group Conference on), 126
Spectrum : From Radar to Spectroscopy—New Regions of the (Prof. D. J. E. Ingram), 1146
Spectrum of Galactic Radio Emission (Dr. F. Graham Smith), 1381
Speech, Handedness and Cerebral Dominance, *review* by Sir Russell Brain, 840
Speed (High) : Ignition of Gas by Sparks produced by Breaking Wires at (Dr. R. L. Gordon, Dr. H. Lord and D. W. Widginton), 1085
Spencer, Prof. B. : The Training of Biochemists (Biochemistry more fitted to be a Postgraduate subject), 955
Spencer, Dr. D. A. : At the Maxwell Colour Centenary Conference (A Hundred Years of Colour Photography), 10
Spenser, Prof. I. D. (Gear, J. R., and) : Biosynthesis of Hydrastine, 1393
Sperm (Mammalian) : Isolation and Properties of Deoxyribonucleic Acid from (Dr. E. Borenfreund, E. Fitt and Dr. A. Bendich), 1375
Spermathecae (Double and Single), in Fleas (Siphonaptera), (Hon. Miriam Rothschild), 1322
(Spica) Nebula : The Alpha Virginis (Walter A. Feibelman), 1083
Spider *Latrodectus geometricus* C. Koch : Cycle and Rhythm in the Ovulation of the (A. Bouillon and R. Lekie), 620
Spiegel, Herbert E., and others : Effects of Drugs on Uptake of Isotopic Norepinephrine by Cat Tissues, 816
Spiegelberg, Dr. Herbert : The Phenomenological Movement, *review* by F. I. G. Rawlins, 5
Spielmann, W., and others : Investigation into Blood Groups in a Paternity Case, 753
Spike-Discharges (Spontaneous), of Vascular Smooth Muscle (Dr. S. Funaki), 1102
Spike and Wave Discharges and Alterations of Conscious Awareness (L. Goldie and J. M. Green), 200
Spikes (X-Ray Diffraction), of Diamond (Y. Yoneda), 1187 ; (Prof. F. C. Frank), 1188
Spin Resonance : Electron (Chemical Society Symposium on), 755
Spin Resonance (Electron) : Spatial Distribution of Free Radicals as studied by (Robert C. Smith and S. J. Wyard), 897
Spin Resonance (Electron), of Adenosine Triphosphate (D. B. Russell and S. J. Wyard), 65
Spin Resonance Absorptions (Electron), induced by Light in Adsorbed Layers of Cyanine Dyes on Silver Bromide Microcrystals (Dr. W. C. Needler, Dr. R. L. Griffith and Dr. W. West), 902
Spin Resonance and Electrical Conductivity of Indole–Iodine (B. Smaller, Dr. I. Isenberg and S. L. Baird, jun.), 168
Spinal Dog : Stimulation of the Heart of the, by Phenoxybenzamine (B. G. Benfey and D. R. Varma), 919
Spinal Motoneurones : Changes in the Membrane during Recurrent Disinhibition of (Dr. Victor J. Wilson and Paul R. Burgess), 918
Spinal Neurones of some Teleosts : Pigment Bodies of the (Prof. A. Stolk), 924
Spinels (Oxide) : Dependence on Temperature of the Distribution of Cations in (Ranajit K. Datta and Prof. Rustum Roy), 169
Spiracle Control in Dragonflies (Dr. P. L. Miller), 621
Splanchnic Nerve : Effect of Bilateral Section of the, on Erythropoiesis (Fumimaro Takaku, Kunitake Hirashima and Prof. Shigeo Okinaka), 500
Spleen Preparations (Cell-free), Incubated *in vitro* : Rise of Antibody Titre in (Dr. Dieter H. Sussdorf), 770
'Split-Brain' Monkey : Changes in Visual Gnostic Functions and Emotional Behaviour following Unilateral Temporal Pole Damage in the (Dr. J. L. deC. Downer), 50
Splitting of Serum Albumin during Electrophoresis on Paper (J. V. McLoughlin), 1305
Sponge : Flimmer-flagellum of the (Björn A. Afzelius), 1318
Sponge Larvae : Inclusion of Parental Somatic Cells in (Frederick E. Warburton), 1317
Spontaneous Development of *L* Forms of Streptococci requiring Secretions of other Bacteria or Sulphydryl Compounds during Normal Growth (Ayala Frenkel and Dr. Willy Hirsch), 728
Spontaneous Miniature Potentials from Insect Muscle Fibres (P. N. R. Usherwood), 814
Spontaneous Plasma Fibrinolysis: Determination of, by a Practical Procedure (Dr. H. Yatzidis), 604
Spontaneous Spike-Discharges of Vascular Smooth Muscle (Dr. S. Funaki), 1102
Spore Dispersal in *Chaetomium globosum* (Kunze), (P. A. Dixon), 1418
Spore Liberation and Dispersal of Coffee Rust *Hemileia vastatrix* B. et Br. (R. W. Rayner), 725
Sporulation (Anti-) Effect of Copper Fungicides : Significance of the, in the Control of Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach (E. Evans, H. L. S. Bevington and E. P. Whitaker), 476
Sprau, F., and Suesz, A. : Nematology (Radio-tracer Techniques using Phosphorus-32 to indicate that *Longidorus maximus* feeds on the Roots of Beet and is thus a Parasite), 1049
Spray Method for the Simultaneous Detection of Iodide and Iodinated Amino-Acids on Filter Paper Chromatograms (Dr. A. Van Zyl and M. Bhaga), 275
Spraying with Gibberellic Acid : Effect of, on Yield and Composition (Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid), (R. Stevens, J. B. Roberts and I. H. Williams), 360
Spring Areas (Hot), with Acid–Sulphate–Chloride Waters (Dr. A. J. Ellis and S. H. Wilson), 696
Springell, Dr. P. H. : An Unreactive Tyrosine Residue in Insulin and the Exclusive Iodination of the *A* Chain, 1372
Springer, Prof. G. F. : Blood Groups in Infra-human Species (Immune Origin of so-called Natural Anti-human *B* Antibodies), 654
Springer, Prof. G. F., and Stalder, Dr. Karlheinz : Action of Influenza Viruses, Receptor-destroying Enzyme and Proteases on Blood Group Agglutinogen *M*, 187
Sproson, W. N. : Some Aspects of Optical Lens Performance, Part 2, The Design of Lens Hoods (B.B.C. Engineering Monograph No. 36), 1355
Sproull, Dr. D. H. : The Physiology, Pharmacology and Pathology of Muscle, *review*, 948
St. Bartholomew's Hospital Medical College, London : Title of professor emeritus of bacteriology conferred on Prof. K. P. Garrod, 546
  Appointment of J. P. Quilliam to the chair of pharmacology, 1349
Stability of Coastal Inlets (P. Brunn and F. Gerritsen), *review* by W. J. Reid, 426
Stability of Freeze-dried Anti-haemophilic Globulin (F. Nour-Eldin), 503
Stability Constant of Copper (II) Complexes of Reduction Products of 2,2'-Dipyridyl (Dr. M. T. Beck and M. Halmos), 1090
Stability Theory of Colloids : Discreteness-of-Charge Effect and the (Dr. S. Levine, D. Calvert and G. M. Bell), 699
Stability (Metabolic), of Body Constituents (Dr. A. N. Davison and Dr. J. Dobbing), 844
Stabilization of Natural Soil Aggregates : Role of Polysaccharides in (Dr. D. J. Greenland, G. R. Lindstrom and Dr. J. P. Quirk), 1283
Stabilizing Selection for Heading Time in a Bulk-Hybrid Population of Barley (Dr. S. K. Jain), 1123
Stacey, Dr. F. D. (Stott, Dr. P. M., and) : Stress Effects on Thermoremanent Magnetization, 585
Stacey, Prof. M. and others : Mannosyl D-Glucosamine Hydrochloride Isomers—*N*-Mannosyl D-Glucosamine Hydrochloride, 142
  Mannosyl D-Glucosamine Hydrochloride Isomers—6-*O*-Mannosyl D-Glucosamine Hydrochloride, 143
Stacey, Prof. M., and others (edited by) : Advances in Fluorine Chemistry, Vol. 1, *review* by Dr. Richard Dresdner, 319
Stafford, J. L. (Hawkey, Christine, and) : Influence of Serum Factors on the Species-specificity of Tissue Thromboplastin, 920
Stages (Early), of Propylthiouracil Treatment in the Rat : Increase of the Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171

Stained (Feulgen-) Somatic Cell Nucleus as a Homogeneously Absorbing Object (Dr. Simon Iversen), 150
Staining of Sulphated Mucopolysaccharides (Dr. I. D. Heath), 1370
Staining (Differential), of Nucleic Acids : Mechanism of (D. J. Goldstein), 407
Staining Components (Non-Specific), from Fluorescein-labelled Conjugates : Simplified Procedure for removing (G. D. Johnson), 70
Staining Reaction of the Pit Membrane of Wood Cells (R. K. Bamber), 409
Stainless Steel : Damage in, due to Fission Fragments (P. R. B. Higgins and A. C. Roberts), 1158
Stalder, Dr. Karlheinz (Springer, Prof. Georg F., and) : Action of Influenza Viruses, Receptor-destroying Enzyme and Proteases on Blood Group Agglutinogen M^g, 187
Standard Telephones and Cables, Ltd. (Mathematics Department of Queen's College, St. Andrews, in collaboration with) : Symposium on Digital Computers, their Use and Control, 554
Standardization (Rennet) : An Invariant Casein Substrate for, derivable from Diverse Sources of Cows' Milk (Dr. G. W. Scott Blair and J. C. Oosthuizen), 697
Standardized Internal Sources of Polonium-210 : Radiolytic Oxidation of Ferrous Solutions with (J. Steyn and D. van As), 903
Standards : Drinking-Water (Nora H. Johnson), 1261
Standards, United States National Bureau of : Handbook No. 78, Report of the International Commission on Radiological Units and Measurements, 28
  Appointment of Martin T. Decker as chief of the Tropospheric Measurements Section, 334
  Appointment of John L. Hague as chief of the Standard Reference Materials Section, 334
  Appointment of Dr. John K. Taylor as chief of the Applied Analytical Research Section of the Analytical and Inorganic Chemistry Division, 334
  Appointment of Dr. David M. Gates as editor of Section D, Radio Propagation, of the *Journal of Research*, 856
  Appointment of Dr. Harry C. Allen, jun., as chief of the Analytical and Inorganic Chemistry Division, 1346
  Monograph No. 19, Atomic Energy Levels in Crystals (J. L. Prather), 1348
Standards, United States National Bureau of (Informations System Branch of the Office of Naval Research Information Centre) : Symposium on Optical Character Recognition, 1145
Standards, United States National Bureau of (Research Information Centre and Advisory Service on Information Processing) : Report on Computer Systems for Searching Chemical Literature (E. C. Medam and H. R. Koller), 23
Standards (European), for Drinking Water (United Nations, World Health Organization), 1261
Standing : Deterioration of Solid-coated Capillary Columns on (George M. Rouayheb and William C. Hamilton), 801
Stanley, Prof. John : Sterile Crosses between Mutations of *Tribolium confusum* Duv., 934
Stanworth, Dr. J. E. : Appointed director of research of Associated Electrical Industries, Ltd., 1142 ; work of, 1142 ; *erratum*, 1349
Staphylococcal Infections (Lethal), in Mice : Biochemical Changes in (George W. Counts, Dr. Ian M. Smith, Dr. Joseph I. Routh, Dr. Elia Ch. Hazard and J. F. McTavish), 783
Staphylococcus pyogenes : Metabolic and Genetic Investigations of Nutritionally Exacting Strains of (Janice B. Edgar and Dr. B. A. D. Stocker), 1121
Staphylococcus Infection : Development in Mice of Prolonged Non-specific Resistance to Sarcoma Implant and, following Repository Injection of Lipopolysaccharide (Samuel J. Prigal), 1111
Stapp, C. : Bacterial Plant Pathogens (translated by A. Schoenfeld), *review* by Dr. W. J. Dowson, 4
Starch : International Convention on (Dr. E. Dux), 236
Starch-Gel : Electrophoresis in a Thin Layer of, followed by a Plastifying Treatment (J. Groulade, Dr. J. M. Fine and C. Ollivier), 72
Starch-Gel Electrophoresis : Comparison of the Serum Protein Fractions of the Newly Hatched Chick with those of Adult Birds using (A. Amin), 708
Stark, Dr. Lawrence, and Hermann, Dr. Howard T. : Transfer Function of a Biological Photoreceptor, 1173
Starr, L. D. (Finger, G. C., and) : 2,6-Difluoropyridine, 595
Stars : Production of Neon in (H. E. Gove, A. E. Litherland and M. A. Clark), 1381
Stars (Radio) : Apparent Recession of Nebulae and the Nature of (Dr. N. S. Japolsky), 480
Starved Stationary Phase *Aerobacter aerogenes* : Induced Enzyme Synthesis in Aqueous Suspensions of (R. E. Strange), 1272
State (Welfare) : Industrial Relations in the, 1129
State and the Trade Unions (D. F. Macdonald), 1129
State Forestry in Great Britain : Four Decades of (Prof. M. L. Anderson), 656
State Forestry in New Zealand : Four Decades of, 451
Statics (Applied) : Energy Principles in (T. M. Charlton), *review* by Prof. R. C. Coates, 1134
Stationary or Moving Objects : Measuring Visual Constancy for (S. M. Anstis, C. D. Shopland and R. L. Gregory), 416
Stationary Phase *Aerobacter aerogenes* : Induced Enzyme Synthesis in Aqueous Suspensions of Starved (R. E. Strange), 1272
Statistical Calculations : Rapid (M. H. Quenouille), *review* by M. J. R. Healy, 1236
Statistical Illustrations (Quantum) : Thermodynamics with (Prof. P. T. Landsberg), *review* by Dr. W. E. Parry, 1236
Statistical Quality Control and Acceptance Sampling (Birmingham College of Advanced Technology Course on), 337
Statistical Return for the First Quarter of 1961 (Registrar General), 1347
Statistical Society, Royal : Election of Officers, 126
Statistics in the University of London, 1247
Statistics : Mental Health, 855
Statistics of Deadly Quarrels (Dr. Lewis F. Richardson), edited by Quincy Wright and C. C. Leinau, *review* by Sir Cyril Burt, 427
Statistics (Medical), at Belfast, 20
Statistics (Population), in the United Kingdom, 1347
Statistics (U.K. Petroleum Industry), relating to Consumption and Refinery Production, 1959 and 1960 (Petroleum Information Bureau), 123
Status of the Norfolk Broads, 751
Steady Motion : Fluid–Fluid Interfaces in (Prof. Walter Rose), 242
Steady Universe with Charge Excess (Ll. G. Chambers), 262
Steckerl, Dr. F., and others : Effect of Alpha-Amylase on the Ehrlich Mouse Ascites Tumour, 1113
Steel (Stainless) : Damage in, due to Fission Fragments (P. R. B. Higgins and A. C. Roberts), 1158
Stein, Prof. G. : Effects of Ionizing Radiation on Organic and Biological Solids (Role of Oxygen in Radiation Damage), 1150
Stein, Dr. W. D. : Dimer Formation and the Transfer of Glycerol across the Erythrocyte Membrane, 352
  Dimer Formation and Glucose Transfer across the Membrane of the Red Blood Cell, 1277
Stein–Leventhal Syndrome : Physiology and Pathogenesis of the (Dr. Virendra B. Mahesh and Dr. Robert B. Greenblatt)—Urinary Androsterone, Etiocholanolone, Pregnanetriol and Δ^5-Pregnenetriol of Ovarian Origin in a Patient with Stein–Leventhal Syndrome, 888 ; Role of FSH in the Pathogenesis of the Stein–Leventhal Syndrome, 889
Stell, W. K. : Elected to a Research Studentship in physiology at Churchill College, Cambridge, 856
Stem Flow Run-off : Light Rainfall and Plant Survival—Measurement of (M. D. Gwynne and J. Glover), 1321
Stemerding, S. : Nematology (Changes in Nematode Populations—*Tylenchoryhnchus dubius* and *Rotykenchus robustus*), 1048
Stendahl : Selected Journalism from the English Reviews by, with Translations of other Critical Writings (edited by G. Strickland), 1031
Stent, Gunther S. (selected by) : Papers on Bacterial Viruses, *review* by L. B. Quesnel, 320
Stentor : The Biology of (Vance Tartar), *review* by Dr. J. A. Kitching, 1133
Stephens, W. H. : Ballistic Missile and Space Technology, *review*, 1131
Stereochemistry of Natural Phenolic Compounds (Plant Phenolics Group Symposium on), 234
Sterile Crosses between Mutations of *Tribolium confusum* Duv. (Prof. John Stanley), 934
Sterile Culture : Growth of Fern Embryos in (Prof. Augustus E. DeMaggio and Prof. Ralph H. Wetmore), 94
Sterilizing Materials for Hospitals (Brian Watkin), 1044
Stern, Kurt : Principles of Human Genetics, second edition, *review* by Dr. G. Ainsworth Harrison, 110
Sternlicht, B. : At the Second International Measurement Conference (Measuring Film Thickness on Bearings), 1351
Steroids : Biochemistry of (Erich Heftmann and Erich Mosettig), *review* by Dr. D. T. Elmore, 111
Steroids as Ice Nucleators (Richard B. Head), 1058
Steroids and Cancer, *review* by E. Reid, 1235
Steroids in relation to Cancer : Biological Activities of—Proceedings of a Conference sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, United States Department of Health, Education and Welfare (edited by Gregory Pincus and Erwin P. Vollmer), *review* by E. Reid, 1235
Sterol Esters : Isolation of, from Human Faeces (Francis Aylward and Pamela Wills), 1397
Sterol Requirements of the Silkworm, *Bombyx mori* (Dr. Toshio Ito), 882
Stetson, Dr. C. A. (Basch, Dr. R., and) : Blood Groups in Infra-human Species (Assay Method for those Antigens of Mice which elicit Cytotoxin and Haemagglutinin Production), 654
Stevens, P. J. (Lock, C. M., and) : Connexion between Micropulsations and the Ionosphere, 584
Stevens, R. : Formation of Phenethyl Alcohol and Tyrosol during Fermentation of a Synthetic Medium lacking Amino-acids, 913
Stevens, R., and others : Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid—Effect of Spraying with Gibberellic Acid on Yield and Composition, 360
Steward, Dr. E. G. : Graphite (Crystallographic Changes which occur during Graphitization), 28
Steward, Prof. F. C., and others : Determination by Mass Spectrometry of the Structure of Proline Derivatives from Apples, 380
Stewart, D. J. : A Micro-method for Performing the Phenylalanine Test on Cultures from Multi-carbohydrate Media, 521
Stewart, Doreen L., and others : Effect of *p*-Fluorophenylalanine on the Multiplication of Foot-and-Mouth Disease Virus, 414
Stewart, J., and others : Enzymic Conversion of Tetrahydrofolic Acid to Folinic Acid by Plant Protein, 179
Stewart, Dr. Richard D., and others : Rapid Infra-Red Determination of Acetone in the Blood and the Exhaled Air of Diabetic Patients, 1008
Steyn, J., and van As, D. : Radiolytic Oxidation of Ferrous Solutions with Standardized Internal Sources of Polonium-210, 903
Stickleback (*Gasterosteus aculeatus* L.) : Significance of the Regeneration of Lateral Plates of the (Tadeusz Penczak), 621
Stigmatic Cuticula of Flowers : Enzymic Breakdown of (W. Heinen and H. F. Linskens), 1416
Stiles, Dr. W. S., and Wyszecki, Dr. G. W. : At the Maxwell Colour Centenary Conference (The Spectrum divided into *M* Small Wavelength Intervals), 10
Still More Nucleic Acids, *review* by Dr. Roy Markham, 742
Stiller, Mary, and Vennesland, Prof. Birgit : Photophosphorylation and the Hill Reaction, 677
Stimulant Properties (Anti-Depressant or), of Impramine : Demonstration of, in Experimental Animals (Dr. D. R. Maxwell and Helen T. Palmer), 84
Stimulants of Cell Division in Developing Fruits (D. S. Letham and E. G. Bollard), 1119
Stimulation by Aldosterone of Sodium Transport in the Loop of Henle (Dr. J. Crabbé), 817
Stimulation of the Heart of the Spinal Dog by Phenoxybenzamine (B. G. Benfey and D. R. Varma), 919
Stimulation by Heparin of Parenchymal Liver Cell Proliferation in Normal Adult Rats (Dr. M. Zimmerman and Evemarie Celozzi), 1014
Stimulation of Lipogenesis in Thyroid Tissue *in vitro* by Free Fatty Acids (Prof. Norbert Freinkel, Dr. Augusto D. Litonjua and Edward C. Saef), 804
Stimulation of Motor Nerve Terminals (Dr. J. I. Hubbard and Robert F. Schmidt), 1103
Stimulation of Turnip Yellow Mosaic Virus Protein Production by 2-Thiouracil (Dr. R. I. B. Francki and Dr. R. E. F. Matthews), 1078

Stimulation (Growth), by High Indole-3-Acetic Acid Concentrations (T. A. Truelsen), 1410
Stimuli (Subliminal) : Response to Series of—an Ethological Method for Investigating the Properties of Nervous Systems (A. J. Pontin), 512
Stimulus-Response Compatibility in the Paired-Associate Learning of Nonsense Syllables (A. D. Baddeley), 1327
Stochastic Population Models in Ecology and Epidemiology (Prof. M. S. Bartlett), *review*, 843
Stocker, Dr. B. A. D. (Edgar, Janice B., and) : Metabolic and Genetic Investigations of Nutritionally Exacting Strains of *Staphylococcus pyogenes*, 1121
Stokes, Dr. John B. : Metabolites of Vitamin B_{12} in Bile after Parenteral Administration of the Vitamin, 807
Stolk, Prof. A. : Pathological Parthenogenesis in a Viviparous Toothcarp, 507
  Pigment Bodies of the Spinal Neurones of some Teleosts, 924
Stomatal Behaviour in *Xanthium pennsylvanicum* : Photoperiodic Effects on (Dr. T. A. Mansfield and Prof. O. V. S. Heath), 974
Stone, Dr. F. G. A. : Appointed to the University of London readership in inorganic chemistry at Queen Mary College, London, 229
Stone, Dr. W. H. : Blood Groups in Infra-human Species (Blood Groups in Cattle), 655
Stoneley, Dr. R. : The Interior of the Earth (Presidential Address to Section A (Mathematics and Physics) of the British Association for the Advancement of Science), 983
Stoppani, Prof. A. O. M., and others : Role of the Pentose Phosphate Pathway in *Pasteurella multocida*, 727
Storage of Grain : Buildings for the Drying and (Bibliography of Research Publications on Farm Buildings, Section 5), (Agricultural Research Council), 440
Storage and Retrieval of Biological Information (Dr. G. C. Ainsworth), 12
Stormont, Dr. C. : Blood Groups in Infra-human Species (Blood Groups in Cattle), 654
Storms (Magnetic), in Interplanetary Space as observed by *Pioneer V* (Eugene W. Greenstadt), 329
Stott, B. M. H., and others : Possible Method for Increasing the Signal-Noise Ratio in the Detection of the First Motion of a Refracted *P*-Wave, 1382
Stott, Dr. P. M., and Stacey, Dr. F. D. : Stress Effects on Thermoremanent Magnetization, 585
Stotz, J. K. : At the Second International Measurement Conference (Measuring Systems for Flight Testing of Aircraft), 1350
Stotzky, G., and others : A Sealing Compound for Use in Biological Work, 410
Stove, E. R., and others : Some Novel Acid-stable Penicillins, 1091
Stover, Dr. Betsy J., and others : Failure of a Dog to Discriminate between Strontium-90 and Calcium given Orally, 713
Strain (Avirulent), of *Salmonella typhimurium* : Resistance to Infection with *Salmonella paratyphi C* in Mice parasitized with a relatively (Dr. J. G. Howard), 87
Strain (Inbred), of Mice : Effect of Hybridization on the Primary Polydipsic Trait of an (Dr. Emanuel Silverstein), 523
Strains (Inbred) : An Iso-Antigen ($\gamma B A$) of Mouse γ-Globulin present in (A. Kelus and J. K. Moor-Jankowski), 1405
Strains (Inbred Mouse) : Social Dominance in (Prof. G. Lindzey, H. Winston and M. Manosevitz), 474
Strains (Nutritionally Exacting), of *Staphylococcus pyogenes* : Metabolic and Genetic Investigations of (Janice B. Edgar and Dr. B. A. D. Stocker), 1121
Strains (Resistant and Susceptible), of Cotton : Leaf Curl Virus and the Flavonoid Content of (Dr. Esam M. Moustafa), 415
Strange, R. E. : Induced Enzyme Synthesis in Aqueous Suspensions of Starved Stationary Phase *Aerobacter aerogenes*, 1272
Strategy of Conflict (Prof. Thomas C. Schelling), *review* by Sir Cyril Burt, 427
Strategy for the War on Hunger (Sir Joseph Hutchinson and Prof. A. H. Bunting), 539
Stratosphere : World-Wide Circulation of Air within the (P. Goldsmith and F. Brown), 1033
Strauss, Prof. Bernard S. : Specificity of the Mutagenic Action of the Alkylating Agents, 730
  An Outline of Chemical Genetics, *review* by Dr. Charlotte Auerbach, 841
Strawberry (*Fragaria*) : Identity of L-Ascorbic Acid formed from D-Glucose by the (Dr. Frank A. Loewus and Stanley Kelly), 1059
Street, Philip : Mammals of the British Isles, *review* by B. J. G. Maitland, 110
Streptococci requiring Secretions of other Bacteria or Sulphydryl Compounds for Normal Growth : Spontaneous Development of *L* Forms of (Ayala Frenkel and Dr. Willy Hirsch), 728
Streptomyces scabies : Apparent Cytoplasmic Inheritance of Tyrosinase Competence in (Prof. K. F. Gregory and Wei-jen Shyu), 465
Streptomycin : Influence of Salts on the Uptake of, by *Escherichia coli* (Paul H. Plotz, Donald T. Dubin and Bernard D. Davis), 1324
Stress : Influence of Moisture Changes on Deformation of Wood under (L. D. Armstrong and G. N. Christensen), 869
Stress Effects on Thermoremanent Magnetization (Dr. P. M. Stott and Dr. F. D. Stacey), 585 ; (J. M. Hall), 586
Stresses and Friction (Contact) : Effective (Dr. D. H. Trollope), 376
Stretch, K. L. : Neuton 'Flux', 60
N–H Stretching Frequencies of Primary Amides : A New Relation between the (Prof. P. G. Puranik and K. Venkata Ramiah), 796
Strickland, E. H., and others : Oxygen Electrode for Measurement of Tissue Slice Respiration, 969
Strickland, G. (edited by) : Selected Journalism from the English Reviews by Stendahl, with Translations of other Critical Writings, 1031
Stride, A. H., and Bowers, R. : Two Unexplored Records of Quaternary History, 1001
Striker, Prof. Gy. : At the Second International Measurement Conference (Possibilities of a Long-range Planning of Instrumentation in a Planned Economy), 1350
Stripping (Anodic), using a Rotating Mercury Drop (Use of the Rotating Hanging Mercury Drop in Analysis), (Dr. J. J. Engelsman and A. M. J. M. Claassens), 240
Strontium-90 in Milk and Agricultural Materials in the United Kingdom, 1959-1960 (Agricultural Research Council, Report No. 4), 20
Strontium (Calcium and) : Transfer of, across Biological Membranes (United States Atomic Energy Commission, United States National Institutes of Health, United States National Science Foundation and the New York State Veterinary College Conference on), 1250
Strontium-90 and Calcium given Orally : Failure of a Dog to Discriminate between (Dr. Betsy J. Stover, M. Goldman and A. Ç. Andersen), 713
Stroud, A. N., and Brues, A. M. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Differences in Synthesis of DNA Response in Two Strains of Pig- and Monkey-Kidney Cells), 341
Stroud, D. B. E., and others : Mannosyl D-Glucosamine Hydrochloride Isomers—6-*O*-Mannosyl D-Glucosamine Hydrochloride, 143
Structural Analysis : Basic Approaches to, *review* by Prof. R. C. Coates, 1134
Structural Component of some Mammalian Tissue Cell Surfaces : Sialic Acid as a (Dr. Leonard Weiss), 1108
Structure of Bacterial Walls : Teichoic Acids and the (Dr. A. R. Archibald, J. J. Armstrong, Prof. J. Baddiley and J. B. Hay), 570
Structure of Feather Keratin (the late Prof. W. T. Astbury and E. Beighton), 171
Structure of Industry in Britain (Prof. G. C. Allen), 1332
Structure of the Moon's Surface (Dr. Gilbert Fielder), *review* by D. H. P. Jones, 424
Structure of the Northern Apennines (Sir Edward Bailey and Prof. W. J. McCallien), 1136
Structure of Paper : The Formation and (Technical Section of the British Paper and Board Makers' Association Conference on), 126
Structure of δ-Plutonium (J. A. C. Marples and J. A. Lee), 1391
Structure of Proline Derivatives from Apples : Determination by Mass Spectrometry of the (Dr. K. Biemann, G. G. J. Deffner and Prof. F. C. Steward), 380
Structure of Small Peptides : Chemical Aspects of the (Dr. Dorothy Wrinch), *review* by Prof. H. N. Rydon, 105
Structure in Ultra-Thin Sections of Vitrinite in Coal : Electron Microscopic Observations of (J. T. McCartney, Sabri Ergun and R. E. Walline), 1361
Structure and Action of the Sucker of *Echeneis* (Prof. B. Bonnell), 403
Structure and Dynamics of the Psyche (C. G. Jung), translated by R. F. C. Hull, *review* by Dr. C. Allen, 1235
Structure and Function of Muscle (edited by Dr. G. H. Bourne), Vol. 2, Biochemistry and Physiology ; Vol. 3, Pharmacology and Disease, *review* by Dr. D. H. Sproull, 948
Structure and Properties of Ionic Melts (Faraday Society General Discussion on), 338, 443
Structure (Age), and Marriage amongst the Groote Eylandt Aborigines : Classification of Kin, (Dr. Frederick G. G. Rose), *review* by Dr. R. Needham, 6
Structure (Atomic), of Graphite and Graphitization (Dr. G. E. Bacon), 27
Structure (Crystal and Molecular), of Diphenyl (Dr. G. B. Robertson), 593
Structure (Electronic), of Metals : The de Haas-Van Alphen Effect and the (Dr. D. Shoenberg), 126
Structure (Genetic Fine), of the ad_7 Locus of *Schizosaccharomyces pombe* : Distribution of X-Ray- and Nitrous Acid-induced Mutations in the (Dr. Herbert Gutz), 1127
Structure (Magnetoplumbite) : Fluoride-compensated Substitution of Bivalent Cations in the (Prof. R. Banks and M. Robbins), 1387
Structure (Protein) : Haemoglobin *F* and the Genetic Control of (Prof. V. M. Ingram), 608 ; (Dr. Wolf W. Zuelzer and Abner R. Robinson), 609
Structure (Sub-Chromatid), and Chromosome Duplication in *Vicia faba* (W. J. Peacock), 832
Structure (Surface) : A Relation between the Reactivity of Human Red Cells to Antiglobulin Tests and their (Judith E. Davis, Prof. H. N. Green and Pamela W. Tymms), 923
Structures of Polymeric Titanium Ethoxide and Titanium Oxide Ethoxides (Prof. D. C. Bradley and A. H. Westlake), 273 ; (R. L. Martin and G. Winter), 274
Structures (Deoxyribonucleic Acid), in the Nucleolus (Dr. Elsa H. J. O'Donnell), 1325
Structures (Surface), of Cylindrical Animal and Plant Cells : Circumscribing Irradiation of, with Polonium-210 α-Particles (R. M. Bergström, R. F. Blåfield and M. W. Brenner), 1165
Stuart, Dr. A. E. (Cooper, Dr. G. N., and) : Sensitivity of Mice to Bacterial Lipopolysaccharide following Alteration of Activity of the Reticulo-Endothelial System, 294
  Non-specific Immunity in Pneumococcal Infection of Mice, 295
Stubblefield, Dr. C. J., and Trotter, Dr. F. M. : Coal in Oxfordshire, 953
Stubbs, Bruce T., and others : Rapid Infra-Red Determination of Acetone in the Blood and the Exhaled Air of Diabetic Patients, 1008
Student's Prize of the Institution of Chemical Engineers awarded to J. P. Catchpole, 338
Studied (A Much-) Protozoon, *review* by Dr. J. A. Kitching, 1133
Studies in Management (G. Hunter), 1332
Studies (Adsorption), by Electron Bombardment : Evaporated Carbon Films of Large Area for (A. E. Bell, J. Pritchard and Prof. K. W. Sykes), 487
Studies (Histochemical and Homogenization), of Mitochondrial α-Glycerophosphate Dehydrogenase in the Nervous System (R. Hess and Dr. A. G. E. Pearse), 718
Studies (Lunar) : New Handbooks for, *review* by D. H. P. Jones, 424
Studies (Management), in Britain, 1251
Studies (Mathematical), in the Psychology of War, *review* by Sir Cyril Burt, 427
Studies (Social), in Britain, 1354
Studies (X-Ray Diffraction), of Bacterial Viruses (Dr. A. C. T. North and Prof. Alexander Rich), 1242
Study in Linear and Non-Linear Programming : Methods of Feasible Directions—a (Dr. G. Zoutendijk), *review* by Dr. M. V. Wilkes, 9
Study of the Preservation and the Restoration of Cultural Property, International Centre for the (Rome) : Works and Publications, 3, Climatology and Conservation in Museums, 1050
Study and Conservation of Nature, International Youth Federation for the : Course in Conservation, by the Verein Naturpark in the Lüneburger Heide, Germany, 1045
Study (Algological), on the Eastern Coasts of the Americas, *review* by Prof. Lily Newton, 1234
Study (Folk-life), Society for : Formation of a, 544

Study (Immunological), of Experimentally Induced Extrahepatic Cholestasis (Dr. Roy G. Shorter and R. G. Lack), 610
Study (An Immunological), of a Human Pituitary Interstitial Cell-stimulating Hormone (N. R. Moudgal and Prof. Choh Hao Li), 192
Study (Mathematical), of the Causes and Origins of War : Arms and Security—a (Dr. Lewis F. Richardson), edited by Nicolas Rashevsky and Ernesto Trucco, *review* by Sir Cyril Burt, 427
Studying the Pyrolysis of Polymers : Differential Thermogravimetry versus Differential Thermometry in (Hugh C. Anderson), 1088
Stumps (Pine) : Inoculation of, against Infection by *Fomes annosus* (Dr. J. Rishbeth), 826
Stutts, Patricia, and others : Biochemical Aspects of Resistance to 6-Mercaptopurine in Human Epidermoid Carcinoma Cells in Culture, 469
Style (Modern), in Mathematical Analysis, *review* by Prof. L. S. Goddard, 6
Styles, H. E. : Appointed director of research, London Transport, 751 ; work of, 751
Stylet-Sheath Material of a Pentatomid : Extraction of Lipids from the Precursor of the (P. W. Miles), 911
Sub-Bituminous Coals (Alberta), as Drilling Fluid Thinners (E. J. Jensen), 1144
Sub-Cellular Fractions of the Brain of the Rat during 'Metrazol' Convulsions : Changes in the Pentose–Nucleic Acid Content of (Prof. G. P. Talwar, B. Sadasividu and V. S. Chitre), 1007
Sub-Cellular Material : Passive Transfer of Contact Sensitivity to Picryl Chloride in Guinea Pigs, with (Dr. J. L. Turk), 915
Sub-Cellular Particles : Ethylene Evolution and (Prof. Stanley P. Burg and Ellen A. Burg), 967
Sub-Chromatid Structure and Chromosome Duplication in *Vicia faba* (W. J. Peacock), 832
Sub-Fractionation of Human Serum Leucine Aminopeptidase (Prof. Clyde A. Dubbs, Charlotte Vivonia and Jean M. Hilburn), 1203
Sub-Unit of Ribosomal Ribonucleic Acid from Yeast (Eiko Otaka, Yukito Oota and Syozo Osawa), 598
Sub-Units : Kinetics of the Enzymic Degradation of Deoxyribonucleic Acid into (Dr. Giorgio Bernardi and Prof. Charles Sadron), 809
Subcutaneous Phycomycosis in Uganda : Two Phycomycetes isolated from (Dr. A. M. M. Wilson), 612
Subjects living at High Altitude : Aldosterone Excretion and Potassium Retention in (P. J. Ayres, Rona C. Hurter, Dr. E. S. Williams and J. Rundo), 78
Sublimation Technique : Adducts of Urea and Thiourea obtained by means of a (B. Casu), 802
Subliminal Stimuli : Response to Series of—an Ethological Method for Investigating the Properties of Nervous Systems (A. J. Pontin), 512
Submarine Irradiance : Time-integrated Measurements of (Ronald Currie and Laurence Draper), 661
Submerged Plants : Inhibition of Growth and Photosynthesis in, with Tetramethylthiuram Disulphide and Sodium Dimethyldithiocarbamate and Reversal of this Inhibition of Photosynthesis (P. E. Björn Lindahl), 51
Substance (Antifungal), from *Pisum sativum* L. : Pisatin, an (Dawn R. Perrin and W. Bottomley), 76
Substance (Single) : Multiple Inhibitions of Cell Metabolism by a (M. Harris and Dr. G. A. Morrison), 1276
Substances (Antimicrobial), from Ferns (Prof. Jasper C. Maruzzella), 518
Substances (*M*- and *N*-Blood Group) : Products of Mild Acid and Alkaline Hydrolysis of (Dr. Elzbieta Romanowska), 1408
Substances (Gibberellin-like), in Plants (Margaret Radley)—Leguminous Root Nodules, 684 ; Seaweed (*Fucus vesiculosus*), 685
Substances (Lipid and Lipidophilic), as Adjuvants : Effectiveness of (D. W. Dresser), 1169
Substances (Organic) : Inhibition of Ultrasonic Decolorization of Rhodamine *B* in Presence of (Prof. Satya Prakash and Sheo Prakash), 1292
Substances (Pharmacologically Active), present in the Central Nervous System (Dr. Paul V. Buday), 245
Substances (Radioactive) : Safe Handling of, 1257
Substitution (Fluoride-compensated), of Bivalent Cations in the Magnetoplumbite Structure (Prof. E. Banks and M. Robbins), 1387
Substrate : Dependence on, of the Electrical Potential across the Isolated Gut (Dr. T. W. Clarkson, A. C. Cross and S. Toole), 501
Substrate (An Invariant Casein), for Rennet Standardization, derivable from Diverse Sources of Cows' Milk (Dr. G. W. Scott Blair and J. C. Oosthuizen), 697
Substrates (One-Degree Orientated) : Epitaxial Growth of Crystals on (Dr. A. Goswami), 160
Subtropical Coasts (Eastern Tropical and), of the Americas : Marine Algae of the (Prof. William Randolph Taylor), *review* by Prof. Lily Newton, 1234
Succinate : Synthesis of, from Acetate (D. D. Davies), 1093
Succinate (Œstriol) : Treatment of Thrombocytopaenic Haemorrhage with (Dr. Hubert Poliwoda), 400
Succinic Dehydrogenase : Nucleolar Localization of, in Normal Rat Hepatic Cells (P. De, R. Chatterjee and Dr. S. Mitra), 925
Succinic Dehydrogenase during Chemical Carcinogenesis : Tissue Dilution Artefact—a Re-interpretation of Variations in Levels of (G. R. N. Jones, L. Bitensky, J. Chayen and Prof. G. J. Cunningham), 1203
Sucker of *Echeneis* : Structure and Action of the (Prof. B. Bonnell), 403
Suckled and Unsuckled Female Rats : Induction of Cancer in (Prof. Giuliano Briziarelli), 921
Suckling of various Species : Correlation between the Growth-Rate of the, and the Percentage of Total Calories from Protein the Milk (Dr. F. W. Bernhart), 358
Sucrases in *Phaseolus vulgaris* (R. A. Cooper and R. N. Greenshields), 601
Sucrose from Fat : Metabolic Production of (Prof. H. Beevers), 433
Suesz, A. (Sprau, F., and) : Nematology (Radiotracer Techniques using Phosphorus-32 to indicate that *Longidorus maximus* feeds on the Roots of Beet and is thus a Parasite), 1049
'Sugar Pump' in the Intestinal Epithelium : Localization of the (Dr. T. Z. Csáky and G. W. Fernald), 709
Sugar Transport in Human Red Cells : Common Carrier System for (Dr. L. Lacko and Dr. M. Burger), 881
Sugar Transport (Mediated) : Persistence in Erythrocyte Ghosts of (Paul G. LeFevre), 970
Sugars (Selected) : Relative Sweetness of α- and β-Forms of (Rose Marie Pangborn and Sandra C. Gee), 810
Sugars (Alcohols and) : Regulation of Pyrogallol Oxidations by (B. Z. Siegel, S. M. Siegel and N. S. Goodman), 180
Suginome, Dr. H. : Awarded a Japanese fellowship in chemistry for 1961-62 by the Ramsay Memorial Trust, 337
Sullivan, Kathleen (McManus, Mary Annunciata, and) : Effects of Kinetin and Indole-3-acetic Acid on Multiplication Rates of *Paramecium*, 619
Sulphate in *Blattella germanica* : Metabolism of the Sulphur Amino-Acids and of (Richard J. Block and S. Mark Henry), 392
Sulphated Mucopolysaccharides : Staining of (Dr. I. D. Heath), 1370
Sulphide Ore : Black Shale, Oil and (Prof. K. C. Dunham), 985
Sulphur Amino-Acids : Metabolism of the, and of Sulphate in *Blattella germanica* (Richard J. Block and S. Mark Henry), 392
Sulphydryl Compounds for Normal Growth : Spontaneous Development of *L* Forms of Streptococci requiring Secretions of other Bacteria or (Ayala Frenkel and Dr. Willy Hirsch), 728
Sulphydryl Group, and the Catechol Amines : Interaction of Hydrocortisone, the (Dr. Sidney Roston), 490
Summer School on Corrosion—Corrosion Problems in the Electrical Power Industry (Battersea College of Technology), 651
Summerhayes, V. S. : Proceedings of the Third World Orchid Conference, *review*, 213
Suomalainen, Dr. Heikki, and Keränen, A. J. A. : The First Anthocyanins appearing during the Ripening of Blueberries, 498
Supek, Z. (Jović, M., and) : Formation of 5-Hydroxytryptamine in an Aqueous Solution of 5-Hydroxytryptophan treated with X-Rays, 383
Supercontraction of Iodinated Wools (O. A. Swanepoel), 908
Supplies (Drug), during the American Revolution (George B. Griffenhagen), 761
Supply (Excess), of Heavy Metals to Barley during Germination : Effect of, with special reference to Catalase and Peroxidase (Dr. S. C. Agarwala, A. Kumar and C. P. Sharma), 726
Supported Beam : Initial Vibrations of a (Dr. R. V. Sharman and D. White), 692
Supposed Formation of Hydrogen Peroxide during the Autoxidation of Haemoglobin : Reactions of Haemoproteins with Hydrogen Peroxide and the (Prof. D. Keilin), 769
Surface of the Moon (V. A. Firsoff), *review* by D. H. P. Jones, 424
Surface (Moon's) : Structure of the (Dr. Gilbert Fielder), *review* by D. H. P. Jones, 424
Surface Area (High) : Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of, using a Continuous Flow Calorimeter (G. H. Bell and A. J. Groszek), 1184
Surface Structure : A Relation between the Reactivity of Human Red Cells to Antiglobulin Tests and their (Judith E. Davis, Prof. H. N. Green and Pamela W. Tymms), 923
Surface Structures of Cylindrical Animal and Plant Cells : Circumscribing Irradiation of, with Polonium-210 α-Particles (R. M. Bergström, R. F. Blåfield and M. W. Brenner), 1165
Surface Symmetry and Cell Dimensions of Layer Lattice Silicates (E. W. Radoslovich), 67
Surface Waves in Anisotropic Elastic Media (V. T. Buchwald and A. Davis), 899
Surface Wax on Leaves : Physiological Significance of (D. M. Hall and R. L. Jones), 95
Surface and Underground Waters : Uranium in British (J. D. Peacock), 1189
Surfaces (Dry or Moist) : A Method of replicating, for Examination by Light Microscopy (Joan Sampson), 932
Surfaces (Mammalian Tissue Cell) : Sialic Acid as a Structural Component of some (Dr. Leonard Weiss), 1108
Surfen, a Quinoline with Oncogenic and Heparin-neutralizing Properties (DeWitt T. Hunter, jun., and Joseph M. Hill), 1378
Surgeons of England, Royal College of : Election of Sir Howard Florey and Dr. Owen H. Wangensteen as Honorary Fellows, 126
Surkevicius, H., and others : Reactivity of Calcium Sulphate Hemihydrate, 704
Survey : Land-Use—Nicaragua (Dr. John Grainger), 1259
Survey in Manufacturing Industry on Merseyside : Ageing and the Semi-Skilled—a (Alistair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine Cunningham), 1249
Survey (Gravity), on the Aira Caldera, Kyusyu, Tokyo (Izumi Yokoyama), 966
Surveys (U.H.F. and V.H.F. Television) : A Mobile Laboratory for (E. W. Taylor and L. C. Munn), 1355
Survival (*in vivo*) : Recovery and, of Rabbit Erythrocytes (Dr. G. F. Doebbler, R. G. Buchheit and A. P. Rinfret), 1405
Survival (Plant) : Light Rainfall and—Measurement of Stem Flow Run-off (M. D. Gwynne and J. Glover), 1321
Survival Curves and Target Theory : New Considerations on (Dr. E. L. Powers), 1045
Surwillo, Dr. Walter W. : Frequency of the 'Alpha' Rhythm, Reaction Time and Age, 823
Susceptibility to Induced Pulmonary Tumours in Mice : Inheritance of (Dr. D. S. Falconer and Dr. Joyce L. Bloom), 1070
Susceptibility to Leaf Blight, caused by *Cochliobolus heterostrophus*, in Nigerian Varieties of Maize (C. L. M. van Eijnatten), 515
Susceptible Strains (Resistant and), of Cotton : Leaf Curl Virus and the Flavonoid Content of (Dr. Esam M. Moustafa), 515
Suspensions (Aqueous), of Starved Stationary Phase *Aerobacter aerogenes* : Induced Enzyme Synthesis in (R. E. Strange), 1272
Sussdorf, Dr. D. H. : Awarded the David Anderson Berry Prize for 1961 of the Royal Society of Edinburgh, 229
  Rise of Antibody Titre in Cell-free Spleen Preparations Incubated *in vitro*, 770
Sustained Oscillations in a Catalytic Chemical System (R. A. Spangler and F. M. Snell), 457
Sutherland, Dr. N. S. : Facts and Theories of Shape Discrimination, 581
Sutton, Dr. D. A. : The British Gelatine and Glue Research Association—Twenty-first Meeting of the Research Panel, 857
Sutton, E. : Parasite Biology (Role of Arthropods as Vectors of Parasitic Infections), 134
Suzuki, Akio, and others : Isolation of Anthraquinone from Humus, 415
Svensson, Ingvar (Boman, Hans G., and) : Evidence for a New Enzyme connected with Protein Synthesis, 674
Swallow, Dr. A. J. : Radiation Chemistry of Organic Compounds, *review* by Dr. S. J. Thomson, 317

- Swallowing (Air–), a Mechanism in Photic Reversal of the Bettle *Trypodendron* (Prof. Kenneth Graham), 519
- Swanepoel, O. A. : Supercontraction of Iodinated Wools, 908
- Swarming of the Polychaete *Odontosyllis phosphorea* Moore, var. *Nanaimoensis* Berkeley, near Nanaimo, B.C. (E. Berkeley), 1321
- Sweet Potato (*Ipomoea batatas* (L.) Lam.) : Evolution of the (Dr. D. E. Yen), 93
- Sweetness (Relative), of α- and β-Forms of Selected Sugars (Rose Marie Pangborn and Sandra C. Gee), 810
- Swierszczewski, Dr. S. : Appointed Research Fellow in nuclear physics in the University of Birmingham, 337
- Swimming Ability of Non-aquatic Reptiles (R. R. Tercafs), 125
- Swinton, Dr. W. E. : Appointed head of the Division of Life Sciences at the Royal Ontario Museum, Toronto, 24
- Swisher, Dr. S. N. : Blood Groups in Infra-human Species (Blood Group Systems in the Dog), 653
- Sydney–Hawkesbury District : Rock Engravings of the (F. D. McCarthy), 854
- Sykes, Dr. Charles : New Creep Laboratory of the British Electrical and Allied Industries Research Association opened by, 1049
- Sykes, G. : Elected honorary president of the Society for Applied Bacteriology, 754
- Sykes, J. R. (Cooper, Marion I., and) : Demonstration by Paper Electrophoresis of an Auto-Immunological Precipitin possibly due to Fibrinolysins, 1212
- Sykes, Prof. K. W., and others : Evaporated Carbon Films of Large Area for Adsorption Studies by Electron Bombardment, 487
- Syllables (Nonsense) : Stimulus-Response Compatibility in the Paired-Associate Learning of (A. D. Baddeley), 1327
- Symbiotic Nitrogen Fixation : Root Temperature and (A. H. Gibson), 1080
- Symmetry (Surface), and Cell Dimensions of Layer Lattice Silicates (E. W. Radoslovich), 67
- Sympathetic Transmission : Lack of Evidence for a Cholinergic Mechanism in (Dr. J. E. Gardiner and Dr. J. W. Thompson), 86
- Symptoms of Barley Leaf-Spot Disease : Production of, by Culture Filtrate of *Helminthosporium sativum* (S. K. Gayed), 725
- Synaptic Transmission in Neurosecretory Cells (Hiromichi Morita, Takaaki Ishibashi and Satoru Yamashita), 183
- Syndrome (Grey Speck), of Oats : Manganese and Magnesium in the (P. B. Vose and Dr. D. J. Griffiths), 299
- Syndrome (Malabsorption), and Exudative Enteropathy : Calcium Metabolism in Man with Calcium-45— (Dr. G. Milhaud and Dr. P. Vesin), 872
- Syndrome (Stein–Leventhal) : Physiology and Pathogenesis of the (Dr. Virendra B. Mahesh and Dr. Robert B. Greenblatt)—Urinary Androsterone, Etiocholanolone, Pregnanetriol and Δ^5-Pregnenetriol of Ovarian Origin in a Patient with Stein–Leventhal Syndrome, 888 ; Role of FSH in the Pathogenesis of the Stein–Leventhal Syndrome, 889
- Syndromes (Nephrotic) : Haptoglobinuria in (A. Marnay), 74
- Synge, J. L. : Relativity, the General Theory, *review* by Dr. G. J. Whitrow, 424
- Synthesis of Adenine from Hydrogen Cyanide under Possible Primitive Earth Conditions : Mechanism of (Prof. J. Oró), 1193
- Synthesis of Succinate from Acetate (D. D. Davies), 1093
- Synthesis (Adenosine Triphosphate), in Erythrocytes : A Positive Feedback Mechanism of (Makoto Nakao, Takashi Motegi, Toshiko Nakao, Saburo Yamazoe and Haruhisa Yoshikawa), 283
- Synthesis (A Convenient), of Muramic Acid and other 3-O-Ethers of D-Glucosamine (Dr. Roy Gigg and Patricia M. Carroll), 495
- Synthesis (Haemoglobin) : Control of Specificity in (Dr. J. Bishop, Dr. G. Favelukes, Prof. R. Schweet and Dr. E. Russell), 1365
- Synthesis (Induced Enzyme), in Aqueous Suspensions of Starved Stationary Phase *Aerobacter aerogenes* (R. E. Strange), 1272
- Synthesis (Protein) : Evidence for a New Enzyme connected with (Hans G. Boman and Ingvar Svensson), 674
- Synthesis (Urea), in the Living Rat Brain (R. K. Dacies, A. J. Defalco, D. Shander, A. Kopelman and Prof. J. Kiyasu), 288
- Synthesis and X-Ray Analysis of Diamond (H. Ll. D. Pugh, J. Lees and J. A. Bland), 865
- Synthetic Corundum : A New Technique for the Production of (E. A. D. White), 901
- Synthetic Medium lacking Amino-acids : Formation of Phenethyl Alcohol and Tyrosol during Fermentation of a (R. Stevens), 913
- System : A New Universal Buffer (Dr. D. A. Ellis), 1099
- System (Catalytic Chemical) : Sustained Oscillations in a (R. A. Spangler and F. M. Snell), 457
- System (Central Nervous) : Enzymic Activity of the (Neurochemical Commission of the World Federation of Neurology International Symposium on), 1349
  - Pharmacologically Active Substances present in the (Dr. Paul V. Buday), 245
  - Tryptamine Receptors in the (Dr. J. R. Vane, Dr. H. O. J. Collier and Dr. S. J. Corne, and Dr. E. Marley and Dr. P. B. Bradley), 1068
- System (Central Nervous), of an Insect (*Periplaneta americana* L.) : Exchanges of Sodium Ions in the (J. E. Treherne), 1223
- System (Common Carrier), for Sugar Transport in Human Red Cells (Dr. L. Lacko and Dr. M. Burger), 881
- System (Electron Transport), and Phosphoproteins of Mitochondria (K. Ahmed, J. D. Judah and C. H. Gallagher), 1309
- System (Human Haematopoietic) : Effects of Ionizing Radiation on the—Report of the Subcommittee on Haematologic Effects, Committee on Pathologic Effects of Atomic Radiation (United States National Academy of Sciences—National Research Council, Publication No. 875), 556
- System ('Hunger Motivational') : Evidence of a, in the Lateral Hypothalamus of the Rat (Dr. P. J. Morgane), 672
- System (Nervous) : Chemistry and the, *review* by Dr. R. V. Coxon, 318
  - Histochemical and Homogenization Studies of Mitochondrial α-Glycerophosphate Dehydrogenase in the (R. Hess and Dr. A. G. E. Pearse), 718
- System (Neurosecretory), of the Desert Locust : Induced Changes in the Amounts of Material in the (Dr. K. C. Highnam), 199
- System (Rat Lens Enzyme) : Catalysis of Incorporation of ^{14}C-Uridine into Rat Lens Ribonucleic Acid by (Dr. Anima Devi and Dr. N. K. Sarkar), 1094
- System (Reticulo-Endothelial) : Sensitivity of Mice to Bacterial Lipopolysaccharide following Alteration of Activity of the (Dr. G. N. Cooper and Dr. A. E. Stuart), 294
- System (Reticulo-Endothelial), of Mice : Effect of Added Vitamins on the Phagocytic Activity of the (J. Cordingley and Prof. T. Nicol), 82
- System (Short-term Dating) : Carbon-14 from Nuclear Explosions as a—Use to determine the Origin of Heartwood (Dr. A. T. Wilson), 714
- Systematics Association and the Challenger Society : Symposium on Speciation in the Sea, 229
- Systemic Insecticides : Enhanced Plant-Growth with Two (M. M. H. Wallace), 513
- Systems (Cell-free) : Biosynthesis of Riboflavin in (Prof. T. W. Goodwin and Dr. A. A. Horton), 772
- Systems (Computer), for Searching Chemical Literature : Report on (E. C. Medam and H. R. Koller), (United States National Bureau of Standards Research Information Centre and Advisory Service on Information Processing), 23
- Systems (Computing) : Redundancy Techniques for (Information Systems Branch of the United States Office of Naval Research Symposium on), 1349
- Systems (Dimethyl Sulphoxide–Water) : Interaction of Lipids and Trypsin in (Prof. A. Lukton, H. Donohue and Prof. F. A. Bettelheim), 565
- Systems (Electrodialysis) : Thermo-electric Phenomena in (Dr. Lloyd H. Shaffer), 591
- Systems (Heterocyclic), with Bridgehead Nitrogen Atoms (William L. Mosby), Part 1, *review* by Dr. G. L. Buchanan, 425
- Systems (Nervous) : Response to Series of Subliminal Stimuli—an Ethological Method for Investigating the Properties of (A. J. Pontin), 512
- Systems Engineering : Operations Research and (Charles D. Flagle, William H. Huggins and Robert H. Roy), *review* by Prof. L. S. Goddard, 105
- Szenberg, Dr. Aleksander, and Warner, N. L. : Large Lymphocytes and the Simonsen Phenomenon, 920
- Szenberg, Dr. Aleksander (Dineen, Dr. J. K., and) : Regeneration of Elements of Donor Origin in Orthotopically Grafted Skin following the Homograft Response, 153
- Szentágothai, Prof. János (edited by Prof. Ferenc Kiss and) : Atlas of Human Anatomy, Vol. 1, Osteology, Syndesmology, Myology ; Vol. 2, Splanchnology, Endocrine Glands, Heart ; Vol. 3, Nervous System, Vascular System, Lymphatic System, Sense Organs, *review* by Prof. J. M. Yoffey, 1232
- Szepsenwol, J. : The Effect of various Diets upon Sexual Maturity of Mice, 1112
- Szewczuk, Dr. A., and others : Histochemical Demonstration of Gamma-Glutamyl Transpeptidase, 767
- Szporny, L., and others : Amino-derivatives of 11β, 17α, 21-Trihydroxy-3, 20-Dioxo-1,4-Pregnadiene, 607
- Sztaricskai, F. (Uri, J., and) : Simple, Quick Methods for Detection of 6-Amino-penicillanic Acid, 1223
- Szwarc, Dr. H. : Effects of Ionizing Radiation on Organic and Biological Solids (Use of Electron-Spin Resonance to Demonstrate the Rapid Recombination of Radicals which occurs on Heating Irradiated Cyclohexane through its Transition Point at 186° K.), 1150
- Szymanski, Herman A., and Povinelli, Richard : Infra-Red Spectra of Water-soluble Carbonates, 64

- T-PHASE : Appearance of the (R. Green), 997
- Tables and Nomograms : A Collection of, for the Processing of Observations made on Artificial Earth Satellites (I. D. Zhongolovich and V. M. Amelin), *review* by D. G. King-Hele, 1315
- Tadpole (Ascidian) : Electron Microscope Observations of the Receptors in the Sensory Vesicle of the (Noël Dilly), 786
- Taha, M. A., and others : Tuber Bulking in the Potato Crop, 782
- Takaku, Fumimaro, and others : Effect of the Bilateral Section of the Splanchnic Nerve on Erythropoiesis, 500
- Takauji, Masa, and others : Controlling Mechanism of the Mechanical Activity of Muscle and that of Blood Coagulation, 80
- Take-Over Bid in Acoustical Training (Prof. E. J. Richards), 748
- Takeyama, N. : Paths of Inter-Molecular Energy Transfer in Photo-sensitized Oxidation-Reduction, 1359
- Talanti, Prof. S., and Hopsu, Prof. Väinö K. : Leucine Aminopeptidase in the Mammary Gland of the Cow and Rat, 86
- Taliaferro, W. H. : Immunity to Protozoa (Cellular Basis of Immunity), 551
- Talwar, Prof. G. P., and others : Changes in the Pentose–Nucleic Acid Content of Sub-Cellular Fractions of the Brain of the Rat during 'Metrazol' Convulsions, 1007
- Tamuro, Hiroko, and others : Chromatographic Fractionation of Hog Thyroglobulin, 1199
- Tancredi, F., and others : Blockage of Transformation of Tryptophan to Nicotinic Acid by Experimental Hepatitis in Mice, 601
- Taneja, G. C., and Gowe, R. S. : Effect of Varying Doses of Undiluted Semen on Fertility in the Domestic Fowl, 828
- Tanganyika : Age of Bed I, Olduvai Gorge (Dr. L. S. B. Leakey, Dr. J. F. Evernden and Dr. G. H. Curtis), 478
- Tapetum Lucidum of the Eye of the Opossum, *Didelphis virginiana*: Cholesterol in the (Antoinette Pirie), 708
- Tapeworms, *Hymenolepis nana* (Cestoda : Hymenolepididae) : A Natural Population of Anomalous Branched, in a Colony of *DBA*/1 Mice (Dr. Donald Heyneman), 297
- Tappel, A. L. (Kumta, U. S., and) : Radiation Damage to Proteins, 1304
- Tapping of *Hevea brasiliensis* : Effect of Ethylene Oxide on the (D. H. Taysum), 1319
- Target Theory : New Considerations on Survival Curves and (Dr. E. L. Powers), 1045
- Tarnay, K. : At the Second International Measurement Conference (Method for Measuring Small Capacities of about 100 pF., with an Accuracy of 1 per cent), 1350
- Tartar, Vance : The Biology of Stentor, *review* by Dr. J. A. Kitching, 1133
- Tarte, P. : Infra-Red Spectroscopic Evidence of Four-fold Co-ordination of Titanium in Barium Orthotitanate, 1002
- Tarutani, Osamu, and others : Chromatographic Fractionation of Hog Thyroglobulin, 1199
- Taste Fibres in the Carp : Function of (Jihei Konishi and Prof. Yngve Zotterman), 286

Taste Thresholds and Food Dislikes (Dr. R. Fischer, F. Griffin, S. England and S. M. Garn), 1328
Tata Memorial Trust : Awards for 1961-62 for research on leukaemia and allied diseases, 228
Tatchell, Dr. J. A. H. : Pulmonary Tumours and Group VII in the House-Mouse, 1125
Tatchell, R. J. : Parasite Biology (Application of New Laboratory Techniques), 135
Tatlow, Prof. J. C., and others (edited by) : Advances in Fluorine Chemistry, Vol. I, *review* by Dr. Richard Dresdner, 319
Tatsuno, T., and others : Toxicology of *Penicillium islandicum*, 864
Tauc, L. (Hughes, Dr. G. M., and) : The Path of the Giant Cell Axons in *Aplysia depilans*, 404
Tavolga, Dr. W. N. (edited by Dr. W. E. Lanyon and) : Animal Sounds and Communication, *review* by Dr. W. H. Thorpe, 741
Tax, Sol., and Callender, Charles (edited by) : Evolution after Darwin, Vol. 3, Issues in Evolution, *review* by Dr. P. G. Fothergill, 425
Taxonomy of the Earthworm in India (G. E. Gates), 227
Taxonomy of *Xanthomonas* and *Pseudomonas* (R. R. Colwell and Prof. J. Liston), 617
Taxonomy (Plant), as a Scientific Discipline (Dr. R. E. Holttum), 990
Taylor, A. L. : Nematology (Use of Nematicides in the United States), 1049
Taylor, A. L. (paper read by) : Nematology (Holotype and Allotype Species of *Meloidogyne* Species and Sub-species), (B. G. Chitwood), 1048
Taylor, Dr. B. W. : Estudios Ecologicos para el Aprovechamiento de la Tierra en Nicaragua (Ecological Land Use Surveys in Nicaragua), 1259
Taylor, E. W., and Munn, L. C. : A Mobile Laboratory for U.H.V. and V.H.F. Television Surveys (B.B.C. Engineering Monograph No. 34), 1355
Taylor, Dr. James : Solid Propellant and Exothermic Compositions, *review* by Dr. W. B. Littler, 3
Taylor, Dr. Joan : Bacteria of the Intestine (Host Specificity and Enteropathogenicity of *Escherichia coli*), 1152
Taylor, Dr. John K. : Appointed chief of the Applied Analytical Research Section of the United States National Bureau of Standards Analytical and Inorganic Chemistry Division, 334 ; work of, 334
Taylor, M. G. : Biorheology and Microcirculation, 758
Taylor, Dr. S. J. : Appointed to the University of London readership in mathematics at Westfield College, London, 1349
Taylor, Prof. William Randolph : Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas, *review* by Prof. Lily Newton, 1234
Taysum, D. H. : Effect of Ethylene Oxide on the Tapping of *Hevea brasiliensis*, 1319
Tchervyakova, V. I. : At the Second International Measurement Conference (Electrical Measuring Instruments based on the Photo-compensating Amplifier), 1351
Tea : The Concept of Kind of (W. Wight and R. C. J. H. Gilchrist), 14
Tea Plant : Classification of the (Dr. H. H. Mann), 409
Teacher Preparation for Health Education (United Nations, World Health Organization, Technical Report Series No. 193—Report of a joint WHO/Unesco Expert Committee), 441
Teachers' Point of View : Science and Education—the, 555
Teachers (Mathematics) : Shortage of, in Britain, 127
Teachers (Science), in Training (Arthur Guinness Son and Co., Ltd., Awards for), 338
Teachers (Scientific) : Shortage of, in the United Kingdom, 123
Teaching of Mathematics (Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom Conference of professors of mathematics from the Universities of Great Britain to consider the), 1145
Teaching of Psychiatry and Mental Health (United Nations, World Health Organization, Public Health Papers No. 9), 1248
Teaching (Biology) : A New Approach to (Dr. L. H. Finlayson), 554
Techne (Cambridge), Ltd. : Correct price of the 'Tempunit' Temperature Controller, 856
Technical Assistance to Overseas Countries (June 17, 1039), *errata*, 24
Technical Books : Recent Scientific and, Supp. iv, July 29 ; Supp. iv, August 26 ; ccccxciii, September 30
Technical College (Federal), Zurich : Appointment of Dr. W. F. Berg as professor of the Photographic Institute, 1043
Technical Conferences (Scientific and) : Forthcoming International (Department of Scientific and Industrial Research, List No. 29), 959
Technical Co-operation, Department of : Work of the, 440
Technical Co-operation in Africa, Commission for (International Union for the Conservation of Nature and the) : Conference on the Conservation of Nature and Natural Resources in Modern African States, 958
Technical Education : Apprenticeship and, 737
Technical Education Abstracts from British Sources (National Foundation for Educational Research in England and Wales), Vol. 1, No. 1, September–December 1960, 21
Technical Reports of the United States Armed Forces, 1347
Technical Writing : The Technique of (Borough Polytechnic, London, lectures on), 755
Technique of Technical Writing (Borough Polytechnic, London, lectures on), 755
Technique (Light Depolarization) : Crystallization of Isotactic Polypropylene using a (J. H. Magill), 1092
Technique (A New), for the Production of Synthetic Corundum (E. A. D. White), 901
Technique (A New Photometric), using a Variable Shutter Device (Dr. O. C. Jones and Dr. F. J. J. Clarke), 1290
Technique (Peak-Shift), in Gas–Liquid Chromatography—Trimethylsilyl Ether Derivatives of Alcohols (Stanley H. Langer and Peter Pantages), 141
Technique (Sublimation) : Adducts of Urea and Thiourea obtained by means of a (B. Casu), 802
Techniques : Management Sciences, Models and—Proceedings of the Sixth International Meeting of the Institute of Management Sciences, Paris 1959 (edited by Prof. C. West and Prof. Michel Verhulst), *review* by T. H. Hawkins, 209
    Rheological (British Society of Rheology and the Non-Destructive Testing Group of the Institute of Physics and the Physical Society Joint Conference on), 24
Techniques in Tropical Pathology (Prof. B. G. Maegraith, Prof. W. E. Kershaw and D. Dagnall), *review* by Prof. P. C. C. Garnham, 1236
Techniques (Redundancy), for Computing Systems (Information Systems Branch of the United States Office of Naval Research Symposium on), 1349
Technological Awards, National Council for : Report for 1960-61, 754
Technological Education, Regional Advisory Council for, London and Home Counties : Regional Development Plan for Courses for the Diploma in Technology, 1044
Technologists : Science for Scientists and, 311
Technology : Explosives, *review* by Dr. W. B. Littler, 3
    Regional Development Plan for Courses for the Diploma in (Regional Advisory Council for Technological Education, London and Home Counties), 1044
    Solar Energy, 1954-1959 (T. Brimelow), 228
Technology, Battersea College of : Summer School on Corrosion (Corrosion Problems in the Electrical Power Industry), 651
Technology, Bradford Institute of : Course on Recent Developments in the Chemistry and Technology of Polymers, 1349
Technology, Illinois Institute of (Chicago) : International Symposium on Photoelasticity, 755
Technology, Liverpool College of : Postgraduate Courses in Nuclear Studies, 338
Technology of Chemical Processing, *review* by R. H. Villet, 1131
Technology of Polymers : Recent Developments in the Chemistry and (Bradford Institute of Technology Course on), 1349
Technology (Advanced), in Britain : Control of Colleges of, 20
Technology (Advanced), Birmingham College of : Course on Statistical Quality Control and Acceptance Sampling, 337
Technology (Advanced), Welsh College of (Dr. A. Harvey), 127
Technology (Chemical) : Encyclopedia of (edited by Raymond E. Kirk and Donald F. Othmer), second Supplement Volume edited by Anthony Standen, *review* by R. H. Villet, 1131
Technology (*Coal Tar*), *Review of* (Coal Tar Research Association), Vol. 12, Part 1, January–June 1960, 22
Technology (Food) : European Symposium on, 959
Technology (Nuclear Reactor) : Analytical Chemistry in (Oak Ridge National Laboratory Fifth Conference on), 651
Technology (Science and), Manchester College of : Appointment of Ronald Beresford Dew as visiting professor of industrial administration, 651
Technology (Science and), National Lending Library for : *N.L.L. News*, No. 1, 1347
Technology (Science and), Royal College of (Glasgow) : Appointment of Dr. David W. A. Sharp as senior lecturer in the Department of Chemistry, 443
    Appointment of Dr. Graham R. Knox as lecturer in the Department of Chemistry, 443
    Endowment of £800 made by Mrs. Hilda M. Beilby, to be known as the Kelvin Prizes, 1349
Technology (Space) : Ballistic Missile and—Proceedings of the Fifth Symposium held at Los Angeles, 1960 (edited by Donald P. LeGalley), Vol. 1, Bioastronautics and Electronics, with Invited Addresses ; Vol. 2, Propulsion and Auxiliary Power Systems ; Vol. 3, Guidance, Navigation, Tracking and Space Physics ; Vol. 4, Re-entry and Vehicle Design, *review* by W. H. Stephens, 1131
Technology and Geography (Prof. S. H. Beaver), 986
Tegge, G., and Kempf, W. : At the International Starch Conference (Effect of certain Cations on Starch), 237
Teichoic Acids and the Structure of Bacterial Walls (Dr. A. R. Archibald, J. J. Armstrong, Prof. J. Baddiley and J. B. Hay), 570
Telecommunication Union, International : European Conference on Broadcasting, 449
Teleost Fish (Marine), *Pollachius virens* : Pharmacological Characterization of the Oxytocic Peptides in the Pituitary of a (Prof. H. Heller, B. T. Pickering, J. Maetz and F. Morel), 670
Teleosts : Pigment Bodies of the Spinal Neurones of some (Prof. A. Stolk), 924
Telescope (Benelux Radio) : Cross-Antenna of the Proposed (Prof. W. N. Christiansen and Dr. J. A. Högbom), 215
Television : Educational—a Survey and a Plan (John Wellens), 226
    Science and, 20
Television Signal-to-Noise Ratio : An Instrument for measuring (S. M. Edwardson), 1355
Television Surveys (U.H.F. and V.H.F.) : A Mobile Laboratory for (E. W. Taylor and L. C. Munn), 1355
Television (British) : Isaac Shoenberg—Silver Jubilee of, 751
Temperature : Dependence on, of the Distribution of Cations in Oxide Spinels (Ranajit K. Datta and Prof. Rustum Roy), 169
Temperature (High) Compounds : Sintered (Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals Symposium on), 1250
Temperature (Low) : Inhibition by Potassium of the Mechanical Responses of Frog Muscles at (H. Lorković), 1011
    Recoil-Atom Reactions at (Slobodanka Veljković and Dr. Garman Harbottle), 1287
Temperature (Root), and Symbiotic Nitrogen Fixation (A. H. Gibson), 1080
Temperature (Viscosity–) Relationships for Dilute Solutions of High Polymers (Dr. W. R. Moore), 1292
Temperatures (Soil), in Iceland (Dr. Bjarni Helgason), 939
Temporal Pole Damage (Unilateral), in the 'Split-Brain' Monkey : Changes in Visual Gnostic Functions and Emotional Behaviour following (Dr. J. L. deC. Downer), 50
'Tempunit' Temperature Controller : Correct price of the (Techne (Cambridge), Ltd.), 856
Tercafs, R. R. : Swimming Ability of Non-aquatic Reptiles, 125
Terminals (Motor Nerve) : Stimulation of (Dr. J. I. Hubbard and Robert F. Schmidt), 1103
Terminology : Neutron (Dr. W. R. Harper), 60
Terms (Petroleum) : Glossary of (edited by George Sell), third edition (Institute of Petroleum), 22
Ternary Compound Formation in the System $CaO{-}Al_2O_3$—MgO (J. H. Welch), 559
Terpene Chemistry, *review* by Prof. B. C. L. Weedon, 1234
Terpenes : The Chemistry of the (Dr. A. R. Pinder), *review* by Prof. B. C. L. Weedon, 1234
Terrestrial Accretion from the Solar Wind (Dr. Ronald Frith), 1182 ; (Dr. E. R. Harrison), 1182 ; (C. M. de Turville), 1183

Terrestrial Exosphere at Heights of 1,000-1,700 km. (Max Römer), 238
Tertiary in Southern England : Tropical Floras in the, *review* by Prof. John Walton, 209
Tertiary Floras (Lower), of Southern England (Marjorie Elizabeth Jane Chandler), I, Palaeocene Floras, London Clay Flora (Supplement), *review* by Prof. John Walton, 209
Terzaghi, K., Founder of the Science of Soil Mechanics, *review* by Kevin Nash, 210
Terzaghi, Karl (selections from the Writings of) : From Theory to Practice in Soil Mechanics, *review* by Kevin Nash, 210
Terzi, Dr. M. : Comparative Analysis of Inactivating Efficiency of Radiation on Different Organisms, 461
Test (A New), for Detecting Anaphylactic Sensitivity—the Basophil Reaction (Prof. Walter B. Shelley and Lennart Juhlin), 1056
Test (Phenylalanine), on Cultures from Multi-carbohydrate Media : A Micro-method for Performing the (D. J. Stewart), 521
Test (Positive Antiglobulin Consumption) : Nature of the Coating Globulin causing a (D. Nelken), 1110
Testing Materials, American Society for (Committee E-14), (Mass Spectrometry Group of the Institute of Petroleum and the) : Conference on Mass Spectrometry, 24
Testing Textiles, *review* by W. J. Onions, 106
Testing (Textile), and Quality Control : Handbook of (Prof. Elliot B. Grover and Prof. D. S. Hamby), *review* by W. J. Onions, 106
Testis Follicles in Miridae : The Number of (Dennis Leston), 93
Tests (Antiglobulin) : A Relation between the Reactivity of Human Red Cells to, and their Surface Structure (Judith E. Davis, Prof. H. N. Green and Pamela W. Tymms), 923
Tests (Quantal Neutralization) : Evaluation of (Prof. S. Fazekas de St. Groth), 891
Testut, R. J. : Appointed president of the sixth International Mineral Processing Congress, 545
^{14}C-Tetrachloroethylene in Mice : Urinary Metabolites of (Dr. Sven Yllner), 820
Tetracycline : Action of, on the Level of Properdin (Vl. Kubelka and M. Rybák), 820
  Mediation of Divalent Metal Ions in the Binding of, to Macromolecules (Dr. Kurt W. Kohn), 1156
Tetragonal Mullite-like Phase from Co-precipitated Gels (J. Ossaka), 1000
Tetrahydrofolic Acid to Folinic Acid : Enzymic Conversion of, by Plant Protein (E. H. Byers, J. Stewart and T. J. Bond), 179
1,3,7,9-Tetramethyluric Acid and Pyrene : Molecular Geometry of a 1:1 Crystalline Complex between (F. De Santis, E. Giglio, Dr. A. M. Liquori and A. Ripamonti), 900
Tetramethylthiuram Disulphide and Sodium Dimethyldithiocarbamate : Inhibition of Growth and Photosynthesis in Submerged Plants with, and Reversal of this Inhibition of Photosynthesis (P. E. Björn Lindahl), 51
Textile Engineering at Leeds, 1346
Textile Research : Polish, 1045
Textile Testing and Quality Control : Handbook of (Prof. Elliot B. Grover and Prof. D. S. Hamby), *review* by W. J. Onions, 106
Textiles : Testing, *review* by W. J. Onions, 106
Thailand, Government of, and others : To set up and operate an Aeronautical Research Laboratory and three Civil Aviation Training Centres, 1043
Thain, Dr. E. M., and others : Biosynthesis of the Pyrethrins, 281
Thalassaemia in an English and in a German Family (Dr. F. Vella and S. A. Ibrahim), 822
Thalassaemia and the Abnormal Human Haemoglobins (Dr. Harvey A. Itano and Prof. Linus Pauling), 398
Thames River (Conn.) : Gross β-Activity in the (D. M. Skauen, T. Hatfield, D. Hanlon and J. S. Rankin, jun.), 1017
Tharp, Marie, and others : The Floors of the Oceans, I, The North Atlantic—Text to accompany the Physiographic Diagram of the North Atlantic (Geological Society of America, Special Paper 65), *review* by Dr. G. E. R. Deacon, 531
Thaung, Dr. Maung Mya : Use of Radio-isotopes in Biological Work in Burma, 875
Theorem (Minimum Energy), for Flow of Dry Granules through Apertures (R. L. Brown), 458
Theoretical Expression for Solubility Product (Prof. B. N. Bhar), 1004 ; (Prof. K. Krishnamurti), 1004
Theoretical Meteorology, *review* by Dr. Robert P. Pearce, 314
  Introduction to (Prof. Seymour L. Hess), *review* by Dr. Robert P. Pearce, 314
Theoretical and Applied Mechanics : Proceedings of the Fifth Congress on ; and the Symposium on Non-Linear Physical Problems (Indian Society of Theoretical and Applied Mechanics), 855
Theoretical and Applied Mechanics (Indian Society of), and Unesco : Symposium on Non-Linear Physical Problems, 855
Théories de l'Évolution des Vertébrés (Erik Jarvik), traduction du Prof. J. P. Lehman, *review* by Dr. E. Trewavas, 945
Theories (Facts and), of Shape Discrimination (Dr. P. C. Dodwell), 578 ; (Dr. N. S. Sutherland), 581
Theory : Abstract Set (Prof. Abraham Fraenkel), second edition, *review* by Dr. R. G. Cooke (June 17, 1047), *erratum*, 126
Theory of Detonation (Ia. B. Zeldovich and A. S. Kompaneets), *review* by Dr. W. L. Murray, 1236
Theory of Markov Processes (E. B. Dynkin), translated from the Russian by D. E. Brown ; edited by T. Kövåry, *review* by D. G. Kendall, 321
Theory to Practice : Fraom, in Soil Mechanics—Selections from the Writings of Karl Terzaghi, *review* by Kevin Nash, 210
Theory of Queueing : Mathematical Methods in the (A. Y. Khintchine), translated by D. M. Andrews and M. H. Quenouille, *review* by Prof. L. S. Goddard, 211
Theory of the Red Shift and the Nature of Planck's Constant (L. Infeld), 1184
Theory of Transition-Metal Ions (Prof. J. S. Griffith), *review* by Prof. C. A. Coulson, 1233
Theory (Filter) : Perceptual Defence and (Neville Moray), 940
Theory (Stability), of Colloids : Discreteness-of-Charge Effect and the (Dr. S. Levine, D. Calvert and G. M. Bell), 699
Theory (Target) : New Considerations on Survival Curves and (Dr. E. L. Powers), 1045
Therapy : Neutron Capture, 1258
Thermal Isomerization of Alpha-Keto-Acid Hydrazones : Application of the Nickel Reaction and, for their Chromatographic Analysis (A. M. Tokushige and Dean E. S. Cook), 797
Thermochemistry and Reactivity of related Free Radicals : Isoelectronic Principle, (Dr. Peter Gray and J. C. J. Thynne), 1357
Thermodynamics and Osmoregulation (Prof. Leonard B. Kirschner), 815
Thermodynamics with Quantum Statistical Illustrations (Prof. P. T. Landsberg), *review* by Dr. W. E. Parry, 1236
Thermoelastic Relaxation induced by Ultrasonics : Crystallization Kinetics on Polyvinylchloride and (Dr. V. Nardi), 563
Thermo-electric Phenomena in Electrodialysis Systems (Dr. Lloyd H. Shaffer), 591
Thermoelectric Power of Single Crystals of Gallium (P. Horner), 58
Thermogravimetry (Differential), versus Differential Thermometry in Studying the Pyrolysis of Polymers (Hugh C. Anderson), 1088
Thermometry (Differential) : Differential Thermogravimetry versus, in Studying the Pyrolysis of Polymers (Hugh C. Anderson), 1088
Thermophysical Properties Research Literature : Retrieval Guide to, Vol. I (edited by Y. S. Touloukian), Book 1, Part A, Guide to Substance Classification and Numerical Codes ; Book I, Part B, Dictionary of Synonyms and Trade Names ; Book I, Part C, Dictionary of Substances ; Book 2, Classified Search Index ; Book 3, Master Bibliography, Author Index, *review* by Prof. A. R. Ubbelohde, 109
Thermoreceptors : The Electrophysiology of (Prof. Herbert Hensel), 1143
Thermoremanent Magnetization : Stress Effects on (Dr. P. M. Stott and Dr. F. D. Stacey), 585 ; (J. M. Hall), 586
'Theta' Pinch : A New 'Wedge' Type Instability observed in a (J. K. Wright, R. D. Medford, J. D. Herbert and R. C. Pottinger), 1054
Thiabendazole, a Highly Effective Anthelmintic for Sheep (Hugh McL. Gordon), 1409
Thiazine Leucobases : Photo-Sensitivity of (D. W. Menzies), 505
Thickness of Nickel-Iron Films : Variation of Anisotropy Axis Dispersion with (H. Clow), 996
Thilo, Prof. E. : Inorganic Polymers, 1046
Thin Layer of Starch-Gel : Electrophoresis in a, followed by a Plastifying Treatment (J. Groulade, Dr. J. M. Fine and C. Ollivier), 72
Thinking (New), in School Mathematics, 335
Thinners (Drilling Fluid) : Alberta Sub-bituminous Coals as (E. J. Jensen), 1144
Thiocyanate Group in Inorganic Compounds : Co-ordination of the (A. Turco and C. Pecile), 66
Thio-diglycollic Acid : Anticancer Properties of (M. B. Sahasrabudhe, M. V. Narurkar, L. B. Kotnis, B. D. Tilak, L. C. Shah and V. N. Gogte), 388
Thioglycolic Acid : Chromium, Uranium, Tungsten and Nickel Complexes of (V. M. Bhuchar), 489
2-Thiouracil : Stimulation of Turnip Yellow Mosaic Virus Protein Production by (Dr. R. I. B. Francki and Dr. R. E. F. Matthews), 1078
Thiourea : Effect of Thyroxine plus, on the Early Development of the Chum Salmon (*Oncorhynchus keta*), (Prof. M. A. Ali), 1214
Thiourea (Urea and) : Adducts of, obtained by means of a Sublimation Technique (B. Casu), 802
Thistlethwaite, F. : Appointed first vice-chancellor of the University of East Anglia, Norwich, 959
Thoday, Prof. J. M. : Location of Polygenes, 368
Thomas, J. A. : Trans-Equatorial Backscatter Observations of Magnetically Controlled Ionization, 792
Thomas, Dr. J. H., and Whittle, Dr. E. : Gas-Phase Reactions of the Halogens and their Compounds, 129
Thomas, Robert : Colorimetric Detection of Penicillins and Cephalosporins on Paper, 1161
Thompson, Ltd., J. Langham : Industrial Research Fellowships, 545
Thompson, Dr. J. W. (Gardiner, Dr. J. E., and) : Lack of Evidence for a Cholinergic Mechanism in Sympathetic Transmission, 86
Thompson, R. H. (Todd, J. R., and) : Methaemoglobin in Chronic Copper Poisoning of Sheep, 89
Thomson, Arthur : Effect of Attention in Peripheral Vision, 732
Thomson, Sir Landsborough : Birds of Eastern and North-Eastern Africa, *review*, 9
Thomson, Dr. S. J. : Radiation Chemistry of Organic Compounds, *review*, 317
Thorium Content of Natural Materials : Measurement of the, by a γ-Ray Counting Method (Prof. J. H. J. Poole and F. N. Byrne), 62
Thorpe, Dr. W. H. : Sounds used by Animals, *review*, 741
Thow, R. F. : Appointed to a lectureship in agriculture at the University of Edinburgh, 545
Three Ectoparasitic Species : Simultaneous Eradication of, from a Colony of Laboratory Mice (Dr. Nigel Bateman), 721
Thresholds (Taste), and Food Dislikes (Dr. R. Fischer, F. Griffin, S. England and S. M. Garn), 1328
Thrombocytopaenic Haemorrhage : Treatment of, with Œstriol Succinate (Dr. Hubert Poliwoda), 400
Thromboplastic Activity of Red Cells (Dr. James A. Inglis and Dr. June W. Halliday), 821
Thromboplastin (Tissue) : Influence of Serum Factors on the Species-specificity of (Christine Hawkey and J. L. Stafford), 920
Thulium Oxide : Rapid Determination of Ytterbium in Aqueous Solutions and in, by Neutron Activation (Minoru Okada), 1090
Thwaites, Bryan (edited by) : Incompressible Aerodynamics, *review* by Prof. Leon Trilling, 315
Thymus Cortex (Mouse) : Periodic Acid–Schiff Positive Giant Cells in the (D. Metcalf and M. Ishidate), 305
Thymus Histone (Calf) : Effect of, on Rats bearing Transplantable Acute Myeloid Leukaemia (Dr. Lubomir Hnilica and Dr. Viktor Holoubek), 922
Thymus Influence in Leukaemogenesis : Analysis of the (Dr. J. F. A. P. Miller), 248
Thynne, J. C. J. (Gray, Dr. Peter, and) : Isoelectronic Principle, Thermochemistry and Reactivity of related Free Radicals, 1357
Thyroglobulin (Dr. A. D. Litonjua)—Effect of Acid, 356 ; Evidence of Thyroid Protease Action, 357
Thyroglobulin (Hog) : Chromatographic Fractionation of (Prof. Nobuo Ui, Osamu Tarutani, Yoichi Kondo and Hiroko Tamura), 1199
Thyroid Feeding : Augmentation of Toxicity of Monoamine Oxidase Inhibitor by (Richard N. Carrier and Dr. Paul V. Buday), 1107
Thyroid Function in Active, Sleeping and Torpid Hummingbirds (Prof. C. J. Shellabarger, R. C. Lasiewski and G. E. Hyncik), 1318

Thyroid Protease Action : Thyroglobulin—Evidence of (Dr. A. D. Litonjua), 357
Thyroid Tissue *in vitro* : Stimulation of Lipogenesis in, by Free Fatty Acids (Prof. Norbert Freinkel, Dr. Augusto D. Litonjua and Edward C. Saef), 804
Thyroidal Iodine-131 : Increase of the Rate of Release of, and of Circulating Thyrotrophic Activity at Early Stages of Propylthiouracil Treatment in the Rat (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171
Thyrotrophic Activity (Circulating) : Increase of the Rate of Release of Thyroidal Iodine-131 and of, at Early Stages of Propylthiouracil Treatment in the Rat (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171
Thyroxine and 2,4-Dinitrophenol : Effect of, on the Rate of Utilization of Glucose (Dr. L. Macho), 604
Thyroxine plus Thiourea : Effect of, on the Early Development of the Chum Salmon (*Oncorhynchus keta*), (Prof. M. A. Ali), 1214
Tice, Linwood F., and others (edited by) : Remington's Practice of Pharmacy, twelfth edition, *review* by Dr. J. H. Gaddum, 8
Tierra en Nicaragua : Estudios Ecologicos para el Aprovechamiento de la (Dr. B. W. Taylor), 1259
Tilak, B. D., and others : Anticancer Properties of Thio-diglycollic Acid, 388
Tilapia nigra and *Tilapia leucosticta* : A Natural Hybrid of, from Lake Naivasha, Kenya Colony (H. Y. Elder and D. J. Garrod), 722
Till, J. E. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Generalized Dose-dependent Prolongation of all three stages of the first Synthetic Cycle following Exposure), 341
Timber Engineering (University of Southampton first International Conference on), 338
Time of Antonio Vallisneri : Experimental Method in Biology from the, to the Present (University of Padua Symposium on), 546
Time of Generation of Living Cells : Estimation of (R. J. M. Fry, S. Lesher and H. I. Kohn), 290
Time (Heading), in a Bulk-Hybrid Population of Barley : Stabilizing Selection for (Dr. S. K. Jain), 1123
Time (Minimum), in Gas Chromatography : Role of Pressure Gradients in obtaining (Prof. J. Calvin Giddings), 1291
Time (Reaction), and Age ('Alpha' Rhythm) : Frequency of the (Dr. Walter W. Surwillo), 823
Time-integrated Measurements of Submarine Irradiance (Ronald Currie and Laurence Draper), 661
Times (Recovery) : A Method of Estimating Ionospheric Absorption Heights from (Syed Ziauddin), 1084
S.48 Timothy (*Phleum pratense* L.) : Effects of Light Intensity on Reproduction in (Dr. G. J. A. Ryle), 196
Tinley, G. H. : Effect of Ferric Dimethyldithiocarbamate on the Rooting of Cuttings of *Hevea brasiliensis*, 1217
Tipped Refuse : Pollution of Water by—Report of the Technical Committee on the Experimental Disposal of House Refuse in Wet and Dry Pits (Ministry of Housing and Local Government), 864
Tips (Refuse) : Pollution from (Nora H. Johnson), 864
Tissue (Human Aortic) : Degree of Polymerization of Hyaluronic Acid isolated from (Dr. Sv. Bertelsen and K. Marcker), 386
Tissue (Human Bone) : Blood-Typing Compact (H. E. Ezra-Cohn and Prof. S. F. Cook), 1267
Tissue (Insect Nerve) : Activity of Glutamic Acid Decarboxylase in (Dr. N. Frontali), 178
Tissue (Liver) : Separation and *in vitro* Culture of Cells from (Dr. Justine S. Garvey), 972
Tissue (Non-Photosynthetic Plant) : Triphosphopyridine Nucleotide-linked Glyceraldehyde Phosphate Dehydrogenase in a (C. R. Benedict and Dr. H. Beevers), 71
Tissue (Thyroid), *in vitro* : Stimulation of Lipogenesis in, by Free Fatty Acids (Prof. Norbert Freinkel, Dr. Augusto D. Litonjua and Edward C. Saef), 804
Tissue Cell Cultures : Immunofluorescence of Pleuropneumonia-like Organisms isolated from (Major W. F. Malizia, M. F. Barile and D. B. Riggs), 190
Tissue Cell Surfaces (Mammalian) : Sialic Acid as a Structural Component of some (Dr. Leonard Weiss), 1108
Tissue Dilution Artefact—a Re-interpretation of Variations in Levels of Succinic Dehydrogenase in Chemical Carcinogenesis (G. R. N. Jones, L. Bitensky, J. Chayen and Prof. G. J. Cunningham), 1203
Tissue Slice Respiration : Oxygen Electrode for Measurement of (E. H. Strickland, F. D. Ziegler and Prof. A. Anthony), 969
Tissue Thromboplastin : Influence of Serum Factors on the Species-specificity of (Christine Hawkey and J. L. Stafford), 920
Tissue Transplantation (University of Chile, and Societies of Santiago and Valparaiso, International Symposium on), 24
Tissue and Organ Cultures (Animal Cell,), in Radiobiology : Use of (New York Academy of Sciences Symposium on), 340
Tissues : Distribution of Sodium and Potassium in certain Cells and (Dr. Norman R. Joseph, Dr. Milton B. Engel and Dr. Hubert R. Catchpole), 1175
  Paper Electrophoresis of Proteins while Preserving the Pattern of their Localization in (Dr. Zh. A. Medvedev), 1021
Tissues of Early Embryos : Amino-Acid Activation in (Dr. Elizabeth M. Deuchar), 1006
Tissues (Cat) : Effects of Drugs on Uptake of Isotopic Norepinephrine by (Hans J. Dengler, Herbert E. Spiegel and Elwood O. Titus), 816
Tissues (Different) : Influence of Polyvalent Ions on Histones isolated from (I. P. S. Agrell and E. G. Christensson), 284
Tissues (Excitable) : Outflux of Inorganic and Organic Phosphate during Membrane Depolarization of (Dr. L. G. Abood, Prof. K. Koketsu and I. Koyama), 395
Tissues (Irradiated Normal) : Effect of, on the Growth of Mouse Carcinoma (Dr. Arthur Compton and Prof. Charles A. Pannett), 1101
Titanium : Infra-Red Spectroscopic Evidence of Four-fold Co-ordination of, in Barium Orthotitanate (P. Tarte), 1002
Titanium Ethoxide (Polymeric), and Titanium Oxide Ethoxides : Structures of (Prof. D. C. Bradley and A. H. Westlake), 273 ; (R. L. Martin and G. Winter), 274
Titanium Oxide Ethoxides : Structures of Polymeric Titanium Ethoxide and (Prof. D. C. Bradley and A. H. Westlake), 273 ; (R. L. Martin and G. Winter), 274
Titre (Antibody) : Rise of, in Cell-free Spleen Preparations Incubated *in vitro* (Dr. Dieter H. Sussdorf), 770
Titterton, Prof. E. W. : Future of Nuclear Physics in Australia, 639
Titus, Elwood O., and others : Effect of Drugs on Uptake of Isotopic Norepinephrine by Cat Tissues, 816
Tobacco Manufacturers' Standing Committee : Research Paper No. 4, Cigarette Smoke Condensate—Preparation and Routine Laboratory Estimation (H. R. Bentley and J. G. Burgan), second edition, 336
Tobacco Mosaic Virus (TMV) : Chromatography of, on Chitin Columns (P. M. Townsley), 626
Tobacco Mosaic Virus (TMV) Lesion Number and Concentration : Relationship of, to the Rate of Lesion Production on Pinto Bean (John Piacitelli and Prof. Vincent Santilli), 624
Tobacco Smoke : Natural Radioactivity in Tobacco and (Dr. V. C. Runeckles), 322
Tobacco and Tobacco Smoke : Natural Radioactivity in (Dr. V. C. Runeckles), 322
α-Tocopherol : Myopathy affecting the Rottnest Quokka (*Setonix brachyurus*) reversed by (B. A. Kakulas), 402
Todd, Sir Alexander : Inorganic Polymers, 1046
Todd, Dr. Audrey : Rigidity Factor of Gelatin Gels, 567
Todd, J. R., and Thompson, R. H. : Methaemoglobin in Chronic Copper Poisoning of Sheep, 89
Token (Type-) Mathematics, a Textbook of Mathematical Linguistics (Dr. Gustav Herdan), *review* by Dr. G. H. Toulmin, 211
Tokushige, A. M., and Cook, Dean E. S. : Application of the Nickel Reaction and Thermal Isomerization of Alpha-Keto-Acid Hydrazones for their Chromatographic Analysis, 797
Tolmach, L. J. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Radiation affects Cell Division much more than Cell Growth), 340
Tomasek, Dr. V., and others : Paper Chromatography (Film showing the Technique of Paper Chromatography of Amino-Acids), 759
Tombs (Megalithic), of France, *review* by Prof. C. F. C. Hawkes, 637
Tombs (Prehistoric Chamber), of France (Dr. Glyn Daniel), *review* by Prof. C. F. C. Hawkes, 637
Tomcsik, Prof. Josef : Elected a Member of the Hygiene Section of the Leopoldina German Academy of Sciences, 122
Tomcufcik, A. S., and others : N,N'-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds—a New Series of Anti-tumour Agents, 611
Tomkeieff, Dr. S. I. : English–Russian Geological Dictionary, *review*, 9
  Russian–English Geological Dictionary, *review*, 9
  Obituary of Dr. N. S. Shatsky, 542
  Obituary of Prof. A. F. Kapustinsky, 647
Tomlinson, Dr. Muriel L. : Awarded a Royal Society and Nuffield Foundation Commonwealth Bursary, 126
To-morrow : Hopes of, 1030
Toole, S., and others : Dependence on Substrate of the Electrical Potential across the Isolated Gut, 501
Tools (Prehistorical) : Eoliths and, 23
Toothcarp (Viviparous) : Pathological Parthenogenesis in a (Prof. A. Stolk), 507
Topology of Linear Neutron Kinetics (L. G. Kemeny), 263
Torpid Hummingbirds (Active, Sleeping and) : Thyroid Function in (Prof. C. J. Shellabarger, R. C. Lasiewski and G. E. Hyncik), 1318
Total Calories from Protein in the Milk : Correlation between Growth-Rate of the Suckling of various Species and the Percentage of (Dr. F. W. Bernhart), 358
Tóth, J., and others : Amino-derivatives of 11β, 17α, 21-Trihydroxy-3,20-Dioxo-1,4-Pregnadiene, 607
Toulmin, Dr. G. H. : Mathematics and Language, *review*, 211
Toulmin, Dr. Stephen, and Goodfield, June : The Fabric of the Heavens, *review* by Dr. M. A. Hoskin, 840
Touloukian, Y. S. (edited by) : Retrieval Guide to Thermophysical Properties Research Literature, Vol. 1 ; Book 1, Part A, Guide to Substance Classification and Numerical Codes ; Book 1, Part B, Dictionary of Synonyms and Trade Names ; Book 1, Part C, Directory of Substances ; Book 2, Classified Search Index ; Book 3, Master Bibliography, Author Index, review by Prof. A. R. Ubbelohde, 109
Tower, Donald B. (edited by Roscoe O. Brady and) : The Neurochemistry of Nucleotides and Amino-Acids—a Symposium of the Section on Neurochemistry, American Academy of Neurology, *review* by Dr. R. V. Coxon, 318
Townsend, Harry : Appointed to the University of London readership in economics at the London School of Economics and Political Science, 651
Townsley, P. M. : Chromatography of Tobacco Mosaic Virus (TMV) on Chitin Columns, 626
Toxicity of Monoamine Oxidase Inhibitor : Augmentation of, by Thyroid Feeding (Richard N. Carrier and Dr. Paul V. Buday), 1107
Toxicity (Ammonia) : Prevention of, by Amino-acids concerned in the Biosynthesis of Urea (Dr. Francesco Salvatore and Dr. Vincenzo Bocchini), 705
Toxicology of *Penicillium islandicum* (K. Uraguchi, T. Tatsuno, M. Tsukioka, Y. Sakai, F. Sakai, Y. Kobayahi, M. Saito, M. Enomoto and M. Miyake), 864
Toxin (A Selective), produced by *Periconia circinata* (R. P. Scheffer and R. B. Pringle), 912
Toyoura, Etsuko, and others : A Method for Isolating Bacteria capable of Producing 6-Aminopenicillanic Acid from Benzylpenicillin, 1122
Trabold, Dr. N. : Blood Groups in Infra-human Species (Blood Group Systems in the Dog), 653
Trace Analysis with a Rotating Hanging Mercury Drop (Use of the Rotating Hanging Mercury Drop in Analysis), (Dr. M. Van Swaay and R. S. Deelder), 241
Tracers (Radioactive), in Chemistry and Industry (Pascaline Daudel), translated by Dr. Ulli Eisner, *review* by Prof. J. R. Partington, 637
Trade in European Birds for Caging, 124
Trade Unions : The State and the (D. F. Macdonald), 1129
Tradition, Science and Practice in Dietetics (D. F. Hollingsworth), 1343
Traffic Control : Modern Methods of (Exhibition at the Science Museum, London, by the Westinghouse Brake and Signal Co., Ltd.), 1044
Training : Apprenticeship and (House of Commons Debate on), 737
Training of Biochemists (Biochemical Society Colloquium on), 24, 955
Training for Management in Britain, 1331

Training (Acoustical) : A Take-Over Bid in (Prof. E. J. Richards), 748
Trait (Primary Polydipsic), of an Inbred Strain of Mice : Effect of Hybridization on the (Dr. Emanuel Silverstein), 523
Trajectories : Space—a Symposium sponsored by the American Astronautical Society, the Advanced Research Projects Agency and Radiation Incorporated (edited by the Technical Staff, Research Division, Radiation Incorporated), *review* by D. G. King-Hele, 1334
Trajectories (Re-entry) : Space-Flight and (International Academy of Astronautics Symposium on), 960
trans(*cis* and) Dichloro- and Dibromo-ethylenes : Ionization Potentials of (Dr. J. Momigny), 1089
Trans-Equatorial Backscatter Observations of Magnetically Controlled Ionization (J. A. Thomas), 792
Transaminase (Rat Liver Glutamic-Oxalacetic) : Intracellular Distribution Pattern of (Dr. Herbert J. Eichel and Josef Bukovsky), 243
Transaminase Activity in the Cactus Plant : Diurnal Variation in (S. K. Mukerji, G. G. Sanwal and Prof. P. S. Krishnan), 516
Transfer of Calcium and Strontium across Biological Membranes (United States Atomic Energy Commission, United States National Institutes of Health, United States National Science Foundation and the New York State Veterinary College Conference on), 1250
Transfer of Glycerol across the Erythrocyte Membrane : Dimer Formation and the (Dr. W. D. Stein), 352
Transfer of Virulence in *Rhizobium trifolii* (Dr. Hans Ljunggren), 623
Transfer (Cross-modal), in Man (M. Cole, S. L. Chorover and Dr. E. Ettlinger), 1225
Transfer (Electron and Hydrogen) : Coupling of Phosphorylation to, by a Chemi-Osmotic Type of Mechanism (Dr. Peter Mitchell), 144
Transfer (Glucose), across the Membrane of the Red Blood Cell : Dimer Formation and (Dr. W. D. Stein), 1277
Transfer (Inter-Molecular Energy), in Photo-sensitized Oxidation-Reduction : Paths of (N. Takeyama), 1359
Transfer (Passive), of Contact Sensitivity to Picryl Chloride in Guinea Pigs, with Subcellular Material (Dr. J. L. Turk), 915
Transfer Function of a Biological Photoreceptor (Dr. Lawrence Stark and Dr. Howard T. Hermann), 1173
Transferins (Serum), in the Genus *Macaca*—Species Distribution of Nineteen Phenotypes (Prof. Morris Goodman and Emily Poulik), 1407
Transform (Laplace) : Inversion of a, arising from a Problem in Applied Chemical Kinetics (T. Wood), 589
Transformation of Cathodically Impregnated and Cooled Austenite (E. G. Ramachandran and C. Dasarathy), 998
Transformation in Plants of Sodium Dimethyldithiocarbamate into other Fungitoxic Compounds (H. M. Dekhuijzen), 198
Transformation of Tryptophan to Nicotinic Acid : Blockage of, by Experimental Hepatitis in Mice (M. Piazza, F. Tancredi and Prof. E. Quagliariello), 601
Transition : Universities in (Prof. H. C. Dent), 1027
Transition-Metal Chemistry, *review* by Prof. C. A. Coulson, 1233
  An Introduction to—Ligand-Field Theory (Dr. Leslie E. Orgel), *review* by Prof. C. A. Coulson, 1233
Transition-Metal Ions : The Theory of (Prof. J. S. Griffiths), *review* by Prof. C. A. Coulson, 1233
Transmission (Arthropod), of Micro-organisms (Dr. John Macleod), 885
Transmission (Cholinergic), in Fish Muscle (K. Krnjević), 1403
Transmission (Experimental), of *Borrelia anserina* (Sakharoff 1891) by *Aedes aegypti* (J. A. Roberts), 1225
Transmission (Sympathetic) : Lack of Evidence for a Cholinergic Mechanism in (Dr. J. E. Gardiner and Dr. J. W. Thompson), 86
Transmission (Synaptic), in Neurosecretory Cells (Hiromichi Morita, Takaaki Ishibashi and Satoru Yamashita), 183
Transpeptidase (*γ*-Glutamyl) : Histochemical Demonstration of (Prof. Z. Albert, Dr. Marian Orlowski and Dr. A. Szewczuk), 767
Transplantable Acute Myeloid Leukaemia : Effect of Calf Thymus Histone on Rats bearing (Dr. Lubomir Hnilica and Dr. Viktor Holoubek), 922
Transplantation : Tissue (University of Chile, and Societies of Santiago and Valparaiso, International Symposium on), 24
Transplantation of Murine Leukaemia by Unconventional Routes (Dr. Harold B. Hewitt), 1213
Transplants (Cell), of Avian Lymphomatosis treated with Hydrogen Peroxide (Dr. V. L. Sanger), 1406
Transport (Active Cation), as a Pacemaker of Respiration (Dr. R. Whittam), 603
Transport (Electron) : Quinones in (Ciba Foundation Symposium on), (edited by G. E. W. Wolstenholme and Cecilia M. O'Connor), *review* by A. I. Scott, 321
Transport (London) : Retirement of A. T. Wilford as director of research, 751
  Appointment of H. E. Styles as director of research, 751
Transport (Mediated Sugar) : Persistence in Erythrocyte Ghosts of (Paul G. LeFevre), 970
Transport (Sodium), in the Loop of Henle : Stimulation by Aldosterone of (Dr. J. Crabbé), 817
Transport (Sugar), in Human Red Cells : Common Carrier System for (Dr. L. Lacko and Dr. M. Burger), 881
Transport System (Electron), and Phosphoproteins of Mitochondria (K. Ahmed, J. D. Judah and C. H. Gallagher), 1309
Trauner, Prof. Richard : Elected a Member of the Stomatology Section of the Leopoldina German Academy of Sciences, 122
Trautwein, Prof. K. (Urbaschek, B., and) : Occurrence of an *Rh*-like Antigen in Red Cells of the Sheep, 193
Travel Scholarship : Bowles Memorial (Royal Horticultural Society), 1349
Travelling Disturbance in the *F* Region of the Ionosphere : Group and Phase Velocities of a (Dr. L. H. Heisler and Dr. J. D. Whitehead), 157
Travelling Wave Tubes, their Characteristics and Operation (English Electric Valve Co., Ltd.), 1348
Travis, D. N., and others : Rotational Analysis of Absorption Bands of Lead Monoxide, 374
Treating Asbestos Filter Pads for Virus Filtration : A Method of (N. St. G. Hyslop), 305
Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid—Effect of Spraying with Gibberellic Acid on Yield and Composition (R. Stevens, J. B. Roberts and I. H. Williams), 360 ; Effect on Growth and Development (I. H. Williams), 361
Treatment of Thrombocytopaenic Haemorrhage with Œstriol Succinate (Dr. Hubert Poliwoda), 400
Treatment of Varicose Veins (Yùtaka Kato and Robert A. Nabatoff), 335
Treatment (High-Pressure) : A New Phase formed by—Face-centred Cubic Molybdenum Monocarbide (E. V. Clougherty, K. H. Lothrod and J. A. Kafalas), 1194
Treatment (Plastifying) : Electrophoresis in a Thin Layer of Starch-Gel, followed by a (J. Groulade, Dr. J. M. Fine and C. Ollivier), 72
Treatment (Propylthiouracil), in the Rat : Increase of the Rate of Release of Thyroidal Iodine-131 and of Circulating Thyrotrophic Activity at Early Stages of (Dr. F. Escobar del Rey, G. Morreale de Escobar, M. D. García García and J. Mouriz García), 1171
Treatment and Examination (Water), Society for : Autumn Meeting, 856
Tree (Jamaican Shade), *Pithecolobium samath* Benth. : Pharmacology of the Alkaloidal Fraction from the Bark of the (B. E. Leonard and Dr. H. S. A. Sherratt), 287
Treherne, J. E. : Exchanges of Sodium Ions in the Central Nervous System of an Insect (*Periplaneta americana* L.), 1223
Treloar, Dr. L. R. G. : Awarded the Colwyn Medal of the Institution of the Rubber Industry, 755
Trémolières, Dr. J. : At the Third International Congress of Dietetics (Diet and Atherosclerosis), 1343
Trends (Limnological), in Lake Kariba (D. Harding), 119
Trends (New), in Atomic Research and their International Significance (Dr. L. Kowarski), 853
Trethowan, Prof. William Henry : Appointed to the chair of psychiatry in the University of Birmingham, 545
Trewavas, Dr. E. : Vertebrate Phylogeny, *review*, 945
Tri-*o*-Cresyl Phosphate Metabolite : Biological Activity of a (Prof. John E. Casida, Morifusa Eto and Ronald L. Baron), 1396
Triassic Sediment : Fossil Chlorins in a (Max Blumer and Gilbert Omenn), 161
Tribe (Chibcha Indian) : New *Rh* Phenotype, *Dcceieif*, found in a (Dr. Miguel Layrisse, Zulay Layrisse, Esperanza García, Dr. Johannes Wilbert and Joaquín Parra R.), 503
Tribolium confusum Duv. : Sterile Crosses between Mutations of (Prof. John Stanley), 934
Triethylenetetraamine-hexaacetic Acid: Removal of Internally Deposited Radionuclides by (Prof. A. Catsch and D. Schindewolf-Jordan), 715
Trifolium repens L. (Kent Wild White Clover) : Flower Initiation in, under Controlled Environmental Conditions (R. J. Haggar), 1120
11*β*, 17*α*, 21-Trihydroxy-3,20-Dioxo-1,4-Pregnadiene : Amino-derivatives of (J. Tóth, Z. Tuba and L. Szporny), 607
Trikojus, Prof. V. M. : Biochemistry at the University of Melbourne, 1238
Trilling, Prof. Leon : Incompressible Aerodynamics, *review*, 315
Trimethylsilyl Ether Derivatives of Alcohols : Peak-Shift Technique in Gas–Liquid Chromatography— (Stanley H. Langer and Peter Pantages), 141
Trinity College, Dublin : Appointment of Dr. D. C. Pepper to the newly established chair of physical chemistry, 333
Triphosphate (Adenosine) : Electron Spin Resonance of (D. B. Russell and S. J. Wyard), 65
Triphosphate (Adenosine) Synthesis in Erythrocytes : A Positive Feedback Mechanism of (Makoto Nakao, Takashi Motegi, Toshiko Nakao, Saburo Yamazoe and Haruhisa Yoshikawa), 283
Triphosphopyridine Nucleotide-linked Glyceraldehyde Phosphate Dehydrogenase in a Non-Photosynthetic Plant Tissue (C. R. Benedict and Dr. H. Beevers), 71
Triplet→Singlet Emission Spectra : Polarization of (V. G. Krishna and Prof. Lionel Goodman), 800
Trisaccharides (Two Serologically Active), isolated from Human Blood-Group *A* Substance (I. A. F. Lister Cheese and Prof. W. T. J. Morgan), 149
Triterpenoid Sapogenin (A New), of Animal Origin (Sea Cucumber), (Takao Matsuno and Toshihiko Yamanouchi), 75
Tritiated Pyrimidine-Nucleosides : Effect of Ribonuclease and Deoxyribonuclease on Incorporation of, into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture (Dr. L. E. Feinendegen, V. P. Bond and R. M. Drew), 1398
Trollope, Dr. D. H. : Effective Contact Stresses and Friction, 376
Trophoblasts during the Post-Partum Period : Antibodies to (J. F. Hulka, K. C. Hsu and S. M. Beiser), 510
Tropical Animal Ecology, Nuffield Unit of : Appointment of Dr. R. M. Laws as senior investigator, 651
Tropical Ecology, International Society for : Proceedings of the Symposium on Forest Ecosystem (Montreal, 1959), 755
Tropical Floras in the Tertiary in Southern England, *review* by Prof. John Walton, 209
Tropical Pathology : Techniques in (Prof. B. G. Maegraith, Prof. W. E. Kershaw and D. Dagnall), *review* by Prof. P. C. C. Garnham, 1236
Tropical Seeds : Dispersal of, by Ocean Currents (R. Mason), 408
Tropical West African Estuary : Seasonal Fluctuations and Distribution of Nitrite in a (J. C. D. Watts), 929
Tropical and Subtropical Coasts (Eastern), of the Americas: Marine Algae of the (Prof. William Randolph Taylor), *review* by Prof. Lily Newton, 1234
Trotman, C. G. : Design and Use of Respirators (History of Respirators), 655
Trotter, Dr. F. M. (Welch, Dr. F. B. A., and) : Geology of the Country around Monmouth and Chepstow (Memoirs of the Geological Survey of Great Britain—England and Wales), 227
Trotter, Dr. F. M. (Stubblefield, Dr. C. J., and) : Coal in Oxfordshire, 953
Trowell, O. A. : Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (Influence of various Gas Mixtures on the Action of Radiation on Lymphocytes from Rat Mesenteric Lymph Nodes), 340
Trucco, Ernesto (edited by Nicolas Rashevsky and) : Arms and Security, a Mathematical Study of the Causes and Origins of War (Dr. Lewis F. Richardson), *review* by Sir Cyril Burt, 427
Truelsen, T. A. : Growth Stimulation by High Indole-3-Acetic Acid Concentrations, 1410
Trump, H. J. : Appointed director of adult education in the University of Southampton, 443
Trypan Blue (Commercial) : Chromatographic Separation of Biologically Active Components from (J. Dijkstra and J. Gillman), 803
Trypanosoma rhodesiense : Cyclical Infection of *Glossina mortisans* with Culture Forms of (the late Prof. R. M. Gordon and J. K. Miller), 1317
Trypondendron (Beetle) : Air-Swallowing, a Mechanism in Photic Reversal of the (Prof. Kenneth Graham), 519

Trypsin : Interaction of Lipids and, in Dimethyl Sulphoxide–Water Systems (Prof. A. Lukton, H. Donohue and Prof. F. A. Bettelheim), 565
Tryptamine Receptors in the Central Nervous System (Dr. J. R. Vane, Dr. H. O. J. Collier and Dr. S. J. Corne, and Dr. E. Marley and Dr. P. B. Bradley), 1068
Tryptophan to Nicotinic Acid : Blockage of Transformation of, by Experimental Hepatitis in Mice (M. Piazza, F. Tancredi and Prof. E. Quagliariello), 601
Tryptophan-Pyrrolase : Effect of 5-Fluorouracil on Hepatic Induced Increase of (Sancia Gaetani and M. Antonietta Spadoni), 1296
Tsukioka, M., and others : Toxicology of *Penicillium islandicum*, 864
Tuba, Z., and others : Amino-derivatives of 11β, 17α, 21-Trihydroxy-3,20-Dioxo-1,4-Pregnadiene, 607
Tube (Elastic) : Fatigue in a Fluid-filled Pulsating (Group-Capt. John Erskine Malcolm), 1012
Tuber Bulking in the Potato Crop (R. W. Radley, M. A. Taha and P. M. Bremner), 782
Tuberculous and Non-tuberculous Conditions : Mycobacteriophages isolated from Persons with (Dr. E. Mankiewicz), 1416
Tubes : Travelling Wave—their Characteristics and Operation (English Electric Valve Co., Ltd.), 1348
Tubules (Malpighian) : Excretion of Inulin by (Dr. J. A. Ramsay and Dr. J. A. Riegel), 1115
Tubules (Single Perfused Proximal), of Rat Kidneys : Comparison of Short-Circuit Current and Net Water Movement in (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Tudor Times : Doctors and Disease in (W. S. C. Copeman), *review* by Sir Russell Brock, 428
Tuft, Dr. Peter : A Morphogenetic Effect of Beta-Mercaptoethanol—Distribution of Water in the Embryo, 1072
Tuft, Dr. Peter (Jurand, A., and) : A Morphogenetic Effect of Beta-Mercaptoethanol—Distribution of Water in the Chick Blastoderm, 1073
Tumor Research (Experimental) : Progress in (edited by F. Homburger), Vol. I, *review* by Prof. F. Bergel, 1231
Tumour (Ehrlich Mouse Ascites) : Effect of Alpha-Amylase on the (Dr. F. Steckerl, A. Ofodile, R. R. Campbell and G. H. Friedell), 1113
Tumour Cells (Human and Mouse) : Selective Uptake of Specifically Bound Cobalt-58 Vitamin B_{12} by (Dr. Bernard A. Cooper and Dr. William Paranchych), 393
Tumour Metabolism, *review* by Prof. F. Dickens, 1335
Tumour Research : Progress Reports and, *review* by Prof. F. Bergel, 1231
Tumour (Anti-) Agents : N,N'-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds—a New Series of (A. S. Tomcufcik, S. D. Willson, A. W. Vogel and A. Sloboda), 611
Tumours : The Glycolysis and Respiration of (Dr. Alan C. Aisenberg), *review* by Prof. F. Dickens, 1335
    Induction of, by Polyoma Virus—Enhancement by Chemical Agents (K. E. K. Rowson, F. J. C. Roe, J. K. Ball and Dr. M. H. Salaman), 893
Tumours (Induced Pulmonary), in Mice : Inheritance of Susceptibility to (Dr. D. S. Falconer and Dr. Joyce L. Bloom), 1070
Tumours (Pulmonary), and Group VII in the House-Mouse (Dr. Joyce L. Bloom), 1124 ; (Dr. J. A. H. Tatchell), 1125
Tunable Source of Radiation for the Far Infra-Red using Beats between Optical Masers : A Proposal for a (Dr. D. C. Lainé), 795
Tungsten and Nickel Complexes (Chromium, Uranium,), of Thioglycolic Acid (V. M. Bhuchar), 489
Turcan, Dr. J. R. : Appointed Research Fellow in the Department of Engineering Production, University of Birmingham, 337
Turco, A., and Pecile, C. : Co-ordination of the Thiocyanate Group in Inorganic Compounds, 66
Turk, Dr. J. L. : Passive Transfer of Contact Sensitivity to Picryl Chloride in Guinea Pigs, with Subcellular Material, 915
Turnbull, Prof. H. W. : *Obituary* by Prof. A. C. Aitken, 223
Turner, B. M. : Grant of £2,284 from the Paul Instrument Fund, for the further development of micro-scale chromatography and electrophoresis techniques, 225
Turner, J. H. : Appointed lecturer in pathology at the University of Edinburgh, 337
Turner, J. R. G., and others : Genetics of a Difference in the Male Genitalia of East and West African Stocks of *Papilio dardanus* (Lep.), 935
Turnip Yellow Mosaic Virus Protein Production : Stimulation of, by 2-Thiouracil (Dr. R. I. B. Francki and Dr. R. E. F. Matthews), 1078
Turtle : Cytochrome c in the Heart of the (Prof. Gunnar Biörck and Dr. Sven Paléus), 712
Turumi, Dr. Koh-Iti, and others : Hexosamine and Sialic Acid Contents in Cells, 596
Tutin, Prof. T. G. : Flora Europaea, 447
Twins (Monozygotic) : The Diego Factor in a Puerto Rican Family—a Case of Anti-Diego in (Dr. Hubert J. Van Peenen, Dr. John Scudder, James A. Jack and Erika Awer), 188
Two Hundred Precious Metal Years—a History of the Sheffield Smelting Company, Ltd., 1760-1960 (Ronald E. Wilson), *review* by Prof. R. W. K. Honeycombe, 108
Two Kinds of Arbor Virus Group *B* : Infectious Ribonucleic Acids derived from Mouse Brains infected with (Prof. Masahiro Nakamura), 624
Two Phycomycetes isolated from Subcutaneous Phycomycosis in Uganda (Dr. A. M. M. Wilson), 612
Two Sense-Modalities : Learning in (Dr. G. Ettlinger), 308
Two Serologically Active Trisaccharides isolated from Human Blood-Group *A* Substance (I. A. F. Lister Cheese and Prof. W. T. J. Morgan), 149
Two-Stage Method (A New), for Cross-linking Proteins (Dr. Felix Borek), 1293
Two Unexplored Records of Quaternary History (A. H. Stride and R. Bowers), 1000
Tymms, Pamela W., and others : A Relation between the Reactivity of Human Red Cells to Antiglobulin Tests and their Surface Structure, 923
Type (Different), of Fluorescent Material as an Optical Maser : Use of a (Jordan J. Markham and Dickron Mergerian), 586
Type-Token Mathematics, a Textbook of Mathematical Linguistics (Dr. Gustav Herdan), *review* by Dr. G. H. Toulmin, 211
Types of Bacteria (Numbers and), from the Rhizosphere of Pasture Plants—Possible Occurrence of *L*-Forms (G. Jagnow), 1220
Typing (Blood-) Compact Human Bone Tissue (H. E. Ezra-Cohn and Prof. S. F. Cook), 1267
Tyrosinase Competence in *Streptomyces scabies* : Apparent Cytoplasmic Inheritance of (Prof. K. F. Gregory and Wei-jen Shyu), 465
Tyrosine Residue (An Unreactive), in Insulin and the Exclusive Iodination of the *A* Chain (Dr. P. H. Springell), 1372
Tyrosol (Phenethyl Alcohol and) : Formation of, during Fermentation of a Synthetic Medium lacking Amino-acids (R. Stevens), 913
Tyulina, N. I., and others : A Soviet View of British Libraries (translated by A. Thompson), (Library Association, Pamphlet No. 22), 752

UBBELOHDE, Prof. A. R. : Graphite, 27
    The Retrieval of Published Information, *review*, 109
Uchida, Kohki, and others : Controlling Mechanism of the Mechanical Activity of Muscle and that of Blood Coagulation, 80
Uemura, Teijiro (Hayashibe, Masaya, and) : Release from the Feedback Inhibition controlling the Biosynthesis of Isoleucine, 1417
Uganda : Education in (Dr. B. E. Lawrence), 990
    Two Phycomycetes isolated from Subcutaneous Phycomycosis in (Dr. A. M. M. Wilson), 612
Ui, Prof. Nobuo, and others : Chromatographic Fractionation of Hog Thyroglobulin, 1199
Úlehla, Jiří : Correlative Inhibition of Formation of Chlorophyll in the Leaves of Etiolated Pea Plants, 613
U.H.V. and V.H.F. Television Surveys : A Mobile Laboratory for (E. W. Taylor and L. C. Munn), 1355
Ultrabasic Liquids (R. H. Clark and Prof. W. S. Fyfe), 158
Ultracentrifugal Differences in Soybean Protein Composition (W. J. Wolf, G. E. Babcock and A. K. Smith), 1395
Ultrasonic Absorption of Polymer Solutions (Dr. G. Gooberman), 693
Ultrasonic Decolorization of Rhodamine *B* : Inhibition of, in Presence of Organic Substances (Prof. Satya Prakash and Sheo Prakash), 1292
Ultrasonic Velocity in Aqueous Silver Nitrate Solution (D. S. Allam and W. H. Lee), 1388
Ultrasonic Velocity in Aqueous Solutions of some Electrolytes (M. G. Seshagiri Rao and Prof. R. Ramachandra Rao), 164
Ultrasonic Waves : Aniline Oxidation initiated by (Dr. Wiktor Wawrzyczek), 701
Ultrasonics : Crystallization Kinetics in Polyvinylchloride and Thermoelastic Relaxation induced by (Dr. V. Nardi), 563
Ultra-Thin Sections of Vitrinite in Coal : Electron Microscopic Observations of Structure in (J. T. McCartney, Sabri Ergun and R. E. Walline), 1361
Ultra-Violet : A Catalogue of the, *review* by Dr. E. G. Kellett, 107
Ultra-Violet (Far) : Irradiated Lithium Fluoride as an Optical Filter in the (J. L. Weeks, S. Gordon and G. M. A. C. Meaburn), 1186
Ultra-Violet Absorption Spectra of 3,4-Benzopyrene : Effect of Ozone on the Fluorescence and (Z. Morlin and K. M. Sáringer), 907
Ultra-Violet Absorption Spectra of Proteins in Deuterium Oxide (Dr. G. H. Beaven and Elizabeth Bailey), 914
Ultra-Violet Irradiation : Effect of, on the Electrical Conductivity of Cathodes coated with Barium Oxide (Dr. C. H. B. Mee), 1383
Unconventional Routes : Transplantation of Murine Leukaemia by (Dr. Harold B. Hewitt), 1213
Undercurrent (Eastward Equatorial), in the Atlantic : Evidence of an, from Measurements of Current Shear (Arthur D. Voorhis), 157
Underground Waters (Surface and): Uranium in British (J. D. Peacock), 1189
Underwater Acoustics : Advanced Study Institute on (Imperial College of Science and Technology, London), 229
Undiluted Semen : Effect of Varying Doses of, on Fertility in the Domestic Fowl (G. C. Taneja and R. S. Gowe), 828
Unesco : Science Study Fellowships, 1250
Unesco (Indian Society of Theoretical and Applied Mechanics and) : Symposium on Non-Linear Physical Problems, 855
Unexplored Records (Two), of Quaternary History (A. H. Stride and R. Bowers), 1000
Ungnade, Herbert E. (edited by) : Organic Electronic Spectral Data, Vol. 2, 1953-1955, *review* by Dr. E. G. Kellett, 107
Unified Europe : The Non-Ferrous Metals Industry in a (Baron Snoy et d'Oppuers), 546
Uniformity (Non-) of Cross-linking in Ion-Exchange Polymers (Dr. E. Glueckauf and R. E. Watts), 904
Unilateral Temporal Pole Damage in the 'Split-Brain' Monkey : Changes in Visual Gnostic Functions and Emotional Behaviour following (Dr. J. L. deC. Downer), 50
Unilever, Ltd., jointly with the Department of Economics of the University of Manchester : To set up a Centre for Business Research, 337
Unions (Trade) : The State and the (D. F. Macdonald), 1129
United Arab Republic, Government of the, and others : To set up and operate an Aeronautical Research Laboratory and three Civil Aviation Training Centres, 1043
United Kingdom : Population Statistics in the, 1347
    Shortage of Scientific Teachers in the, 123
    Strontium-90 in Milk and Agricultural Materials in the, 1959-1960 (Agricultural Research Council, Report No. 4), 20
United Kingdom, Committee of Vice-Chancellors and Principals of the Universities of the : Conference of professors of mathematics from the Universities of Great Britain, to consider the Teaching of Mathematics, 1145
United Kingdom Atomic Energy Authority : Progress on the Advanced Gas-cooled Reactor at Windscale, 853
    Seventh Annual Report (1960-61), 857
United Kingdom Atomic Energy Research Establishment : Course on the Principles of Radiation Protection, 856
United Kingdom Courses in Nuclear Energy, 125
United Kingdom Petroleum Industry Statistics relating to Consumption and Refinery Production, 1959 and 1960 (Petroleum Information Bureau), 123
United Nations : Demographic Yearbook for 1960, 1346
United Nations (Food and Agriculture Organization) : Forum in Tokyo on the Fishing Research Vessel, 229
    Meetings on Nutrition in Latin America, 959
    Appointment of Dr. Fabian Recalde as a nutrition officer, 1045
    International Conference on Fish in Nutrition, 1045
    Technical Meeting on Plant Exploration and Introduction, 1240

United Nations (Food and Agriculture Organization), and others : Conference on the Use of Radioisotopes in Animal Biology and the Medical Sciences, 959
United Nations (World Health Organization) : Technical Report Series No. 215, The Planning of Public Health Services—Fourth Report of the Expert Committee on Public Health Administration, 441
  Technical Report Series No. 193, Teacher Preparation for Health Education—Report of a joint WHO/Unesco Expert Committee, 441
  Technical Report Series No. 221, Scientific Meeting on Rehabilitation in Leprosy—Report, 1248
  Public Health Papers No. 9, Teaching of Psychiatry and Mental Health, 1248
  Public Health Papers No. 10, Control of Soil-transmitted Helminths (Prof. Paul C. Beaver), 1249
  WHO Chronicle, Vol. 15, No. 7, Health in Yugoslavia, 1260
  European Standards for Drinking Water, 1261
United Nations (World Health Organization), and others : Conference on the Use of Radioisotopes in Animal Biology and the Medical Sciences, 959
United States : Cactus Virus in the (the late Irene M. Sammons and Prof. M. Chessin), 517
  Chemical Composition of Precipitable Water Vapour over the (Dr. Henry M. Papée and Dr. Alberto Montefinale), 136
  Goals for Science in the, 312
  Improving Biological Communications in the, 23
  Water Resources of the (Prof. W. G. V. Balchin), 444
United States Air Force Cambridge Research Laboratories (Thermal Radiation Laboratory of the Geophysics Research Directorate) : Conference on Electrically Exploded Wires, 1250
United States Armed Forces : Technical Reports of the, 1347
United States Atomic Energy Commission, and others : Conference on Transfer of Calcium and Strontium across Biological Membranes, 1250
United States Department of Health, Education and Welfare : Public Health Service Publication No. 815, Highlights of Research Progress in General Medical Sciences (1960), 658
  Public Health Service Publication No. 854, Research Highlights in Ageing, 1145
United States National Academy of Sciences : Election of foreign associates, 18
United States National Academy of Sciences—National Research Council : Grant of 56,000 dollars from the National Science Foundation, for a one-year study of Chemical Notation Systems, 23
  Report on Science in Space, 124
  Sector-Focused Cyclotrons—Proceedings of an Informal Conference, Sea Island, Georgia, 1959 (edited by F. T. Howard), (Nuclear Science Series, Report No. 26, Publication No. 656), *review* by M. Snowden, 532
  Publication No. 848, Effects of Inhaled Radioactive Particles, 556
  Publication No. 849, Long-Term Effects of Ionizing Radiations from External Sources, 556
  Publication No. 875, Effects of Ionizing Radiation on the Human Haematopoietic System, 556
United States National Bureau of Standards : Handbook No. 78, Report of the International Commission on Radiological Units and Measurements, 28
  Appointment of Martin T. Decker as chief of the Tropospheric Measurements Section, 334
  Appointment of John L. Hague as chief of the Standard Reference Materials Section, 334
  Appointment of Dr. John K. Taylor as chief of the Applied Analytical Research Section of the Analytical and Inorganic Chemistry Division, 334
  Appointment of Dr. David M. Gates as editor of Section D, Radio Propagation, of the *Journal of Research*, 856
  Appointment of Dr. Harry C. Allen, jun., as chief of the Analytical and Inorganic Chemistry Division, 1346
  Monograph No. 19, Atomic Energy Levels in Crystals (J. L. Prather), 1348
United States National Bureau of Standards (Informations System Branch of the Office of Naval Research Information Centre) : Symposium on Optical Character Recognition, 1145
United States National Bureau of Standards (Research Information Centre and Advisory Service on Information Processing) : Report on Computer Systems for Searching Chemical Literature (E. C. Medam and H. R. Koller), 23
United States National Cancer Institute, and others (sponsored by) : Proceedings of a Conference on Biological Activities of Steroids in relation to Cancer (edited by Gregory Pincus and Erwin P. Vollmer), *review* by E. Reid, 1235
United States National Institutes of Health, and others : Conference on Transfer of Calcium and Strontium across Biological Membranes, 1250
United States National Museum : Bulletin No. 225, Drug Supplies during the American Revolution (George B. Griffenhagen), 761
United States National Research Council (National Academy of Sciences) : Grant of 56,000 dollars from the National Science Foundation, for a one-year study of Chemical Notation Systems, 23
  Report on Science in Space, 124
  Sector-Focused Cyclotrons—Proceedings of an Informal Conference, Sea Island, Georgia, 1959 (edited by F. T. Howard), (Nuclear Science Series, Report No. 26, Publication No. 656), *review* by M. Snowden, 532
  Publication No. 848, Effects of Inhaled Radioactive Particles, 556
  Publication No. 849, Long-Term Effects of Ionizing Radiations from External Sources, 556
  Publication No. 875, Effects of Ionizing Radiation on the Human Haematopoietic System, 556
United States National Science Foundation : Grant of 56,000 dollars to the National Academy of Sciences—National Research Council, for a one-year study of Chemical Notation Systems, 23
  Establishment of an Office of Antarctic Programs, 125
  Appointment of Dr. Thomas O. Jones as head of the Office of Antarctic Programs, 125
  Reviews of Data on Research and Development, No. 27, Report on Scientists and Engineers engaged in research and development in Colleges and Universities in the United States for 1958, 225
  Reviews of Data on Research and Development, No. 28, Capital Expenditure of Colleges and Universities in the United States for research and development in the Natural and Social Sciences in 1958, 545
  Science Information Personnel—the New Profession of Information combining Science, Librarianship and Foreign Languages (Leonard Cohen and Kenneth Craven), 452
United States National Science Foundation, and others : Conference on Transfer of Calcium and Strontium across Biological Membranes, 1250
United States Naval Observatory : Reprint No. 19, Resources and Treasures of the Library (Marjorie S. Clopine), 22
United States Nuclear Power Projects : Fact Sheets on, third edition, 958
United States Office of Naval Research (Information Systems Branch) : Symposium on Redundancy Techniques for Computing Systems, 1349
United States Public Health Service : Research Grants in Radiological Health, 442
United Steel Companies, Ltd. : Appointment of David Owen as head of the Department of Operational Research and Cybernetics, 443
Units (Sub-) : Kinetics of the Enzymic Degradation of Deoxyribonucleic Acid into (Dr. Giorgio Bernardi and Prof. Charles Sadron), 809
Units and Measurements (Radiological) : Report of the International Commission on (United States National Bureau of Standards, Handbook No. 78), 28
Universal Buffer System : A New (Dr. D. A. Ellis), 1099
Universe (The Steady), with Charge Excess (Ll. G. Chambers), 262
Universe (An Unsteady), with Excess of Charge (Ll. G. Chambers), 1082
Universities in Britain, 1027
Universities of the British Commonwealth, Association of : Commonwealth Universities Yearbook 1961, *review*, 843
Universities in Transition (Prof. H. C. Dent), 1027
Universities of the United Kingdom, Committee of Vice-Chancellors and Principals of the : Conference of professors of mathematics from the Universities of Great Britain, to consider the Teaching of Mathematics, 1145
Universities (British) : Scientific Research in, 1960-61 (Department of Scientific and Industrial Research), 228
Universities (Colleges and), in the United States : Report on Scientists and Engineers engaged in research and development in, for 1958 (United States National Science Foundation), 225
  Capital Expenditure of, for research and development in the Natural and Social Sciences in 1958, 545
Universities Council for Adult Education : Report for 1959-60, 861
Universities Federation for Animal Welfare : Annual Summer Congress, 755
  Symposium on the Assessment of Pain, 1353
Universities Yearbook : Commonwealth, 1961 (Association of Universities of the British Commonwealth), *review*, 843
University of Arizona : Lunar and Planet Laboratory Contributions, No. 1, Orthographic Atlas of the Moon (compiled by D. W. G. Arthur and E. A. Whitaker ; edited by Gerard P. Kniper), Edition 'A' showing the Standard Orthographic Coordinate Grid, Part 1, Central Area, *review* by D. H. P. Jones, 424
University of Baghdad : Grant of £7,500 from the Gulbenkian Foundation, for the Faculty of Engineering, 754
University of Birmingham : Appointment of Dr. L. Riddiford as senior lecturer in the Department of Physics, 337
  Appointment of G. Martelli as lecturer in the Department of Physics, 337
  Appointment of Dr. S. Swierszczewski as Research Fellow in nuclear physics, 337
  Appointment of Dr. J. H. Broadhurst as senior research associate in nuclear physics, 337
  Appointment of D. E. Kidd as research associate in nuclear physics, 337
  Appointment of Dr. C. A. Barson and Dr. A. E. Pedler as senior Research Fellows in the Department of Chemistry, 337
  Appointment of R. J. Heitzmann as Research Fellow in the Department of Chemistry, 337
  Appointment of R. G. Bird as lecturer in the Department of Civil Engineering, 337
  Appointment of B. K. Gazey as Research Fellow in the Department of Electrical Engineering, 337
  Appointment of Dr. B. Henderson as Research Fellow in the Department of Physical Metallurgy, 337
  Appointment of G. R. Ogram and B. Walker as Research Fellows in the Department of Industrial Metallurgy, 337
  Appointment of Dr. A. T. Kolnai as Lloyd-Muirhead Fellow in social philosophy, 337
  Appointment of J. R. Turcan as Research Fellow in the Department of Engineering Production, 337
  Appointment of Dr. B. J. Key as lecturer in neuropharmacology, 337
  Appointment of Dr. R. R. Dils as lecturer in the Department of Medical Biochemistry and Pharmacology, 337
  Appointment of D. Rimmer as lecturer in the Department of Economics, 337
  Appointment of Prof. William Henry Trethowan to the chair of psychiatry, 545
  Gulbenkian Foundation Conference on the Biology Syllabus for Sixth Forms, 554
  Appointment of J. T. Allanson to the second chair of electrical engineering, 647
University of Bristol : Long Ashton Research Station, Bristol (R. W. Marsh), 547
  Third International Conference on Neurosecretion, 856
University of Cambridge : Grant of £150 for three years from the Gulbenkian Foundation, in aid of the Arctic Canada expedition, 754
  Grant of £100 for three years from the Gulbenkian Foundation, in aid of the expedition to Nagir, Karakoram, 754
  Grant of £50,000 from the Gulbenkian Foundation, for three fellowships at Churchill College, 855
  Grant of £5,500 from the Gulbenkian Foundation, to the Pepys Library, Magdalene College, for preparation and publication of a catalogue, 855
  Election of Dr. A. Hewish to a Fellowship in Churchill College, on appointment as College lecturer in physics, 856
  Election of J. A. Miller and R. M. Loynes to Junior Research Fellowships in Churchill College, 856

  Election of N. W. Ashcroft, S. J. Bernau, G. R. G. Lewison, D. McCormack Smyth and W. K. Stell to Research Studentships in Churchill College, 856
  Election of S. Chatterjee and E. A. Kohll to Postgraduate Studentships in Churchill College, 856
University of Chile, and others : International Symposium on Tissue Transplantation, 24
University of Dublin : Appointment of Dr. D. C. Pepper to the newly established chair of physical chemistry in Trinity College, Dublin, 333
University of Durham : Fifteenth Report of the Appointments Board, 649
  Establishment by the Medical Research Council of a Research Group on Demyelinating Diseases, 1143
University of East Anglia : Appointment of F. Thistlethwaite as first vice-chancellor, 959
University of Edinburgh : Appointment of J. H. Turner as lecturer in pathology, 337
  Appointment of Dr. I. Bihler, Dr. J. Newham, C. McGreavy, Herman Wen-Geun Chew and Dr. A. S. Milton as I.C.I. Research Fellows, 337
  Appointment of Dr. Eric Samuel as reader in radiodiagnosis, 443
  Appointment of Dr. H. M. Adam as reader in pharmacology, 443
  Appointment of Dr. J. S. Robson as reader in therapeutics, 443
  Appointment of Dr. N. A. Mitchison as reader in zoology, 443
  Appointment of Dr. P. P. Brown as lecturer in bacteriology, 443
  Appointment of Dr. W. P. Doyle and Dr. J. L. Holmes as lecturers in chemistry, 443
  Anonymous gift of £1,000 to the Department of Radiotherapy, 443
  Appointment of Juris Reinfelds and Imre Leszlo as I.C.I. Research Fellows, 545
  Appointment of R. F. Thow to a lectureship in agriculture, 545
  Appointment of Dr. L. M. Muggleton to a lectureship in electrical engineering, 545
  Appointment of Benjamin Mitchell to a lectureship in veterinary surgery, 545
University of Glasgow : Appointment of Dr. John Lamb to the James Watt chair of electrical engineering, 224
  Appointment of Dr. David W. A. Sharp as senior lecturer in the Department of Chemistry in the Royal College of Science and Technology, 443
  Appointment of Dr. Graham R. Knox as lecturer in the Department of Chemistry in the Royal College of Science and Technology, 443
  Appointment of Dr. T. R. F. Nonweiler to the Mechan chair of aeronautics and fluid mechanics, 853
  Endowment of £800 made by Mrs. Hilda M. Beilby to the Royal College of Science and Technology, Glasgow, to be known as the Kelvin Prizes, 1349
University of Hamburg : European Symposium on Vitamin B_{12} and Intrinsic Factor, 1154
University of Leeds : Appointment of Dr. J. W. Craggs to the newly created chair of mathematics, 122
  Grant of £16,600 a year for seven years from the Foundation for Management Education, 228
  Appointment of Dr. A. Chadwick to a lectureship in zoology, 228
  Appointment of Dr. B. L. Shaw to a lectureship in inorganic and structural chemistry, 228
  Appointment of F. P. G. Whitaker as director of management studies in the Department of Economics and Commerce, 228
  New Courses leading to honours and ordinary B.Sc. degrees in Food Science, 546
  Appointment of Dr. P. Grosberg to a research chair of textile engineering, 650
  Appointment of Dr. P. R. Brook and Dr. G. A. Morrison as lecturers in the Department of Organic Chemistry, 650
  Appointment of R. S. Davidson as Brotherton Research Fellow in the Department of Organic Chemistry, 650
  Appointment of Dr. I. G. Gass as a lecturer in the Department of Geology, 650
  Appointment of W. Houghton-Evans as lecturer in architectural engineering, 650
  Appointment of M. Kirk as a lecturer in the Department of Social Studies, 650
  Appointment of P. Levi as Bernard Hickson Fellow in the Department of Botany, 650
  Appointment of Dr. G. A. Salmon as J. W. Wootton Research Fellow in the Department of Chemistry, 650
  Appointment of Dr. Jean E. Wilkinson as Medical Research Council Research Fellow in the Department of Medicine, 650
  Grant of £1,000 from the Yorkshire Council of the British Empire Cancer Campaign to the Department of Physical Chemistry, 650
  Grant of £15,000 a year for seven years from the Anglo-American Corporation of South Africa, Ltd., to continue the work of the Research Institute of African Geology, 650
  Grant of £13,362 during two years from the Department of Scientific and Industrial Research to the Department of Geology, for an investigation into African rocks, 650
  Grant of £1,000 from Courtaulds, Ltd. to the Department of Textile Industries, 650
  Grant of £1,000 during two years from W. L. Ingle, Ltd. to the Procter Department of Food and Leather Science, 650
  Grant of £2,470 from the Ministry of Aviation for a research project in the Department of Metallurgy, Houldsworth School of Applied Science, 650
  Grant of £2,897 during two years from the Department of Scientific and Industrial Research, for a research project in the Department of Biochemistry, 650
  Appointment of Dr. P. Grosberg to the chair of textile engineering, 1346
University of Leicester : Grant of £200 for three years from the Gulbenkian Foundation, in aid of the expedition to East Greenland, 754
University of Liverpool : Appointment of Dr. A. S. King as professor of veterinary anatomy, 648
University of London : Appointment of Dr. J. S. Webb to the University chair of applied geochemistry at the Imperial College of Science and Technology, 229
  Appointment of Dr. F. G. A. Stone to the University readership in inorganic chemistry at Queen Mary College, 229
  Title of reader in biology in the University (at the Royal Free Hospital School of Medicine) conferred on Dr. Agnes M. Hughes, 229
  Appointment of Prof. J. N. P. Davies to the University readership in morbid anatomy at the Postgraduate Medical School of London, 229
  Appointment of H. D. Ritchie to the University readership in surgery at the London Hospital Medical College, 229
  Title of reader in dental anatomy in the University (at the London Hospital Medical College) conferred on R. W. Fearnhead, 229
  Title of reader in organic chemistry in the University (at Royal Holloway College) conferred on Dr. T. G. Bonner, 229
  Advanced Study Institute on Underwater Acoustics, at the Imperial College of Science and Technology, 229
  Appointment of Dr. M. Gordon to the University readership in physical chemistry at the Imperial College of Science and Technology, 337
  Title of reader in demography in the University (at the London School of Economics and Political Science) conferred on J. Hajnal, 337
  The Chester Beatty Research Institute of the Institute of Cancer Research, Royal Cancer Hospital (Prof. A. Haddow), 430
  Appointment of Dr. C. B. Alcock as reader in chemical metallurgy at the Imperial College of Science and Technology, 443, 1349
  Appointment of Dr. J. N. Hunt as reader in physical oceanography at the Imperial College of Science and Technology, 443, 1349
  Appointment of Dr. J. A. Kitchener as reader in the science of mineral processing at the Imperial College of Science and Technology, 443, 1349
  Appointment of S. B. Watkins to the University readership in chemical engineering at King's College, 546
  Title of reader in pure mathematics (at Queen Mary College) conferred on Dr. K. W. Gruenberg, 546, 651
  Title of professor emeritus of geography (at the London School of Economics and Political Science) conferred on Prof. R. O. Buchanan, 546
  Title of professor emeritus of bacteriology (at St. Bartholomew's Hospital Medical College) conferred on Prof. L. P. Garrod, 546
  Title of professor emeritus of medical statistics (at the London School of Hygiene and Tropical Medicine) conferred on Sir Austin Bradford Hill, 546
  Title of professor emeritus of chemistry (at University College) conferred on Sir Christopher Ingold, 546
  Title of professor emeritus of pharmaceutical chemistry (at the School of Pharmacy) conferred on Prof. W. H. Linnell, 546
  Title of professor emeritus of psychology (at Birkbeck College) conferred on Prof. C. A. Mace, 546
  Title of professor emeritus of chemical engineering (at the Imperial College of Science and Technology) conferred on Prof. D. M. Newitt, 546
  Title of professor emeritus of pharmacology (at University College) conferred on Prof. F. R. Winton, 546
  Appointment of Prof. W. D. Gill to the chair of oil technology at the Imperial College of Science and Technology, 651
  Appointment of S. B. Watkins to the University readership in chemical engineering at King's College, 651
  Appointment of Dr. Brian Abel-Smith to the University readership in social administration at the London School of Economics and Political Science, 651
  Appointment of L. P. Foldes to the University readership in economics at the London School of Economics and Political Science, 651
  Appointment of Harry Townsend to the University readership in economics at the London School of Economics and Political Science, 651
  Title of professor of civil engineering (at King's College) conferred on J. K. T. L. Nash, 651
  Title of professor of powder technology (at King's College) conferred on Dr. H. E. Rose, 651
  Title of reader in economics (at the London School of Economics and Political Science) conferred on J. Wiseman, 651
  Title of reader in engineering science (at King's College) conferred on Dr. J. M. Zarek, 651
  Grant of £15,000 from the Gulbenkian Foundation to the School of Oriental and African Studies, 754
  Grant of £10,000 from the Gulbenkian Foundation, to establish a Gulbenkian Fund at King's College, 855
  Fourteenth International Instruction Course at the Imperial College of Science and Technology by Carl Zeiss, Jena, on Fine Measuring Instruments, 1045
  Appointment of James Durbin to the University chair of statistics at the London School of Economics and Political Science, 1247
  Appointment of J. P. Quilliam to the chair of pharmacology at St. Bartholomew's Hospital Medical College, 1349
  Appointment of Dr. S. J. Taylor to the University readership in mathematics at Westfield College, 1349
  Title of professor of electrical engineering in the University (at the Imperial College of Science and Technology) conferred on Dr. J. H. Westcott, 1349
  Appointment of Dr. J. R. Butler to the readership in pure geochemistry at the Imperial College of Science and Technology, 1349
  Appointment of J. C. G. Coleridge to the readership in physiology at the Royal Free Hospital School of Medicine, 1349
  Appointment of Prof. J. N. Phillips to the readership in morbid anatomy at the Postgraduate Medical School of London, 1349
University of London School of Pharmacy : Title of professor emeritus of pharmaceutical chemistry conferred on Prof. W. H. Linnell, 546
University of Manchester : Appointment of Dr. F. Koenigsberger to the newly created chair of machine tool engineering, 19
  Unilever, Ltd., jointly with the Department of Economics, to set up a Centre for Business Research, 337
  Appointment of R. H. Kantorowich as professor of town and country planning, 443
  Appointment of Dr. F. Ursell to the Beyer professorship of applied mathematics, 647
  Appointment of Ronald Beresford Dew as visiting professor of industrial administration at the Manchester College of Science and Technology, 651
University of Melbourne : Biochemistry at the (Prof. V. M. Trikojus), 1238
University of Michigan : Studies, Scientific Series Vol. 21, Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas (Prof. William Randolph Taylor), *review* by Prof. Lily Newton, 1234

University of Nottingham : International Symposium on Inorganic Polymers, 1046
  Proceedings of the Seventh Easter School in Agricultural Science—Digestive Physiology and Nutrition of the Ruminant (edited by Dr. D. Lewis), *review* by Dr. I. W. McDonald, 1334
University of Oporto : Grant from the Gulbenkian Foundation, in aid of biochemistry, 754
University of Oxford : First award of the J. H. Burn fellowship (in the Department of Pharmacology) for 1961-62 made to H. P. Rang, 229
  Grant of £100 for three years from the Gulbenkian Foundation, in aid of the expedition to the Andes of Peru, 754
  Grant of £100 for three years from the Gulbenkian Foundation, in aid of the expedition to the Atlas Mountains of Southern Morocco, 754
  Grant of £100 for three years from the Gulbenkian Foundation, in aid of the expedition to the South Caspian, 754
  Grant of £50 for three years from the Gulbenkiah Foundation, in aid of the women's expedition to the Madeiras, 754
  Grant of £22,000 from the Gulbenkian Foundation, for lecture rooms in the New Law Library, 855
  Retirement of Sir Wilfrid Le Gros Clark from the chair of anatomy, 957
  Appointment of Prof. G. W. Harris to the chair of anatomy, 957
University of Padua : Symposium on the Experimental Method in Biology from the Time of Antonio Vallisneri to the Present, 546
University of Reading : Annual Report for 1960 of the National Institute for Research in Dairying, 237
  Annual Report for 1960 of the Museum of English Rural Life, 544
University of Roorkee : Theoretical and Applied Mechanics at the, 855
University of St. Andrews (Mathematics Department of Queen's College), in collaboration with Standard Telephones and Cables, Ltd. : Symposium on Digital Computers, their Use and Control, 554
University of Sheffield : Appointment of Dr. L. Mirsky to a readership in pure mathematics, 229
  Appointment of Dr. V. R. Pickles to a readership in physiology, 229
  Appointment of Dr. F. A. Benson to a readership in electronics, 229
  Appointment of G. Lederer as a lecturer in pure mathematics, 229
  Appointment of Dr. P. B. N. Neal as senior Research Fellow in mechanical engineering, 229
  Appointment of A. B. Hedley to the Central Electricity Authority research fellowship in fuel technology, 229
  Appointment of Dr. N. H. March to the newly created second chair of physics, 337, 543
  Appointment of Dr. V. C. L. Hutson as lecturer in applied mathematics, 337
  Appointment of J. W. Ross as junior Research Fellow in physics, 337
  Appointment of Dr. I. Hawkes as lecturer in the Postgraduate School of Mining, 337
  Appointment of Dr. C. J. Ball as lecturer in metallurgy, 337
  Appointment of I. Livingstone as lecturer in economic statistics, 337
  Appointment of P. E. Brown as senior lecturer in preventive medicine and public health, 337
  Appointment of W. T. Baker as health physicist, 337
  Contributions to the Development Fund now total £480,975, 651
University of Southampton : First International Conference on Timber Engineering, 338
  Appointment of B. M. Bland to the University librarianship, 443
  Appointment of H. J. Trump as director of adult education, 443
  Appointment of Dr. J. P. Jones to the Westland readership in helicopter engineering, 443
  Title of reader in chemistry conferred on Dr. I. G. M. Campbell, 443
University of Sussex : Appointment of Dr. C. Eaborn as professor of chemistry, 1349
  Appointment of Dr. R. J. Blin-Stoyle as professor of theoretical physics, 1349
  Appointment of Dr. K. F. Smith as professor of experimental physics, 1349
University of Wales : Retirement of Prof. V. C. Morton from the chair of pure mathematics in the University College of Wales, Aberystwyth, 333
  Appointment of Dr. W. B. Pennington to the chair of pure mathematics in the University College of Wales, Aberystwyth, 333
  Retirement of Prof. E. J. Roberts from the chair of agriculture in University College of North Wales, Bangor, 439
  Appointment of Ian A. McK. Lucas to the chair of agriculture in University College of North Wales, Bangor, 439
  Grant of £200 for three years from the Gulbenkian Foundation, in aid of the expedition to Mount Sabalan, 754
University (Australian National), Canberra : Hon.D.Sc. degree awarded to Sir Rudolph Peters and to Dr. J. L. Pawsey, 443
University (Hebrew) : Seminar at the Medical School on Biorheology and Microcirculation, 757
University (National), of Ireland : Appointment of Dr. Eva M. Philbin to the chair of organic chemistry in University College, Dublin, 439
University College, Dublin : Appointment of Dr. Eva M. Philbin to the chair of organic chemistry, 439
University College, London : Title of professor emeritus of chemistry conferred on Sir Christopher Ingold, 546
  Title of professor emeritus of pharmacology conferred on Prof. F. R. Winton, 546
University College of North Staffordshire : To become Keele University in 1962, 229
  Appointment of H. Liebeck to a lectureship in mathematics, 229
  Appointment of Dr. M. Asgar Ali to an I.C.I. research fellowship, 229
  Appointment of M. J. A. Smith to the Mullard Research Fellowship in physics, 229
  Appointment of Dr. W. P. McCann to be Leverhulme research officer, 229
University College of North Wales, Bangor : Retirement of Prof. E. J. Roberts from the chair of agriculture, 439
  Appointment of Ian A. McK. Lucas to the chair of agriculture, 439
University College of Rhodesia and Nyasaland : Grant of £5,000 from the Gulbenkian Foundation, for an archaeological laboratory, 855
University College of Wales, Aberystwyth : Retirement of Prof. V. C. Morton from the chair of pure mathematics, 333
  Appointment of Dr. W. B. Pennington to the chair of pure mathematics, 333
University Extra-Mural Libraries in Great Britain : Aims, Organization and Methods of (E. P. Pritchard), 649
University Selection, *review* by Dr. W. H. King, 947
University Service : The World (Hilda M. Browne), 1140
Unreactive Tyrosine Residue in Insulin and the Exclusive Iodination of the A Chain (Dr. P. H. Springell), 1372
Unsteady Universe with Excess of Charge : An (Ll. G. Chambers), 1082
Unsuckled Female Rats (Suckled and) : Induction of Cancer in (Prof. Giuliano Briziarelli), 921
Unusual Base Composition of the Ribonucleic Acid of Wild Cucumber Mosaic Virus (H. Yamazaki and Prof. Paul Kaesberg), 96
Upper Arm Bone (Australopithecine) : An Australopithecine Scoop made from a Right (Prof. Raymond A. Dart), 372
Upper Atmosphere : Density of the, determined from Satellite Orbits, and its Variation during 1957-61 (D. G. King-Hele and D. M. C. Walker), 114
Upper Plastic Limit of Clays : Role of Interparticle Forces in Development of (Dr. Raymond Yong and E. T. Selig), 938
Uptake of Isotopic Norepinephrine by Cat Tissues : Effects of Drugs on (Hans J. Dengler, Herbert E. Spiegel and Elwood O. Titus), 816
Uptake of Streptomycin by *Escherichia coli* : Influence of Salts on the (Paul H. Plotz, Donald T. Dubin and Bernard D. Davis), 1324
Uptake (Selective), of Specifically Bound Cobalt-58 Vitamin B_{12} by Human and Mouse Tumour Cells (Dr. Bernard A. Cooper and Dr. William Paranchych), 393
Uracil (6-Aza-) : Chromosomal Elimination induced by (M. Collette Mahoney, Dr. E. R. Witkus and Dr. C. A. Berger), 627
Uraemia (Acute Experimental) : Elimination of Bovine Serum Albumin in (Dr. G. Gowland, F. G. Smiddy, Dr. R. G. Burwell and F. M. Parsons), 1210
Uraguchi, K., and others : Toxicology of *Penicillium islandicum*, 864
Uranin Solution : Average Extinction Coefficient for Re-absorption of Fluorescence Radiation through (Dr. K. K. Rohatgi and G. S. Singhal), 799
Uranium in British Surface and Underground Waters (J. D. Peacock), 1189
Uranium as Pyrophosphate : Determination of (Dr. F. M. Brewer), 1290 ; (J. S. Wright, T. J. Hayes and J. A. Ryan), 1291
Uranium, Tungsten and Nickel Complexes (Chromium,), of Thioglycolic Acid (V. M. Bhuchar), 489
Uranium Disilicide (Allan Brown and J. J. Norreys), 61
Urbaschek, B., and Trautwein, Prof. K. : Occurrence of an *Rh*-like Antigen in Red Cells of the Sheep, 193
Urea : Phosphate-dependent Degradation of (R. C. Valentine and Prof. R. S. Wolfe), 925
  Prevention of Ammonia Toxicity by Amino-acids concerned in the Biosynthesis of (Dr. Francesco Salvatore and Dr. Vincenzo Bocchini), 705
Urea Cycle in Rat Liver : Effect of Adrenalectomy on the Activity of Enzymes of the (Patricia McLean), 1302
Urea Synthesis in the Living Rat Brain (R. K. Davies, A. J. Defalco, D. Shander, A. Kopelman and Prof. J. Kiyasu), 288
Urea and Thiourea : Adducts of, obtained by means of a Sublimation Technique (B. Casu), 802
Uresch, F., and others : Chemical Character of the Pigments of a New Nitrogen-fixing Micro-organism, 1413
Urgency of International Co-operation, 943
Uri, J., and Herpay, Zs. : Use of Desertomycin Medium in Routine Selective Isolation of Fungi Pathogenic to Man, 1015
Uri, J., and Sztaricskai, F. : Simple, Quick Methods for Detection of 6-Aminopenicillanic Acid, 1223
^{14}C-Uridine : Catalysis of Incorporation of, into Rat Lens Ribonucleic Acid by Rat Lens Enzyme System (Dr. Anima Devi and Dr. N. K. Sarkar), 1094
Urinary Assay : Assessment of Chronic Exposure to Radiostrontium by (Dr. C. L. Comar and Jay Georgi), 390
Urinary Concretions forming on Zinc Implants in Rat Bladders : Incorporation of Phosphorus-32 into (Dr. Richard F. Keeler and Dr. Stuart A. Lovelace), 715
Urinary Excretion of Histamine : Relationship between the Decrease in, and the Initiation of the Labour Process (Dr. Halina Wiskont-Buczkowska), 290
Urinary Excretion (Decreased), of 2-Aminoethanol by Irradiated Human Beings (Dr. Ralph R. Cavalieri, M. T. Van Metre and W. I. Sivertsen), 1303
Urinary Inhibitors of Hyaluronidase (P. J. Knudsen and J. Koefoed), 1306
Urinary Metabolites of ^{14}C-Tetrachloroethylene in Mice (Dr. Sven Yllner), 820
Urine : Determination of Lead in, by Atomic Absorption Spectroscopy (J. B. Willis), 381
Urine (Normal Human) : Quantitative Determination of Metadrenaline and Normetadrenaline in (Dr. Kaoru Yoshinaga, Dr. Chuichi Itoh, Dr. Nozomu Ishida, Dr. Tatsuo Sato and Dr. Yasuo Wada), 599
Urine (Porcine) : Neutral 17-Ketosteroids in (L. G. Huis in't Veld, B. Louwerens and W. Reilingh), 175
Urine Analysis : Determination of Caesium-137/Potassium Ratios in Diet and in the Human Body by (A. Morgan and Gloria M. Arkell), 1100
Urine Nitrogen in Kwashiorkor : Partition of (Dr. J. C. Edozien and E. J. Phillips), 47
Ursell, Dr. F. : Appointed to the Beyer professorship of applied mathematics at the University of Manchester, 647 ; work of, 647
Urwick, L. F. : The Manager, 1332
Use of Animal Cell, Tissue and Organ Cultures in Radiobiology (New York Academy of Sciences Symposium on), 340
Use in Biological Work : A Sealing Compound for (G. Stotzky, W. Culbreth and Dr. L. B. Mish), 410
Use of Cellulose Acetate Membranes for Detecting Penicillinase-producing Organisms (Prof. R. Knox and J. T. Smith), 926
Use of Desertomycin Medium in Routine Selective Isolation of Fungi Pathogenic to Man (J. Uri and Zs. Herpay), 1015
Use of a Different Type of Fluorescent Material as an Optical Maser (Jordan J. Markham and Dickron Mergerian), 586
Use of Glacial Acetic Acid for Killing Parasitic Nematodes for Collection Purposes (Bjørn Berland), 1320
Use in Identification : Essential Oil from Fresh *Cannabis sativa* and its (L. Martin, D. Morison Smith and C. G. Farmilo), 774

Use in Lake Victoria : A Phytoplankton Multi-Sampler and its (Dr. J. H. Evans), 53
Use of Organic Reagents in Inorganic Analysis (A. I. Busec and N. G. Polianskii), translated from the Russian by J. T. Greaves, *review* by Dr. D. T. Lewis, 1336
Use to determine the Origin of Heartwood : Carbon-14 from Nuclear Explosions as a Short-term Dating System— (Dr. A. T. Wilson), 714
Use of Radioisotopes in Animal Biology and the Medical Sciences (International Atomic Energy Agency, the Food and Agriculture Organization and the World Health Organization of the United Nations, Conference on), 959
Use of Radioisotopes in Biological Work in Burma (Dr. Maung Mya Thaung), 875
Use of Respirators : The Design and (Ergonomics Research Society and the British Occupational Hygiene Society Discussion on), 655
Use in Ribonucleic Acid Fractionation : Modified Cellulose Ion Exchangers and their (Dr. L. Bosch, G. van der Wende and Dr. H. Bloemendal), 349
Use of the Rotating Hanging Mercury Drop in Analysis—Anodic Stripping using a Rotating Mercury Drop (Dr. J. J. Engelsman and A. M. J. M. Claassens), 240 ; Trace Analysis with a Rotating Hanging Mercury Drop (Dr. M. van Swaay and R. S. Deelder), 241
Use of Scientific Literature (B. C. Vickery), 1347
Use of Veronal Buffers in Formalin Fixatives (Dr. S. J. Holt and Dr. R. Marian Hicks), 832
Use (The Economic), of Brains (Prof. C. F. Carter), 987
Use (Proposed), of Magnetic Fields in Electron Microscopic Radioautography (Lucien G. Caro), 1188
Use and Control : Digital Computers, their (Mathematics Department of Queen's College, St. Andrews, in collaboration with Standard Telephones and Cables, Ltd., Symposium on), 554
Uses of Castorseed, 753
Usherwood, P. N. R. : Spontaneous Miniature Potentials from Insect Muscle Fibres, 814
U.S.S.R. : Scientific Research in the, 123
  Women Engineers in the, a Study of the Education, Employment and Prospects of Women Engineers in the U.S.S.R. (L. S. Souter and R. Winsdale), 30
U.S.S.R. Academy of Sciences : Mathematical Tables, Vol. 12, A Collection of Tables and Nomograms for the Processing of Observations made on Artificial Earth Satellites (I. D. Zhongolovich and V. M. Amelin), *review* by D. G. King-Hele, 1135
Uterine Activity : Effect of Bradykinin on (Dr. B. Berde and Dr. K. Saameli), 83
Utilizability of Viral Protein during Multiplication of Fowl Plague Virus : Influence of *p*-Fluorophenylalanine on the Production of Viral Ribonucleic Acid and on the (Dr. Christoph Scholtissek and Rudolf Rott), 1023
Utilization of Glucose : Effect of Thyroxine and 2,4-Dinitrophenol on the Rate of (Dr. L. Macho), 604
Utilization (Formation and), of Poly-β-Hydroxybutyric Acid by Knallgas Bacteria (*Hydrogenomonas*), (Prof. H. G. Schlegel, G. Gottschalk and R. von Bartha), 463

VACANCIES (Cation) : Quenching of, in Doped Crystals of Sodium Chloride (the late Sir K. S. Krishnan and S. C. Jain), 162
Vacek, Dr. Z., and others : Role of the Lung in Metabolism of Fat during Ontogeny of the Rat, 85
Vacuole in *Nitella* : Relative Volumes of Cytoplasm and (Dr. Paul Saltman and Dr. Tyge Christensen), 825
Valentine, Prof. D. H. : Flora Europaea (Hybrids in Flora Europaea), 448
Valentine, R. C., and Wolfe, Prof. R. S. : Phosphate-dependent Degradation of Urea, 925
Vallisneri, Antonio : Experimental Method in Biology from the Time of, to the Present (University of Padua Symposium on), 546
Value (Adaptive), of Regenerative Ability (Dr. A. E. Needham), 720
van As, D. (Steyn, J., and) : Radiolytic Oxidation of Ferrous Solutions with Standardized Internal Sources of Polonium-210, 903
van Eijnatten, C. L. M. : Susceptibility to Leaf Blight, caused by *Cochliobolus heterostrophus*, in Nigerian Varieties of Maize, 515
Van Landuyt, J. F., and others : Polarization induced by Radiation in 'Perspex', 595
Van Meter, Clarence T., and others (edited by) : Remington's Practice of Pharmacy, twelfth edition, *review* by Dr. J. H. Gaddum, 8
Van Metre, M. T., and others : Decreased Urinary Excretion of 2-Aminoethanol by Irradiated Human Beings, 1303
van Oene, H., and Cragg, Prof. L. H. : Elimination of the Absorption Correction in the Viscometry of Dilute Solutions of High Polymers, 1160
Van Peenen, Dr. Hubert J., and others : The Diego Factor in a Puerto Rican Family—a Case of Anti-Diego in Monozygotic Twins, 188
Van Proosdy, Dr. C. : Smoking—its Influence on the Individual and its Role in Social Medicine, *review* by Dr. W. Carruthers, 108
van Swaay, Dr. M., and Deelder, R. S. : Use of the Rotating Hanging Mercury Drop in Analysis—Trace Analysis with a Rotating Hanging Mercury Drop, 241
Van Zyl, Dr. A., and Bhaga, M. : A Spray Method for the Simultaneous Detection of Iodide and Iodinated Amino-Acids on Filter Paper Chromatograms, 275
van de Mond, Th. (Mars, P., and) : Selectivity of Palladium Catalysts for the Oxidation of Carbon Monoxide in the Presence of Hydrogen, 1003
Van den Berghs, Ltd. : Memoir to mark the Centenary of the birth of Sir Frederick Gowland Hopkins (Prof. Ernest Baldwin), 439, *erratum*, 546
van den Brande, Prof. J. : Presidential Address at the sixth International Symposium on Nematology, 1048
van der Bij, Dr. R. J. : At the International Starch Conference (Quantitative Estimation of Ether and Ester Groups in Starch Derivatives), 237
van der Grinten, P. M. E. M., and Dijkstra, Dr. A. : Integration with Ionization Detectors in Gas Chromatography, 1195
van der Horst, Dr. C. J. G. : Keto-Acids and Amines in Rumen Liquid of Cattle, 73
Van der Veen, J., and others : Selenomethionine as an Antioxidant, 1201
van der Wende, G., and others: Modified Cellulose Ion Exchangers and their Use in Ribonucleic Acid Fractionation, 349
Vane, Dr. J. R., and others : Tryptamine Receptors in the Central Nervous System, 1068
Vanhuyse, Dr. V. J., and others : Polarization induced by Radiation in 'Perspex', 595
Vanpraet, G. J., and others : Polarization induced by Radiation in 'Perspex', 595
Vapour (Precipitable Water), over the United States : Chemical Composition of (Dr. Henry M. Papée and Dr. Alberto Montefinale), 136
Vapours (Organic) : Measurements of Heats of Adsorption of, on Adsorbents of High Surface Area using a Continuous Flow Calorimeter (G. H. Bell and A. J. Groszek), 1184
Variability (Inter-Individual), in Pigmentation (André Leguebe), 1326
Variable Shutter Device : A New Photometric Technique using a (Dr. O. C. Jones and Dr. F. J. J. Clarke), 1290
Variants (Denatured Haemoglobin), in Acid Buffers : Solubility of (I. D. Fraser and Dr. A. B. Raper), 355
Variates (Infinitely Many) : An Introduction to (Prof. Enders A. Robinson), *review* by Prof. D. V. Lindley, 214
Variation of Anisotropy Axis Dispersion with Thickness of Nickel-Iron Films (H. Clow), 996
Variation (Diurnal), in Transaminase Activity in the Cactus Plant (S. K. Mukerji, G. G. Sanwal and Prof. P. S. Krishnan), 516
Variation (Morphological), in *Ferrobacillus ferrooxidans* related to the Rate of Iron Oxidation (M. P. Silverman and M. H. Rogoff), 1221
Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogenesis : Tissue Dilution Artefact—a Re-interpretation of (G. R. N. Jones, L. Bitensky, J. Chayen and Prof. G. J. Cunningham), 1203
Variations (Seasonal), of Caesium-137 in Air at Ground-Level (K. G. Vohra, V. S. Bhatnagar and C. Rangarajan), 747
Varicose Veins : Treatment of (Yutaka Kato and Robert A. Nabatoff), 335
Varieties (Nigerian), of Maize : Susceptibility to Leaf Blight, caused by *Cochliobolus heterostrophus*, in (C. L. M. van Eijnatten), 515
Varma, D. R. (Benfey, B. G., and) : Stimulation of the Heart of the Spinal Dog by Phenoxybenzamine, 919
Varying Doses of Undiluted Semen : Effect of, on Fertility in the Domestic Fowl (G. C. Taneja and R. S. Gowe), 828
Vascular Smooth Muscle : Spontaneous Spike-Discharges of (Dr. S. Funaki), 1102
Vegetative Propagation in the Coconut (*Cocos nucifera* L.) : Possibilities of (S. R. Gangolly and K. M. Pandalai), 1218
Veins (Varicose) : Treatment of (Yutaka Kato and Robert A. Nabatoff), 335
Veljković, Slobodanka, and Harbottle, Dr. Garman : Recoil-Atom Reactions at Low Temperature, 1287
Vella, Dr. F., and Ibrahim, S. A. : Thalassaemia in an English and in a German Family, 822
Velocities (Group and Phase), of a Travelling Disturbance in the *F* Region of the Ionosphere (Dr. L. H. Heisler and Dr. J. D. Whitehead), 157
Velocity of Cardiac Contraction and Size of the Heart : Relationship between—a Hypothesis (Dr. H. Feinberg), 1373
Velocity (Group) : Wave Propagation and (Dr. Léon Brillouin), *review* by Prof. M. H. L. Pryce, 104
Velocity (Molar Sound), in Molten Hydrated Salts (P. R. K. L. Padmini and Prof. B. Ramachandra Rao), 684
Velocity (Ultrasonic), in Aqueous Silver Nitrate Solution (D. S. Allam and W. H. Lee), 1388
Velocity (Ultrasonic), in Aqueous Solutions of some Electrolytes (M. G. Seshagiri Rao and Prof. R. Ramachandra Rao), 164
Vennesland, Prof. Birgit (Stiller, Mary, and) : Photophosphorylation and the Hill Reaction, 677
Venom (Bee) : Effect of, on Concentration of Blood Glucose and Liver Glycogen in Rabbits (Prof. A. H. Mohamed and K. Zaki), 605
Ventricle (Lateral), of Brain : Antibody Response in Rabbits following Injection of Sheep Erythrocytes into (Prof. B. D. Janković, Dr. M. Draškoci and Dr. K. Isaković), 288
Verco Medal of the Royal Society of South Australia presented to Dr. R. L. Specht, 1247
Verdonk, Prof. G. : At the Third International Congress of Dietetics (Influence of Nutrition and Ways of Life on Blood Cholestrol and the Prevalence of Atherosclerosis and Coronary Heart Disease among Trappist and Benedictine Monks), 1343 ; (Diet and Atherosclerosis), 1343
Vergence (Eye) : Barbiturates and (Gerald Westheimer and Cyril Rashbass), 833
Verhulst, Prof. Michel (edited by Prof. C. West and) : Management Sciences, Models and Techniques—Proceedings of the Sixth International Meeting of the Institute of Management Sciences, Paris, 1959, *review* by T. H. Hawkins, 209
Vermiculite : Complexes of, with Amino-Acids (G. F. Walker and W. G. Garrett), 1389
Vernon, Dr. Leo P. : Appointed director of research of the Charles F. Kettering Laboratory, Yellow Springs, Ohio, 229
Veronal Buffers : Use of, in Formalin Fixatives (Dr. S. J. Holt and Dr. R. Marian Hicks), 832
Vertebrate Phylogeny, *review* by Dr. E. Trewavas, 945
Vertébrés : Théories de l'Évolution des (Erik Jarvik), traduction du Prof. J. P. Lehman, *review* by Dr. E. Trewavas, 945
V.H.F. (U.H.F. and) Television Surveys : A Mobile Laboratory for (E. W. Taylor and L. C. Munn), 1355
Very-Low-Frequency Modulation of Discrete Frequency Solar Noise Bursts (Dr. Jules Aarons, Santimay Basu, William Kidd and Richard Allen), 56
Vesicle (Sensory), of the Ascidian Tadpole : Electron Microscope Observations of the Receptors in the (Noël Dilly), 786
Vesin, Dr. P. (Milhaud, Dr. G., and) : Calcium Metabolism in Man with Calcium-45—Malabsorption Syndrome and Exudative Enteropathy, 872
Veterinary Anatomy at Liverpool, 648
Veterinary College (New York State), and others : Conference on Transfer of Calcium and Strontium across Biological Membranes, 1250
Vibrations (Initial), of a Supported Beam (Dr. R. V. Sharman and D. White), 692
Vice-Chancellors and Principals of the Universities of the United Kingdom, Committee of : Conference of professors of mathematics from the Universities of Great Britain, to consider the Teaching of Mathematics, 1145

Vicia faba : Sub-Chromatid Structure and Chromosome Duplication in (W. J. Peacock), 832
Vickerman, K. (Baker, Dr. J. R., and) : Parasite Biology (Significance of the Trypanosomatid Kinetoplast), 134
Vickers, K. U., and McClean, R. : Furunculosis in Lough Neagh Pollan *Coregonus pollan*, Thompson, 930
Vickery, B. C. : The Use of Scientific Literature, 1347
Victoria, Museum of Applied Science of (Melbourne) : Report for 1959-60, 124
Victoria, Royal Society of : First award of the Research Medal made to Dr. George Baker, 1247
Victoria (Lake) : A Phytoplankton Multi-Sampler and its Use in (Dr. J. H. Evans), 53
Victorian American : The—Lithographs from the Harry T. Peters 'American on Stone' Collection (Antony N. B. Garvan and Peter C. Welsh), 226
Vidal-Beretervide, Kempis (De Vries, Juan X., and) : Some New Esters of Chromone-2-Carboxylic Acid, 171
View : Science and Education—the Teachers' Point of, 555
View (A Soviet), of British Libraries (V. I. Shunkov, G. G. Firsov and N. I. Tyulina), translated by A. Thompson, (Library Association, Pamphlet No. 22), 752
Viewing and Listening : Facts and Figures about (British Broadcasting Corporation), 1051
Villet, R. H. : Technology of Chemical Processing, *review*, 1131
Vilstrup, Dr. Th., and others : Displacement Potentials and Bending of Rod-like Polyelectrolytes, 484
Viral Infection in the Chick Embryo : Mechanism of Recovery from (Dr. Samuel Baron and Alick Isaacs), 97
Viral Protein during Multiplication of Fowl Plague Virus : Influence of *p*-Fluorophenylalanine on the Production of Viral Ribonucleic Acid and on the Utilizability of (Dr. Christoph Scholtissek and Rudolf Rott), 1023
Viral Ribonucleic Acid : Influence of *p*-Fluorophenylalanine on the Production of, and on the Utilizability of Viral Protein during Multiplication of Fowl Plague Virus (Dr. Christoph Scholtissek and Rudolf Rott), 1023
α-Virginis (Spica) Nebula (Walter A. Feibelman), 1083
Virtual Absence of Glutathione Instability of the Erythrocytes among Armenians in Iran (Dr. J. E. Bowman and Dr. D. G. Walker), 221
Virulence in *Rhizobium trifolii* : Transfer of (Dr. Hans Ljunggren), 623
Virus (Arbor), Group *B* : Infectious Ribonucleic Acids derived from Mouse Brains infected with Two Kinds of (Prof. Masahiro Nakamura), 624
Virus (Cactus), in the United States (the late Irene M. Sammons and Prof. M. Chessin), 517
Virus (Foot-and-Mouth Disease) : Effect of *p*-Fluorophenylalanine on the Multiplication of (F. Brown, D. N. Planterose and Doreen L. Stewart), 414
Virus (Fowl Plague) : Influence of *p*-Fluorophenylalanine on the Production of Viral Ribonucleic Acid and on the Utilizability of Viral Protein during Multiplication of (Dr. Christoph Scholtissek and Rudolf Rott), 1023
Virus (Leaf Curl), and the Flavonoid Content of Resistant and Susceptible Strains of Cotton (Dr. Esam M. Moustafa), 415
Virus (Leukaemogenic) : Leukaemia in the Progeny of Mice inoculated with a (Dr. M. H. Salaman and J. J. Harvey), 509
Virus (Polio-) : Chromatographic Analysis of Infectious Ribonucleic Acid from (C. Cocito, A. Prinzie and P. De Somer), 573
Virus (Polio-) Multiplication : Effects of Heavy Water on—Results and Speculations on Mechanism (Dr. David Kritchevsky, Dr. Richard I. Carp and Dr. Hilary Koprowski), 250
Virus (Polyoma) : Induction of Tumours by—Enhancement by Chemical Agents (K. E. K. Rowson, F. J. C. Roe, J. K. Ball and Dr. M. H. Salaman), 893
Virus (Tobacco Mosaic) (TMV) : Chromatography of, on Chitin Columns (P. M. Townsley), 626
Virus (Tobacco Mosaic) (TMV) Lesion Number and Concentration : Relationship of, to the Rate of Lesion Production on Pinto Bean (John Piacitelli and Prof. Vincent Santilli), 624
Virus (Wild Cucumber Mosaic) : Unusual Base Composition of the Ribonucleic Acid of (H. Yamazaki and Prof. Paul Kaesberg), 96
Virus Diseases : Research into (Pfizer Group Laboratories for), 20
Virus Diseases (Potato) : Proceedings of the Fourth Conference on (Braunschweig, 1960), edited by J. Brandes, R. Bartels, V. Völk and C. Wetter (Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig), 657
Virus Filtration : A Method of Treating Asbestos Filter Pads for (N. St. G. Hyslop), 305
Virus Protein Production (Turnip Yellow Mosaic) : Stimulation of, by 2-Thiouracil (Dr. R. I. B. Francki and Dr. R. E. F. Matthews), 1078
Viruses : Plant (Dr. Kenneth M. Smith), third edition, *review* by Dr. B. D. Harrison, 111
Viruses (Bacterial) : Papers on (selected by Gunther S. Stent), *review* by L. B. Quesnel, 320
  X-Ray Diffraction Studies of (Dr. A. C. T. North and Prof. Alexander Rich), 1242
Viruses (Influenza), Receptor-destroying Enzyme and Proteases : Action of, on Blood Group Agglutinogen M^g (Prof. Georg F. Springer and Dr. Karlheinz Stalder), 187
Viscometry of Dilute Solutions of High Polymers : Elimination of the Absorption Correction in the (H. van Oene and Prof. L. H. Cragg), 1160
Viscosity–Temperature Relationships for Dilute Solutions of High Polymers (Dr. W. R. Moore), 1292
Vision (Human) : Limits of (Dr. R. A. Weale), 471
Vision (Peripheral) : Effect of Attention in (Arthur Thomson), 732 ; (B. Babington Smith), 732
Vision Research, Vol. 1, Nos. 1-2, 650
Visit of Russian Scientists to Harwell, 957
Visual Constancy : Measuring, for Stationary or Moving Objects (S. M. Anstis, C. D. Shopland and R. L. Gregory), 416
Visual Gnostic Functions and Emotional Behaviour : Changes in, following Unilateral Temporal Pole Damage in the 'Split-Brain' Monkey (Dr. J. L. deC. Downer), 50
Vitamin: Metabolites of Vitamin B_{12} in Bile after Parenteral Administration of the (Dr. John B. Stokes), 807
Vitamin-Levels in Blood and Serum (H. Baker, O. Frank, I. Pasher, Dr. H. Sobotka and S. H. Hutner), 78
Vitamin B_{12} : Gel Filtration of Sea-Water—Separation of Free and Bound Forms of (Dr. K. W. Daisley), 868
  Metabolites of, in Bile after Parenteral Administration of the Vitamin (Dr. John B. Stokes), 807
Vitamin B_{12} (Specifically Bound Cobalt-58) : Selective Uptake of, by Human and Mouse Tumour Cells (Dr. Bernard A. Cooper and Dr. William Paranchych), 393
Vitamin B_{12} and Intrinsic Factor : European Symposium on, 1154
Vitamins (Added) : Effect of, on the Phagocytic Activity of the Reticulo-Endothelial System of Mice (J. Cordingley and Prof. T. Nicol), 82
Vitreous Silica : Permeation of Gaseous Oxygen through (Dr. Francis J. Norton), 701
Vitrinite in Coal : Electron Microscopic Observations of Structure in Ultra-Thin Sections of (J. T. McCartney, Sabri Ergun and R. E. Walline), 1361
Vivian, H. E. (Lawrence, M., and) : The Action of Calcium Chloride on Mortar and Concrete, 125, *erratum*, 1145
Vivian, H. E. (Roberts, J. A., and) : The Widening of Expansion Cracks in Mortar by Salt Solutions, 125, *erratum*, 1145
Viviparous Toothcarp : Pathological Parthenogenesis in a (Prof. A. Stolk), 507
Vivonia, Charlotte, and others : Subfractionation of Human Serum Leucine Aminopeptidase, 1203
Vlasov, N. A. (edited by) : Fast-Neutron Spectroscopy (R. V. Rybakov and V. A. Sidorov), translated from the Russian, *review* by Dr. P. A. Egelstaff, 8
Vlček, Augustin (Cook, Margaret J., and) : Sex-Ratio in Mice, 89
Vogel, A. W., and others : N,N'-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds—a New Series of Anti-tumour Agents, 611
Vohra, K. G., and others : Seasonal Variations of Caesium-137 in Air at Ground-Level, 747
Volcanoes in the West Indies : Research on, 225
Völk, V., and others (edited by) : Proceedings of the Fourth Conference on Potato Virus Diseases, Braunschweig, 1960 (Biologische Bundesanstalt für Land- und Forstwirtschaft), 657
Vollmer, Erwin P. (edited by Gregory Pincus and) : Biological Activities of Steroids in relation to Cancer—Proceedings of a Conference sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, United States Department of Health, Education and Welfare, *review* by E. Reid, 1235
Voltage-Clamp Experiments with *Nitella* (G. P. Findlay), 812
Volumes (Relative), of Cytoplasm and Vacuole in *Nitella* (Dr. Paul Saltman and Dr. Tyge Christensen), 825
von Albertini, Prof. A. : Elected a member of the Pathology Section of the Leopoldina German Academy of Sciences, 122
von Bartha, R., and others : Formation and Utilization of Poly-β-Hydroxybutyric Acid by Knallgas Bacteria (*Hydrogenomonas*), 463
von Kármán, Prof. Theodore : Space-Flight and Re-entry Trajectories, 960
Voorhis, Arthur D. : Evidence of an Eastward Equatorial Undercurrent in the Atlantic from Measurements of Current Shear, 157
Voprosy Psikhologii (translations from, to be published in *Problems of Psychology*), 958
Vose, P. B., and Griffiths, Dr. D. J. : Manganese and Magnesium in the Grey Speck Syndrome of Oats, 299
Voss, Dinor O. (Bonner, jun., Dr. Walter D., and) : Some Characteristics of Mitochondria Extracted from Higher Plants, 682
Vyskočil, František (Novotny, Dr. Ivan, and) : Inhibition by Physostigmine of the Increase of Oxygen Consumption induced by Potassium in Muscle, 916

WADA, Dr. Yasuo, and others : Quantitative Determination of Metadrenaline and Normetadrenaline in Normal Human Urine, 599
Waelsch, Prof. Heinrich, and others : Incorporation of Histamine into Liver Protein *in vivo*, 1204
Waight, Dr. E. S. : Infra-Red Arts, *review*, 315
Wajda, Isabel, and others : Incorporation of Histamine into Liver Protein *in vivo*, 1204
Waldi, Dr. D. : Paper Chromatography (Separation of particular families of Compounds—Chromatographic Separation of some two hundred Alkaloids on Formamide-treated Paper), 759
Wales : Report on the Water Resources of (Welsh Advisory Water Committee), 135
Wales (England and), National Foundation for Educational Research in : *Technical Education Abstracts from British Sources*, Vol. 1, No. 1, September–December 1960, 21
Walker, B. : Appointed a Research Fellow in the Department of Industrial Metallurgy, University of Birmingham, 337
Walker, C. H. M., and others : Amino-acid Composition of Serum Mucoprotein in Rheumatic Fever, 922
Walker, Prof. D. : Awarded a Royal Society and Nuffield Foundation Commonwealth Bursary, 126
Walker, D., and others : Amino-acid Composition of Serum Mucoprotein in Rheumatic Fever, 922
Walker, Dr. D. G. (Bowman, Dr. J. E., and) : Virtual Absence of Glutathione Instability of the Erythrocytes among Armenians in Iran, 221
Walker, D. M. C. (King-Hele, D. G., and) : Density of the Upper Atmosphere determined by Satellite Orbits, and its Variation during 1957-61, 114
Walker, G. F., and Garrett, W. G. : Complexes of Vermiculite with Amino-Acids, 1389
Walker, P. J. : Parasite Biology (Significance of the Trypanosomatid Kinetoplast), 134
Wallace, H. R. : Nematology (Orientation of *Ditylenchus dipsaci* to Physical Stimuli), 1048
Wallace, M. M. H. : Enhanced Plant-Growth with Two Systemic Insecticides, 513
Walline, R. E., and others : Electron Microscopic Observations of Structure in Ultra-Thin Sections of Vitrinite in Coal, 1361
Wallis, D. I. : Response of the Labellar Hairs of the Blowfly, *Phormia regina* Meigen, to Protein, 917

Wallis, Dr. F. S. : The Museums Association, 553
  Museums and Galleries, 862
Walls (Bacterial) : Teichoic Acids and the Structure of (Dr. A. R. Archibald, J. J. Armstrong, Prof. J. Baddiley and J. B. Hay), 570
Walters, Dr. S. M. : Flora Europaea (Problems of Abstracting and Indexing European Taxonomic Literature), 448
Walton, E., and others : Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine, 1312
Walton, Prof. John : Tropical Floras in the Tertiary in Southern England, *review*, 209
Wangensteen, Dr. Owen H. : Elected an Honorary Fellow of the Royal College of Surgeons of England, 126
War : Arms and Security, a Mathematical Study of the Causes and Origins of (Dr. Lewis F. Richardson), edited by Nicolas Rashevsky and Ernesto Trucco, *review* by Sir Cyril Burt, 427
  Mathematical Studies in the Psychology of, *review* by Sir Cyril Burt, 427
War on Hunger : A Strategy for the (Sir Joseph Hutchinson and Prof. A. H. Bunting), 539
Warburton, Frederick E. : Inclusion of Parental Somatic Cells in Sponge Larvae, 1317
Ward, Prof. A. G. : At the Twenty-first Meeting of the Research Panel of the British Gelatine and Glue Research Association (Rupture Properties of Gelatine Gels), 857
Warm-water Species in the Plankton off Newfoundland during Winter Months (V. Bainbridge), 1216
Warner, Brian : Holistic Approach to Selenology, 586
Warner, N. L. (Szenberg, Dr. Aleksander, and) : Large Lymphocytes and the Simonsen Phenomenon, 920
Warren, Diana M., and Wilkins, Malcolm B. : An Endogenous Rhythm in the Rate of Dark-Fixation of Carbon Dioxide in Leaves of *Bryophyllum fedtschenkoi*, 686
Warren, Sir Hugh : *Obituary* by L. J. Davies, 1042
Watanabe, Hiroshi, and others : Isolation of Nigerose and Kojibiose from Dextrans, 278
Water : Pollution of, by Tipped Refuse—Report of the Technical Committee on the Experimental Disposal of House Refuse in Wet and Dry Pits (Ministry of Housing and Local Government), 864
Water in the Chick Blastoderm : Distribution of (A Morphogenetic Effect of Beta-Mercaptoethanol), (A. Jurand and Dr. Peter Tuft), 1073
Water in the Embryo : Distribution of (A Morphogenetic Effect of Beta-Mercaptoethanol), (Dr. Peter Tuft), 1072
Water in Great Britain : Conservation of, 421
Water from the Skin of the Ox : Increase of Evaporative Loss of, in response to Local Heating of the Hypothalamus (Dr. D. L. Ingram, J. A. McLean and Dr. G. C. Whittow), 81
Water (Drinking) : European Standards for (United Nations, World Health Organization), 1261
Water (Drinking-) Standards (Nora H. Johnson), 1261
Water (Fresh-) Eutrophic Lakes of India : Hydrogen Ion Concentration, Plankton and Fish in (Dr. S. M. Das), 511
Water (Heavy) : Effects of, on Poliovirus Multiplication—Results and Speculations on Mechanism (Dr. David Kritchevsky, Dr. Richard I. Carp and Dr. Hilary Koprowski), 250
Water (New Zealand Rain-) : Barium-140 in (G. S. McNaughton and R. N. Woodward), 1344
Water (Old Sea-) : A Lake in British Columbia containing (Dr. P. M. Williams, Prof. W. H. Matthews and Prof. G. L. Pickard), 830
Water (Sea-) : Gel Filtration of—Separation of Free and Bound Forms of Vitamin B_{12} (Dr. K. W. Daisley), 868
Water (Sea-) and Chloride Solutions : Formation of Calcium Chloraluminate by Interaction of Portland Cement with (Dr. L. Heller and Dr. M. Ben-Yair), 488
Water (Warm-) Species in the Plankton off Newfoundland during Winter Months (V. Bainbridge), 1216
Water Committee, Welsh Advisory : Report on the Water Resources of Wales, 135
Water Movement (Net) : Comparison of Short-Circuit Current and, in Single Perfused Proximal Tubules of Rat Kidneys (Dr. Erich E. Windhager and Dr. Gerhard Giebisch), 1205
Water Resources of the United States (Prof. W. G. V. Balchin), 444
Water Resources of Wales : Report on the (Welsh Advisory Water Committee), 135
Water Treatment and Examination, Society for : Autumn Meeting, 856
Water Vapour (Precipitable), over the United States : Chemical Composition of (Dr. Henry M. Papée and Dr. Alberto Montefinale), 136
Water-soluble Carbonates : Infra-Red Spectra of (Herman A. Szymanski and Richard Povinelli), 64
Water and Alcohol Consumption : Comparison between, in Six Animal Species in Free-choice Experiments (Dr. Alpo Arvola and Dr. Olof Forsander), 819
Waterman, Talbot H. (edited by) : The Physiology of Crustacea, Vol. 2, Sense Organs, Integration and Behaviour, *review* by Dr. J. Green, 428
Waters, A. H. : Vitamin B_{12} and Intrinsic Factor (Relationships between Vitamin B_{12} and Folic Acid), 1156
Waters, Dr. W. A. : Physical Methods of Organic Chemistry, *review*, 317
Waters (Acid–Sulphate–Chloride) : Hot Spring Areas with (Dr. A. J. Ellis and S. H. Wilson), 696
Waters (Near-shore), of Porto Novo, South India : Occurrence of *Eutrichocheles modestus* (Herbst) in the (K. Balasubrahmanyan and Dr. J. Jacob), 830
Waters (Surface and Underground) : Uranium in British (J. D. Peacock), 1189
Watkin, Brian : Sterilizing Materials for Hospitals, 1044
Watkins, Dr. J. C. (Curtis, Dr. D. R., and) : Analogues of Glutamic and γ-Amino-*n*-butyric Acids having Potent Actions on Mammalian Neurones, 1010
Watkins, S. B. : Appointed to the University of London readership in chemical engineering at King's College, London, 546, 651
Watson, Dr. D. G. : Glasgow fellowship in chemistry renewed for 1961-62 by the Ramsay Memorial Trust, 337
Watson, Prof. H. E. : The Development of the Neon Glow Lamp (1911-61), 1040
Watson, T. E. (Roff, H. E., and) : Job Analysis (Institute of Personnel Management), 336
Watson, Prof. W. H. : A Concept of Atomic Existence, 658
Watson-Munro, Prof. C. N. : *Obituary* of Dr. G. C. J. Dalton, 1142
Watt-Carter, D. E., and Corke, R. L. : Effect of External Conditions on the Phase of Radio Signals, 1286
Watts, J. C. D. : Seasonal Fluctuations and Distribution of Nitrite in a Tropical West African Estuary, 929
Watts, R. E. (Glueckauf, Dr. E., and) : Non-Uniformity of Cross-linking in Ion-Exchange Polymers, 904
Wave (*P*-) (Refracted) : Possible Method for Increasing the Signal-Noise Ratio in the Detection of the First Motion of a (F. A. Key, J. K. Wright, E. W. Carpenter and B. M. H. Stott), 1382
Wave Discharges (Spike and), and Alterations of Conscious Awareness (L. Goldie and J. M. Green), 200
Wave Propagation and Group Velocity (Dr. Léon Brillouin), *review* by Prof. M. H. L. Pryce, 104
Waves' ('Blood) : Brain Waves and (Henry R. Pantek), 577
Waves (Brain), and 'Blood Waves' (Henry R. Pantek), 577
Waves (Surface), in Anisotropic Elastic Media (V. T. Buchwald and A. Davis), 899
Waves (Ultrasonic): Aniline Oxidation initiated by (Dr. Wiktor Wawrzyczek), 701
Wawrzyczek, Dr. Wiktor : Aniline Oxidation initiated by Ultrasonic Waves, 701
Wax (Surface), on Leaves : Physiological Significance of (D. M. Hall and R. L. Jones), 95
Weald (Western) : The Land and Man in the (Prof. S. W. Wooldridge), 132
Weale, Dr. R. A. : Limits of Human Vision, 471
Weathered Karroo Dolerites : Climate and (Dr. H. H. Weinert), 325
Weaver, Dr. Warren : A Great Age for Science, 312
Weaver-Finches (*Quelea quelea*) : Melanism in Captive (Dr. B. Lofts), 993
Webb, Prof. D. A. : Flora Europaea (Problems of Abstracting and Indexing European Taxonomic Literature), 448
Webb, Dr. J. S. : Appointed to the University of London chair of applied geochemistry at the Imperial College of Science and Technology, 229
Weber, Prof. K., and others : Inhibition of Chemi-Luminescence of Luminol by means of Oximes, 177
Weddell, Dr. A. G. M. : Assessment of Pain (Structure of the Nerve Terminals in the Skin as they appeared in Electron and Optical Microscopy), 1353
'Wedge' Type Instability (A New), observed in a 'Theta' Pinch (J. K. Wright, R. D. Medford, J. D. Herbert and R. C. Pottinger), 1054
Weedon, Prof. B. C. L. : Terpene Chemistry, *review*, 1234
Weeds in Britain, *review* by Dr. Alec Lazenby, 842
Weeds and Aliens (Sir Edward Salisbury), *review* by Dr. Alec Lazenby, 842
Weeks, J. L., and others : Irradiated Lithium Fluoride as an Optical Filter in the Far Ultra-Violet, 1186
Weenink, R. O., and others : Lipids of the Common Pea (*Pisum sativum* L.) with special reference to the Occurrence of Galactolipids, 1301
Weiner, Prof. Murray, and others : Lack of Effect of Coenzyme Q_{10} on Coumarin-induced Hypoprothrombinaemia, 711
Weinert, Dr. E.: Flora Europaea (Problems of Abstracting and Indexing European Taxonomic Literature), 448
Weinert, Dr. H. H. : Climate and Weathered Karroo Dolerites, 325
Weinman, Dr. J., and Manoach, Dr. M. : Biorheology and Microcirculation (Photoelectric Techniques for Studying the Peripheral Circulation in Man), 758
Weinshelbaum, Dr. Edward I., and Wool, Dr. Ira G. : Effect of Adrenalectomy and Corticosteroids on Distribution of Radioactivity in Protein of Cell Fractions from Myocardial Slices, 1401
Weischer, B. : Nematology (Discovery of Two Species of Desmoscolecidae in Vineyard Soils from Bad Ems), 1048
Weiss, Dr. A. D. (Kay, Prof. H., and) : Relationship between Simple and Serial Reaction Times, 790
Weiss, J. B. (Smith, Dr. I., and) : Paper Chromatography, 759
Weiss, Prof. Joseph J., and others : Radiation-induced Formation and Reactions of the Formyl Radical Ion, 1386
Weiss, Dr. Leonard : Sialic Acid as a Structural Component of some Mammalian Tissue Cell Surfaces, 1108
Weissberger, Arnold (edited by) : Physical Methods of Organic Chemistry, third edition, Parts 3 and 4, *review* by Dr. W. A. Waters, 317
Weissberger, Arnold, and others (edited by) : Organic Analysis, Vol. 4, *review* by Dr. D. T. Lewis, 741
Weitz, B. : Immunity to Protozoa (Immunity in Trypanosomiasis), 552
Welbourn, B. : *Obituary*, 542
Welch, Dr. F. B. A., and Trotter, Dr. F. M. : Geology of the Country around Monmouth and Chepstow (Memoirs of the Geological Survey of Great Britain—England and Wales), 227
Welch, J. H. : Ternary Compound Formation in the System $CaO–Al_2O_3–MgO$, 559
Welding Research Association (British) : New Laboratory (R. P. Newman), 1147
Welfare State : Industrial Relations in the, 1129
Welfare (Animal), Universities Federation for : Annual Summer Congress, 755
  Symposium on the Assessment of Pain, 1353
Wellcome Research Fellowships (Animal Health Trust Awards), 1250
Wellcome Trust (Carlsberg Foundation and the) : Award of Carlsberg–Wellcome Research Fellowships for 1960-61, 754
Wellens, John : Educational Television—a Survey and a Plan, 226
Welsh, Peter C. (Garvan, Antony N. B., and) : The Victorian American—Lithographs from the Harry T. Peters 'American on Stone' Collection (Smithsonian Institution, Publication 4466), 226
Welsh Advisory Water Committee : Report on the Water Resources of Wales, 135
Welsh College of Advanced Technology (Dr. A. Harvey), 127
Wentworth, J. W. : At the Maxwell Colour Centenary Conference (Colour Television), 11
Werner, Dr. A. E. : At the sixty-seventh Annual Conference of the Museums Association (Problems of Conservation), 553
West, Prof. C., and Verhulst, Prof. Michel (edited by) : Management Sciences, Models and Techniques—Proceedings of the Sixth International Meeting of the Institute of Management Sciences, Paris, 1959, *review* by T. H. Hawkins, 209
West, Dr. G. B. (Harris, J. M., and) : Anaphylactoid Reaction in Rats, 399
West, Dr. W., and others : Electron Spin Resonance Absorptions induced by Light in Adsorbed Layers of Cyanine Dyes on Silver Bromide Microcrystals, 902

West African (East and) Stocks of *Papilio dardanus* (Lep.): Genetics of a Difference in the Male Genitalia of (J. R. G. Turner, C. A. Clarke and P. M. Sheppard), 935
West African Estuary (Tropical) : Seasonal Fluctuations and Distribution of Nitrite in a (J. C. D. Watts), 929
West Ford Project : Long-Range Radio Communication by Satellite Microwave Dipoles—the, 1237
West Indies : Research on Volcanoes in the, 225
West Riding of Yorkshire, *review* by Prof. H. C. Darby, 208
  The Place-Names of the (Prof. A. H. Smith), Part I, Lower and Upper Strafforth and Staincross Wapentakes ; Part 2, Osgoldcross and Agbrigg Wapentakes ; Part 3, Morley Wapentake, *review* by Prof. H. C. Darby, 208
Westcott, Dr. J. H. : Title of professor of electrical engineering in the University of London (at the Imperial College of Science and Technology) conferred on, 1349
Western Australian Museum : Annual Report for 1959-60, 335
Western Weald : The Land and Man in the (Prof. S. W. Wooldridge), 132
Western and Central Europe : Introduction of the Potato into (V. S. Lekhnovitch), 518
Westfield College, London : Appointment of Dr. S. J. Taylor to the University of London readership in mathematics, 1349
Westheimer, Gerald, and Rashbass, Cyril : Barbiturates and Eye Vergence, 833
Westinghouse Brake and Signal Co. Ltd. : Exhibition at the Science Museum, London, of Modern Methods of Traffic Control, 1044
Westlake, A. H. (Bradley, Prof. D. C., and) : Structures of Polymeric Titanium Ethoxide and Titanium Oxide Ethoxides, 273
Westphal, Prof. Otto : Elected a Member of the Biochemistry Section of the Leopoldina German Academy of Sciences, 122
Wet Condition (Dry and) : Radiation Sensitivity of *Escherichia coli* to β-Rays in (S. B. Bhattacharjee and N. N. Das Gupta), 1015
Wetmore, Prof. Ralph H. (DeMaggio, Prof. Augustus E., and) : Growth of Fern Embryos in Sterile Culture, 94
Wettability and the Capture, Carriage and Deposition of Particles by Raindrops (R. R. Davies), 616
Wetter, C., and others (edited by) : Proceedings of the Fourth Conference on Potato Virus Diseases, Braunschweig, 1960 (Biologische Bundesanstalt für Land- und Forstwirtschaft), 657
Whaley Skull : The (D. R. Brothwell), 854
What is Basic Science ?, *review* by Sir George Pickering, 530
Wheeler, Prof. T. S., and Partington, Prof. J. R. : The Life and Work of William Higgins, Chemist (1763-1825), *review* by Frank Greenaway, 533
Whipple, Dr. Fred L. : The Dust Cloud about the Earth, 33
Whitaker, E. A. (compiled by D. W. G. Arthur and) : Orthographic Atlas of the Moon (edited by Gerard P. Kniper), Edition 'A' showing the Standard Orthographic Coordinate Grid, Part I, Central Area, *review* by D. H. P. Jones, 424
Whitaker, E. P., and others : Significance of the Anti-Sporulation Effect of Copper Fungicides in the Control of Sigatoka Disease of Bananas caused by *Mycosphaerella musicola* Leach, 476
Whitaker, F. P. G. : Appointed director of management studies in the Department of Economic and Commerce, University of Leeds, 228
Whitby, James L., and others : Increase in Specific Bactericidal Antibodies after Administration of Endotoxin, 296
White, A. M. (Arnstein, H. R. V., and) : Vitamin B_{12} and Intrinsic Factor (Biological Function of Vitamin B_{12}), 1155
White, D. (Sharman, Dr. R. V., and) : Initial Vibrations of a Supported Beam, 692
White, E. A. D. : A New Technique for the Production of Synthetic Corundum, 901
White Clover (Kent Wild), *Trifolium repens* L. : Flower Initiation in, under Controlled Environmental Conditions (R. J. Haggar), 1120
White Laboratory Rats : Leptospirosis of (Dr. M. Füzi and R. Csóka), 1123
Whitehead, Dr. J. D. (Heisler, Dr. L. H., and) : Group and Phase Velocities of a Travelling Disturbance in the *F* Region of the Ionosphere, 157
Whitrow, Dr. G. J. : A New Approach to General Relativity, *review*, 424
Whittaker, Mary (Harris, Prof. H., and) : Differential Inhibition of Human Serum Cholinesterase with Fluoride—Recognition of Two New Phenotypes, 496
Whittam, Dr. R. : Active Cation Transport as a Pace-maker of Respiration, 603
Whittle, Dr. E. (Thomas, Dr. J. H., and) : Gas-Phase Reactions of the Halogens and their Compounds, 129
Whittow, Dr. G. C., and others : Increase of Evaporative Loss of Water from the Skin of the Ox in response to Local Heating of the Hypothalamus, 81
Whyte, Lancelot Law : The Atomic Problem—a Challenge to Physicists and Mathematicians, *review* by Prof. R. Eisenschitz, 214
Whyte, Dr. R. O. : Plant Exploration and Introduction, 1240
Widening of Expansion Cracks in Mortar by Salt Solutions (J. A. Roberts and H. E. Vivian), 125, *erratum*, 1145
Widginton, D. W., and others : Ignition of Gas by Sparks produced by Breaking Wires at High Speed, 1085
Wieser, Dr. W. : Copper in Isopods, 1020
Wigglesworth, Prof. V. B. : Sound Production and Sound Reception by Insects, *review*, 428
Wiggs, P. K. C. : Graphite (Elastic Properties of Graphite), 28
Wight, W., and Gilchrist, R. C. J. H. : The Concept of Kind of Tea, 14
Wilbert, Dr. Johannes, and others : New *Rh* Phenotype, *Dcce^{t}e^{t}f*, found in a Chibcha Indian Tribe, 503
Wild Cucumber Mosaic Virus : Unusual Base Composition of the Ribonucleic Acid of (H. Yamazaki and Prof. Paul Kaesberg), 96
Wild White Clover (Kent), *Trifolium repens* L. : Flower Initiation in, under Controlled Environmental Conditions (R. J. Haggar), 1120
Wildlife (African) : New Hope for, 958
Wilford, A. T. : Retirement as director of research, London Transport, 751 ; work of, 751
Wilkes, Dr. M. V. : Digital Computers and Nuclear Reactor Calculations, *review*, 9
  Linear Programming, *review*, 9
  Methods of Feasible Directions, *review*, 9
Wilkins, Malcolm B. (Warren, Diana M., and) : An Endogenous Rhythm in the Rate of Dark-Fixation of Carbon Dioxide in Leaves of *Bryophyllum fedtschenkoi*, 686
Wilkinson, Dr. Jean E. : Appointed Medical Research Council Research Fellow in the Department of Medicine in the University of Leeds, 650
Williams, Dr. E. S., and others : Aldosterone Excretion and Potassium Retention in Subjects living at High Altitude, 78
Williams, Dr. H. H. : Parasite Biology (Marine Parasitology), 134
Williams, I. H. : Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid—Effect on Growth and Development, 361
Williams, I. H., and others : Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid—Effect of Spraying with Gibberellic Acid on Yield and Composition, 360
Williams, M. O. (Hopkins, Dr. C. A., and) : Parasite Biology (Application of New Laboratory Techniques), 135
Williams, Dr. P. M., and others : A Lake in British Columbia containing Old Sea-Water, 830
Williams, Prof. W. T., and Lambert, J. M. : Nodal Analysis of Associated Populations, 202
Williams-Wynne, Colonel J. F. : Appointed a member of the National Parks Commission, 546
Willis, Dr. A. J. : Further Observations on a Filamentous Saprophyte from Wookey Hole Caves, 724
Willis, Dr. Edwin R. (Roth, Dr. Louis M., and) : The Biotic Associations of Cockroaches, *review* by David R. Ragge, 106
Willis, J. B. : Determination of Lead in Urine by Atomic Absorption Spectroscopy, 381
Wills, Pamela (Aylward, Francis, and) : Isolation of Sterol Esters from Human Faeces, 1397
Willson, S. D., and others : N,N'-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and related Compounds—a New Series of Anti-tumour Agents, 611
Wilson, Dr. A. M. M. : Two Phycomycetes isolated from Subcutaneous Phycomycosis in Uganda, 612
Wilson, Dr. A. T. : Carbon-14 from Nuclear Explosions as a Short-term Dating System—Use to determine the Origin of Heartwood, 714
Wilson, Dr. C. : Assessment of Pain (Factors affecting the Placebo Response), 1354
Wilson, Dr. C. W. (Hewitt, Dr. H. B., and) : Awarded the David Anderson Berry Prize for 1961 of the Royal Society of Edinburgh, 229
Wilson, Leslie : The National Reference Library of Science and Invention, 733
Wilson, M. H. : At the Maxwell Colour Centenary Conference (Colour as Light and Colour as Darkness), 11
Wilson, Ronald E. : Two Hundred Precious Metal Years—a History of the Sheffield Smelting Company, Ltd., 1760-1960, *review* by Prof. R. W. K. Honeycombe, 108
Wilson, Dr. S., and Dixon, Dr. G. H. : A Comparison of Cod and Bovine Insulins, 876
Wilson, S. H. (Ellis, Dr. A. J., and) : Hot Spring Areas with Acid–Sulphate–Chloride Waters, 696
Wilson, Dr. Victor J., and Burgess, Paul R. : Changes in the Membrane during Recurrent Disinhibition of Spinal Motoneurones, 918
Wilson, W. E. (Berg, W. G., and) : Formation of Boron Nitride in Diborane-Hydrazine Flames, 380
Wind (Solar) : Terrestrial Accretion from the (Dr. Ronald Frith), 1182 ; (Dr. E. R. Harrison), 1182 ; (C. M. de Turville), 1183
Windhager, Dr. Erich E., and Giebisch, Dr. Gerhard : Comparison of Short-Circuit Current and Net Water Movement in Single Perfused Proximal Tubules of Rat Kidneys, 1205
Winitz, Dr. Milton (Greenstein, Dr. Jesse P., and): Chemistry of the Amino Acids, *review* by Sir Hans Krebs, 1132
Winkler, E. J., and others : A Nemin Assay Procedure, 155
Winkler, Dr. S. : At the International Starch Conference (Effect of certain Cations on Starch), 237
Winsdale, R. (Souter, L. S., and) : Women Engineers in the U.S.S.R., a Study of the Education, Employment and Prospects of Women Engineers in the U.S.S.R. (Caroline Haslett Memorial Trust), 30
Winston, H., and others : Social Dominance in Inbred Mouse Strains, 474
Winter, G. (Martin, R. L., and) : Structures of Polymeric Titanium Ethoxide and Titanium Oxide Ethoxides, 274
Winter Months : Warm-water Species in the Plankton off Newfoundland during (V. Bainbridge), 1216
Winton, Prof. F. R. : Title of professor emeritus of pharmacology (at University College, London) conferred on, 546
Wires : Electrically Exploded (Thermal Radiation Laboratory of the Geophysics Research Directorate of the United States Air Force Cambridge Research Laboratories Conference on), 1250
Wires at High Speed : Ignition of Gas by Sparks produced by Breaking (Dr. R. L. Gordon, Dr. H. Lord and D. W. Widginton), 1085
Wiseman, J. : Title of reader in economics (at the London School of Economics and Political Science) conferred on, 651
Wiskont-Buczkowska, Dr. Halina : Relationship between the Decrease in Urinary Excretion of Histamine and the Initiation of the Labour Process, 290
Wissenschaften, Deutsche Akadamie der, zu Berlin : Völkerkundliche Forschungen, Band 3, Classification of Kin, Age Structure and Marriage amongst the Groote Eylandt Aborigines (Dr. Frederick G. G. Rose), *review* by Dr. R. Needham, 6
Witkus, Dr. E. R., and others : Chromosomal Elimination induced by 6-Aza-Uracil, 627
Witt, Dr. H. T., and others : Experimental Evidence for the Mechanism of Photosynthesis, 194
Witts, Prof. L. S. : Vitamin B_{12} and Intrinsic Factor, 1156
Woad : Indigotin Content of (C. Berkeley), 1414
Woese, C. R. : Non-Random Occurrence of Amino-Acid Replacements, 1196
Wokes, F. : Vitamin B_{12} and Intrinsic Factor (B_{12} Deficiency characterized by Low Serum B_{12}), 1155
Wolf, W. J., and others : Ultracentrifugal Differences in Soybean Protein Composition, 1395
Wolfe, Prof. R. S. (Valentine, R. C., and) : Phosphate-dependent Degradation of Urea, 925
Wolff, H. S. : Design and Use of Respirators (Applications of Respirators in Physiological Research), 655
Wolstenholme, G. E. W., and O'Connor, Cecilia M. (edited by) : Ciba Foundation Symposium on Quinones in Electron Transport, *review* by A. I. Scott, 321
Wolters, Prof. A. W. P. : *Obituary* by Prof. R. C. Oldfield, 646

Women (Pregnant) : Oxytocinase Activity of Plasma of (Stig E. J. Melander), 176
Women Engineers in the U.S.S.R., a Study of the Education, Employment and Prospects of Women Engineers in the U.S.S.R. (L. S. Souter and R. Winsdale), 30
Women Science Teachers, Association of (Science Masters' Association and the) : Science and Education, a Policy Statement, 555
  Chemistry for Grammar Schools, 555
  Biology for Grammar Schools, 555
  Physics for Grammar Schools, 555
Womersley, Dr. H. B. S. : Awarded a Royal Society and Nuffield Foundation Commonwealth Bursary, 126
Wood, R. B., and others : Conversion of L-Galactono-γ-lactone into L-Ascorbic Acid by Plants, 282
Wood, T. : Inversion of a Laplace Transform arising from a Problem in Applied Chemical Kinetics, 589
Wood under Stress : Influence of Moisture Changes on Deformation of (L. D. Armstrong and G. N. Christensen), 869
Wood (Pulp-) : Management of Pine Stands for (J. J. Kotzé), 855
Wood Cells : Staining Reaction of the Pit Membrane of (R. K. Bamber), 409
Wood Extractives (Plant Phenolics Group Meeting on), 229
Woodall, Dr. Mary : Elected president of the Museums Association, 554
Woodlice : Rectal Glands in (Dr. Minati Gipta), 406
Woodliff, Dr. H. J. : Awarded a grant from the Lady Tata Memorial Trust, for research on leukaemia and allied diseases, 228
  Cytochemistry of *L*1210 Cells, 1313
Woods, Prof. D. D. : The Training of Biochemists (The recently devised Four-year Biochemistry Course at Oxford), 956
  Vitamin B_{12} and Intrinsic Factor (Relationships between Vitamin B_{12} and Folic Acid), 1156
Woodward, R. N. (McNaughton, G. S., and) : Barium-140 in New Zealand Rainwater, 1344
Wookey Hole Caves : Further Observations on a Filamentous Saprophyte from (Dr. A. J. Willis), 724
Wool, Dr. Ira G. (Weinshelbaum, Dr. Edward I., and) : Effect of Adrenalectomy and Corticosteroids on Distribution of Radioactivity in Protein of Cell Fractions from Myocardial Slices, 1401
Wool Keratin : Crystallization in (Dr. Allan Skertchly), 138
Wooldridge, Prof. S. W. : The Land and Man in the Western Weald (Presidential Address to the Annual Congress of the South-Eastern Union of Scientific Societies), 132
Wools (Iodinated) : Supercontraction of (O. A. Swanepoel), 908
Work : The Motivation of (F. Herzberg), 1332
Work (The Life and), of William Higgins, Chemist (1763-1825), (Prof. T. S. Wheeler and Prof. J. R. Partington), *review* by Frank Greenaway, 533
World (Changing) : Birds in a (Phyllis Bond), 133
World (Industrial) : Management in the (Prof. F. Harbison and Prof. C. A. Myers), 1332
World (Mechanized) : Growing Old in a (F. Le Gros Clark), *review* by Prof. L. S. Hearnshaw, 532
World Communications (Sir Gordon Radley), 987
World Federation for Mental Health : Grant from the Gulbenkian Foundation, 754
World Federation of Neurology (Neurochemical Commission) : International Symposium on Enzymic Activity of the Central Nervous System, 1349
World Health Organization (United Nations) : Technical Report Series No. 215, The Planning of Public Health Services—Fourth Report of the Expert Committee on Public Health Administration, 441
  Technical Report Series No. 193, Teacher Preparation for Health Education—Report of a joint WHO/Unesco Expert Committee), 441
  Technical Report Series No. 221, Scientific Meeting on Rehabilitation in Leprosy—Report, 1248
  Public Health Papers No. 9, Teaching of Psychiatry and Mental Health, 1248
  Public Health Papers No. 10, Control of Soil-transmitted Helminths (Prof. Paul C. Beaver), 1249
  WHO Chronicle, Vol. 15, No. 7, Health in Yugoslavia, 1260
  European Standards for Drinking Water, 1261
World Health Organization (United Nations), and others : Conference on the Use of Radioisotopes in Animal Biology and the Medical Sciences, 959
World Orchid Conference (sponsored by the American Orchid Society, the British Orchid Growers' Association and the Royal Horticultural Society, London, 1960) : Proceedings of the Third, *review* by V. S. Summerhayes, 213
World Population Trends, 1346
World Problem : Public Health as a, 441
World University Service (Hilda M. Browne), 1140
World-Wide Circulation of Air within the Stratosphere (P. Goldsmith and F. Brown), 1033
Worshipful Society of Apothecaries of London : Second Annual Report and List of Members (1960-61) of the Faculty of the History of Medicine and Pharmacy, 21
  Maccabaean Prize and Medal for 1962, 650
Wright, J. K., and others : A New 'Wedge' Type Instability observed in a 'Theta' Pinch, 1054
  Possible Method for Increasing the Signal-Noise Ratio in the Detection of the First Motion of a Refracted *P*-Wave, 1382
Wright, J. S., and others : Determination of Uranium as Pyrophosphate, 1291
Wright, Dr. Norman C. : At the Third International Congress of Dietetics (International Collaboration in the Solution of Problems of Human Diet), 1343
Wright, Quincy, and Leinau, C. C. (edited by) : Statistics of Deadly Quarrels (Dr. Lewis F. Richardson), *review* by Sir Cyril Burt, 427
Wright, Prof. W. D. : The Maxwell Colour Centenary, 10
Wrinch, Dr. Dorothy : Chemical Aspects of the Structure of Small Peptides, *review* by Prof. H. N. Rydon, 105
Writing (Technical) : The Technique of (Borough Polytechnic, London, lectures on), 755
Wyard, S. J. (Russell, D. B., and) : Electron Spin Resonance of Adenosine Triphosphate, 65
Wyard, S. J. (Smith, Robert C., and) : Spatial Distribution of Free Radicals as studied by Electron Spin Resonance, 897
Wyatt, Dr. H. V. : Biology in Britain, 960
Wye and Crundale Downs Nature Reserve (Nature Conservancy), 1248
Wynne-Jones, Prof. W. F. K. : Graphite (Physical and Chemical Studies of the Graphitization Process), 28
Wyszecki, Dr. G. W. (Stiles, Dr. W. S., and) : At the Maxwell Colour Centenary Conference (The Spectrum divided into *M* Small Wavelength Intervals), 10

X-CHROMOSOME of *Drosophila melanogaster* : Recombination in the (C. N. Law), 1180
X-Irradiation *in utero* : Leukaemia in the *AkR* Mouse after (Donald Porteous), 291
X-Ray Analysis of Diamond : Synthesis and (H. Ll. D. Pugh, J. Lees and J. A. Bland), 865
X-Ray Diffraction Spikes of Diamond (Y. Yoneda), 1187 ; (Prof. F. C. Frank), 1188
X-Ray Diffraction Studies of Bacterial Viruses (Dr. A. C. T. North and Prof. Alexander Rich), 1242
X-Ray Microscopy (Dr. V. E. Cosslett and Dr. W. C. Nixon), *review* by Prof. Arne Engström, 1332
X-Ray- and Nitrous Acid-induced Mutations in the Genetic Fine Structure of the ad_7 Locus of *Schizosaccharomyces pombe* : Distribution of (Dr. Herbert Gutz), 1127
X-Rays : Chromosome Rearrangements induced by, in Immature Germ Cells of *Drosophila* (Seymour Abrahamson), 523
  Formation of 5-Hydroxytryptamine in an Aqueous Solution of 5-Hydroxytryptophan treated with (M. Jović and Z. Supek), 383
Xanthium pennsylvanicum : Photoperiodic Effects on Stomatal Behaviour in (Dr. T. A. Mansfield and Prof. O. V. S. Heath), 974
Xanthomonas and *Pseudomonas* : Taxonomy of (R. R. Colwell and Prof. J. Liston), 617
Xenopsylla cheopis Roths. : An Abnormal Form of Female Rat Flea (M. I. D. Sharma and G. C. Joshi), 727

YAGI, Prof. Kunio, and others : Hydrolysis of Fatty Acid Esters of Riboflavin by Pancreatic Lipase, 174
Yalow, Dr. Rosalyn S. (Berson, Dr. Solomon A., and) : Immunochemical Distinction between Insulins with Identical Amino-Acid Sequences, 1392
Yamaguchi, Saburo, and others : Controlling Mechanism of the Mechanical Activity of Muscle and that of Blood Coagulation, 80
Yamamoto, H., and others : Incorporation of 5,10,15 ^{14}C-Farnesol Pyrophosphate into *Phycomyces carotenoids*, 1299
Yamamoto, Yaskazu Lucas : Biological Effectiveness of Thermal Neutrons and of Heavy Particles from the $^{10}B(n,\alpha)^{7}Li$ Reaction, 1258
Yamamoto, Yoshiko, and others : Hydrolysis of Fatty Acid Esters of Riboflavin by Pancreatic Lipase, 174
Yamanouchi, Toshihiko (Matsuno, Takao, and) : A New Triterpenoid Sapogenin of Animal Origin (Sea Cucumber), 75
Yamashita, Satoru, and others : Synaptic Transmission in Neurosecretory Cells, 183
Yamazaki, H., and Kaesberg, Prof. Paul : Unusual Base Composition of the Ribonucleic Acid of Wild Cucumber Mosaic Virus, 96
Yamazoe, Saburo, and others : A Positive Feedback Mechanism of Adenosine Triphosphate Synthesis in Erythrocytes, 283
Yannas, J. B., and Gonzalez, R. N. : A Clear Instance of Rheopectic Flow, 1384
Yasuda, Masahide, and others : Controlling Mechanism of the Mechanical Activity of Muscle and that of Blood Coagulation, 80
Yatzidis, Dr. H. : Determination of Spontaneous Plasma Fibrinolysis by a Practical Procedure, 604
Yeast : Changes of Permeability of, with Sodium Deoxycholate (Dr. L. Kováč), 698
  Sub-Unit of Ribosomal Ribonucleic Acid from (Eiko Otaka, Yukito Oota and Syozo Osawa), 598
Yeast Protoplasts : Fixation of, for Electron Microscopy (Dr. P. F. Elbers), 1022
Yellow A^{y}/a Mice : 5-Methyltryptophan and Darkening of the Hair in (Dr. Vivianne T. Nachmias), 934
Yen, Dr. D. E. : Evolution of the Sweet Potato (*Ipomoea batatas* (L.) Lam.), 93
Yield and Composition : Effect of Spraying with Gibberellic Acid on (Treatment of Hops (*Humulus lupulus* L.) with Gibberellic Acid), (R. Stevens, J. B. Roberts and I. H. Williams), 360
Yllner, Dr. Sven : Urinary Metabolites of ^{14}C-Tetrachloroethylene in Mice, 820
Yoffey, Prof. J. M. : Illustrations of Human Anatomy, *review*, 1232
Yokoyama, H., and others : Incorporation of 5,10,15 ^{14}C-Farnesol Pyrophosphate into *Phycomyces carotenoids*, 1299
Yokoyama, Izumi : Gravity Survey on the Aira Caldera, Kyusyu, Japan, 966
Yoneda, Y. : X-Ray Diffraction Spikes of Diamond, 1187
Yong, Dr. Raymond, and Selig, E. T. : Role of Interparticle Forces in Development of Upper Plastic Limit of Clays, 938
Yonge, Prof. C. M. : Life and Environment on the Bed of the Sea (Presidential Address to Section D (Zoology) of the British Association for the Advancement of Science), 986
Yorkshire : The Place-Names of the West Riding of (Prof. A. H. Smith), Part 1, Lower and Upper Strafforth and Staincross Wapentakes ; Part 2, Osgoldcross and Agbrigg Wapentakes : Part 3, Morley Wapentake, *review* by Prof. H. C. Darby, 208
  The West Riding of, *review* by Prof. H. C. Darby, 208
Yorkshire Philosophical Society : Report for 1960, 442
Yoshikawa, Haruhisa, and others : A Positive Feedback Mechanism of Adenosine Triphosphate Synthesis in Erythrocytes, 283
Yoshinaga, Dr. Kaoru, and others : Quantitative Determination of Metadrenaline and Normetadrenaline in Normal Human Urine, 599
Young, Dr. L. E. : Blood Groups in Infra-human Species (Blood Group Systems in the Dog), 653
Youth Federation (International), for the Study and Conservation of Nature : Course in Conservation, by the Verein Naturpark in the Lüneburger Heide, Germany, 1045
Ytterbium in Aqueous Solutions and in Thulium Oxide : Rapid Determination of, by Neutron Activation (Minoru Okada), 1090

Yudkin, Prof. J. : At the Third International Congress of Dietetics (Diet and Cardiac Ischaemia), 1344
Yugoslavia : Health in (United Nations, World Health Organization, *WHO Chronicle*, Vol. 15, No. 7), 1260

ZAJDELA, F., and others : A New Family of Potent Carcinogens—Benzopyridocarbazoles, 1005
Zaki, K. (Mohamed, Prof. A. H., and) : Effect of Bee Venom on Concentration of Blood Glucose and Liver Glycogen in Rabbits, 605
Zangwill, Prof. O. L. : Cerebral Dominance and its relation to Psychological Function, *review* by Sir Russell Brain, 840
  Assessment of Pain (Assessment of Pain in Man), 1353
Zarek, Dr. J. M. : Title of reader in engineering science (at King's College, London) conferred on, 651
Zeiss, Carl (Jena) : Fourteenth International Instructional Course at the Imperial College of Science and Technology, London, on Fine Measuring Instruments, 1045
Zeldovich, Ia. B., and Kompaneets, A. S. : Theory of Detonation, *review* by Dr. W. L. Murray, 1236
Zenk, Dr. M. H. : 1-(Indole-3-acetyl)-β-D-Glucose, a New Compound in the Metabolism of Indole-3-acetic Acid in Plants, 493
Zero-valent Platinum : Complex Compounds of (Dr. J. Chatt and G. A. Rowe), 1191
Zhongolovich, I. D., and Amelin, V. M. : A Collection of Tables and Nomograms for the Processing of Observations made on Artificial Earth Satellites, *review* by D. G. King-Hele, 1135
Zhukovsky, Prof. P. N. : Plant Exploration and Introduction (Gene Resources of the World), 1241
Ziauddin, Syed : A Method of Estimating Ionospheric Absorption Heights from Recovery Times, 1084
Ziegler, F. D., and others : Oxygen Electrode for Measurement of Tissue Slice Respiration, 969
Zimmer, Dr. K. G. : Effects of Ionizing Radiation on Organic and Biological Solids (Role of Oxygen in Radiation Damage), 1150
Zimmerman, Dr. M., and Celozzi, Evemarie : Stimulation by Heparin of Parenchymal Liver Cell Proliferation in Normal Adult Rats, 1014
Zinc (High-purity) : Determination of Indium in (Naïm Košaric and the late G. Leliaert), 703
Zinc Implants in Rat Bladders : Incorporation of Phosphorus-32 into Urinary Concretions forming on (Dr. Richard F. Keeler and Dr. Stuart A. Lovelace), 715
Zinnemann, Dr. K. : Manual of Bacteria and Actinomyces, *review*, 1333
Zirconium and Hafnium Oxides : Reduction of (F. K. McTaggart), 1192
Zoological Society of London : Annual Awards, 649
Zoologie, Société Royale de (Anvers) : Bulletin No. 20, Swimming Ability of Non-aquatic Reptiles (R. R. Tercafs), 125
Zotterman, Prof. Yngve : Assessment of Pain (Specificity of Cutaneous Nerve Fibres), 1353
Zotterman, Prof. Yngve (Konishi, Jihei, and) : Function of Taste Fibres in the Carp, 286
Zoutendijk, Dr. G. : Methods of Feasible Directions, a Study in Linear and Non-Linear Programming, *review* by Dr. M. V. Wilkes, 9
Zuckerman, Avivah : Immunity to Protozoa (Immunity in Malaria), 552
Zuckerman, B. M. : Nematology (Radiotracer Techniques using Carbon-14 to estimate the amount of Plant Fluid ingested by a single Nematode), 1049
Zuelzer, Dr. Wolf W., and Robinson, Abner R. : Haemoglobin *F* and the Genetic Control of Protein Structure, 609
Zuppinger, K., and others : Diagnostic Significance of Heterogeneous Lactic Dehydrogenases in Malignant Effusions, 507

Subscriptions

The charges for direct postal subscriptions to "NATURE" are

12 months	... ...	£10	0	0
6 months (26 issues)	... ...	£5	0	0
3 months (13 issues)	... ...	£2	10	0

Postage paid, to any part of the world

* * *

All subscriptions are payable in advance

* * *

Volumes begin in January, April, July and October of each year, but subscriptions may commence with any issue

* * *

Payment from abroad should be made by
International Money Order or by Cheque
payable at a London Bank to Macmillan & Co., Ltd.

* * *

MACMILLAN & CO., LTD.,
St. Martin's Street, London, W.C.2

Printed in Great Britain by FISHER KNIGHT & CO. LTD., The Gainsborough Press, St. Albans,
and published by MACMILLAN & CO. LIMITED, at St. Martin's Street, London, W.C.2,
and ST. MARTIN'S PRESS, INC., 103 Park Avenue, New York 17, N.Y., U.S.A.
Saturday, December 9, 1961

PHYSICS AND ELECTRONICS LIBRARY

THE NATIONAL REFERENCE LIBRARY FOR SCIENCE AND TECHNOLOGY

ABOUT a year ago, on May 31, 1960, the Minister for Science, in reply to a question in the House of Lords, announced that the National Reference Library of Science and Invention, which he had already stated on July 30, 1958, was to be housed in a new Patent Office building to be erected on the South Bank in London, is to be formed as part of the British Museum Library; it was to be housed as a separate unit, and scientific publications at present in the British Museum Library would be incorporated in the new Library. Lord Hailsham then said that the terms of a lease of the site were being settled between the Ministry of Works and the London County Council, and that it was hoped to start building in the spring of 1963, with the view of completion by the end of 1965. Some further information was supplied by the Parliamentary Secretary for Science, Mr. Denzil Freeth, in replying for the Government in an adjournment debate on the subject on March 14, 1961; further, the Association of Special Libraries and Information, in view of the importance of the issues involved, has devoted almost the whole of the March 1961 issue of the *Journal of Documentation* to a symposium of important papers on the subject.

In the first of these, Prof. R. S. Hutton, writing with the authority of his experience on the Council of the Association of Special Libraries and Information Bureaux, and as a user of the Patent Office Library for nearly sixty years, reviews the contribution which that Library has made to the needs of the scientist and technologist, its decline in standards since 1914, and the official procrastination both before and after the Second World War. The difficulties and problems which any such library must face are fairly though briefly stated, but Prof. Hutton lays the responsibility for the present situation unequivocably where it belongs—on the shoulders of successive Governments which have refused to face the issues clearly presented to them by their own advisers. In the second paper, Mr. E. M. Nicholson, director-general of the Nature Conservancy and chairman of the Scientific Library and Technical Information Committee of the Advisory Council on Scientific Policy, reviews in greater detail the steps which, after the abandonment of the proposal for a 'science centre' in London, issued in the present project. He is no more inhibited than Prof. Hutton in his criticism of Government delays, and it is significant that, in welcoming the physical link of the new library with the Patent Office in occupying the same building, he gives as one reason the fact that the London County Council has made it a condition of providing this site that building should begin in the near future. This, Mr. Nicholson quietly remarks, provides the best possible safeguard against a continuation of procrastination in getting on with the job. For all the lip service which Governments of both political parties have paid to the need to support scientific and technical research and its application, and to expand the number of scientists and technologists, it is, in fact, difficult to parallel the record of neglect, indifference and waste of public money and talent which has characterized official policy in this field during the past fifteen years.

A second reason which Mr. Nicholson gives for welcoming the new site is its proximity with a great complex of offices of one of the largest industrial concerns in Europe. This, he rightly points out, should make contact with users easier. Further, he suggests that had adequate contact been maintained between scientific libraries and their users, these libraries would not, alone among scientific institutions, have failed to benefit by the greatly increased resources recently made available for science. At the top level, this lack of contact has meant that even the high prestige and assiduous efforts of the Advisory Council on Scientific Policy have barely been able to neutralize the apathy and inertia which beset almost every effort to secure greater resources for the national institutions of Britain in the library field.

That is putting it generously, for not every informed observer would concede as much. Moreover, Mr. Nicholson emphasizes that information and advice have had in consequence to be obtained from a much more limited range of sources than would be expected in view of the number of interests and institutions potentially concerned; and he suggests that there is virtually no body of senior people professionally trained both as scientists and librarians who could form a source of man-power. That proposition is more open to debate, however, though admittedly on any definition there are few enough such senior people. Responsibility, however, lies to some extent with the profession itself: the perennial discussions on training intelligence or information officers and librarians have not always been either so profitable or constructive as they might have been.

Whether the work that has gone into the launching of the National Lending Library for Science and Technology will be of some benefit to the Reference Library also remains to be seen; but Mr. Nicholson is possibly a little optimistic in looking to the advisory committee under Sir Lindor Brown's chairmanship. It is precisely in respect of the representation of industrial users that the composition of that committee has been criticized. Prof. Hutton, who equally stresses the importance of the users expressing their point of view on the full-scale needs of these libraries, looks rather to the Association of Special Libraries and Information Bureaux, and urges that advisory committees for each library, and opportunities for those who use the libraries to voice their needs, are most desirable. Mr. Freeth, on March 14, explicitly committed the Government to the establishment of an advisory committee for the new library.

Mr. Nicholson lays some stress on the recruitment of suitably qualified scientific staff with adequate competence in librarianship, and is disturbed that recruitment should seriously be contemplated of librarians who know no science or scientists with no training in librarianship. On this point there has been some criticism of the policy adopted for the National Lending Library, on which Dr. D. J. Urquhart does not appear to have entirely satisfied his critics. On Mr. Nicholson's further point that, in organizing the accessibility and communication of this vast harvest of scientific research, we should be virtually devoid of research results and advice in the provision and organization of scientific libraries, some reassurance is to be found in Mr. Freeth's reply on March 14. The Government is already considering how best research into information techniques can be promoted, and it is fully agreed that the new Library should co-operate in this kind of research in any way in which it may be able.

In the meantime, however, the National Science Foundation in the United States is very actively promoting such investigations, and a touchstone of the Government's sincerity in this particular respect may well be found in the extent to which the Association of Special Libraries and Information Bureaux is enabled to undertake similar investigations. This, of course, is not to deny the importance, recognized by Mr. Freeth, of sound judgment in the selection of projects. The formulation and selection of projects, moreover, need not await the coming into operation of the new Library, and, as Mr. Nicholson's paper sufficiently indicates, there are many matters on which we are as yet too inadequately informed for sound decisions as to the equipment and operation of a first-class modern scientific library. That could well be totally different from libraries as even many librarians now visualize them.

In view of the importance of co-operation between user and library, the remaining two papers in the symposium are of special interest. Miss Maysie Webb, who in April 1960 was appointed head of the Patent Office Library with this preparatory work in mind, reviews the pattern of usage of the present Library and the plans for the future; and Mr. A. H. Chaplin, keeper in the Department of Printed Books, British Museum, to which the new reference Library will be attached as a division, considers some of the problems arising out of that relation. Mr. Freeth, on March 14, also referred to the work being done on the internal arrangement of the new Library by Mr. Wilson, of that Department, and by Sir Frank Francis, director of the British Museum, who himself is a member of the library committee of the Advisory Council for Scientific Policy. He rejected for the present Mr. Austen Albu's suggestion of a special advisory committee, and recommended that suggestions which scientists, industrialists, librarians or others may have for the new Library should be sent direct to the people he had named as concerned with its planning.

Miss Webb begins by directing attention to three broad principles on which the operation of the Patent Office Library has been based in the past. First, it has been recognized that in an economy increasingly geared to science and technology, it is prudent to make freely available a store of such knowledge, thus providing the scientist with his raw materials, stimulating invention and facilitating the work of the technical community in general. This outlook is reflected in the 'open-access' facilities, and Miss Webb states explicitly that the whole of the new South Bank library premises will be designed with open access as a basic requirement. This facility has been a most valuable feature of the Patent Office Library; while Miss Webb also indicates that the Library staff will play a more active part in exploiting the resources of the new Library and in helping users at all levels, her paper will be warmly welcomed by scientists and technologists for this firm assurance alone, with its corollary that seating accommodation for at least half the expected number of readers, close to or among the bookshelves, is to be provided.

Miss Webb, moreover, is well aware of the frustration which can arise from the lack of immediacy in a closed-access system, and there is already some uneasiness on this score in view of the fact that this new reference library is to be associated with the British Museum. It was recently pointed out in the House of Commons that, while every user of the British Museum Reading Room greatly appreciates the efficiency, courtesy and ability of the staff, it still takes nearly an hour and a half to obtain a book compared with 12 or 15 min. at the Bibliothèque Nationale in Paris. Miss Webb's paper offers some assurance on this point, and it is evident, too, from Mr. Chaplin's paper that the importance of the time factor in the new arrangements is fully appreciated on the British Museum side. Although many administrative questions have still to be resolved, the final arrangements will be such as to support fully the open access principle and provide rapid and frequent transport between the two libraries for handling the marginal material or older material of only occasional usefulness transferred to the British Museum itself.

The third principle emphasized by Miss Webb is that the Patent Office Library has been concerned in the past with the field of invention, catering in general for the graduate or practising scientist. The new library will broaden the range and thus alter the balance, and in discussing plans for the future Miss Webb refers to the possibility of increasing the depth as well as the range of subjects covered, particularly in respect of material in foreign languages, technical trade literature and translations. It is also hoped that much extra material will be available as a result of the link with the British Museum, though legislation will be needed before this material could be available for consultation at short notice.

This aspect is discussed more fully by Mr. Chaplin, who visualizes the British Museum playing an important part in research in the history of science, provided the legislation referred to by Miss Webb is enacted and that the British Museum is assured of sufficient storage space to receive the material discarded by the Reference Library. Moreover, while he emphasizes the need for rapid and frequent transport between the two Libraries, he recognizes that there are other means of meeting urgent demands which require attention; such devices as the telegraphic transmission of photo-copies or closed-circuit television may be instanced. Like Miss Webb, Mr. Chaplin recognizes that documentation activities should have an important place in the programme of the new library, and he visualizes this as a more specialized extension of work already undertaken by, or in close association with, the British Museum. Miss Webb also referred to the preparation of subject bibliographies and selective reading lists, and it is clear that she contemplates that the new Reference Library will be staffed so as to admit of such activities to a certain extent.

How far such activities will materialize remains to be seen. At present only two of the staff of the Patent Office Library are science graduates and nine professional librarians. While it is not expected that specialists in all the subjects to be covered will be recruited, even in the planning period of the next few years it would seem that there is room for a good deal of improvement in the proportion of academic and professional staff. Miss Webb appears to be well aware of the problem, however, and to recognize that such staff is needed long before the new Library begins to operate.

Meanwhile, Miss Webb puts the emphasis on a detailed research programme, and it should be noted that she looks to find some of the answers to questions about the new Library and its readership in co-operation with its users. The Advisory Council on Scientific Policy has already recommended that research into the whole problem of collation and dissemination of scientific information should be undertaken under the auspices of the new National Lending and Reference Libraries for Science and Technology, and Miss Webb recognizes that, given all the co-operation of users, this work should be proceeding already and requires the recruitment of staff for the purpose.

On that point Mr. Freeth was less reassuring than he might have been. He agreed that the new Library needed a sufficient staff with academic training capable of extending their fields of knowledge in a scientific direction, and that it should have an expert staff capable of providing a first-rate information service for those using the library. But he appeared to think that the matter of research into information techniques and the like could be left until the new Library is operating. As already indicated, this will be a test of the reality of the Government's interest in the matter, and in this connexion the concluding paragraph of Mr. Nicholson's paper should be considered.

"Let us hope", he wrote, "that those who now have to put into execution this vital project will succeed in realizing their opportunities to the full. Let us also remember that until we see the National Science Reference Library opened we can never feel actively confident that there will not be some other slip between the cup and the lip. Anyone who may be tempted to jog the arm that lifts the cup should, therefore, think twice about it." From one who has been so closely associated with the Scientific Library and Technical Information Committee of the Advisory Council on Scientific Policy, such a caution is the more significant. It is clear that Miss Webb and those associated with her in planning the new Library and its services have sensible views of its functions and possibilities, that they are aware of the potentialities of new techniques, as well as of the solid advantages of some practices of the past, and that they are anxious to secure the co-operation of all potential users. Such co-operation should be given generously and imaginatively, but more than constructive criticism is required. Let it not be forgotten that Government parsimony and short-sightedness could yet once again jettison the whole scheme, that procrastination in dealing with needs as they arise could multiply costs once again out of all proportion and destroy efficiency. It is for users, therefore, to co-operate also in seeing that at all levels the Government is kept aware of the magnitude and urgency of the problem, and the needs of the new Library are clearly and continuously presented throughout the planning period and when it is fully operational. In that task not only the individual scientists and technologist but also their professional associations, and such bodies as the Association of Special Libraries and Information Bureaux, the Parliamentary and Scientific Committee and the Advisory Council for Scientific Policy, have a responsibility which should be vigilantly and resolutely discharged.

EXPLOSIVES TECHNOLOGY

Solid Propellent and Exothermic Compositions.
By Dr. James Taylor. (Newnes Technical Surveys). Pp. vii + 153 + 12 plates. (London: George Newnes, Ltd., 1959). 25s. net.

THIS book provides a very useful and interesting survey of the development of exothermic chemical reactions in the solid state as sources of energy for many applications and devices. It contains broad reviews of modern developments in initiators, explosives and propellants. The author, Dr. James Taylor, has spent the greater part of his working life in the explosives industry; he has made many personal contributions to the developments described, and has drawn freely on the work of his colleagues in the Nobel Division of Imperial Chemical Industries, Ltd.

There is a chapter devoted to the history of gunpowder, its applications as a propellant and its present-day use in ignition and delay systems, fireworks and military pyrotechnics. Explosives are rather briefly reviewed—the author dealt comprehensively with the performance of commercial blasting explosives in his monograph *Detonation in Condensed Explosives* (1950). There is a description of the various types of initiating explosives (detonators, fuse-heads and percussion primers) and the modern types of propellants used in small arms and guns are reviewed; this is followed by an interesting account of the gas-producing reactions that have been used in devices to improve the safety of blasting operations. The chapter on rocket motors is particularly well written, and there is an extremely good review of "power cartridges", starting with the "humane cattle killer", which was developed just after the First World War, and leading up to the numerous devices which have been developed in the past thirty years for operations such as starting aircraft engines, inflating life-jackets, propelling model aircraft, etc. All these applications call for a high level of reliability and this has been achieved in spite of the complexity of many of the systems. In each of the fields covered, the thermochemical and ballistic aspects are very adequately dealt with for a proper understanding of the design principles and working of the applications described.

It is, of course, unfortunate that the contributions by Government research organizations towards many of the developments described cannot be dealt with because much of the subject-matter is of a classified nature and this leads, as the author points out in the preface, to some lack of balance in the over-all treatment. However, the book achieves its aim and is warmly commended to those seeking an introduction to branches of explosives technology that have greatly increased in importance in recent years.

W. B. LITTLER

SOME BACTERIAL PLANT DISEASES OF CENTRAL EUROPE

Bacterial Plant Pathogens

By C. Stapp. Translated by A. Schoenfeld. Pp. xviii+292. (London: Oxford University Press, 1961.) 42*s*. net.

DURING the past sixty years the knowledge of plant diseases has been enlarged at an ever-increasing rate, mainly due to the careful investigations of German plant pathologists, which were collected together by Paul Sorauer and appeared in 1928 in the third edition of his *Handbuch der Pflanzenkrankheiten*. As new knowledge accumulated, larger editions were called for, and in each one Dr. C. Stapp contributed the section dealing with bacterial diseases of plants. In the 6th edition, issued in 1956, the material to be dealt with was so great that Dr. Stapp's account was published as a separate volume of the *Handbuch* entitled *Bakterielle Krankheiten*. This great work by so well-known and so highly esteemed an investigator contained accounts of, and references to, all recorded diseases up to 1955 and was admirably illustrated.

The advances in this subject made in the past ten years, the great interest shown by both university and high-school teachers, and the prohibitive price of his *Bakterielle Krankheiten* induced Dr. Stapp in 1958 to bring out a less-expensive book based largely on his previous comprehensive work. It also included accounts of some twenty-four diseases occurring in Europe, with references up to 1957. It was printed on highly glazed white paper on which the numerous photographic illustrations were clearly reproduced and it concluded with a very complete index. The title was *Pflanzenpathogene Bakterien*.

Now, in 1961, this admirable work has been rendered into English because, as Dr. Stapp indicates in the English preface (also dated 1958), besides the rising interest in Germany, a like desire for information on the bacterial diseases of plants has manifested itself in "other Central and Western European countries and also in some countries outside Europe". It is certainly flattering to know that plant pathologists of these "other" countries apparently prefer English to German. The translator has produced an elegant, though not always accurate, rendering of Dr. Stapp's German and includes his very few errors such as *Pseudomonas delphinii* being pathogenic only to the Cruciferae (Stapp, p. 12, Schoenfeld, p. XII) and *Prunus devaricata* for *P. divaricata* (Stapp, p. 177, Schoenfeld, p. 188).

The English version follows exactly the arrangement of the German edition, the chapters, headings and sectional paragraphs are in the same order, and, as in the original, there are two parts, a general part concerned with methods and identification and a special part describing in detail some twenty-four plant diseases. Both parts are equally instructive. In the first we have Dr. Stapp's own bacteriological methods, some of which are rather unusual. He prefers steaming to autoclaving, neutralizes his media either with sodium carbonate or with dilute phosphoric acid and stains his smears on cover-slips, not on slides. The method, which is illustrated, of making transfers of pure cultures whereby both tubes and their extracted plugs are manœuvred into the same hand is not a common practice in Britain nor is it universal in Germany. A slight error occurs in both the German and English editions in the description of sterilizing Crosse and Bennett's selective medium in which the authors state (*Trans. Brit. Myc. Soc.*, 38, 83; 1955) "and sterilized by bringing the pressure slowly (3–5 min.) up to 7 lb." Dr. Stapp's version (p. 26) is "Druck langsam auf 1 atü [that is, 1 atmosphere which equals 15 lb. not 7] bringen und darauf 3–4 Minuten halten". In the reference the abbreviation *Trans.* (*Transactions*) is omitted in both editions (Schoenfeld, p. 10).

In the special part the descriptions of the diseases and their pathogens vary in length, that dealing with crown gall (*Agrobacterium tumefaciens*) being the longest, and containing clear accounts of the various theories of tumour-formation. In the chapter on black-leg of potato nearly 5 pages are devoted to Dr. Stapp's arguments for naming the pathogen *Erwinia phytophthora* (Appel) Holland in preference to *E. atroseptica* (van Hall) Jennison. Of most interest perhaps at the moment is the chapter on fireblight caused by *E. amylovora*, which has only recently been recognized in southern England. The account of plum and cherry canker caused by *Pseudomonas mors-prunorum* (spelt in one word—*morsprunorum*) is thoroughly up to date; the wilt of beans due to *Corynebacterium flaccumfaciens* is also included although it does not occur in Germany.

Some of the phrases used by the translator are apt to be misleading and some are quite inaccurate. As examples, on p. 2 we read about "Koch's steam-pot", a literal translation of "Koch'schen Dampftopf" (p. 19), meaning steam sterilizer or just steamer: on p. 3 is "if the nutrient medium is not bottled immediately", but Dr. Stapp writes (p. 19) "nicht gleich in Röhrchen [that is, tubes] abgefüllt": on p. 4, "The meat infusion is measured and weighed", whereas Dr. Stapp would do one or the other (p. 21, "abgemessen oder abgewogen"). On p. 16 is "breed pathogens from" for isolate in pure culture (German p. 31 "reinzuzüchten") and in the footnote on the same page we find "incubated . . . for the first time" for isolated . . . for the first time (German p. 32, "erstmalig . . . herausgesüchtet"). On the same page occurs "Koch's dishes" for the German (p. 31) "Koch'schen Platterverdünnungen" (that is, Koch's dilution plates). On p. 19 "woodland fruits" is the translation of "Obstgehölze" meaning fruit trees, and later on the same page is "the inserted graft is covered with phloem tissue" for the German (p. 34) "Bast" meaning bast fibre (used by horticulturists).

At the end of section 6 this occurs—"every biologist should acquire during his working life", which is not quite the same as Dr. Stapp's statement (p. 35) "jedem Biologen eigen sein sollte" which might be better rendered—every biologist should naturally possess.

In many places when describing the action of bacteria in milk the German word "peptonisiert" becomes "peptone formed", which may be biochemically correct, but the more common English term would be peptonized or digested or even cleared. On p. 21 the German (p. 37) "auf der letzten Platte" (on the last plate) is rendered "at the bottom of the last plate" and on p. 22 we find the German (p. 37) "rechtwinklig" (right angle) becomes "rectangle"! On p. 55 it is actually stated that "freshly diseased blackleg potato shoots are easily obtainable from the fields throughout the year . . ." an utter impossibility! But Dr. Stapp writes "alljährlich", that is, every year.

Apart from incorrect and misleading statements it would seem that the translator is not too well acquainted with English bacteriological terminology.

A few printing errors have been noted, for example, on p. iv "bakterian" appears for "bakterien" and on p. 146 "See p. 9" should be "See p. 17", p. 236 is followed by p. 137 and in Table 4 on the same pages, *Nicotiana lustica* should be *N. rustica.*

The book concludes with a very comprehensive index after Dr. Stapp's style, but a misprint occurs on p. 286 where *Pseudomonas populans* is printed for *P. papulans*. The numerous references in the text do not all appear at the end of each chapter and for the missing ones recourse has to be made to Dr. Stapp's large volume *Bakterielle Krankheiten.*

Although beautifully produced, the illustrations are not as clear as those of Paul Parey, probably owing to the less highly glazed surface of the cream-coloured paper.

This is not a book which may be recommended for the inexperienced worker as his only source of information. W. J. Dowson

AN APPRAISAL OF PHENOMENOLOGY

The Phenomenological Movement

A Historical Introduction. By Dr. Herbert Spiegelberg. (Phænomenologica, Vols. 5 and 6.) Vol. 1: Pp. xxxii + 392. Vol. 2: Pp. ix + 393–735. (The Hague: Martinus Nijhoff, 1960.) 47.50 guilders the two volumes.

These two volumes, erudite and well produced, set out to offer a historical introduction to that branch of philosophy commonly known as the phenomenological movement. Its chief exponent, Edmund Husserl (1859–1938), would have preferred some such terms as 'circle' to 'movement', but to English-speaking students the difference is immaterial. In fact, the author is at pains to present his work in a form most helpful to readers in the United States: they seem to need a bridge to span the gap between their own intellectual climate and that of Europe. Not that they lack competent exponents; yet the background is very different from what is basically an admixture of *Gestalt* psychology and *Geisteswissenschaften.* Biographers need no reminding that Husserl started as a pupil of the great Weierstrass, and therefore cannot be said to have been devoid of mathematical sensitivity. It is probably true, however, that in claiming phenomenology as fundamental and prior to all scientific thinking he gave an impression of detachment from techniques which might, in a more narrow sense, be said to belong to the natural sciences as we know them. Nevertheless, "back to the things themselves" is not a bad imperative for any laboratory, especially when to it is added the need to orientate ourselves towards phenomena in front of which theoretical patterns have become too obtrusive. That, in brief, is the phenomenologist's aim. Several schools of thought have wrestled with this problem before, but the present attempt is focused more sharply.

Although somewhat negative, one important characteristic of the *Methodik* must be mentioned. It is the critical outlook which emerges in relation to Occam's razor. Probably few devices have been more misconceived and misconstrued. Simplicity and parsimony are all very well as disciplines, but the former is to be distrusted if it means little else than an artificial scraping to the bone of our concepts, and the latter when it leads to a degree of abstraction utterly at variance with the true pattern of Nature. Incidentally, this is why the razor is not the correct tool for—say—rheology and morphology. Here, odd as it may seem, parameters and constants can well be introduced liberally, even if later on their number can be substantially reduced and their scope narrowed. As Dr. Spiegelberg remarks, reverence and the lens are more fitting instruments than a bulldozer. The age-long parable of the wheat and the tares is directly applicable—"Let both grow together until the harvest".

All of which is but to recognize that every type of perception should be given a chance, including even those not necessarily involving the presence of a sense-organ for their registration. Of course, the eye, as the window of the mind, remains paramount, but *gestalten* other than visual are known, and are fully worthy of a place.

The 'feel' of these pages has been deliberately presented in advance of a factual statement of their content. This is because there are almost as many phenomenologies as there are phenomenologists, and to expect a crisp formula describing the labours of the latter would be to ask the impossible. Nevertheless, by a sound system of classification of his material, the author prevents the reader from straying too far from the historical track.

Thus, after some introductory material, the first part deals with the preparatory phase; Part 2 with the German phase. Then comes a very accomplished 'lead-in' as to how and why the movement spread to France (Part 3), where it achieved considerable support and success. Which done, we are asked in Part 4 to assess the position at mid-century, and incidentally the cool reception of the subject in Great Britain stands out clearly. Finally, in Part 5, principles and appraisals give the inquirer as good an idea (outlined in the first part of this review) of what it is all about, as can be required of anybody reporting on so difficult a theme.

Lastly, there are several pages of charts surveying the development of phenomenology in Germany and in France, followed by indexes and a glossary.

All this is a most valuable addition to the literature of the subject. One could make minor criticisms of style, like the irritating repetition of the phrase "to be sure" and the graceless abbreviation "photo". But these are trifles in an excellent treatment. Edmund Husserl remains at the centre of the stage, around him grouped both disciples and critics united, whatever their differences, to do him honour.

F. I. G. Rawlins

MODERN STYLE IN MATHEMATICAL ANALYSIS

Foundations of Modern Analysis

By J. Dieudonné. (Pure and Applied Mathematics: a Series of Monographs and Textbooks, Vol. 10.) Pp. xiv+361. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 8.50 dollars.

In recent decades tremendous changes have come about in many branches of pure mathematics. Not only have content and emphasis altered, but in addition there has been a fundamental change in the interpretation of large sections of classical mathematics. Abstraction and generalization, leading to the unification of previously scattered concepts, have played a powerful part in this process. One of the most vigorous protagonists of this activity has been the Bourbaki school of French mathematicians. The Bourbaki point of view on mathematical analysis is admirably presented in the book by Prof. J. Dieudonné. For every notion the concept itself is emphasized rather than the manipulation involved in using the notion, and this applies to the large number of problems as well as the text itself.

The book starts with an account of the theory of sets and the properties of real numbers and the real line. Then come two very important chapters, on metric spaces and normed spaces. The contents of these chapters form the backbone of the whole work, and are fundamental for all that follows. The important notions in a metric space are completeness, compactness and connectedness. These are carefully introduced, after a good deal of preliminary terminology, which deals essentially with geometrical ideas that remain unaltered under 'deformations'. For a normed space the fundamental results are the continuity criterion and a theorem of F. Riesz characterizing finite dimensional spaces, the latter being vital in spectral theory. The next two chapters deal with Hilbert spaces and with spaces of continuous functions, including the Stone–Weierstrass approximation theorem and some applications. It is at this stage that the reader comes to a presentation of the differential calculus. The theorems are familiar to all mathematicians, but are presented, against the background of the previously developed ideas, in a way which will be new to many readers. This formulation, involving greater abstraction and more mental effort, is considered worth while because it prepares the reader for the ideas of the calculus on a differentiable manifold. There follows a chapter on plane topology (the method of Eilenberg). Then analytic functions with an appendix on applications. A chapter on existence theorems presents results in the theory of differential equations, these results being linked with the notion of completeness. Finally, there is a chapter on spectral analysis, a subject chosen because it draws on all the material presented earlier in the book and leads to one of the main branches of functional analysis.

Apart from its actual mathematical content, the book is especially important because of the impact it will have on mathematical analysts, as to what should be taught to students of pure mathematics and how it should be presented. Prof. Dieudonné is particularly outspoken about several matters. For example, there is no mention of the theory of the 'Riemann integral', and the author states: "It may well be suspected that, had it not been for its prestigious name, this would have been dropped long ago, for (with due reverence to Riemann's genius) it is certainly quite clear to any working mathematician that nowadays such a 'theory' has at best the importance of a mildly interesting exercise in the general theory of measure and integration. Only the stubborn conservatism of academic tradition could freeze it into a regular part of the curriculum, long after it had outlived its historical importance". For another example, it may be noted that in the chapter on analytic functions the common idea of a 'multiple-valued' function is not introduced, and reasons are given why this idea is limiting and undesirable.

It is important that the reader should understand the aim of this book as well as its contents. For it is a book that lives: it has orientation as well as content and the orientation is justified. At the present time, when there is considerable heart-searching as to what should constitute a curriculum in pure mathematics and how it should be presented, it is particularly fitting that this book should appear, for it may help to clear the way in a somewhat twisted jungle of ideas and prejudices. It may be helpful to quote a part of Prof. Dieudonné's introduction:

"My opinion is that the graduate student of to-day must, as soon as possible, get a thorough training in this abstract and axiomatic way of thinking, if he is ever to understand what is currently going on in mathematical research. This volume aims to help the student to build up this 'intuition of the abstract' which is so essential in the mind of a modern mathematician.

"It is clear that students must have a good working knowledge of classical analysis before approaching this course."

L. S. Goddard

AGE AND KINSHIP IN AUSTRALIA

Classification of Kin, Age Structure, and Marriage amongst the Groote Eylandt Aborigines

A Study in Method and a Theory of Australian Kinship. By Frederick G. G. Rose. (Deutsche Akademie der Wissenschaften zu Berlin. Völkerkundliche Forschungen, Band 3.) Pp. xii+572. (397 Abbildungen.) (Berlin: Akademie-Verlag; London: Pergamon Press, 1960.) 100s. net.

This book concentrates on two highly important matters in the analysis of descent systems: the significance of relative age, and the factual documentation of the constitution and working of particular systems. The society dealt with is the

Ingura or Wanindijaugwa of Groote Eylandt, off the northern coast of Australia. The account is based on about four months research in 1941, at which time the population numbered between 300 and 350 individuals. They were organized into eleven localized totemic patriclans, these being grouped into unnamed exogamous moieties.

The major argument is that 'gerontocracy' (the practice of marrying young girls to considerably older men) leads to the abandonment of the general Australian rule of bilateral cross-cousin marriage, and thence to the adoption of the matrilateral rule. The author tries to demonstrate this by assessing the ages of individuals and relating the resultant age-distribution by sex to possible rules of marriage. He shows, for example, that there is an average difference of 15 years between 'full cross-cousins', the patrilateral being the older. It is therefore argued that under gerontocracy this relative could not be married, so that neither patrilateral nor bilateral cross-cousin marriage is possible in this society. But this conclusion relates only to first cousins, whereas typically in a lineal descent system it is a more extensive class of relative, not defined primarily in genealogical terms, which is covered by the marriage regulations. If, then, we consider the ages of all members of the indigenous categories concerned, we have reason to expect a different situation; and in fact the case of individual No. 6, selected solely according to sex (male) and age (23), gives the following results: seven female patrilateral cross-cousins, average age 37; fifteen matrilateral, average age 36. If this case is typical, as on general grounds we may presume it to be, it shows that it is simply the author's European prejudice towards a genealogical definition of status which frames his argument and lends it apparent support.

Moreover, the account of the system is defective. As might be expected with such a short investigation, and an imperfect knowledge of the language, the ethnography is slight and unsatisfactory. The relationship terms seem, on the whole, to indicate an eight-section system, but the genealogical specifications reported are insufficient to establish the precise features of the actual system. Certain positions are not covered by the specifications, and there is an untypical distinction of patrilateral from matrilateral cross-cousin. It is clear, however, that the form of social classification is, contrary to the author's odd assertion, neither 'Kariera' nor 'Karadjeri' (he thinks the 'Aranda' type of system never existed). Also, the normal marriage is said to be with the mother's mother's brother's daughter's daughter who is a 'distant' mother's brother's daughter; but this cannot be reconciled with the system apparently framed by the terminology. Moreover, the abundant tabular data, occupying 220 pages of the book, are no substitute for genealogies, details of group-composition, and territorial distribution, let alone the indigenous ideology, and they cannot yield the evidence which is necessary to structural investigations of the kinds suggested by the author's argument.

In view of the interest of the issues he deals with, Dr. Rose's study is particularly exasperating. His record of ages is precisely the kind of exact evidence which is essential to the comprehension of Australian societies, and he is right to deplore its absence from other accounts; but without a comprehensive structural examination, conducted primarily in terms of the categories employed by the people themselves, this record is of depressingly little sociological use. It is possible, however, that it may serve certain purposes; and the work also includes percipient and forceful observations on a variety of other topics in the study of aboriginal Australian society (see, for example, his criticisms of Radcliffe-Brown's statements about the Kariera). Inadequate though it must be judged, and fallacious as its central argument almost certainly is, Dr. Rose's monograph is a singular (if eccentric) contribution to the field and deserves a place in any library of social anthropology.

R. Needham

SOME ASPECTS OF HÆMATOLOGY

Lectures on Haematology

Edited by F. G. J. Hayhoe. Pp. viii+247+24 plates. (Cambridge: At the University Press, 1960.) 60*s*. net.

The chapters in this book represent a course of thirteen lectures delivered to an audience of post-graduate clinicians and medical research workers. Most of the information has been published elsewhere, but this is in no sense a text-book of hæmatology. Each chapter is a survey, by an expert, of some rapidly expanding or controversial topic in the field. The accounts are of necessity brief, and only some of the most recent work is covered in detail. A good bibliography at the end of each chapter brings in the earlier literature.

The first five chapters deal with abnormalities in production and reactions of erythrocytes. J. V. Dacie writes on acquired hæmolytic anæmia, W. M. Davidson on iron metabolism and iron-deficiency anæmia and R. H. Girdwood on the pathogenesis and differential diagnosis of megaloblastic anæmias. The hazards of blood transfusion are discussed by G. T. Discombe, and J. C. White gives an account of hæmoglobin variants.

Although these chapters are directed primarily to the audience concerned they will provide useful information for a much wider circle of readers. Anyone concerned with the preparation or administration of blood for transfusion would profit from reading this entertainingly written and eminently practical account of the hazards involved. Especially since, as the author mentions, there is at present no comprehensive review of the subject in the English language.

Chapters 6–10 deal with various aspects of leukæmia. F. G. J. Hayhoe, who is also the editor of the book, considers the clinical and cytological recognition and differentation of the leukæmias. G. Wetherley-Mein writes on the myeloproliferative syndromes, and R. Doll on the incidence of leukæmia and the significance of environmental factors in its causation. The chemotherapy of leukæmia is discussed, as a practical problem, by D. A. G. Galton, and H. E. M. Kay writes on the potentialities of marrow transplantation in the treatment of leukæmia.

The last three chapters deal with abnormalities in hæmostasis. R. M. Hardisty discusses the contribution of blood and blood vessels in the hæmostatic process. Congenital and acquired disorders of coagulation are reviewed by G. I. C. Ingram and the pathogenesis of purpura by J. F. Ackroyd.

A great merit of the book is that it is easy to read, for in most cases the lively and personal approach of a lecture has survived in the printed account.

M. E. Nutt

Fast-Neutron Spectroscopy

By B. V. Rybakov and V. A. Sidorov. Edited by N. A. Vlasov. Translated from the Russian. (Supplement No. 6 of the *Soviet Journal of Atomic Energy,* 1958.) Pp. ix+121. (New York: Consultants Bureau, Inc.; London: Chapman and Hall, Ltd., 1960.) 17.50 dollars.

DURING the past few years neutron spectroscopy in the energy-range of 0·3–30 MeV. has made enormous progress, and in this book we have a review of the field up to 1958. Since this is a Russian work the English translation has not appeared until recently and so is already partly out of date. Nevertheless, it has especial interest because of the coverage it gives of fast-neutron spectroscopy in the U.S.S.R.

The most powerful technique which has been introduced into this field recently is the time-of-flight method. Development of fast pulsed sources and fast detectors and electronic equipment has given this method its present overall usefulness. Approximately two-thirds of the book is given to a valuable discussion of these techniques. The authors have been working with the cyclotron at the U.S.S.R. Institute of Atomic Energy and have considerable experience of time-of-flight work. Their description of this field covers their own equipment and experiments, in addition to others.

There are as well chapters covering methods which have a more specialized application. Such methods as proton-recoil, helium-3 spectrometer and scintillation counter techniques are covered adequately.

This review is intended for people already working in this field or about to do so; a graduate background of physics is assumed. The style is fair, some clarity and emphasis being lost in translation. However, the actual technical translation errors do not seem to be many. From the aesthetic point of view the cover design is depressing and the print is tiring. Probably most scientists would find it a relatively expensive book.

In conclusion, this book could be recommended as good reading, but scarcely worth buying for an individual scientist's library. P. A. EGELSTAFF

Atomic and Nuclear Physics

By Prof. Robert S. Shankland. Second edition. Pp. xv+665. (New York and London: The Macmillan Company, New York, 1960.) 49*s.*

THIS is the second edition of Prof. Shankland's undergraduate text-book. It is cast in the form of fairly brief lecture notes with a minimum of mathematics and perhaps one typical experiment for each topic. The range of physics covered is very broad and admirably up to date. Electron physics, molecular physics, solid state, nuclear and high-energy physics and reactor physics are all included. Although the underlying unity of microscopic physics is implied by including them all in one book, the clear demonstration of this is seriously impeded by the rather traditional and individual presentation of each field. That is, the partial emphasis on historical development is not sufficient to make this aspect very clear, but makes it difficult to see the elegant unity which has been achieved with the help of quantum mechanics.

This is a criticism of the overall organization of the book. The individual topics are treated clearly and simply and in each case carefully selected references to the original literature can lead the student to the details of the work. The experiments described for illustrative purposes are generally very modern ones. About 350 problems are collected at the end of the book.

This text is on the whole a very good example of its kind, especially if accompanied closely by lectures in experimental physics and quantum mechanics. E. B. PAUL

Mammals of Northern Rhodesia

A Revised Check List with Keys, Notes on Distribution, Range Maps, and Summaries of Breeding and Ecological Data. By W. F. H. Ansell. Pp. xxxi+180+7 plates. (Lusaka: Government Printer, 1960.) 12*s.* 6*d.*

FAR more than a checklist, this is really an admirably produced pocket guide, into which the maximum amount of information has been compressed without losing simplicity of arrangement and ease of reference. With identification keys, notes on habits, habitats and distribution, as well as seven useful colour plates by P. Dandelot, it is a model of its kind. Both the introduction, with its stress on the need for further collecting before the numbers and range of the mammals are irrevocably reduced by human activity, and the discussion of problems of habitat, ecology and distribution, are well calculated to stimulate or arouse interest in these aspects of the subject. The book will also be of much practical value to the professional working on the mammals of Central Africa. Sections listing rejected species, and species likely to be included as a result of further investigation, help to complete the work, and separate indexes of English, scientific and vernacular names help easy reference.

The only criticism relates to the unfortunate printing of the seventeen full-page distribution maps sideways without a corresponding adjustment of the captions. In two cases, zebra and lechwe, where cross-hatching indicates the distribution of local races, the result is the opposite of what was obviously intended. R. W. HAYMAN

Remington's Practice of Pharmacy

Edited by Eric W. Martin, E. Fullerton Cook, E. Emerson Leuallen, Arthur Osol, Linwood F. Tice and Clarence T. Van Meter. Twelfth edition. Pp. xii+1866. (Easton, Penn.: Mack Publishing Company, 1961.) n.p.

DR. FULLERTON COOK has now played an important part in producing successive editions of this large standard work for more than 60 years. It deals with the whole of American pharmacy from history and ethics to poison control. It contains useful information about all the sciences which a pharmacist must understand, and there are more than 600 pages devoted to the properties of the drugs used in medicine. These drugs are, for the first time, classified pharmacologically, in recognition of the fact that pharmacists are now consulted about the actions of drugs, but a summary of the chemical classification is also included. Among the subjects dealt with in the various parts of the book are statistics, rheology, chromatography, sterilization, methods of manufacture, sustained action medication, aerosols, parasiticides, pesticides, diagnostic drugs, allergenic extracts, radiopharmacy, analytical pharmacy, immunization and many other things. The information on all these topics is full and up to date. The total number of pages has increased by 160 since the last edition was published five years ago. The six editors, 56 assistant editors and more than 160 contributors are to be congratulated on keeping this reference book up to date. J. H. GADDUM

Sofiano (T.A.) English–Russian Geological Dictionary

Edited by I. D. Borneman *et al.* State Publ. Office Techn. Theor. Literature, Moscow, 1957. Pp. 525. Price 17r. 90k.

Sofiano (T.A.) Russian–English Geological Dictionary

Edited by A. P. Lebedev and V. E. Khain. Central Editorial Board, Foreign Language Scient. Techn. Dictionaries, Fizmatgiz, Moscow, 1960. Pp. 559. Price 18r. 60k. (old roubles), 1r. 86k. (new roubles).

THESE two dictionaries, well printed and bound, and of a convenient size (21 × 14 × 3 cm.), contain some 35,000 terms each. The majority of these terms consist of one word, but quite a number of them are adjectival terms, such as, for example, 'deltaic fan', or even more complex phrases like 'planar flow structure'. Besides purely geological, petrological and mineralogical terms there is a number of mining, civil engineering and allied physical, chemical and biological terms. Some adjectival terms are placed under their main term. Thus the term 'sand' is followed by 'arkose sand', 'coral sand', etc. On the whole the compiler of these two dictionaries, Madame T. A. Sofiano, has been rather generous in her collection of terms, which is all to the good. As with other dictionaries, there are occasional mistranslations. The English–Russian dictionary has also a list of English abbreviations, which is not very satisfactory, but there is no list of Russian abbreviations. In spite of these minor defects these two dictionaries represent extremely valuable auxiliary reference books for geologists. S. I. TOMKEIEFF

Linear Programming

Methods and Applications. By Saul I. Gass. Pp. xii + 223. (London: McGraw-Hill Publishing Company, Ltd., 1958.) 50*s*. 6*d*.

Methods of Feasible Directions

A Study in Linear and Non-Linear Programming. By Dr. G. Zoutendijk. Pp. vi + 126. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1960.) 30*s*.

THE first book is a formal mathematical text on linear programming and is to be distinguished from the many general accounts on the subject that have appeared. It contains much information that could in the past be obtained only from original papers. Although writing for those with mathematical leanings, the author has a constant eye on the practical applications of the methods he describes. The book is divided into three parts. The first contains a general introduction and some necessary mathematical background, the second describes the theoretical and computational methods available for use, and the third discusses applications. The book has already become well known in the short period that has elapsed since its publication and is to be highly recommended.

The second book has the sub-title *A Study in Linear and Non-Linear Programming*, and is in the nature of a research monograph. Its appreciation requires a knowledge of present-day linear programming principles and techniques such as might be obtained from the foregoing book. The first half is concerned with linear programming, and in particular with a series of improvements to the product-form algorithm. In favourable circumstances these enable substantial economies to be made in the amount of computation required.

The second half of the book is concerned with methods of solving the general convex programming problem. In all these methods one starts with a feasible solution, determines a feasible direction of advance which will increase the value of the solution, and takes a step of appropriate length in that direction. There are various methods of determining the feasible direction of advance and the length of the step to be taken. Some of these described reduce in the case of linear or quadratic problems to methods which are already well known. M. V. WILKES

Digital Computers and Nuclear Reactor Calculations

By Ward C. Sangren. Pp. xi + 208. (New York and London: John Wiley and Sons, Inc., 1960.) 8.50 dollars; 68*s*.

PROSPECTIVE readers might not gather from the title of this book that the first half contains a valuable general introduction to digital computers written by a very experienced user. This is a pity, since others than nuclear physicists and engineers might profit from this part of the book. The chapter on programming in particular has an authentic ring about it. The chapter on numerical analysis is perhaps less successful being rather too compressed for the novice and neither sufficiently comprehensive nor sufficiently critical to appeal to the expert.

The last four chapters are concerned with the solution of technical problems arising in reactor design and in other branches of nuclear engineering; their scope is indicated by their titles: "A Code for Fission-Product Poisoning", "Diffusion and Age-Diffusion Calculations", "Transport Equation—Monte Carlo", "Additional Reactor Calculations". There is a useful selected bibliography. The author remarks that the problems he describes are good examples of problems in mathematical physics that can be 'solved' sensibly with digital computers: he considers, therefore, that they should interest not only reactor specialists but also applied mathematicians, physicists, and persons professionally interested in computer applications. M. V. WILKES

Birds of Eastern and North Eastern Africa

By C. W. Mackworth-Praed and C. H. B. Grant. Second edition. (African Handbook of Birds, Series 1, Volume 2.) Pp. viii + 1113 + plates 54–96 + photographs 7–19. (London: Longmans Green and Co., Ltd., 1960.) 50*s*. net.

THIS is the indispensable work of reference for all who are interested in the birds of East Africa, and it is not surprising that the demand for copies has called for a second edition. The two volumes were originally published in 1952 and 1955; a second edition of Volume 1 has already appeared and the revision is thus now complete. The work having been reproduced by photo-lithography, the changes are necessarily limited to additional matter inserted in spaces which had been left for the purpose and on some extra pages at the end. A supplementary index to the addenda and corrigenda is conveniently provided. LANDSBOROUGH THOMSON

THE MAXWELL COLOUR CENTENARY

AFTER all the recent questioning caused by Dr. Edwin Land with his demonstrations of two-colour projection, the principles of three-colour reproduction came into their own again at the Maxwell Colour Centenary Conference held at the Imperial College of Science and Technology, London, during May 16–18, under the joint auspices of the Colour Group (Great Britain) and the Inter-Society Color Council (U.S.A.), and attended by about 220 members from twelve countries. The particular centenary to be celebrated was the lecture delivered by Maxwell at the Royal Institution on May 17, 1861, when he gave the first public demonstration of three-colour reproduction by projecting images in red, green and blue light in superposition on a screen.

The Conference was fortunate to be able to arrange a centenary discourse at the Royal Institution itself exactly a hundred years after Maxwell's lecture. The subject of the discourse was "A Hundred Years of Colour Photography", and it was delivered by Dr. D. A. Spencer (Kodak, Ltd., Harrow). One of the most interesting items in the lecture was the report of a piece of detective work by Dr. R. M. Evans, of the Eastman Kodak Company in Rochester, U.S.A., leading to the explanation of how Maxwell had succeeded in obtaining an acceptable colour reproduction at a time when photographic emulsions were sensitive only to wave-lengths extending from the blue part of the spectrum downwards into the ultra-violet. Evans provided convincing evidence to show that, in securing his red separation negative, Maxwell had unwittingly taken advantage of the ultra-violet transmission common to many red dyes and filters. Further, the green filter must have had just sufficient transmission at the long-wave end of the blue part of the spectrum to enable Maxwell to secure a distinctive exposure for the green separation negative. By these fortuitous circumstances Maxwell was thus able to produce a three-colour photograph some twenty years before photographic emulsions sensitive to the green and red had been produced.

The theme of the Conference itself progressed from a study of the facts of three-colour mixture and their biological interpretation to their application in photography, television and printing, with a final day devoted to a discussion of colour appearance. The programme was not overburdened with papers, three introductory talks in each half-day being the normal arrangement, followed by informal discussion. Not all the speakers kept to their allotted 20 min., but at most of the sessions there was a satisfactory division between prepared and extemporary contributions.

The paper by Dr. D. B. Judd (National Bureau of Standards, Washington) on the opening morning had a detective flavour similar to that of Dr. Evans's study, since he had made a fresh analysis of the colour mixture curves obtained by Maxwell and was able to show that they were in closer accord with the Commission International d'Eclairage standard curves than had generally been realized. Maxwell's theoretical ideas, too, had anticipated many of the basic concepts of 'modern' colorimetry, and Dr. B. H. Crawford (National Physical Laboratory, Teddington) reported some new results from the National Physical Laboratory on large-field colour matching which had been inspired by Maxwell's original experiments. In Maxwell's method, colour mixture curves can be derived by mixing two fixed wave-lengths with a third, variable, wave-length so as to match a constant white, thus providing fixed adaptation conditions for all the matches. Crawford found a significant difference between the mixture data calculated from this type of observation compared with those derived directly from more orthodox experiments in which three fixed red, green and blue wave-lengths are mixed to give a match of each wave-length in the spectrum in turn after suitable desaturation. This disagreement may provide a possible clue to the small, but significant, discrepancies in the field tests made on the large-field colour mixture functions prepared by the Colorimetry Committee of the Commission Internationale de l'Eclairage from the Stiles–Burch–Speranskaya data. However, if Crawford's results are confirmed, they imply in effect a failure of additivity in large-field colorimetry, a possibility that has already been the subject of some discussion and which will no doubt be investigated more fully in the near future.

Perhaps the most original contribution to the meeting came from Dr. W. S. Stiles (National Physical Laboratory, Teddington) and Dr. G. W. Wyszecki (National Research Council, Ottawa), when they considered the spectrum as divided into M small wave-length intervals. They then showed that there was a statistical limit to the number of different selections of these M elements which would combine together to reproduce a given colour, ranging from a minimum number of combinations for colours in the spectrum to a maximum for a white surface. Lights of different spectral composition which have the same colour are known as metamers, and Stiles and Wyszecki showed that 'counting metamers' might prove of value, for example, in assessing the colour-rendering properties of two illuminants or comparing the merits of two sets of colour-matching functions.

In the biological session, we were reminded once again, by Dr. W. A. H. Rushton (Trinity College, Cambridge), Prof. Yves Le Grand (Natural History Museum, Paris) and Prof. R. W. Ditchburn (University of Reading), of the complexities of the visual process. However, the ophthalmoscopic arrangement used both by Rushton and by Weale for measurements of the reflectivity of the fundus has led to some surprisingly direct information about the density changes in the visual pigments during bleaching and regeneration, and about their spectral sensitivity. Rushton, indeed, is obtaining gratifying confirmation of the type of colour-mixture curves associated with the classical three-components theory of colour vision, and as derived, for example, by Pitt from work on the dichromatic form of colour defect.

Nevertheless, it is clear from the work on eye movements and on the stabilized retinal image at the University of Reading under Prof. Ditchburn, that the scanning of the retinal image plays a vital part in colour discrimination. Indeed, it seems almost certain that time-coding of the neural information must occur at some stage in its transmission from retina to visual cortex, and Ditchburn suggested that the colours we see may be determined very largely by edge effects occurring at the boundaries separating one area of colour from another in the retinal image.

However that may be, we can agree with Le Grand that the one certainty that emerges at the moment is the complexity of the visual processes and their interactions at all stages. Yet to a question about the possibility of the retinal receptors behaving like wave-guides and of their colour sensitivity being determined by their physical structure, Rushton was prepared categorically to deny this on the evidence of the colour of light scattered sideways into the receptors from other illuminated parts of the interior of the eyeball.

May 17 was devoted to discussions of problems of colour reproduction, and it was fascinating to compare and contrast the differing situation facing photographers, television engineers and printers. Colour photography depends on subtractive colour mixtures using yellow, magenta and cyan dyes; but the dyes that have to be used possess unwanted and overlapping absorptions. Hence no simple relation exists between exposure and density of colour, so that some colour distortion is inevitable. Defects of colour quality in one direction have therefore to be offset by deliberate distortions in the opposite direction; but the enormous popularity of colour photography is a tribute to the successful compromise which has been achieved. Here, as Mr. R. G. Horner (Ilford, Ltd., Brentwood) acknowledged, the eye is very accommodating and tries its best to see things as the brain thinks they should be seen.

Colour photographic prints present greater problems than colour transparencies, and Dr. R. W. G. Hunt (Kodak, Ltd., Harrow) explained how manufacturers had been able to take advantage of the fact that the light from the great majority of colour pictures integrates to grey. Photo-electric methods of controlling the colour balance can therefore be used, although the 'integrating to grey' method is sometimes criticized because of the colour distortion that occurs with special subjects. Mr. L. C. Jesty (Sylvania–Thom Colour Television Laboratories, Ltd.) jokingly congratulated Dr. Hunt because he had described this limitation as 'colour failure', whereas to the disappointed customer it is commonly described as 'subject failure'.

In many ways the situation in colour television is more straightforward. The picture is reproduced by additive colour mixture, the laws and data of trichromatic colorimetry can be fed directly into the system, and transformations of the colour information to a form best suited for transmission and reception can be achieved by the electronics engineer in a box which he labels 'matrix'. All this was admirably surveyed by Mr. J. W. Wentworth (Radio Corporation of America); but the television engineer is still left with the major problem of simplifying and reducing the cost of the colour receiver. The most expensive item, of course, is the colour tube which, in its more common form, comprises a three-electrode gun, a shadow mask and a phosphor mosaic. It would be ironic if someone invented a cheaper receiver based on subtractive colour mixture, and so deprived television of some of the theoretical simplicity of an additive system.

Colour printing is in a different position still, since pigment mixture, as Dr. D. R. Duncan (Paint Research Station, Teddington) emphasized, involves both absorption and scattering and is therefore neither purely subtractive nor purely additive. Hence, as was made clear both by Mr. D. C. Gresham (McCorquodale, Ltd., London) and Mr. W. L. Rhodes (Rochester Institute of Technology) colour printing is very much an empirical process that does not lend itself to any neat theoretical or mathematical solution. Moreover, for various technical reasons, including the need to sharpen the fine detail, it is often necessary to include a black printer. Colour printing then becomes a four- instead of a three-colour process. The skill of the craftsman ensures that excellent colour prints can be made, but the demand for mass printing makes automatic or semi-automatic colour correction a very attractive proposition, and Mr. V. C. Hall (Time, Inc., Springdale) described how this can be done with scanning machines and scanner computers. However, so far, only some 10 per cent of colour prints are made in this way.

The subject for the third day of the Conference could be summed up as 'colour as we see it'. If any physicists present were still under the delusion that the physical stimulus was all that mattered they were rudely disabused by the lively discussion that developed on colour appearance and how it could be studied. Miss Dorothy Nickerson (U.S. Department of Agriculture, Washington) was concerned with the possibility of exploiting the Munsell variables of hue, value and chroma as the co-ordinates in a system of appearance specification, with the colour-rendering properties of light sources particularly in mind. Dr. Crawford also discussed the problem of colour rendering, and he showed how the remembered appearance of known objects, or memory assessment, had proved a valid method of study. Prof. H. Helson (University of Texas) described some experiments in which the appearance of coloured shadows was in fact expressed in terms of the Munsell colour chips from measurements made by a binocular matching technique. Mr. W. C. Granville (Granville Color Service, Libertyville) demonstrated the critical selection of colours which we make in designing a colour scheme, especially in schemes involving near-neutral colours. He considered that in the colour atlases in common use many more samples of low saturation were required in the neighbourhood of the neutral axis.

Mr. M. H. Wilson (Goethean Science Foundation, Stowbridge) took us back to Goethe and treated us to some very effective demonstrations of colour as light and colour as darkness. His description of additive mixture as the mixing of coloured lights and subtractive mixture as the mixing of coloured darknesses gave the audience just the mental shock necessary to keep a conference alive. No doubt to the orthodox physicist such phrases sound like nonsense, but the subject of colour is one where the physicist soon has to leave much of his orthodoxy behind him. Certainly one of the most valuable aspects of the meeting was its success in bringing colour experts of very different backgrounds and experience together into intimate and informal discussion.

For the Colour Group it was an auspicious start to its new existence as an independent body, and Dr. Hunt, the present chairman of the Group, paid a fitting tribute to the Institute of Physics and the Physical Society both for help in organizing the meeting and for the manner in which the Group was enabled to separate from the parent body and launch into an orbit of its own.

W. D. Wright

STORAGE AND RETRIEVAL OF BIOLOGICAL INFORMATION*

By DR. G. C. AINSWORTH

Commonwealth Mycological Institute, Kew, Surrey

MANY problems of storage and retrieval of biological information are common to those of scientific information in general, and to give perspective in a connotation where 'information' is usually thought of as synonymous with 'the literature' certain pecularities of scientific literature must be noted before considering some characteristic features of biological information.

Much of the best scientific literature is ephemeral. Its classics are seldom read. Scientific work is firmly based on the findings of other scientists, and is brought to a right conclusion only when the results are made generally available. In other words, there must be communication, and the modern tempo requires this communication to be rapid. Further, while much literature in the ordinary sense is national, science, and hence scientific literature, are international, and the prestige value of science coupled with the growth of nationalism during recent years has increased the scatter of the languages in which scientific results are reported; but this has been counteracted by the desire of authors to be widely read. The amount written or summarized in English continues to increase.

For biology, as for science in general, the size of the literature and the current output are not known with any certainty. In February this year *Biological Abstracts*, begun in 1926, published its millionth abstract. This comprehensive abstracting journal for all branches of biology except clinical medicine is at present increasing its coverage. This year 87,000 abstracts are promised, and by next a rate of 100,000 abstracts a year is to be attained. But such a coverage would be incomplete, for a recent World Health Organization survey[1] has estimated that the annual output for biology and medicine approaches 400,000 articles and more medical and veterinary literature than non-medical biologists like to think has biological interest (and there are 4,000–5,000 medical periodicals containing about 230,000 papers each year[2]), while biologically relevant data are reported in the fields not only of biochemistry and biophysics but also of chemistry and physics, statistics and semantics, anthropology, psychology, and so on. Admittedly the biological data are few in some of these studies; but probably more fruitful connexions could be made if some specialists were less specialized.

The extent of the literature on some specific biological topics is more accurately known. For example, in 1953, for plant protection in the wide sense, the latest (1960) issue of the annual *Bibliographie der Pflanzenschutzliteratur* records 15,000 items obtained by scanning some 3,000 periodicals, and for fungus diseases of higher animals and man a census of the publications for 1957 has so far yielded 1,105 items. Further, as an example of an estimated total, there are not less than a quarter of a million books and papers on fungi.

As for science in general, the bulk of biological literature is stored as journal articles, and here a rather curious state of affairs may be noted. Although any investigation is only completed when the results are communicated and although State (or other) funds are usually available, if on a modest scale, for scientific investigation, very little money indeed is made available for the costs of publication, as Mellanby[3] has pointed out. Biologists have to unite in co-operatives (or 'publishing societies') and arrange for the publication of their own work. The State recognizes the value of such societies by allowing remission of income tax on an individual's subscriptions to approved societies, by permitting more altruistic members to covenant their subscriptions, and by an annual grant-in-aid of scientific publications (administered by the Royal Society) to enable societies to publish more without going bankrupt. There is a feeling that biological societies have not always received a fair share of this grant-in-aid. For 1959–60, however, biological societies received half the grant distributed[4], but as more than half remained undistributed there seems scope for more importunity by the smaller societies, particularly those with a taxonomic bias, all of which have at times had to restrict their activities owing to printing and publishing costs.

This traditional method of publication has disadvantages. It is wasteful, for the economics of many periodicals depend on the subscribers, both individual and institutional, receiving much material they would never otherwise have bought, thus preventing its availability to others who need it. This has long been realized, and a recent Unesco report and bibliography by Phelps and Herling[5] lists more than a hundred papers and reports on alternatives to the scientific periodical. But nothing is done. The traditional method still works. The position is not yet intolerable.

Biology differs from most other branches of science in that an important part of its information is stored not as literature but as specimens. Even the simplest objects of a biologist's study are complex, and while great progress has been made in describing living things in words, by illustrations, and by musical notation there are frequent uncertainties of identification or problems of affinity which can be resolved only by the comparison of specimens or tape recordings: hence the many collections of specimens, from those of the large national herbaria and zoological and geological museums to the personal collections of individuals. The specimens need not, however, be dead or fossilized. There are national parks and nature reserves, zoological and botanical gardens with their diverse regional and exotic contents, culture collections of micro-organisms of all groups, and one cannot exclude collections of cultivated plants and domesticated animals which shade off into the accumulations of fanciers, whether of tropical fish or alpine plants. All in their different ways contribute to the store of basic biological information.

The retrieval of specimens, which is not always easy (for their cataloguing often leaves much to be desired), is a topic which cannot be given detailed consideration here. It must be sufficient to indicate present trends by recalling that since 1952 the *Index*

---

* Substance of a lecture to the London Branch of the Institute of Biology, April 27.

ACADEMIC PRESS New York and London

Protein Structure

By Harold A. Scheraga, *Cornell University, Ithaca, New York*

August 1961, *about* 310 *pp.*, 64*s*.

The primary aim of this book is to present some of the quantitative aspects of the physicochemical approach to the protein structure problem, including both gross and internal structure. In discussing gross structure, polymer principles have been applied. In considering the more detailed aspects of internal structure, attention has been directed to interactions between polar groups and nonpolar ones. The material is developed from first principles, assuming only a background of elementary physical chemistry.

The book has evolved from a series of lectures given for C.S.I.R.O. in Melbourne, based on research carried out in the author's laboratory during the past decade. It will be of interest to all students and research workers engaged in the study of the structure of biological activity of proteins. It will also be of value to workers in other biological sciences in which the principles of protein structure and activity are of interest.

CONTENTS:

Hydrodynamic Properties of Protein Solutions
Internal Structure; Effect of Hydrogen Bonding on Side-Chain Reactivity
Limited Proteolysis
Denaturation
Limited Proteolysis and Aggregation in the Fibrinogen-Fibrin Conversion
Some Experimental Methods
Configurational Studies of Insulin, Lysozyme, and Ribonuclease
REFERENCES—SUBJECT INDEX.

Protein Biosynthesis

Proceedings of the Symposium held under the auspices of U.N.E.S.C.O., at Wassenaar, August 29–September 2, 1960

Edited by R. J. C. Harris, *Imperial Cancer Research Fund, London*

June 1961, 409 *pp.*, 95*s*.

Contents listed in Nature, March 11, 1961, *also available upon request.*

This international symposium was sponsored jointly by U.N.E.S.C.O., C.I.O.M.S., and the Netherlands Cancer Institute. This book includes not only the papers but also the major contributions to the discussion. It provides the first authoritative and complete account of progress in this rapidly expanding field.

The treatment is primarily biochemical. All major research groups in this field were represented. This volume will be useful not only to biochemists but also to those cytologists, biophysicists, and chemists who are interested in such topics as the function of nucleic acids, the structure of cytoplasm, the properties of ribosomes, the biosynthesis of viruses, and the synthesis of antibodies.

Amino Acids, Proteins, and Cancer Biochemistry

Papers presented at the JESSE P. GREENSTEIN MEMORIAL SYMPOSIUM, Division of Biological Chemistry, American Chemical Society, September 16, 1959

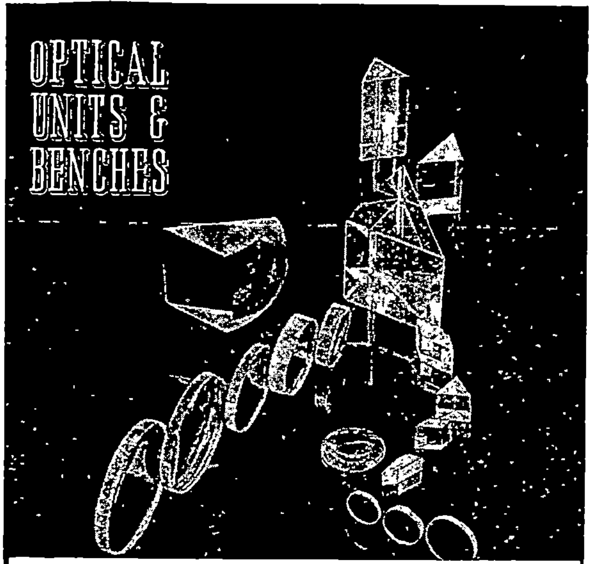
Edited by John T. Edsall, *Harvard University*
Preface by Sidney W. Fox and Julius Schultz

1960, 244 *pp.*, 56*s*.

"We believe that this book, which includes all of the papers given at the Symposium, will make an important contribution in portraying some of the major current developments in amino acid and protein chemistry and in the biochemistry of cancer—the two great fields in which Greenstein's work was preeminent." —*From the Preface*

Phone : WHItehall 6631

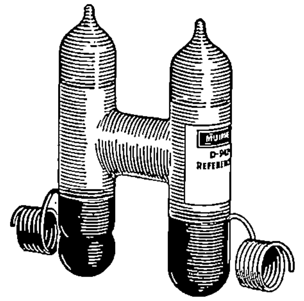
111 FIFTH AVENUE, NEW YORK 3 17 OLD QUEEN STREET, LONDON, S.W. 1



*ESSENTIAL FOR RESEARCH
AND EXPERIMENT*

Full Catalogue from

R. & J. BECK Ltd.
69/71, Mortimer Street, London, W.1.



WESTON

CELLS

**STANDARD
AND
INDUSTRIAL
REFERENCE
TYPES**

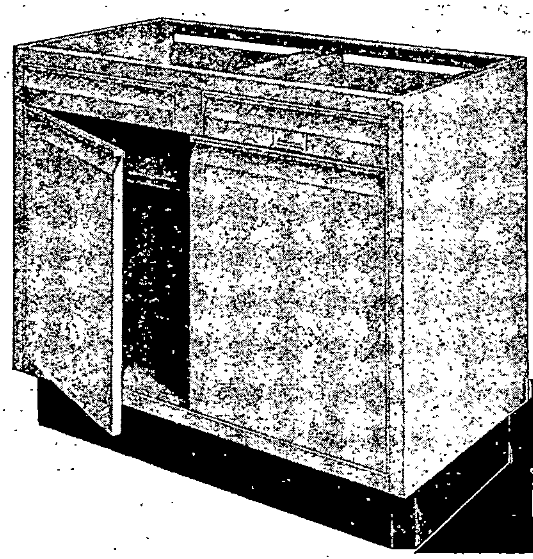
MUIRHEAD Weston Cells are accurate fundamental standards of voltage. More than twelve Saturated and Unsaturated types available—choose one to suit your application. Ask for Publications 425 and 430.

MUIRHEAD

PRECISION ELECTRICAL INSTRUMENTS

MUIRHEAD & CO. LIMITED, BECKENHAM, KENT, ENGLAND
Telephone: Beckenham 4888
MUIRHEAD INSTRUMENTS LIMITED, STRATFORD, ONTARIO, CANADA
Telephone: 271-3880
MUIRHEAD INSTRUMENTS INC., 441 LEXINGTON AVE., NEW YORK 17, N.Y., U.S.A.
Telephone: Murray Hill 2-8131
514

The Griffin-Grundy 'TEN-POINT' All Metal Laboratory Furniture

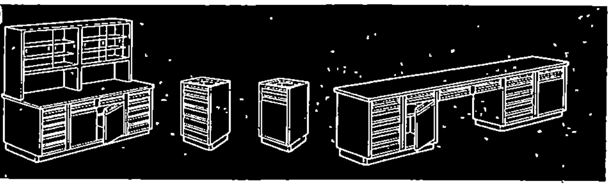


1 Front and rear welded frames provide maximum rigidity.
2 Removable back gives access to services.
3 Metal drawer tracks and slides incorporate a cam adjustment.
4 Rubber buffers to drawers and doors ensure quiet operation.
5 Recessed door and drawer-handles improve appearance.
6 Adjustable shelves in cupboard units.
7 Easily adjusted levelling feet fitted with P.V.C. shoes protect floor.
8 Detachable toe plinths.
9 Zinc coated mild steel construction for added protection.
10 Stoved melamine-base enamel inside and outside for easy cleaning.

Units dismantle for shipment resulting in
lower freight charges, and easy assembly on site.
Full instructions are provided with each installation.
Write for literature to-day.

Griffin & George
(LABORATORY CONSTRUCTION) LTD

Morden Road Mitcham Surrey Mitcham 9201
Walsall Road . Perry Barr Birmingham 22B Birchfields 5002
In association with Grundy Equipment Limited



MEMBER OF GRIFFIN & GEORGE GROUP • LONDON • BRISTOL • BIRMINGHAM • MANCHESTER • NEWCASTLE-UPON-TYNE • GLASGOW

Herbariorum[6] (already in its fourth edition) has provided a handy directory to the herbaria of the world (and this work is in the course of supplementation by a list of collectors represented in public herbaria) and that the Unesco-sponsored International Federation of Culture Collections of Micro-organism at Lausanne, founded in 1947, attempts to locate for inquirers any cultures wherever they may be available.

The storage of biological literature should be integrated with the retrieval pattern, and so it is convenient to consider the rationale of storage and the retrieval pattern together.

The most frequently employed retrieval method can only be noted. All 'reading habits of scientists surveys' are in agreement that the first reaction of any scientist needing information is to ask a colleague who it is thought may know the answer. Only when this fails are other avenues explored.

Retrieval can be divided, if somewhat arbitrarily, into retrieval of current literature and retrieval from literature of the past. Retrieval from current literature begins with the arrival of members' copies of society journals. This is supplemented by visits to the departmental library, reprints from others working in the same field, abstract journals, etc. From such sources every research worker notes papers of current interest which he stores in his head or in a more or less convenient and more or less haphazard manner, depending on his temperament and the quality of his secretarial support. For some, noting information becomes obsessional. For most it is a drudgery to evade.

It does seem that a primary requirement for rapid retrieval is an efficient 'contents of current journals' service. *Current Contents*, issued by the Institute for Scientific Information, Philadelphia, with the collaboration of journal proprietors, offers a weekly list of the contents pages of more than six hundred journals in the "chemical, pharmaco-medical, and life sciences". More analytical, but not necessarily more useful, monthly surveys are provided by the well-subsidized and therefore relatively inexpensive *Bibliography of Agriculture* (based at the Library of the U.S. Department of Agriculture, Washington) and the *Index Medicus* (National Library of Medicine, Washington). At the beginning of 1960 the last of these was re-organized and its production mechanized. Each of these periodicals lists 100,000 or more titles a year and each is integrated with a photocopying service. Descriptive zoology is covered in a more leisurely manner by the *Zoological Record,* veterinary science by the *Index Veterinarius,* and more in this way might be done. At the moment suggestions for an *Index Biologicus* are being canvassed.

The next step is provided by the diverse abstracting organizations which scan the literature independently, usually with particular audiences in mind. Much could be said about these services. One generalization is that any item abstracted is likely to be abstracted more than once. The World Health Organization survey already mentioned estimated that 246 journals from 10 countries publish more than one and three-quarter million abstracts a year, that is, more than four times the total output of medical and biological articles.

Retrieval from the past is fraught with difficulties. Memories are short, many special libraries are young, and the searching of annual and cumulative indexes is tedious and very uncertain of results. (One inevitable imperfection of indexing is that indexers can never be sure what posterity will find relevant.)

Biology has one major advantage over other branches of science in having a built-in, internationally used, basic retrieval code (which also reflects a more or less internationally acceptable classification) in the scientific names applied to living things, especially at the generic and specific levels. The truly international character of the binomial names of species is not always fully appreciated. Such Latin names are frequently the only means by which a Western biologist can ascertain the subject of writings in Russian, Japanese, and Chinese. When this is realized, the importance of using names in line with what may sometimes seem to be the arbitrary requirements of the three *International Codes of Nomenclature* (those for zoology, botany and bacteriology) should be apparent.

Scientific names are also the basic grid references by which one experimental biologist is able to relate his observations to those of others. There are, of course, pitfalls. Changes in names necessitated by taxonomic studies tend to give taxonomists an unjust reputation for irresponsibility. Many taxonomists are well aware of the undesirability of disturbing traditional nomenclature. Unfortunately the cataloguing of organisms is far from complete, and many necessary taxonomic changes (and hence changes in names) will have to be made.

One thing that taxonomists are not required to do under the *Codes* is to register their nomenclatural innovations. It is left to others to find the published announcements, and that may take decades. Many names are thus duplicated, and unnecessary confusion results. Even the latest supplement (that for 1950–55) to the *Index Kewensis* (which since 1895 has been compiling the names of the genera and species of flowering plants) has a spread of more than a hundred years. (The average lag in retrieval by current content and abstract journals of, say, 75 per cent of the literature noticed is probably about 9–12 months, a period which approximates to the interval between the submission of a paper to a journal and its subsequent publication.)

There is another outstanding need. While there are lists of varying length and varying degrees of incompleteness of the genera and species of most of the main groups, there are very few complete lists, and no general compilation. A list of the genera proposed for all organisms would be a most valuable tool not only for biologists but also for librarians, information officers, and so on. By consulting such a list, information could be correctly classified for filing, the direction in which to look for further information indicated, and the duplication of names would be less likely. The systematic position of each name could be briefly coded (on the lines suggested by Mullins and Nickerson[7]) and the work, which should be internationally sponsored, need not be larger than one of the sectional volumes of the *London Telephone Directory*—and might be of similar quality and format, for such a compilation would need to be under constant revision, and it would be rational when the latest arrived to discard the previous edition.

This raises the question of the length of life of biological literature. The average life of a physics paper is said to be 2–3 years. Biological literature, much of it still being descriptive, is rather longer-lived although some useful material scarcely survives birth.

During the past two years more than a hundred papers have been published to show that 1 gm. daily of griseofulvin taken orally effectively controls infection of man by diverse dermatophytes in all five continents. Many of these papers were necessary. Some are of high quality and beautifully printed on good paper. This should not mislead. They are mostly already as dead as last night's evening newspaper. They will not be consulted again, but they will survive for long in indexes and bibliographies and so tend to obscure still useful data on a notable antibiotic.

At the other extreme taxonomic biological literature is long-lived. A comprehensive taxonomic paper may have an effective life of anything from fifty to a hundred and fifty or more years and at centres where general taxonomic investigation is undertaken works a century old are in constant use at the bench.

For science in general the small use to which much of the literature is put has been brought recently into prominence by the survey by Urquart and Bunn[8] of the use made of the Science Library serial holdings during 1956. They found that, of the total of 9,120 holdings, 350 titles accounted for 50 per cent of the total use, 1,200 for 80 per cent, and they consider that for perhaps half the present titles the total use is less than 10 times per 10 years. The relevance of this in planning storage is obvious. For some journals one run would be sufficient for the needs of the whole country—provided photocopies or microfilms were loaned—while for bulky, little-used, or rare material microfilms, microcards, or fiche could be used to reduce storage and acquisition costs.

Microtexts (which have recently been most lucidly reviewed by Davison[9]) have many advantages, and to taxonomists it is their retrieval value which appeals so strongly. One of the banes of taxonomic work is one taxonomist copying data from publications not seen from the work of another (who as likely as not copied from a predecessor who, even if he did see the original, probably interpreted what he read; only a very experienced taxonomist can copy what an author actually wrote). A number of useful taxonomic journals and scarce out-of-print books and monographs are already available on cards or fiche. But their production is slow and unplanned, and their cost, the demand being so much below what it might be, relatively high, although cheap compared with originals. In part the small demand is due to prejudice, in part to the lack of a mass-produced reading device, and in large part just ignorance.

One of the chief omissions in this brief survey is a consideration of systems of classification for information storage and for indexing the information stored. Another omission is any mention of the coding and mechanical searching for data. Mechanization begins with the identification of, say, timber-attacking fungi by hand-sorting punched cards with a knitting needle[10], and proceeds via elaborate mechanical sorting of thousands—even tens or hundreds of thousands—of Hollerith or IBM cards to systems of coding on magnetic tape which can be searched at incredible speeds. Some of these methods are of proved value, others are of great potential value. There does, however, seem to be a very real danger of treating comprehensive systems for storage and retrieval as ends in themselves. Much effort can be wasted in coding and storing information for which there proves to be little or no demand. The most urgent present need for biology is for some much simpler aids to information storage and retrieval.

---

[1] *W.H.O. Library News*, 12, Supp., 1959 (*Biol. Abst.*, 35 (6), xii; 1960).

[2] Brodman, E., and Taine, S. I., Reprints of papers for Intern. Conf. on Scientific Information, Area 2, 121, 1958 (*Biol. Abst.*, 33 (5), xii; 1959).

[3] Mellanby, K., *J. Inst. Biol.*, 7, 83 (1960).

[4] *Year-book of the Royal Society of London*, 274 (1961).

[5] Phelps, R. H., and Herling, J. P., *Unesco Bull. Lib.*, 14, 89 (1960); *Alternatives to the Scientific Periodical. A Bibliography* (Unesco, 1959).

[6] Published by International Association for Plant Taxonomy and Nomenclature, Utrecht.

[7] Mullins, L. J., and Nickerson, W. J., *Chronica Botanica*, 12, 211 (1951).

[8] Urquart, D. J., and Bunn, R. M., *J. Document.*, 15, 21 (1959).

[9] Davison, G. H., *Library Assoc. Rec.*, 63, 69 (1961).

[10] Findlay, W. P. K., *Trans. Brit. Mycol. Soc.*, 31, 106 (1947).

THE CONCEPT OF KIND OF TEA

By W. WIGHT and R. C. J. H. GILCHRIST

Tocklai Experimental Station and Jorehaut Tea Co., Cinnamara, Assam

BY tea we mean the 'black tea' usually consumed in Great Britain and elsewhere which is made from the fermented shoots of *Camellia sinensis* (L.) O. Kuntze, and its hybrids. Our remarks do not refer to unfermented 'green tea' or to partially fermented 'oolong tea'.

When one speaks of tea 'quality' two meanings can be conveyed: to the uninitiated the expression suggests the overall value or acceptability of a tea, in the sense of a summation of its desirable attributes; but in the trade, 'quality' is a particular attribute which, by itself, does not determine acceptability.

Attributes of tea recognized by professional tasters have been described in a recent report[1]. Quality and strength are important, and a full understanding of their separate meaning is necessary if tea markets are to be expanded. In our investigations quality has been defined as a dulcet freshness, like that of a recently caught fish, in contrast with one that has been in cold storage. The quality of tea enhances one's appreciation of its odour. A crude analogy is the sugar that imparts a quality to sauce without making it noticeably sweet. A fresh hazel nut (*Corylus* sp.) has the sweetness of quality. Some tasters might disagree with these definitions, but it is usual for their ordination of teas on a 'quality' [*sic*] basis to be very similar. Strength is a bitterness like bitter beer contrasted with mild beer.

Relative intensities of quality and strength, measured by tasters' scores[2], vary from plant to plant. Between populations the two attributes are often positively associated, with a correlation coefficient of the order of 0·6. The morphological feature of pubescence is positively correlated with quality[3]; and under the same conditions a similar correlation can be expected between pubescence and strength. Whereas pubescence makes roughly equal contributions to these two attributes, other morphological features make unequal contributions: for example, the vascular bundle when defined as the ratio of xylem cells to phloem cells (per unit area of cross-section), makes a positive contribution to quality and a negative contribution to strength, in accordance with the statistically significant ($P < 0{\cdot}01$) equations:

$$\text{Quality} = -27 + 0{\cdot}06C + 5{\cdot}5H + 0{\cdot}16Pi + 50Vb$$
$$\text{Strength} = 22 + 0{\cdot}11C + 4{\cdot}5H + 0{\cdot}05Pi - 22Vb$$

where C is the crop yield of the bush, H is its pubescence, Pi is phloem index[4], and Vb is vascular bundle. C varied from 86 to 171, H from 1·30 to 3·23, Pi from 20 to 116, and Vb from 0·40 to 0·66. The factors are derived from 10 jat populations. The equations are intended only to indicate the nature of the situation within the limits of *C. sinensis* var. *assamica*. It is not possible to enter into details in the present article. It is, however, important to note that vascular bundle (Vb) differs greatly between biotypes.

It is misleading to say that "The various types of tea in commercial use result mainly from differences in manufacture"[5]. All black tea is not alike, and many differences are due to genetical factors. For example, the kinds of tea called 'Ceylon', 'Darjeeling' and 'Assam', are well known and will serve as examples. In common parlance, these teas 'taste' differently; but much of this effect is due to odours the perception of which is independent of taste buds. Thus it is necessary to say that each tea has a characteristic odour.

Good-quality Assam tea that is lacking in strength would lose some cash value, merely because strength is an accepted feature of Assam tea. The strength of good Assam tea is almost quinine-like in its bitterness. Such high strength would be unacceptable to those who drink either Darjeeling or Ceylon tea, and would therefore tend to reduce the cash value of the tea. Thus the value of a tea depends on the market in which it is to be sold. Similarly, prices in auctions depend on a tea conforming to the attributes expected from its designation, as 'Assam tea', 'Ceylon tea', and so on.

These examples show that a certain odour, and a certain balance of quality and strength, define a kind of tea. Odour is decided in the first place by the botanical nature of the plant. It is to be noted that 'aroma', used in an earlier paper[1], was meant to be synonymous with 'odour' used here and by food technologists[6].

The kind of tea known as 'Assam' is made from *C. sinensis* var. *assamica*. It cannot be made from var. *sinensis*[7]. Hybridity with *sinensis* introduces unwanted odours; but hybridity with the southern form of plant seems to introduce desirable odours.

Darjeeling tea is made from populations with pronounced features of the type variety (var. *sinensis*). Some of the populations are hybrids between distinct species[8]. Odours of *sinensis* seem to be needed in Darjeeling tea[7]; but recent investigations strongly suggest that decisive odours have also been contributed by other taxa like *C. irrawadiensis* or *C. taliensis*, or even more distantly related species.

Plants of the Assam variety (var. *assamica*) grown in Darjeeling do not make Darjeeling tea. Representative Darjeeling plants, brought as seed to Assam, do not make Assam tea. Tea from similar plants, established in Assam as the descendants of seed imported directly from China and Formosa, has been accepted as Darjeeling tea by professional tasters.

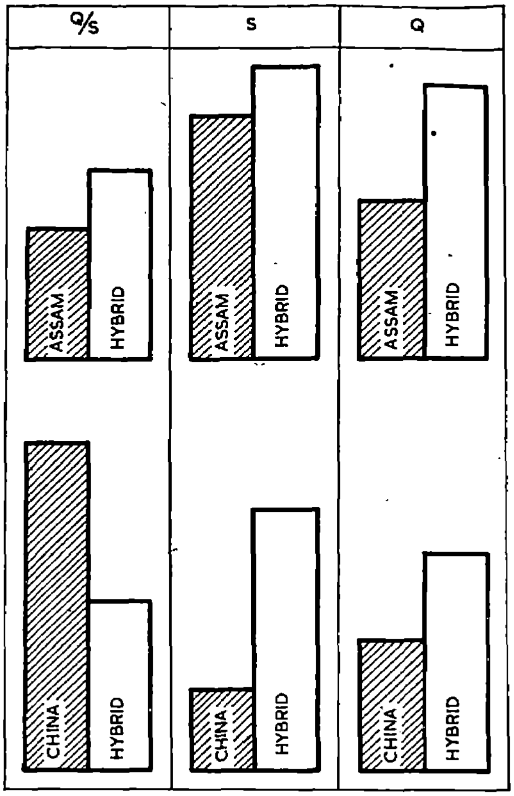


Fig. 1. Nature of the tea made from putative hybrids between *Camellia sinensis* var. *sinensis* and *C. sinensis* var. *assamica*. Column heights indicate the tasters' perception of quality (Q) and strength (S), and the ratio of these measures (Q/S). 'Assam' and 'China' indicate plants not appreciably different from the respective types. 'Hybrid' means plants with some features of the other taxon, but assignable to the type with which they are coupled in the figure

Ceylon tea, like Darjeeling tea, is made from *sinensis* hybrids; but there are more features of the southern form in Ceylon than in Darjeeling[9]. It seems likely that genetical factors derived from the southern form play a large part in determining the characteristic odour of Ceylon tea.

It has not been found possible to make Assam tea from plants of the southern form growing in Assam; but open-pollinated seed of these plants, taken to high elevations in Ceylon, produced plants (of the southern form) that made good Ceylon tea. The possibility of making Ceylon tea in Assam has not been investigated; but the evidence clearly indicates that at least three kinds of tea, which take their names from regions, must be determined primarily by genotype and, possibly, by interactions of genotype with environment. Interactions in terms of 'phloem index' (which is associated with quality[1]) have been demonstrated in transplant experiments[10].

In addition to odour, the balance of quality and strength differs between taxonomic types. Fig. 1 shows that quality relative to strength is high in *sinensis*; conversely, high strength, relative to quality, characterizes *assamica*. Fig. 1 also shows that quality is improved by hybridity—a point that was amplified recently[1]. The figure further shows that the strength of nominal *sinensis* plants is increased by slight hybridity with *assamica*; but, in this connexion, it is to be remembered that strength may not be particularly desirable in tea that can be made from *sinensis*. Comparable data for hybrids with the southern form are lacking, but the quality to strength ratio appears to be the same as that of *assamica*.

New kinds of tea could be a great asset to tea exporters; but it is evident that new odours would be needed. However, the odours in plants of the southern form are sufficiently variable to make them most desirable for breeding new kinds of tea. The form needs more study. It is the conceptual type[11] of material received from Tran-ninh by courtesy of M. du Pasquier who attached importance to the tea plants of that region[12]. Primary selection might lead to a stock as valuable to tea as the Barberton *U*4 strain was to cotton[13]. This would be due to odour. But the need, or otherwise, for strength, depending on prospective markets, would have to be kept in mind. Quality is always needed and is a concomitant of hybridity between distinct types[1]. In short, odour classification[6] and the separate definition of quality and strength seem essential to a progressive tea-breeding programme.

[1] Wight, W., and Gilchrist, R. C. J. H., *Rep. Tocklai Exp. Sta. 1959*, 69 (1961).

[2] Wight, W., *World Crops*, **8**, (7), 263 (1956).

[3] Wight, W., and Barua, P. K., *Nature*, **173**, 630 (1954).

[4] Wight, W., and Barua, D. N., *Curr. Sci.*, **23**, 78 (1954).

[5] Monopolies and Restrictive Practices Commission, *Rep. Supply of Tea* (H.M.S.O.).

[6] Crocker, E. C., *Flavour* (New York, 1945).

[7] Wood, D. J., *Rep. Tocklai Exp. Sta. 1957*, 176 (1958).

[8] Barua, P. K., and Dutta, A. C., *Phytomorph.*, **9** (4), 372 (1960).

[9] Green, M. J., *Rep. Tocklai Exp. Sta. 1958*, 62 (1959).

[10] Wight, W., *Rep. Tocklai Exp. Sta. 1959*, 87 (1961).

[11] Wight, W., *Nature*, **183**, 1726 (1959).

[12] du Pasquier, R., *Extrait Bull. Econ. Indochine*, No. 169, N.S., 6 (Hanoi, 1924).

[13] Hutchinson, J., *The Application of Genetics to Cotton Improvement* (Cambridge, 1959).

NORWICH MEETING OF THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

It can never be sufficiently stressed that the elaborate annual meetings of the British Association for the Advancement of Science, for which the Association is best known, are intended primarily to disseminate scientific knowledge among the lay public. The Association's one-hundred and twenty-third annual meeting: to be held in Norwich during August 30–September 6, may serve to underline this fact, for although Norwich is a historic provincial capital, it is the centre of a predominantly rural community in which scientific processes are not of the widest application. Furthermore, although in the near future it will be, Norwich is not at present a university town, so that it will not call on university resources to assist the meeting, as do the majority of the Association's hosts. Instead, it is calling on the staffs of the City College and schools in Norwich to help in organizing the sectional programmes, on schools and civic buildings to house the meetings and on its civic officers for the general organization.

The meeting will follow the customary pattern of an inaugural meeting: two evening discourses, a full programme of sectional lectures and excursions to many parts of East Anglia, some 45 excursions to places of general interest (in which the area is unusually rich) and a number of social functions, including four garden parties in settings which should prove ideal. There will also be a young people's programme which will include a 'Science in Schools' exhibition and an 'Open Forum' for both adults and young people at which scientists will answer questions impromptu. The presidential address to be given at the inaugural meeting by Sir Wilfrid Le Gros Clark will be entitled "The Humanity of Man".

There are, however, two features in the programme for the coming meeting which are novel. One will be a public demonstration of the new Swiss colour television technique known as Eidophor. Eidophor is a form of large-screen television projection invented by the late Dr. Fritz Fischer, of Zurich. Since his death in 1947 it has been continually developed by Prof. Baumann, and the costly and delicate apparatus used will be brought by road from London and installed in St. Andrew's Hall. It will relay to a smaller hall the proceedings at the inaugural meeting and on subsequent evenings will give demonstrations. This is likely to prove a fascinating feature of the meeting, and Norwich is indeed fortunate that it has been possible to arrange these demonstrations. The other novel feature is also in the sphere of the visual arts: this is a daily programme of scientific films from all nations which will be shown in the Noverre Cinema. There will be a different programme each day, and it is hoped that so comprehensive a collection of outstanding scientific films will prove a great attraction.

As is common, the majority of the branches of scientific study which are catered for at the Association's annual meetings are well reflected in Norwich and Norfolk. Norwich, by historic tradition, beginning with the growth of the the weaving industry, enjoys a great reputation as a home of handicraftsmanship. The decline of the medieval textile industries was offset by the craftsman's inherent versatility, and the change-over to the manufacture of shoes, in particular, during the past century, enabled the city to retain its reputation in this direction. In more recent times the development of light engineering has sustained native potential, and it is with this background that the subject of one of the discourses, "The Impact of Automation", to be given by Dr. B. V. Bowden, principal of the Manchester College of Science and Technology, assumes a particular interest. In the same way as physicists and engineers, the biologists, geologists and archæologists are provided with a fruitful research field, since East Anglia is a distinctive part of the insular land-mass of Great Britain. Once part of a terrain which was joined to the European continent and then largely inundated, its flora and fauna are objects for rewarding comparative study. The coasts and the Broads will be visited on many occasions both by people with specialized interests and those who wish to enjoy their scenic beauty. The subject of the other discourse, which will be given by Field-Marshal Viscount Alanbrooke, will be "Birds of Spain", and this discourse, in an area notable for its wild life, is expected to draw a large audience. Archæologically, Norfolk's records of the historic ages prior to the Roman occupation are of absorbing interest, and of the Roman period itself they are probably unsurpassed in Britain.

Forestry and agriculture are perhaps the two sciences to which most justice can be done, since the Forestry Commission owns many acres in west Norfolk where afforestation is intensively pursued; and in the sphere of agriculture it would be hard to refute Norfolk's claim as the cradle of modern farming methods. From the time of Coke of Norfolk onwards, highly developed farming has been the major industry, and local agricultural experimental stations have made significant contributions to the progress of farming throughout the country.

With these factors in mind, and with the backcloth of a gracious cathedral city, the organizers of the Association's 1961 meeting hope that it will be even more successful than its predecessors in Norwich in 1868 and 1935. Every effort will be made to welcome visitors from outside the area and to ensure that they derive all possible pleasure and benefit from the meeting. D. D. Aldridge

OBITUARIES

Mr. J. Walter Ryde, F.R.S.

John Walter Ryde died at Marlborough, Wiltshire, on May 15, after a short illness. He was sixty-three years old. He was born in Brighton on April 15, 1898, the son of Mr. W. W. Ryde, and was educated at St. Paul's School and in France. Plans for him to continue his studies in Germany and then go to the University of Cambridge were interrupted by the First World War. He returned from Berlin to England just before the outbreak of war and entered the City and Guilds Technical College, Finsbury, where he studied under Prof. Sylvanus Thompson and Prof. Charles R. Darling.

Ryde joined the Royal Engineers voluntarily in 1916, at the age of eighteen, on the completion of his course at City and Guilds, and was trained in anti-aircraft work in Monmouthshire and on the Norfolk coast before being drafted to France during the last few months of the War. He continued to serve in France until his demobilization in 1919.

Ryde joined the Research Laboratories of the General Electric Company, Ltd., as a physicist in 1919, going directly from his war service to the newly established research unit directed by Clifford C. Paterson in the Company's lamp factory at Hammersmith. He was a leading member of the scientific staff of the Laboratories from the start.

In the early days at Hammersmith, and again after the Laboratories had moved to their new premises at Wembley in 1923, the variety of Ryde's research work was indicative of the breadth of interests which was so characteristic of him. They included pure spectroscopy, thermionics and the gaseous electrical discharge. Under his direction the first practical high-pressure mercury vapour lamp was developed—the foundation of a new industry to which he made many subsequent contributions.

His theoretical work on the scattering of light, first applied in the 'twenties to optical diffusing media in glasses, was extended by him during the Second World War to classic studies of the attenuation and the radar echoes produced by meteorological phenomena at centimetre wave-lengths. (A footnote in this paper includes the following: "The author (J. W. R.) and Mrs. Ryde were responsible for the theory and calculation of the effects caused by liquid and solid particles,——".) He was an early worker on crystal valves and quickly recognized the potentialities of germanium as a semiconducting material. Other researches included velocity modulation tubes, luminescent materials and optical projection systems. He contributed much to various research projects undertaken for the United Kingdom Atomic Energy Authority, and he took part in work on the *Fido* fog-dispersal system. It was typical of Ryde to design, in his leisure hours, a pocket illumination chart for use by the Services. It showed levels of illumination, by day and by night, at various latitudes, and was used extensively in military operations.

Ryde was elected a Fellow of the Royal Society in 1948. He was appointed chief physicist at the G.E.C. Research Laboratories—recently named the Hirst Research Centre—in 1950 and chief scientist in 1953. During these recent years he had overall responsibility for the programmes of pure scientific research of the Centre, and his particular interests lay in the fields of new materials, crystal growing and solid-state physics.

He was a member of the Royal Institution of Great Britain since 1935 and chairman of the Davy Faraday Laboratory of the Institution since 1951. He served on the Board of Managers of the Institution and always took a great interest in its affairs. He was a Fellow of the Institute of Physics and the Physical Society, a Fellow of the Royal Astronomical Society, and a member of the Geological Association, the Wiltshire Archæological Society and the Athenæum.

Ryde married, in 1930, Dorothy Ritchie, daughter of the then chief illuminating engineer of the General Electric Co. There is one son.

The Hirst Research Centre has lost a senior man who earned the admiration and respect not only of his colleagues but also of eminent men both in Great Britain and overseas. He lived his life for science, and his leisure activities were very largely extensions of his life's work. Archæology was a persistent interest—at an early age he learned to read hieroglyphic writing and ancient scripts. He was a profound student of astronomy, but, in recent years, microscopy became a dominant hobby. He was particularly interested in diatoms, and his beautifully mounted specimens are evidence of the elegant experimental skill which he retained to the end.

His memory was prodigious and visual. He never forgot the details of work which he had done and could sketch from memory some experimental arrangement or the detailed shape of a specimen long afterwards. Such a memory, supplemented with the many carefully prepared and indexed note-books which he compiled throughout his working life, made Ryde a powerful authority indeed in the world of science.

In all his work he displayed great ability and unfailing perseverance, together with brilliant insight and inspiration. His colleagues will always remember his patient readiness to discuss and assess the problems of the day and his helpful elucidation of critical experiments, often elegant in their simplicity, which he designed to solve them. O. W. HUMPHREYS

Dr. P. A. Gorer, F.R.S.

THE unexpected death of Dr. Peter Alfred Gorer on May 11 will be deeply regretted by all who knew him. Born in 1907, he was educated at Charterhouse before taking up medicine at Guy's Hospital. During this period he developed an interest in hibernation, and his earliest papers were on this subject. After qualification, he left Guy's for the Department of Genetics at University College, London, under J. B. S. Haldane. It was here, and later at the Lister Institute, that he first began a lifelong study of the genetic and immunological implications of tumour transplantation. At this time only the genetic basis of tumour transplantation had been established. Gorer proposed that genetically determined antigens present in neoplastic tissues were the immediate arbiters of the fate of such grafts. If the transplant introduced antigens not possessed by the recipient animal, rejection of the graft would follow. Between 1937 and the outbreak of the Second World War, he produced solid evidence in support of this idea. During hostilities he acted as assistant morbid anatomist, and hæmatologist, at Guy's Hospital, and remained afterwards as reader in the Department of Experimental Pathology. Only this year he had been chosen to fill the chair of a newly created Department of Immunology at Guy's Hospital.

On returning to the subject of his researches after the War, he spent several years developing the serological methods necessary before any further progress was possible. The defeat of these technical difficulties enabled him to perform a detailed analysis of the major system of tissue antigens in mice, known as the H-2 system. His latest paper on this subject indicated the progress which has been made in mapping the genes which dictate the presence of the H-2 antigens.

But Gorer was probably best known for his work on the part played by humoral antibody during the rejection of foreign tissue grafts. He established that such antibodies were present in the serum of mice within three days of receiving a graft of alien tumour; furthermore, normal tissues such as skin were similarly able to excite the formation of humoral antibodies. The function of these antibodies is still the subject of research, but it is clear from his evidence that humoral antibodies have several effects; which one predominates varies according to the target graft, and to a lesser extent the recipient.

At an early stage Gorer was aware of the probability of antigenic differences between normal and malignant tissues. The occurrence of antigenic loss had been implied by much genetic evidence, and he confirmed this by serological means. Few investigations distinguished clearly between normal tissue antigens possessed by tumour cells, and antigens peculiar to the neoplastic state. In 1956, in collaboration with one of his pupils, D. B. Amos, he discovered the first of a series of antigens specific to mouse lymphatic leukæmia cells. Later work showed that such antigens were detectable in spontaneous as well as induced leukæmias. It is sad indeed that he was unable to start on the important investigation of the significance of these antigens.

One cannot account for the pre-eminent position of Peter Gorer merely as a result of his brilliant researches and thoughtful reviews. He had great charm and wit, a breadth of interest rarely found, and perhaps the most striking attribute of all was an ability to discuss his own subject dispassionately. It gave great pleasure to his many friends and pupils when he was elected Fellow of the Royal Society in 1960. We shall greatly miss him.

He leaves a widow, a son and a daughter.

J. R. BATCHELOR

NEWS and VIEWS

U.S. National Academy of Sciences: Foreign Associates

THE following have been elected foreign associates of the U.S. National Academy of Sciences: Prof. K. E. Bullen, professor of applied mathematics in the University of Sydney; Prof. B. Ephrussi, professor of genetics in the Sorbonne, and director of the Genetics Laboratory of the National Centre for Scientific Research at Gif-sur-Yvette; Dr. W. K. Heisenberg, director of the Max Planck Institute for Physics and Astrophysics, Munich; Prof. V. Prelog, professor of organic chemistry and director of the Laboratory, Federal Institute of Technology, Zurich.

Prof. K. E. Bullen, F.R.S.

PROF. BULLEN is a native of New Zealand, born in Auckland in 1906. His interest in seismology was aroused by New Zealand's Hawke's Bay earthquake of 1931 and encouraged by Sir Harold Jeffreys, whom he met the same year, during Bullen's studies in Cambridge. Together they produced the Jeffreys–Bullen tables of seismic travel times, which have been used since 1940 in preparing the *International Seismological Summary*. Bullen's pressure-incompressibility hypothesis, issued in 1946, led to the inference that the Earth's inner core is solid (in the sense that it can transmit seismic waves), although surrounded by a fluid outer core; it also led him to construct his Earth model B, a widely used mathematical model of the Earth's seismic functions. In addition, Prof. Bullen is the author of several papers on the internal structures of Venus and Mars and on the origin of the Moon.

Prof. B. Ephrussi

PROF. EPHRUSSI was born in Moscow in 1901. On completing his secondary education there, he went to Paris in 1919 and continued his studies at the Sorbonne, where he received a Rockefeller Foundation fellowship and obtained the D.Sc. degree in 1932. During 1930–34 he undertook investigations in the field of chemical embryology and tissue culture, becoming in 1931 an assistant to Prof. Faure-Fremiet at the Institut de Biologie Physico-Chimique. Among his numerous writings on these subjects, his book on tissue culture, published in 1932, is now an acknowledged classic. An interest in genetics, occasioned by his studies of tissue culture, led him to apply for a second Rockefeller Foundation fellowship to work with Prof. T. H. Morgan at California Institute of Technology during 1934–35. In the course of his experiments there in genetics and tissue culture, he observed that a certain combination of genes which causes death in embryonic mice is not necessarily lethal at the cellular level, since individual tissue cultures from these embryos will survive far beyond the embryo's ten-day life-span. In 1935 Ephrussi returned to France, accompanied by Prof. G. W. Beadle. Together they undertook a series of eye-disk transplantation experiments in *Drosophila* which resulted in the first experimental proof of the genetic control of biochemical reactions. After the Second World War he began his post-war studies on yeast, and soon discovered that mutations caused by acridine dyes transform aerobic into anaerobic strains. His subsequent classic investigations probed the biochemical interplay between cytoplasmic and nuclear elements, and resulted in a second book dealing with the significance of nuclear and cytoplasmic genetic variation in embryological development. He is now entering on a study of the genetics of mammalian somatic cells in tissue culture.

Prof. W. K. Heisenberg, For.Mem.R.S.

PROF. HEISENBERG won the Nobel Prize for Physics in 1932, for the creation of quantum mechanics. His was a fundamental approach to the problems of atomic mechanics, based on observable physical properties such as the frequencies and intensities of spectral lines emitted by atoms and molecules; this system, known as matrix mechanics, dispensed with unwarranted assumptions inherent in earlier atomic theory, arising from pictorial representations of planetary orbits within the atom. In 1927 he solved the puzzle posed by the existence of two separate spectra of helium gas, showing that one of them arises when the two electrons of the helium atom are paired with opposite spin and the other when the spins are in the same direction. He then predicted, by analogy, that the hydrogen molecule should exist in two forms: one in which the paired hydrogen nuclei spin in the same direction, and another in which they spin in opposite directions. Two years later the two forms of hydrogen were experimentally shown to exist. It was also in 1927 that Heisenberg formulated his famous principle of indeterminacy (or uncertainty principle), which maintains that regardless of the nature of the experiment there is an inherent theoretical limit to the precision with which both position and momentum of a particle can be established simultaneously. He showed that this ultimate limit of physical measurement is defined by a fundamental universal constant that had been introduced into physics by Max Planck at the turn of the century.

Prof. V. Prelog

PROF. Prelog was born in Sarajevo, Yugoslavia, in 1906. He studied chemistry and chemical engineering at the Prague Institute of Technology, where in 1929 he received the degree of doctor of technical sciences. He headed the research laboratory of the G. J. Driza Co. in Prague until 1935, when he joined the Department of Technology of the University of Zagreb as an associate professor. In 1942 he joined the Department of Organic Chemistry of the Swiss Federal Institute of Technology (Eidgenossische Technische Hochschule) in Zurich, where he became associate professor in 1947, professor in 1950, and director of the Department in 1957. He is the author of some 270 scientific papers on such topics as alkaloids and steroids, heterocyclic and many-membered-ring compounds, antibiotics, and stereochemistry.

Applied Mathematics at Belfast: Dr. A. Dalgarno

DR. A. DALGARNO, though only thirty-three years old, has been appointed to a chair of quantum mechanics in the Department of Applied Mathematics at the Queen's University of Belfast. He began his brilliant research career at University College, London, where in 1951 he obtained a Ph.D. degree. In the same year he moved to Belfast as an assistant lecturer, and was promoted successively to lecturer in 1952 and reader in 1956. Last year he was made director of the newly established Digital Computing Laboratory—an appointment he still holds. Since 1951 he has paid a number of prolonged visits to the United States as guest research worker at the Massachusetts Institute of Technology, and as consultant to Air Force Cambridge Research Center and to the Geophysics Corporation of America. Dr. Dalgarno's research interests include the methods of quantum mechanics (especially perturbation theory), atomic and molecular structure, collision processes and upper atmospheric physics. His skill at mathematical analysis is exemplified by his classical paper with Dr. J. T. Lewis (*Proc. Roy. Soc.*, A, 233, 70; 1956) on the calculation of long-range forces between atoms. This paper gives the exact solution to an important problem which had been treated by approximate methods for more than a quarter of a century. As well as being very creative, Dr. Dalgarno is a stimulating supervisor of research students. His appointment will consolidate the strength of the postgraduate school at Belfast.

Machine Tool Engineering at Manchester: Prof. F. Koenigsberger

DR. FRANZ KOENIGSBERGER has been appointed to the newly created chair in machine tool engineering in the Faculty of Technology in the University of Manchester. Dr. Koenigsberger received his engineering training in the Technische Hochschule, Berlin-Charlottenburg, where he qualified for the degree of Dipl.Ing. in 1931 with honours. He obtained the degree of M.Sc.Tech.(Manchester) in 1952 and the degree of D.Sc.(Manchester) in 1954. After a period of research experience under Prof. Schlesinger in Berlin, he obtained industrial experience in Berlin and later in Belgium. During 1935–38 he was chief engineer and chief designer in the Machine Tool Department of Ansaldo, Genoa, and in 1939 became chief mechanical engineer for Messrs. Cooke and Ferguson, Ltd., a post he held until he was appointed lecturer in mechanical engineering in the Manchester College of Science and Technology in November 1947. He was appointed senior lecturer in production engineering in 1955, and reader in machine tools and production processes in May 1957. Dr. Koenigsberger is well known for his work in the field of production engineering, and is a member of the International Institution for Production Engineering Research.

Medical Statistics at Belfast:

Prof. E. A. Cheeseman

Dr. E. A. Cheeseman was recently appointed to a personal chair of medical statistics in the Department of Social and Preventive Medicine at the Queen's University of Belfast.

Dr. Cheeseman was educated at the William Ellis School and the University of London. He obtained his B.Sc. (Economics) in 1938, and Ph.D. (Medicine) in 1947. He was a member of the Territorial Army at the outbreak of war and until early 1945 served with the Royal Artillery. After six months as a statistical staff officer with H.Q. 21 Army Group he was demobilized with the honorary rank of Captain. After the War he joined the Department of Epidemiology and Vital Statistics at the London School of Hygiene as a member of the Medical Research Council's Statistical Research Unit under Prof. A. Bradford Hill. In 1948 he became lecturer in medical statistics in the Department of Social and Preventive Medicine at the Queen's University of Belfast, and adviser in medical statistics to the Northern Ireland Hospitals Authority. He was appointed reader in 1952. He is a member of the Statistical Committee of the Medical Research Council and of the Statistics Sub-Committee of the Registrar-General of England and Wales, and has been the author or part-author of numerous publications in the field of human genetics and the statistical aspects of medical research. His chief professional interests are advising medical colleagues on the design and analysis of experiments and trying to convince medical undergraduates and postgraduates that statistics are not as difficult as they appear to be.

Control of Colleges of Advanced Technology in Britain

On June 22, Sir David Eccles, Minister of Education, made a statement in the House of Commons on the development of colleges of advanced technology. Control of these colleges, which have been maintained by local education authorities, should be transferred to independent governing bodies. These would receive direct grants from the department of education. His proposal stems from the observation that the success of these colleges in developing as national institutions of higher education has engendered new problems of planning and staffing. Though local government has given good service in the past, to-day the colleges are too big for local management. Bearing in mind the quest of the Robbins Committee and the consideration it might be giving to the future of colleges of advanced technology, the Minister is writing to associations of local authorities and teachers to propose this new status. To preserve links with the local authorities, who have been so helpful, it is suggested that the authorities continue to be represented on the new governing bodies.

The Colonial Microbiological Research Institute, Trinidad

The Colonial Microbiological Research Institute, Port of Spain, Trinidad, was opened on July 5, 1948, by Lord Hankey, chairman of the Colonial Products Research Council. The function of the Institute was to devote its energies to fundamental research in microbiology, to the application of the results of its investigations and to assisting agricultural and other industries in improving their products. The Institute maintained a collection of micro-organisms, the Hankey Culture Collection, in which microbes of industrial or agricultural importance received special attention. It has now been decided to close the Institute as from April 30. It had been financed from Colonial Development and Welfare Research funds up to March 31, 1960, and by the Government of Trinidad and Tobago during the past year. The buildings had become the property of the Government of Trinidad and Tobago. They have since been given to the University College of the West Indies and leased by the College to the Trinidad Regional Virus Laboratory in furtherance of closer association between these two institutions. The Hankey Culture Collection is now housed in the Faculty of Agriculture, University College of the West Indies, St. Augustine, Trinidad, and is under the charge of a curator.

Science and Television

The first attempt to provide a connected and long-term series of science programmes on television for adults has been announced by the B.B.C. Broadcast on Saturday mornings, the series has been grouped under the headings of "The Structure of Matter", "Physics in the Service of Man", "Human Biology" and "Science for Medicine", and will continue until the end of the year. Each programme has been broadcast previously on one or other of the B.B.C. programmes, but they have been re-arranged to provide a new basis for study and to illustrate definite themes. The speakers include many well-known men of science from Great Britain and the Commonwealth, and should enrich a collection of programmes which should bring pleasure to many viewers. It will be interesting, later, to see which members of the community have followed the broadcasts. Full details of the whole series have been set out by the B.B.C. in a recent pamphlet entitled *Saturday Morning Television introduces Science on Saturday* (Pp. 16. London: British Broadcasting Corporation, 1961), which can be obtained from 35 Marylebone High Street, London, W.1.

Pfizer Virus Research Laboratories

The latest aid to vaccine research by the Pfizer Group at Sandwich is the provision of a new specially designed building for research into virus diseases. It has extensive laboratory and animal accommodation, and is designed to preclude the possibility of infectious organisms escaping to the outside as well as preventing any contamination entering the building. The unit consists of three sections: a group of laboratories and two groups of animal blocks of four wards each, all completely separate and designed so that movement of personnel and air between each is controlled. Each animal ward and laboratory has its own high-intensity ultra-violet lock entrance. Sterile air is supplied to individual rooms and temperatures can be adjusted individually. One of the most important research projects to take place in this building will be the investigation of trachoma, a virus disease which causes blindness in Africa and Asia. The Pfizer Group are co-operating with the British Medical Research Council's Trachoma Research Group in this project.

Strontium-90 in Milk, 1960

A number of investigations have already indicated that the levels of strontium-90 in milk produced in the United Kingdom have been determined in large measure by the rate of fall-out occurring at or near the time of milk production. Consequently, a fall in the rate of deposition should result in a downward trend of the activity-levels in milk. This is borne out in a report by the Agricultural Research Council on the strontium-90 levels in milk samples, representative of a substantial fraction of United Kingdom milk-production during the period January–June 1960 (Report No. 4, *Strontium-90 in Milk and Agricultural Materials in the United Kingdom*, 1959–1960. Pp. xiii + 81, 34 tables, 14 figures. London: H.M.S.O., 1961. 5*s*.). Thus it appears, on one hand, that the deposition in the first six months of 1960 was less than 25 per cent of that in the same period of 1959, and, on the other, that the strontium-90 levels in milk at June 1960 were about one-half of those observed twelve months previously. Complete information on strontium-90 levels for other items of the diet in 1960 is not yet to hand, but the milk results give a preliminary indication that the mean level in the total mixed diet of the population was appreciably lower for 1960 than for the previous year. In addition to a full discussion of the milk results, the report assesses the results of a survey of strontium-90 in permanent pastures and animal bone carried out during 1959. It also includes useful summaries of the results of experimental investigations designed to elucidate the factors controlling the passage of fission products through food chains. Throughout, the discussion is lucid, and the experimental results are well presented.

Technical Education Abstracts

The place of technical education in the national economy is now so fundamental that any journal devoted exclusively to its promotion deserves the warmest of greetings. In the case of the newly launched *Technical Education Abstracts*, however, some qualification is necessary. (The National Foundation for Educational Research in England and Wales. *Technical Education Abstracts from British Sources*, September–December, 1960 (Vol. 1, No. 1). Pp. 57 + xxviii. (London: National Foundation for Educational Research in England and Wales, 1961.) Subscriptions: 42*s*. per volume of four quarterly issues.) Many of the abstracts necessarily relate to articles which are largely persuasive, and, though compelling interest at the time of publication, lack much of the force of the original articles both because of the condensed form and because they refer to topics which may have lost their urgency. This criticism may be offset if the publishers can, as they suggest, reduce the time between abstraction and the date of publication. A further improvement could be made in the selection of abstractors. Few of these are known in the field of technical education and this may explain why so many of the abstracts are taken from relatively few journals. The range could be widened if more help were obtained from industrial correspondents, many of whom would give their services voluntarily.

History of Medicine and Pharmacy at London

The Faculty of History of Medicine and Pharmacy was founded under the auspices of the Worshipful Society of Apothecaries of London (which received its Charter from King James I in 1617) on April 3, 1959. The aims of the Faculty are to foster and extend more general interest in medical history by enlisting the support of all medical corporations, schools, societies, clubs and individuals. The Faculty also welcomes the interest and support of general historians in forging closer links between medical and pharmaceutical history and general, social and economic history. The Society's ancient Hall in the City of London serves as a general meeting place for lectures, discussions and other meetings held in pursuance of these aims. In the second annual report (*The Worshipful Society of Apothecaries of London: Faculty of the History of Medicine and Pharmacy. Second Annual Report and List of Members*, 1960–61. Pp. 20. London: The Worshipful Society of Apothecaries of London, 1961) the Faculty describes some of the considerable progress during the past year. Membership now stands at 304, and, on the initiative of the Faculty, St. Thomas's Hospital Medical School has appointed "a Society of Apothecaries Lecturer in the History of Medicine", and the governing body of King's College Medical School has already approved the establishment of a similar appointment. The Faculty's endowed lectures have now been named; the annual students' lecture will henceforth be known as "The Osler Lecture", and the autumn lecture will be named "The Sydenham Lecture", after the great English clinician of the seventeenth century, Thomas Sydenham. Dr. Kenneth Dewhurst has accepted an invitation to give the first "Sydenham Lecture" on November 22, 1961, at Apothecaries' Hall; his subject will be Thomas Sydenham. The second British Congress on the History of Medicine is to be held in London during September 28–29.

The Library Association

The annual report of the Council of the Library Association for the year ended December 31, 1960, presented at the annual meeting in Hastings, 1961, describes the steps taken to press for early action by Parliament on the proposals of the Roberts Committee for the reform of the public library service (Pp. 28. London: Library Association, 1961). A memorandum on the status and training of reference librarians has been adopted by the Council and distributed to all chief librarians, and, at the request of the Select Committee on Estimates, a memorandum regarding the House of Commons Library was presented to the Select Committee, and a deputation later gave oral evidence. An investigation was initiated into ways and means of helping the developing Commonwealth countries, particularly in Africa and Asia, to establish more adequate library services. As a first step a survey is being made of existing services in these countries. A comprehensive and detailed guide to British periodicals is being compiled, and suggestions for improving the classification and expansions to the schedules have been sent to the editor of the Dewey Decimal Classification. The Association is taking steps to establish a National Advisory Council for Micro-reproduction following a meeting at which all types of libraries, learned societies and institutions were represented; a steering committee has been appointed.

The Brown Library, Liverpool

An illustrated booklet issued by the City of Liverpool Libraries, Museums and Arts Committee as a guide to the rebuilt Brown Library and the new links with the Picton and Hornby Libraries gives a concise history of the Brown Library, and then describes briefly the services which the rebuilt library offers in commerce, science and technology (Pp. 32. Liverpool: Brown, Picton and Hornby Libraries, 1961). Besides the special research services to science and industry offered by Ladsirlac, these include a

R326

commercial and social sciences library, technical library, science library, technical documents unit, patents library and microfilm unit. In the arts the library includes a Commonwealth library, an American library, international library, biography library, reference issue foyer, and a record office and local history library. The booklet displays well the regional character of the libraries and their services, and it should be noted that they are depository libraries for the publications of the American, British and European Atomic Energy Commissions, and for the publications of the United Nations Organization and the United Nations Educational, Scientific and Cultural Organization.

Library of the U.S. Naval Observatory

AN article by Marjorie S. Clopine, librarian of the United States Naval Observatory, reprinted from *Special Libraries* (52, No. 2, February 1961) and issued by the Observatory as Reprint No. 19, describes the resources and treasures of the Library, which are of particular interest for astronomers. By 1960 the Library contained more than 55,000 catalogued volumes and also many sets of periodicals, the mathematics collection being especially strong in analysis and noted for its value in reference work. There is a collection of 500 rare books printed before 1800. Besides long sets of nautical almanacs, the Library has a valuable collection of star catalogues and charts, observatory publications, bibliographical reference works in astronomy, the complete works of many astronomers and mathematicians, and mathematical tables.

Chemical and Allied Industries at the Science Museum Library

UNDER the title *Books on the Chemical and Allied Industries* the Science Museum Library has issued, for the Ministry of Education, a short-title list of books published in 1930 and onwards on the chemical industry, with books on mining and metallurgy, and the paper, leather and textile industries and received in the Science Library up to the end of 1959 (Pp. ix + 118. London: H.M.S.O., 1961. 12*s*. 6*d*. net). The list has been compiled by L. R. Day from the subject catalogue in the Library and is the second in a series of catalogues, each carrying a large division of the Science Library's collection. Entries are arranged in broad subject groups selected from the Universal Decimal Classification, and both an author index and a subject index are included. While intended primarily to enable users to take advantage of books available in the Library it is hoped that the list will assist other libraries in both the selection and classification of works in these fields. A Supplement, issued as Science Library Bibliographical Series No. 781, includes books received in the Science Library in 1960 (Pp. 10. London: Science Museum, 1961).

New American Electricity Plant

THE largest power plant in the western hemisphere has recently begun to operate on the United States–Canadian border. It harnesses the power of the River Niagara, which drops 325 ft. over cataracts and falls in its 34-mile course between Lake Erie and Lake Ontario. The new hydro-electric development, one of the world's largest, is the Robert Moses Niagara Power Project. The power output of its two generating plants is 2,190,000 kilowatts, exceeding the giant United States' Grand Coulee Dam. The new Niagara River project began power production three years to a day after contracts for its construction were signed. Construction has been rushed and limited operation started because of a power shortage that has existed since 1956, when a power plant was destroyed by a rock-slide at Niagara Falls. Only two of the plant's 13 generators are now operating, but the remaining 11 will be completed and placed in use at the rate of one each five months. The Robert Moses Niagara Power Project, built by the New York State Power Authority and named after its first chairman, is a 720,000,000-dollar non-profit undertaking, financed through the sale of bonds to private investors. Cost of the plant and interest to investors will be paid through the sale of electricity. Several utility companies have contracted to buy the plant's electrical output. Because of limitations of water-diversion, the United States' share of river-flow during daylight in summer is not sufficient for continuous operation of the new plant. To ensure a constant water supply, the Robert Moses project includes a 1,880-acre storage reservoir which will be filled through diversion of Niagara River water during the winter. The reservoir, however, is more than an alternative source of water for operation of the power plant. It has been equipped with a reversible pump-generating system, and is used for production of 240,000 kilowatts of electricity.

Coal Tar Technology

Review of Coal Tar Technology, for the first half of 1960, presents the customary authoritative survey of international research, development, production, purification and patents relative to coal tar and its products covering that period. (January–June, 1960, 12, Part 1. Pp. ii + 63. Gomersea, Leeds: Coal Tar Research Association, 1960. 20*s*. per volume.) Section 1 reviews the then current work on coal tar itself, the primary fractions light oils, benzole, pitch and bitumen; tar acids; tar bases; monocyclic and polycyclic aromatic hydrocarbons; also utilization, which ranges from road tar, paints, pipecoatings, solid and liquid fuels, lubricants, plastics and resins, to wood preservatives and pesticides. Section 2 is devoted to general chemistry and chemical technique of coal tar and products; space is taken up chiefly with catalytic processes, for example, alkylation, isomerization, hydrogenation, oxidation, desulphurization, cracking. Physical chemistry of these substances concerns mainly rheology, vapour pressure and thermal properties. The paragraphs on analytical work include spectroscopic and chromatographic applications to coal tar and products, while a concluding category of chemical techniques describes recent work in connexion with distillation and crystallization problems.

Glossary of Petroleum Terms

THE *Glossary of Petroleum Terms*, first published in 1951, with a second edition in 1955, has now been revised and enlarged, bringing it up to date. (Third edition, revised and enlarged. Edited by George Sell. Pp. vii + 39. London: Institute of Petroleum, 1961. 5*s*.) The petroleum industry is no exception to the trend of many others in developing over the years of its evolution a colloquial vocabulary understood only by those engaged in its day-to-day pursuits. In fact, especially in English-speaking international environments, the particular 'jargon' involved is, to the layman, probably more incomprehensible than in the case of many other modern, comparable commercial undertakings. Such words as 'bean', 'bund', 'Christmas tree', 'feedstock', 'fishing', 'go-devil', 'overhead', 'packer', 'sweating', 'wildcat', to quote only a few, have quite different meanings in this industry from those ordinarily understood in general conversation. There is need for such specialized guidance as this, especially for the non-technical people in the industry, but this diminutive pamphlet (it is scarcely more than that, for of its 39 pages, 29 are devoted to the 'glossary' itself, 7 to an appendix on petroleum terms in U.K. legislation, 3 to abbreviations of titles of some official bodies, for example, API, DTD, IP, NCB, SAE, etc.) provides only modest fulfilment. Petroleum technology to-day is such an enormously ramified subject that nothing short of a comprehensive dictionary devoted to its tenets can cover the ground of its words and meanings adequately. The task has been attempted elsewhere some years ago, but even then on a rather limited scale. Whether the time is now ripe for conception and execution of a bold enterprise of this nature is a matter of judgement, policy and cost, but it is one to which the Institute of Petroleum, responsible for this *Glossary*, might well give careful consideration before the next edition is due for publication.

Improving Biological Communications in the United States

Establishment of a Biological Serials Record Centre is a principal recommendation of the Biological Communications Project, supported by the National Science Foundation, with the objective of collecting and disseminating detailed information on all biological serial and periodical publications, estimated at 25,000–50,000. The Centre will assess what proportion of this material is available to biologists in the United States, and will also study the general effectiveness of present periodicals as a system of communication. The National Science Foundation has also made a grant of 56,000 dollars for a one-year study of chemical notation systems, to be conducted by the National Academy of Sciences–National Research Council, and is supporting for a second year the Chemical Abstracts Service of the American Chemical Society's project to provide a centralized information service for chemists. A survey of computer systems for searching chemical literature has been completed by the National Bureau of Standards Research Information Centre and Advisory Service on Information Processing, and its results are available in a report by E. C. Medam and H. R. Koller, obtainable at 2.25 dollars from the Office of Technical Services, U.S. Department of Commerce, Washington 25, D.C.

Eoliths and Prehistorical Tools

As far back as 1911 there were furious quarrels among British prehistorians as to whether or not the Tertiary eoliths found by Reid Moir were really fashioned by man. To some extent the quarrel was theological: Was it conceivable that mankind could be as old as was suggested by the finds? Much research was undertaken, and at the time it was determined that Nature could chip, the blows coming from one or many directions, but not from two, or, better, three directions only. Further, if a piece of tabular flint was boldly chipped along one edge and then the aretes between the flake scars carefully removed by subsequent flaking so as to produce a sharp, flaked edge, then Nature could not have been responsible for the work. Briefly, if it were possible to demonstrate design then the chipped object had been fashioned by man. Later the study of prehistory spread to other continents, but the above criteria were largely forgotten. Much chipped material claimed to be man's handiwork was unearthed and this material in turn became the yardstick for the study of still more flaked objects. Now the matter is being carefully reconsidered, and Dr. Desmond Clark has undertaken considerable research work, especially as regards materials other than flint. In a recent monograph (*Bull. Brit. Mus.* (*Nat. Hist.*), 5, No. 4. Pp. 71–90 + plates 15–23. London: British Museum (Natural History). 17*s.* 6*d.*), Dr. Clark concerns himself with specimens of very early Pleistocene age from certain beds in Israel. His conclusions are that probably every one of the specimens in question has been flaked by natural agencies, and these agencies he describes in some detail. Of course, should man have been in existence at the time in what is now Israel there was nothing to stop him picking up a naturally chipped object and using it as a tool. But that is not the question under consideration. The article has a wider interest than merely whether certain specimens from Israel were fashioned by Nature or by man; the detailed studies of how Nature can work will be very useful to prehistorians in other areas when they have to determine whether or not their early finds are really genuine tools or *lusi naturae*.

The Institute of Fuel

Dr. James Burns, deputy chairman, North Thames Gas Board, has been elected president of the Institute of Fuel, and will take office in October. Sir Harold Hartley has been made an honorary member of the Institute. Sir Harold was chairman of the Fuel Research Board of the Department of Scientific and Industrial Research during 1932–47; chairman of the British National Committee and of the International Executive Council of the World Power Conference during 1935–50; and president of the Conference during 1950–56.

Commonwealth Scientific and Industrial Research Organization Chemical Research Laboratories

Following the appointment of the director of the Commonwealth Scientific and Industrial Research Organization Chemical Research Laboratories, Dr. I. W. Wark, to the Executive, it has been decided that, in future, overall management of these Laboratories will be the responsibility of a committee of the chiefs and officers-in-charge of the various divisions and sections at Fishermen's Bend, Melbourne. The first chairman is Dr. A. L. G. Rees, chief of the Chemical Physics Division.

A New Computing Centre

On June 7 the IBM Co. opened a new data-processing centre at 58 Newman Street, London. This centre is equipped with type 7090 and type 1401 computers; the former machine is one of the most powerful at present in operation, and the latter forms a suitable second echelon support device. Users of the centre are expected to do their own programming, but this is relatively easy because the *Fortran* automatic programming system is available and this is backed by the *Share* user co-operation system. The centre staff will be available for basic training and also to give advice to users in particular cases.

The Selby Fellowship

THE Selby fellowship has been established to bring graduates of high promise and proved capacity for research to work in the laboratories of universities or research institutions in Australia. The fellowship may be awarded in any branch of physical or biological science, and is tenable at any university or research institution in Australia, normally for one full year of work. Candidates must have taken their first university degree, and have had preliminary research experience, at some university or universities in a country other than Australia. In general, they should hold a Ph.D. degree or have equivalent status and experience, but the fellowship will not normally be given to those who have already held a senior research award. Applicants should be less than thirty years of age on July 1 in the year in which his application is received. The fellowship will carry a stipend of £1,500 (Australian) per annum. The costs of the Fellow's travel to Australia, and back to his country of origin at the conclusion of the fellowship, will be provided, up to a limit of £A500, including dependants, if any. Applications should be sent to the Assistant Secretary, Gordon Street, Canberra City, A.C.T., before July 30.

Challenger Society Grants for Marine Biology or Oceanography

THE Challenger Society has announced that it is prepared to make small grants for research in marine biology or oceanography at a recognized laboratory during the year June 1961–62. Applications, accompanied by details of the proposed research, should reach the Honorary Secretary, Challenger Society, National Institute of Oceanography, Wormley, Godalming, Surrey, not later than July 14.

The Training of Biochemists

THE Biochemical Society is organizing a colloquium on "The Training of Biochemists", on July 13, during the course of the Society's two-day meeting in Oxford (July 13–14). Included in the programme will be the following topics and speakers: biochemistry as an honours degree subject (Prof. S. V. Perry); the graduate biochemist and industry (Dr. W. F. J. Cuthbertson); university and departmental entrance requirements (Dr. Margaret Kerly); the teaching of biochemistry at Oxford (Prof. D. D. Woods); teaching in a small non-professorial department (Dr. G. R. Tristram); the M.Sc. course in biochemistry (Dr. E. M. Crook); summarizing remarks (Prof. F. C. Happold). Further information can be obtained from Dr. K. S. Dodgson, Department of Biochemistry, Physiology Institute, University College of South Wales and Monmouthshire, Newport Road, Cardiff.

Announcements

DR. W. E. SWINTON, of the Department of Palæontology, British Museum (Natural History), South Kensington, has been appointed head of the Division of Life Sciences at the Royal Ontario Museum, Toronto; Dr. Swinton will take up his new appointment in September.

MR. B. L. J. HART has succeeded Mr. Frank Worsfold as principal of the Computer Application Training Centre, of International Computers and Tabulators, Ltd., at Bradenham Manor. Mr. Hart was formerly a member of the International Computers and Tabulators' Computer Group, and is well known for his work on management and production control.

THE British Society of Rheology and the Non-Destructive Testing Group of the Institute of Physics and the Physical Society are holding a joint conference on "Rheological Techniques" in Llandudno during September 20–22. Further information can be obtained from Dr. Peter Lord, Royal College of Advanced Technology, Peel Park, Salford 5.

AN international conference on "Embryology", the fifth of its kind, is being sponsored by the editorial board of the *Journal of Embryology and Experimental Morphology*, and will be held in London at the Middlesex Hospital Medical School during September 18–21. Further information can be obtained from Dr. L. Brent, Department of Zoology, University College, Gower Street, London, W.C.1.

JOINTLY the Science Council, the Physical Society and the Crystallographic Society of Japan are organizing an international conference on "Magnetism and Crystallography" at Kyoto during September 25–30. Further information can be obtained from either Prof. T. Nagamiya or Prof. S. Miyake, Organizing Committee of the International Conference on Magnetism and Crystallography, Science Council of Japan, Ueno Park, Tokyo, Japan.

AN informal conference organized jointly by the European Organization for Nuclear Research and the Max Planck Institute, Heidelberg, on "Fission and Spallation Phenomena and their Application to Cosmic Rays", will be held at the Organization's Laboratories, Meyrin (Geneva), during September 26–29. Further information can be obtained from the Scientific Conference Secretariat (E. W. D. Steel), CERN, Geneva 23, Switzerland.

THE Mass Spectrometry Panel of the Institute of Petroleum, in conjunction with the American Society for Testing Materials (Committee *E*-14), is organizing a conference on "Mass Spectrometry" at the University of Oxford during September 12–15. Further information can be obtained from the organizing secretary, Mr. W. J. Brown, Instrumentation Division, Associated Electrical Industries (Manchester), Ltd., Trafford Park, Manchester 17.

THE University of Chile and various societies of Santiago and Valparaiso are organizing an international symposium on "Tissue Transplantation", to be held in Santiago and Valparaiso, Chile, during August 30–September 2. The programme will cover: the genetical basis; immunological problems; present status and prospective applications of tissue transplantation. Further information can be obtained from Dr. Alberto P. Christoffanini, secretary of the organizing committee of the symposium, Maximo Humbser 567, Santiago, Chile.

ERRATA. In the article on "Technical Assistance to Overseas Countries" in *Nature* of June 17, p. 1039, par. 4, lines 5 and 6, for "directorates of the Overseas Services and the Overseas Geological Surveys" read "Directorate of Overseas Surveys and the Overseas Geological Surveys".

THE sixth International Nematology Symposium will be held in Ghent during July 24–28. It is regretted that on p. 963 of the June 10 issue of *Nature* the word "Hæmatology" was printed for "Nematology".

NEW RESEARCH LABORATORIES FOR THE PAINTS DIVISION OF IMPERIAL CHEMICAL INDUSTRIES LTD.

It is no exaggeration to say that, within the past twenty-five to thirty years, a revolution has taken place in the paint industry which has changed paint-making from an art into a science. As a result, the simple linseed oil/natural resin paints which had been in use for centuries have been displaced almost entirely by a wide range of synthetic coatings, each of which is specially formulated for the conditions under which it will be applied, and for the performance it will have to give, whether this is on a house, a high-speed aircraft, a motor-car, a household appliance or on one of the many other surfaces to which paints and lacquers are now applied.

This rapid development of modern paint coatings is the result of the interplay between the increasingly stringent requirements of the users and the research and development work carried out within the industry to anticipate these needs. The applied research necessary to produce the required products has in turn been nourished and stimulated by the fundamental research on polymers carried out within the industry and at universities and research institutions, and by the increasing range of synthetic raw materials that have become available from the chemical industry.

The Paints Division of Imperial Chemical Industries Ltd., which was one of the first paint companies in the United Kingdom to establish a research department some thirty years ago, has always placed considerable emphasis on research directed towards improving its existing products and to the production of new ones. To improve the accommodation for its Research Department, a five-storey block of laboratories has been erected recently on the Division's headquarters site at Slough, Bucks.

The new building, which is 48 ft. wide × 160 ft. long, provides about 30,000 sq. ft. of working space for laboratories, offices and the library of the Division.

It is constructed of *in situ* concrete faced with Leicester golden brown and Vandyke brown bricks, ornamented with blue mosaic column facings and artificial stone copings, and is planned on a 5-ft. longitudinal module. On all floors, the main corridor has been offset from the centre line, giving 22-ft. wide rooms on the north side and 15-ft. wide rooms on the south side. On the lower floors, where it is unlikely that the function or size of rooms will need to be altered, the walls are generally of solid construction; but on the higher floors, where changes are more likely to occur, 'Holoplast' partitioning has been used.

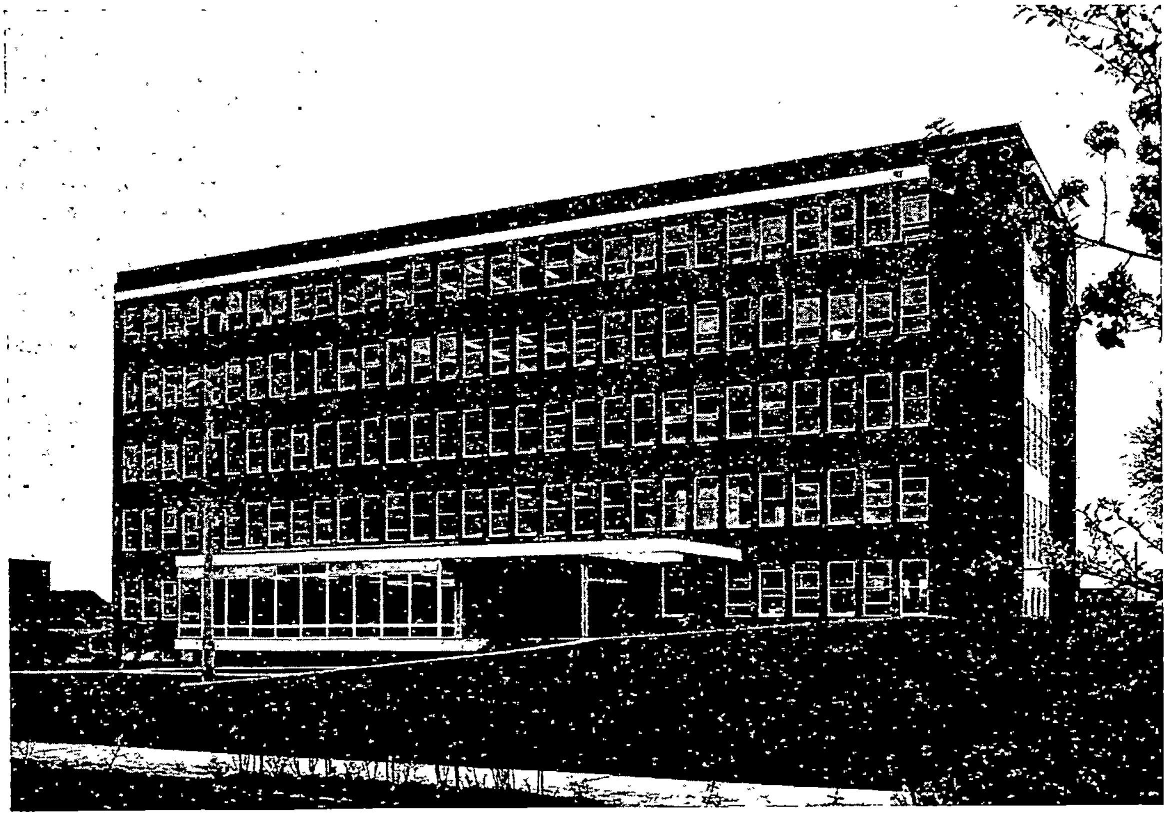


Fig. 1. North and west sides of I.C.I. Paints Division's new research laboratories at Slough, Bucks., showing the entrance and library reading room on the left

Floors generally are covered with large grey linoleum tiles finished with a polyurethane lacquer. The 5-ft. spacing of the columns on the outside walls has allowed for the provision of large sash windows, and the false ceilings, above which are carried the horizontal service distributions for the floor above, are rebated on the window side to increase the window height to the maximum.

Space heating is carried out by means of hot-water convectors built into the walls below the window sills. Window ventilation is relied on for offices, but in the laboratories, the fume-cupboard system provides the main ventilation. The fume-cupboard fans are run continuously throughout the day, warmed replacement air, cleaned by electrostatic precipitation, being supplied to all laboratories through a system of trunking concealed in the corridor ceiling.

To enable the extract ducts from fume cupboards on the lower floors to be carried up to the roof without interfering with the design of laboratories on the upper floors, the majority of the fume cupboards are recessed into a 4-ft. wide longitudinal strip which has been provided on all floors between the corridor and the laboratories on the wide side. Vertical ducts located in this strip carry the extract trunking from the different groups of fume cupboards up to the exhaust fans on the roof. Similar ducts carry the main risers for the piped and electrical services and the laboratory drainage. Access to all these ducts and to the backs of fume cupboards is provided from doors in the corridor, thereby minimizing interference with work in the laboratories when maintenance is necessary.

In general, 5-ft. wide double-sided island benches at right-angles to the windows have been adopted as the standard with a 5-ft. walk-way between, giving a 10-ft. centre-to-centre spacing between benches, corresponding to the 10-ft. tap-off systems on the ring mains and the 5-ft. columns on the window walls. With the present staff, this type of layout provides an average of about 15 ft. of bench per person. A special feature of the benches is the grouping of the service outlets into towers. Electrical sockets are mounted vertically on the fronts of these towers, with the water-control valves immediately above them, the corresponding water outlets being located at the side of the towers over a central lead-lined trough drain. The outlets for gas services (town gas, compressed air or nitrogen) are at the base of each tower, with extended control spindles to handles mounted on the fascia of the bench. The tops of the towers are recessed to carry the ends of over-bench shelves, beneath and on each side of which are mounted lengths of 'Unistrut' channel. On these slide specially designed clamps for the erection of bench scaffolding, thus eliminating the need to use retort stands for the support of apparatus. Similar 'Unistrut' channels have been provided on the walls in many laboratories for the erection of tall apparatus, and in these locations wall service outlets have also been provided.

Bench tops are either of teak, stainless steel or 'Formica' according to the particular type of work being carried out on them. The underbench furniture is of painted softwood and consists of 3-ft. 4-in. long interchangeable cupboard and drawer units.

The laboratories on the top floor of the building are devoted to organic chemical work directed towards the discovery of new polymers which may be of value as surface coatings, while those on the second and third floors are concerned with the further development of these polymers into paints and with their evaluation in comparison with standard products. The second floor also provides accommodation for the research management, administration, a central typing service and a conference room. The first floor is almost entirely occupied by the Physical Chemistry and Analytical Sections, both of which make extensive use of physical techniques such as infra-red and ultra-violet spectroscopy, polarography and chromatography, etc. The Physics Section, which is housed on the ground-floor, is concerned with problems involved in the dispersion and physical behaviour of pigments and with the physical characteristics of paint films and their effect on such properties as gloss, hardness, adhesion and durability. The remainder of the ground-floor is occupied by the Division Library and Information Service, which, in addition to housing a library of some 20,000 volumes dealing with a wide range of scientific interests, provides information in the form of bulletins, literature surveys, translations, etc.

The basement, as well as housing the main engineering services for the building, also provides accommodation for two air-conditioned rooms used for physical tests on paint films.

J. W. DORLING

NORTH BORNEO EXPEDITION 1961

MOUNT KINABALU (13,455 ft.) is the highest peak between the Himalayas and the Snow Mountains of New Guinea, and, though not a snow-mountain itself, it has an alpine flora relating these two parts of the world. It was first climbed in 1851 by Sir Hugh Low and has since attracted many botanists, zoologists and geologists. It is now known to have one of the richest floras of any mountain, but a great part of it remains to be explored.

To further the biological exploration of North Borneo, which will also assist the Government of North Borneo in its plans for the establishment of a national park on this famous mountain, the Royal Society set up in 1960 a committee under the chairmanship of Sir Joseph Hutchinson. This year, in co-operation with the Government of North Borneo, the Society has sent out a reconnaissance expedition under the leadership of Mr. E. J. H. Corner to investigate the practically unexplored eastern side of the mountain, where the forest extends down to the lowlands. In particular, the vegetation and soils are being studied as indicators of land which should be conserved within the boundaries of the national park. It is well known that in these precipitous regions of high rainfall, the clearing of luxuriant primary forest may lead to irreparable impoverishment of the soil with erosion and consequent silting and flooding of the rivers.

As soil-scientist, Mr. G. P. Askew (Wye College, University of London) is accompanying the expedition to work in conjunction with the Soils Division of the Department of Agriculture in North Borneo. Mr. Adam Stainton, who has taken part in the botanical exploration of Nepal, organized through the British

Museum (Natural History), is assisting in botanical and photographic work. Other personnel have been drawn from Malaya, Sarawak and North Borneo, with the intention of developing regional interest in the project. The Government of Singapore has made available from the staff of the Singapore Botanic Gardens the services of the botanist, Dr. Chew Wee Lek, and of two plant collectors, Md. Shah and Kadim b. Tassim. Much scientific work on the flora of Borneo has been done at these Gardens, the herbarium of which contains a very valuable set of Bornean plants. Md. Shah is the son of the late Haji Md. Nur, who contributed so much to the scientific work of the Gardens. General vertebrate zoology is in the charge of Prof. J. L. Harrison (Nanyang University), who joined the expedition for a month during June 17–July 22, and Mr. Chin Phui Kong (assistant fisheries officer, North Borneo). Mr. B. E. Smythies (conservator of forests, Sarawak), the author of the recently published book *Birds of Borneo*, is joining the expedition as ornithologist in July. The Forest Department, North Borneo, is assisting in opening up the intended trail up the eastern shoulder of the mountain and across the so-called Pinosuk Plateau, which is of great scenic and biological interest, but apparently of little use for economic development. Various members of the Department are visiting the expedition in its different stages.

The members of the expedition passed through Singapore to Jesselton at the end of May and are spending from June until mid-September on the slopes of the mountain. It is intended to use a portable aluminium sheet hut as a base camp at an altitude of 5,000 ft., whence local expeditions will be made with tents up the unexplored eastern approaches of the mountain. Since exact localization will often be necessary, a set of aerial photographs is being used in conjunction with the War Office and Air Ministry survey maps (1 : 50,000).

Among the many objects of the expedition, special attention is being given to the soil conditions in the mossy forest at 5,000–7,000 ft. altitude and in the areas of ultrabasic rock, to the occurrence of the great parasitic flower *Rafflesia*, to the extraordinary variety of wild figs, and to the opportunity for obtaining photographs to illustrate Mr. Corner's durian theory of tropical forests; Borneo being the centre of the remarkable genus of durian trees.

Local arrangements are being made through the office of the Resident, West Coast Residency, North Borneo, and Messrs. Harrisons and Crosfield have undertaken to act as the Expedition's agents in North Borneo. The botanical and zoological results will be co-ordinated by the staffs of the Royal Botanic Gardens, Kew, and the British Museum (Natural History) respectively.

ATOMIC STRUCTURE OF GRAPHITE AND GRAPHITIZATION

An informal discussion on graphite, under the auspices of the Faraday Society, was held on January 26 in the Chemical Engineering and Chemical Technology Department of the Imperial College of Science and Technology, London. About 120 people were present, including five from France and one or two others from the Continent.

The programme was opened by the chairman, Prof. A. R. Ubbelohde (Imperial College of Science and Technology), who outlined the scope of the papers to be presented and indicated the kind of problems which it was hoped would be clarified by the papers and subsequent discussion. The first paper, by Prof. C. A. Coulson (Mathematical Institute, University of Oxford), was on the electronic band structure of graphite and the results of a calculation of the energy-levels by conventional tight-binding methods. For the case of a single graphite plane, it is found that the π electrons have energy-levels which fall into two half-bands, just touching one another at the ends where the density of states is zero. Under the influence of neighbouring layers the two half-bands broaden and overlap, so that the valance band is not quite full and the upper (conduction) band is not quite empty. From this energy-level distribution, both the bond-length and the shape of the X-ray emission band can be calculated and they are in good agreement with experimental results. Other properties, such as the electrical conductivity and the de Haas–Van Alphen effect, depend only on the nature of the top occupied levels, and reasonable agreement with the calculation in found in practice. Finally, Prof. Coulson discussed the electronic behaviour at the edges of a graphite layer and the possible situations when either neighbouring sites or a single separated site are involved. Both adsorption experiments and quenching by oxygen are explicable if it is supposed that there are unpaired electrons at a few sites around the edges.

In the following paper, Dr. G. E. Bacon (Atomic Energy Research Establishment, Harwell) reviewed the outcome of the study of graphite by diffraction methods in terms of: (1) the limited crystallographic perfection; (2) the distribution of electrons around the carbon atoms; (3) the texture of artificial graphites; (4) the effect of irradiation by neutrons. The characteristic features of X-ray powder photographs could be explained in detail in terms of random displacements of the carbon planes about the *c*-axis, with a measureable difference in the inter-layer spacing between oriented and mis-oriented planes. The proportion of rhombohedral modification can be varied by mechanical working or by treatment with strong acids or bromine, but the detailed manner in which the transformation takes place is not understood. A combination of neutron diffraction data, careful measurement of X-ray intensities and theoretical calculations has established that the departures from spherical symmetry in the electronic distribution are very small. Irradiation in a nuclear reactor produces significant changes in macroscopic properties, such as elasticity and bulk dimensions, and although these can be explained in general terms as due to interstitial carbon atoms and vacancies, the detailed nature of these defects is still uncertain.

Under the title "Defauts Cristallins dans le Graphite", Prof. J. Mering (Institut National de Recherche Chimique Appliquée, Paris) gave a systematic classification of the various types of defect which can be found in graphite crystals, and discussed their detection and observation and their influence on chemical and physical properties. At the outset he emphasized the outstanding difficulties in this kind of work—the non-existence of a perfect graphite crystal as a reference standard, and the impossibility of introducing or removing in any simple way faults of a given type. The main classification was into point, line and planar faults. Point faults are the simplest type, but they are difficult to detect by direct means, although they produce relatively simple effects on physical properties (such as electrical resistivity and the Hall coefficient) and on the chemical properties, in particular the tendency to form lamellar compounds. These point faults can be subdivided in terms of substitution atoms, vacancies and interstitials. Faults associated with dislocations and with surfaces are the least simple to define and interpret, but they are detectable more directly by diffraction methods and optical and electron microscopy. Planar faults were subdivided into 'surfaces of dislocations' (such as occur between the not-quite-parallel domains in so-called single crystals of natural graphite or between hexagonal and rhombohedral regions) and errors in layer stacking (such as the orientation faults, which are particularly noticeable in artificial graphites, or random mistakes in an *AB* or *ABC* stacking sequence).

The second session of the meeting dealt with slightly more technological aspects of graphite and was opened with a paper by Mr. P. K. C. Wiggs (Morgan Crucible Co., Ltd., London) on "Elastic Properties of Graphite". Single crystals of graphite have a Young's modulus parallel to the basal planes which is about three times as high as for steel, but parallel to the *c*-axis the modulus is many times smaller. The easiest mode of deformation is, as would be expected, a shear in which neighbouring planes slide over one another. The properties of polycrystalline graphite bear little resemblance to those of the ideal single crystal, and are determined very largely by the disordered material at the junctions between crystallites and by internal voids: such anisotropy as exists is a consequence of the texture which results from the nature of the starting materials and mode of manufacture. As a consequence of this, it is found that all the elastic moduli of polycrystalline graphite are of the same order of magnitude as the low-shear modulus for the sliding of adjacent planes in the single crystal. Only for the more recent developments of pyrolytic graphite and graphite whiskers, where stresses cannot be relieved by sliding, do the high modulus and strength of the single crystal parallel to the planes become apparent. At temperatures greater than 2,000° C., electric furnace graphite is deformed plastically to a very significant extent, but on heating up to this temperature there is a two-fold increase in the elastic moduli on account of the increasing thermal motion of the carbon atoms, which reduces the ability of one plane to slide over another.

A paper by Dr. E. G. Steward (Northampton College, London) described the crystallographic changes which occur during graphitization. There is still considerable doubt about the structural details of poorly crystallized material, which gives very diffuse X-ray diffraction patterns. Recently, there has been a revival of suggestions of non-planar structures, such as a diamond-type of bonding, particularly from electron diffraction observations. As graphitization proceeds and the crystallites grow, the interpretation is much clearer: a progressive modulation of the two-dimensional bands and a general sharpening of the reflexions demonstrates the approach towards the ideal Bernal structure. The ease of graphitization depends on the structure of the crystal nuclei and whether these are separated by cross-linkages. Initial growth occurs by incorporation of non-organized carbon at the crystallite edges, but further progress depends on the existence of near-parallel orientation between neighbouring crystallites. An important characteristic of the final product may be the size of the quasi-perfect macro-units which are much larger than the regions of higher structural perfection revealed by X-ray diffraction. Recent developments in the work on pyrolytic carbons and the examination of moiré fringe patterns in the electron microscope were also discussed.

The final paper, by Prof. W. F. K. Wynne-Jones (King's College, Newcastle upon Tyne), dealt with physical and chemical studies of the graphitization process.

A number of questions and general discussion followed each of the sessions. One of the principal topics was the lack of understanding of the transformation from ordinary to rhombohedral graphite and the energy relations between the two forms. It was also evident that we still have little precise knowledge of the intergranular material which bonds together the individual crystallites, and which is so important in determining the physical and chemical properties of ordinary non-ideal material. G. E. Bacon

RADIOLOGICAL UNITS AND MEASUREMENTS

RADIATION dosimetry is an immensely complicated subject and many of its problems have been only partially resolved. The difficulties are of a fundamental nature and arise: (*a*) from the complexity of the interactions of neutrons and electromagnetic radiations with matter; (*b*) from the still greater complexity of interaction of charged particles, especially electrons, with matter. So long as the understanding of these basic processes remains incomplete, there will be outstanding problems in the dosimetry of ionizing radiations. There is consequently a recurring need—it amounts to a necessity—for authoritative pronouncements on this developing subject to maintain world-wide uniformity in the units, standards and measurements of radiation and radioactivity. The National Bureau of Standards *Handbook 78** is intended to satisfy this need. It supersedes the 1956 report of the International Commission on Radiological Units and Measurements, and the chief interest lies in the difference between the two reports. There has been no major revision and the differences consist almost entirely of accretion and expansion; the new handbook is in fact twice as long as the old. The chief aim according to the preface is ". . . to improve the accuracy of the evaluation of absorbed dose in all places of interest in a patient or other objects". To this end, there is a greatly expanded section on clinical and biological applications, including a new and detailed treatment of energy absorption in and near bone. An important new recommendation for radiotherapists is that the routine recording of bone doses and of tumour doses should now include a statement of maximum and minimum absorbed doses in rads. There is a section entitled "Report on an Inquiry on Current Errors in Clinical Dosimetry" which is somewhat disturbing; it should be carefully studied by all concerned.

---

* United States Department of Commerce: National Bureau of Standards. Handbook No. 78: Report of the International Commission on Radiological Units and Measurements (ICRU), 1959. Pp. x+90. (Washington, D.C.: Government Printing Office, 1961.) 65 cents.

In Part 3 ("Physical Aspects of Dosimetry") linear energy transfer and neutron dosimetry are treated in much greater detail, and there are entirely new sections on chemical, solid state, calorimetric and photographic dosimetry. The appendixes contain some interesting and often impressive accounts of international comparisons of various radioactivity standards, and it is noteworthy that Hönigschmid's weighings of radium standards, in which he estimated an error of about 0·02 mgm. (in around 20 mgm.), have now been accurately confirmed by ionization and calorimetric measurements. There are new appendixes on focal spots and the estimation of integral absorbed dose and the final appendix states the International Commission on Radiological Units and Measurements Position Plan adopted at Geneva in September 1958.

Many of the larger criticisms that could be raised against this report are now under active debate in preparation for the major revision referred to in the preface. In a composite production of this nature it is very difficult to preserve consistency. Thus, in referring to the calculated values of mass stopping power ratios (s_m) in Table 8·3, it is stated in footnote 24 "... that an uncertainty of the order of 1 per cent exists in s_m ..." yet later in the same section on page 37 it is recognized that the basis of these calculations is incorrect. The earlier admission on page 1 of errors "... of the order of a *few* per cent ..." would appear to convey the requisite degree of caution. The symbolism is not always consistent and in some places (for example, p. 40 and Figs. 8·6 *b–d*), 'E' is used to denote particle kinetic energy, whereas in other places (for example, p. 51 and Figs. 8·3 *a–c*), 'T' is used for the same purpose.

It is almost superfluous to add that this *Handbook* is essential reading for all radiation physicists, radiotherapists, radiologists and radiobiologists. Unfortunately it is rather unattractively produced, it has several misprints and grammatical errors, and the numerous footnotes are scarcely legible without the aid of magnification.

P. R. J. Burch

THE RED DEER COMMISSION

THE Red Deer Commission was constituted in October 1959 with the general aim of furthering the conservation and control of red deer in Scotland. Its setting up marked the first comprehensive attempt to deal with a controversial problem which has vexed the Highlands for the best part of two centuries.

The introduction of large-scale sheep farming into the Highlands in the latter half of the eighteenth century, replacing the old population of farmers with their primitive breeds of cattle and sheep, resulted, towards the end of that century, in the reduction of deer stocks to probably their lowest level. At the beginning of the nineteenth century another influence was introduced by the development of the sport of stalking, giving a new value to the deer. When prices of wool and mutton declined towards the end of that century, the area given over to deer forests increased rapidly, from less than two million acres in 1883 to more than three and a half million acres at its peak in 1912. In 1952, however, the area of deer forests was estimated to have been reduced to about three million acres.

The influence of these developments on the range and population of deer affected every aspect of highland life, causing deep dissensions. Between 1872 and 1952 six different committees or commissions were appointed by Parliament to make recommendations with regard to conservation and control, but little or nothing was achieved until 1939. The Deer and Ground Game (Scotland) Bill introduced in that year provided powers to control deer, to be exercised by the Department of Agriculture with the assistance of an Advisory Committee and local committees. Developments were prevented by the War, and it was not until 1959 that an Act of Parliament was passed to introduce close seasons for deer in Scotland.

The Act is divided into five parts. Part 1 deals with the setting up of a Commission and its powers. Part 2 specifies close seasons for stags during October 21–June 30 and for hinds during February 16–October 20, to come into force on October 21, 1962. The Commission has now issued its first report*, and, in it, states that, "we consider that if viable stocks of deer are to be maintained throughout the traditional deer forest areas of the Highlands they should not be broken up into isolated fragments. At the same time, if they are not to continue as a serious liability to agriculture and forestry, they must be kept under control. In other words, as grazing animals they should be subject to reasonable standards of management."

The numbers and population densities of deer, unlike those of many other wild animals, do not appear to be self-limiting. In the absence of natural predators such as wolves, or of sufficient killing, deer will increase until they overgraze and damage their habitat, die in large numbers from starvation, or overspill on to agricultural land. Persistent overgrazing must lead to the progressive impoverishment of the hill pastures, on which they depend for their food, and a declining productivity. For the past two centuries, man has provided the only check against this deterioration. The fact that he has not everywhere been exercising sufficient control is the prime reason for the setting up of the Red Deer Commission, with certain over-riding powers, including authority to go on private land in the exercise of its functions.

During the period under review, the Commission has been concerned primarily with the control of marauding deer. Experience this year has shown that it is both widespread and continuous. Where estates are under good management, effective measures are taken to protect tenants and neighbours from damage. There are, however, areas where much more could be done to protect those who suffer. In general, where the laird or factor is permanently resident, satisfactory co-operation and understanding are most easily achieved. Where there is no one of sufficient authority to deal with it on the spot, and the estate concerned is factored from an office outside the Highlands, it is inevitably more difficult.

---

* The Red Deer Commission. Report for the period 1st October 1959, to 31st December, 1960. Pp. 22. (London: H.M. Stationery Office, 1961.) 1*s*. 3*d*. net.

In some areas where marauding is prevalent there are not enough stalkers and gamekeepers available to deal with the situation. The Commission believes that the widespread marauding taking place throughout the year is probably a symptom of overstocking on recognized deer land in the vicinity, and that the root of all deer 'trouble' is lack of adequate management. The annual kill of hinds, for example, is generally too low; the only way to reduce overlarge stocks is in the culling of hinds. When it is left to an inadequate staff of stalkers or gamekeepers this most important aspect of management is apt to go by default. Sometimes, excessive stocks of deer have been maintained to make allowance for the slaughter done by gangs of poachers. Whatever justification there may have been for this practice in the past, it no longer exists.

Estates in some areas are seriously handicapped in stag shooting by the incursion of the public in the later summer and autumn. If such estates are to be enabled to carry out their obligations during the open (stalking) season, means will have to be found to dissuade the public from disturbing the ground and possibly courting danger to themselves.

WOMEN ENGINEERS IN THE U.S.S.R.

WOMEN form a much higher proportion of engineers and technologists in the U.S.S.R. than in Western Europe or the United States. A study arranged by the Caroline Haslett Memorial Trust was designed to throw light on the factors giving rise to this high proportion, on the capability of women to benefit from a technical education, and on the employment prospects of women so trained. The study included an investigation in the U.S.S.R. itself, and this was undertaken by Miss L. S. Souter, head of the Magnetic Materials Section of the Associated Electrical Industries Research Laboratory, Harlow, and Miss R. Winslade, assistant instruments group manager, Research and Control Instruments, Ltd.

In their report* the authors show that, under the national educational system of the U.S.S.R., co-education of boys and girls is the general practice. In 1943 the sexes were separated into different schools, but, after a ten-year trial, the experiment was abandoned under pressure of public opinion. Only one trace of the separation remains; while boys of 11–14 are having carpentry lessons, girls are being taught domestic science. For all other subjects boys and girls attend the same classes, and, with few exceptions, girls have the same choice of specialities as boys. Comparisons of the performance of men and women as engineers have been made easier by this common educational background. Although the right of entry into university was not fully extended to women until after the 1917 revolution, the limited right of attendance at higher educational courses (some specially set up for women) was granted in 1905 as a result of revolutionary developments at that time and, by 1907, a number of technical institutions were open. There are records of women attending engineering classes at polytechnics during 1907–9, but the numbers were comparatively small.

Equality of opportunity brought the proportion of women in some of the universities up to 50 per cent in 1920. While at the higher medical schools 80–85 per cent of the students were women, the entry into the engineering schools appears to have been much slower. Even as late as 1928, only 1 per cent of the students of thermal power were women; the proportions of women students taking chemistry, physics and mathematics courses was much higher.

The rate of entry of girls into engineering at a lower level appears to have been somewhat faster, and was encouraged by the all-out drive to make good the technical backwardness of the country as a whole. By 1929, 37·1 per cent of pupils at the technicums or trade schools were girls.

In the middle 1930's the entry of girls into engineering institutes increased gradually and then rapidly, partly as a result of the drive to develop industry throughout the country. By 1938 about 28 per cent of the engineering students were women, and by the end of the decade the 30 per cent mark appears to have been passed. Young men were sharing engineering classes with a high proportion of girls and were taught by staffs which in 1939 included 23 per cent women. They came to take it for granted that engineering was not only a possible but also a suitable profession for women.

The increasing proportion of women students in engineering institutes continued after the Second World War, but the rate of increase was falling; the numbers are now increasing slowly, and there has been negligible change in the 33–35 per cent level reached in the late 1940's. There has, however, been a considerable increase in the number of women holding higher engineering qualifications. The industries which attract women engineers show marked differences from Great Britain. Here, industries with a long tradition of women operatives are slow to attract (or accept) women engineers; in the U.S.S.R. it is the textile and food industries which have the highest proportion of women engineers. The high proportion of women engineers in the U.S.S.R. was so much taken for granted that the reasons for the Souter–Winslade study were frequently called into question and led to no little amusement.

Throughout the U.S.S.R. women are forbidden to take up any branch of engineering which is considered to be prejudicial to health. Some examples are: (1) at trade-level—girls are not permitted to service television sets because of the danger of radiation and because of the heavy weights of the sets themselves; they are permitted to train as telephone service engineers, but not allowed to carry out work involving the climbing of roofs or poles, or to work on underground cables; (2) at professional level—girls are permitted to study as mining engineers and may exercise their profession in surface jobs, but they are forbidden to work underground.

---

* Caroline Haslett Memorial Trust. *Women Engineers in the U.S.S.R.: a Study of the Education, Employment and Prospects of Women Engineers in the U.S.S.R.*, carried out under the auspices of the Caroline Haslett Memorial Trust. By L. S. Souter and R. Winslade. Pp. 28. (London: The Caroline Haslett Memorial Trust, 25 Foubert's Place, W.1, 1960).

Strong attempts are made to discourage girls from some types of work which are regarded as unsuitable although not forbidden by law. These posts fall into the categories of physically dangerous or arduous, such as forging or rolling processes, or unpleasant, such as some of the processes of chemical engineering. As a general rule, there are fewer women in heavy industry than in the textiles, food, watch and clock industries.

The Krasny Oktyabr sweet factory appears to be typical of the food industry, and here a women occupies the post of director, and women are in charge of the departments concerned with planning (engineering economist), costing (economist), analytical planning, technical control of output, mechanical engineering and design. The women acting in a supervisory capacity are: the deputy chief mechanical engineer, 15–20 design engineers and 2–4 production shop supervisors. Women head most of the ancillary departments, including book-keeping, library, polyclinic and canteen. Men occupy the posts of commercial director (combined with the post of vice-director), chief engineer, chief mechanical engineer, head of wages department and 2–4 of the production shop supervisors. There was general agreement that girls and women show a particular preference for engineering economics, engineering design, laboratory work and teaching. Engineering economics, in particular, is popular with girls, and, until the current year, made up 95 per cent of the students of faculty in the Leningrad Polytechnic. Engineering economics includes the economy of distribution and supply of energy, metallurgical production and processing, mechanical engineering, machine construction and mechanization of working processes by automation.

Preference for engineering design work was illustrated in the teaching institutes by the number of women students in courses on airfield construction, road, bridge and tunnel design at the Institute of Ways of Communication; the figure of 35 per cent compared with 10 per cent in the mechanical faculties.

The attraction of research work was evident, and the proportion of women in all types of laboratories, such as those concerned with fundamental research, development, works-aid, and material or process control, was much higher than the 28 per cent average. Almost all the research laboratories of the Likhachiov Motor Works are headed by women and some completely staffed by women. 50 per cent of the staff of the Research Institute of Common Technologies are women, and here women head a fair proportion of the sections. 60 per cent of the staff of the Research Institute for Communications (covering telecommunications) are women.

There is a general shortage of teachers, and there is a drive to make more junior teaching posts more attractive. Engineers are released from industry to the teaching institutions on a part-time basis; no extra hours per week are worked and extra pay is given. Nevertheless, it is difficult to attract people to teaching. In spite of the fact that teaching is said to be fairly high in order of popularity with women, and most teachers of mathematics and physics are women, they comprise only about 20 per cent of the teaching staffs of these institutions, that is, less than the national average among engineers.

Men and women receive equal pay, and there is no reason why women employees should be preferred on this score. The minimum age for retirement for women is 55 compared with 60 for men, but both men and women often continue working for up to ten years after the minimum age.

It was not possible to make a comparable study of the distribution of girls between various trades which are taught in the technicums or of the girls obtaining technical qualifications at junior technical colleges. The authors considered the question of whether or not women in the U.S.S.R. are allowed and encouraged to enter the engineering professions and also whether they achieve promotion to top positions.

In 1957 women formed 10 per cent of the directors, chief engineers and other leading specialists in the national economy, as opposed to 38 per cent employed as engineers. The total figures for all ranks of engineers show that 28 per cent of the positions are occupied by women.

In the U.S.S.R. Miss Souter and Miss Winslade also examined the objections put up to exclude women from senior positions in engineering in Great Britain. They found that girls were putting up performances in mathematics and physics comparable with boys; although the proportion of prizes going to girls varied from year to year; over a ten-year period they averaged a share of the prizes in relation to the total numbers. There is a slight departure from the general trend in Kharkov where, in the Institute's entrance examination, girls put up a slightly better performance than boys in physics, chemistry and foreign languages, while boys do better in mathematics and Russian. At all stages the adequacy of the mental capacity of women was so taken for granted that some difficulty was experienced in bringing discussion with scientists in the U.S.S.R. to a factual level. Schoolgirls appear to have no inhibitions about tackling technical work, and 40–45 per cent of the technical streams of secondary schools are girls. These girls show much enthusiasm for all types of technical work and, in some schools, have the reputation of being more accurate with machine tools than boys.

THE DUST CLOUD ABOUT THE EARTH

In *Nature*, 189, 127 (1961), Dr. F. L. Whipple discusses four possible explanations for the concentration of dust near the Earth. Of these the most favourable consideration is given to the hypothesis that it originates in collisions between meteorites and the Moon.

I would like to propose as at least equally tenable a fifth hypothesis: that the dust originates from meteorites that graze the Earth's atmosphere. Clearly meteorites that strike the Earth's atmosphere at too large an angle (or with too small an impact parameter) will be burnt up in the conventional way. Clearly, too, meteorites that approach the Earth with too large an impact parameter will not be slowed enough to have their orbits greatly changed. Between these limits, however, must be some the velocity of which, on re-emergence from the atmosphere, has been reduced to within the range of 7–11 km./sec. which will lead to their capture by the Earth's gravitational field. Those larger and faster will of course be broken up, liquefied or even vaporized in the process. Particles with an initial speed of 15–20 km./sec. and of masses up to perhaps 10^{-5} gm., however, might be expected to radiate well enough to dissipate their excess energy without complete disintegration. Even if the impact parameter for any one particle must lie between limits only 1 km. apart, perhaps in the region of 100 km. above the Earth's surface, the cross-section available for interplanetary material will be nearly one-tenth the total cross-section of the Moon. All this material will have been captured by the Earth's gravitational field, whereas the fraction of material splashed off the Moon to be so captured must be exceedingly minute.

Of course, most of the captured material will be lost in the atmosphere at the very next perigee of its new orbit. The Moon is still required for this hypothesis although not as an actual source of supply. Its importance is that it will distort the paths of those particles which have long elliptical orbits approaching it at all closely ; roughly half these distortions will make atmospheric capture even quicker by reducing the particle's energy, but the other half will give a gain of energy which will increase the radius of approach to the Earth at perigee and, in some cases, allow the production of the very long-lived orbits required.

The range of initial orbits, from which such quasi-permanent capture is possible, is difficult to estimate, but it seems to me to be at the very least as probable a process as that described by Dr. Whipple.

Consideration of the method of slowing down and of capture suggests the possibility that a higher proportion of iron–nickel particles might be found in the local than in the interplanetary dust and that there might be a preponderance of particles revolving about the Earth in the same sense as does the Moon. The former, though not the latter, suggestion would be reversed according to the lunar-collision hypothesis.

J. H. Fremlin

Department of Physics,
University of Birmingham.

Dr. Whipple[1] has given an excellent summary of rocket, satellite and space-probe measurements demonstrating the existence of a high concentration of interplanetary dust near the Earth. It is the purpose of this article (1) to show in more detail than presented elsewhere[2] how this dust blanket may reasonably be expected to arise, (2) to correct the unfortunate although quite justifiable misinterpretation Whipple[1] has made of my theory[2], which was described too concisely, and (3) to show that Whipple's[1] different spatial dependence of the observations are not in disagreement with the theory presented here in greater detail.

Wyatt and Whipple[3] have shown that the most minute cometary debris acquires nearly circular orbits due to the relativistic aberration of solar radiation pressure[4], and that the debris spirals into the Sun with a radial velocity of twenty to six hundred Earth radii a year at the position of the Earth's orbit, depending on the effect of solar corpuscular radiation[5]. By solving the Lagrangian equations of motion of the particles[2] in the combined solar and terrestrial gravitational fields, one may determine the orbits which the particles follow as they approach the planets during their inward motion. It is important to note that, independent of angular momentum considerations, the planets will attract and concentrate the dust in the ecliptic plane as is readily determined by solving the equation of motion in a direction perpendicular to the ecliptic plane. As discussed in a previous paper[2], inclusion of this effect will cause the concentration of dust in the plane of the ecliptic to vary as the inverse three-halves power of the solar distance which is observed[2] rather than the inverse first power which would be predicted in the absence of the planets[2].

The results for motion within the ecliptic plane may be much more simply understood, however, if one examines their motion in an Earth-centred co-ordinate system and (except for the relative earth-particle velocity due to their differing solar distances) neglects the motion of the Earth and particles about the Sun. This neglect may be shown to be inconsequential when one transforms the exact Lagrangian to an Earth-centred co-ordinate system. Under these conditions the particles encounter the Earth in an initially hyperbolic orbit and a negligible quantity of them enters captured orbits even temporarily. The theory assumes only hyperbolic orbits about the Earth. Even those few which are captured are soon lost due to the effects of radiation pressure, which is a major force on such slight objects as to be affected by the Poynting–Robertson radiation effect[3,4].

Whether or not the particles encounter the Earth (that is, impinge on the atmosphere and are present at low satellite altitudes of a few thousand kilometres) is determined by the conservation of their angular momentum. At infinity, their velocity with respect to the Earth is at least that due to their different solar distance, r :

$$v_\infty = \sqrt{\frac{GM_\odot}{r}} - \sqrt{\frac{GM_\odot}{D}} \sim \frac{1}{2}\sqrt{\frac{GM_\odot}{D}}\,\frac{a}{D} \qquad (1)$$

where $a = r - D$, D is the radius of the Earth's orbit, G the universal gravitational constant, and $M_\odot$ the mass of the Sun. If they are in a circular orbit about the Sun, their moment arm with respect to the Earth is initially $\sqrt{(a^2 + h^2)}$ where h is the perpendicular distance of the particles from the ecliptic plane. (Since the dust is mainly concentrated in the ecliptic plane, most of it has negligible inclination to the ecliptic.) The velocity of the particles at perigee is due to their fall in the Earth's gravitational potential, if one assumes their initial velocity to be slight. Hence in order to reach a distance, b, from the Earth's surface, a and h must satisfy the condition:

$$\frac{1}{2}\sqrt{\frac{GM_\odot}{D}}\,\frac{a}{D}\sqrt{(a^2 + h^2)} \leqslant b\sqrt{2\frac{GM_\oplus}{b}} \qquad (2)$$

in order for the particle's angular momentum about the Earth to be conserved. $M_\oplus$ is the mass of the Earth. Hence, in order to reach the surface of the Earth:

$$a\sqrt{(a^2 + h^2)} \leqslant (2D)^{3/2}\sqrt{M_\oplus/M_\odot} = 1{\cdot}76 \times 10^4 \qquad (2a)$$

where distances have been expressed in terms of the radius of the Earth. Fig. 1 illustrates the maximum

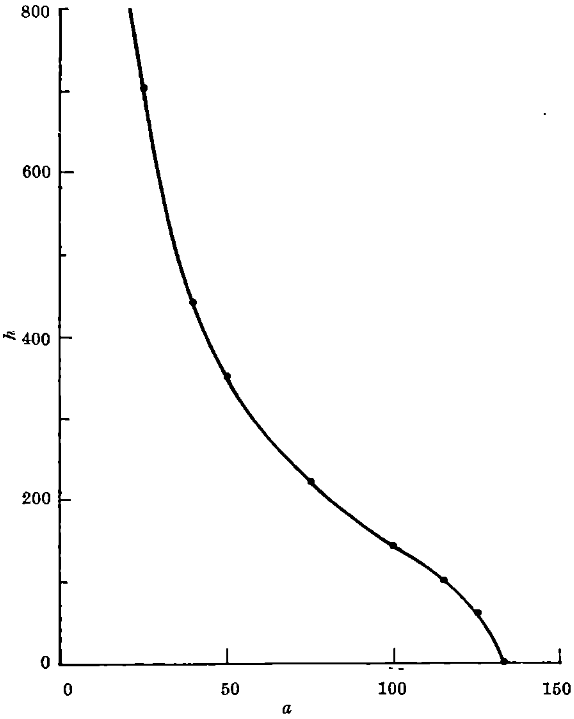


Fig. 1. Maximum permissible solar distance, r, different from the Earth's orbital radius, D, for interplanetary dust initially in a circular orbit about the Sun to reach the Earth's surface. The abscissa, $a = r - D$, and ordinate, h, the distance from the plane of the Earth's orbit, are expressed in terms of the Earth's radius. Values of h and a to the right of the curve require the incident dust to have too much angular momentum to reach the surface of the Earth ($a = 1$)

distance from the Earth in the plane perpendicular to the Earth's orbit from which circularly orbiting dust may be captured. The effect of radiation pressure would be to shift the curve to larger positive values of a, but much the same effective capture cross-section would result. All particles within a radius where their terrestrial gravitational potential exceeds their kinetic energy with respect to the Earth will have their orbits materially affected by the Earth's pull, but they will not be captured (that is, encounter the Earth's atmosphere) unless equations 2 and 2a are satisfied.

The radial velocity of the dust (with respect to the Earth) as a function of distance from the centre of the Earth, l, may be found by assuming a particle's total kinetic energy is due to its fall in the Earth's field and its angular momentum $\mathbf{l} \times \mathbf{v}$ is a constant equal to the left-hand side of equation 2.

$$v_l = \sqrt{2\frac{GM_\oplus}{l}(1 - b/l)} \sim \sqrt{2GM_\oplus/l} \qquad (3)$$

is the equation for the radial velocity of a particle in a hyperbolic orbit, where b is the perigee distance.

The total flux of dust entering a sphere of radius l must be a constant given by:

$$4\pi l^2 v_l n_l = \text{constant} \qquad (4)$$

If the angular dependence is neglected, one obtains an average density for dust approximately equal to:

$$n_l \sim n_0(l_0/l)^{3/2} \qquad (5)$$

where l_0 is the effective range of the Earth's gravitational field, and n_0 is the particle density in the absence of planetary condensation. For $l \sim b, n_l$ would vary as l^{-2} or faster. This effect is compensated in part by those particles within the Earth's gravitational pull the initial positions of which exceed the condition expressed in equation 2 for b equal to the radius of the Earth. Those particles populate the region next to a planet, but are not captured.

Excellent observations of dust-scattered light in the solar corona show that the concentration of dust in interplanetary space[2] is 10^{-14}–10^{-15} particles per c.c. at the position of the Earth's orbit. Yet space-vehicle observations[1] give flux densities of 10^{-12} particles/cm.^{2} at a height of 3,000 km. The dust most probably has a velocity of only $\sim$ 11 km./sec. [6]. Thus, these observations provide the best available evidence for planetary condensation of dust forming a blanket about the planets. Since n_0 is most likely 10^{-15}/c.c., l_0 would have to be about 100 Earth radii.

Rocket observations at about 100 km. give a higher concentration of dust still consistent with the largest estimates of the theory presented here, but indicating a more rapid decrease with height than $l^{-3/2}$ (ref. 1). A more rapid increase in dust concentration with decreasing height than the $l^{-3/2}$ prediction is to be expected from equations 3 and 4 for l approximately equal to perigee, b. Thus if much of the dust had a perigee close to one Earth radius, $n_l \sim (l - b)^{-1/2}$ would be more in agreement with the observations. Furthermore, space-vehicle measurements are of momentum, not mass, and detector thresholds might be expected to decrease rapidly with height in some cases, because particle gravitational energy decreases with height ; since particle number decreases rapidly with particle mass, detectors would count smaller, more numerous particles at lower altitudes in those cases when the relative velocity of particle and vehicle depends appreciably on height. De Jager and Hoffmeister[7] have written interesting, although quite different accounts, of the same subject.

I am pleased to thank Dr. F. S. Johnson for his interest in and support of this work and Dr. F. L. Whipple for pointing out the necessity for a more complete discussion of the theory.

DAVID B. BEARD*

Lockheed Missiles and Space Division,
Palo Alto, California.

* Permanent address : University of California, Davis.

[1] Whipple, F. L., *Nature*, **189**, 127 (1961).
[2] Beard, D. B., *Astrophys. J.*, **129**, 496 (1959).
[3] Wyatt, S. P., and Whipple, F. L., *Astrophys. J.*, **111**, 134 (1950).
[4] Robertson, H. P., *Mon. Not. Roy. Astro. Soc.*, **97**, 423 (1937).
[5] Whipple, F. L., *Astrophys. J.*, **121**, 750 (1955).
[6] Öpik, E. J., *Irish Astro. J.*, **4**, 84 (1956).
[7] Hoffmeister, C., *Veröff Berlin Babelsburg*, **10**, 1 (1932); *Astro. Nacht.*, **271**, 49 (1940).

DR. FREMLIN's suggestion of a contribution to the dust cloud near the Earth by meteorites that graze the Earth's atmosphere cannot be disproved. On the other hand, estimates of the total contribution to the Earth by meteorites, for example, G. S. Hawkins[1], add up to only a few tons a day in orbits hyperbolic with respect to the Earth. The total input[2], however, appears to add up to hundreds or even possibly thousands of tons a day. Hence, I should expect the fractional contribution by meteorites to be minor.

In Dr. Beard's preceding article I am pleased to see my statement of his point of view corrected. His conclusion, however, is subject to question. Though the dust particles producing the zodiacal light may well move in nearly circular orbits about the Sun this fact is not yet demonstrated. That their orbital inclinations to the ecliptic are negligible is really very doubtful, unless collisions among them can be proved to reduce the inclinations markedly. Haug[3], for example, finds that the light in the zodiacal cloud is fairly well represented by the distribution of orbital inclinations found for photographic meteors. Thus, the mean velocities of the particles with respect to the Earth and hence the effective gravitational cross-section of the Earth for the particles may not be much greater than its geometrical cross-section, rendering Beard's process ineffective.

From acoustical impact measurements on the satellite *Explorer I*, Hibbs[4] finds, in contrast to the statement in my earlier article, but in agreement with my suggestion, that the evidence strongly indicates that the particles are moving in orbits about the Earth. I am glad of the opportunity to make this correction to the earlier article and to note that particles ejected from the Moon may move in all types of orbits about the Earth. Thus, my predicted concentration towards the Moon's orbit in direct motion may easily be wrong, depending sensitively on the velocity distribution of particles ejected in lunar impact explosions.

Singer[5] finds that particles passing by the Earth isotropically in hyperbolic orbits should demonstrate a shadowing effect of the Earth such that impact-rates on surfaces very close to the Earth's surface will be somewhat smaller than at a distance of a few thousand kilometres. The observational data concerning the number of meteoritic dust impacts made by rockets are highly uncertain and may be much over-estimated. Thus the increase of concentration very near the Earth may well be smaller than I concluded in my earlier article, but still very high. It is clear that more observational data and more detailed theory are needed to clarify these matters.

Fred L. Whipple

Smithsonian Astrophysical and
Harvard College Observatories.

[1] Hawkins, G. S., *Astro. J.*, **65**, 318 (1960).
[2] Öpik, E. J., *Irish Astro. J.*, **4**, 84 (1956).
[3] Haug, U., *Z. Astrophysik*, **44**, 71 (1958).
[4] Hibbs, A. R., *J. Geophys. Res.*, **66**, 371 (1961).
[5] Singer, S. F. (private communication).

ANALYSIS OF POLARIZED LIGHT BY THE BEE'S EYE

By Prof. DONALD KENNEDY*

Zoology Department, Syracuse University, Syracuse, and Marine Biological Laboratory, Woods Hole, Mass.

AND

Dr. EDWARD R. BAYLOR

Woods Hole Oceanographic Institution, Woods Hole, Mass.

Bees, like some other arthropods, can orient various movements according to the plane of polarization of the incident light[1]. Such performances have been explained widely on the basis that the compound eye contains an analyser for polarized light. Another view has been proposed, namely, that environmental clues (for example, reflectance patterns) which depend on plane of polarization are perceived instead[2,3].

If the bee's eye is in fact an analyser for polarized light, the analysis could occur either in the dioptric apparatus of the eye or in the receptors (rhabdomeres) themselves. All efforts to locate the analyser in the dioptric apparatus by examining cornea and crystalline cone for dichroism have, however, met with failure. Because of this, and since disagreement exists about the behavioural evidence for or against extra-ocular analysis, it is thought that the receptors themselves are polarization analysers.

The method by which this is most often thought to occur was originally described, as a hypothetical model, by Autrum, and adopted by von Frisch[1] to explain bee orientation to polarized light. Later it was given some physiological substance by Autrum and Stumpf[4]. Briefly, the theory proposes that the opposite pairs of retinula cells with their rhabdomeres, which are shaped in ommatidial cross-section like the segments of an orange, have specific 'sensitive planes' of polarization, owing to their structure or to the orientation of pigment molecules within them (Fig. 1). The latter explanation, in particular, is favoured by Stockhammer[5]. Some support for the notion that the rhabdomeres can act as analysers has come from the demonstration that fixed rhabdomeres are doubly refracting[6,7]; they are, however, more weakly so when fresh, and are not dichroic in any circumstances[8]. The mere existence of molecular orientation would in any event scarcely prove that the structure is used in polarization analysis. With the arrangement of rhabdomeres shown in Fig. 1, in which the arrows in the individual rhabdomeres indicate the 'sensitive planes' of polarization, a polarized light stimulus will always stimulate the same number of receptors regardless of its plane. Thus the electrical output of the ommatidium or of a population of ommatidia should be independent of the plane of polarization. Autrum and Stumpf have shown that this is true for the bee's eye. De Vries *et al.*[9], however, were able to find no 'ripple' in the electro-retinogram from single ommatidia or the whole eye in *Calliphora*, when the plane of polarization was rotated at various frequencies.

A somewhat different sort of electro-physiological test has been applied to this problem more recently. It has yielded results which, we believe, are inconsistent with the Autrum model, like those of de Vries *et al.* The experiments to be described were actually done in 1958 and communicated in the form of abstracts[10]. We report them now in detail because the Autrum model is so widely invoked to explain the orientation of polarized light[5], and even as an aid in interpreting the fine structure of insect rhabdomeres[11]. Von Frisch *et al.*[12], in a recent review, have treated our preliminary note in a surprising manner. They describe it, quite correctly, as being without methodological detail, and then reject its conclusions by citing a personal communication from

---

* Present address: Stanford University, Stanford, California.

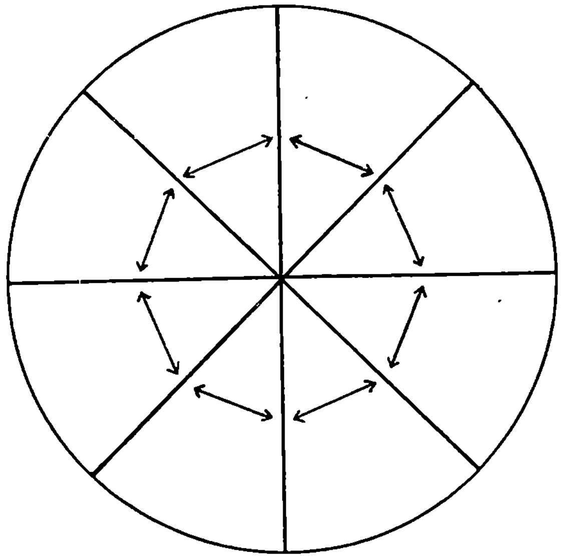


Fig. 1. The model of the bee's ommatidium proposed by Autrum and Stumpf (ref. 4). The arrow located in each rhabdomere indicates its 'sensitive plane' of polarization; opposite pairs of receptors are identical in this respect

Autrum, who suspects that we had not taken certain sources of error into account. Although this is possible, it is difficult to know how Prof. Autrum could have a reasonable basis for such a technical criticism, since no experimental detail was supplied in our abstract. Nevertheless, von Frisch *et al.*[12] had sufficient confidence in this opinion to state, in their summary: "Electrophysiological, polarization-optical and electron-microscopical studies have indicated unanimously that polarized light is analysed within the compound eyes of insects, in the rhabdomeres of the visual cells".

The technique used in our experiments was that of selective adaptation, a method which has been applied with success to other problems in sensory physiology in which the aim is to separate sub-modalities from a compound response. The retinal action potential or electro-retinogram of the honey-bee's eye was recorded from the isolated head preparation with sharpened steel needle electrodes, amplified with a capacitance-coupled pre-amplifier (time-constant of 1 sec., sufficiently long not to distort the responses to brief flashes) and displayed on a cathode-ray oscilloscope from which photographic records were made. The adapting and the stimulating light occupied nearly coincident optical axes, approximately normal to the corneal surface at its centre. The adapting source was a 300-W. tungsten projection bulb, the stimulating source a 20-W. microscope lamp. Stimuli were delivered through a photographic shutter, and more than covered the corneal surface. In the normal experimental procedure, a test-flash was delivered to the dark-adapted eye through a 'Polaroid' filter. The eye was then adapted to light for 30 sec. with the 'Polaroid' in the same position; after this, the course of dark-adaptation was followed by exposing the eye to test-flashes of the same intensity, duration and polarization plane as that which preceded the adaptation. After dark-adaptation was complete, the experiment was repeated with only one difference: the 'Polaroid' filter was rotated 90° for the adaptation period only. The first procedure will be referred to as a 'control' and the second as an 'experimental' adaptation. In effect, then, the procedure tested whether adaptation in a particular plane of polarization spares any significant proportion of the population of receptor units. According to the Autrum hypothesis, those rhabdomeres having 'sensitive planes' different from the planes of polarization of the adapting light should be less affected by it. Therefore, one would expect less light-adaptation, and/or faster recovery in the dark, when the planes of polarization of adapting light and test flash differ.

The intensity of all test-flashes used in these experiments was 125 ft.-candles; that of the adapting light was 3,200 ft.-candles. Test-flash durations were usually 0·1 sec. The intensity of the test-flashes was chosen so as to be in the region where, for most preparations, the change in electro-retinogram amplitude is large for a small intensity increment. Electro-retinogram amplitudes were measured on responses photographed on moving paper from the oscilloscope trace. In all experiments, test-flashes were delivered 7, 12, 17, 22, 32 and 42 sec. following the end of light-adaptation. Dark-adaptation was virtually complete within 1 min., in agreement with the observations originally made by Autrum[13] that the eyes of certain insects (including the bee) are 'fast' in terms of adaptation-rate and the ability to follow flickering stimuli. In plotting the time-course of dark-adaptation, successive amplitudes are expressed as a percentage of the amplitude of the response to the initial flash, before adaptation. In most, adaptation is not quite complete at 42 sec.; but two series, in which the response amplitude at 42 sec. was less than 80 per cent of the dark-adapted value and in which, therefore, some overall change in sensitivity had clearly occurred, were rejected.

Table 1 shows the results of experiments on the extent of light-adaptation produced by the two procedures described here. The figures given are amplitude of the electro-retinogram taken 7 sec. after the end of light-adaptation and expressed as percentages of the initial dark-adapted value. The experiments have been done in pairs, the control either immediately preceding or following the experimental one in the adjacent column. In all pairs of experiments, the difference between adaptation in the same plane of polarization or the perpendicular one is small. The paired experiments naturally differ from one another, since the relationship between the test-flash and adapting intensities used and the sensitivity of the electro-retinogram may vary from preparation to preparation. The important aspect of the results is the high degree of internal consistency shown by the paired experiments. The average reduction for the two groups is identical.

Fig. 2 shows complete dark-adaptation curves, averaged from data such as those shown in Table 1. Six control and six experimental runs made on four

Table 1. Extent of Light-Adaptation, tested by Stimuli in the same Plane of Polarization as that of the Adapting Light (Control) and in the Opposite Plane

Amplitude of responses recorded 7 sec. following the light-adaptation, and expressed in per cent of the amplitude before adaptation

Preparation	Experiment No.	Control	Experimental
2	1	79	67
2	2	58	62
3	1	80	80
3	2	77	77
4	1	74	82
4	2	72	64
5	1	69	66
	Average	73	73

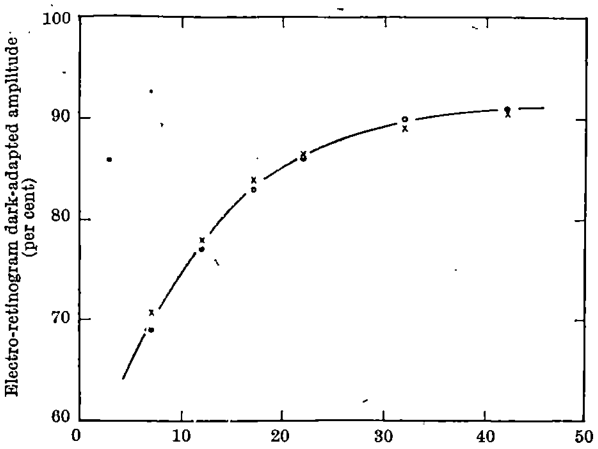


Fig. 2. Dark-adaptation curves for the bee's eye. Each point is the average of six runs on four preparations. 'Control' adaptations are indicated by crosses, 'experimental' adaptations by filled circles

different preparations have been averaged. The two average curves are virtually identical.

These results show that there is no population of receptors contributing to the electro-retinogram which can be adapted selectively to a measurable extent by exposure to intense plane-polarized light. It may be noted that 'contribute' in the sense used above does not mean 'generate'; the receptor cells may or may not play a large part in generating the electro-retinogram, but they surely trigger activity in those post-synaptic units which do generate it directly. For our purposes, the distinction is meaningless, because the effects of adaptation are felt at all levels, not just at the primary one. The electro-retinogram is used only because it is a convenient test of the sensitivity of a system.

The results seem to us inconsistent with the Autrum hypothesis. Since our experiments were performed, however, new evidence in support of the hypothesis has been produced. Some investigators[14,15] have recently obtained intracellular records from two dipteran eyes, *Lucilia* and *Calliphora* respectively. Both groups assumed that the responses, 'generator' potentials without spikes, originate from retinula cells; both show that, in some units at least, the amplitude of the generator potential depends on the plane of polarization of the stimulating light. In the Burkhardt and Wendler experiments, the difference between the response produced by the most effective and least effective planes of stimulus polarization was equivalent to a 46 per cent difference of intensity.

There is no question that these results provide strong support for the Autrum model. In attempting to resolve our own failure to obtain, by selective adaptation, results consistent with the model, three possibilities exist: (1) the inconsistency is explained by a real difference in property between the bee's eye and the dipteran eyes investigated; (2) our experiments lacked the sensitivity required to reveal a differential polarization sensitivity; or (3) the results on recording from single retinula cells do not represent a real act of physiological polarization analysis by the sensory cell. Possibility (1) cannot be evaluated at this point, but does not seem likely. We cannot rule out possibility (2); but it would be surprising if a peripheral mechanism producing, under the favourable conditions of our experiment, a differential polarization sensitivity too small for us to detect, could be useful under natural conditions, where the bee must analyse rather weakly polarized light from the sky. If each retinula cell in the bee's eye actually responded to its 'sensitive plane' of polarization as though it were twice as bright as that 90° away, a selective adaptation experiment of the kind we performed would surely have revealed sensitivity differences; furthermore, steady illumination in one plane of polarization abruptly changed to a plane differing by 90° would be expected to recruit an additional response from less-adapted elements. We have not been able to show such 'shift' responses in the electro-retinogram in our own experiments, nor could de Vries *et al.*[9] with somewhat different methods in *Calliphora*.

The third possibility, that the results from intracellular recording do not indicate real polarization analysis by the sense cell, must at least be considered. In the first place, the responses in question are only inferred to come from retinula cells; there is no way, at present, of being sure that they do. Since such cells are certainly the most likely source of the records, however, it seems fair to assume that they do. Burkhardt and Wendler show records from a single cell, and state that the difference in amplitude of the potential produced by light stimuli having polarization planes 90° apart is equivalent to a 46 per cent difference of intensity. They say that a "large number" of penetrated cells showed such differences, but do not specify what the number is, what percentage of the total observations it represents, or what is the average amplitude difference for different stimulus planes of polarization. Even if one concludes that, in all cells showing the phenomenon, it had the same magnitude as the illustrated case, one has no idea how many cells failed to show it at all. The authors state, indeed, that some did not; and in fact their conclusion that the property of polarization analysis may be restricted to certain retinula cells already involves a departure from the Autrum model in its original form. Those cells in the Burkhardt and Wendler experiments which did show differential responses to different planes of polarization exhibit one rather peculiar property: the difference is dependent on wave-length, appearing only with short-wave-length stimuli. If orientation of the photo-sensitive pigment within the ultrastructure of the rhabdomere is the critical factor in polarization analysis, as proposed by Stockhammer[7], it is difficult to understand why the vibration planes of blue light would be critical in determining absorption and the vibration planes of red light unimportant.

It is well known that structures optically interposed between a source of polarized light and a detector can create a kind of polarization sensitivity by differentially refracting, reflecting or absorbing light of particular *e* vectors. It is possible, though by no means certain, that the intracellular micro-pipette introduced into the retinula cell could act in just this way; if it did, then the results could be explained as an act of polarization analysis by the recording micro-electrode. The absence of the effect in some cells, and its presence in others, might thus be related to the depth of penetration, or to the orientation of the micro-electrode with respect to the stimulating light. Electrodes with tip diameters of less than 0·5μ, the kind used for these experiments, are able to record extracellular potentials only very locally. It may be that a solution to the problem raised by the possible optical action of the penetrating micro-electrode might be to record outside the cells. In some circumstances at least, the micro-electrode should record dominantly the output of one retinula cell, and if the latter indeed varies with stimulus polarization, the recorded extracellular potential should vary too. Burkhardt and Wendler refer briefly to extracellular recordings in connexion with the experiments and state that their amplitude did not change with plane of polarization. It would be an exceptional circumstance if an extracellular micro-pipette of the diameter used recorded equally from all members of a given ommatidial cluster of retinula cells.

At this point, it is evidently impossible to decide which of the three possibilities is correct. The Autrum model continues to be the most attractive explanation of polarized-light sensitivity in insects. Our results are, however, not consistent with it; and it seems reasonable to ask that before it is given the kind of total acceptance accorded it by von Frisch *et al.*[12] and other behavioural workers, direct and unambiguous evidence be brought in its favour.

This investigation was supported in part by a grant from the National Science Foundation.

[1] von Frisch, K., *Bees: Their Vision, Chemical Senses and Language* (Cornell Univ. Press, Ithaca, New York, 1950).
[2] Smith, F. E., and Baylor, E. R., *Ecology*, **140**, 360 (1960).
[3] Kalmus, H., *Nature*, **182**, 1526 (1958).
[4] Autrum, H., and Stumpf, H., *Z. Naturforsch.*, **5 b**, 116 (1950).
[5] Stockhammer, K., *Ergbn. Biol.*, **21**, 23 (1959).
[6] Menzer, G., and Stockhammer, K., *Naturwiss.*, **38**, 190 (1951).
[7] Stockhammer, K., *Z. vergl. Physiol.*, **38**, 30 (1956).
[8] de Vries, H., and Kuiper, J., *Ann. N.Y. Acad. Sci.*, **74**, 196 (1958).
[9] de Vries, H., Jielof, R., and Spoor, A., *Physica*, **19**, 419 (1953).
[10] Baylor, E. R., and Kennedy, D., *Anat. Rec.*, **132**, 411 (1958).
[11] Fernandez-Moran, H., *Exp. Cell. Res.*, Supp. 5, 586 (1958).
[12] von Frisch, K., Lindauer, M., and Daumer, K., *Experientia*, **16**, 289 (1960).
[13] Autrum, H., *Z. vergl. Physiol.*, **32**, 176 (1950).
[14] Kuwabara, M., and Naka, N., *Nature*, **184**, 455 (1959).
[15] Burkhardt, D., and Wendler, L., *Z. vergl. Physiol.*, **43**, 687 (1960).

ENHANCEMENT OF INHIBITION OF INTESTINAL ABSORPTION BY CETRIMIDE–PHLORIDZIN COMBINATION

By J. A. NISSIM

Department of Pharmacology, Guy's Hospital Medical School, London, S.E.1

THE inhibitory effects of trimethylhexadecylammonium bromide (cetrimide) and stearate on the intestinal absorption of glucose in the rabbit, and of glucose, sodium butyrate and methionine in the rat were described by me earlier[1,2]. Later, the anæsthetized mouse was used as a test preparation for the study of intestinal absorption as well as for the rapid assay of substances capable of influencing this function. In this animal, cetrimide was found to exhibit its greatest inhibitory effect on the absorption of glucose, while sodium butyrate and methionine absorption were inhibited to a moderate extent. Phloridzin, on the other hand, which was also found to inhibit the absorption of glucose markedly, and that of butyrate moderately, had no effect whatever on the absorption of methionine. Differences shown by these and other species in the actions and activities of cetrimide and of phloridzin suggested that the effects were enzymic in nature, but further elucidation of the site and mode of action of these substances was desirable. It was considered that the effects of simultaneous intestinal perfusion of these two compounds might throw additional light on this problem, particularly with regard to such matter as synergism, potentiation and antagonism, as well as effect on phosphatase activity. A preliminary report of the results of this investigation is given in this article, while a more detailed account will be published elsewhere.

The effects of cetrimide, phloridzin and combinations of the two substances on the intestinal absorption of glucose, sodium butyrate and methionine were compared by means of the mouse assay method, using both the half-hour and the five-minute intestinal perfusion techniques, which will be described elsewhere. Pure interbred mice of C_3H and *CBA* strains were used. Cetrimide and phloridzin were perfused in concentrations of 0·001, 0·002 and 0·01 per cent singly and in combination, on groups of four animals each, while the concentration of three nutrients investigated was always 0·2 per cent. Chemical estimation of the three substances in the perfusate at the end of the experiment was carried out and their percentage absorption was calculated.

Striking enhancement of the inhibition of the intestinal absorption of both glucose and of butyrate was obtained when equal concentrations of cetrimide and phloridzin were perfused simultaneously. This effect was most noticeable in the case of glucose absorption in the half-hour perfusion experiments, and the results are given in detail in Table 1. In the range of concentrations used, the regression of response on the logarithm of the drug concentration was linear in all three cases (Fig. 1) for half-hour experiment and highly significant, while deviations from regression were not significant.

Table 1. PERCENTAGE ABSORPTION OF GLUCOSE, IN EACH OF FOUR MICE, FROM INTESTINAL PERFUSION FLUID CONTAINING DIFFERENT CONCENTRATIONS OF CETRIMIDE, PHLORIDZIN OR CETRIMIDE + PHLORIDZIN

The fluid was perfused for half an hour in each experiment

Concentration of drug (per cent)	Cetrimide	Phloridzin	Cetrimide + phloridzin
0·001	44·1	18·8	10·2
	40·6	35·7	11·1
	51·9	32·4	3·9
	49·9	39·5	2·7
	Mean 46·625	31·6	6·975
0·002	23·3	19·2	9·4
	27·0	19·6	5·8
	41·3	16·8	2·8
	29·2	17·9	2·6
	Mean 30·02	18·375	5·15
0·01	16·8	10·2	−3·6
	36·5	1·7	−4·4
	28·4	7·9	1·7
	14·6	10·5	−0·2
	Mean 24·075	7·575	−1·625

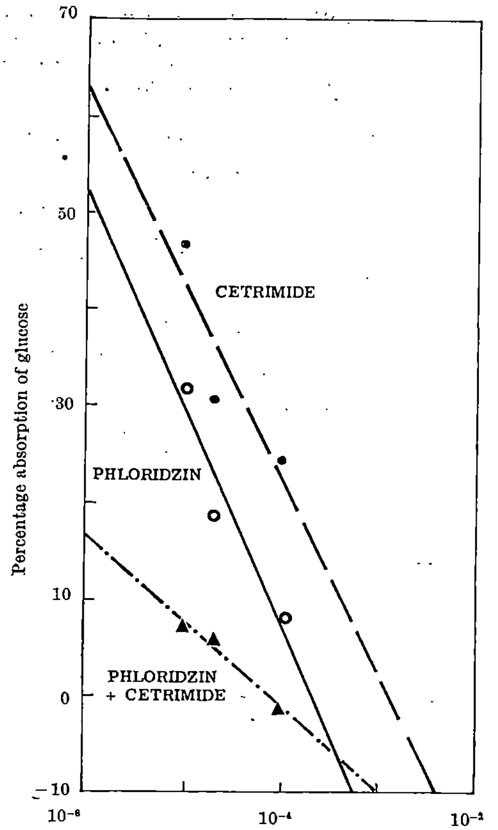


Fig. 1.

The slopes of the dose-response lines were practically the same for the two drugs when these were used singly. When the drugs were used in combination, however, the slope was significantly different. The main statistical results together with a skeleton analysis of variance appear in Table 2.

Phloridzin was about five times as potent in inhibiting the intestinal absorption of glucose in the mouse as cetrimide, while the two substances perfused together were about ten times as active as phloridzin alone. Since the slope of the cetrimide + phloridzin dose-response line differed from that of phloridzin or of cetrimide alone, the potency ratio assumes different values at different concentration-levels, and would be given by the antilogarithm of the distance between any two points taken parallel to the concentration co-ordinate. The value 10·45 of this ratio given in Table 2 was calculated on the assumption of parallelism, and would represent an average. The concentration of cetrimide which produces this enhancement was only 1/10 × 1/5 or 1/50 of that which would be required of this substance to produce the same inhibition of glucose absorption when used alone.

The combination of the two drugs also gave enhanced-inhibition of butyrate absorption. Though the butyrate absorption figures were considerably scattered, so that precise determination of the fiducial limits of the potency ratio was not possible, the potency ratio value of twelve was of the same order as that obtained in the case of glucose. No enhancement of the inhibitory effect on methionine absorption was obtained, however.

The five-minute assays gave an enhancement value of six for the inhibition of glucose absorption. The lower figure obtained in these experiments was the result of the relatively long time required by cetrimide before it exhibits its full effect.

The inhibition of methionine absorption was unchanged, though the inhibition of glucose and of butyrate absorption became much more pronounced when the two drugs were combined. Hence, it seems more likely that the basis of this increase in effect is an enhancement of the action of phloridzin by cetrimide rather than the opposite. This enhancement occurred at a considerable range of concentrations, and its magnitude is probably among the greatest known in pharmacology.

The synergism shown between cetrimide and phloridzin in the inhibition of intestinal absorption is not simply additive. From this it may be concluded that when the drugs act independently, their effects are exerted at different sites in the absorptive mechanism. The degree of enhancement in the combined action is also greater than can be explained by assuming two different sites of inhibition in the same biochemical chain of reaction, though this may in fact represent their basic mode of action. A third mechanism has to be invoked to explain the magnitude of the effect. The formation of a cetrimide–phloridzin complex, which is more active than either compound alone, though theoretically possible, does not seem probable. A conserving action of the cetrimide on the phloridzin appears more likely. This may be due to the protection of the latter drug by the former from rapid destruction or clearance, but the fact that there is no fall in the activity of either compound in half-hour as compared with five-minute perfusion experiments points strongly against such a hypothesis. A suggestion which seems to be compatible with all the facts hitherto

Table 2. ANALYSIS OF VARIANCE, DOSE-RESPONSE LINES, DIFFERENCE BETWEEN SLOPES, AND POTENCY RATIO WITH FIDUCIAL LIMITS FOR CETRIMIDE, PHLORIDZIN, AND CETRIMIDE + PHLORIDZIN IN HALF-HOUR INTESTINAL PERFUSION ASSAYS IN THE MOUSE

	Cetrimide	Phloridzin	Cetrimide + phloridzin
Analysis of variance			
Source of variation:			
Linear regression	$P < 0·01$	$P < 0·001$	$P < 0·01$
Deviation from regression	$P > 0·05$	$P > 0·05$	$P > 0·2$
Dose-response line	$y = 62·55 - 20·17\,x$	$y = 51·46 - 22·51\,x$	$y = 16·11 - 8·79\,x$
x = log concentration			
Difference between slopes	2·34		13·72
	$P < 0·8$		$P < 0·01$
Potency ratio	4·8		10·45
Range of potency ratio at $P = 0·01$	26·7 – 1·9		70·2 – 3·8

established is that cetrimide may exert a conserving effect by displacing the phloridzin from 'silent receptors', where the drug has no action, to 'active receptors', so that the effective concentration is greatly increased. A further possibility is that the surface-active cetrimide may facilitate the penetration of phloridzin into the cell to its site of action. It may prove possible to put this hypothesis to the test by investigating other surfactants of different composition.

The present investigation raises a number of intriguing questions; for example, whether the synergistic action of cetrimide would also extend to the inhibitory effect of phloridzin on glucose re-absorption by the renal tubules, and whether phloridzin in turn would exhibit synergism with respect to the germicidal activity of cetrimide. Answers to such questions are being sought. In preliminary investigations there has been no obvious synergism between the two substances with regard to inhibition of intestinal alkaline phosphatase, though both seem to be equipotent in this respect.

[1] Nissim, J. A., *Nature*, **187**, 308 (1960).
[2] Nissim, J. A., *Nature*, **187**, 305 (1960).

PARTIAL ACID-HYDROLYSIS OF A MUCOPOLYSACCHARIDE WITHOUT APPRECIABLE *N*-DEACETYLATION OF HEXOSAMINE

By Dr. T. J. PAINTER and Prof W. T. J. MORGAN, C.B.E., F.R.S.
Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1

IN the partial acid-hydrolysis of polysaccharides which contain residues of *N*-acetyl-hexosamine, scission of the glycosidic linkages is accompanied by *N*-deacetylation of the hexosamine residues[1-3]. This complication is avoided in partial acetolysis[4,5], but during subsequent *O*-deacetylation of the acetolysate, extensive degradation of the oligosaccharides may occur[5,6]. A partial acid-hydrolysate can be re-*N*-acetylated[7]; but we report here some results which show that this step may not always be necessary.

In a previous communication[8], one of us reported the conversion of inulin into oligosaccharides in high yield, by hydrolysis with water-soluble polystyrenesulphonic acid. This was accomplished by dialysing the oligosaccharides continuously from the reaction mixture as they were liberated; they thereby escaped further hydrolysis, since the acid was non-dialysable. It seemed likely that, if applied to a polysaccharide containing *N*-acetyl-hexosamine residues, the same technique would not only improve the yield of oligosaccharides, but also diminish the extent of *N*-deacetylation of the products.

To illustrate this possibility, methyl 2-acetamido-2-deoxy-β-D-glucopyranoside was chosen as a model substance. A solution of this compound (20 mgm.) in 0·2 *N* hydrochloric acid (20 ml.) was heated at 86·5 ± 0·5°, and the liberation of 2-acetamido-2-deoxy-D-glucose was followed by a modification[9] of the Morgan–Elson method. The yield rose to a maximum of 0·77 mole/mole after 100 min., and then slowly decreased. The liberation of 2-amino-2-deoxy-D-glucose was also followed, and the yield of this product after 100 min. was 0·18 mole/mole. (This was measured as reducing power, using Nelson's reagents[10]; with the help of calibration curves, the concentrations of 2-amino-2-deoxy-D-glucose were determined, after subtracting from the total reducing power the contributions of the previously determined concentrations of 2-acetamido-2-deoxy-D-glucose.) Since methyl 2-amino-2-deoxy-β-D-glucopyranoside was hydrolysed to a negligible extent under the same conditions[6], it followed that the yield of 2-acetamido-2-deoxy-D-glucose after 100 min. would have been about 95 per cent, if it had been possible to remove this compound continuously from the reaction mixture as it was liberated.

Experiments were then carried out with a mucopolysaccharide prepared from hog gastric mucin. This material contained residues of fucose (10·2 per cent), galactose (23·5 per cent), 2-acetamido-2-deoxy-glucose (26·8 per cent) and 2-acetamido-2-deoxy-galactose (16·3 per cent); the remainder of the macromolecule consisted essentially of peptide material. (These analytical figures are subject to the usual errors involved in mucopolysaccharide analysis, which arise from incomplete release of monosaccharides by acid-hydrolysis[1-3].) The preparation of the water-soluble polystyrenesulphonic acid, and the general procedure for the hydrolysis, are described elsewhere[8].

To a solution of the mucopolysaccharide (300 mgm.) in water (40 ml.) was added polystyrenesulphonic acid (0·0404 *N*; 20 ml.), and 'Amberlite *IR*-120' (H^+ form) resin (5 gm.). (The 'Amberlite' resin was included to prevent gradual neutralization of the dilute acid by basic substances which were liberated during hydrolysis, and absorbed from the laboratory atmosphere. The 'Amberlite' resin was not an efficient catalyst when used alone, probably owing to the difficulty with which the large mucopolysaccharide molecules penetrated the resin particles.) The reaction mixture (*p*H 2·2) was dialysed at 86 ± 1° against distilled water (800 ml.). The initial effective area of membrane ('Visking' 32/32 tubing) was 240 cm.^{2}. At intervals, the dialysates were replaced by fresh distilled water[8]; the dialysates were evaporated separately, and the residues were weighed. The results are recorded in Table 1.

Table 1

Time (hr.)	1·5	4·0	7·0	11·0	21·0	50·0
Total weight dialysed (mgm.)	43·7	106·7	143·9	177·2	217·7	243·0

These residues were examined on paper chromatograms, which were developed in ethyl acetate/pyridine/water (10:4:3 v/v), and sprayed with silver nitrate–sodium hydroxide reagents[11]. The four above-named monosaccharides were detected, together with at least thirteen different oligosaccharides. Five of the oligosaccharides appeared from their chromatographic mobilities to be the five disaccharides previously isolated from human blood-group *A* substance in this laboratory[12]. Free hexosamine was not detected, either on these chromatograms or on similar chromatograms treated with ninhydrin[13]. None of the oligosaccharides produced a colour with ninhydrin. Most of the oligosaccharides gave a purple colour after treatment with alkali, followed by Ehrlich's reagent[14], indicating the presence of N-acetyl-hexosamine residues.

From the solution remaining in the dialysis bag, 76 mgm. of material was isolated, after neutralization and removal[8] of the polystyrenesulphonic acid and resin. This material consisted largely of free amino-acids, but also contained residues of hexosamine (4·2 per cent) and galactose (3·3 per cent). After taking into consideration the addition of the elements of water to each fragment during hydrolysis, and possible analytical errors, it was evident that about 90 per cent of the total carbohydrate moiety of the mucopolysaccharide had been converted into mono- and oligo-saccharides having their N-acetyl groups still intact.

In an attempt to rationalize this result, the rate of N-deacetylation of 2-acetamido-2-deoxy-D-glucose under the same hydrolytic conditions was studied. This compound (13·5 mgm.) was heated at 86 ± 1° in 0·0135 N polystyrenesulphonic acid (6 ml.). At intervals, samples were withdrawn and neutralized, and their content of 2-amino-2-deoxy-D-glucose was estimated with ninhydrin[15]. The results are shown in Table 2.

Table 2

Time (hr.)	1	2	3	5	7	10	13	16
N-deacetylation (per cent)	1·4	2·8	4·6	7·3	10·0	13·7	17·5	21·2

When the hyperbolic logarithms of the concentrations of 2-acetamido-2-deoxy-D-glucose were plotted against time, a straight line was obtained, having slope $k = 1{\cdot}5 \times 10^{-2}$ hr.$^{-1}$. Similarly, from the results in Table 1, the hyperbolic logarithms of the amounts of carbohydrate material remaining in the dialysis bag were plotted against time. An approximate straight line was obtained, having slope $K = 10{\cdot}5 \times 10^{-2}$ hr.$^{-1}$. Assuming that all the N-acetyl-hexosamine residues in the mucopolysaccharide were N-deacetylated at the same rate, irrespective of their state of combination, and that the proportion of these residues in the diffusible material remained constant throughout the hydrolysis, we can write $C_t = C_0 e^{-(k + K)t}$, where C_0 is the initial concentration of N-acetyl-hexosamine residues in the dialysis bag, and C_t is the concentration after time t. It may be deduced that the percentage N-deacetylation after time t is given by $\frac{100k}{k + K}(1 - e^{-(k + K)t})$. When no provision is made for dialysis, $K = 0$, and the expression simplifies to $100(1 - e^{-kt})$. In Table 3, these expressions have been used to calculate the percentage of N-deacetylation to be expected at various times, with and without the use of a membrane.

Table 3

Time (hr.)	N-deacetylation (per cent)	
	With dialysis	Without dialysis
2	2·7	2·9
5	5·7	7·2
10	8·8	13·8
20	11·4	26·0
50	12·5	52·8
100	12·6	77·7
∞	12·6	100·0

The maximum possible extent of N-deacetylation to be expected when the membrane was used is seen to be 12·6 per cent. Although these assumptions are unlikely to be strictly valid, this calculated result accounts satisfactorily for the observed high yield of N-acetylated mono- and oligo-saccharides.

The residues obtained from the various dialysates were combined and separated into mono- and oligosaccharide fractions by selective displacement from a charcoal–'Celite' column[16]. The yield of oligosaccharides represented about 40 per cent of the original weight of the mucopolysaccharide. This yield was more than three times as great as the maximum yield which we were able to obtain by hydrolysis with mineral acid. Separation and identification of the oligosaccharides are now in progress, details of which will be reported later.

Financial support from the Nuffield Foundation and the Medical Research Council is gratefully acknowledged.

[1] Moggridge, R. C. G., and Neuberger, A., *J. Chem. Soc.*, 745 (1938).
[2] Foster, A. B., Horton, D., and Stacey, M., *J. Chem. Soc.*, 81 (1957).
[3] Johansen, P. G., Marshall, R. D., and Neuberger, A., *Biochem. J.*, **77**, 239 (1960).
[4] Masamune, H., Sinohara, H., and Okuyama, T., *Tôhoku J. Exp. Med.*, **68**, 165 (1958).
[5] Barker, S. A., Foster, A. B., Stacey, M., and Webber, J. M., *J. Chem. Soc.*, 2218 (1958).
[6] Painter, T. J., and Morgan, W. T. J. (unpublished results).
[7] Barker, S. A., Heidelberger, M., Stacey, M., and Tipper, D. J., *J. Chem. Soc.*, 3468 (1958).
[8] Painter, T. J., *Chem. and Indust.*, 1214 (1960).
[9] Aminoff, D., Morgan, W. T. J., and Watkins, W. M., *Biochem. J.*, **51**, 379 (1952).
[10] Nelson, N., *J. Biol. Chem.*, **153**, 375 (1944).
[11] Trevelyan, W. E., Procter, D. P., and Harrison, J. S., *Nature*, **166**, 444 (1950).
[12] Côté, R. H., and Morgan, W. T. J., *Nature*, **178**, 1171 (1956).
[13] Payne, W. J., and Keiber, R. J., *Arch. Biochem. Biophys.*, **52**, 1 (1954).
[14] Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).
[15] Moore, S., and Stein, W., *J. Biol. Chem.*, **176**, 367 (1948).
[16] Whistler, R. L., and Durso, D. F., *J. Amer. Chem. Soc.*, **72**, 677 (1950).

INTERMEDIATE POOL BETWEEN AMINO-ACIDS AND GLOBIN IN DUCK BLOOD *IN VITRO*

By L. BIANCO, G. GIUSTINA* and E. LAZZARINI
Istituto di Fisica del Politecnico, Milano

If duck red cells, previously incubated *in vitro* in their plasma with 2-^{14}C glycine, are washed and re-incubated in unlabelled plasma, the activity of hæmoglobin increases with time (re-incubation time) according to an exponential law[1]. These data are shown in Table 1. We find that inside red cells there is a pool of radioactive substances which cannot be removed completely by washing and which are used for hæmoglobin synthesis. The following equation fits the experimental points:

$$\alpha = 1 - A \exp(-\lambda t) \qquad (1)$$

---

* Istituto di Patologia Medica dell'Università, Milano.

Table 5

Exp.	Sample	a_G	Molarities of unlabelled amino-acids		
			Glycine	Serine	Alanine
1	1	162 ± 4	—	—	$0\cdot9 \times 10^{-3}$
	2	176 ± 4	$0\cdot25 \times 10^{-2}$	$0\cdot40 \times 10^{-2}$	$0\cdot9 \times 10^{-3}$
	3	182 ± 3	$0\cdot5 \times 10^{-1}$	$0\cdot80 \times 10^{-1}$	$0\cdot9 \times 10^{-3}$
2	1	72 ± 4	—	—	$0\cdot9 \times 10^{-3}$
	2	72 ± 5	$0\cdot5 \times 10^{-2}$	$0\cdot38 \times 10^{-2}$	$0\cdot9 \times 10^{-3}$
	3	64 ± 4	$0\cdot52 \times 10^{-1}$	$0\cdot38 \times 10^{-1}$	$0\cdot5 \times 10^{-1}$
	4	66 ± 2	$1\cdot06 \times 10^{-1}$	$0\cdot76 \times 10^{-1}$	$1\cdot0 \times 10^{-1}$

a = specific activity in dis./min./mgm.

in the second. They were different for amounts of inactive glycine, serine and alanine, carriers added. Except for 2-^{14}C glycine, which was not added, all other conditions were the same as described for hæmolysate incubation experiments.

From the data reported (Table 5) the following conclusions can be deduced: (*a*) Globin can absorb radioactivity from labelled ultrafiltered substances. (*b*) This radioactivity taken up is not due to free radioactive glycine or serine or alanine, which can be formed from glycine conversion[3], because the added carriers were not effective in reducing globin specific activity. Therefore, we suggest a free peptide pool as globin precursor in duck blood *in vitro*.

Further experiments are in progress to prove precursor product relationships between the transient part of the microsomes and these peptides and globin, and to find the molecular weights of these peptides. Indeed, we know only an upper limit, because almost all radioactivity of ultrafiltrate can dialyse.

We wish to thank Prof. S. Ranzi of the University of Milan for the ultracentrifugations and Mr. F. Bamonte for technical assistance in collecting experimental data.

[1] Breccia, A., Fantola, A. L., Giustina, G., and Lazzarini, E., *Nature*, **184**, 1878 (1959).

[2] Dintzis, H. M., Borsook, H., and Vinograd, J., "Microsomal Particles and Protein Synthesis", *First Symp. Biophys. Soc.* (Pergamon Press, New York, 1958).

[3] Breccia, A., Fantola, A. L., Giustina, G., and Lazzarini, E., *Energia Nucleare*, **7**, 99 (1960).

[4] Giustina, G., and Lazzarini, E., *Nature*, **181**, 1285 (1958).

[5] Scheiger, H. G., Rapoport, S., and Schölzel, E., *Nature*, **178**, 141 (1956).

DIDEOXYSUGARS OF *PASTEURELLA PSEUDOTUBERCULOSIS*-SPECIFIC POLYSACCHARIDES, AND THE OCCURRENCE OF ASCARYLOSE

By Dr. D. A. L. DAVIES

Microbiological Research Establishment, Experimental Research Establishment, Porton

THE isolation and composition of the type-specific polysaccharides of the five serotypes of *Pasteurella pseudotuberculosis* have previously been described[1]. The identity of the 3,6-dideoxy-hexoses present in these materials, and to which the serological specificity may be largely due, was, however, left in some doubt.

Sugars of the 3,6-dideoxy-hexose series (paratose, abequose, colitose and tyvelose) are known to occur in polysaccharides of Enterobacteria[2], where they occupy the positions of non-reducing end-group residues and are the specific serological determinants responsible for some 'antigens' of the Kauffmann–White scheme. One other sugar of this series, ascarylose, is known to occur in the egg cell membranes of *Ascaris* worms[3].

It was previously reported that *P. pseudotuberculosis* type II polysaccharide contains abequose (3,6-dideoxy-D-*xylo*-hexose); this was based on chromatographic data only. This identification has been confirmed in the following way, using a serological cross-reaction which has long been known[4]. Specific polysaccharide extracted from *Salmonella salinatis* (which contains abequose, responsible[5] for Kauffmann–White 'antigen' 4) was adsorbed on to sheep erythrocytes[6], which thus became agglutinable not only by homologous *Salmonella Gp. B* antisera but also by *P. pseudotuberculosis* type II antiserum. Similarly, erythrocytes sensitized with *P. pseudotuberculosis* type II polysaccharide could be agglutinated by both antisera. The hæmagglutination of both kinds of sensitized sells by the heterologous antisera could be inhibited by abequose (isolated fron *S. salinatis* polysaccharide) but not by any other sugar tested, including those most closely related structurally to abequose (for example, D-fucose, D-galactose, D-glycero-D-galactoheptose, tyvelose). Sufficient dideoxy-sugar was isolated from *P. pseudotuberculosis* type II polysaccharide to test in this system, and inhibition was again obtained, leaving no doubt that this sugar is abequose.

It was previously reported[1] that *P. pseudotuberculosis* type IV polysaccharide contains tyvelose (3,6-dideoxy-D-*arabino*-hexose), but again only on chromatographic evidence. It is already known[7] that this type is related serologically to *Salmonellae* of group *D*, due to Kauffmann–White antigen 9, and that this 'antigen' is due to tyvelose end-groups in the group *D* specific polysaccharide[8]. The cross-reaction could be demonstrated readily by hæmagglutination as already described, but in this case the reactions could not be inhibited by tyvelose (isolated from *S. typhosa* polysaccharide), ascarylose (its optical enantiomorph), or any other sugar tested. The dideoxy-sugar was therefore isolated from *P. pseudotuberculosis* type IV polysaccharide; material was hydrolysed for 20 min. in *N* sulphuric acid at 100°, neutralized with barium hydroxide and the salt-free hydrolysate concentrated to a thin syrup. The leading component was isolated by elution from chromatograms run on Whatman 3-mm. paper irrigated with butanol/pyridine/water (6 : 4 : 3); the eluates were concentrated to a syrup, which gave a strong positive rotation. Since ascarylose has a negative rotation, the *P. pseudotuberculosis* type IV sugar is tyvelose.

The polysaccharide of *P. pseudotuberculosis* type V contains a sugar which cannot be distinguished chromatographically from tyvelose[1]. In view of the different specificities of the type IV and V materials, the sugar would be either tyvelose differently linked in the two instances or alternatively the type V material might contain the optical isomer ascarylose (3,6-dideoxy-L-*arabino*-hexose). The dideoxy-sugar of type V polysaccharide was isolated chromatographically as previously described and the syrup, after filtration through charcoal, gave a strong negative rotation ($[\alpha]_D = -20°$, about 2 in water), approaching that of crystalline ascarylose ($[\alpha]_D = -25°$). This identity was confirmed by preparing the dideoxy-hexitol[3]; the syrup (approx. 25 mgm.) was reduced in aqueous solution with $NaBH_4$ (25 mgm.) and left at room temperature overnight, excess $NaBH_4$ was then destroyed with a little *N* acetic acid. The solution was evaporated to dryness over phosphorus pentoxide and sodium hydroxide pellets and the residue extracted with methanol; the methanol solution was dried and extracted several times with boiling acetone, the acetone solution reduced in volume and left in the cold to crystallize. After recrystallization from acetone the dideoxy-hexitol gave $[\alpha]_D = +38 \pm 3°$ (in methanol) and had an m.p. of 112–113°. This is clearly the optical isomer of tyvelitol and the *P. pseudotuberculosis* type V sugar is ascarylose.

For polysaccharides of *P. pseudotuberculosis* types I and III, chromatograms indicated that the dideoxy-sugars differed from abequose and tyvelose (or their optical isomers colitose and ascarylose), and migrated in the position of paratose (3,6-dideoxy-D-*ribo*-hexose)[9]. The chromatographic separation of these sugars differed from abequose and tyvelose (or their separation could be obtained on paper electrophoresis in borate buffer[10]. In this system the sugars could be separated completely from one another in a 2-hr. run, with R_{rhamnose} values of 1·03 for abequose/colitose, 1·22 for tyvelose/ascarylose and 1·38 for paratose. The types I and III dideoxy-sugars gave R_{rhamnose} 1·38 but the different specificities of the polysaccharides required a demonstration of whether the sugars were indeed identical or again a pair of optical isomers.

The sugars were isolated as described above. The specific rotation of paratose is only +10° and the sugar syrups both gave positive rotations; with the amount of material available, however, the absolute readings were too small to be convincing. From the solutions, dideoxy-hexitols were prepared by reduction with $NaBH_4$ as described above, and in both cases the hexitols gave $[\alpha]_D$ approx. −20° (paratitol $[\alpha]_D$ −18°). It is clear, therefore, that paratose is present in both type I and III polysaccharides and is presumably linked in a different way in each instance to give the different specificities.

The situation is thus, that all the known 3,6-dideoxy-hexoses occur in bacterial polysaccharides[11] and these include all the configurations except the L-*ribo*- and D- and L-*lyxo*- forms.

[1] Davies, D. A. L., *J. Gen. Microbiol.*, **18**, 118 (1958).

[2] Westphal, O., *Ann. Inst. Pasteur*, **98**, 789 (1960).

[3] Fouquey, C., Polonsky, J., and Lederer, E., *Bull. Soc. Chim. Biol.*, **40**, 315 (1958).

[4] Schütze, H., *Arch. Hyg.*, **100**, 181 (1928).

[5] Staub, A. M., Tinelli, R., Lüderitz, O., and Westphal, O., *Ann. Inst. Pasteur*, **96**, 303 (1959).

[6] Davies, D. A. L., Crumpton, M. J., Macpherson, I. A., and Hutchison, A. M., *Immunology*, **1**, 157 (1958).

[7] Knapp, W., *Zent. Bakt., I Orig.*, **164**, 57 (1955); *Z. Hyg.*, **146**, 315 (1960).

[8] Staub, A. M., and Tinelli, R., *Bull. Soc. Chim. Biol.*, **39**, Supp. 1, 65 (1957).

[9] Davies, D. A. L., Staub, A. M., Fromme, I., Lüderitz, O., and Westphal, O., *Nature*, **181**, 822 (1958).

[10] Foster, A. B., *Chem. Indust.*, 1050 (1952).

[11] Davies, D. A. L., *Adv. Carbohydrate Chem.*, **15**, 271 (1960).

SIALIC ACIDS AND THE ELECTROKINETIC CHARGE OF THE HUMAN ERYTHROCYTE

By G. M. W. COOK, Dr. D. H. HEARD and Dr. G. V. F. SEAMAN*

Department of Radiotherapeutics, University of Cambridge

IT has long been recognized that strongly acidic groups are responsible for the net negative charge of the erythrocyte. Furchgott and Ponder[1] suggested that the charge was derived from a phospholipid, possibly cephalin. Winkler and Bungenberg de Jong[2] came to similar conclusions from charge reversal spectra work.

Recent evidence indicates that the electrokinetic behaviour of the erythrocyte may derive from mucosubstances. Hirst[3] suggested, from erythrocyte virus adsorption and elution studies, that the sites of adsorption may have a mucoprotein character. Hanig[4] showed that adsorption of virus with concomitant agglutination of the red cells followed by elution of the virus was associated with a very marked decrease in the microelectrophoretic mobility of the red cells. Stone and Ada[5] examined more fully the relationship between changes in the microelectrophoretic mobility of red cells and their agglutination after treatment with viral enzymes and discussed the changes in terms of the receptor gradient of Burnet[6].

The specificity of an enzyme derived from *Vibrio cholerae* cultures (receptor-destroying enzyme) was examined by Gottschalk and Lind[7], and afterwards recognized as a glycosidase by Gottschalk[8] and termed 'neuraminidase' by him[9] because of its ability to liberate acylated neuraminic acids by cleavage of an α-glycosidic linkage[10].

Klenk and Uhlenbruck[11] have found that the receptor-destroying enzyme will liberate acylated neuraminic acids from erythrocyte stroma of various mammalian species. Furthermore, Klenk and Lempfrid[12] found that the sialic acid liberated by the receptor-destroying enzyme from human erythrocyte stroma was N-acetylneuraminic acid (NANA). Klenk[13] suggested that the negative charge of the erythrocyte could be ascribed to an acylated neuraminic acid.

Trypsin releases a sialomucopeptide[14] which is thought to be related to the decrease in the electrophoretic mobility of the treated erythrocyte[15,16].

---

* Beit Memorial Research Fellow.

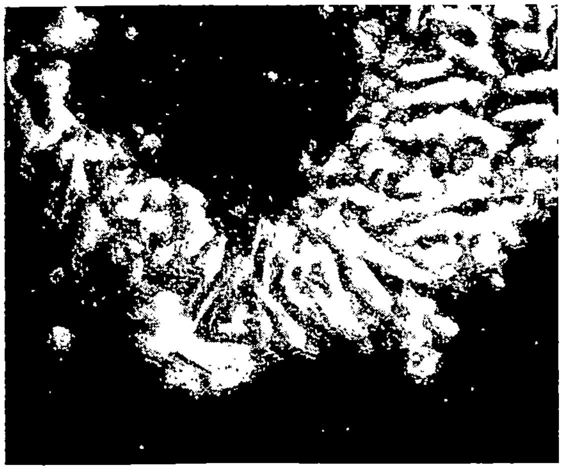


Fig. 1. Photograph of sialic acid crystallized from the supernatant of erythrocytes treated with receptor-destroying enzyme using methanol/diethyl ether/light petroleum mixture. Dark ground illumination. (× *c.* 150)

It is shown here that the decrease in erythrocyte charge observed after treatment by crystalline neuraminidase is brought about by the release of an N or O,N-acetylated neuraminic acid, the carboxyl group of which is in consequence removed from the electrophoretic plane of shear. The reduction in the electrokinetic charge of the erythrocyte is shown to be essentially a loss of anionic groups rather than the generation of a significant proportion of cationic groups.

Standard washed human erythrocytes[16] phenotypes (*ARh*+ve, *ORh*+ve and −ve) were used in the experimental work. Packed red cells (about 1 c.c. per experiment) were treated with receptor-destroying enzyme prepared according to the method of Ada and French[17]. The receptor-destroying enzyme solution 1 vol. (activity 1,500 units per ml. [1 mgm. receptor-destroying enzyme protein = 10^7 units] in 0·145 *M* aqueous sodium chloride : 0·005 *M* aqueous calcium chloride buffered to *p*H 7 with 0·5 *M* aqueous sodium bicarbonate) was incubated for 1 hr. at 37° with 1 vol. of human erythrocytes (packed 2,000*g* for 15 min.). The erythrocyte suspensions treated with receptor-destroying enzyme were centrifuged (2,000*g* for 10 min.) and the supernatant fluids removed for analysis.

The receptor-destroying enzyme-treated and the appropriate control red cells were given a standard wash in 0·145 *M* aqueous sodium chloride[16] and the treated red cells examined by microelectrophoresis[18]. Samples of standard washed red cells subjected to the receptor-destroying enzyme were treated with a 2 per cent v/v acetaldehyde solution in 0·145 *M* aqueous sodium chloride buffered to *p*H 7·5 with 0·5 *M* aqueous sodium bicarbonate solution[19]. The receptor-destroying enzyme/acetaldehyde-treated red cells were stored for not less than twenty days, given a standard wash in 0·145 *M* aqueous sodium chloride solution and then examined by microelectrophoresis. The *p*H of the 0·145 *M* aqueous sodium chloride solution was adjusted by means of isotonic aqueous hydrochloric acid or sodium hydroxide.

The supernatant fluid from the red cells treated with receptor-destroying enzyme was deproteinized by the addition of 1 vol. of 10 per cent w/v aqueous trichloroacetic acid solution to 1 vol. of supernatant fluid. After flocculation and centrifugation (2,000*g* for 10 min., 4° C.) the clear supernatant fluid was assayed for sialic acids by the resorcinol-hydrochloric acid method of Svennerholm[20] using NANA as a standard. Sialic acids found in the control supernatant fluids were negligible.

The sialic acid released was characterized microscopically by its crystalline form and by paper chromatography. The supernatant fluid from 2-c.c. packed erythrocytes treated with receptor-destroying enzyme was deproteinized with trichloroacetic acid, filtered through a Whatman No. 50 paper and the clear filtrate extracted with diethyl ether. The aqueous phase was not desalted so as to avoid unnecessary loss of any labile sialic acids, but was concentrated directly under reduced pressure at 50° C. and an equal volume of methanol added. The sialic acid was crystallized by adding a few drops of diethyl ether : light petroleum (60–80° C.) (1 : 2 by vol.) mixture and leaving at 4° C. for 24 hr. Clusters of fluffy needle-like crystals (about 200 μgm.) were obtained (Fig. 1). The mixture from which sialic acids could be crystallized was examined by descending paper chromatography (Whatman No. 1 paper) using: (*a*) butan-1-ol : acetic acid : water (4 : 1 : 5), organic phase; (*b*) butan-2-ol : acetic acid : water (4 : 1 : 5). The air-dried chromatograms were dipped in orcinol–trichloroacetic reagent (3 : 5-dihydroxytoluene (0·5 gm.) trichloroacetic acid (15 gm.) dissolved in water-saturated butan-1-ol (100 ml.)) and developed at 105° C. The chromatographic behaviour of NANA (in solvent *b*) was examined in the presence of the same quantity of sodium chloride as in the experimental samples (Fig. 2).

It has been suggested[21] that the decrease in the electrophoretic mobility of erythrocytes after treat-

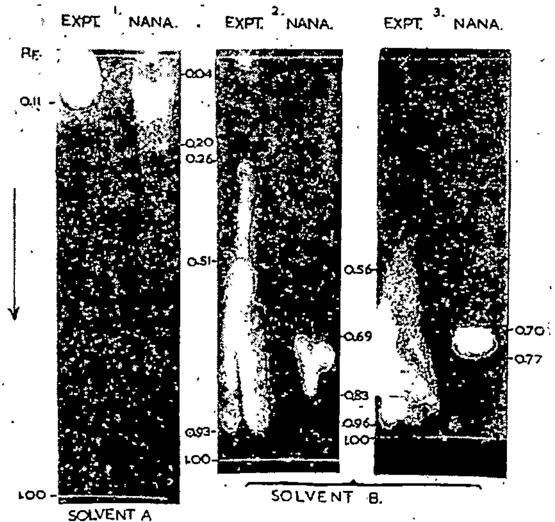


Fig. 2. Photostat copies of chromatograms developed with orcinol/trichloroacetic acid reagent

(1) Solvent *A* (butan-1-ol: acetic acid: water (4 : 1 : 5) descending); material released from erythrocytes by the receptor-destroying enzyme (R_F 0·11); N-acetylneuraminic acid (50 μgm.) (R_F 0·04–0·20)

(2) Solvent *B* (butan-2-ol: acetic acid: water (4 : 1 : 5) descending); material released from erythrocytes by receptor-destroying enzyme (R_F 0·26–0·51) (0·51–0·93); N-acetylneuraminic acid (50 μgm.) (R_F 0·69–0·83)

(3) Solvent *B*. N-acetylneuraminic acid (50 μgm.) and sodium chloride (12 mgm. equiv. to sodium chloride present in Exp. 2 above). (R_F 0·56–0·96); N-acetylneuraminic acid (50 μgm.) (R_F 0·70–0·77)

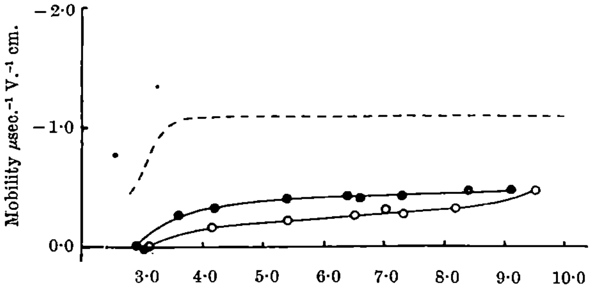


Fig. 3. *p*H-mobility relationships for untreated, receptor-destroying enzyme-treated, and receptor-destroying enzyme/acetaldehyde-treated red cells in 0·145 *M* aqueous sodium chloride at 25° C. – – –, untreated cells; ○, red cells treated with receptor-destroying enzyme; ●, red cells treated with receptor-destroying enzyme/acetaldehyde

ment with the *PR*8 strain of influenza virus, results from the appearance of cationic groups. The titration studies of Curtain[22] on urinary inhibitor, which showed that treatment of this inhibitor with receptor-destroying enzyme yielded a substance possessing cationic groups of about *p*K 11, supported this view. Recent work on the specificity of neuraminidases by Gottschalk[8] has shown that the receptor-destroying enzyme is an α-glycosidase, and therefore that its action is unlikely to yield cationic groups.

Aldehyde treatment of normal human erythrocytes produces no detectable change in their electrophoretic behaviour[19], which from the specificity of aldehydes for potential cationic groups[23] suggests the absence of cationic groupings effective at the electrophoretic slip plane[19]. The relatively small change in mobility of human erythrocytes subjected to receptor-destroying enzyme after treatment with acetaldehyde, in conjunction with constancy of the isopotential *p*H (Fig. 3) indicates that only a small part of the reduction in charge of the red cell treated with receptor-destroying enzyme could be attributed to cationic groups.

The possibility that the changes in electrokinetic behaviour of the red cell observed after treatment with receptor-destroying enzyme could be brought about by the irreversible adsorption of the enzyme or by impurities in the receptor-destroying enzyme is considered to be unlikely for the following reasons: (1) Purification of the crystalline receptor-destroying enzyme normally involves its adsorption and facile elution from packed washed human erythrocytes. (2) The receptor-destroying enzyme has been shown from hæmagglutination studies[24] to be eluted from red cells and the red cells to be incapable of further adsorption of enzyme[24]. (3) There is not likely to be sufficient material present as an impurity in the receptor-destroying enzyme (total protein 0·1 μgm./ml.) to produce significant coverage of 10^{10} red cells.

The *p*H-mobility relationships in 0·145 *M* aqueous sodium chloride for normal, receptor-destroying enzyme treated and receptor-destroying enzyme/acetaldehyde treated human red cells given in Fig. 3 show: (1) For the normal red cell the *pKa* of the surface ionogenic groups is at about 2·8 (the *pKa* of NANA is 2·6 (ref. 25)). (2) For the receptor-destroying enzyme/acetaldehyde-treated red cells there is only a small increase in the mobility compared with the corresponding red cells treated with receptor-destroying enzyme. The cationic character of the treated red cell is too small to account for the reduction in mobility of the normal red cell on treatment with receptor-destroying enzyme; these observations are consistent with those of Stone and Ada[5].

Assuming the sialic acid released by the action of the receptor-destroying enzyme to be free NANA[12] of mol. wt. 309, and when bound in the membrane of the erythrocyte that its carboxyl group is effective at the electrophoretic plane of shear, the theoretical quantity of a sialic acid expected to be released per 10^{10} human erythrocytes for a given reduction in surface charge density was calculated. The carboxyl group of each sialic acid moiety when bound in the red-cell membrane was assumed to correspond to one electron charge on the surface of the erythrocyte. The red-cell charge densities were calculated from the Gouy–Chapman equation which at 25° C. for uni-univalent electrolytes reduces to:

$$\sigma = 3{\cdot}52 \times 10^4 . I^{1/2} . \sinh(\zeta/51{\cdot}3)$$

where σ is the charge density in e.s.u. cm.$^{-2}$, *I* is the ionic strength[26] and ζ the zeta potential in mV. The

Table 1

Sub-ject	Pheno-type	Experi-ment	Total No. of erythro-cytes per experi-ment	Erythrocyte		Reduction in charge density $\Delta\sigma$ e.s.u. cm.$^{-2}$	No. of electrons lost per 10^{10} erythro-cytes	Theor-etical yield of NANA per 10^{10} erythro-cytes (μgm.)	Experi-mental yield of NANA per 10^{10} erythro-cytes (μgm.)	Ratio experi-mental/theor-etical yield of NANA	Remarks
				Mobility μsec.$^{-1}$ V.$^{-1}$ cm.	Charge density σ e.s.u. cm.$^{-2}$						
1	*ARh*+ve	R.D.E.	1×10^{10}	−0·38	1,276	2,404	$8{\cdot}15 \times 10^{16}$	41·8	89·2	2·13	Red cell counts and hæmatocrits performed in duplicate on each system using standard techniques
		Control	1×10^{10}	−1·08	3,680	—	—	—	—	—	
2	*ORh*+ve	R.D.E.	$1{\cdot}2 \times 10^{10}$	−0·39	1,311	2,369	$8{\cdot}03 \times 10^{16}$	41·2	81·9	1·98	
		Control	$1{\cdot}2 \times 10^{10}$	−1·08	3,680	—	—	—	—	—	
3	*ORh*+ve	R.D.E.	1×10^{10}	−0·82	2,753	927	$3{\cdot}14 \times 10^{16}$	16·1	42·8	2·66	
		Control	1×10^{10}	−1·08	3,680	—	—	—	—	—	
4*a*	*ORh*−ve	R.D.E.	$8{\cdot}4 \times 10^{9}$	−0·32	1,074	2,606	$8{\cdot}83 \times 10^{16}$	45·3	92·8	2·05	
		Control	$8{\cdot}4 \times 10^{8}$	−1·08	3,680	—	—	—	—	—	See below
4*b*	*ORh*−ve	R.D.E. 1	$1{\cdot}2 \times 10^{10}$	−0·41	1,377	2,303	$7{\cdot}81 \times 10^{16}$	40·0	73·2	1·83	Repeat experiment on same subject, 18 days interval between 4*a* and 4*b*
		2	$1{\cdot}2 \times 10^{10}$	−0·48	1,611	2,069	$7{\cdot}01 \times 10^{16}$	36·0	68·0	1·89	
		Control	$1{\cdot}2 \times 10^{10}$	−1·08	3,680	—	—	—	—	—	
5	*ORh*+ve	R.D.E. 1	$6{\cdot}85 \times 10^{9}$	−0·38	1,276	2,404	$8{\cdot}15 \times 10^{16}$	41·8	108·1	2·59	
		2	$6{\cdot}85 \times 10^{9}$	−0·38	1,276	2,404	$8{\cdot}15 \times 10^{16}$	41·8	96·2	2·30	
		Control	$6{\cdot}85 \times 10^{9}$	−1·08	3,680	—	—	—	—	—	

R.D.E., receptor-destroying enzyme.

2·18
Mean ratio exp./theor.
yield of NANA

Gouy–Chapman equation may yield low charge densities for an ion-penetrable system, such as a red cell. Haydon[27] has pointed out that for such a system the following equation would be more likely to yield a reliable value for σ:

$$\sigma = (1 + \sqrt{1 - \alpha})\, 3{\cdot}52 \times 10^4 . I^{1/2} . \sinh(\zeta/51{\cdot}3)$$

where α is the fraction of the total space within the surface which is not available for counter ions. For $\alpha = 1$ the Gouy–Chapman equation is obeyed; for $\alpha = 0$ the actual surface charge density will be twice that found from the Gouy–Chapman equation. At present, in the case of a red cell, only an arbitrary value can be ascribed to α.

The experimental yields of sialic acids are given in Table 1 and are about twice those expected from the Gouy-Chapman theory. Even though the overall accuracy of the experimental results is unlikely to be better than about ± 20 per cent, because of the error involved in red cell counts and the unavoidable slight hæmolysis during the treatment with receptor-destroying enzyme, not all the variation between the experimental results is likely to be accounted for by this error. The Haydon factor α is unlikely to account completely for the discrepancy between the theoretically calculated yields of sialic acid and those found experimentally. The divergence between the theoretical and experimental figures indicates that the sites of neuraminidase action may not be sterically localized in the regions of the cell surface where the carboxyl group of the sialic acids are electrokinetically fully effective. It is therefore probable that some sialic acid has arisen from regions deep to the electrophoretic plane of shear and that this portion of the total sialic acid liberated by the receptor-destroying enzyme may depend on stereospecific factors and therefore be liable to variation from person to person, thus possibly accounting for the variation in the experimental results.

The chromatographic behaviour of the material released from the red cell by treatment with receptor-destroying enzyme (Fig. 2) is indicative of NANA with possibly some O,N-acetylated neuraminic acids present; the NANA could have originated from the decomposition of a labile O,N-acetylated neuraminic acid.

The results described in this article indicate that the dominant ionogenic group at the surface of the human erythrocyte is the carboxyl group of a bound N or O,N-acetylated neuraminic acid.

We wish to thank the Medical Research Council for financial assistance; Dr. G. Ada for samples of crystalline receptor-destroying enzyme; Prof. G. Blix, Mr. M. J. Barnes, Prof. E. Klenk and Dr. L. Svennerholm for samples of various sialic acids; Prof. W. T. J. Morgan for a sample of sialo-mucopolysaccharide and Mr. E. W. Mitchell for the photography.

[1] Furchgott, R. F., and Ponder, E., *J. Gen. Physiol.*, **24**, 447 (1941).

[2] Winkler, K. C., and Bungenberg de Jong, H. G., *Arch. Neerl. Physiol.*, **25**, 431, 467 (1940–41).

[3] Hirst, G. K., *J. Exp. Med.*, **87**, 301 (1948).

[4] Hanig, M., *Proc. Soc. Exp. Biol. Med.*, **68**, 385 (1948).

[5] Stone, J. D., and Ada, G. L., *Brit. J. Exp. Path.*, **33**, 428 (1952).

[6] Burnet, F. M., *Austral. J. Exp. Biol. Med. Sci.*, **20**, 81 (1942).

[7] Gottschalk, A., and Lind, P. E., *Nature*, **164**, 232 (1949).

[8] Gottschalk, A., *Biochim. Biophys. Acta*, **20**, 560 (1956).

[9] Gottschalk, A., *Biochim. Biophys. Acta*, **23**, 645 (1957).

[10] Blix, G., *Chemistry and Biology of Mucopolysaccharides*, 304 (A Ciba Foundation Symposium; J. and A. Churchill, Ltd., 1958).

[11] Klenk, E., and Uhlenbruck, G., *Hoppe-Seyl. Z.*, **311**, 227 (1958).

[12] Klenk, E., and Lempfrid, H., *Hoppe-Seyl. Z.*, **307**, 278 (1957).

[13] Klenk, E., *Chemistry and Biology of Mucopolysaccharides*, 311 (A Ciba Foundation Symposium; J. and A. Churchill, Ltd., 1958).

[14] Cook, G. M. W., Heard, D. H., and Seaman, G. V. F., *Nature*, **188**, 1011 (1960).

[15] Ponder, E., *Blood*, **6**, 350 (1951).

[16] Seaman, G. V. F., and Heard, D. H., *J. Gen. Physiol.*, **44**, 251 (1960).

[17] Ada, G. L., and French, E. L., *Nature*, **183**, 1740 (1959).

[18] Bangham, A. D., Flemans, R., Heard, D. H., and Seaman, G. V. F., *Nature*, **182**, 642 (1958).

[19] Heard, D. H., and Seaman, G. V. F. (in preparation).

[20] Svennerholm, L., *Biochim. Biophys. Acta*, **24**, 604 (1957).

[21] Bateman, J. B., Zellner, A., Davis, M. S., and McCaffrey, P. A., *Arch. Biochem. Biophys.*, **60**, 384 (1956).

[22] Curtain, C. C., *Austral. J. Exp. Biol. Med. Sci.*, **31**, 623 (1953).

[23] French, D., and Edsall, J. T., *Adv. Protein Chem.*, **2**, 277 (1945).

[24] Buzzell, A., and Hanig, M., *Adv. Virus Res.*, **5**, 289 (1958).

[25] Gottschalk, A., *The Chemistry and Biology of Sialic Acids and Related Substances*, 26 (Camb. Univ. Press, 1960).

[26] Lewis, G. N., and Randall, M., *J. Amer. Chem. Soc.*, **43**, 1112 (1921).

[27] Haydon, D., *Biochim. Biophys. Acta* (in the press).

PARTITION OF URINE NITROGEN IN KWASHIORKOR

By Dr. J. C. EDOZIEN and E. J. PHILLIPS

Department of Chemical Pathology, University College, Ibadan

It has been known for many years that the level of dietary protein intake greatly affects the partition of nitrogen in the urine[1]. In particular, low protein diets are associated with reduced relative excretion of urea nitrogen and with increased relative amounts of ammonia and of "undetermined nitrogen"[1,2]. Recent investigations[3] have shown that there is an increase in the excretion of free amino-acids in severe protein deficiency in children (kwashiorkor), from which it may be inferred that part of the increase in the "undetermined nitrogen" of urine in subjects on poor protein diet is caused by increased amounts of free amino-acid nitrogen. During investigation of the excretion of free amino-acids in kwashiorkor, we observed that in many of our patients the formol titration method of determining amino-acids in urine showed a much higher degree of amino-aciduria than is evident from chromatograms of the free amino-acids of the urines. It was suspected that there may be an increased excretion of other urine constituents which are estimated as 'amino-acids' by the formol titration procedure. We therefore examined the amino-acids of hydrolysed urines and we present here a preliminary report on observations on the partition of urine nitrogen and the excretion of bound amino-acids in kwashiorkor.

Urine ammonia was estimated by direct nesslerization of diluted urine: the results do not differ significantly from values obtained by the microdiffusion method of Conway[4]. Urea plus ammonia was determined by nesslerization after incubating with urease. Urea was obtained by difference. Total nitrogen was determined by the micro-Kjeldahl method. Creatinine, and creatinine plus creatine, were measured by the alkaline picrate method, before and after boiling with picric acid[5]. Creatine was obtained by difference. Uric acid was determined by the uricase method[6] and amino-acid nitrogen by formol titration with subtraction of ammonia nitrogen[7]. Two-dimensional chromatography was carried out in butanol/pyridine/water and phenol/ammonia/water according to the procedure described by Smith[8]. For free amino-acids, electrolytically desalted urine was applied to the paper, aliquots containing 250 μgm. nitrogen being used for normal urines. Free plus bound amino-acids were examined after the urine was hydrolysed by refluxing 1 ml. of protein-free urine with 9 ml. of 6 *N* hydrochloric acid for 24 hr., removing the hydrochloric acid by evaporating to dryness and re-dissolving the residue in 4 ml. water and desalting. A four-fold dilution of the original urine was thus made during this process. The excretion of bound amino-acids was assessed by noting qualitative and quantitative changes in the chromatogram of the hydrolysed urine as compared with that of unhydrolysed urine containing an equivalent amount of total nitrogen.

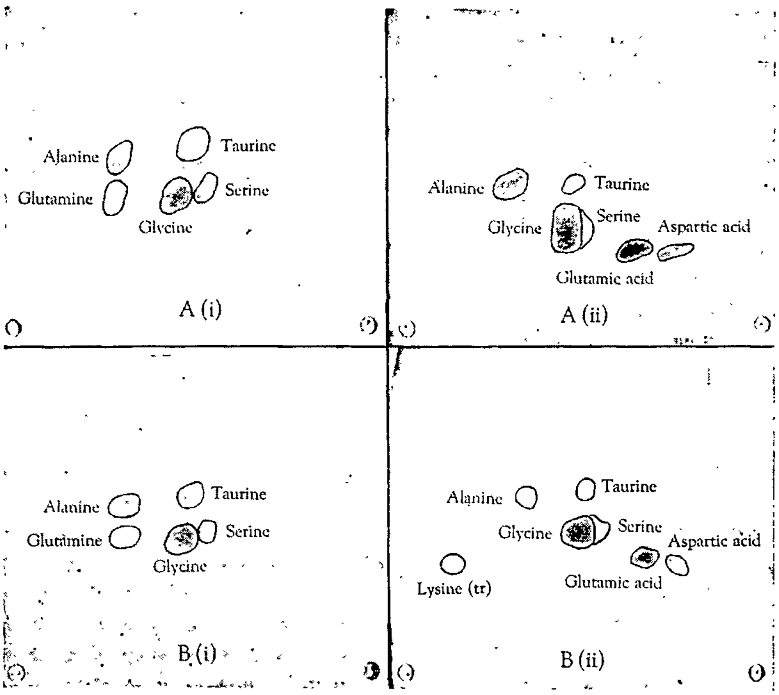


Fig. 1. Amino-acid excretion in healthy children
A (i) and *B* (i), free amino-acids in urine.
A (ii) and *B* (ii), free plus bound amino-acids after acid hydrolysis. All samples contain 250 μgm. nitrogen.

The partition of urine nitrogen in healthy Nigerian children presumed to be on normal protein diets, in healthy Nigerian children presumed to eat low protein diets and in children with kwashiorkor are shown in Table 1. The results confirm earlier observations that in kwashiorkor there is an increase in the relative amounts of ammonia and of amino-acids. They also show that there is an increase in the relative excretion of creatine and of uric acid. It should be emphasized, however, that the increases are in relative amounts, and do not necessarily indicate increased daily output. In kwashiorkor, urine volume is suppressed to about half to one-third of normal, and since the concentration of total nitrogen is also reduced to about a half, only those substances which show an increase in relative amounts of more than approximately four times are likely to show increase in absolute amount per 24 hr.

The relative excretion of urea is very significantly less than normal: there is very little change in the relative concentration of creatinine. The absolute amounts of these two substances per 24 hr. are very much below normal.

Table 1. PARTITION OF NITROGEN IN URINE IN HEALTHY CONTROLS AND IN KWASHIORKOR
Values expressed as percentage of total nitrogen, unless stated otherwise

	Controls: *A* (12)	Controls: *B* (13)	Kwashiorkor (25)
Total nitrogen (gm./litre)	8·0	7·0	3·8
Ammonia	4·5	7·5	20·2
Urea	79·5	54·9	41·2
Creatinine	4·1	3·4	3·8
Creatine	0·2	—	2·5
'Uric acid'	3·5	—	6·8
'Amino-acids'	3·2	—	25·3

Controls *A*: Healthy children, being children of African members of the academic staff of the University College and presumed to eat normal protein diets.
Controls *B*: Healthy children from Imesi, a village about 100 miles from Ibadan and presumed to eat the traditional low-protein diet.

Fig. 1 shows photographs of chromatograms of free and total amino-acids in two healthy children on normal protein diets. Serine, glycine, taurine, alanine and histidine (sometimes methyl histidine) are usually present, but essential amino-acids (leucine plus *iso*leucine plus phenylalanine in one spot, valine plus methionine plus tryptophan in one spot, arginine, lysine, tyrosine, tryptophan) and other spots (asparagine, ornithine) are occasionally observed, though when present they are excreted in only trace amounts. The hydrolysed urines usually show an increase in the intensity of glycine and alanine and the appearance of aspartic acid and glutamic acid. Glutamine is destroyed in the process. Traces of other amino-acids may appear in some cases.

Kwashiorkor urines usually show varying degrees of amino-aciduria (Fig. 2). Chromatograms of free amino-acids comparable with those for normal children can usually be produced by spotting a volume of urine containing 2–15 (usually 4) times less nitrogen than we apply in the case of normal urines, indicating that there is an approximately four-fold increase in the proportion of free amino-acid nitrogen to total nitrogen in the urine of kwashiorkor patients. Essential amino-acids are not involved in this increase: in the majority of cases essential amino-acids (with the exception of lysine) could not be identified in chromatograms of the urine. The almost invariable over-excretion of β-amino*iso*butyric acid and the appearance of ethanolamine in several cases are other features of the amino-aciduria of kwashiorkor. Bound amino-acids also show considerable increase. The increase in bound amino-acids in most cases appears to be more than the increase in the excretion of free amino-acids. Clinical recovery is associated with

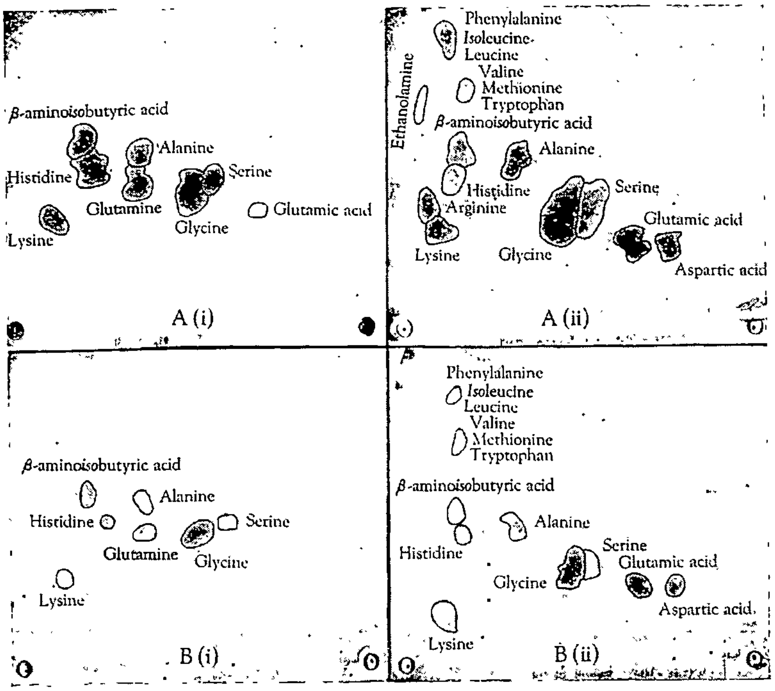


Fig. 2. Amino-acid excretion in a child with kwashiorkor

A (i), free amino-acids, and *A* (ii), free plus bound amino-acids, after acid hydrolysis. Urine spotted contained 250 μgm. nitrogen.

B (i), free amino-acids, and *B* (ii), free plus bound amino-acids, after acid hydrolysis. Urine spotted contained 62·5 μgm. nitrogen.

When this is compared with Fig. 1, it shows that there was more than a four-fold increase in both free and bound amino-acids.

return of the excretion of free and total amino-acids to normal, but over-excretion of β-amino*iso*butyric acid may persist for some time.

Creatine, creatinine and uric acid were also determined in blood serum. The results are shown in Table 2.

Table 2. Serum Concentration in mgm./100 ml.

Determination	Control children (30)	Kwashiorkor (25)
Creatine	1·41 ± 0·38 (0·8 – 2·3)	1·50 ± 0·79 (0·4 – 3·6)
Creatinine	0·75 ± 0·18 (0·5 – 1·2)	0·60 ± 0·23 (0·4 – 1·0)
'Uric acid'	3·95 ± 1·3 (1·6 – 6·3) In only 1/30 serum-level > 6·0 mgm./100 ml.	8·5 ± 4·3 (1·3 – 23·3) In 17/25 serum-level > 6·0 mgm./100 ml.

The increased output of ammonia is probably the result of increased excretion of organic acids[9] and of failure of the kidneys to produce acid urine[10]. Dean[11] has suggested that the excretion of large amounts of ammonia may show that ammonia is being produced that cannot be carried to urea because the xanthine–citrulline–arginine cycle is defective: it should be pointed out, however, that normally urinary ammonia is not derived from blood ammonia, but is produced in the kidneys as a defensive mechanism for body cation. The creatinuria is associated with increased breakdown of muscle. The fall in total creatinine excretion is probably due to the diminished mass of muscle; decreased renal clearance of creatinine has been postulated as an additional factor, but, as our results show that there is no increase in creatinine-level in plasma, we do not consider this likely. The etiology of the amino-aciduria of kwashiorkor has been discussed in a previous communication[3], in which it was also shown that ethanolaminuria is caused by deficiency of methyl donors.

The daily urinary excretion of uric acid is not a very sensitive index of the total uric acid turnover, because extra-renal excretion eliminates a considerable portion of the uric acid produced each day[12]. The level of uric acid in the plasma is a more reliable index, and the substantial rise in serum uric acid observed in kwashiorkor points clearly, in the absence of severe renal damage, to over-production of uric acid in this disease. Dean[11] has observed that in kwashiorkor the urine contains increased quantities of purine metabolites other than uric acid; these purines contribute to the urine as much nitrogen as uric acid, and thus account for part of the "undetermined nitrogen". We have been able to account for practically all the "undetermined nitrogen" without allowing for these purines; it may well be that uricase is not specific for uric acid and that part of the increase which we have noted is due to these abnormal purines. The accumulation of purine metabolites points, like the over-production of β-amino*iso*butyric acid, to rapid cellular breakdown.

The bound amino-acids present in urine include peptides and amino-acids conjugated with other urine constituents. It can readily be demonstrated by treating, with biuret reagent, urine which has been de-colorized with activated charcoal and deproteinized with zinc sulphate and sodium hydroxide, that much of the increase in the bound amino-acids of kwashiorkor urine is due to increased peptide excretion. It is not yet clear whether this peptiduria is due to rapid tissue breakdown, or whether it is associated with failure of protein synthesis caused either by enzyme deficiencies or by deficiency of key amino-acids required to link the peptide chains together. Some of these peptides may be biologically active and may relate to the retention of fluid and to the circulatory collapse which sometimes supervenes in the course of severe kwashiorkor.

[1] Folin, O., *J. Amer. Med. Assoc.*, 69, 1209 (1917).

[2] Platt, B. S., and Heard, C. R. C., *Proc. Nutrition Soc.*, 17, ii (1958).

[3] (*a*) Schendel, H. W., Antonis, A., and Hansen, J. D. L., *Pediat.*, 23, 662 (1959). (*b*) Edozien, J. C., Phillips, E. J., and Collis, W. R. F., *Lancet*, i, 615 (1960). (*c*) Vis, H., Dubois, R., Loeb, H., Vincent, M., and Bigwood, E. J., *Ann. Soc. Belge Med. Trop.*, 38, 991 (1958). (*d*) Dubois, R., Vis, H., Loeb, H., and Vincent, M., *Helv. Paediat. Acta*, 14, 13 (1959).

[4] Conway, E. J., *Microdiffusion Analysis and Volumetric Error* (Crosby, Lockwood and Son, Ltd., London, fourth ed., 1957).

[5] Bonsnes, R. W., Taussky, H. H., *J. Biol. Chem.*, 158, 581 (1945).

[6] Freichtmeir, T. V., and Wrenn, H. T., *Amer. J. Clin. Path.*, 25, 833 (1955).

[7] King, E. J., and Wooton, I. D. P., *Microanalysis in Medical Biochemistry*, 159 (J. and A. Churchill, Ltd., London, 1956).

[8] Smith, I., *Chromatographic Technique* (William Heinemann, Ltd., London, 1958).

[9] Edozien, J. C., *Nature*, 184, 1150 (1959).

[10] Smith, R., *Lancet*, i, 764 (1959).

[11] Dean, R. F. A., Panel IV. Fifth Int. Cong. Nutrition, 39 (Washington, 1960).

[12] Sorenson, L. B., *Metabolism*, 8, 687 (1959).

CHANGES IN VISUAL GNOSTIC FUNCTIONS AND EMOTIONAL BEHAVIOUR FOLLOWING UNILATERAL TEMPORAL POLE DAMAGE IN THE 'SPLIT-BRAIN' MONKEY

By DR. J. L. deC. DOWNER

Scientific Staff, Medical Research Council, Department of Anatomy, University College, London

DURING the course of investigating inter-hemispheric transfer of visual discrimination habits in monkey (*Macaca mulatta*), it was found in three animals that transfer of monocularly learned visual discrimination habits did not occur following mid-sagittal division of optic chiasma, corpus callosum, anterior commissure and that part of the hippocampal commissure lying subjacent to the corpus callosum[1,2]. Transfer of these habits did occur in a fourth animal in which the anterior commissure remained intact although optic chiasma, corpus callosum and hippocampal commissure had been sectioned. Since the anterior commissure is the major commissure between the temporal poles[3–5,7] these observations suggested that the temporal poles might play some part in the transfer of visual gnostic behaviour.

A right unilateral temporal pole resection was carried out in an animal (*D*-26) that had previously completed a visual discrimination training sequence following mid-sagittal sectioning of optic chiasma and cerebral commissures. Following a recovery period of two weeks duration, the eye ipsilateral to the temporal pole lesion was left open and the opposite eyelids sutured shut. There appeared immediately many of the behavioural patterns described by Kluver and Bucy[6,7] in animals with bilateral temporal pole removals. The animal seemed unable to recognize edible from non-edible objects by visual cues alone; it would pick up the objects and spend considerable time sniffing and biting before either eating or rejecting them. The post-operative changes in affective behaviour were of especial interest. Prior to the temporal pole removal this animal was wild and intractable. When observers looked into its cage the animal would bare its teeth and dash forward, jumping at the door and attempting to bite and claw. Following the temporal pole removal the animal responded placidly to such visual stimulation and would approach the experimenter peacefully and take raisins from his hand. It is important to note that this placid behaviour occurred only in response to visual stimuli. If one attempted to touch the animal or grasp its arm, a full aggressive reaction would occur.

Opening the eye projecting to the undamaged side and closing the other eye resulted in an abrupt change in behaviour back to the pre-operative level. There were no signs of visual agnosia, and the animal showed a persistent aggressive response at the appearance of the experimenter.

The question then arose as to whether or not the change in emotional behaviour to visual stimulation was merely a reflexion of the visual agnosia or due to damage to sub-cortical structures, in particular the amygdaloid complex, which had been removed during the unilateral temporal pole ablation. In an attempt to answer this, another animal (*D*-28) that had previously undergone mid-sagittal sectioning of optic chiasma and cerebral commissures was subjected to unilateral amygdalectomy. To avoid damaging the temporal neocortex, the amygdaloid area was approached from beneath the right frontal pole and removed using the aspiration technique. During the course of surgery, it was found necessary to section the right olfactory tract in order to obtain adequate exposure. Post-operative recovery was uneventful.

This animal showed no signs of visual agnosia irrespective of which eye was open. Similarly, there did not appear to be much change in affective behaviour although, superficially, it seemed as if the animal was a little more placid when the eye ipsilateral to the side of the amygdalectomy alone was open. Another craniotomy was then carried out. The left hemisphere was gently retracted from the falx, and the incision along the length of the corpus callosum was reopened. In addition, the hippocampal, habenular and posterior commissures were completely sectioned. The incision was extended to include the massa intermedia. The supra-optic recess was entered, and the tip of the knife passed between the medial walls of the hypothalamus until the sphenoid bone was reached. Post-operative recovery revealed a transient left abducens nerve palsy and a mild but persistent right hemiparesis.

Following the second operation, this animal did not differ from the typical wild and aggressive monkey when observed with both of its eyes open. At the sight of an observer, it would grimace, bare its teeth and jump around the cage. Closing the eye on the side of the amygdalectomy did not result in any observable change. However, opening this eye and closing the opposite eye produced a dramatic change in behaviour. The animal showed no signs of aggression or fear at the sight of human observers and would approach the front of the cage to take raisins from the experimenter's hand.

As in the instance of the animal with the entire temporal pole removal, this placidity occurred only in response to visual stimulation; touching or prodding the animal's arm would produce a momentary aggressive reaction. The placid response to visual stimuli promptly disappeared when the eyelids on the side of the amygdalectomy were sutured shut and the other eye opened. These alternations in affective behaviour were observed over a period of three months, after which the animal was killed and its brain prepared for histological examination.

Although these observations are of interest in themselves, their primary importance lies in illustrating an approach to the investigation of the behavioural correlates of central nervous system lesions which has the advantage of permitting repeated control and experimental observations of the same animal. In the 'split-brain' animal, a unilateral lesion is functionally comparable with a symmetrical bilateral ablation (with respect to visual stimulation) when the eye ipsilateral to the lesion is open and the opposite eyelids sutured shut. When the eye contralateral to the lesion is open and the ipsilateral eyelids closed, the animal behaves with respect to visual stimuli as in its immediate pre-operative state. This suggests that one can, in effect, 'remove' brain tissue and 'replace' it by opening and closing the appropriate eye. Among the several possible extensions of this approach comparing the effects of different lesions performed in opposite hemispheres in the same animal may prove to be especially intriguing.

[1] Downer, J. L. deC., *Fed. Proc.*, **17**, 37 (1958).
[2] Downer, J. L., deC., *Brain*, **82**, 2 (1959).
[3] Rundles, R. W., and Papez, J. W., *J. Comp. Neurol.*, **68** (1938).
[4] Fox, C. A., Fisher, R. R., and Desalva, S. J., *J. Comp. Neurol.*, **89**, (1948).
[5] Poblete, R., Ruben, R. J., and Walker, E. A., *J. Neurophysiol.*, **22**, (1959).
[6] Kluver, H., and Bucy, P. C., *Amer. J. Physiol.*, **119** (1937).
[7] Bucy, P. C., and Kluver, H., *Arch. Neurol. Psychiat.*, **44** (1940).

INHIBITION OF GROWTH AND PHOTOSYNTHESIS IN SUBMERGED PLANTS WITH TETRAMETHYLTHIURAM DISULPHIDE AND SODIUM DIMETHYLDITHIOCARBAMATE AND REVERSAL OF THIS INHIBITION OF PHOTOSYNTHESIS

By P. E. BJÖRN LINDAHL
Institute of Plant Physiology, University of Uppsala

THE biological action of certain dithiocarbamyl compounds has been reviewed by Goksøyr[1]. Their fungicidal action and the inhibition of certain aldehyde dehydrogenases caused by 'Antabuse' account for most of their biological effects. According to Green *et al.*[2] photosynthesis in *Chlorella* is markedly inhibited by sodium diethyldithiocarbamate (Na-DEDT). This effect is related to a similar one of other inhibitors, which like Na-DEDT are denoted "Cu-poisons" by these authors. The inhibition of the oxygen production of isolated chloroplasts by 5×10^{-3} *M* Na-DEDT is about 30–50 per cent[3]. On the other hand, the photosynthetic phosphorylation in isolated chloroplasts is not inhibited by 1×10^{-3} *M* Na-DEDT[4].

No inhibition of photosynthesis by tetramethylthiuram disulphide (TMTD) or homologous substances has been described so far. In a Dutch horticultural paper, however, a gardener reports that he has cleared the soil from algal fouling with TMTD[5].

In the present investigation the action of different concentrations of TMTD and sodium dimethyldithiocarbamate (Na-DMDT) on the growth of 2–5 mm.-long thalli of the green alga *Enteromorpha linza* was investigated using a non-sterile procedure of cultivation. Considerable inhibition of growth was obtained with both substances (Fig. 1). The relatively large dispersion of the replicates results from the inhomogeneity of the inoculated material (cf. controls) and on the instability of the applied chemicals. It is known that the presence of trace elements in the nutrient solution causes the concentration of DMDT⁻ to drop below the intended level, and that metal-DMDT-complexes, which are possibly toxic, are formed[1]. The number of equivalents necessary in order to induce inhibition seems to be the same for the two substances (Fig. 1).

Starting from the growth inhibitions, caused by TMTD and Na-DMDT, the action of these substances on photosynthesis and respiration in the dark was investigated. Photosynthesis was measured with no correction for the simultaneous light-respiration. The determinations were made using both *p*H measurements and a Warburg-method. Respiration in the dark was determined only with the Warburg method.

The first method involves measurements of the changes in *p*H caused by photosynthesis in submerged plants. Each experiment includes measurements of simultaneous changes in two media, one with and one without inhibitor. When inhibition of photosynthesis occurs, the *p*H is changed to a smaller extent in the solution containing the inhibitor than in the other solution. In this a suitable quantity of algae at adequate temperature and light changes the *p*H from 8 to 9 or more during 2–3 hr. of photosynthesis. Non-protolytes or substances, nearly completely protolysed at *p*H 8, can be tested for inhibitory effect with this method. In this investigation the method has been used to determine the extent of inhibition of photosynthesis by TMTD in different species of submerged plants. The degree of inhibition of photosynthesis is taken from a nomo-

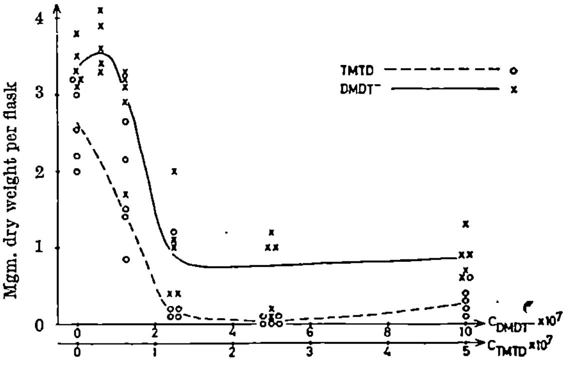


Fig. 1. Growth of thalli of *Enteromorpha linza* at different concentrations (moles per litre) of TMTD and Na-DMDT. Medium: sea water with addition of 0·01 per cent sodium nitrate, 0·005 per cent dipotassium hydrogen phosphate, 0·1 per cent *tris*-buffer, and trace elements chelated with Na_3-versenol (medium *ASP6* (ref. 6)). Initial *p*H 7·6. In each flask 5–10 thalli 2–5 mm. long. Light intensity about 4,000 lux. Temperature 18° C. The TMTD series was gathered after 14 days, and the Na-DMDT series after 13 days. Thalli were dried at 105° C. and weighed

gram which has on a theoretical basis been calculated for each kind of medium applied. Sea water was used as medium for marine algae and fresh water, rich in bicarbonate, for submerged higher plants. The conditions were as follows:

(*A*) Sea water of salinity 32 thousandths. The algae were stored in sea water one day and night before the experiment. The sea water, which was saturated with TMTD, also contained small amounts of DMDT-. The experiments were performed at a light intensity of about 650 lux and at a temperature of about 18° C.

(*B*) Sea water of salinity 25 thousandths. The algae were partly cultivated and partly stored in the medium given in the legend of Fig. 1. The experiment was carried out at an illumination of about 7,000 lux and at a temperature of 18° C. $C_{\mathrm{TMDT}} = 0{\cdot}3 \times 10^{-4}\ M$.

(*C*) Fresh water with an addition of 1 m.mol. sodium bicarbonate and 0·05 m.mol. sodium hydroxide per litre. The plants were acclimatized and cultivated in this medium for 1 and 20 days respectively. The illumination was about 7,000 lux and the temperature 18° C. $C_{\mathrm{TMTD}} = 0{\cdot}7 \times 10^{-4}\ M$.

About equal quantities of plants were placed in four test tubes containing 10 ml. solution, two of the tubes with TMTD and two without inhibitor. No corrections have been introduced in order to compensate for the differences in the photosynthesis before the addition of the TMTD solution.

The extent of inhibition (Table 1) is given in per cent of photosynthesis without inhibitor.

Table 1

	Condition	Inhibition (per cent)
Chlorophyceae		
Ulva lactuca	*B*	70, 85
Enteromorpha compressa	*A*	100, 105
Enteromorpha linza (not cultured)	*B*	85, 105
Enteromorpha linza (cultured)	*B*	110, 115
Cladophora rupestris	*A*	70, 80
Acrosiphonia centralis	*B*	30, 50
Phaeophyceae		
Pylaiella littoralis (cultured)	*B*	60, 75
Ectocarpus confervoides	*A*	80, 80
Rhodophyceae		
Ceramium strictum	*A*	100, 100
Ceramium rubrum	*A*	95, 100
Callithamnion corymbosum	*A*	90, 100
Polysiphonia urceolata	*B*	45, 50
Submerged moss		
Campylium sp.	*C*	70, 80
Submerged higher plants		
Utricularia intermedia	*C*	40, 55
Hydrilla verticillata	*C*	95, 105

According to this the photosynthesis of submerged plants seems generally to be inhibited by TMTD.

The concentrations of TMTD in the water solutions used have been analysed according to a method worked out by Åkerström and based on some of his investigations[7]. Work by Åkerström and me on the effect of derivatives of TMTD and of related substances on photosynthesis is progressing.

Enteromorpha linza used in the experiments with the Warburg method has been taken partly from a non-sterile culture, *E.l.*(cult), in the nutrient solution (cf. legend to Fig. 1) and partly from the sea, *E.l.*(sea), off the west coast of Sweden. The *E.l.*(sea), before being used, was kept in light in the nutrient solution for different lengths of time.

The manometric experiments have been carried out in conical vessels of about 17 ml. at 18° C. and with a light intensity of 6,800 lux at the bottom of the vessel. The bicarbonate solution described in the legend of Fig. 1 was used, but without addition of Na_3-versenol and trace elements. Its *p*H was adjusted to 8·3–8·4, after which the solution was brought to equilibrium with atmospheric carbon dioxide by aeration, and 3·0 ml. of this medium with or without inhibitor was pipetted into the main compartment and 0·5 ml. into each side-arm. Readings were taken every 5 min. for 100–140 min. The quantity of algae in each vessel was chosen so as to make the increase in pressure not to exceed 70 mm. Brodie solution.

In order to measure a possible inhibition of endogenous respiration experiments were performed in the dark. These differed from the above only as regards the duration of the experiments and the illumination. Because of the low respiratory-rate, readings, were taken every 30 min. for 280–340 min. The quantity of algae in each vessel was chosen so as to produce a decrease in pressure not less than 25 mm. Brodie solution. The ratio of the oxygen production in light and the oxygen uptake in the dark of the same thallus varies from 35/1 to 25/1. From this it is obvious that the inhibition of the photosynthesis studied cannot be interpreted as a consequence of stimulation of respiration.

TMTD and Na-DMDT inhibit photosynthesis in both *E.l.*(cult) and *E.l.*(sea). Fig. 2 shows the extent of this inhibition in *E.l.*(cult) at different concentrations of both substances. TMTD has evidently a much stronger effect than Na-DMDT. A comparison between Figs. 1 and 2 reveals that the ranges of concentration in which the inhibition of photosynthesis occurs do not coincide with the corresponding range of concentration for inhibition of growth. In the dark no inhibition of the endogenous respiration in *E.l.*(cult) is obtained either by $0{\cdot}38 \times 10^{-5}\ M$ TMTD or by $1 \times 10^{-4}\ M$ Na-DMDT.

The inhibition of photosynthesis by $1 \times 10^{-4}\ M$ Na-DMDT in *E.l.*(sea) can be lowered by addition of equivalent quantities of certain metal ions, namely, Cd^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+} and Au^{3+}. In some experiments with Cu^{2+} complete reversions of the inhibitions were obtained. No reduction of the inhibition is obtained by the same concentration of Mn^{2+}, Sn^{2+} and Fe^{3+}, while at the same concentration of Zn^{2+} photosynthesis is further diminished. Besides, the inhibition is decreased by $1 \times 10^{-4}\ M$ glutathione and $2 \times 10^{-4}\ M$ cysteine, whereas $1 \times 10^{-4}\ M$ of the latter substance does not reduce the inhibition.

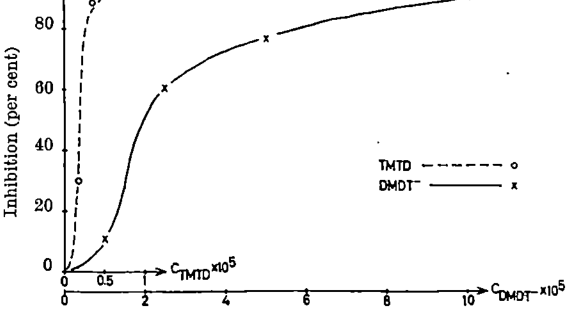


Fig. 2. Inhibition of photosynthesis in *Enteromorpha linza* (cultured) at different concentrations (moles per litre) of TMTD and Na-DMDT

In a corresponding manner, an inhibition induced by TMTD has been investigated. The inhibition in *E.l.*(cult) by 0·69 × 10^{-5} *M* TMTD is not lowered by addition of 1 × 10^{-4} *M* Cu^{2+}, whereas this is the case in the presence of 1 × 10^{-4} *M* Au^{3+}. The inhibition of photosynthesis in *E.l.*(sea) by 0·54 × 10^{-4} *M* TMTD and in *E.l.*(cult) by 0·36 × 10^{-4} *M* TMTD is considerably diminished by the addition of 1 × 10^{-4} *M* glutathione, and in the former material also by 2 × 10^{-4} *M* cysteine.

It appears from the above that the inhibition of photosynthesis caused by both TMTD and Na-DMDT is almost completely reversible. The reduction of the Na-DMDT-induced inhibition by different metal ions can be explained by the drop in concentration of $DMDT^-$, caused by the formation of metal-DMDT-complexes. The fact that the TMTD-induced inhibition is not diminished by addition of 1 × 10^{-4} *M* Cu^{2+} could, however, be due to the circumstance that $DMDT^-$ ions *per se* are not responsible for this inhibition. Under the presumption that the photosynthesizing plant is able both to oxidize $DMDT^-$ and to reduce TMTD, the observed phenomena may be regarded as effected by TMTD only. In keeping with this is the fact that addition of glutathione or cysteine diminishes inhibition caused both by Na-DMDT and TMTD. Further, the reversal of the inhibition with metal ions, when this is induced by $DMDT^-$, could thus be due to the ability of the plant to reduce earlier formed TMTD to $DMDT^-$, which is then complexed.

A full description of the present investigations will appear in *Svensk Botanisk Tidskrift* and *Physiologia Plantarum*.

I am indebted to Prof. N. Fries, Dr. K.-G. Nyholm, Prof. P. E. Lindahl, and Dr. S. Åkerström for their interest and help with this work.

[1] Goksøyr, J., *Physiol. Plant.*, **8**, 719 (1955).

[2] Green, L. F., McCarthy, J. F., and King, C. G., *J. Biol. Chem.*, **128**, 447 (1939).

[3] Andreeva, T. F., and Zubkovich, L. E., *Trudy Inst. Fiziol. Rastenij im. K. A. Timirjazeva Akad. Nauk S.S.S.R.*, **8**, 1, 67 (1953).

[4] Wessels, J. S. C., *Biochim. Biophys. Acta*, **29**, 113 (1958).

[5] Myrstad, A., *Gartneryrket*, **33**, 556 (1959).

[6] Provasoli, L., McLaughlin, J. J. A., and Droop, M. R., *Arch. Mikrobiol.*, **25**, 392 (1956–57).

[7] Åkerström, S., *Arkiv Kemi*, **14**, 403 (1959).

A PHYTOPLANKTON MULTI-SAMPLER AND ITS USE IN LAKE VICTORIA

By Dr. J. H. EVANS
Royal Holloway College, Englefield Green, Surrey

FROM April until August 1960, many phytoplankton samples were taken with a Ruttner sampler from the northern end of Lake Victoria, especially in the north-west corner of Pilkington Bay of Buvuma Island (Fig. 1). The main constituents of the phytoplankton were *Melosira nyassensis* var. *victoriae*, *M. granulata*, *M. varians*, *M. agassizii*, *Aphanocapsa elachista*, *Lyngbya circumcreta*, *Anabaena circinalis* and *Merismopedia elegans*. Certain other diatoms and blue-green algae were observed sporadically as were twelve species of the Chlorococcales. Very wide variations in the horizontal and vertical distributions of phytoplankton were found in Pilkington Bay, and in the north-west corner of the bay there was a sharp overall increase in phytoplankton during May. The variations in distribution, and the May increase, although the latter might have been a reflexion of seasonal change, were possibly due to water movements. The possible association between water movements and variations in plankton density has been mentioned by Talling[1], and Fish[2] has referred to exchanges between deep and shallow water and the possible increase in fertility of the main lake resulting from the outward movement of fertile inshore waters.

To investigate water movements and the carriage of phytoplankton near the surface, a multi-sampler was designed and constructed (Fig. 2). This consisted of a 'Dexion' No. 112 alloy-angle framework with four empty, sealed 1-gallon cans acting as floats. Eight phytoplankton nets held on galvanized steel loops were attached to the frame. The narrow end of each net was tied tightly on to a connecting tube of plastic, which in turn led into a collecting tube which, kept in place only by a close-fitting rubber sleeve, could

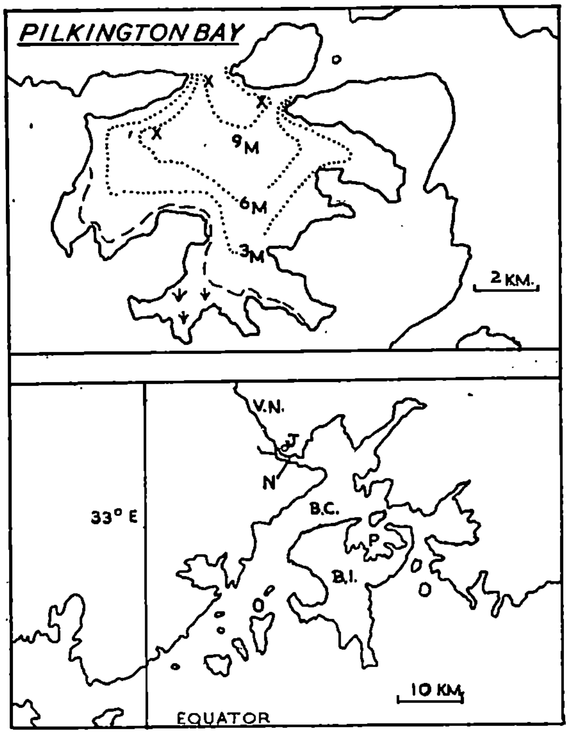


Fig. 1. Pilkington Bay with depth contours in metres; *x*, approximate positions that phytoplankton multi-sampler was used. Lower map shows the position of the bay (*P*) in Buvuma Island (*B.I.*) at the northern end of Lake Victoria. *V.N.*, Victoria Nile; *J*, Jinja; *N*, Napoleon Gulf; *B.C.*, Buvuma Channel

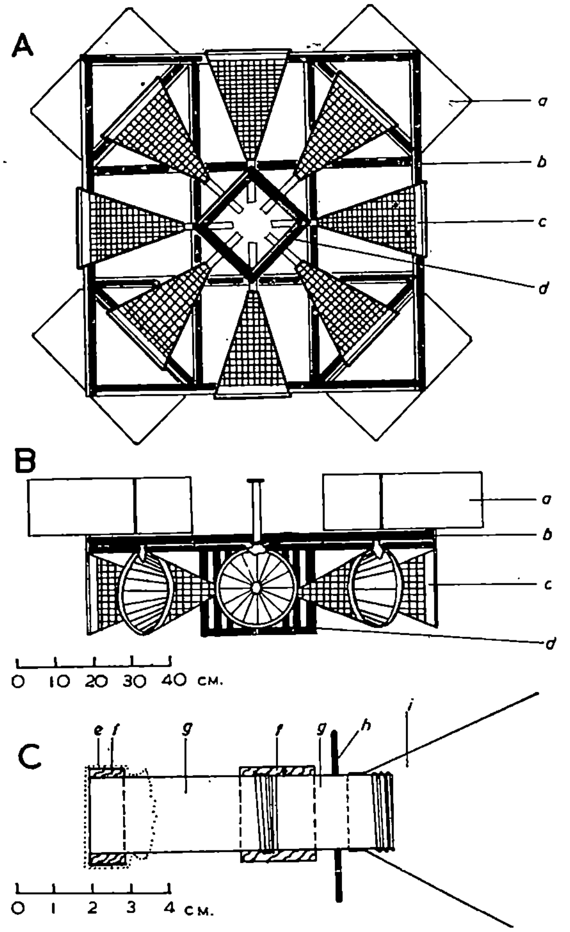


Fig. 2. The phytoplankton multi-sampler. *A*, plan view, from beneath; *B*, elevation; *C*, collecting tube in half-section. *a*, Float; *b*, framework of 'Dexion' No. 112 alloy-angle; *c*, phytoplankton net on wire loop; *d*, centre-piece of framework; *e*, fine-mesh phytoplankton netting; *f*, rubber tubing as sleeve; *g*, plastic tube; *h*, edge of alloy angle upright of centre-piece; *i*, inner end of phytoplankton net

easily be detached. Each collecting tube was closed at its outer end (away from the net) by a piece of phytoplankton mesh retained over a short length of rubber sleeving by a rubber band. Wide-mouth, screw-cap, 9 × 5 cm. plastic bottles, each containing about 10 ml. sterile, distilled water and each marked *N*, *N.W.*, *W*, etc., were carried for storing the collecting tubes.

The multi-sampler was placed in the water with the centre of each net opening at about 15 cm. below the surface. With the floats marked *N*, *S*, *E* and *W*, the multi-sampler was orientated in a north–south direction and held in position from a small launch or from a dinghy at anchor, usually for 10 min. At the end of this time the collecting tubes were detached carefully and each placed quickly in the appropriate bottle. In the laboratory, the contents of each collecting tube were washed into a 50 ml. measuring cylinder, made up to a known volume and, after shaking, a sample taken for a quantitative determination of the phytoplankton by the sedimentation technique[3,4]. The results, after corrections for dilutions and the time of each run, were recorded for each net as units/min. All species of *Melosira* were counted together as cells, species of *Pediastrum* and *Scenedesmus* as cœnobia, *Anabaena circinalis* as cells, *Lyngbya circumcreta* as coil turns, *L. limnetica* as filaments, *Aphanocapsa elachista* as colonies, *Merismopedia elegans* as groups of eight cells and all other algae as individual cells. During each use of the multi-sampler, three surface-water samples were taken to determine the general concentration of phytoplankton in the vicinity of the apparatus.

The first trial run was in the Napoleon Gulf where there was known to be a current of about 20 cm./sec. In parallel with the multi-sampler, an Ekman current meter was used. The multi-sampler results can be plotted as histograms and vector diagrams, and four of them are shown in Fig. 3. The resultant component indicates the general flow direction during the sampling period, and this was found to be close to that given by the Ekman current meter (Fig. 3*A*). The resultant component can also be regarded as being approximately related to the rate of flow.

As there are practical difficulties involved, such as local turbulences, net resistances at higher rates of flow and net clogging in high concentrations of phytoplankton, it is not proposed here to attempt to calculate accurately absolute velocities of currents from the multi-sampler results. It is believed, however, that the multi-sampler results, in comparison with the phytoplankton concentrations in the water samples taken in parallel, do give some indication of current velocities and a comparison of several such results with actual rates of flow as determined with the Ekman current meter (Table 1) supports this. For the sake of simplicity, however, the result for a single net, that which collected the most phytoplankton, has been used, rather than the resultant component of the vector diagram, in calculating the current velocity. This method, although possibly less precise, is believed to be sufficiently accurate for such approximations. In two of the results included in Table 1, only counts of *Melosira* were considered as all the blue-green algae on those occasions were restricted to the water surface. These results suggest that provided there are no serious turbulences there is a relationship between current velocity and the quantity of phytoplankton trapped by the net most closely facing the prevailing current.

That is:

$$F = P.k \text{ cm./sec.}$$

where F = calculated rate of flow;

Table 1. RELATIONSHIPS BETWEEN RATES OF FLOW AS DETERMINED BY EKMAN CURRENT METER AND FROM PHYTOPLANKTON COUNTS

Time and place	E = rate of flow in cm./sec. measured from an Ekman current meter	$P = \dfrac{\text{phytoplankton units/min.}}{\text{phytoplankton units/ml.}}$	$k = E/P$
(1) Napoleon Gulf 1600–1610 30.5.60	19.5	13	1·50
(2) Pilkington Bay 1420–1430 30.8.60	< 6·0	4	< 1·50
(3) Pilkington Bay 1730–1740 24.6.60	5·0	4	1·25

For (1) and (3) only *Melosira* counts, as cells/min. and cells/ml. were included in the calculations as, on these occasions, the Cyanophyceae were restricted to the water surface above the level of the nets.

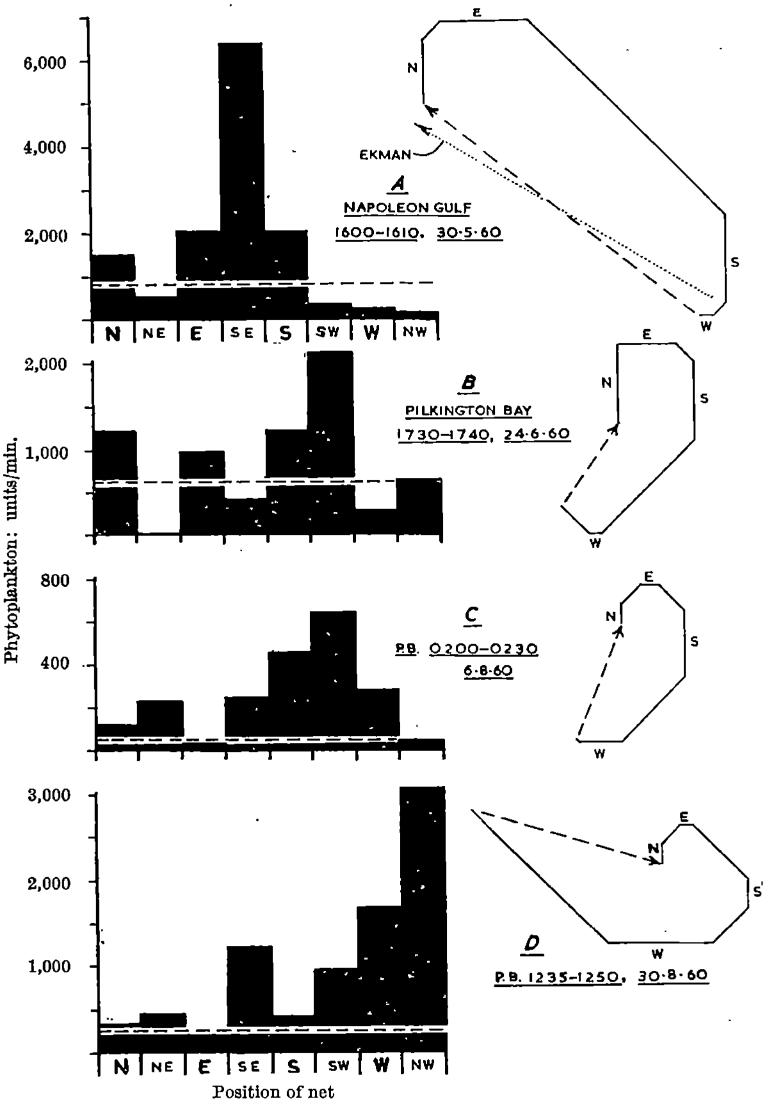


Fig. 3. Some results obtained with the phytoplankton multi-sampler. For the histograms, the broken horizontal line represents the mean density of phytoplankton, in units/ml., in the surface water during the sampling period. For the vector diagrams, on the right, the results have been plotted in the eight directions of the compass, but the diagrams are not all to the same scale. The broken line of the vector diagrams is the resultant component, and indicates the general flow direction. For the top figure (*A*), the flow direction indicated by an Ekman current meter is included for comparison. For *D*, the multi-sampler was at the mouth of the bay

$$P = \frac{\text{phytoplankton units/min.}}{\text{phytoplankton units/ml.}} \text{ in water sample ;}$$

k is a value in the range 1·25–1·50.

As is to be expected, with decreasing velocities there was a closer and closer approach of calculated rate of flow to actual rate of flow. Below a rate of flow of about 5 cm./sec. the Ekman current meter will not register over a short period of time. The multi-sampler, however, will give results, within 10 min., at rates of flow of less than 5 cm./sec. These results may be used comparatively, and, bearing in mind that they are still approximations, some indication of absolute rate of flow may be determined. In one series of samples, for example, the calculated rate of flow was in the range 1·1–1·3 cm./sec., and it is not unreasonable to conclude that the actual rate was in the range 1·0–1·5 cm./sec.

The area under investigation is well removed from the Lake Victoria outflow, and the major cause of surface water currents was the wind (cf. Hutchinson[5]) which, until about midday on most days, tended to blow gently into the Bay from a northerly direction and then change abruptly to a stronger wind from a southerly direction. The results for Fig. 3*D* were obtained, however, at a time when a strong wind from west-north-west produced a surface flow of about 15 cm./sec. Surface water currents from, or near, the south-west, south or south-east would tend to carry phytoplankton from shallow inshore regions to the mouth of the Bay and through into the open, deeper water of the Buvuma Channel and so, possibly, act as an inoculum of algae for the open water of the lake north of Buvuma Island. At the same time there would be an inflow of water, below the surface, into the Bay, which might result ultimately in the return of algae, such as *Melosira*, sunk deep in the Buvuma Channel, to the photic zones in the shallower, inshore parts of the Bay. An incoming, oblique up-current was once observed, by virtue of a dense concentration of visible clumps of blue-green algae, from a dinghy anchored in the mouth of the Bay. This current was possibly due to a combination of a wind from the north and a local internal *seiche* movement.

The generally slower surface currents from northerly directions carried some algae into the Bay, and during one sampling (Fig. 3*D*) about 95 per cent of the phytoplankton from the north-west was *Melosira*. The current veered round completely during the sampling period, however, and it was found that currents from the south and south-east, and from the north-east, contained 70–90 per cent Cyanophyceae. This suggests the occurrence of localized areas of *Melosira* outside the Bay to the north-west and of Cyanophyceae to the north-east and inside the Bay.

A near replica of the phytoplankton multi-sampler has been constructed for trials in Britain, and an entirely new, more compact multi-sampler is being devised for later use.

My thanks are due to the Colonial Office for the award of a research fellowship, and to the staff of the East African Fisheries Research Organization for much practical help and encouragement during this and other work carried out in Central East Africa.

[1] Talling, J. F., *Proc. Roy. Soc.*, B, **147**, 57 (1957).

[2] Fish, G. R., *Fish. Publ., Lond.*, **10**, 1 (1957).

[3] Utermohl., H., *Verh. Int. Ver. Limnol.*, **5**, 567 (1931).

[4] Lund, J. W. G., Kipling, C., and Le Cren, E. D., *Hydrobiol.*, **11**, 143 (1958).

[5] Hutchinson, G. E., *A Treatise on Limnology* (New York, 1957).

LETTERS TO THE EDITORS

RADIOASTRONOMY

Very-Low-Frequency Modulation of Discrete Frequency Solar Noise Bursts

In the radio-frequency spectrum, solar radio bursts have been classified according to their dynamic structure. Information on the velocity of shock waves or the solar corpuscular stream as well as on the physical mechanism of the radiation has been obtained by this recording method. Another technique for studying the mechanism of solar radio radiation is to observe the modulation structure of bursts and the quiet Sun at discrete frequencies. Jager and Veer[1] studied solar radio transients by recording 200 Mc./s. radio noise on fast paper tape. Their observations were concerned with the time duration and distribution of radio-pips, their band-width and the presence of the echo phenomena.

In an effort to study the very-low-frequency details of different types of solar radio bursts, observations were made at the Sagamore Hill Radio Observatory, Massachusetts, on three discrete frequencies, 220 Mc., 400 Mc. and 3,000 Mc./s., with an 84-ft parabolic antenna. The magnetic tape records, taken to observe d.c. to 600 c./s. fluctuations, were analysed over 5–550 c./s. to detect enhancements or absorption in this range. The power spectra of the modulation envelope show that the high-frequency end of the very-low-frequency band is accentuated.

Type II and type IV bursts reveal marked attenuation over certain very-low-frequency bands. An isolated burst at 220 Mc./s. was associated with a sharp and fast development on one particular band.

The spectra of random noise, limited to a narrow intermediate-frequency band of centre frequency f_0, may be written as:

$$x(t) = x_i(t)\cos 2\pi f_0 t + x_q(t)\sin 2\pi f_0 t \qquad (1)$$

where $x_i(t)$, $x_q(t)$ are in-phase and quadrature components respectively at any instant and are totally independent of each other.

Assuming square-law detection, it can be shown that the power spectra of the envelope of narrow band-limited random noise will be triangular. The triangular spectrum when sampled over a very small band compared with the band limit of the noise will yield a flat spectrum. In the present investigation the very-low-frequency band extended from 5 to 550 c./s., which is very small compared with the intermediate-frequency band-widths of at least 1 Mc./s. In this case, therefore, the modulation spectrum of the random noise in the envelope is expected to be flat.

Solar radio radiation was received at 220 Mc., 400 Mc. and 3,000 Mc./s. The intermediate-frequency band-widths varied, ranging from 1 Mc. at 220 Mc. to 200 Mc. at 3,000 Mc./s. (using travelling-wave tubes in the latter system). The intermediate-frequency signal, approximately a square bandpass, was detected in square-law detectors and recorded on a frequency-modulated magnetic tape. The solar event of August 11, 1960, provided an opportunity to record all types of bursts. Using the dynamic spectra taken at Fort Davis, Texas, by A. Maxwell and A. R. Thompson (private communication), particular periods of type II, type III and type IV bursts could be selected and the very-low-frequency spectra analysed within the range of 5–550 c./s. The records were normalized against noise tube and noise diode spectra.

Theoretically, the spectrum of the noise generator envelope within the very-low-frequency band was expected to be flat. Fig. 1, showing this spectrum, illustrates the relatively flat frequency response of the entire receiver, recorder, and analysing system. The spectrum of quiet Sun emission is also observed to be flat, as illustrated in Fig. 2.

However, the envelope spectra of all types of bursts when normalized against noise samples show definite structure (Fig. 3, *a–d*). The enhanced response at 60 c./s. and its harmonics may be disregarded as possible power frequency pick-up. The high end of the band is accentuated in all three frequency channels. Type II and type IV burst envelopes display certain bands with marked attenuation (Fig. 3, *a–d*). These spectral regions are distributed throughout the analysed band and have a maximum band-width of approximately 60 c./s. The spectrum of the envelope of an isolated burst shows sharp and fast development of structure around two narrow very-low-frequency bands (arrows in Fig. 4). This is followed by a gradual widening of the feature. The time-interval between succeeding spectra is 4 sec.

The emphasis on the high-frequency end of the very-low-frequency power spectra of the envelope indicates that the solar noise bursts do not fit the

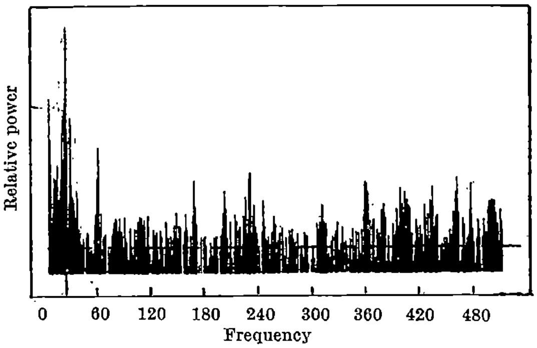


Fig. 1. Envelope spectra of random noise generator at 220 Mc./s. The dashed line shows the average power spectra. Enhanced response at 60 c./s. and 120 c./s. is due to power frequency pick-up

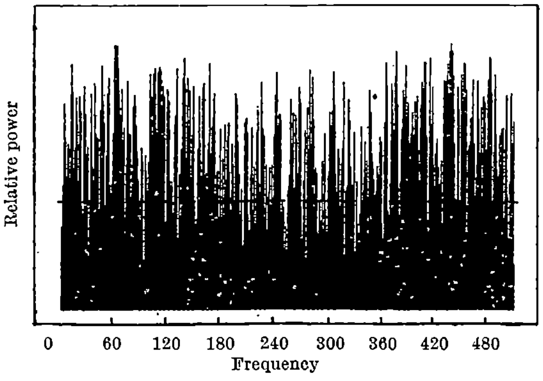


Fig. 2. Envelope spectra of radio noise from the quiet Sun at 220 Mc./s. on March 10, 1961, at 1756 U.T.

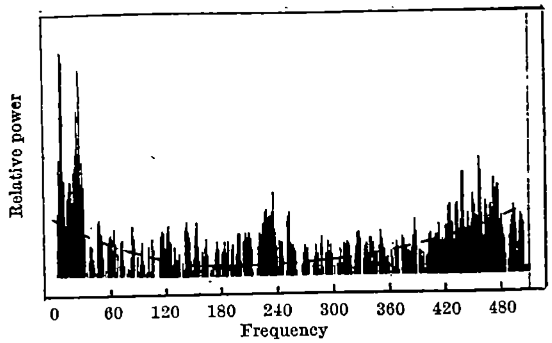


Fig. 3*a*. Envelope spectra of slow drift burst from the Sun at 220 Mc./s. on August 11, 1960, at 1929 U.T.

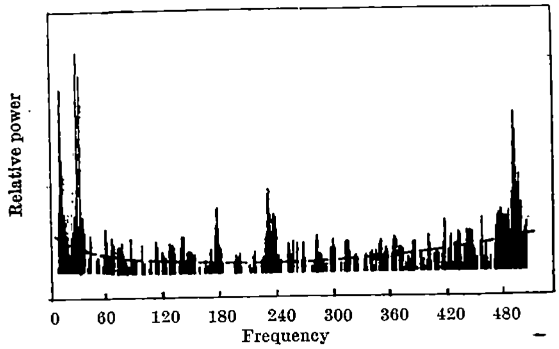


Fig. 3*b*. Envelope spectra of continuum emission from the Sun at 220 Mc./s. on August 11, 1960, at 2003 U.T.

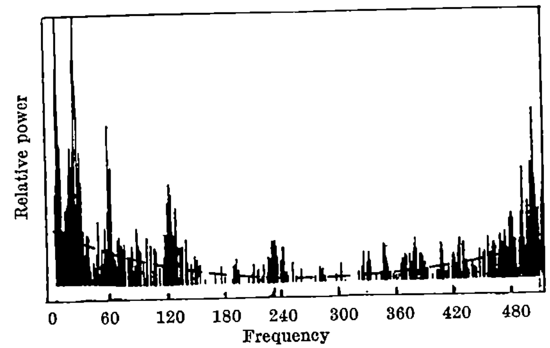


Fig. 3*c*. Envelope spectra of continuum emission from the Sun at 400 Mc./s. on August 11, 1960, at 2003 U.T.

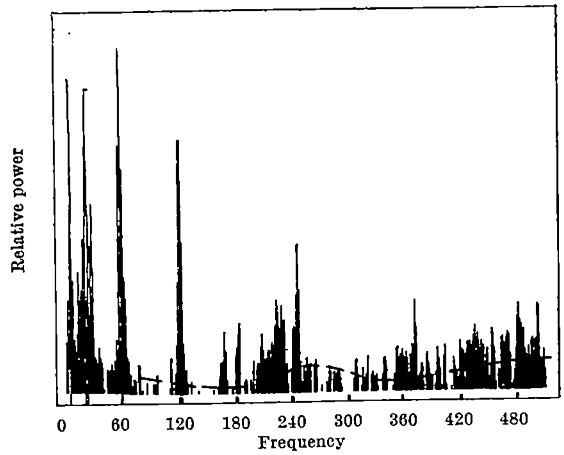


Fig. 3*d*. Envelope spectra of continuum emission from the Sun at 3,000 Mc./s. on August 11, 1960, at 2003 U.T.

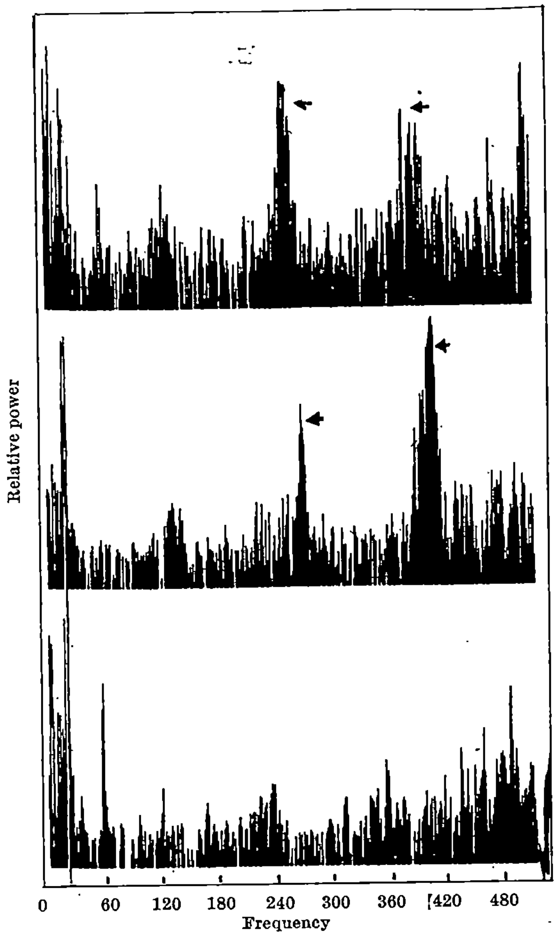


Fig. 4. Development of a fast narrow-band structure in the envelope spectra of an isolated burst from the Sun at 400 Mc./s. on August 11, 1960, at 2052 U.T.

normal law of random functions. The development of a narrow-band structure in the case of an isolated burst may be explained on the basis of a model in which the source is situated above the zero-refractive-index layer and a delayed interaction exists between the direct and the reflected ray. The consequent phase delay might give rise to a definite very-low-frequency modulation structure. A proper distance scale between the source and the reflector might account for the attenuation bands in type II and type IV spectra. The possibility of an inherent modulation at the source from the shock wave mechanism also exists.

A more detailed study of the modulation spectra over a wider band should be undertaken before any hypothesis is advanced on the envelope spectra of different types of solar bursts. A new technique for observing solar mechanisms might result, particularly if the effect of the magnetic field around sunspots as well as a general magnetic field could be observed by the envelope studies of the modulation process.

JULES AARONS
SANTIMAY BASU
WILLIAM KIDD
RICHARD ALLEN

Air Force Cambridge Research Laboratories,
Office of Aerospace Research (U.S.A.F.),
L. G. Hanscom Field, Bedford, Massachusetts.

---

[1] Jager, C. de, and Veer, F. Van't, *I.A.U. Symp.*, No. 4 (August 1955).

Lunar Occultation of a Radio Source

At present only a small number of radio sources have been satisfactorily identified with photographic objects. The important task of establishing further identifications faces a serious difficulty because of the uncertainty in the measured positions of the radio sources. This uncertainty usually amounts to several minutes of arc, and it is only for a few intense sources that the error has been reduced to less than one minute of arc.

It has been suggested by several authors, for example by Link and Neuzil[1], that observations should be made of the occultation of radio sources by the Moon, and this technique has already been used to study some intense sources of large angular diameter[2–5]. It is the purpose of this communication to direct attention to the high precision with which source positions can be measured by observing lunar occultations with equipment which can track the Moon continuously. The results of a preliminary test of the technique on a radio source are described here.

On December 8, 1960, the 250-ft. radio telescope at Jodrell Bank was used for observing the occultation by the Moon of the radio source 3*C* 212 (ref. 6). The measurements were made at a frequency of 237 Mc./s. using a receiver with a band-width of 2 Mc./s. and a post-detector time constant of 4 sec. The observations were carried out by recording the output of the receiver with the telescope in automatic motion following the centre of the Moon. The beam-width of the aerial was about 1·2° between half-power points and therefore covered an area considerably larger than the Moon's disk.

The occultation was observed as a sharp fall in the received power as the source was covered by the Moon, followed later by a sharp rise as the source reappeared. The measured times at which these events occurred are:

Time of disappearance 08h. 03m. 52·5s. ±5s. U.T.
Time of reappearance 08h. 40m. 49s. ±5s.

These times correspond to the passage of the source across the limb of the Moon and they have been derived from the records on the assumption that the angular size of the source is small compared with the diffraction pattern of the Moon's limb.

H.M. Nautical Almanac Office has kindly calculated the celestial co-ordinates of the source from the observed times which define two possible positions of the source, one on either side of the centre of the Moon on a line perpendicular to its direction of motion. In principle this ambiguity can be resolved by observations of a subsequent occultation; however, in the present case, the position given in the Cambridge 3*C* Catalogue is sufficiently precise to allow one of the two possible positions to be chosen. The calculated mean place of the source for 1950·0 is given below together with the data from the Cambridge 3*C* Catalogue.

Position computed from occultation (1950.0)
Source 3*C* 212: *R.A.* 08h. 55m. 55·7s. ± 0·3s.
Dec. 14° 21′ 27″ ± 2″

Catalogue data (6)
Source 3*C* 212: *R.A.* 08h. 56m. 00s. ± 5s.
Dec. 14° 25′ ± 8′
Flux 21×10^{-26} W. m.$^{-2}$ (c./s.)$^{-1}$ at 160 Mc./s.

The quoted errors in the computed position have been estimated only from the errors in measurement of the time of immersion and emersion, and neglect any possible refraction in a lunar ionosphere or the effect of irregularities in the Moon's limb. The high degree of accuracy achieved is due to the circumstances of the occultation. The closest approach of the source to the centre of the Moon was about 12 minutes of arc, and under these conditions an error of one minute in the duration of the occultation introduces an error of only 12″ of arc in the declination. If the source had passed close to the centre of the Moon the accuracy would have been considerably reduced, as in this case a similar error in duration would have produced a declination error of about three minutes of arc.

The measurement reported here probably represents the most accurate determination yet made of the position of a radio source. The method used has the great advantage that it is not subject to uncertainties introduced by refraction in the Earth's troposphere or ionosphere. It is also free from the effects of confusion, as the solid angle subtended by the Moon's diffraction pattern is only of the order 1/100 sq. degree. A significant source of error is likely to lie in the assumption that the angular diameter of the source is negligible. However, a preliminary comparison of the observed occultation curves with the theoretical diffraction pattern shows that the diameter of the source must be less than or equal to eight seconds of arc, and hence the resultant error in declination will not be greater than approximately four seconds of arc. It may therefore be concluded that, taking into account all sources of error, the estimated position of the source is probably accurate to within about five seconds of arc.

The work described here was carried out as part of a programme designed to measure accurate positions of a number of radio sources by the method of lunar occultations. A detailed description of the method together with an analysis of all the data so far obtained will be published later.

I wish to thank Sir Bernard Lovell and Prof. R. Hanbury Brown for their interest in the work, and Mrs. Flora McBain Sadler of H.M. Nautical Almanac Office for the calculation of the position of the source.

C. HAZARD *
University of Manchester,
Nuffield Radio Astronomy Laboratories,
Jodrell Bank.

---

* Present address: School of Physics, University of Sydney, New South Wales.

[1] Link, F., and Neuzil, L., *Bull. Astro. Insts. Csl.*, **5**, 112 (1954).
[2] Elsmore, B., and Whitfield, C. R., *Nature*, **176**, 447 (1955).
[3] Boischot, A., Blum, E. J., Ginat, M., and le Roux, E., *C.R. Acad. Sci., Paris.*, **242**, 1849 (1956).
[4] Rishbeth, H., *Austral. J. Phys.*, **9**, 494 (1956).
[5] Rishbeth, H., and Little, A. G., *The Observatory*, **77**, 897 (1957).
[6] Edge, D. O., Shakeshaft, J. R., McAdam, W. B., Baldwin, J. E., and Archer, S., *Mem. Roy. Astro. Soc.*, **68**, 37 (1959).

PHYSICS

Thermoelectric Power of Single Crystals of Gallium

As part of a programme to investigate the anisotropy of non-cubic crystals, the thermoelectric power of single crystals of the orthorhombic metal gallium has been examined. The aim of the programme is the explanation of the large magnitude of the anisotropies of the physical properties of gallium.

The single crystals were prepared from gallium of at least 99·99 per cent purity, and they were grown in the form of cylindrical rods encased in a glass tube. The length of the specimens was 10–13 cm. and their diameter was about 0·35 cm. The single crystals were grown from seed by passing a freezing plane through the super-cooled melt and three crystals were produced such that the rod axis in each case was aligned parallel to a principal crystallographic axis. In each case the rod axis and crystallographic axis coincided to one degree or better.

The three crystals were set up so that their upper ends protruded into a 'Perspex' container. Commercial copper wire, 20 s.w.g., was used as the reference material after being annealed to cherry red heat. A copper lead was welded to each end of the crystal to give a good mechanical and electrical contact. During this procedure, care was taken to minimize constraints in the annealed copper. The three sets of leads were connected to the three channels of a Cambridge vernier potentiometer which was used to measure the Seebeck e.m.f.'s. A Hilger-Watts *F.A.*1 galvanometer-amplifier was used as a null detector for the potentiometer, which was graduated in 0·1-μV. steps. By galvanometer interpolation it was found possible to estimate potentials to 0·02 μV.

The lower ends of the specimens were immersed in a Dewar flask of alcohol. The upper 'Perspex' container was filled with a mixture of ice and water and the temperature of the lower bath altered by the addition of solid carbon dioxide, the temperature being recorded by an alcohol-in-glass thermometer. In this manner the Seebeck e.m.f. (e) of the three crystals with respect to copper was measured with one junction maintained at 0° C., while the lower junction was maintained at temperatures + 30° C. to −70° C.

Fig. 1 shows the results obtained for the three crystals aligned along the (100), (010) and (001) crystallographic axes. With the resources available, it was not found possible entirely to eliminate spurious thermal e.m.f.'s set up in the measuring circuit, but the results near the origin of Fig. 1 indicate that these were fairly small compared with the magnitudes measured. The normal convention as regards the sign of the Seebeck e.m.f.'s was used, that is, the e.m.f. is considered positive if the electrical current produced by the temperature gradient flows from the variable to the ice-cold junction through the specimen.

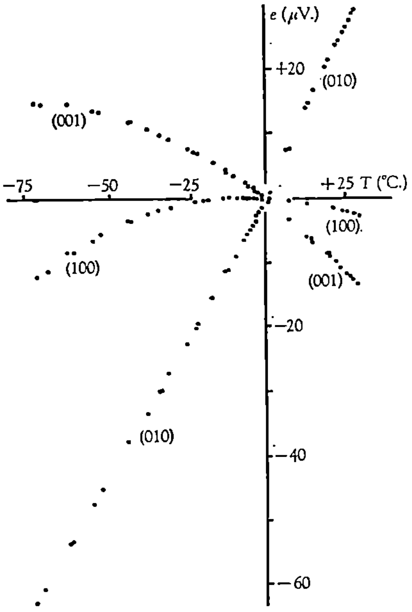


Fig. 1. The variation of the Seebeck e.m.f. (μV.) with temperature of the variable junction (° C.) for three gallium crystals aligned along the principal axes

Table 1 gives, with respect to copper, details of the thermoelectric power (Q) of the specimens as calculated from Fig. 1, that is, $Q = (de/dT)$. As is usual, the variation of Q with temperature is approximately linear, being of the form $Q = A + BT$. The respective values of the coefficients A and B are shown in Table 1.

Table 1. GALLIUM SINGLE CRYSTALS—THERMOELECTRIC PARAMETERS WITH RESPECT TO COPPER

Crystal orientation	(100)	(010)	(001)
Thermoelectric power (Q) at 0° C. in μV. deg.$^{-1}$ C. (A)	$-0{\cdot}02_3$	$+0{\cdot}96_2$	$-0{\cdot}38_0$
Temperature coefficient of Q in μV. deg.$^{-2}$ C. (B)	$-0{\cdot}005_2$	$+0{\cdot}003_8$	$-0{\cdot}004_7$

Though the scale of Fig. 1 is too small to show the details in the region of the origin, the results for the (100) crystal exhibit, with respect to copper, a neutral temperature at about − 3° C. and an inversion temperature at about − 6° C.

The absolute thermoelectric power of copper has been determined by several workers and the latest results[1] give good agreement with previous values. These authors found that the absolute thermoelectric power (Q^*) of copper was not significantly affected by impurities above 100° K., and that it could be represented by the expression:

$$Q^*_{\mathrm{Cu}} = +\,5{\cdot}4 \times 10^{-3}\,\theta\,.$$

where θ is in degrees absolute. Using this information, the present results can be corrected to absolute values. Again, expressing the results in the form $Q = A + BT$, where T is in degrees centigrade, this gives:

$$Q^*_{\mathrm{Ga}(100)} = 1{\cdot}46 + 0{\cdot}000_2 T$$

$$Q^*_{\mathrm{Ga}(010)} = 2{\cdot}44 + 0{\cdot}009_2 T$$

$$Q^*_{\mathrm{Ga}(001)} = 1{\cdot}10 + 0{\cdot}000_7 T$$

These results are interesting in that the anisotropy ratios of the coefficients A are not as large as those of the electrical or thermal conductivity[2]. The B coefficients are also interesting in that the coefficient for the (010) axis is considerably larger than those for the (100) and (001) axes. For the latter axes, the value of B is practically zero, which indicates that the Thomson coefficients ($T(dQ^*/dT)$) for these two directions are practically zero for the range of temperature under consideration. It is considered that these effects may be of fundamental importance and it is hoped to discuss them more fully later.

This programme on anisotropy is under the general direction of Prof. A. J. Woodall, to whom thanks are due for encouragement and advice. The Dean of the Royal Military College of Science is thanked for permission to publish this communication.

P. HORNER

Physics Branch,
Royal Military College of Science,
Shrivenham, Swindon, Wiltshire.

---

[1] Gold, A. V., MacDonald, D. K. C., Pearson, W. B., and Templeton, I. M., *Phil. Mag.*, **5**, 765 (1960).

[2] Powell, R. W., *Proc. Roy. Soc.*, A, **209**, 525 (1951).

Neutron Terminology

Weinberg and Wigner[1] have made a plea for a more scholarly exposition of reactor technology; Ghose and Young[2] suggest that the proposed introduction of a unit of neutron flux be made an opportunity to make customary the use of the word 'flux' in this context. Their suggestion, however, involves an over-simplification, through neglecting to make allowance for the two concepts of neutron current and neutron flux, the importance of which I have emphasized elsewhere[3].

In reactor terminology, neutrons traversing unit area in unit time are measured as neutron current, not as neutron flux. It would be more correct to say neutron current density, but there is no good reason to prefer flux density. Neutron flux has, conventionally, the same dimensions as neutron current, but arises from a different point of view: whereas neutron current is a rate of crossing a surface by neutrons, neutron flux is a rate of track formation by the neutrons in a volume, being equal to the neutron density (in the sense of concentration) times the mean speed. If Ghose and Young's suggestion of calling this quantity 'flux density' were adopted, then flux would become total number of neutrons times mean speed—a change that would make confusion worse confounded. It should be noted that though a unit of flux would, on dimensional considerations alone, be valid for neutron current also, this extension of its use would lead to a possibility of error because, in going from flux to current, a factor of $\frac{1}{2}$ appears (for random motion) as a result of the inclination of the line of flight to a surface becoming involved.

While feeling that the meaning at present attached to 'neutron flux' can be justified, there is another usage which cannot. Whereas a microscopic cross-section has dimensions L^2, the so-called macroscopic cross-section has dimensions L^{-1}: it is not a cross-section at all. It can, for example, be an absorption coefficient, and has the correct dimensions for one. Terms such as 'absorption coefficient' are already well known, are less cumbersome, and using them instead of macroscopic cross-section would permit the word 'microscopic' to be dropped when referring to a true cross-section.

It would, furthermore, lead to greater clarity if 'multiplication constant' were reserved for a regular lattice of fuel and moderator of infinite extent, and not used for the most important variable in a real reactor. For the latter, 'reproduction ratio' seems the appropriate choice.

W. R. HARPER

67 Burton Court,
London, S.W.3.

---

[1] Weinberg, A. M., and Wigner, E. P., *The Physical Theory of Neutron Chain Reactors* (Univ. Chicago Press, 1958).

[2] Ghose, S. C., and Young, J. C., *Nature*, **190**, 621 (1961).

[3] Harper, W. R., *Basic Principles of Fission Reactors* (Interscience Pub., New York and London, 1961).

Neutron 'Flux'

It is the title, not the definition, of 'neutron flux' which is ambiguous, particularly the application of a vector term to a scalar quantity. The title should imply some connexion with the flow of neutrons; in fact, as the velocities are integrated regardless of direction, the function defines the rate of generation of neutron track-lengths per unit volume at a given point. This is important in reaction problems because, as the elementary particles are treated as spherical and hence presenting a fixed cross-section to any neutron, it measures the collision potential. It is nothing whatever to do with the flow of neutrons, except in such special cases as collimated beams or by indirect calculation when the distribution pattern has been determined; yet the phrase gives this impression and misleads many entrants to the nuclear field.

The mere fact that neutron flux, like the pressure or temperature of a gas, is caused by the movement of the particles involved, is no good reason for applying to a scalar a title which in all other branches of science is reserved for vectors; my own preferred alternative is neutron (collision) potential, but even a synthetic proposal would be better than the present term.

K. L. STRETCH

College of Advanced Technology,
Gosta Green, Birmingham 4.

CRYSTALLOGRAPHY

'Effective' Ionic Charge in Crystals

A RECENT communication by Mooser and Pearson[1] has directed attention to the confusion which can result from two distinct usages of the term 'ionic'. As an example they cite discussions of the bonding in zinc blende, where the tetrahedral bonds may be said to arise from resonance involving (i) $Zn^{2-}S^{2+}$ and (ii) $Zn^{2+}S^{2-}$. The first is the extreme covalent structure which gives each atom four valence electrons; the second is conventionally called the fully ionic structure since both atoms have closed-shell configurations. As Mooser and Pearson point out, both (i) and (ii) are physically ionic, and the convention which describes 'neutral bonded' Zn^0S^0 as 50 per cent ionic, because it involves equal resonance contributions from (i) and (ii), can be most misleading.

The question of the ionic charge in compounds isomorphous with zinc blende, a class which includes several important semi-conductors, has been much discussed, since for such purposes as energy-band calculations or the calculation of electronic levels at a vacancy it is necessary to know the electrostatic potential in the crystal. There is considerable confusion in the literature on the question of how this ionic charge may be measured. The confusion is due partly to the situation to which Mooser and Pearson have directed attention, but is also a result of the use of the term 'effective ionic charge'. Since these authors also use this expression without defining it, their timely article will not decrease the confusion on this point. As an example of the way in which effective ionic charge can be determined physically, they instance the dipole moment μ of a hydrogen halogenide molecule for which the nuclear separation is r_0. The effective ionic charge is then taken to be $Ze = \mu/r_0$, from which it is apparent that the definition of effective ionic charge which they intend may be "the value for the ionic charge deduced from a physical measurement on the basis of an over-simplified model or theory". The purpose of the present communication is to point out that the effective charge which can be deduced from certain physical measurements on crystals is clearly not the ionic charge, although it has been used as such[2].

I shall confine attention to crystals isomorphous with zinc blende. Methods of measuring the ionic charge which have been suggested may be classed as: (*a*) miscellaneous, (*b*) X-ray diffraction, and (*c*) those depending for their interpretation on the theory of lattice dynamics. Under (*a*) we may instance studies of microcleavage[3], precise measurements of the wave-lengths of the $K\alpha_1$ and $K\alpha_2$ lines of sulphides[4], and paramagnetic resonance experiments[5] on zinc blende–manganese. None of these gives a quantitative result. Accurate X-ray measurements on zinc blende have been made by Jumpertz[6], who claims to have shown that the zinc atom carries a charge of $1\cdot29|e|$. This result can be disregarded since it was obtained by deriving individual atomic scattering factors $f(s)$ from the measured structure factors, and is therefore entirely dependent on the way in which the empirical curve for $f_{\mathrm{Zn}}(s)$ is extrapolated to $s = 0$.

The ionic charge can in principle be determined unambiguously by X-ray diffraction only when a boundary can be drawn around the ion so as to pass through regions of the unit cell in which the electron density is small compared with the reciprocal of the volume enclosed. This will seldom, if ever, be the case: it can never be the case in a crystal in which there is appreciable covalent bonding. It may, of course, be possible to measure the charge in some arbitrarily specified volume, and to relate this to the ionic charge on a theoretical basis.

There remain the methods which we may class as 'lattice dynamical'. These include the comparison of measured and calculated cohesive energy, dielectric constant, elastic constants, piezoelectric modulus and infra-red absorption and reflectivity. The theory involves several approximations; in particular it is usually assumed either that the electronic polarizability of ionic crystals can be neglected, or that the electronic dipole moment of an atom is directly proportional to the Coulomb field[7]. On either assumption; (*a*) the elastic constants C_{11}, C_{12}, C_{44}; (*b*) the piezoelectric modulus e_{14}; (*c*) the difference $\varepsilon_s - \varepsilon_e$ between static and high-frequency dielectric constants: (*d*) the ratio ω_L/ω_T of the frequencies of longitudinal and transverse optic modes of vibration (which determines the width of the band of high reflectivity in the infra-red); (*e*) the occurrence of infra-red absorption at the frequency ω_T, all involve the ionic charge Ze. Either assumption, however, leads to unsatisfactory results when the frequencies of the normal modes of vibration are calculated for alkali halides, and results in considerably better agreement with experiment[8] are obtained for sodium iodide if it is assumed that the dipole moments also depend on the short-range exchange forces between the atoms, and conversely. Similar calculations have recently been made[9] for crystals of the zinc blende type, and show that the charge determining (*c*), (*d*) and (*e*) is an effective charge $Z'e$ which differs from the ionic charge by an amount which depends on the difference of the electronic polarizabilities of the two atoms concerned, and on the values of parameters describing the short-range interaction, as for the alkali halides. It is therefore possible to have $Z = 0$, but $Z' \neq 0$; that is, for a physically non-ionic crystal to exhibit properties which have hitherto been regarded as a test of ionicity. This unexpected conclusion is confirmed by the recent work of Tolpygo[10], whose earlier work in this field[11] has been overlooked in more recent theories[12] of the relation between Z and Z' in alkali halides. In general the piezoelectric modulus is found not to be directly proportional to Z, although it may be under certain conditions. Only the elastic constants C_{11} and C_{12} are found to be independent of the electronic polarizability and of the parameters which describe the short-range interaction between electronic dipole moments and atomic displacements. Detailed results will not be given here; but it seems worth mentioning that the values of the elastic constants of gallium arsenide and of similar III–V compounds, and a comparison of these values with those of germanium, indicate that $\mathrm{Ga^0As^0}$ is a much better physical description than is $\mathrm{Ga^+As^-}$, and that the values of Z' which have been reported[13] are not inconsistent with this conclusion.

Finally, we may note that, in at least one other branch of solid-state physics, ionic charges are deduced by gross over-simplification. It appears to be generally assumed that the spontaneous polarization of a ferroelectric is given by:

$$P = \sum_j Z_j e u_j$$

where u_j is the displacement of the jth atom from its position in the unit cell of the phase of higher symmetry. It has, however, been shown[14] that the charges defined by this equation are apparent charges the relation of which to the ionic charges is quite complicated.

W. COCHRAN

Crystallographic Laboratory,
Cavendish Laboratory,
Cambridge.

[1] Mooser, E., and Pearson, W. B., *Nature*, **190**, 406 (1961).
[2] Birman, J. L., *Phys. Rev.*, **109**, 810 (1958).
[3] Wolff, G. A., and Broder, J. D., *Acta Cryst.*, **12**, 313 (1959).
[4] Faessler, A., and Goehring, M., *Z. Phys.*, **142**, 558 (1955).
[5] van Wieringen, J. S., *Disc. Farad. Soc.*, **19**, 118 (1955).
[6] Jumpertz, E. A., *Z. Electrochem.*, **59**, 425 (1955).
[7] Born, M., and Huang, K., *Dynamical Theory of Crystal Lattices* (Oxford Univ. Press, 1954).
[8] Woods, A. D. B., Cochran, W., and Brockhouse, B. N., *Phys. Rev.*, **119**, 980 (1960).
[9] Cochran, W. (to be published). Cowley, R. A. (to be published).
[10] Tolpygo, K. B., *Fizika Tverdogo Tela*, **2**, 2655 (1960). Translation: *Soviet Physics, Solid State*, **2**, 2367 (1961).
[11] Tolpygo, K. B., *J. Exp. Theoret. Phys.* (U.S.S.R.), **20**, 497 (1950).
[12] Dick, B. J., and Overhauser, A. W., *Phys. Rev.*, **112**, 90 (1958).
[13] Picus, G., Burstein, E., Henvis, B. W., and Haas, M., *J. Phys. Chem. Solids*, **8**, 282 (1959).
[14] Cochran, W., *Advances in Physics*, **9**, 387 (1960).

Uranium Disilicide

A URANIUM–SILICON phase with a hexagonal *C*32-type structure, described by Zachariasen[1] as stoichiometric β-$\mathrm{USi_2}$ and thought by Kaufmann, Cullity and Bitsianes[2] to be deficient in silicon, was shown by us[3] to be $\mathrm{USi_{1\cdot67}}$($\mathrm{U_3Si_5}$). At the same time attention was directed to the existence of another phase with the *C*32 ($\mathrm{AlB_2}$)-type structure which was shown to be stoichiometric $\mathrm{USi_2}$. This phase is isostructural with β-$\mathrm{ThSi_2}$.

A further phase described as α-$\mathrm{USi_2}$ by both Zachariasen and Kaufmann *et al.* has been found by us to occur at approximately 65 atomic per cent silicon. Arc-melted alloys containing 66·7 atomic per cent silicon consisted of this phase and $\mathrm{USi_3}$, in the ratio of about 5 : 1 by weight. These results suggest that this phase is non-stoichiometric and has the formula $\mathrm{USi_{1\cdot88}}$. This composition corresponds to one vacant silicon lattice-site in each pair of tetragonal structure cells.

Further experiment has shown that hexagonal $\mathrm{USi_2}$, prepared by reaction of the elements in liquid bismuth, is stable only up to 450° C. Above this temperature $\mathrm{USi_3}$ is obtained as the product of reaction in the liquid. When hexagonal $\mathrm{USi_2}$ is heated alone above 450° C., mixtures of $\mathrm{USi_{1\cdot88}}$ and $\mathrm{USi_3}$ are obtained. The relative proportions of the two phases are the same as in the arc-melted alloy

Table 1

Phase	Composition in at. percentage silicon	Structure type and space group	Structure cell dimensions (Å.)
$USi_{1\cdot67}$	62·5	Hexagonal defect $C32$-type $C6$/mmm.	$a_0 = 3\cdot843 \pm 0\cdot001$ $c_0 = 4\cdot069 \pm 0\cdot001$
$USi_{1\cdot88}$	65	Tetragonal defect C_c-type I 4/amd.	(i) heat treated below 650° C. $a_0 = 3\cdot930 \pm 0\cdot001$ $c_0 = 14\cdot06 \pm 0\cdot01$ (ii) arc-melted alloy $a_0 = 3\cdot948 \pm 0\cdot002$ $c_0 = 13\cdot67 \pm 0\cdot01$
USi_2	66·7	Hexagonal $C32$-type $C6$/mmm.	$a_0 = 4\cdot028 \pm 0\cdot001$ $c_0 = 3\cdot852 \pm 0\cdot001$

containing 66·7 atomic per cent silicon. No transformation comparable with that of β- to α-$ThSi_2$ [3,4] has been found as a result of heat-treating hexagonal USi_2.

Slight differences have been noted between the X-ray powder patterns of $USi_{1\cdot88}$ heat-treated above and below 650° C. This is considered to be a consequence of differences in ordering of the silicon atoms on the available lattice sites.

Crystallographic data for the three related uranium–silicon phases are summarized in Table 1.

This work was supported by the United Kingdom Atomic Energy Authority, Harwell, and we wish to thank the Director for permission to publish the results.

ALLAN BROWN*
J. J. NORREYS

Central Research Laboratories,
The General Electric Co., Ltd.,
Hirst Research Centre,
Wembley.

---

* Present address: Institute of Chemistry, Uppsala University, Uppsala, Sweden.

[1] Zachariasen, W. H., *Acta Cryst.*, **2**, 94 (1949).
[2] Kaufmann, A. R., Cullity, B., and Bitsianes, G., *Trans. Amer. Inst. Min. Met. Eng.*, **209**, 203 (1957).
[3] Brown, A., and Norreys, J. J., *Nature*, **183**, 673 (1959).
[4] Brown, A., and Norreys, J. J., *J. Inst. Met.*, **89**, 238 (1961).

GEOCHEMISTRY

Measurement of the Thorium Content of Natural Materials by a γ-Ray Counting Method

RECENT work by S. Raboy and C. C. Traul[1] on the background of sodium iodide scintillation counters suggested to us that it should be possible to measure the thorium content of a rock directly by counting the 2·62 MeV. γ-ray emitted in the decay of thorium C'' (^{208}Tl) to stable lead-208, since this γ-ray is more energetic than any in the uranium series. Through the generosity of the American Government a large sodium iodide crystal and suitable photomultiplier and the necessary electronic equipment were available to us to try out the method.

In an ordinary igneous rock the thorium content is usually about three times the uranium content. Fig. 1 shows the high-energy end of the γ-ray spectra of the thorium and uranium families, there being three times as much thorium as uranium. It will be seen that by using a wide-window single-channel analyser with its lower gate set at channel 25 on the spectrum, and its upper gate at channel 39, practically only the thorium C'' γ-ray will be counted. It was found experimentally that for a typical rock with a thorium/uranium ratio of three about 3 per cent of the net count in the chosen window was due to the uranium family. If the uranium content of the rock is known, the necessary correction to the observed net count can readily be made.

The sodium iodide crystal used was 4⅝ in. in diameter and 1·8 in. deep, coupled to an E.M.I. 9530*B* fused silica photomultiplier with a cathode 4·4 in. in diameter. A special container was made so that the bottom and sides of the crystal could be surrounded by a ½-in. layer of powdered material. This container held about 600 gm. of powdered rock.

It was found that there was a large thorium count in the background, apparently due to the thorium content of the granite walls of the building. By surrounding the crystal and photomultiplier with 4 in. of lead this was practically eliminated, but a trace was left, probably due to the small amount of thorium in the materials inside the shield.

The apparatus had to be standardized by using a sample of known thorium content. This was made by mixing pure thorium oxide containing 2 gm. of thorium with 1 kgm. of a sand of low thorium content. The oxide came from Joly's laboratory and is at least 50 years old. Since mesothorium-1 is the longest-lived daughter element in the thorium family with a half-life of 6·7 years, the oxide must be practically in radioactive equilibrium. It was found that a certain quantity of thoron escaped from this standard, consequently the standard sample holder was hermetically sealed. The count obtained per gm. of thorium is about 1,370 counts/min. 1 gm. thorium-232 produces $2\cdot16 \times 10^5$ disintegrations/min., but since only 34 per cent of these lead to the thorium C'' branch, the total theoretical 2·62-MeV. γ-ray emission should be $7\cdot35 \times 10^4$/min. Thus only about 2 per cent of this theoretical total is counted in the 2·62-MeV. peak. This result is in reasonable agreement with the efficiency as determined by K. J. Van Oostrum and A. C. Meijer[2] for a large sodium iodide crystal and E.M.I. photomultiplier.

The background is about 4·0 counts/min., and 600 gm. of a rock containing 10^{-5} gm. thorium/gm. gives a net count of 8·2/min., that is, about double the background count. The minimum quantity of thorium which can be measured is assumed to be that which will give a net count equal to four times its standard deviation. This gives a value of $0\cdot04 \times 10^{-5}$ gm. thorium/gm. of sample if 600 gm. of the specimen is counted for 20 hr.

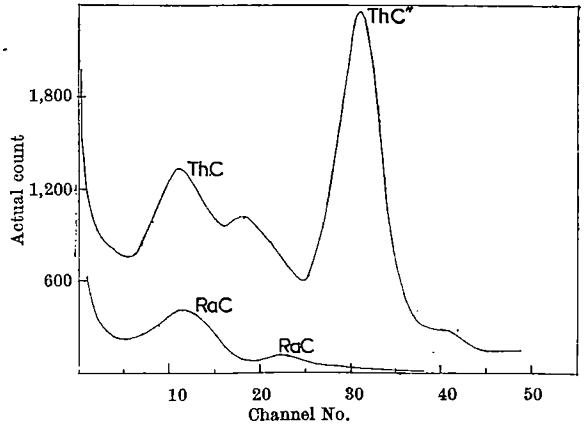


Fig. 1

Table 1

Material	Thorium 10^{-5} gm./gm.	
	γ-Ray method	Flow method
Average for six Deccan basalts	$0\cdot25 \times 10^{-5}$	$0\cdot46 \times 10^{-5}$
Basalt from Idaho Falls, Snake River, Oregon	$0\cdot19 \times 10^{-5}$	$0\cdot50 \times 10^{-5}$
Rapakivi Granite, Finland	$3\cdot03 \times 10^{-5}$	$6\cdot00 \times 10^{-5}$
Eclogite, Eppenreuth, Bavaria	$< 0\cdot03 \times 10^{-5}$	$0\cdot30 \times 10^{-5}$
Wicklow Granite, Ireland	$1\cdot15 \times 10^{-5}$	—
Globigerina Ooze, 2,493 fm., 48° 17′ N., 39° 49′ W.	$0\cdot32 \times 10^{-5}$	—
Globigerina Ooze, 720 fm. 38° 46′ W., 28° 31′ N.	$0\cdot41 \times 10^{-5}$	—

There are two chief objections to this method: first, a comparatively large sample is required; secondly, the requisite apparatus though entirely standard is moderately expensive. Unfortunately, most of the specimens the thorium content of which had already been measured by flow methods were too small to use. Some of the results we have obtained so far are given in Table 1.

The values given for the flow method were those obtained on the same specimens before 1930 and confirmed the modern view that such values were too high. The results for the Wicklow granite and for the Deccan basalts agrees very well with the modern estimate for the average thorium contents of such rocks.

We include the figures for the Globigerina oozes, since they seem to show that thorium is present in the oceanic deposits. The first specimen is situated west of the Atlantic Ridge, and the second is on the ridge and quite close to the Azores. It will be noticed that the shallower water specimen gives a considerably higher value These samples were dredged up by the cable ship *Faraday* in 1907. E. Picciotto and S. Wilgain[3] have given values varying from $0\cdot41 \times 10^{-5}$ gm. thorium/gm. to $0\cdot85 \times 10^{-5}$ gm. thorium/gm. for red clays from the Central Pacific cores of the Swedish Deep-sea Expedition 1947–48.

In conclusion, we may mention that P. M. Hurley[4] has also used a γ-ray spectrometer method for estimating the thorium content of rocks. He, however, worked at the lower-energy end of the spectrum, which means that the calculation of the results is much more complicated due to the greater contribution of the uranium series at these levels. It is of interest to note, however, that his result of $0\cdot28 \times 10^{-5}$ gm. thorium/gm. for a Deccan basalt agrees very closely with ours.

J. H. J. POOLE
F. N. BYRNE

Physical Laboratory,
Trinity College,
Dublin.

---

[1] Raboy, S., and Traul, C. C., *Nuc. Inst. and Methods*, **9**, 145 (1960).

[2] Van Oostrum, K. J., and Meijer, A. C., *Nuc. Inst. and Methods*, **10**, 31 (1961).

[3] Picciotto, E., and Wilgain, S., *Nature*, **173**, 632 (1954).

[4] Hurley, P. M., *Bull. Geol. Soc. Amer.*, **67**, 395 (1956).

METALLURGY

Resistivity Changes due to Formation of *G. P.* Zones (Clusters rich in Solute)

An anomalous increase in resistivity has been observed during formation of *G. P.* zones (clusters rich in solute) in several aluminium alloys[1,2]. Mott[3] suggested that this effect may be due to a critical scattering of the conduction electrons when the zone grows to dimensions of the wave-length of the conduction electrons. Matyas[4] showed on theoretical grounds that a maximum should occur for the resistivity change during zone formation.

The changes in resistance during zone formation in an aluminium–zinc alloy were discussed extensively in a recent paper by Panseri and Federighi[5]; the authors assumed that a zone of critical size is associated with the peak in resistance, and that the magnitude of the peak goes up with increasing number of zones.

The object of our research has been to test whether a zone of definite size is associated with the maximum in resistance which occurs during ageing. An alloy of aluminium–5·3 at. per cent zinc has been studied, using both resistance measurements and small-angle scattering of X-rays. For the resistance measurements, the resistance of a specimen 0·005 in. thick was measured at the temperature of liquid nitrogen. The specimen was quenched from 300° C. into water at 25° C. and aged at 0° C. or 30° C. Fig. 1 shows the variation in resistance with time. The resistance maximum occurs at 2 min. for ageing at

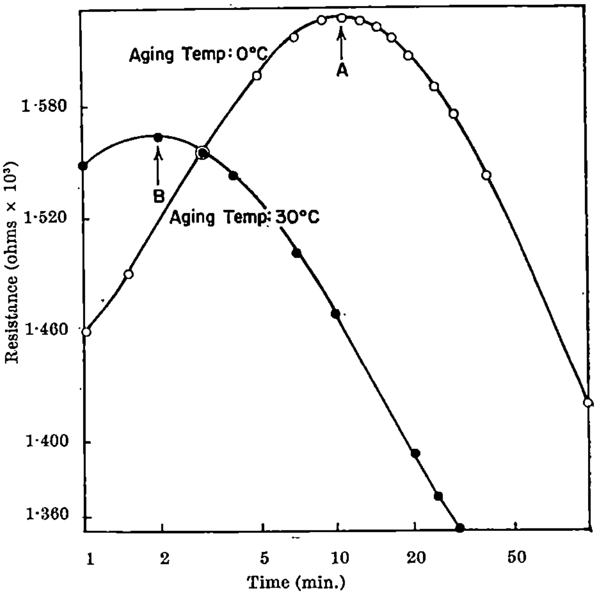


Fig. 1. Resistance in ohms versus log time for ageing at (*A*) 0° C. and (*B*) 30° C. after quenching from 300° C.; aluminium–5·3 at. per cent zinc

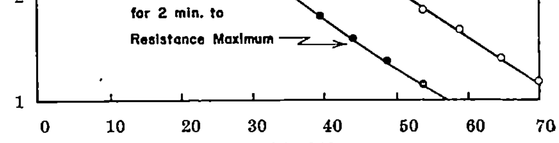


Fig. 2. Log intensity in counts/sec. versus ε^2, where ε is the scattering angle, 2θ, in radians. The specimens were aged to the time of the resistance peaks at (*A*) 0° C. and (*B*) 30° C.; aluminium–5·3 at. per cent zinc

30° C., and at 11 min. for ageing at 0° C. The magnitude of the peak at 0° C. is some 4 per cent higher than that of the peak at 30° C. Fig. 2 shows the small-angle scattering obtained from a specimen 0·005 in. thick of the same alloy and given treatments identical with the specimens used for resistance measurements. Ageing of the specimen was halted at the time of the peak in resistance by bringing it to −195° C. (liquid nitrogen), at which temperature the X-ray data were obtained. Balanced filters were used to obtain monochromatic copper $K\alpha$ radiation; step-scanning was employed due to the long exposures required. (The results are plotted as log I in counts/sec. versus ε^2, where ε is the scattering angle, 2θ, in radians.) The slope of the linear region to the right of the maximum gives the radius of gyration, R_g, of the spherical zone[6]. R_0, the zone radius, is given by $R_0 = (5/3)^{1/2} R_g$. R_0 is an average which emphasizes the largest zones, and since the scattering curves are not linear to the right of the peak (Fig. 2) there must be a range of sizes. The average radius can be expected to be about $\frac{1}{2} R_0$ for any reasonable distribution ; this is 5 Å. for both the 0° C. and 30° C. resistance peaks. The maximum probable uncertainty in radius due to counting statistics and stability of X-ray output and counter was about 3 per cent.

The resistance maxima are then associated with zones of an average size of 10 Å. in diameter. Matyas's equation applied to the case investigated predicts about 7 Å. for the zone diameter. This is in reasonable agreement with what has been observed here experimentally ; the results confirm the relation between the resistance maximum and zone size.

Re-ageing after reversion yields sizes closely the same as those quoted above, despite the fact that the ageing kinetics are different. These results will be part of a more detailed publication.

This investigation was sponsored by the U.S. Air Force Office of Scientific Research under contract No. *AF* 49(638)–524.

H. HERMAN
J. B. COHEN

Department of Materials Science,
The Technological Institute,
Northwestern University,
Evanston, Illinois.

[1] Federighi, T., and Passari, L. *Acta Met.*, **7**, 422 (1959).
[2] Gayler, M. L. V., *J. Inst. Metals*, **60**, 249 (1937).
[3] Mott, N. F., *J. Inst. Metals*, **60**, 267 (1937).
[4] Matyas, Z., *Phil. Mag.*, **40**, 324 (1949).
[5] Panseri, C., and Federighi, T., *Acta Met.*, **8**, 217 (1960).
[6] Guinier, A., *Ann. Phys.* (Paris), **12**, 90 (1939).

Porous Nickel Oxide

IN the course of an investigation of the oxidation properties of nickel strip used in the manufacture of electron-tube cathodes, a phenomenon was observed which may be of general interest.

Two samples of nickel in the form of strip 0·002 in. thick were oxidized by heating in air for 5 min. at 1,000° C. Dilute nitric acid was used to dissolve the metal and provide small pieces of coherent oxide film about 0·0001 in. thick. Under microscopic examination, the film from one sample was found to be apparently homogeneous and entire (Fig. 1*a*), while the other contained pores or holes about 1μ in diameter (Figs. 1*b*, *c*). The only distinguishing characteristic of the strip which produced this porous oxide film was that it contained about 0·02 per cent aluminium ; the other strip contained about one-tenth of that amount.

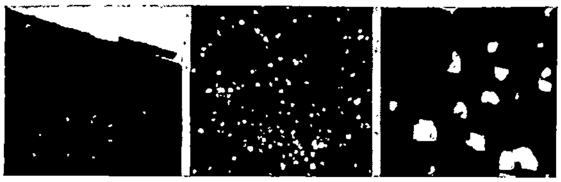


Fig. 1. Electronmicrographs of nickel oxide films formed on: (*a*) nickel containing 0·002 per cent aluminium (× *c*. 390); (*b*) nickel containing 0·02 per cent aluminium (× *c*. 390); (*c*) nickel containing 0·02 per cent aluminium (× *c*. 1,880)

It is possible that small crystals of alumina are formed similar to those found by Allison and Samelson[1] when they oxidized nickel in wet hydrogen. These crystals may have fallen out of the nickel oxide layer when the nickel was dissolved, leaving the sharp-cornered holes in the oxide.

I am indebted to Dr. E. P. Bertin for the electron-micrographs.

I. S. SOLET

Radio Corporation of America,
Electron Tube Division,
Harrison, New Jersey.

[1] Allison, H. W., and Samelson, H., *J. App. Phys.*, **30**, 1419 (1959).

CHEMISTRY

Infra-Red Spectra of Water-soluble Carbonates

INFRA-RED spectra of some carbonates have been reported by Hunt, Wisherd and Bonham[1], Underwood, Toribara and Neuman[2] and by Miller and Wilkins[3]. Hunt *et al.* suggested that the position of the band near 11μ was a function of the atomic weight of the cation, and also pointed out that Miller and Wilkins's work substantiated this suggestion with the exception of lithium carbonate. We have evidence that this is not so. Further, Underwood *et al.* reported the 11μ band for sodium and potassium at different positions from that reported by Miller and his workers. Although they indicated their results were in general agreement with Miller and Wilkins, no attempt to point out or explain this discrepancy was made. Finally, the role of water in influencing both band positions and broadness appears to have been neglected in interpreting spectra of water-soluble inorganic compounds and this will be discussed here.

We find that for the soluble carbonates (sodium, potassium, rubidium and cæsium), this band appears at 11·4μ when they are in the anhydrous state and 11·6μ when they are hydrated. The band is therefore not a function of the atomic weight of the cation (at least for the soluble carbonates) but of the degree of hydration. As the degree of hydration is increased the band decreases in intensity and disappears when complete solution is obtained. No attempt was made to study which hydrate exists when the band shifts from 11·4 to 11·6μ, but it has been found that only slight traces of water are required, especially for the more soluble carbonates of rubidium and cæsium, to produce this shift. Decius[4] has related this position of the band for calcium carbonate to its crystal structure.

The band at 6·95μ for the anhydrous carbonates is shifted to 7·2 when complete solution is obtained. The intermediate states of hydration, however, give both bands, and, depending on the concentration of water, one appears as a shoulder on the other, or they are broadened into one band appearing midway between the two. Thus the broadness and position of this band depend to a great extent on the water content, a fact apparently not recognized by many workers: although Miller and Wilkins, in their work, suggested that this possibility existed, they carried out no further investigation of it.

Attention should be directed to a band in the 9–10μ region for some of the carbonates. This band appears in strontium and lead carbonates in the work reported by Hunt *et al.* and also appears at 9·55μ in the spectra of sodium, potassium, rubidium and cæsium carbonate. According to Gatehouse *et al.*[5], this band appears only for carbonates having the aragonite structure.

These results were obtained in 'Nujol' and fluorcarbon mulls, potassium bromide pellets, powders dispersed on salt plates (for sodium carbonate only) and using physically sorbed water of synthetic zeolites as a solvent. This latter technique is useful for studying varying concentrations of water on carbonate spectra and was carried out in apparatus described elsewhere[6]. The problem of exchange in all these media was also studied and will be reported later.

We thank David Teloh for recording some of the spectra used in this work. One of us (R. P.) received a grant from the Petroleum Research Fund of the American Chemical Society.

HERMAN A. SZYMANSKI
RICHARD POVINELLI

Department of Chemistry,
Canisius College,
Buffalo 8, N.Y.

[1] Hunt, J. M., Wisherd, M. P., and Bonham, L. C., *Anal. Chem.*, 22, 1478 (1950).
[2] Underwood, A. L., Toribara, T. Y., and Neuman, W. F., *J. Amer. Chem. Soc.*, 77, 317 (1955).
[3] Miller, F. A., and Wilkins, C. H., *Anal. Chem.*, 24, 1254 (1952).
[4] Decius, J. C., *J. Chem. Phys.*, 22, 1946 (1954); 23, 1290 (1955).
[5] Gatehouse, B. M., Livingstone, S. E., and Nyholm, R. S., *J. Chem. Soc.*, 3137 (1958).
[6] Szymanski, H. A., Povinelli, R., Stamires, D., and Lynch, G., *Anal. Chem.*, 31, 2110 (1959).

Electron Spin Resonance of Adenosine Triphosphate

IT has been suggested that in adenosine triphosphate (ATP) the phosphate chain and the adenine are linked by the pentose, so that the two terminal phosphates touch the two nitrogen atoms at positions 6 and 7 in the adenine ring[1]; and that, since triphosphate compounds might be electron acceptors and adenine is a good electron donor, the adenine group might donate an electron to the triphosphate group[2]. I. Isenberg and A. Szent-Györgyi[2] obtained an electron spin resonance signal from ATP (di-sodium salt) which they attributed to internal charge transfer between the adenine and the triphosphate. They also suggested that there was a possibility that the signal could be due to a transition metal impurity in the form of a transition metal – ATP complex. In view of the great importance of ATP in living processes, we have re-examined the electron spin resonance of this compound to decide between these possibilities.

The electron spin resonance spectrometer used in these experiments operates at 9·42 kMc./s. and has a conventional phase-sensitive detector system with a field modulation of 280 c./s. In these experiments the spectrometer sensitivity is 10^{13} ΔH spins, where ΔH is the line-width of the resonance signal of the material under investigation.

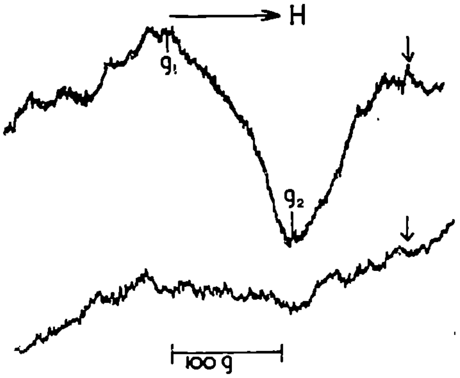


Fig. 1. First derivative of electron spin resonance absorption. Upper curve is commercial ATP, lower curve is purified ATP. The arrow indicates the free electron resonance

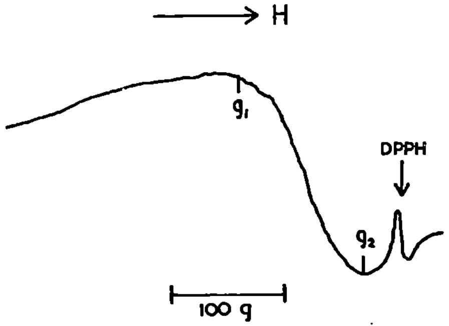


Fig. 2. First derivative of the electron spin resonance absorption of copper phthalocyanine

When commercial ATP (di-sodium salt) similar to that used by I. Isenberg and A. Szent-Györgyi was examined in the spectrometer, an anisotropic line of several hundred gauss line-width was obtained (Fig. 1). The full line shape could not be recorded as the largest field swept was 460 ± 20 gauss. Two arbitrary g values, $g_1 = 2{\cdot}128 \pm 0{\cdot}010$ and $g_2 = 2{\cdot}064 \pm 0{\cdot}004$, where g_2 is approximately equal to $g_\perp$ and g_1 is related to $g_\parallel$, were measured. These values are significantly higher than the free electron value of 2·0023. The position and shape of the signal indicated the presence of copper bound in some way to the nitrogen atoms of the adenine group. The ATP was therefore analysed for copper, which was estimated colorimetrically at 435 mμ with diethyldithiocarbamate[3], and the amount found was 75·2 ± 4·0 μgm. copper/gm. ATP.

Copper phthalocyanine was prepared to test whether copper bonded to nitrogen was responsible for the electron spin resonance signal observed in ATP. The copper phthalocyanine gave a spectrum of the same anisotropy as the ATP spectrum. Two arbitrary g values were measured as before, $g_1 = 2{\cdot}087 \pm 0{\cdot}004$ and $g_2 = 2{\cdot}025 \pm 0{\cdot}001$. The differences between the values of g_1 and g_2 for ATP and copper phthalocyanine are the same, which suggests that the resonance signal observed in ATP is due to copper, the differences in the absolute values of g_1 and g_2 in the two cases being due to slight differences in the chemical bonding of the copper.

To confirm this suggestion, a known weight of copper phthalocyanine, diluted with aluminium oxide, was placed in a quartz tube, so that the length of the sample was the same length as the ATP sample, and a spectrum recorded. The amount of copper present in the ATP was calculated from the relative signal sizes, assuming that the shapes of both signals were the same. The value obtained was 83·5 ± 13·5 μgm. copper/gm. ATP, which agrees within experimental error with the figure of 75·2 ± 4·0 μgm. copper/gm. ATP obtained by the diethyldithiocarbamate method. A second sample of commercial ATP gave the values 71·5 ± 4·0 μgm. copper/gm. ATP by the diethyldithiocarbamate method and 70·4 ± 10·1 μgm. copper/gm. ATP from electron spin resonance measurements. A sample of ATP purified by the method of Dubois, Albaum and Potter[4] was found to contain 17·8 ± 4·0 μgm. copper/gm. ATP by the diethyldithiocarbamate method, which agrees with that obtained by electron spin resonance measurements (17·8 ± 2·9 μgm. copper/gm. ATP).

These results show that the electron spin resonance signal observed in ATP is most probably due to copper impurity present as a metal-ATP complex. Other work indicates that the complex is very stable (Russell, D. B., unpublished work) and that ATP forms stable complexes with iron (Russell, D. B., unpublished work) and manganese[5]. If charge transfer does occur between the adenine and the triphosphate, there is insufficient unpairing of electron spin for this to be observed on the electron spin resonance spectrometer used.

We thank Dr. P. J. Warren, of the Department of Chemical Pathology of this hospital, who estimated the copper content of the ATP samples by the diethyldithiocarbamate method.

This work was supported by the British Empire Cancer Campaign.

D. B. Russell
S. J. Wyard*

Physics Department,
London Bridge, S.E.1.

---

* Present address: Biophysics Department, Stanford University, Stanford, California, U.S.A.

[1] Szent-Györgyi, A., *Introduction to a Submolecular Biology* (Academic Press, 1960).

[2] Isenberg, I., and Szent-Györgyi, A., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1232 (1959).

[3] Eden, A., and Green, H. H., *Biochem. J.*, **34**, 1202 (1940).

[4] Dubois, K. P., Albaum, H. G., and Potter, V. R., *J. Biol. Chem.*, **147**, 699 (1943).

[5] Cohn, M., and Townsend, J., *Nature*, **173**, 1090 (1954).

Co-ordination of the Thiocyanate Group in Inorganic Compounds

There has been a rapid growth of interest in the study of the thiocyanate ion in inorganic compounds.

Previously we have investigated the vibrational behaviour of the thiocyanate group in several inorganic compounds[1]. Similar investigations have been carried out independently and with different compounds by Chamberlain and Bailar[2], Kinell and Strandberg[3] and Mitchell and Williams[4]. However, the last two authors have limited their work to the CN-stretching vibration.

There are three ways in which the di-functional thiocyanate ion can be attached to a metal cation, namely, *M*–SCN, *M*–SCN–*M* and *M*–NCS. They can be differentiated by the frequencies assigned to the fundamental stretching modes of the triatomic group, approximating to CN- and CS-stretching. In fact, we find that the CS-stretching frequency is more diagnostic, particularly in the absence of *M*–SCN–*M* 'bridges'. Moreover, the observation of the 5μ region, where the CN bands occur, is often particularly difficult when working with solid samples.

Table 1. Fundamental Stretching-Frequencies (cm.$^{-1}$) of the SCN Group in Some Thiocyanate Complexes*

Compound	ν_3 (SCN)	ν_1 (SCN)	Structure	Ref.
$Hg(SCN)_2$	2,118	716	—	*a*
$K_2Hg(SCN)_4$	2,115	716	Hg–S	*b*, *k*
$K_2Co(NCS)_4.4H_2O$	2,074 bd	822, 818	Co–N	*b*
$K_2Pt(SCN)_4$	2,089, 2,120	697 700 sh, 690 sh	Pt–S	*b*
$K_2Pd(SCN)_4$	2,118, 2,086 bd	703 707 sh, 696 sh	—	
cis-$Pt(NH_3)_2(SCN)_2$	2,116	703	Pt–S	*c*
trans-$Pd(NH_3)_2(SCN)_2$	2,116, 2,100	701	—	
$Pt(PEt_3)_2(NCS)_2$	2,113	855	—	
$Pt(PPr^i)_2(NCS)_2$	2,106	854	—	
$Pd(PEt_3)_2(NCS)_2$	2,089	846, 842 sh	—	
$Pd(PPr_3^i)_2(NCS)_2$	2,094	852 sh, 844	—	
$Zn(N_2H_4)_2(NCS)_2$	2,060 bd	790	Zn–N	*d*
$Zn\ py_2(NCS)_2$	2,096, 2,075	847	—	
$Mn(N_2H_4)_2(NCS)_2$	2,065	794	Mn–N	*d*
$Ni[SC(NH_3)_2]_2(NCS)_2$	2,080	810	Ni—N	*e*
$Ni[CH_3S(CH_2)SCH_3](NCS)_2$	2,085	805	—	*f*
$Ni(PR_3)_2(NCS)_2$	2,095	860	—	*j*
$K_2Pt(SCN)_6$	2,125, 2,115	694	Pt–S	*b*, *l*
$Cu\ py_4(NCS)_2$	2,094	824	—	
$Co\ py_4(NCS)_2$	2,066	800	Co–N	*g*
$Ni(NH_3)_4(NCS)_2$	2,102	778	Ni–N	*h*
$Ni\ py_4(NCS)_2$	2,080	798	Ni–N	*g*
$(NH_4)[Cr(NH_3)_2(NCS)_4]$	2,110, 2,042	828	Cr–N	*i*

* Spectra were observed in 'Nujol' mulls and measured with sodium chloride and calcium fluoride optics on Perkin–Elmer spectrometers (21 and 112 respectively).

(*a*) The X-ray structure determination of $Hg(SCN)_2.KSCN$ indicates Hg–S bonds and absence of a bridge structure; for ref. see under (*b*).

(*b*) Zhdanov, G. S., and Zvonkova, Z. V., *Doklady Mezhdunarod. Kongr. Teoret. i Priklad. Khim.*, XIII Kongr. Stockholm (1953), p. 175.

(*c*) See ref. 11.

(*d*) Braibanti, A., private communication.

(*e*) Nardelli, M., Braibanti, A., and Fava, F., *Gazz. Chim. Ital.*, **87**, 1209 (1957). The 810 cm.$^{-1}$ band reported by Yamaguchi, A., *et al.* (*J. Amer. Chem. Soc.*, **80**, 527; 1958) has been assigned by us to the ν_1 vibration.

(*f*) The 805 cm.$^{-1}$ band reported by Sweeny, D. M., *et al.* (*J. Amer. Chem. Soc.*, **77**, 6521; 1955) is assigned here to the ν_1 vibration.

(*g*) Porai-Koshits, M. A., and Antsishkina, A. S., *Kristallografiya*, **3**, 686 (1958).

(*h*) Yukhno, E. K., and Porai-Koshits, M. A., *Kristallografiya*, **2**, 239 (1957).

(*i*) Saito, Y., Takeuchi, Y., and Pepinsky, R., *Z. Krist.*, **106**, 476 (1955).

(*j*) R = Et, Pr^n, Pr^i, *cyclo*hexyl.

(*k*) These results are in good agreement with those reported by Chamberlain and Bailar (ref. 2).

(*l*) Frequencies reported by Chamberlain and Bailar (ref. 2).

Our recent results are summarized in Table 1, together with those for other compounds examined by us[1].

An analysis of the CS-stretching frequencies ν_1(SCN) in compounds of known structure clearly indicates the infra-red criterion for the characterization of the metal-thiocyanate bond. The symmetrical band ν_1 occurs between 690 and 720 cm.$^{-1}$ for the $M \leftarrow$ SCN compounds, and is in the range 780–860 cm.$^{-1}$ for the $M \leftarrow$ NCS compounds. An extension of this criterion to compounds of unknown structure listed in Table 1 leads to the following conclusions, which may be of general interest for class (*b*) metals.

The CS-stretching frequencies found in $[Pd(SCN)_4]^{2-}$ and $[Pt(SCN)_4]^{2-}$ are in agreement with *M*–S bonds, whereas in the complexes with the phosphines the metal must be bound to the nitrogen atom. It is worth noting that we also verified that sulphur binding in $[Pd(SCN)_4]^{2-}$ and $[Pt(SCN)_4]^{2-}$ places SCN^- in the same position as Cl^- in the spectrochemical series. The shift of the ultra-violet absorption bands towards higher frequencies (as compared with the frequencies of the chloride derivatives) is fairly clear (1,500 cm.$^{-1}$) in the case of $Pd(PPr^i_3)_2(NCS)_2$, but is indistinguishable in $Pd(PEt_3)_2(NCS)_2$.

The change from *M*–S to *M*–N bonds when two SCN groups of $[M(SCN)_4]^{2-}$ are replaced by two phosphine molecules is not observed when the same SCN groups are substituted by two ammonia molecules giving $M(NH_3)_2(SCN)_2$. These results indicate that in complexes where SCN is not the sole ligand, the type of co-ordination of the thiocyanate group may depend on the nature of the second ligand partner. There must be a critical point where, with a particular ligand, change in the co-ordination can occur. It should then be possible, in the case of complexes with general formula $M(CNS)_mX_n$, to prepare two structural isomers of the same compound, depending on the correct choice of the X ligand or alternatively by changing the ratio $m : n$. No two such isomers with any particular metal has yet been reported. It has been suggested that the $[Ag(SCN)_2]^-$ ion in solution is an equilibrium mixture having Ag–N as well as Ag–S bonds[5]. However, further evidence to support this point seems necessary.

Previous workers have pointed out that a given metal ion becomes attached to one end of the SCN group rather than the other[6]. The binding is generally through sulphur in those cases where the stability sequence among the halides is $I^- > Br^- > Cl^- > F^-$ (ref. 7) and the change from *M*–SCN to *M*–NCS bonding coincides approximately in the Periodic Table with the change in relative stability from the above sequence to $F^- > Cl^- > Br^- > I^-$ (ref. 4). Some authors have pointed out that this is due to a change in the relative importance of covalent and ionic bonding in the compounds[4,7], whereas others have stressed the importance of the overlap between particular sets of orbitals in explaining the above stability series[8]. We believe that the π-orbitals of the thiocyanate group should not be neglected as a factor in the stabilization of the *M*–S bond.

There are two sets of antibonding π-orbitals in the thiocyanate ion and both are localized on the sulphur atom ; use of the empty *d*-orbitals of sulphur can confer further acceptor properties on the sulphur end of the group. Interaction of d_π electrons on the metal atom with these empty orbitals will result in additional stability of the *M*–S bond. Whether or not this factor of additional stability will be of importance will depend on the availability of the d_ε electrons of the metal and on their relative energy as compared with that of the antibonding π-orbitals of the thiocyanate.

The change from *M*–S to *M*–N bonds for the complexes of platinum in Table 1 can be explained by considering that strong π-electron acceptors, as tri-substituted phosphines are believed to be, can make the d_ε electrons less available for binding with the π-orbitals of SCN, that is, for a bond on the sulphur end.

In the cases mentioned the effect of the phosphine molecules is to change the co-ordination from *M*–S to *M*–N. However, there are also compounds with the same ligands where the thiocyanate group still retains a distinct bridge configuration. In such cases one finds that the *M*–S bond is weakened, whereas the *M*–N bond apparently is not affected. A Pt–S bond-length (2·44 Å.) greater than the sum of the atomic radii (2·36 Å.) has been found by Owston and Rowe in $[Pt_2(SCN)_2Cl_2(PPr)^n_3)_2]$, and this result has been explained with somewhat similar arguments to those adopted above[9]. Also, in the case of $AgSCN.PPr^n_3$, the Ag–S bond-lengths are considerably greater (2·75 Å.) than expected (2·43 Å. in AgSCN)[10]. These results can be compared with the 'normal' bond-lengths of 2·30 and 2·27 Å. reported in *cis*- and *trans*- $Pt(NH_3)_2(SCN)_2$ (ref. 11).

A broad generalization based on the few cases discussed here is obviously not possible ; more experimental material is required in order to appreciate the factors affecting the change from *M*–SCN to *M*–NCS bonding. Nevertheless, our observations suggest that π-bonding may be of importance in balancing the equilibrium $M\text{–NCS} \rightleftarrows M\text{–SCN}$ far to the right for cations such as Pd(II) and Pt(II). If our interpretation is correct, the same change in co-ordination of the thiocyanate as observed for the complexes with the phosphines may be predicted to occur by different, strong π-electron acceptor ligands.

A. Turco
C. Pecile

Istituti di Chimica Generale e
Chimica Fisica,
University of Padua,
Italy.

[1] Pecile, C., Giacometti, G., and Turco, A., *Atti Accad. Lincei*, **28**, 189 (1960). See also Proc. Fourth Int. meeting on Molecular Spectroscopy, Bologna (1959), *Spectrochim. Acta* (in the press).

[2] Chamberlain, M. N., and Bailar, J. C., *J. Amer. Chem. Soc.*, **81** 6412 (1959).

[3] Kinell, P., and Strandberg, B., *Acta Chem. Scand.*, **13**, 1607 (1959).

[4] Mitchell, P. C. H., and Williams, R. J. P., *J. Chem. Soc.*, 1912, (1960).

[5] Ahrland, S., Chatt, J., Davies, N. R., and Williams, A. A., *J. Chem. Soc.*, 276 (1958).

[6] Schäffer, C. E., Conf. on Co-ordination Chemistry, London, 1959.

[7] Williams, R. J. P., *Ann. Rep. Chem. Soc.*, **56**, 101 (1960).

[8] Ahrland, S., Chatt, J., and Davies, N. R., *Quart. Rev. Chem.*, **12**, 265 (1958).

[9] Owston, P. G., and Rowe, J. M., *Acta Cryst.*, **13**, 253 (1960).

[10] Turco, A., Panattoni, C., and Frasson, E., *Nature*, **187**, 272 (1960).

[11] Bleidelis, Ya.Ya., *Kristollografiya*, **2**, 278 (1957). Bleidelis, Ya.Ya., and Bokii, G. B., *ibid.*, **2**, 281 (1957).

Surface Symmetry and Cell Dimensions of Layer Lattice Silicates

In a previous communication[1] it was pointed out that the ditrigonal surface symmetry observed for a number of layer lattice silicates probably plays an important part in determining polymorphism among the micas. This idea has now been extended considerably by theoretical and experimental studies to be reported elsewhere. It appears to me that the ideal layer lattice structures must have ditrigonal, rather than hexagonal, surface symmetry. That is, the tetrahedral groups apparently can rotate quite readily about axes normal to the sheets to allow the tetrahedral sheets to contract to the dimensions of the octahedral sheets.

These concepts have several important implications. For example : (1) it is shown easily that the average tetrahedral rotation is given by $\alpha = \cos^{-1}(b_{\text{obs.}}/b_{\text{tetr.}})$, where $b_{\text{obs.}}$ is observed *b*-axis, and $b_{\text{tetr.}}$ is *b*-dimension which the given tetrahedral layer would assume, if unconstrained. This gives reasonable agreement to $\alpha_{\text{obs.}}$ for those structures for which the latter is known. Values of $\alpha_{\text{calc.}}$ for a large number of layer lattice silicate structures show that almost all these minerals have more or less ditrigonal surface networks, for example, α for micas = 6–23°.

(2) The substitution of aluminium for silicon in the tetrahedral layer will not increase $b_{\text{obs.}}$ but merely increase α, that is, change the surface configuration. Previous unit cell formulæ giving $b_{\text{obs.}}$ in terms of composition[2] are therefore wrong in this respect. Multiple regression analyses, of *b* against composition,

computed separately for experimental data for kaolins, micas and chlorites have each yielded non-significant regression coefficients for tetrahedral Al^{iv}, confirming the theoretical predictions.

(3) In the case of the micas, the interlayer cations play a major part in determining the unit-cell dimensions, a direct contradiction of current concepts. In general the 'Si'–O tetrahedra rotate until the nearer six oxygens (of the twelve) are 'in contact' with the interlayer cation, as I[3] have shown in the particular case of $2M_1$ muscovite. A large interlayer cation therefore contributes significantly to the value of $b_{\text{obs.}}$; interlayer K^+ has a greater effect than octahedral Fe^{++} for micas. This physical concept has been confirmed by the multiple regression analyses mentioned. A single relation obtained from the mica data, giving b in terms of composition, fits both normal and brittle micas adequately, and both dioctahedral and trioctahedral types. For example, the unusual brittle mica xanthophyllite has $b_{\text{obs.}} = 9{\cdot}01$ and $b_{\text{calc.}} = 9{\cdot}016$, whereas earlier formulæ break down altogether[2].

(4) It is obvious that the relative rigidity of the Si–O tetrahedral group takes control of the b-axis dimensions of those layer minerals for which the octahedral layer would be significantly larger than the tetrahedral layer. It is for this reason that $b_{\text{obs.}} = 9{\cdot}15$ for talc, rather than $\approx 9{\cdot}3$; and the same factor is possibly an important one in distinguishing the chrysotile from the antigorite structures.

Necessary experimental results to support these ideas are now being submitted for publication. Some of these data have been kindly supplied by Dr. K. Norrish of this Division, with whom I have had very profitable discussions of this work.

E. W. RADOSLOVICH

Division of Soils,
Commonwealth Scientific and
Industrial Research Organization,
Adelaide.

[1] Radoslovich, E. W., *Nature*, 183, 253 (1959).

[2] Brindley, G. W., and MacEwan, D. M. C., *Ceramics Symp., Brit. Cer. Soc.*, 15 (1953).

[3] Radoslovich, E. W., *Acta Cryst.*, 13, 919 (1960).

R_M and Solvent Composition in Paper Chromatography

WHEN information concerning the structure of substances is required from paper chromatographic data, R_M values are used rather than R_F as they are more directly related to the different groups of which the molecules of these substances are composed[1]. In cases when one phase forming the chromatographic system is composed of two solvents practically immiscible with the other liquid phase, at any proportion of the component solvents (for example, hexane + chloroform/water; hexane/water + dimethylsulphoxide) and assuming that chromatography is a true counter-current extraction process, following the plate theory, a simple equation can be derived relating the R_M value to the composition of the two-component phase.

In the systems mentioned, under ideal conditions, the partition coefficient of a substance between the mixed two-component phase (1 + 2) and the other phase is related to the volume fractions of the component solvents (u_1 and u_2) and to the partition ratios of the substance between the pure component solvents and the other phase (K_1 and K_2) by Kemula–Buchowski's equation[2]:

$$\log K = u_1 \log K_1 + u_2 \log K_2 \qquad (1)$$

Taking into account that $u_1 + u_2 = 1$ we can write:

$$\log K + \log r = u_1 \log K_1 + u_2 \log K_2 + u_1 \log r + u_2 \log r$$

or

$$\log Kr = u_1 \log K_1 r + u_2 \log K_2 r \qquad (2)$$

(r is volume coefficient or 'paper constant' is ratio of cross-sectional areas of non-polar and polar phase.)

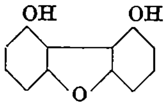
As K_1, K_2 and K are usually defined as ratios of concentrations of substance in the non-polar and polar phase, at equilibrium, then $R_M = -\log Kr$ in conventional paper chromatography and $R_M = \log Kr$ in reversed phase chromatography[1] so that in both cases:

$$R_M = u_1 R_{M1} + u_2 R_{M2} \qquad (3)$$

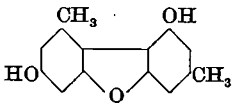
where R_M, R_{M1}, R_{M2} denote the R_M values of the substance in the systems: mixed solvent (1 + 2)/other phase; pure solvent 1/other phase; pure solvent 2/other phase.

If the volume coefficient r is constant and independent of the composition of the mixed solvent (1 + 2), the R_M value of a substance should thus be a linear function of the volume composition of the mixed solvent (this will also apply if r is an exponential function of volume composition, that is, $\log r = ku_1$).

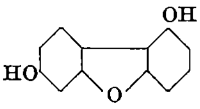
In order to give an experimental illustration of this simple rule, in Fig. 1 some results from other work[3] are given, represented in the form of R_M versus volume composition plots. Experimental details are to be found in ref. 3.



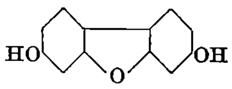
1



2



3



4

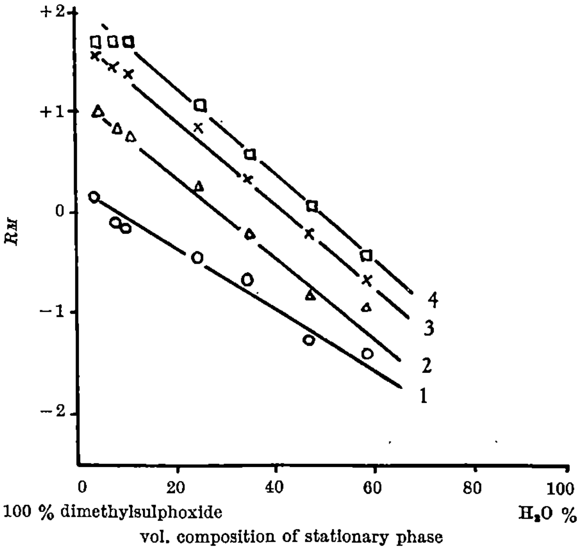


Fig. 1. Mobile phase in these experiments was *iso*propylether

It can be seen that for certain solvent systems results may be obtained in accordance with equation 3. Similar relationships can also be expected in cases when the paper is impregnated with a non-polar liquid (for example, paraffin oil) and developed with mixtures of water and alcohol, etc.

EDWARD SOCZEWIŃSKI

Department of Inorganic Chemistry,
Academy of Medicine,
Lublin, Poland.

[1] Bate-Smith, E. C., and Westall, R. G., *Biochim. Biophys. Acta*, 4, 427 (1950).

[2] Kemula, W., and Buchowski, H., *Roczn. Chem.*, 29, 718 (1955).

[3] Soczewiński, E., and Wachtmeister, C. A. (in preparation).

BIOCHEMISTRY

Separation Methods based on Molecular Sieving and Ion Exclusion

CROSS-LINKED dextran ('Sephadex') has been shown to possess properties of a molecular sieve[1]. The size limits for effective molecular sieving depend on the degree of cross-linking. Molecules or ions capable of entering the gel phase may be subject to specific interaction with the matrix substance. Thus, for example, when certain aromatic compounds are passed through 'Sephadex' columns, a more extensive retardation is found than if the gel acted as a molecular sieve only.

It has now been found that, due to the superimposed sieving and sorption effects (the latter due to aromaticity and charge), polypeptides such as oxytocin and vasopressin are not easily separated from salts, amino-acids and many other substances of low molecular weight (with K_d about 1). It was thought that the separation factors in such cases would be increased if the swelling of the gel grains were more restricted. Following up this idea, we tried solvent systems that contained various proportions of acetic acid, pyridine and water. The swelling of the gel in such solvents is less extensive than in water, and, as expected, some compounds could be effectively excluded while others were distributed to different extents between the gel phase and the free liquid. The distribution is highly dependent on the concentration of acetic acid, which, when large effects are desired, should exceed 40 per cent by volume. In our experiments 15–30 per cent of pyridine was also included both to limit the swelling and avoid too high an acidity of the medium, and also to make effective desalting of peptides possible.

Fig. 1 shows two superimposed diagrams of similar filtration experiments made under the following conditions. Seven grams of low molecular weight substances from posterior lobes of hog pituitaries, prepared by gel filtration as described in an earlier publication[2], were dissolved in 50 ml. of a solvent mixture consisting of 60 parts of acetic acid, 15 parts of pyridine and 25 parts of water (by volume). The solution was filtered through a 'Sephadex' *G* 50 column, 65 cm. × 4·5 cm., packed and pre-washed in the same solvent. The rate of flow was about 25 ml. per hr. Ninhydrin reaction[3] was determined in 0·05-ml. aliquots, from fractions of 12–13 ml. Similar results were obtained with 'Sephadex' *G* 25. It was found by other methods that the substances in fractions 1, 2 and 3 consisted mainly of peptides ; for example, vasopressin was found in peak 2. Fraction 5–7 consisted of amino-acids and other ninhydrin-reactive compounds.

Ion exclusion on xerogels is another principle for fractionation of electrolytes[4]. Using this principle, we have separated peptides on ion exchangers derived from cross-linked dextran (kindly put at our disposal by Dr. P. Flodin, Pharmacia, Uppsala).

As a principle for separation, ion exclusion, with the fixed ionizable groups completely titrated, is

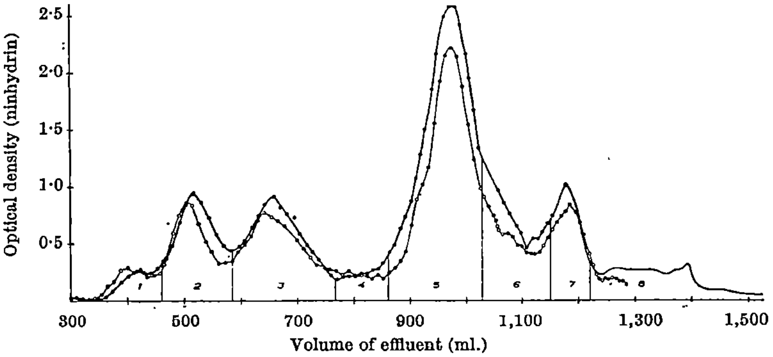


Fig. 1. Distribution diagrams of two similar gel filtration experiments on a 'Sephadex' *G* 50 column. Solvent system: 60 parts of acetic acid, 15 parts of pyridine and 25 parts of water

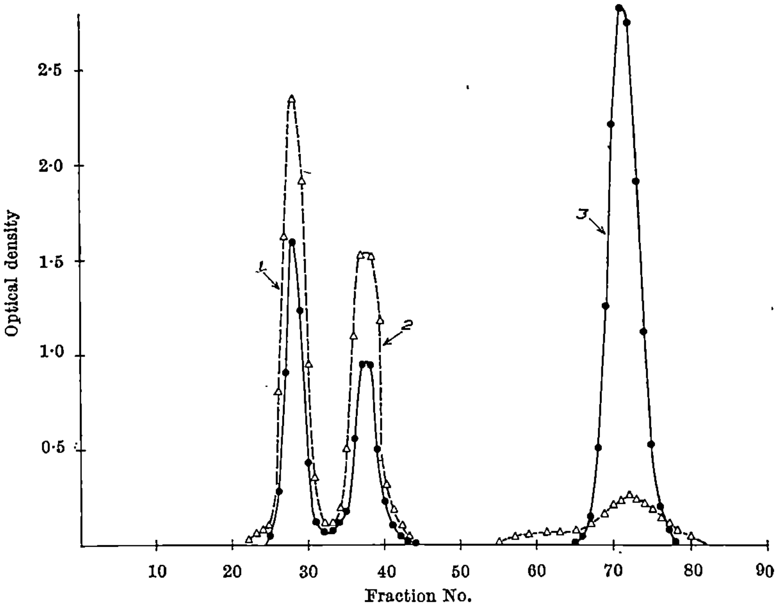


Fig. 2. Ion-exclusion diagrams on a DEAE-'Sephadex' *A* 25 column. Folin–Lowry reaction, -Δ-Δ-Δ-, and ninhydrin, -●-●-●-. Vasopressin is found in peak 1 and oxytocin in peak 2

probably in some respects superior to ion exchange. The contact with the ion exchanger is less active, since intimate interaction with strong adsorption centres is avoided. The capacity appears to be larger and the reproducibility better than is generally the case for ion-exchange chromatography, in which eluants of constant composition are employed. However, the resolution is possibly inferior to what may be achieved in ion-exchange chromatography. The ion or molecular sieving confers a property on the charged gel that converts ion exclusion into something more than merely an inversion of exchange. The sorption or exclusion of ions and molecules is chiefly based on the interplay of restricted diffusion and electrostatic repulsion.

Fig. 2 illustrates ion exclusion of neurohypophyseal peptides that originally were bound to proteins[2]. The experiment was carried out on a diethylaminoethylether derivative of cross-linked dextran (DEAE-'Sephadex' *A* 25, 2·4 m.equiv./N/gm. dry weight : water regain, 5·5 ; particle size, 200–400 mesh). 250 mgm. peptide material were dissolved in 5 ml. of 0·1 molar pyridinium acetate and introduced into a 95 cm. × 3·5 cm. column bed. The chromatogram was developed in 0·1 molar pyridinium acetate at a speed of 30 ml. per hr. After 90 ml. eluent had passed into the column, 7·5 ml. fractions were collected. Aliquots of 0·1 ml. were analysed with ninhydrin and 0·2 ml. aliquots by the Folin–Lowry method[5]. The reproducibility is excellent. Peak 1 contains lysine vasopressin (150–200 international rat blood-pressure units per mgm.), and peak 2 chiefly consists of oxytocin (300–400 international avian blood-pressure units per mgm.). Negatively charged ion species are of course adsorbed and may be eluted at a later stage either with the same eluant (if the sorption forces are weak enough) or by displacement.

The outcome of a number of preliminary experiments made under other conditions and with other substances indicates that the methods described here ought to find applications in many fields.

Further details of this work will be published later.

J. PORATH
E. B. LINDNER

Institute of Biochemistry,
University of Uppsala.

[1] Porath, J., and Flodin, P., *Nature*, **183**, 1657 (1959); *Biochim. Biophys. Acta*, **39**, 193 (1960). Gelotte, B., *J. Chromatography*, **3**, 330 (1960). Flodin, P., *ibid.*, **5**, 103 (1961).

[2] Lindner, E. B., Elmqvist, A., and Porath, J., *Nature*, **184**, 1565 (1959).

[3] Moore, S., and Stein, W. H., *J. Biol. Chem.*, **211**, 907 (1954).

[4] Wheaton, R. M., and Bauman, W. C., *Indust. Eng. Chem.*, **45**, 228 (1953).

[5] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

Simplified Procedure for removing Non-specific Staining Components from Fluorescein-labelled Conjugates

Coons and Kaplan[1] in their description of the fluorescent antibody technique using antisera coupled with fluorescein isocyanate found that the non-specific staining which occurred when such conjugates were applied to tissue sections could be minimized by dialysing the conjugates against 0·01 *M* phosphate buffer solution *p*H 7·0 for several days, followed by treatment with liver powder.

Marshall *et al.*[2] showed that fluorescein isothiocyanate was superior to the isocyanate in this technique, and in their description of the simpler conjugating procedure which is possible using this compound they also dialysed against buffer *p*H 7·0 to remove non-specific staining components from the solution.

It has been found that non-specific staining material in conjugates prepared from fluorescein isothiocyanate can all be removed by serial treatment with liver suspension alone, thus eliminating the long dialysis procedure.

In these experiments the following systems were employed : (1) human group '*A*' stomach sections treated with rabbit anti-'*A*' serum[3] followed by goat anti-rabbit γ-globulin conjugate ; (2) calf thyroid sections treated with lupus erythematosus serum containing antinuclear factor[4] followed by rabbit anti-human γ-globulin conjugate. In addition to these 'sandwich' tests the conjugates were also applied direct to the sections, all of which were cut in a cryostat.

Conjugates of crude globulin preparations were prepared according to the method described by Marshall *et al.*[2], and after overnight mixing in the cold aliquots of the reaction mixture were treated : (1) by dialysis against buffer *p*H 7·0 as described by Coons and Kaplan[1], followed by a single absorption with liver suspension ; (2) by serial treatment with liver suspension. The anti-rabbit conjugate was treated with human liver and the anti-human conjugate with rabbit liver. The liver suspensions were prepared by suspending the dried powder, made by the method of Coons and Kaplan, in *p*H 7·0 buffered saline, centrifuging, and the excess fluid decanted. The use of such a moist suspension eliminates the considerable loss of conjugate which occurs when dry powder is used. Equal volumes of the packed suspensions were added to aliquots of the conjugates and the mixtures slowly rotated for 10 min. at 4° C. The supernate was removed after centrifugation and for serial absorption was treated with a further volume of suspension ; after mixing for 10 min. and centrifuging, the supernate was finally treated with a third volume of suspension.

The results obtained by treating sections with the various conjugate preparations are summarized in Table 1.

Table 1. EFFECT ON NON-SPECIFIC STAINING AND AGGLUTININ TITRE OF DIALYSIS AND/OR TREATMENT WITH LIVER

Treatment	Non-specific staining	Specific staining	Agglutinin titre of anti-human γ-globulin conjugate
Dialysis + 1 liver treatment	Very slight	Bright	1,280
Undialysed 1 liver treatment	Moderate	Bright	1,280
Undialysed 2 liver treatments	Very slight	Bright	1,280
Undialysed 3 liver treatments	Minimal	Moderate	640

It will be seen that two treatments with liver suspension resulted in conjugates which gave satisfactory specific staining and no more non-specific staining than the conventional procedure. Conjugates treated three times with liver showed least non-specific staining but detectable reduction in the brightness of the specific staining. This latter observation was confirmed by titrating the various preparations of the anti-human γ-globulin conjugate for their agglutinating activity against human *Rh*+ erythrocytes sensitized with incomplete anti-*D* antibody. The titres obtained are shown in Table 1.

In the orthodox procedure one of the effects of dialysis is to reduce the *p*H of the mixture from 9·0 to 7·0. It therefore seemed possible that the failure of one liver treatment to bring about an adequate reduction in non-specific staining might be attributable to the higher *p*H of the undialysed solution.

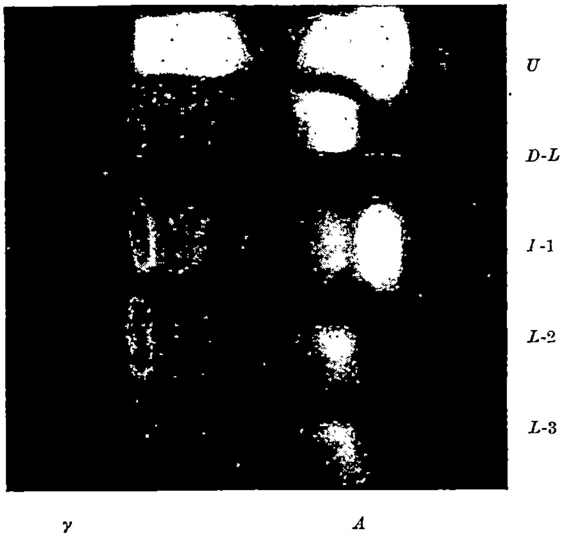


Fig. 1. Electrophoresis of fluorescent conjugate solutions, carried out on cellulose acetate membranes in borate-barbitone buffer, *p*H 8·6 at about 15 V./cm. *A*, albumen position; γ, γ-globulin position; *U*, untreated; *D-L*, dialysed and treated once with liver suspension; *L*-1, *L*-2, *L*-3, one, two and three liver treatments respectively of the undialysed conjugate

That this is not so was shown by adjusting the reaction of an undialysed conjugate to *p*H 7·0 by the addition of phosphoric acid. After one treatment with liver suspension this preparation gave as much non-specific staining as the same conjugate treated with liver at *p*H 9·0.

Electrophoresis of the conjugate preparations was carried out on cellulose acetate membrane at about 15 V./cm. in borate-barbitone buffer, *p*H 8·6 for 1 hr. The strips were photographed in ultra-violet light and the results are shown in Fig. 1. The untreated conjugate gave a serum protein pattern on which two bright zones were superimposed, one just ahead of the albumin, the other in the β–α_2-globulin region. It is evident that dialysis followed by a single liver treatment, and serial liver treatment alone, have similar effects on the electrophoretic patterns obtained, the brightest two zones being considerably reduced by both procedures. It is of interest that the γ-globulin components of the conjugates constitute a very small proportion of the fluorescent material present, although when these strips were stained with protein dyes the γ-globulin stained strongly and was a major component. It therefore appears that the fluorescein reagent has a greater affinity for proteins other than γ-globulin.

On the basis of these results serial treatment with appropriate organ suspension alone appears to give satisfactory conjugate preparations for staining tissue sections.

The electrophoresis was carried out by Dr. R. Consden, and it is a pleasure to acknowledge his help and interest in this work.

G. D. Johnson

Rheumatism Research Unit (Medical Research Council),
Canadian Red Cross Memorial Hospital,
Taplow, Maidenhead.

---

[1] Coons, A. H., and Kaplan, M. H., *J. Exp. Med.*, **91**, 1 (1950).

[2] Marshall, J. D., Eveland, W. C., and Smith, C. W., *Proc. Soc. Exp. Biol.*, **98**, 898 (1958).

[3] Glynn, L. E., Holborow, E. J., and Johnson, G. D., *Lancet*, ii, 1083 (1957).

[4] Holborow, E. J., Weir, D. M., and Johnson, G. D., *Brit. Med. J.*, ii, 732 (1957).

Triphosphopyridine Nucleotide-linked Glyceraldehyde Phosphate Dehydrogenase in a Non-Photosynthetic Plant Tissue

It has been established that, whereas the usual diphosphopyridine nucleotide (DPN)-linked glyceraldehyde-phosphate dehydrogenase is found generally distributed in higher plant tissues, a separate enzyme, specifically requiring triphosphopyridine nucleotide (TPN) as coenzyme, is restricted to leaves[1–4]. The distinctive association of the TPN-glyceraldehyde phosphate dehydrogenase with the photosynthetic apparatus and the specificity for TPN shown in some types of photosynthetic phosphorylation have encouraged the view that the reductive step in hexose synthesis in leaves is mediated by this enzyme.

An active hexose synthesis from acetate which also involves a reversal of the glycolytic sequence occurs in the colourless endosperm tissue of some germinating fatty seedlings. It was therefore of interest to find that in this non-photosynthetic tissue both types of glyceraldehyde phosphate dehydrogenase are present.

Extracts were prepared in sucrose phosphate mixtures from endosperm tissue of castor beans which had been grown in the dark for 4–5 days (see ref. 5). Enzyme activity of the supernatant solution after centrifuging at 18,000*g* was measured by the procedure outlined by Gibbs[6] in which reduction of the coenzyme is followed in the presence of fructose-1,6-diphosphate (to provide substrate through the action of aldolase), arsenate and fluoride. The results in Table 1 show that both TPN and DPN are reduced and that the rate of reduction increases with enzyme concentration. The maximum rates achieved with DPN are considerably higher than those with TPN. The results in Table 2 show the effect of increasing substrate concentration on enzyme activity. It is seen that the TPN enzyme is saturated at lower substrate-levels than the DPN enzyme.

Table 1. EFFECT OF INCREASING THE AMOUNT OF ENZYME EXTRACT ON THE INITIAL RATES OF DPN AND TPN REDUCTION

The reaction mixtures contained, in micromoles: *tris* buffer (*p*H 8·5)' 100; sodium arsenate, 51; sodium fluoride, 60; enzyme extract and (DPN, 0·15 plus fructose-1,6-diphosphate, 2·6) or (TPN, 0·13 plus fructose-1,6-diphosphate, 0·3). Total volume, 3·0 ml.

Enzyme added (ml.)	TPN reduction	DPN reduction
	(Δ O.D.$_{340}$/min.)	
0·025	0·004	0·012
0·050	0·0145	—
0·080	0·025	—
0·10	0·030	0·048
0·20	—	0·081
0·30	—	0·089

Table 2. EFFECT OF INCREASING AMOUNTS OF SUBSTRATE ON DPN AND TPN REDUCTION

Reaction mixtures as in Table 1, with 0·1 ml. enzyme solution and fructose-1,6-diphosphate as indicated

Fructose-1,6-diphosphate (μmoles)	TPN reduction	DPN reduction
	(Δ O.D.$_{340}$/min.)	
0·0	—	0·003
0·056	0·016	—
0·112	0·020	—
0·168	0·0205	—
0·187	—	0·021
0·224	0·0225	—
0·370	—	0·031
1·30	—	0·046
2·60	—	0·049

The possibility that TPN reduction was due to glucose-6-phosphate dehydrogenase rather than glyceraldehyde-phosphate dehydrogenase was eliminated by the following observations:

(*a*) The rate of reduction of TPN was much lower when glucose-6-phosphate was used instead of fructose-1,6-diphosphate. (*b*) The reduction was completely inhibited by 0·001*M* iodoacetate. (*c*) Reduction of TPN was observed when glyceraldehyde-3-phosphate was used directly as substrate.

In crude extracts TPN reduction does not require the addition of arsenate or phosphate (cf. ref. 6). In experiments with a partially purified preparation, which contains relatively greater amounts of the TPN enzyme, arsenate stimulations are observed. Nevertheless, a considerable fraction of the TPN reduction appears to be catalysed by a third (non-reversible) dehydrogenase which was also discovered originally in green leaves[8,9]. The properties and intracellular distribution of the various dehydrogenases will be described in another communication[10].

C. R. Benedict
H. Beevers

Department of Biological Sciences,
Purdue University,
Lafayette, Indiana, U.S.A.

[1] Gibbs, M., *Nature*, **170**, 164 (1952).
[2] Arnon, D. I., *Science*, **116**, 635 (1952).
[3] Hagemann, R. H., and Arnon, D. I., *Arch. Biochem. Biophys.*, **57**, 421 (1955).
[4] Fuller, R. C., and Gibbs, M., *Plant Physiol.*, **34**, 324 (1959).
[5] Beevers, H., and Walker, D. A., *Biochem. J.*, **62**, 114 (1956).
[6] Gibbs, M., *Methods in Enzymology*, edit. by Colowick, S. P., and Kaplan, N. O., **1**, 411 (1957).
[7] Stumpf, P. K., *J. Biol. Chem.*, **192**, 519 (1950).
[8] Axelrod, B., and Bandurski, R. S., *J. Biol. Chem.*, **204**, 939 (1953).
[9] Arnon, D. I., Rosenberg, L. L., and Whatley, F. R., *Nature*, **173**, 1132 (1954).
[10] Yamamoto, Y., Benedict, C. R., and Beevers, H. (in preparation).

Electrophoresis in a Thin Layer of Starch-Gel followed by a Plastifying Treatment

QUANTITATIVE analysis after starch-gel electrophoresis is usually difficult due to : (1) the diffusion of protein fractions, (2) the difference of migration according to the thickness of the gel, and (3) the fragility and opalescence of this migrating medium.

The cutting of the gels accompanied by superficial staining of the surfaces only partially resolved these difficulties. Some progress has been obtained by cutting thin slices in the median zone of the gel, which can be stained and rendered transparent after inclusion in agar and dried by heating[1,2]. On the other hand, immersion in pure glycerol[3] or transformation into 'plastic' by heat only[4] has been tried. However, the results obtained did not permit a true quantitative analysis. The technique described here resolves these problems: (1) the stain penetrates into the gel completely, (2) the gel is transformed into a thin, transparent and supple film.

Smithies's technique[5] is used for the preparation of the starch-gel. The gel is poured into the apparatus giving a homogeneous layer 3 mm. thick[7]. The lateral diffusion of the more concentrated proteins (for example, albumin) is limited by cutting a thin band (2 mm.) of gel between the samples. A sheet of polyethylene protects the gel from the air. The introduction of the sample is done by mixing the serum in some gel in order to obtain a continuous medium.

With this technique, the serum components give strictly parallel bands. After electrophoresis, there are two possible treatments :

(1) It is possible to transform the gel into plastic before staining. The gel is placed for 1 hr. in a bath of an aqueous solution containing 5 per cent acetic acid and 5 per cent glycerol. It is then heated with an infra-red tube of 1,200 W. for 3 hr. at a distance of 65 cm. The plastified and transparent plate is stained in aqueous dye solution during 2 hr. (naphthalene black 0·5 gm., glacial acetic acid 50 ml., distilled water 1,000 ml.). Decoloration is obtained by successive washings in 5 per cent aqueous acetic acid solution. The last bath contains 50 per cent glycerol. The gel is dried with filter paper and is heated by an infra-red lamp for 20 min.

(2) It is also possible to stain the gel directly for 4 hr., then after successive washings it is placed for 1 hr. in an aqueous solution containing 5 per cent acetic acid and 5 per cent glycerol. The gel is heated for 3 hr. with infra-red on a glass plate.

The first technique reduces the risk of deterioration during manipulation. The second results in a more supple plastic film. The transparent band can be measured by direct photometry (Fig 1).

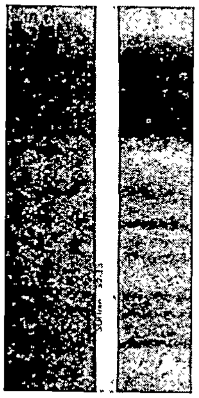


Fig. 1

In addition we noted that Poulik discontinuous buffer system[6] has the advantage of giving a narrow zone (1 cm. wide) of albumin and clearer pre- and post-albumin zones.

J. Groulade

Laboratoire de Biochimie Médicale,
Centre Hospitalier régional de Grenoble.

J. M. Fine
C. Ollivier

Centre National de Transfusion Sanguine,
6 rue Alexandre Cabanel, Paris, 15^{e}.

[1] Fine, J. M., and Waszczenko, Z., *Nature*, **181**, 269 (1958).
[2] Rubinstein, H. J., Owen, J. A., and Larsen, W., *Clin. Chim. Acta*, **5**, 583 (1960).
[3] Gratzer, W. B., and Beaven, G. H., *Clin. Chim. Acta*, **5**, 577 (1960).
[4] Pinteric, in Smithies, O., *Advances in Protein Chemistry* (1959).
[5] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[6] Poulik, M. D., *Nature*, **180**, 1477 (1957).
[7] Groulade, J., and Ollivier, C., *Annales Biol. Clin.*, **18**, 595 (1960).

Changes in Glucose-6-phosphatase Activity in Liver and Kidney at Birth

GLUCOSE-6-PHOSPHATASE catalyses the last step in the chain of reactions by which glycogen is converted to glucose. The enzyme is found only in liver, kidney and intestinal mucosa. Its activity is low in fœtal liver and increases at birth in the guinea pig[1] and the rat[2]. Glucose-6-phosphatase has been measured in human liver only in the first half of gestation. Cori and Schulman[3] found very low levels in an 18-week fœtus. Villee[4] used the production of glucose by liver slices as a measure of glucose-6-phosphatase. Activity appeared about the tenth week of gestation and had reached about half adult levels by 22 wk. Aurichio

Table 1. CHANGES IN GLUCOSE-6-PHOSPHATASE ACTIVITY AT BIRTH

Species		Fœtus at term	24 hr. after birth	Adult
Rat	Liver	4·9	68·6	20·7
	Kidney	3·3	8·6	26·2
Rabbit	Liver	7·9	46·4	16·0
	Kidney	5·3	8·8	16·8
Guinea pig	Liver	3·4	38·6	16·7
	Kidney	6·0	22·4	17·3
Sheep	Liver	5·7	45·7	20·9
	Kidney	6·0	31·4	24·1

Results are expressed in μM glucose-6-phosphate hydrolysed per mgm. N/hr.; each figure is the mean of at least three determinations done in duplicate.

System: Glucose-6-phosphate, 0·02 M; *tris* buffer pH 7·0, 0·1 M; enzyme 0·1 ml. of a 10 per cent homogenate in 0·25 M sucrose. Final volume 0·5 ml. Substrate was omitted from the blank. Incubated for 10 min. at 37° and de-proteinized with 1 ml. 10 per cent (w/v) trichloracetic acid. Inorganic phosphate liberated was determined by the method in ref. 10.

and Rigillo[5] found low, but variable, activity in post-mortem material from 20 to 40 weeks of gestation.

In the rat, activity increases in the first 24 hr. after birth to levels several times those in adult liver[6,7]. Glucose-6-phosphatase activity has now been measured in liver and kidney in a number of species of differing maturity at birth.

Table 1 shows glucose-6-phosphatase activity in liver and kidney of the fœtus at term, the new-born animal 24 hr. after birth and the mother in four different species. The rat is very immature at birth, with closed eyes and no hair. The new-born rabbit is more mature than the rat, but less mature than the lamb and guinea pig which are born with eyes open and able to run about within a short time of birth. Nevertheless, in all these species there is a considerable increase in glucose-6-phosphatase activity in the liver just after birth. Kidney glucose-6-phosphatase behaves rather differently. In the rat and rabbit there was only a small increase at birth and adult activity was reached 2 weeks after birth. In the lamb and guinea pig, however, activity reached adult-levels within 24 hr. after birth. This species difference might be related to differences in the maturity of renal function at birth.

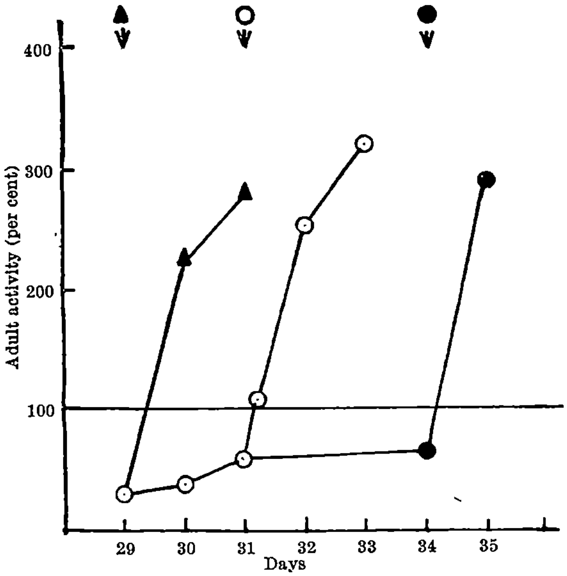


Fig. 1. The effect of premature and postmature delivery on liver glucose-6-phosphatase activity in the new-born rabbit. Results are expressed as a percentage of adult activity (16·0 μM glucose-6-phosphate hydrolysed/mgm. N/hr.). Mature, ○; premature, ▲; postmature, ●. Delivery is indicated by the arrow

The effect of delivery on the increase in activity of glucose-6-phosphatase is shown in Fig. 1. The same rise in activity was found in animals delivered at term naturally and by Cæsarean section. Animals delivered two days before term by Cæsarean section showed a rise within 24 hr. to levels as high as those in 24 hr.-old rabbits delivered at term. When pregnancy was prolonged with chorionic gonadotrophin[8], there was no increase until after delivery, when a rise again occurred within 24 hr.

At the end of gestation large amounts of glycogen have accumulated in the liver in all species which have been examined[9]. One possible factor may be low glucose-6-phosphatase activity, as originally suggested[1]. Immediately after birth, liver glycogen disappears very rapidly, presumably to maintain blood glucose in the new-born animal. At this time, there is a very considerable increase in glucose-6-phosphatase activity in the liver in all species which have been examined. This increase in activity is not related to maturity at birth. The effects of early and late delivery suggest that the stimulus for the increase in activity is related to delivery. Nemeth[8] has described similar findings on the effect of delivery on tryptophan pyrollase activity in rabbit liver.

M. J. R. DAWKINS

Nuffield Institute for Medical Research,
43 Woodstock Road, Oxford.

[1] Nemeth, A. M., *J. Biol. Chem.*, **208**, 773 (1954).
[2] Weber, A., and Cantero, G., *Cancer Res.*, **17**, 995 (1957).
[3] Cori, G. T., and Schulman, J. L., *Ped.*, **14**, 646 (1954).
[4] Villee, C. A., *J. App. Physiol.*, **5**, 437 (1953).
[5] Aurichio, S., and Rigillo, N., *Biol. Neonat.*, **2**, 146 (1960).
[6] Kretchmer, N., *Ped.*, **23**, 606 (1959).
[7] Dawkins, M. J. R., *J. Path. Bact.*, **79**, 289 (1960).
[8] Nemeth, A. M., *J. Biol. Chem.*, **234**, 2921 (1959).
[9] Shelley, H. J., *Brit. Med. Bull.* (in the press).
[10] Fiske, C. H., and Subba Row, Y., *J. Biol. Chem.*, **66**, 375 (1925).

Keto-Acids and Amines in Rumen Liquid of Cattle

AN investigation was made into the occurrence of keto-acids in rumen liquid of cattle; simultaneously the amino-acids and the amines were examined, as electrophoresis at pH 4·5 revealed that basic components occurred in rumen liquid. The following methods were used:

Keto-acids: After adjustment of the pH to 4·7 and a treatment with 'Norit', rumen liquid is passed through a 'Dowex 50*W*' column in order to remove the amino-acids. As mentioned in an earlier communication[1] concerning keto-acids in serum and urine of cattle, the keto-acids are converted into 2,4-dinitrophenyl hydrazones, after which they are reduced by hydrogen to amino-acids and identified as such.

Amino-acids: From the 'Dowex 50*W*' column the amino-acids are eluted with 6 N ammonia. After evaporation to dryness, the residue is submitted to electrophoresis with evaporation at pH 4·5. In this way a good separation in groups up to and including the amines is obtained. The amino-acids are detected by ninhydrin and copper nitrate solution. The red-coloured spots are eluted with methanol and the extinction is measured at 525 mμ. This method is semi-quantitative, but it gives sufficient information about the changes in the concentrations.

Amines: The pH of a suitable quantity of rumen liquid, for example 40 ml., is adjusted to 4·5, after which the liquid is centrifuged. Then sodium hydroxide solution is added and a mixture of salts (sodium sulphate and disodium hydrogen phosphate[2]). The *p*H must be ± 12. The liquid is first extracted with ether in order to remove lipids and other interfering material and then three times with butanol. The butanol solution is concentrated *in vacuo* to a small volume and then submitted to electrophoresis, combined with evaporation at *p*H 11·7. Afterwards the paper (27 × 55 cm.) is chromatographed in propanol/0·2 *N* ammonia (3 : 1). From Fig. 1 it can be seen that a good separation even of cadaverine and putrescine is obtained.

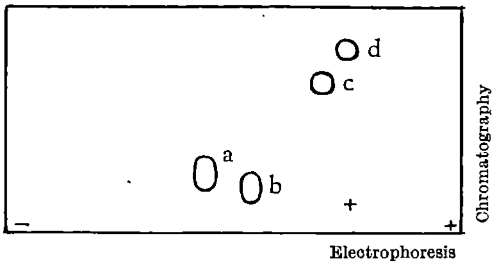


Fig. 1. Separation of amines in the butanol fraction. *a*, Cadaverine; *b*, putrescine; *c*, histamine; *d*, tyramine

In this way the following keto-acids could be shown: pyruvic acid, hydroxypyruvic acid, glyoxilic acid, α-keto-*iso*valeric acid, α-keto-*iso*caproic acid or α-keto β-methylvaleric acid, oxalo-acetic acid and α-keto-glutaric acid. A few components are still under investigation.

It appeared that the concentration of the keto-acids increases within 30 min. after feeding. An examination of hay and grass revealed that these foodstuffs contain keto-acids. Therefore, glucose was added to rumen liquid in order to investigate if keto-acids would be formed. Indeed, it appeared that the concentration of pyruvic acid was now strongly increased. Moreover, it was remarkable that the *p*H decreased noticeably, and that the concentration of the amino-acids and other basic components increased strongly. The fall of the *p*H depends on the time when the sample is taken: shortly after feeding, the decrease of *p*H, after addition of glucose, is at its greatest.

As it was possible that the basic components contain amines, for example, histamine and tyramine, which Dain *et al.*[3] identified in rumen liquid of experimentally over-fed sheep, an investigation in this direction was carried out.

In a first experiment glucose, histidine, tyrosine and tryptophan were added to rumen liquid in order to examine if decarboxylation occurred. Indeed, histamine, tyramine and probably tryptamine could be detected after 24 hr. Afterwards, rumen liquid, to which fodder-cakes, caseine and glucose, and caseine alone were added, was examined. Frequently, histamine and tyramine could be detected, but cadaverine, putrescine and colamine occurred in far greater amounts. If lysine were the most important component of the basic amino-acids, the concentration of cadaverine was high; by addition of arginine to rumen liquid the concentration of putrescine increased strongly. From these results it is postulated that lysine in the rumen forms cadaverine and that arginine, after conversion to ornithine, forms putrescine.

The factors responsible for the formation of the amines and amino-acids are under investigation *in vitro* and *in vivo*. In one case, namely, a heifer, a greater concentration of amines was found in the rumen.

I am much indebted to Prof. G. Wagenaar for putting the cows at my disposal and to Mr. C. G. van der Kamp for technical assistance.

C. J. G. van der Horst

Laboratory of Biochemistry,
Oudwijk 11,
Utrecht, Netherlands.

[1] Horst, C. J. G. van der, *Nature*, **187**, 146 (1960).
[2] McIntire, F. C., Roth, L. W., and Shaw, J. L., *J. Biol. Chem.*, **190**, 537 (1947).
[3] Dain, J. L., Neal, A. L., and Dougherty, R. W., *J. Anim. Sci.*, **14**, 930 (1955).

Haptoglobinuria in Nephrotic Syndromes

As a rule, only traces of haptoglobin can be found, by titrimetric method, in the urine of patients presenting a nephrotic syndrome with considerable proteinuria, as against their serum, which is very rich in α_2 globulin and especially in haptoglobin. Actually, it is so only in the case of patients belonging to Smithies's groups *2·1* and *2·2* (ref. 1), for, when the serum belongs to group *1·1*, the haptoglobin, of lower molecular weight, filtered through the nephrotic kidney, can reach a concentration of several gm. per litre[2,3].

I have had the opportunity of examining the urines of patients whose haptoglobin was type *2·2* in some, while in others it was type *2·1*.

In the urines of patients with haptoglobin of type *2·2*, I could never find any haptoglobin either by Jayle's titrimetric method (measure of the peroxidasic activity of the hæmoglobin–haptoglobin complex), or by electrophoresis in starch-gel, even when the haptoglobin was very much increased and the proteinuria very considerable. For example, in the case of patients with renal amyloidosis, the serum haptoglobin was as high as 10 gm./l., the proteinuria was 20 gm./l.; the urine, very rich in seromucoid α_1, contained no haptoglobin. The same result was found in the case of lipoidic nephrosis belonging to Smithies's group *2·2*, and this, whatever the rate of haptoglobin in the serum.

With patients having haptoglobin of group *2·1*, the titrimetric method has always allowed detection of a small quantity of haptoglobin in the urine, either directly if the proteinuria were important, or by concentrating the urine after dialysis if the proteinuria were rather low. For example, in the case of a patient whose serum haptoglobin remained about 5 gm./l., 0·50 gm. of haptoglobin was found in the urine for a proteinuria of 25 gm./l. When the latter was 7 gm., there was still 0·10 gm. of haptoglobin; when it went down to 0·20 gm./l., I still could, by sufficient concentration of the urine, reveal a small quantity of haptoglobin. In other patients with haptoglobin of the same group *2·1* and haptoglobinæmia of 4·5 gm./l., but with a comparatively low proteinuria (about 2 gm./l.), I found, by concentrating the urine ten times, a very small but still measurable quantity of haptoglobin.

The urine of all these patients was analysed by electrophoresis in starch-gel following Smithies's method, after preliminary addition of hæmoglobin so as to reveal the hæmoglobin–haptoglobin complexes, very small quantities of which can be detected by benzidine staining.

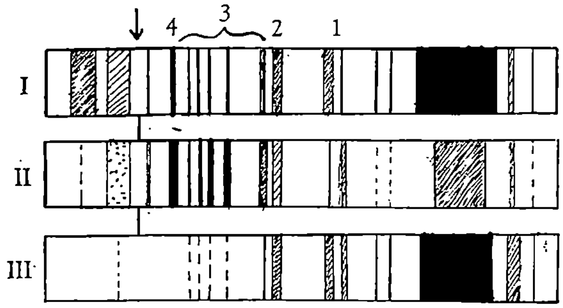


Fig. 1. Amidoblack coloration. I, Normal serum; II, nephrotic serum; III, nephrotic urine. (1) Haptoglobin *1·1*; (2) β globulin *C*; (3) haptoglobin zone; (4) slow α2

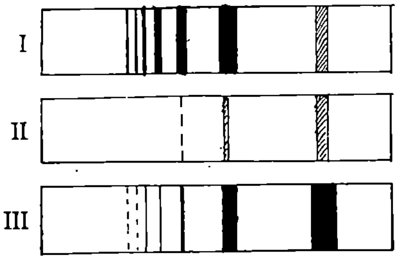


Fig. 2. Haptoglobin *2·1* hæmoglobin complex in nephrotic syndrome; benzidine coloration. I, Serum; II, urine; III, haptoglobin obtained from urine, by saline precipitation

When proteinuria is very low, the urines are concentrated before including them in the starch-gel, or the haptoglobin can also be isolated by salt precipitation.

Results: (*a*) Homozygous patients with haptoglobin *2·2* have no haptoglobin in their urine. (*b*) Heterozygous patients with haptoglobin *2·1* have three distinct bands in their urine. The first corresponds to haptoglobin *1·1*, the second to haptoglobin β and the third to the most anodic band of the αβ haptoglobins (haptoglobin *1·1* and β do not occur in sera of group *2·2*) (Figs. 1 and 2).

It is to be noted that the intensity of haptoglobin *1·1* is the same as in the serum, while that of the following band is much weaker than in the serum. The pattern of urinary haptoglobin is identical, whether the concentration of haptoglobin in the urine is 0·1 gm. or 1 gm./l.

In the case of a patient suffering from renal amyloidosis and whose proteinuria was very high, we separated the urinary haptoglobin by salt precipitation and I obtained 0·8 gm. per 100 ml. solution. The starch-gel electrophoresis pattern showed all the bands of the serum, but their proportion varied greatly in the urine and the serum. A very large quantity of haptoglobin *1·1* and very small quantities of the least anodic components were found. The β component is less considerable than the haptoglobin *1·1*; in serum, on the other hand, it is much more intense.

Table 1

Haptoglobin type	Serum haptoglobin (gm./l.)	Proteinuria (gm./l.)	Urinary haptoglobin (gm./l.)	
			Directly titrated	After concentration
La 2·1	4·9	25	0·50	
	5·5	7	0·10	
	5·0	6		0·60
	5·0	2		0·12
	5·2	1·5		0·10
	5·0	0·2		0·10
B 2·1	4·0	2		0·10
	4·2	2·3		0·14
	5·0	1		0·05
	5·0	0·5		traces
Le 2·1	5·0	20	0·10	
	5·0	24	0·10	
	5·5	18	0·08	
R 2·2	10·0	20	0·00	
Ca 2·2	4·6	24	0·00	
	5·0	8	0·00	0·00

These results confirm that the haptoglobin zones represent distinct protein components, and confirm the difference between the molecular size of these haptoglobins. I find mostly, in nephrotic urine, haptoglobins the molecular weight of which is relatively low and which belong to type *2·1* and not to type *2·2*.

A. Marnay

Laboratoire de Chimie Biologique,
Faculté de Médecine,
45 rue des Saints-Pères, Paris.

---

[1] Smithies, O., *Advances in Protein Chemistry*, **14**, 65 (1959).

[2] Jayle, M. F., Vialatte, S., Lagrue, G., and Boussier, G., *J. Urol. Méd. Chir.*, **59**, 800 (1953).

[3] Jayle, M. F., and Boussier, G., *Bull. Soc. Chim. Biol.*, **36**, 959 (1954).

A New Triterpenoid Sapogenin of Animal Origin (Sea Cucumber)

In earlier papers one of us (T. Y.)[1] reported the isolation, chemical properties, and toxicity to fish and warm-blooded animals, of crystalline saponin from the body wall of the sea cucumber, *Holothuria vagabunda*, and designated this as a new saponin, holothurin.

A similar saponin also named holothurin was independently isolated by Nigrelli *et al.*[2] from *Actinopyga agassizi*, a sea cucumber found in the Bahama Islands. Recently, by the extensive study of Sobotka *et al.*[3], holothurin was shown to be further separable into two components: precipitable and non-precipitable by cholesterol. The former component was designated holothurin *A* and proved to be a mixture of several glycosides each of which contains a steroid aglycone of about 26–28 carbon and 4–5 oxygen atoms, one molecule each of four different sugars and one molecule of sulphuric acid as the sodium salt. As sugar components, glucose, xylose, glucomethylose and 3-O-methyl glucose were identified.

Naturally occurring saponins can be divided into two groups. The first of these gives steroidal sapogenins on hydrolysis, the second triterpenoid sapogenins. Triterpenoid sapogenins, so far as we know, have been isolated only from the plant kingdom. It is, therefore, of considerable biogenetic interest to report in this communication the isolation of a triterpenoid sapogenin of animal origin, sea cucumber.

The main sapogenin which was obtained by acid hydrolysis of holothurin was found to be $C_{30}H_{44}O_5$ (I), m.p. 301°, $[\alpha]_D + 14{\cdot}9°$, giving a brown coloration with tetranitromethane. It appeared to be an unknown triterpene and was named holothurigenin. Both holothurin and holothurigenin showed an infra-red absorption band at 1,767 cm.$^{-1}$ attributable to a five-membered lactone. This would indicate that the lactone ring was present in the animal material rather than produced during the acid hydrolysis of the saponin. The direct proof for its presence was provided by the course of the lithium aluminium hydride reduction of holothurigenin (I), which led to the corresponding diol (II), $C_{30}H_{48}O_5$, m.p. 223°, $[\alpha]_D$ + 62·8°, $\lambda_{max.}$' 244 mμ, log ε 4·22, infra-red: 3,448, 3,509, and 3,636 cm.$^{-1}$.

On mild acetylation with acetic anhydride–pyridine of (I), monoacetate (III), $C_{32}H_{46}O_6$, m.p. 277–8°, $[\alpha]_D$ + 10·3°, $\lambda_{max.}$ 244 mμ, log ε 4·42, infra-red: 1,724, 1,767, and 3,636 cm.$^{-1}$ was obtained, which was unaffected by chromic acid/pyridine. Chromic acid/pyridine oxidation of (I) gave a monoketone (IV), $C_{30}H_{42}O_5$, m.p. 290–1°, $[\alpha]_D$ −40, $\lambda_{max.}$ 244 mμ, log ε 4·28, $\lambda_{max.}$ 285 mμ, log ε 2·65, infra-red: 1,701, 1,767, and 3,636 cm.$^{-1}$, which was identical with monoketone obtained by chromic acid oxidation (equivalent to one oxygen atom) in acetic acid solution of (I) at room temperature. Drastic acetylation of (I) led to the corresponding triacetate (V), $C_{36}H_{50}O_8$, m.p. 248°, $[\alpha]_D$ −26·4, infra-red: 1,724, 1,736, and 1,764 cm.$^{-1}$. (V) was reconverted into the parent triterpene (I) on saponification.

Thus the five oxygen atoms were demonstrated to be present as five-membered lactone ring and three hydroxyl groups which differed in their reactivity. It was clear that two of three hydroxyl groups are oriented in hindered positions.

The ultra-violet absorption of (I) exhibited a primary maximum at 244 mμ (log ε 4·15), with subsidiary maxima at 238 and 255 mμ. Coupled with the colour reaction with tetranitro-methane of (I), this suggested the presence of a conjugated heteroannular diene. By catalytic hydrogenation in acetic acid solution of (I), dihydroholothurigenin (VI), $C_{30}H_{46}O_5$, m.p. 285°, $\lambda_{max.}$ 215 mμ, log ε 3·01, was obtained in which the ultra-violet absorption band observed at 244 mμ had disappeared due to a conjugated double bond.

At this stage of the investigation it was concluded that holothurigenin was a trihydroxy-lactone-diene belonging to the triterpenoid series. The structure of holothurigenin is now being investigated.

TAKAO MATSUNO*
TOSHIHIKO YAMANOUCHI

Kyoto College of Pharmacy,
Kyoto, Japan.

---

* Present address: Department of Medicinal Chemistry, University of Buffalo, Buffalo, New York.

[1] Yamanouchi, T., *Pub. Seto Mar. Biol. Lab.*, 4 (2–3), 183 (1955); *Teikoku Gakushiin Hokoku*, 73 (1942); *Zool. Mag.*, 55, 87 (1943); *Folia Pharmacologica Japonica*, 38, fasc. 2, 115 (1943).

[2] Nigrelli, R. F., *Zoologica* (New York), 37, 89 (1952).

[3] Chanley, J. D., Ledeen, R., Wax, J., Nigrelli, R. F., and Sobotka, H., *J. Amer. Chem. Soc.*, 81, 5180 (1959).

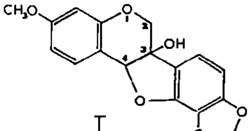
Pisatin: an Antifungal Substance from *Pisum sativum* L.

It is obvious that plants are resistant to attack by fungi that are non-pathogenic to them. However, until recently, the mechanism of this resistance has been obscure. Pisatin, a substance believed to be responsible for such resistance in the garden pea (*Pisum sativum* L.), has been recently isolated from pea pods inoculated with fungal spores[1].

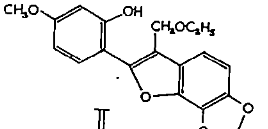
Pisatin has the molecular formula $C_{17}H_{14}O_6$, and, from chemical and spectroscopic evidence, contains one alcoholic hydroxyl, one methoxyl and one methylenedioxy group. Detailed examination of pisatin and some of its degradation products by physico-chemical methods, including nuclear magnetic resonance, ultra-violet and infra-red spectra, indicates that pisatin has structure I.

The location of the methoxyl and methylenedioxy groups is suggested by the very close similarity of the ultra-violet and infra-red spectra to those of pterocarpin[2], although the possibility that the methylenedioxy group is attached to different positions in the same ring cannot be entirely excluded. Pisatin has ultra-violet absorption maxima at 309 mμ (log ε 3·86) and 286 mμ (log ε 3·68) with an inflexion at 281 mμ, while pterocarpin has maxima at 310 mμ (log ε 3·92) and 286 mμ (log ε 3·69) with an inflexion near 280 mμ. Pisatin is optically active with $(\alpha)^{20°}_{578} = +280°$ ($c = 0·11$ in ethanol). Pterocarpin has comparable optical activity, but is lævorotatory[2]. Pisatin appears to differ from pterocarpin only in having a hydroxyl group on carbon 3, and also by possibly having the opposite optical configuration.

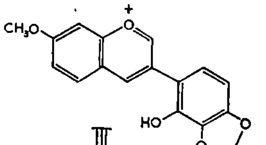
The presence of the hydroxyl group confers some interesting chemical properties on pisatin. Thus in the presence of acid, pisatin readily loses a molecule of water across positions 3 and 4 to form anhydropisatin, $C_{17}H_{12}O_5$ (ref. 1). The disappearance of the band at 3,610 cm.$^{-1}$ in the infra-red spectrum confirms the loss of a hydroxyl group in passing to anhydropisatin. This dehydration extends the conjugation over the whole molecule and leads to a considerable displacement of the ultra-violet absorption maxima towards the visible, with a concomitant intensification of absorption. The ultra-violet absorption spectrum of anhydropisatin ($\lambda_{max.}$ at 339 mμ and 358 mμ; log ε of 4·58 and 4·60) is qualitatively very similar to that reported by Suginome[3] for the closely related substance, anhydrosophorol, formed by cyclodehydration of sophorol, a 2′-hydroxy isoflavanone obtained from *Sophora japonica* L. (Leguminosæ, sub-family Papilionaceae).



I



II



III

When alcoholic solutions of anhydropisatin are heated or exposed to diffuse daylight, fission occurs across the 1 : 2 oxygen–carbon bond giving rise to compound (II). The ultra-violet spectrum of this substance with $\lambda_{max.}$ 269 mμ (log ε 4·18) and 319 mμ (log ε 4·39) is similar to those reported for related 2-phenyl benzofurans[4]. On the other hand, if pisatin itself is irradiated with mercury 253·7 mμ light, it is converted initially to a substance which is spectroscopically very similar to pisatin. This substance differs from pisatin, however, in not forming anhydropisatin on mild acid treatment, and in not showing comparable antifungal activity[5]. Further irradiation followed by acidification gives rise to the isoflavylium compound (III) which is cherry red in strong acid and greenish yellow in weakly acid or neutral solutions (cation has $\lambda_{max.}$ 419, 552 mμ; log ε 4·0, 4·0; neutral molecule has only $\lambda_{max.}$ 419 mμ, log ε 4·0). Spectrophotometric examination shows that this compound is half converted to the cation in 3·4 *M* hydrochloric acid, which indicates that this base is about 100,000 times weaker than the flavylium bases such as anthocyanidins. Compound (III) has similar properties to those reported for the isoflavylium compound prepared by Baker[6].

The ring structure of pisatin occurs in a number of other natural products including pterocarpin and homopterocarpin[2] (from *Pterocarpus santalinus* L.f.), coumestrol[7] (from ladino clover *Trifolium repens* L.), erosnin[8] (from the yam bean *Pachyrrhizus erosus* Rich.), psoralidin[9] (from *Psoralea corylifolia* L.) and wedelolactone[4] (from *Wedelia calendulacea* Less.). It is of interest that in all cases, except *Wedelia calendulacea* (Compositae), the plants, from which this relatively new series of compounds have been obtained, are members of the family Leguminosae (Papilionaceae).

Further details of this work will be published elsewhere.

We wish to thank Mr. B. Moore and Prof. A. J. Birch, of the University of Manchester, for a sample of pterocarpin.

DAWN R. PERRIN
W. BOTTOMLEY

Division of Plant Industry,
Commonwealth Scientific and Industrial Research Organization,
P.O. Box 109, City,
Canberra.

[1] Cruickshank, I. A. M., and Perrin, Dawn, R., *Nature*, **187**, 799 (1960).
[2] McGookin, A., Robertson, A., and Whalley, W. B., *J. Chem. Soc.*, 787 (1940).
[3] Suginome, H., *J. Org. Chem.*, **24**, 1655 (1959).
[4] Govindachari, T. R., Nagarajan, K., and Pai, B. R., *J. Chem. Soc.*, 629 (1956).
[5] Cruickshank, I. A. M., and Perrin, Dawn R., *Austral. J. Biol. Sci.* (in the press).
[6] Baker, W., *J. Chem. Soc.*, 1593 (1929).
[7] Bickoff, E. M., Lyman, R. L., Livingston, A. L., and Booth, A. N., *J. Amer. Chem. Soc.*, **80**, 3969 (1958).
[8] Eisenbiess, J., and Schmid, H., *Helv. Chim. Acta*, **42**, 61 (1959).
[9] Duttagupta P. C., Khastgir, H. N., and Sengupta, P., *Chem. and Indust.*, 937 (1960).

Prodigiosin in an Actinomycete

PRODIGIOSIN, the bright red pigment produced by organisms of the genus *Serratia*, is among the more conspicuous pigments extant in the microbial world. The chemical nature of prodigiosin has been the subject of extensive study and was defined as a tripyrrylmethene by Wrede and Rothhaas in 1934[1]. Subsequent work has demonstrated that the pigment is actually composed of a number of separable components rather than the single entity supposed by earlier workers[1]. Although prodigiosin has been studied predominantly in the genus *Serratia*, the possible presence of the pigment in an actinomycete has been reported[2]. In the course of our isolating vast quantities of actinomycetes from soils collected throughout the world, a small number of orange-red pigmented *Streptomyces* were observed. This communication is concerned with the identification of the pigment produced by these actinomycetes as identical to that produced by *Serratia marcescens*.

The actinomycetes were grown on a medium consisting of soytone, casein hydrolysate, and yeast extract at 0·1 and 3 per cent agar. Plates were inoculated from slants of the actinomycete. After growth for 5 days at 28° C. the cells were washed from the agar surface with acetone/water (50 : 50), dried under vacuum, and extracted with ethanol in a Soxhlet extractor. The pigment was further purified according to the procedure of Williams *et al.*[3].

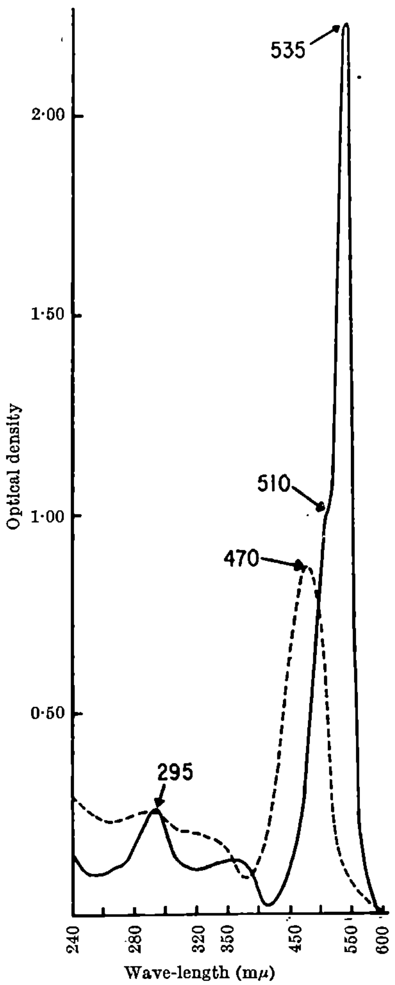


Fig. 1. Spectral properties of purified prodigiosin from an actinomycete. Solid line, when dissolved in absolute ethanol + hydrochloric acid. Broken line, in absolute ethanol + sodium hydroxide

Chromatographic separation of the prodigiosin components was achieved by ascending chromatography using Whatman No. 1 paper and developed with petroleum ether/ethyl ether (2 : 1). Comparison of the chromatographic behaviour of the actinomycete pigment with the bacterial pigment[3] indicated that some differences in the relative quantities of the various components between the two species do exist. The extracts from both actinomycete and *S. marcescens* gave orange-red zones at the following R_F values : 0·92, 0·85, 0·74, 0·46, and a blue spot at the origin.

For the purpose of obtaining ultra-violet and visible spectra of the actinomycete pigment, approximately 100 μgm. of dried pigment was chromatographed. After development, the coloured area at R_F 0·92 was eluted with 10 ml. ethanol. This was the predominant spot on the paper. For a control an equal area of blank paper at the same R_F was also eluted. Spectral analyses were run on a Cary recording spectrophotometer. Acid or alkaline conditions were obtained by adding 1 drop of 1 N hydrochloric acid or 1 N sodium hydroxide to the ethanol extract.

The acid and alkaline spectra of the pigment are presented in Fig. 1. The alkaline spectrum has a peak at 470 mμ. The acid spectrum exhibits peaks at 535 mμ and 295. mμ and a shoulder at 510 mμ. These results are in agreement with published spectra for bacterial prodigiosin[3]. Chromatographic comparison of the bacterial and actinomycete pigment in various solvents[4] showed that the same components were present in each although the relative amounts varied.

The results demonstrate that actinomycetes of the genus *Streptomyces* producing prodigiosin are present in soils. Prodigiosin was isolated, and is qualitatively equivalent to the bacterial pigment, although differences in relative quantities of the components do exist.

JEROME J. PERRY

Kitchawan Research Laboratory,
Brooklyn Botanic Garden,
Ossining, New York.

[1] Wrede, F., and Rothhaas, A., *Hoppe-Seyl. Z.*, **226**, 95 (1934).

[2] Dietzel, E., *Naturwiss.*, **35**, 345 (1948).

[3] Williams, R. P., Green, J. A., and Rappoport, D. A., *J. Bact.*, **71**, 115 (1956).

[4] Rappoport, D. A., Calvert, C. R., Loeffler, R. K., and Gast, J. H., *Anal. Chem.*, **27**, 820 (1955).

PHYSIOLOGY

Vitamin-Levels in Blood and Serum

EXCEPT for folic acid and cyanocobalamin (vitamin B_{12}) only scattered information can be found in the literature on the content of water-soluble vitamins in human blood and serum[1,2]. In order to establish basic values of normal blood and serum vitamin for various clinical investigations, microbiological assays were carried out for a number of vitamins by the methods which we have developed.

Blood was obtained from an antecubital vein and permitted to clot at room temperature for 3 hr. After centrifugation, serum was withdrawn for assay. Care was taken to obtain serum free of any hæmolysis; intracellular contents gave erroneous values. Citrated blood was used for whole-blood assays. Blood and serum were assayed for (*a*) B_{12} with *Ochromonas malhamensis*[3a], (*b*) folic acid with *Lactobacillus casei*[3b], (*c*) unconjugated pteridines with *Crithidia fasiculata*[3c], (*d*) thiamine with *O. malhamensis*[3d], (*e*) nicotinic acid with *Tetrahymena pyriformis*[3f], (*f*) pantothenic acid with *L. plantarum*[3e], and (*g*) vitamin B_6 with *T. pyriformis*[3e]. Table 1 gives the range encountered both in whole blood and in serum of 28 normal subjects. The whole-blood values for folic acid, unconjugated pteridine, thiamine, nicotinic acid, and perhaps for the B_6 group (Table 1) are higher than those for serum; the reverse is true for B_{12}. Nicotinic acid activity almost wholly resides in the cellular elements of the blood.

The folic acid activity of serum, not whole blood, serves as an index of clinical folic acid deficiency[3b]. In blood of normal subjects the cells carry 1/5–1/6 the total B_{12}; in pernicious anæmia their share is 1/2–1/3[4a,b]. Serum B_{12}-levels reflect the tissue supply.

Table 1. VITAMIN ACTIVITIES OF WHOLE BLOOD AND SERUM (μGM./ML.)*

Vitamin	Whole blood	Serum
B_{12}	0·12–0·45	0·14–0·64
Folic acid	14–72	7–27
Unconjugated pteridine	27–70	11–43
Thiamine	20–41	3–15
Nicotinic acid	4,900–9,600	50–80
Pantothenic acid	230–2,075	200–1,650
B_6 group	26–78	26–43

* Based on 28 normal subjects.

In other work, we have observed exceptionally high levels of unconjugated pteridines in sera from patients with gout, while folic acid activity was in the normal range. The normal values presented here have also served as controls in comparative work on the vitamin content in maternal versus fœtal blood[5a,b] and for comparison with the cerebrospinal fluid values in patients with neurologic disease[6a,b].

We thank Dr. T'sai F. Yu of the Department of Medicine, Mount Sinai Hospital, for supplying samples from patients with gout; also we thank the U.S. Public Health Service, the National Association for Mental Health, and the American Cancer Society for financial support.

H. BAKER
O. FRANK
I. PASHER

Seton Hall College of Medicine,
Division of Hepatic Metabolism
and Nutrition,
Jersey City, New Jersey.

H. SOBOTKA

Department of Chemistry,
Mount Sinai Hospital, New York.

S. H. HUTNER

Haskins Laboratories,
New York.

[1] Girdwood, R. H., *Adv. in Clin. Chem.*, edit. by Sobotka, H., and Stewart, C. P., **3**, 235 (Academic Press, 1960).

[2] Grasbeck, R., *Adv. in Clin. Chem.*, edit. by Sobotka, H., and Stewart, C. P., **3**, 299 (Academic Press, 1960).

[3] *a*, Baker, H., Frank, O., Pasher, I., and Sobotka, H., *Clin. Chem.* (to be published). *b*, Baker, H., Herbert, V., Frank, O., Pasher, I., Wasserman, L. R., and Sobotka, H., *ibid.*, **5**, 275 (1959). *c*, Baker, H., Frank, O., Pasher, I., Hutner, S. H., and Sobotka, H., *J. Protozool.*, **8** (to be published). *d*, Baker, H., Pasher, I., Frank, O., Hutner, S. H., Aaronson, S., and Sobotka, H., *Clin. Chem.*, **5**, 13 (1959). *e*, Baker, H., Frank O., Pasher, I, Hutner, S. H., and Sobotka, H., *ibid.*, **6**, 572 (1960). *f*, Baker, H., Frank, O., Pasher, I., Dinnerstein, A., and Sobotka, H., *ibid.*, **6**, 36 (1960).

[4] *a*, Baker, H., Pasher, I., Sobotka, H., Hutner, S. H., Aaronson, S., and Ziffer, H., *Nature*, **180**, 1043 (1957). *b*, Sobotka, H., Baker, H., and Ziffer, H., *Amer. J. Clin. Nutrition*, **8**, 283 (1960).

[5] *a*, Baker, H., Erdberg, R., Pasher, I., and Sobotka, H., *Proc. Soc. Exp. Biol. Med.*, **94**, 513 (1957). *b*, Baker, H., Ziffer, H., Pasher, I., and Sobotka, H., *Brit. Med. J.*, i, 978 (1958).

[6] *a*, Sobotka, H., Christoff, N., and Baker, H., *Proc. Soc. Exp. Biol. Med.*, **98**, 534 (1958). *b*, Sobotka, H., Baker, H., and Frank, O., *ibid.*, **103**, 321 (1960).

Aldosterone Excretion and Potassium Retention in Subjects living at High Altitude

IT has been shown[1] that during a prolonged stay at altitudes above 15,000 ft. there is an increase in the salivary excretion of sodium and a decrease in that of potassium. In one subject this change in salivary electrolyte composition showed a marked acclimatization effect (Fig. 1). These findings suggested that, on arrival at a high altitude, the level of circulating aldosterone was likely to be greatly reduced, but that there would probably be a tendency for it to return slowly towards normal values with passage of time.

During July and August 1960, members of the Middlesex Hospital Medical School High Altitude Physiological Expedition spent twenty-four days at an altitude of 14,300 ft. on Mont Blanc[2]. 24-hr. urine collections were made from seven subjects (four men and three women). These specimens were frozen and sent in 'Styrocell' insulated containers by air to London, where they arrived still solid.

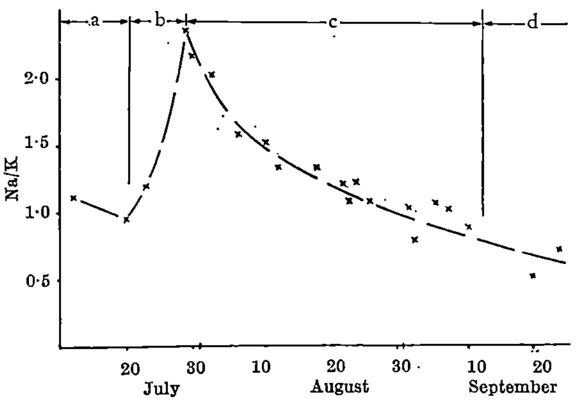


Fig. 1. Salivary sodium/potassium in one individual during an expedition to the Karakoram Mountains. – –, sodium/potassium level in London; *a*, 8,000–10,000 ft; *b*, 10,000–16,000 ft.; *c*, 16,000–20,000 ft.; *d*, below 10,000 ft. Each point is derived from five observations

Assay of urinary aldosterone was carried out by a physico-chemical method modified from that of Ayres *et al.*[3]. The sodium and potassium contents were determined by flame photometry. The results are shown in Fig. 2 and it will be seen that the inference from the earlier work is correct, assuming the validity of urinary aldosterone assay as an index of circulating hormone-levels.

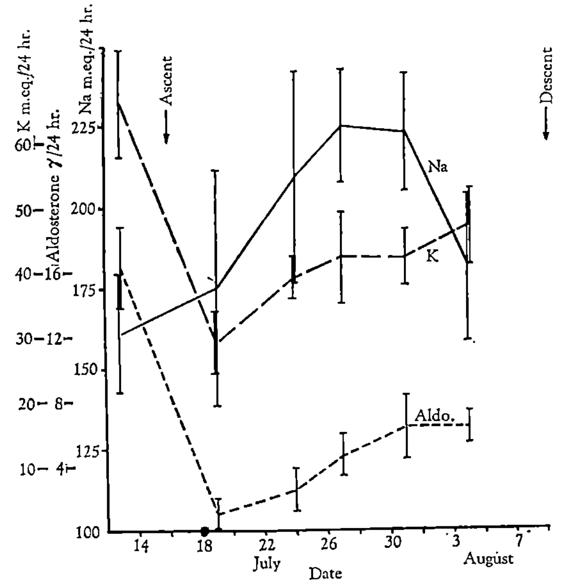


Fig. 2

The decreased potassium excretion apparent from Fig. 2 should result in a demonstrable increase in body potassium content. An attempt was made by one of us (J. R.) to confirm this in three subjects, whose whole body potassium contents were estimated by taking advantage of the naturally radioactive isotope potassium-40, present in potassium to the extent of 0·0119 per cent. The body radioactivity was measured in the human gamma-ray spectrometer[4] at the Atomic Energy Research Establishment, Harwell, before the expedition, and again two days after the descent from the mountain. The results are shown in Table 1. At each measurement the potassium content was calculated from the counting-rates observed in the energy bands 875–1,275 keV. and 1,275–1,675 keV. in the gamma-ray spectrum (potassium-40 emits 1·46 MeV. quanta). The equipment was calibrated by determining the counting-rates in these energy bands from known amounts of pure potassium chloride in aqueous solution in a phantom, and there may be systematic errors of up to about ± 5 per cent in the estimates of the contents of individuals[4]. In all cases the agreement between the two estimates of each subject's content was within the statistical error of counting, indicating the absence of detectable contamination with other radioactive nuclides emitting gamma-rays of energy greater than 0·8 MeV. The values in Table 1 are the weighted means of the pairs of estimates, with statistical standard errors only.

Table 1

Subject	Total potassium content (gm.)		
	Before expedition	After descent	Difference
P. M. ♀	107 ± 3·8	115 ± 3·8	8 ± 5·4
E. W. ♂	146 ± 4·0	152 ± 3·9	6 ± 5·6
W. W. ♀	100 ± 4·2	110 ± 3·8	10 ± 5·7
Total	353 ± 6·9	377 ± 6·6	24 ± 9·6

In only one subject is the increase in potassium content significant at the 90 per cent confidence-level, but the increase in the total content of all three subjects is significant at the 99 per cent confidence-level.

During the stay on the mountain the diet of the subjects consisted largely of dehydrated foodstuffs. In order to establish what effect the diet had on the results obtained, one of the subjects carried out a control experiment at sea-level. 24-hr. urine samples were collected for five consecutive days while he was on a normal diet, and then continued while he was on the same diet as he had eaten on the mountain. These were assayed for sodium and potassium, and three of the five samples relating to the period of normal diet and five of those relating to the period on the special diet were assayed for aldosterone.

The aldosterone results are plotted in Fig. 3 where they are compared with the results from the same subject for the corresponding period on Mont Blanc. It will be observed that a drop in aldosterone

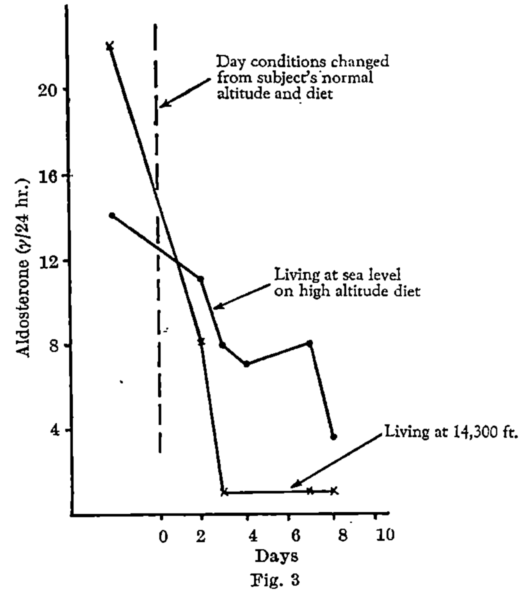


Fig. 3

excretion followed the change in diet, but that this drop was less marked than it was when a change in altitude coincided with the dietary change. If the mean 24-hr. excretion before and after the changed conditions is compared in each case, we see that on the mountain the excretion dropped to 12·5 per cent of its previous mean whereas it dropped to 53·5 per cent when the diet alone was changed.

It is a pleasure to acknowledge the financial support received from the Medical Research Council, from the Clinical Research Committee of the Middlesex Hospital, and from the Middlesex Hospital Club.

P. J. Ayres
Rona C. Hurter
E. S. Williams

Barnato-Joel Laboratories,
The Middlesex Hospital
Medical School,
London, W.1.

J. Rundo

Atomic Energy Research Establishment,
Harwell, Berkshire.

---

[1] Williams, E. S., *Clin. Sci.*, **21**, 37 (1961).

[2] Williams, E. S., and Edwards, R., *Middlesex Hosp. J.*, **60**, 167 (1960).

[3] Ayres, P. J., Garrod, O., Simpson, S. A., and Tait, J. F., *Biochem. J.*, **65**, 639 (1957).

[4] Rundo, J., *Proc. Second Int. Conf. Peaceful Uses of Atomic Energy*, **23**, 101 (1958).

Controlling Mechanism of the Mechanical Activity of Muscle and that of Blood Coagulation

It has long been suggested that there exists a relationship between colloidal behaviour of muscle and that of blood, namely, (1) molecular structure of actomyosin and that of fibrinogen (both belong to the *k–m–e–f* group[1]), (2) coagulative phenomena both in sarcoplasm and in blood[2], and (3) the role of calcium both in contractile activity of muscle and in blood coagulation[2]. Recently, Heilbrunn[2] has suggested that the mechanism of blood coagulation in relation to the mechanism of cellular activity may be significant. The present communication is concerned with the positive results, which were obtained in a model system of the muscle, to demonstrate the close resemblance between the mechanism in mechanical muscular contraction and that in blood coagulation.

As shown in Fig. 1, phospholipids remove the inhibitory effect[3] of muscle granules on adenosine triphosphatase activity of myofibrils prepared from skeletal muscle. The concentration at which the phospholipid effect begins to appear is relatively low. In Fig. 2, it is shown that heparin inhibits the adenosine triphosphatase activity of myofibrils prepared from skeletal and cardiac muscle. The inhibition is not complete, even if considerably higher concentrations of the agent are used, while the effect begins to appear at relatively lower concentrations, especially in the case of skeletal myofibrils. It is interesting that in the case of myofibrils prepared from heart, which is poor in heparin[2], the inhibitory effect is relatively lower, for example, at 100 units/ml. of heparin it is only about 50 per cent; while, under the similar condition, it is considerably higher in the case of skeletal muscle, which is rich in heparin[2]. The development of tension of a single glycerinated fibre of rabbit psoas muscle due to adenosine triphosphate is also inhibited by the addition of heparin. One more interesting point is that adenosine triphosphatase activity of granules prepared from rabbit skeletal muscle is not inhibited by heparin. The inhibitory action of heparin is therefore specific to the kind of adenosine triphosphatase. The inhibitory action cannot be removed by the addition of calcium and appears regardless of concentrations of adenosine triphosphate.

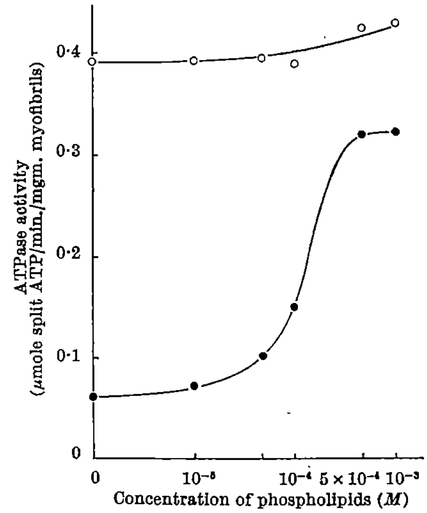


Fig. 1. Phospholipids mixture and the inhibitory action of granules on adenosine triphosphatase activity of myofibrils prepared from rabbit skeletal muscle. ○, Splitting of adenosine triphosphate by fibrils in the absence of granules; ●, that in the presence of granules. The reaction mixture of 3 ml. consisted of 5 m*M* adenosine triphosphate, 5 m*M* magnesium chloride, 5 m*M* potassium oxalate, 360 μgm. protein/ml. of fibrils, 28 μgm. protein/ml. of granules, and 20 m*M* *tris*(hydroxymethylamino)-methane (*p*H 7). Final concentration of potassium in the reaction mixture was 50 m*M*, and the experiments were done at 20° C. Phospholipids mixture used consists of 55 per cent lecithin, 27 per cent colamine-cephalin, 2 per cent serine-cephalin, 5 per cent inositol phosphatid and 5 per cent sphingolipid; and molarity of phospholipids in the reaction solution was calculated as 775 average molecular weight. Concerning myofibrils and granules, see the explanation in Fig. 2

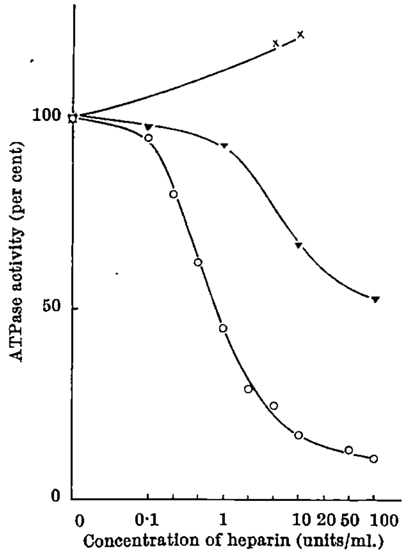


Fig. 2. Effect of heparin on the enzymatic activity of various kinds of adenosine triphosphatase. ○, Myofibrils prepared from rabbit skeletal muscle; ▼, that from dog cardiac muscle; and ×, granules extracted from rabbit skeletal muscle. Myofibrils were obtained according to Perry *et al.* (ref. 8); and granules from 2,600*g* to 35,000*g* were extracted from muscle homogenate, which were obtained in the solution, consisting of 0·1 *M* potassium chloride, 5 m*M* potassium oxalate, and 5 m*M* phosphate buffer (*p*H 7). Conditions of experiments are similar to those of Fig. 1

It is well known that muscle contains various kinds of phospholipids, especially cephalin and lecithin, and heparin[2]. However, their functions are not always clear. Of course, our results are not direct evidence of their significance in the mechanical activity of muscle and do not indicate the dual relationship between the mechanical activity of muscle and blood coagulation, with respect to the processes on which the substances mentioned have an effect. Our results are, however, considered to be very interesting in that substances which play an important part in blood coagulation exert some influence on the function of the contractile element of muscle in the same way as blood coagulation. In view of the liberation of heparin-like substances[2] from skeletal muscle during continued activity and of the existence of lipids[4] in microsome or sarcoplasmic reticulum, it would seem probable from our results that a certain lipid compound, especially a substance related to phospholipids such as cephalin and lecithin, and a certain substance related to heparin, control the activity of the contractile element of muscle. (Our preliminary experiments show that the fatigue substance[2] from frog leg muscles has an inhibitory action on the development of tension of the above fibre due to ATP.)

Many reports[5-7] concerning the mechanism in the excitation-contraction link have been presented. The conclusive solution for this problem has, however, not yet been given. Our results suggest the complexity of the linking mechanism and the resemblance of this mechanism to the mechanism in blood coagulation.

We wish to thank Prof. T. Nagai and Prof. E. Miyazaki for their encouragement in the course of the present work. We are also grateful to Dr. T. Shimojo of the Biochemical Department of Sapporo Medical College for supplying a sample of phospholipids mixture.

Masahiro Fujino
Saburo Yamaguchi
Masa Takauji
Kohki Uchida
Masahide Yasuda
Tatsuaki Matsushima

Department of Physiology,
Sapporo Medical College,
Sapporo, Japan.

[1] Astbury, W. T., *Exp. Cell Res.* Supp. 1, 234 (1949).

[2] Heilbrunn, L. V., *The Dynamics of Living Protoplasma* (Academic Press, New York, 1956).

[3] Bendall, J. R., *Nature*, **181**, 1188 (1958).

[4] Lorand, L., Molnar, J., and Moos, C., *Conf. Chemistry of Muscular Contraction* (Igaku Shoin, Tokyo, 1957).

[5] Kahn, A. J., and Sandow, A., *Science*, **112**, 647 (1950).

[6] Huxley, A. F., and Taylor, R. E., *J. Physiol.*, **144**, 426 (1958).

[7] Fujino, M., Matsushima, T., Muroya, T., Yabu, H., Yamaguchi, S., and Takahashi, M., *Nature*, **186**, 318 (1960).

[8] Perry, S. V., and Grey, T. C., *Biochem. J.*. **64**, 184 (1956).

Increase of Evaporative Loss of Water from the Skin of the Ox in Response to Local Heating of the Hypothalamus

The existence of centres in the hypothalamus which are concerned with thermoregulation has been established for several species of mammals[1]. In the cat and monkey[2,3] local heating of the region between the optic chiasma and the anterior commissure is accompanied by vasodilatation, an increase in respiratory-rate and sweating.

In the ox, heating of a discrete region of the hypothalamus causes panting and vasodilatation[4], but it is not known if such heating causes any change in the rate of vaporization of water from the skin. Nor is it certain that the sweat glands play any part in thermoregulation[5]. The effect of local heating of the hypothalamus of bull calves on vaporization of water from their skin is reported here.

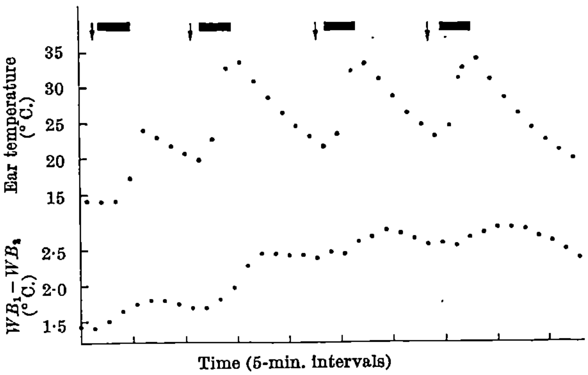


Fig. 1. The figure is taken from a recorder chart on which each temperature was recorded every 80 sec. An increase in the difference between the wet-bulb temperatures ($W.B._1-W.B._2$) indicates an increase in evaporation from the skin. The arrows mark the point at which heating of the hypothalamus was started, while the blocks show the 3-min. periods during which the temperature of the hypothalamus was at 41·5° C.

The brain was heated by means of a radio-frequency voltage applied between two electrodes 1·5 cm. apart on either side of the midline, dorsal to the optic chiasma. A thermocouple midway between the electrodes recorded the temperature to which the brain was heated. Moisture loss from the skin was measured by means of a metal 'sweat cup' through which a stream of air was drawn at a constant rate. The cup was applied to the unshaved skin by means of an elastic belt. Comparison of the wet-bulb temperatures of the air entering and leaving the cup revealed changes in the rate of vaporization of moisture from the skin. Skin temperatures were determined with (40 standard wire gauge) copper-constantan thermocouples applied to the shaved skin of the pinnæ of the ears while skin temperatures under the 'sweat cups' were measured beneath the hair. The rate of respiration was recorded by means of an electro-mechanical counter[6].

Three Ayrshire bull calves, all aged one year, were exposed to room temperatures of 10° C.–20° C. in a climatic room. Three 'sweat cups' were disposed over the thoracic, mid flank and sacral regions of each calf. When the temperature of the hypothalamus was raised to 41·5° C. and held there for 3 min. the rate of loss of moisture in each cup increased in 31 out of 35 instances, although the magnitude of the increase varied. In the four experiments in which there was little or no increase in loss of moisture, the rate of evaporation was already high. The response of one animal (*D*) was considerably lower than that of the other two (*B* and *C*). This animal was much smaller and was known from other work to be inapt in the control of its body temperature in a hot environment.

In all three calves, at the same time as evaporation from the skin increased there was a rise in respiratory-rate, and in those instances in which initially the skin temperatures of the ears were low there was a marked rise in the ear temperature indicating increased blood-flow. Small increases in skin temperature under the 'sweat cups' were also noticed on most occasions. As may be seen in Fig. 1 the rate of loss of moisture increased rapidly while the hypothalamus was being heated and continued to rise after heating had ceased; thereafter evaporation declined slowly. Heating the hypothalamus for less than 3 min. or to less than 41·5° C. produced only a very small increase in evaporation, although a rise in the skin temperature of the ears occurred and respiratory-rate increased.

When the hypothalamus was repeatedly heated to 41·5° C. for 3 min. at intervals, the rate of evaporation from the skin increased progressively (Fig. 1), but the magnitude of the increase tended to decline with successive stimulations.

The results of the present work demonstrate that evaporation from the skin as well as vasodilatation and panting can be stimulated by heating the hypothalamus. It is not, however, suggested that a rise in the temperature of the hypothalamus is a necessary condition for an increase in loss of moisture from the skin.

The increase in evaporation may be related to the active secretion of the sweat glands, or to an increase in diffusion of water through the skin. The progressive increase in evaporation after repeated heating is, however, difficult to account for if evaporative loss is due to simple diffusion.

D. L. INGRAM
J. A. MCLEAN
G. C. WHITTOW

Department of Physiology,
Hannah Dairy Research Institute, Ayr.

---

[1] Ranson, S. W., *Res. Pub. Assoc. Nerv. Ment. Dis.*, **20**, 342 (1940).
[2] Magoun, H. W., Harrison, F., Brobeck, J. R., and Ranson, S. W., *J. Neurophysiol.*, **1**, 101 (1938).
[3] Beaton, L. E., McKinley, W. A., Berry, C. M., and Ranson, S. W., *J. Neurophysiol.*, **4**, 478 (1941).
[4] Findlay, J. D., and Ingram, D. L., *J. Physiol.*, **155**, 72 (1961).
[5] Weiner, J. S., and Hellman, K., *Biol. Rev.*, **35**, 141 (1960).
[6] Nisbet, W., *J. Physiol.*, **144**, 4, *P* (1958).

Effect of Added Vitamins on the Phagocytic Activity of the Reticulo-Endothelial System of Mice

WE have reported that œstrogens stimulate the reticulo-endothelial system in the spleen, liver and lymph nodes and lead to mobilization of the phagocytes into the blood stream and thus probably to an increase of the general defence of the body against infection[1]. We have also shown that antibiotics[2] and sulphonamides[3] have little effect on the phagocytes; but cortisone depresses the reticulo-endothelial system and also reduces the concentration of gamma-globulin in the serum to about half the normal level[4]. Unpublished research in this laboratory also shows that when the reticulo-endothelial system is stimulated with, for example, diethylstilbœstrol, the serum gamma-globulin is increased to about twice the normal level, and the formation of antibody is increased about eight times. Groups of mice and guinea pigs may be protected by this means against challenge with a number of lethal doses of virulent bacteria. Further unpublished results show that, when animals are given a diet free from vitamin *A*, the reticulo-endothelial system becomes greatly depressed and when normal diet (Oxo mouse and rat diet) is resumed the phagocytic index returns to normal in about thirteen days.

The present research was planned to ascertain the effect of added vitamins on the phagocytic activity of the reticulo-endothelial system in normal healthy animals.

Two hundred and ten male white mice (T.O. Swiss strain) of 20–25 gm. weight were used in these experiments. Ten animals were used to test each vitamin. The vitamins used, the dose, route of administration, frequency and duration of dosage are shown in Table 1. The estimated daily vitamin requirements, also shown in Table 1, are those given by Spector[5].

The doses in most of the experiments are greater than the estimated requirement and during the treatment the animals remained on normal diet. Vitamin D was dissolved in olive oil, vitamin E and vitamin K (natural) in arachis oil to which a small amount of 'Teepol' was added, and the remaining vitamins were given in aqueous solution suitably buffered for stability according to information kindly supplied by Roche Products. All the solutions were made up so that one dose was contained in 0·2 ml. The route

Table 1. EFFECT OF ADDED VITAMINS ON THE PHAGOCYTIC ACTIVITY OF THE RETICULO-ENDOTHELIAL SYSTEM

Vitamin used	Estimated daily requirement	Dose	Route	Frequency of dosage	Duration of dosage	Mean phagocytic index (*K* value) ± *S.E.*
Vitamin A palmitate	20 I.U.	70 I.U.	I.P.	2/week	1 week	14 ± 2·0
		70 I.U.	I.P.	2/week	2 weeks	14 ± 4·0
		70 I.U.	I.P.	2/week	3 weeks	12 ± 1·0
Calciferol	2·0 I.U.	10 I.U.	oral	2/week	1 week	11 ± 2·0
		10 I.U.	oral	2/week	3 weeks	11 ± 1·0
Vitamin A + calciferol		Vit. A—20 I.U. Calciferol—2 I.U.	I.P. oral	daily	2 weeks	17 ± 2·0
		,,	,,	daily	4 weeks	16 ± 8·0
Aneurin	0·02 mgm.	0·06 mgm.	oral	daily	1 week	17 ± 2·1
Pyridoxine	0·02 mgm.	0·08 mgm	oral	daily	1 week	19 ± 3·1
Nicotinic acid	0·1 mgm.	0·2 mgm.	oral	daily	1 week	14 ± 1·9
Inositol	?	40·0 mgm.	oral	daily	1 week	18 ± 1·5
Biotin	0·0008 mgm.	0·006 mgm.	oral	daily	1 week	14 ± 0·6
Pantothenol	0·2 mgm.	1·0 mgm.	oral	daily	1 week	15 ± 0·5
Choline	4·0 mgm.	40·0 mgm.	oral	daily	1 week	20 ± 1·8
Cyanocobalamine	0·00008 mgm.	0·0016 mgm.	I.P.	daily	1 week	17 ± 1·2
Ascorbic acid	?	1·0 mgm.	oral	daily	1 week	15 ± 1·2
α-Tocopherol	0·1 mgm.	2·0 mgm.	oral	daily	1 week	16 ± 1·3
Methylnaphthyline di(dihydrogen) phosphate	0·02 mgm.	0·4 mgm.	oral	daily	1 week	15 ± 1·6
Naphthaquinone	0·02 mgm.	0·4 mgm.	oral	daily	1 week	15 ± 1·0
Aneurine Riboflavine Pyridoxine Nicotinic acid Ascorbic acid		0·4 mgm. 0·8 mgm. 0·4 mgm. 2·0 mgm. 1·0 mgm.	oral	daily	1 week	17 ± 1·7
Controls						15 ± 2·0

of administration was either intraperitoneal or by stomach tube.

Two days after completion of the vitamin treatment the phagocytic activity was measured in each group of animals by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood[6], the procedure used being that described in a previous communication[3], and the total body phagocytic activity or phagocytic index being denoted by the symbol K.

Ten control animals, receiving no treatment, showed a phagocytic index or K value of 15 ± 2·0.

The results are shown in Table 1. In the doses administered the vitamins given to normal healthy growing mice had no discernible effect on the phagocytic activity of the reticulo-endothelial system. The above results might well have been expected since, for the most part, vitamin action appears to be related to the enzyme systems of normal cellular metabolism. In contrast, in infection, when the metabolism of the reticulo-endothelial cells increases, the requirement for the various vitamins may also be raised.

In these investigations we gratefully acknowledge advice and gifts of vitamins from Roche Products, Ltd., and Glaxo Laboratories, Ltd., and also financial assistance from the Tobacco Manufacturers' Standing Committee and the Medical Research Council.

J. Cordingley
T. Nicol

Department of Anatomy,
King's College,
London, W.C.2.

[1] Nicol, T., Helmy, I. D., and Abou-Zikry, A., *Brit. J. Surg.*, **40**, 166 (1952).

[2] Sewell, I. A., and Nicol, T., *Nature*, **181**, 1662 (1958).

[3] Nicol, T., and Sewell, I. A., *Nature*, **184**, 1241 (1959).

[4] Nicol, T., Snell, R. S., and Bilbey, D. L. J., *Brit. Med. J.*, ii, 800 (1956).

[5] Spector, W. S., *Handbook of Biological Data*, **1**, 197 (W. B. Saunders Co., Philadelphia, 1956).

[6] Biozzi, G., Benacerraf, B., and Halpern, B. N., *Brit. J. Exp. Path.*, **34**, 441 (1953).

Effect of Bradykinin on Uterine Activity

It was reported recently[1] that plasma kinin is formed spontaneously in human plasma collected during labour. Tested on the isolated rat uterus, the maximum oxytocic activity was stated to be equivalent to several units of oxytocin per millilitre of plasma. It was suggested that kinin formation may be related to the powerful contractions of the human uterus during parturition.

As yet the only kinin which has been chemically defined and is available in a pure form is bradykinin. We therefore investigated the uterine effect of synthetic bradykinin[2] on the human uterus *in situ* (external[3,4] and internal[5] tocography) and *in vitro*[6], and on the uterus *in situ* of anæsthetized non-pregnant rats, cats[7] and rabbits[8].

Single intravenous injections of up to 2 μgm./kgm. bradykinin and intravenous infusions of 0·14, 0·28 or 0·56 μgm./kgm./min. of bradykinin—each dose infused for a period of 5 min.—had no oxytocic effect on the human uterus *in situ* a few hours before or after labour. In one case, 1·4 μgm./kgm./min. infused for 3 min. was followed by a contraction, which may have been connected with the administration of the peptide. Although no uterine effect could be detected, the administration of higher doses of bradykinin elicited a number of symptoms, such as lowering of the systemic blood pressure, hot flushes, sensation of warmth in various parts of the body, etc.

Isolated strips of human myometrium obtained at Cæsarian section contracted only after bradykinin doses as high as several hundred or even thousand μgm./l. Some strips failed to respond even to 10,000 μgm./l., although their sensitivity to oxytocin ('Syntocinon^{R}'), for example, 25–100 milli-units/l. was normal (100 milli-units oxytocin is equal to 0·2 μgm. peptide).

The rat uterus *in situ* usually gave a short-lived contraction with 100 μgm./kgm. bradykinin (intravenous); 10 μgm./kgm. elicited a response only in a few animals. 100 milli-units/kgm. oxytocin consistently produced a response in all the rats.

In the cat uterus *in situ* 100 μgm./kgm. bradykinin (intravenous) invariably elicited a short-lived response. In many animals 33 μgm./kgm. or 10 μgm./kgm. was also effective, and one responded to as little as 3·3 μgm./kgm. All the cats displayed a characteristic uterine reaction to 40–80 milli-units/kgm. of oxytocin.

The sensitivity of the rabbit uterus *in situ* to bradykinin varied greatly. Whereas in some rabbits the intravenous injection of 1 or 10 μgm./kgm. was followed occasionally by a brief contraction of the uterus, others did not react even to 100 μgm./kgm. (intravenous). All rabbits showed a normal sensitivity to methyl-ergometrine ('Methergin^{R}'): 0·15 mgm./kgm. invariably elicited the usual uterotonic reaction.

The isolated rat uterus is known to have a very high sensitivity to bradykinin: a minute dose of 0·00003 μgm./ml. will sometimes elicit a contraction[9]. However, the findings presented in this communication show that bradykinin can scarcely be regarded as oxytocic in the usual sense of the word. The doses of bradykinin required to elicit a contraction of the rat, cat and rabbit uterus *in situ* are usually much higher than those evoking a marked fall of blood pressure. Moreover, the contraction is of a shape different from that produced by a low dose of oxytocin. The amounts of bradykinin which contract the isolated human myometrium are extremely high, and on the human uterus *in situ* bradykinin is virtually devoid of any effect, even in doses producing considerable distress.

These findings do not favour the view that bradykinin might be a physiological oxytocic factor responsible for increased uterine activity during labour in the human being.

B. Berde

Pharmacological Laboratories,
Sandoz, Ltd.,
Basle.

K. Saameli

Department of Obstetrics and Gynæcology,
Cantonal Hospital,
St. Gall, Switzerland.

[1] Armstrong, D. A. J., and Stewart, J. W., *Nature*, **188**, 1193 (1960).

[2] Boissonnas, R. A., Guttmann, St., and Jaquenoud, P.-A., *Helv. Chim. Acta*, **43**, 1349 (1960).

[3] Bainbridge, M. N., Nixon, W. C. W., Schild, H. O., and Smyth, C. N., *Brit. Med. J.*, i, 1133 (1956).

[4] Smyth, C. N., *J. Obstet. Gynæc. Brit. Emp.*, **64**, 59 (1957).

[5] Caldeyro-Barcia, R., and Poseiro, J. J., *Clin. Obstet. and Gynec.*, **3**, 386 (1960).

[6] Berde, B., and Saameli, K., *Acta Endocrinol.*, **32**, 391 (1959).

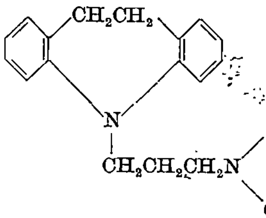
[7] Berde, B., Doepfner, W., and Konzett, H., *Brit. J. Pharmacol.*, **12**, 209 (1957).

[8] Rothlin, E., *Schweiz. med. Wschr.*, **68**, 971 (1938).

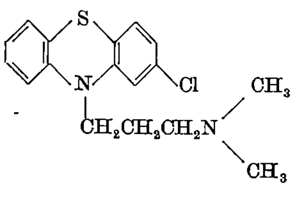
[9] Konzett, H., and Stürmer, E., *Brit. J. Pharmacol.*, **15**, 544 (1960).

Demonstration of Anti-Depressant or Stimulant Properties of Imipramine in Experimental Animals

IMIPRAMINE (I) is a drug that is related chemically to the major tranquillizers of the phenothiazine group such as chlorpromazine (II).



(I)



(II)

As distinct from chlorpromazine, however, imipramine is claimed to be of considerable value in the treatment of depression, particularly of the endogenous type[1-3]. Pharmacologically, imipramine has many of the properties of chlorpromazine although it is quantitatively less potent. Thus in the mouse, imipramine, like chlorpromazine, reduces motor activity, prolongs hexobarbitone sleeping time, and causes a fall in rectal temperature[4,5]. Both imipramine and suitable doses of chlorpromazine counteract the hypothermia, ptosis, bradycardia, and diarrhœa caused by reserpine administration in rats[6], and both drugs produce sedation in the cat and dog[4]. In the curarized cat, both drugs block the electrocortical desynchronization caused by electrical stimulation of the mesencephalic reticular formation[4], although it has been suggested[7] that this action of imipramine is due to atropine-like properties rather than to chlorpromazine-like sedation. Slight qualitative differences have been observed between chlorpromazine and imipramine in their action on the electrical activity recorded from certain sub-cortical areas in the rabbit. The resemblance between the pharmacological actions of these two drugs has led to the view[6] that the differences between the pharmacological effects of chlorpromazine and imipramine are, at present, only quantitative, rather than qualitative. Furthermore, unlike other drugs, such as iproniazid and nialamide, used at present in the treatment of depressive illnesses, imipramine is not an inhibitor of mono-amine oxidase[9]. There appeared, therefore, to be no pharmacological basis for its important clinical anti-depressant properties.

We wish to report on the action of imipramine in two laboratory tests which clearly reveal actions indicative of central stimulant or anti-depressant properties and which are qualitatively different from those of chlorpromazine and related phenothiazine derivatives.

(*a*) *Production of secondary conditioned responses in the rat.* A conditioned avoidance situation similar to that previously described[10] was used, in which rats were trained to climb a vertical rope at the sounding of a buzzer (conditioned stimulus), in order to avoid an electric shock (unconditioned stimulus). The three types of response which conditioned rats may show when placed in this situation are: (1) climbing the rope at the first conditioned stimulus (this is the response shown by most fully conditioned animals); (2) climbing the rope after a number of conditioned stimuli, or not climbing at all (this indicates that the conditioned reflex has been partly or completely inhibited); (3) climbing the rope before the conditioned stimulus is given. This last type of response has been termed a secondary conditioned response[11]. The ability of imipramine and other drugs either to block the conditioned reflex or to induce a secondary conditioned response was determined at intervals after administration. All the rats used in this investigation were selected from those giving consistent performances and climbing on the first conditioned stimulus.

(*b*) *Reversal of reserpine-induced ptosis in the rabbit.* In order to demonstrate an anti-reserpine property of imipramine we used the following method: Reserpine was infused intravenously into conscious rabbits restrained in a stock until a dose of 2·5 mgm./kgm. had been administered. Approximately 30 min. after the

Table 1. ACTIVITIES OF IMIPRAMINE, CHLORPROMAZINE, DEXAMPHETAMINE AND COCAINE IN PRODUCING SECONDARY CONDITIONED RESPONSES IN THE RAT AND IN REVERSING THE ACTION OF RESERPINE IN THE RABBIT

Substance	Conditioned reflex test (rat)				Behavioural changes in the rat	Reserpine reversal. Effective dose intravenous (rabbit) (mgm./kgm.)
	Route	Conditioned reflex	Secondary conditioned response			
		Effective dose for block of conditioned reflex	Dose (mgm./kgm.)	Per cent development of secondary conditioned response		
Dexamphetamine sulphate	s.c.	>4·0	0·25 0·5 1·0 2·0 4·0	20 50 80 60 40	General stimulation, exophthalmos, marked increase in motor activity, jerky head movements, fighting between rats	1·5±0·3
Cocaine hydrochloride	s.c.	>10·0	0·6 2·5 10·0	30 50 70	General stimulation, exophthalmos, no increase in motor activity. Rats show increase in alertness and reactivity. Jerky head movements, some disorientation.	6·8±0·6
Imipramine hydrochloride	p.o.	>40·0	5·0 10·0	20 40	Exophthalmos up to first hr. after dosing. Appearance becoming normal or showing slight ptosis. Initial increase in motor activity. No sedation.	15±1
			20·0 40·0	25 10	Most rats are sedated. Complete or partial ptosis, reduction in motor activity. Resemble chlorpromazine-treated animals.	
Chlorpromazine hydrochloride	s.c.	3·0	—	—	General sedation, complete or partial ptosis, reduction in motor activity, some loss of muscle tone.	>40

reserpine infusion, the rabbits developed symptoms of sedation including loss of reactivity, ptosis, hypothermia, and often loss of the righting reflex. This central depression was marked 1 hr. after the reserpine, and lasted for at least 4 hr. One hour after the administration of reserpine, a dilute solution of the drug under test was infused intravenously at the rate of 0·4 ml./min. The quantity of drug required to reverse the reserpine sedation completely was measured. We found that the degree of ptosis of the animals was the most satisfactory criterion of reserpine-induced sedation, since this followed closely the level of general reactivity and enabled a sharp end-point of the reserpine reversal to be obtained.

Table 1 sets out the activities of imipramine in these two tests in comparison with chlorpromazine, dexamphetamine and cocaine. Inhibitors of mono-amine oxidase, such as iproniazid and nialamide, were inactive in the two procedures.

At low doses in the rat, imipramine showed clear-cut stimulant properties; but as the dose was increased, chlorpromazine-like central depressant properties became more apparent. In the rabbit, complete reversal of reserpine-induced ptosis was observed after intravenous infusions, and if the infusion were continued exophthalmos became prominent. These changes were accompanied by an increase in alertness and general reactivity.

The fact that iproniazid and nialamide are inactive in these tests whereas imipramine shows clear-cut activity suggests that the mechanism of the anti-depressant action of these drugs is quite different. Sigg[4] has suggested, from experiments on the nictitating membrane of the anæsthetized cat, that imipramine bears some resemblance to cocaine in its peripheral actions and that its anti-depressant effect may be due to sensitization of central adrenergic synapses. Although we have no evidence on this point, imipramine would appear to differ from cocaine in that it shows stimulant properties at low doses only, while at high doses a chlorpromazine-like type of sedation is observed.

It is of interest that the effective doses of dexamphetamine, cocaine and imipramine in the tests in the rat and rabbit are of the same order of magnitude and this suggests that the same central property is being measured in the two procedures. Whether this property is the one which in man is responsible for the clinical anti-depressant effects we cannot say. However, these experiments clearly differentiate imipramine from chlorpromazine and show its resemblance to the classical central stimulants.

D. R. Maxwell
Helen T. Palmer

Research Laboratories,
May and Baker, Ltd.,
Dagenham,
Essex.

[1] Kuhn, R., *Schweiz. med. Wschr.*, **87**, 1135 (1957).

[2] Azima, H., and Vispo, R. A., *Amer. J. Psychiat.*, **115**, 245 (1958).

[3] Ball, J. R. B., and Kiloh, L. G., *Brit. Med. J.*, ii, 1052 (1959).

[4] Sigg, E. B., *Canad. Psychiat. Assoc., J.*, **4**. *S* 75 (1959)

[5] Domenjoz, R., and Theobold, W., *Arch. Int. Pharmacodyn.*, **120**, 450 (1959).

[6] Costa, E., Garattini, S., and Valzelli, L., *Experientia*, **16**, 461 (1960).

[7] Bradley, P. B., and Key, B. J., *Brit. J. Pharmacol.*, **14**, 340 (1959).

[8] Van Meter, W. G., Owens, H. F., and Himwich, H. E., *Canad. Psychiat. Assoc. J.*, **4**, *S* 113 (1959).

[9] Pulver, von R., Exer, B., and Herrmann, B., *Arzneim.-Forsch.*, **10**, 530 (1960).

[10] Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M., and Koetschet, P., *Arch. Int. Pharmacodyn.*, **92**, 305 (1953).

[11] Maffii, G., *J. Pharm., Lond.*, **11**, 129 (1959).

Role of the Lung in Metabolism of Fat during Ontogeny of the Rat

Milk of the rat contains up to 20 per cent fat[1], and the gastro-intestinal tract of infant rats absorbs fat more rapidly than that of adult animals[2]. The cells of the intestinal mucosa of infant rats after a meal contain much more neutral fat than those of adult animals where more 'acid lipids' are found[3] (using nile blue). This, together with a lower intestinal esterase activity in infant rat intestine[3], indicates a different absorption and perhaps transport of fat. Since fat transported via lymphatic pathways must pass through the lungs before entering the blood, it was thought pertinent to examine this organ histochemically during ontogeny of the rat.

First, infant rats (1 and 10 days post-natally) taken directly from the mother animal with their stomachs full of milk were compared with animals aged 45 days (post-weaning stage) and adult rats. As is evident from Fig. 1, fat accumulates in the adventitia of the arterioles and arteries and in the walls of the alveoli in infant rats aged 1 and 10 days. No fat is found in the macrophages. In animals aged 45 days and adult rats there is no fat. Only occasionally, macrophages are seen having fat droplets in their cytoplasm. Complete starvation for 24 hr. resulted in disappearance of all fat from the lungs of infant rats. In adult animals no changes were observed.

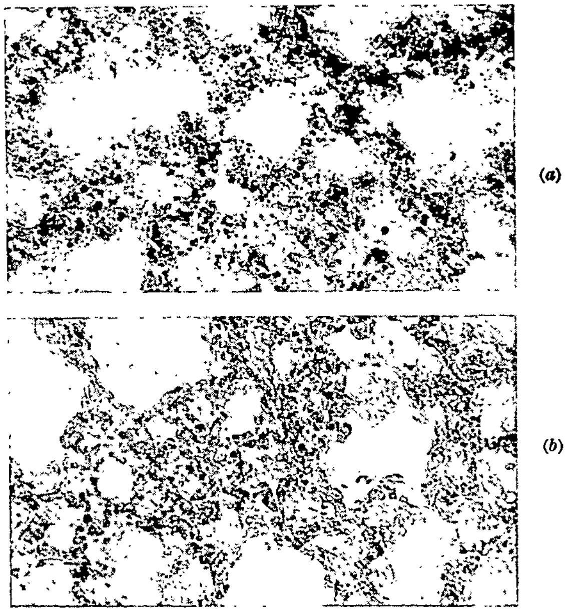


Fig. 1. (*a*) Lung of 10-day-old rat after feeding with milk. Frozen section, oil red 7*B* (× 90). (*b*) Lung of adult rat after feeding. Frozen section, oil red 7*B* (× 90)

In further experiments olive oil or oleic acid (1,000 mgm./100 gm. body-weight) was administered to starving rats and the animals were killed 1 hr. and 3 hr. and, for adult rats, also 7 hr. later. The histological picture was exactly the same as in fed animals. Again fat was found in the lungs of suckling rats and none in adult animals.

Schrade and Biegler[4] also found no fat histologically in the lungs of adult rats following a fatty meal. On the other hand, they found an increase in fat content of the lungs as determined chemically. This might indicate that the fat in the lungs is present in a different form in adult and infant rats, and that this might be due to a difference in transport of fat and in the part played by the lungs in this process. A preliminary experiment using a 5-day-old puppy showed the same picture as in infant rats.

Z. VACEK
P. HAHN
O. KOLDOVSKÝ

Institute of Embryology,
Charles University,
Institute of Physiology,
Czechoslovak Academy of Sciences,
Prague.

[1] Glass, R. L. (dissertation, University of Minnesota, 1956).
[2] Koldovský, O., Hahn, P., Jiránek, J., and Tintěra, J., *Čs. fysiol.*, 8, 211 (1959).
[3] Anisimova, E., Vacek, Z., Koldovský, O., and Hahn, P., *Čs. fysiol.*, 8, 392 (1959).
[4] Schrade, W., and Biegler, R., *Klinische Wochenschrift*, **34**, 1247 (1956).

Lack of Evidence for a Cholinergic Mechanism in Sympathetic Transmission

THE nerve supply to many sympathetic effector organs contains cholinergic nerve fibres as well as adrenergic fibres. Recently, the hypothesis has been put forward that a cholinergic mechanism is involved in sympathetic transmission[1,2]. It has been suggested that acetylcholine released from cholinergic nerve endings liberates from a local tissue store sympathetic transmitter which in turn acts on the effector organ.

One of the ways in which this hypothesis has been tested has been to use a hemicholinium, HC3 (ref. 3), which inhibits the formation of acetylcholine[4,5]. If sympathetic transmission does depend on the release of acetylcholine the effect of stimulating post-ganglionic sympathetic nerves should be blocked by HC3. Rand and Chang[6] showed that HC3 blocked the contractions of the isolated ductus deferens of the guinea pig produced by stimulation of the sympathetic nerve. Later they showed similar effects on a number of other sympathetic effector organs, including the isolated uterus and colon of the rabbit, the perfused rabbit ear, the isolated atria of the cat and the piloerector muscles of the cat's tail, most of which are known to contain both cholinergic and adrenergic fibres in their nerve supply[7].

To determine whether adrenergic transmission is necessarily mediated via a cholinergic mechanism we have used the nictitating membrane of the cat. This can be set up as an isolated nerve–smooth muscle preparation[8], and the evidence is that under these conditions neuromuscular transmission is purely adrenergic[8]. The post-ganglionic nerve fibres were stimulated with supramaximal square-wave shocks of 0·5 msec. duration at frequencies of 5, 10, 20, 25, 50 and 100 c./s. for periods of 7 sec. repeated every minute. HC3 in concentrations of 20–540 μgm./ml. ($=3{\cdot}5 \times 10^{-5}-9{\cdot}4 \times 10^{-4}\,M$) had no effect on the contractions of the muscle in response to nerve stimulation; even when stimulation was carried out in this way for more than 1 hr., there was no effect. These findings confirm those reported by Wilson and Long[9], who tested the action of HC3 on the nictitating membrane *in vivo*.

HC3 prevents the formation of acetylcholine not by inhibiting choline acetylase directly but by preventing the passage of choline through cellular membranes which enclose the enzyme[5]. The blocking effect of HC3 may not therefore become apparent unless the duration and frequency of nerve stimulation are sufficient to deplete the stores of acetylcholine[4]. In our experiments the higher frequencies of intermittent stimulation were greater than those which were required to produce an effect on the test objects used by Chang and Rand[7]. Nevertheless, the possibility remained that HC3 would not block the contractions of the nictitating membrane unless the nerve were stimulated continuously. When this was done at frequencies of 25 c./s. and 100 c./s. the contraction produced began to wane almost immediately, but the presence of HC3 in concentrations of 60–100 μgm./ml. ($=1{\cdot}05 \times 10^{-4}-1{\cdot}75 \times 10^{-4}\,M$) did not alter the rate at which the contraction decayed, even when HC3 was present for as long as 40 min.

Another indication that transmission is cholinergic might be that choline prevents or reverses the waning contraction produced by continuous stimulation at high frequencies. It was found, however, that choline in concentrations of 1·4–200 μgm./ml. ($=10^{-5}-1{\cdot}42 \times 10^{-3}\,M$) did not diminish the rate of decay.

These results do not support the suggestion that a cholinergic mechanism is concerned in sympathetic transmission over adrenergic nerve fibres.

We wish to thank Dr. M. J. Rand and Dr. H. Wilson for generous supplies of HC3. Experiments in which these gifts of HC3 were used gave results identical to those obtained with HC3 prepared by one of us (J. E. G.).

J. E. GARDINER
J. W. THOMPSON

Department of Pharmacology,
Institute of Basic Medical Sciences,
Royal College of Surgeons of England,
Examination Hall,
Queen Square, London, W.C.1.

[1] Burn, J. H., and Rand, M. J., *Nature*, **184**, 163 (1959).
[2] Burn, J. H., and Rand, M. J., *Brit. J. Pharmacol.*, **15**, 56 (1960).
[3] Schueler, F. W., *J. Pharmacol.*, **115**, 127 (1955).
[4] MacIntosh, F. C., Birks, R. I., and Sastry, P. B., *Nature*, **178**, 1181 (1956).
[5] Gardiner, J. E., *J. Physiol.*, **138**, 13–14P (1957).
[6] Rand, M. J., and Chang, V., *Nature*, **188**, 858 (1960).
[7] Chang, V., and Rand, M. J., *Brit. J. Pharmacol.*, **15**, 588 (1960).
[8] Thompson, J. W., Ph.D. thesis, University of London (1960).
[9] Wilson, H., and Long, J. P., *Arch. Int. Pharmacodyn.*, **120**, 343 (1959).

HISTOCHEMISTRY

Leucine Aminopeptidase in the Mammary Gland of the Cow and Rat

MORPHOLOGICALLY the mammary gland is a cutaneous gland, but physiologically it must be classed as an accessory gland of the reproductive system. In the various stages of the life of the female mammals the mammary gland undergoes many structural changes. Up to the age of puberty it is rudimentary in structure. During pregnancy the endocrine factors furnish a stimulus for proliferation of the mammary tissue, resulting in a complete transformation and maturation of the gland. The appearance of fine granulations and fat droplets in the epithelium cells indicates the onset of secretion in advanced pregnancy.

To our knowledge, the activity of the histochemically demonstrable leucine aminopeptidase in the mammary tissue has not been studied previously; therefore the object of our present work was to investigate the leucine aminopeptidase activity in the mammary gland of the cow and rat in the various stages of development.

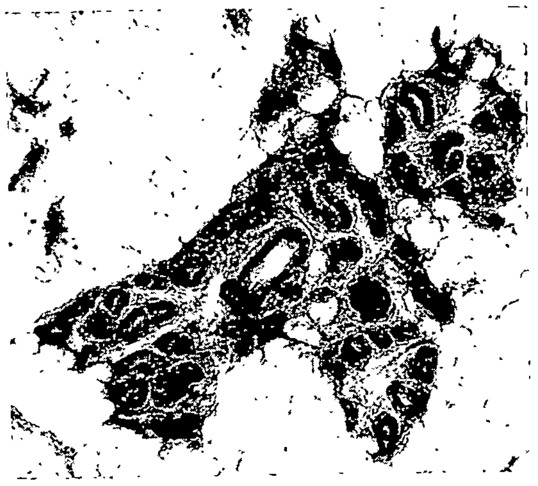


Fig. 1. Great activity of leucine aminopeptidase in the mammary tissue of a pregnant rat. (× 50)

The method employed in the demonstration of the leucine aminopeptidase activity in the tissue was the original method of Nachlas *et al.*[1]. The mammary glands were taken from virgin, pregnant and lactating animals. The parts of glands were cooled with dry ice and sectioned with the Pearse cold microtome[2]. The incubation time was 15–30 min.

The positive areas were characterized by a violet stain. In the mammary glands from virgin and pregnant animals a strong activity of leucine aminopeptidase was observed. This activity was located in the walls of the glandular ducts and also in the epithelium buds representing the glandular tissue in the non-functional gland. These buds were mainly without lumina. An example of this activity is shown in Fig. 1. The connective tissue including adipose tissue was nearly negative. In the lactating gland the activity was very slight (Fig. 2). Its location was the same as that in the non-functional gland. Especially in the alveolar epithelium the activity was minimal, in fact practically absent.

From the physiological point of view, the result is difficult to explain. The role of leucine aminopeptidase in tissues is still obscure. Observations made by Pearse and Tremblay[3] indicate that leucine aminopeptidase participates in the protein synthesis of the parathyroid glands. It also seems likely that the leucine aminopeptidase of the cells of the gastric mucosa is responsible for the pepsin synthesis[4]. This enzyme is, however, not associated with the casein synthesis of the mammary gland during lactation according to the present results. Apparently, the protein synthesis may be independent of the activity of the histochemically demonstrable leucine aminopeptidase.

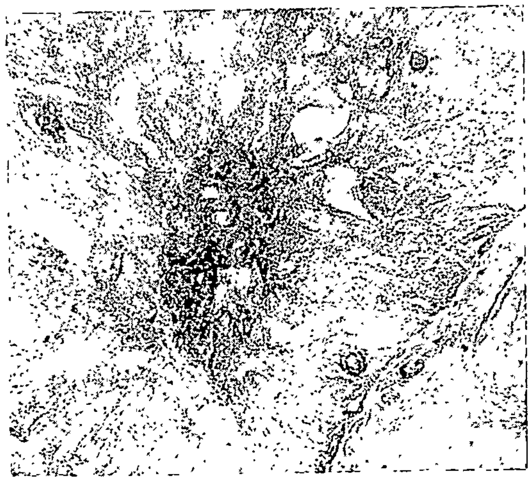


Fig. 2. Mammary gland of a lactating rat. Activity very slight. (× 50)

Further investigations on the effect of various hormones on the leucine aminopeptidase in the mammary tissue are in progress.

S. Talanti

Department of Anatomy,
Veterinary College,
Helsinki.

Väinö K. Hopsu

Department of Anatomy,
University of Turku,
Turku, 3, Finland.

[1] Nachlas, M. M., Crawford, D. T., and Seligman, A. M., *J. Histochem. Cytochem.*, 5, 264 (1957).

[2] Pearse, A. G. E., *Histochemistry: Theoretical and Applied* (Little, Brown and Co., Boston, 1960).

[3] Pearse, A. G. E., and Tremblay, G., *Nature*, 181, 1532 (1958).

[4] Talanti, S., and Hopsu, V. K., *Endocrinol.* (in the press).

PATHOLOGY

Resistance to Infection with *Salmonella paratyphi C* in Mice parasitized with a Relatively Avirulent Strain of *Salmonella typhimurium*

Although previous specific active or passive immunization may prolong slightly the survival-time of mice infected with mouse-pathogenic *Salmonellæ* (for example, *Salm. typhimurium*, *Salm. enteritidis*), the number of long-term survivors is but little improved[1–3]. Similarly, attempts to enhance resistance against mouse typhoid non-specifically, either by previous infection with *Mycobacterium tuberculosis* (strain *BCG*)[4] or by injections of lipid *A* from Gram-negative bacteria[5], have also met with only limited success. On the other hand, Hobson[3] showed that mice were highly resistant to virulent *Salm. typhimurium*, in terms of survival, if they had been infected previously with an avirulent variant of the same species. As *Salm. typhimurium* is a reticulo-endothelial parasite in mice, like *Myco. tuberculosis* and *Brucella abortus*, the possibility arises that this greatly increased resistance might resemble the cellular antibacterial immunity mechanism inferred from investigations with these latter micro-organisms[6–8]. Evidence has been obtained, however, both from *in vitro* macrophage studies and mouse infection experiments, which suggests that 'cellular immunity' following infection with *Myco. tuberculosis* cross-reacts against *Brucellæ* and vice versa[9–12]. The present communication illustrates that there is also a considerable non-specific element in the resistance produced by avirulent *Salm. typhimurium*, for this has been found to be effective against a heterologous *Salmonella* infection. *Salm. enteritidis* was considered unsuitable for use in this role as it shares somatic antigen components with *Salm. typhimurium*. Instead, *Salm. paratyphi C* (6,7,*Vi* : *c* : 1,5) was selected as it shares only one second-phase flagellar antigen with *Salm. typhimurium* (1,4,5,12 : *i* : 1,2) and, although not a natural mouse pathogen, produces an experimental infection similar to mouse typhoid.

A group of 30 female mice of the *LAB.* Grey outbred stock (weight 20–23 gm.) was infected with *Salm. typhimurium* (strain *S2/R*) by injecting intravenously 1,000 bacteria taken from a 6-hr. broth culture. This was the same relatively avirulent strain of *Salm. typhimurium* previously used by Dr. Derek Hobson[3] and kindly provided by him. After 13 days, at which time no deaths had occurred, 5 animals were killed and viable bacterial counts made on the homogenized liver and spleen of each. *Salm. typhimurium* was recovered in all cases with counts varying between 10,000 and 50,000. On the following day (day 14), the phagocytic activity of the reticulo-endothelial system was investigated in a further 5 mice, by following the rate of disappearance of a standard dose of colloidal carbon from the circulating blood according to the method of Biozzi and his colleagues[13]. The results in Table 1 show that the reticulo-endothelial system was more than twice as active, when assessed by the phagocytic index *K*, than in a group of control mice. Furthermore, the mice parasitized with *Salm. typhimurium* showed some relative enlargement of the liver and spleen, although this was pronounced in only 2 out of the 5 animals examined. The remaining 20 mice treated, together with 20 normal animals, were then injected intravenously with *Salm. paratyphi C* (Kauffmann East Africa strain) at a dose of 100,000 bacteria from a 6-hr. broth culture. Whereas 100 per cent mortality occurred in the control group, only one mouse died among those pre-infected with *Salm. typhimurium* during a 30-day period of observation (Table 2). *Salm. paratyphi C* was isolated from the heart blood of this sole fatality.

It seems improbable that such a high degree of resistance, which compares favourably with the result of active immunization against *Salm. paratyphi C* in mice[14], could be explained by the minor flagellar antigenic relationship between these *Salmonella* species (unless, of course, *H* antigens assume an importance in this resistance following parasitization which they do not possess in orthodox immunization procedures). Neither would the raised phagocytic activity of the reticulo-endothelial system alone provide a satisfactory explanation, for greater degrees of stimulation induced by other means have failed to protect against infections of this type[15,16]. In some way, previous parasitization of the reticulo-endothelial cells by *Salm. typhimurium* would seem to inhibit subsequent multiplication within those cells of a secondarily invading *Salmonella*[3], even when this does not necessarily belong to the same or a closely related species.

Table 1. ACTIVITY OF THE RETICULO-ENDOTHELIAL SYSTEM IN MICE INFECTED WITH *Salm. typhimurium* (STRAIN *S2/R*, 1,000 BACTERIA INTRAVENOUSLY) 14 DAYS PREVIOUSLY. THE CLEARANCE OF 16 MGM./100 GM. COLLOIDAL CARBON (*C*11/1431*a*) WAS MEASURED

Group	Phagocytic index *K**	$\frac{W}{WLS}$†
Mice infected with *Salm. typhimurium* 14 days previously	0·036 0·041 0·043 0·056 0·064	15·7 15·5 13·7 8·5 8·9
Mean	0·048	12·5
Controls	0·017 0·018 0·025	23·2 16·5 16·1
Mean	0·020	18·6

* $K = \frac{\log c_1 - \log c_2}{t_2 - t_1}$, where c_1 and c_2 are the concentrations of carbon in the blood at times t_1 and t_2 (min.)

† *W* = body-weight (gm.)
WLS = weight of liver and spleen (gm.)

TABLE 2. THE RESULTS OF INFECTION WITH *Salm. paratyphi C* (100,000 BACTERIA INTRAVENOUSLY) IN NORMAL MICE AND MICE INFECTED WITH AVIRULENT *Salm. typhimurium* (STRAIN *S2/R*, 1,000 BACTERIA INTRAVENOUSLY) 14 DAYS PREVIOUSLY

Group	$\frac{\text{Mice dead}}{\text{Mice injected}}$	Time to death (days)
Mice infected with *Salm. typhimurium* (*S2/R*) 14 days previously	$\frac{1}{20}$	8
Controls	$\frac{20}{20}$	5,5,5,6,6,6,6,6,6,6,6,7,7,7,7,7,7,9,11,11

This investigation was carried out at the Wright-Fleming Institute, St. Mary's Hospital Medical School, London.

J. G. HOWARD

Department of Surgical Science,
University of Edinburgh.

[1] Greenwood, M., Topley, W. W. C., and Wilson, J., *J. Hyg.* (Camb.), **31**, 257 (1931).

[2] Hobson, D., *J. Path. Bact.*, **73**, 399 (1957).

[3] Hobson, D., *J. Hyg.* (Camb.), **55**, 334 (1957).

[4] Howard, J. G., Biozzi, G., Halpern, B. N., Stiffel, C., and Mouton, D., *Brit. J. Exp. Path.*, **40**, 281 (1959).

[5] Howard, J. G., Rowley, D., and Wardlaw, A. C., *Nature*, **179**, 314 (1957).

[6] Lurie, M. B., *J. Exp. Med.*, **75**, 247 (1942).

[7] Suter, E., *J. Exp. Med.*, **97**, 235 (1953).

[8] Pomales-Lebrón, A., and Stinebring, W. R., *Proc. Soc. Exp. Biol., N.Y.*, **94**, 78 (1957).

[9] Elberg, S. S., Schneider, P., and Fong, J., *J. Exp. Med.*, **106**, 545 (1957).

[10] Pullinger, E. J., *J. Hyg.* (Camb.), **36**, 456 (1936).

[11] Nyka, W., *Amer. Rev. Tuberc.*, **73**, 251 (1956).

[12] Henderson, D. W., Lancaster, M. C., Packman, L., and Peacock, S., *Brit. J. Exp. Path.*, **37**, 597 (1956).

[13] Biozzi, G., Benacerraf, B., Stiffel, C., and Halpern, B. N., *C. R. Soc. Biol.* (*Paris*), **148**, 431 (1954).

[14] Archer, G. T. L., and Whitby, J. L., *J. Hyg.* (Camb.), **55**, 513 (1957).

[15] Howard, J. G., M. D. Thesis, University of London (1959).

[16] Biozzi, G. (personal communication).

ANATOMY

Functions of the Mammalian Carotid Rete Mirabile

ALTHOUGH a carotid rete mirabile is present in many mammals, notably the artiodactyla, its function is uncertain. Many workers have suggested that the rete may regulate the cerebral blood-flow[1,2], but emphasis has usually been laid on its effect on the arteries rather than the veins. However, an alternative possibility is that the rete is concerned primarily with assisting the venous return from the interior of the cranium, which is especially liable to be embarrassed in animals that feed with the head dependent. Enhancement of the blood-flow in veins is known to be a function of certain retia mirabilia elsewhere[3].

When present, the carotid rete always lies within a venous lake, usually the cavernous sinus[1]. In man, the expansile pulsations of the internal carotid artery are said to assist the expulsion of venous blood from the sinus[4], but a single thick-walled artery has limited powers of expansion[5]. Replacement of one artery by multiple thin-walled channels must enhance considerably the arterial expansion and, thus, the venous return.

In general, the evidence for such a pumping mechanism is the demonstration of pulsatile flow in the veins draining a part, coincident with the pulsations in the arteries of supply. In the umbilical vein, for example, such coincident pulsations have been recorded[6]; these are certainly due to direct pressure from the companion arteries in the umbilical cord. We have obtained comparable results with respect to the carotid rete of the sheep. In two heads from freshly slaughtered sheep the jugular vein and the common carotid artery were cannulated in the neck, and warm water was perfused via the artery at a pressure of 100 mm. of mercury. The venous pressure settled at 33 cm. of water. Rhythmic occlusion and release of the inflow channel gave rise to coincident pulsations in the venous flow, and the venous pressure rose by 12 cm. and overflowed the manometer tube. In a third sheep's head the vein cannulated was a large one, draining the extracranial tissues only. Rhythmic arterial pulsations had no effect on the flow in this vein, an indication that the venous pulsations noted in the first two experiments were directly transmitted from the arterial vessels to the venous lake within the cavernous sinus, the rete mirabile acting as a venous pump.

C. H. Barnett
C. D. Marsden

Department of Anatomy,
St. Thomas's Hospital Medical School,
London, S.E.1.

---

[1] Daniel, P. M., Dawes, J. D. K., and Pritchard, M. M. L., *Phil. Trans. Roy. Soc.*, B, **237**, 173 (1953).

[2] Ask-Upmark, E., *Acta Psychiat., Kbh.*, Supp. **6**, 1 (1935).

[3] Barnett, C. H., Harrison, R. J., and Tomlinson, J. D. W., *Biol. Rev.*, **33**, 442 (1958).

[4] Johnston, T. B., Davies, D. V., and Davies, F., *Gray's Anatomy*, thirty-second ed. (Longmans, London, 1958).

[5] Bramwell, J. C., Dowling, A. C., and Hill, A. V., *Heart*, **10**, 289 (1923).

[6] Reynolds, S. R. M., *Amer. J. Physiol.*, **166**, 25 (1951).

BIOLOGY

Sex-Ratio in Mice

The impression gained from glancing through our mouse record cards suggested to us an unusual sex-ratio in some of our strains; some strains appeared to be consistently producing more males than females or vice versa. As impressions are notoriously misleading, it was decided to carry out a brief analysis.

Mice are produced and recorded at the Laboratory Animals Centre, Carshalton, in the manner described by Bartlett *et al.*[1]; under our system mice are sexed not at birth but at weaning. Because of this, the only litters included in the sex analysis were those where all the mice survived until nineteen days of age (that is, until weaning). Total numbers of litters per strain were considered to ascertain whether the proportion analysed could be correlated with abnormal sex-ratio. The period under review was March 1955, until February 1959.

Results obtained from this analysis are recorded in Table 1. Columns 2, 3 and 4 give the figures necessary for analysis of the litters, and columns 5, 6 and 7 those necessary for the sex analysis of the mice from litters where there was 100 per cent survival. The expected numbers of each sex for each strain were calculated on the basis that the male : female ratio equals 1 : 1 and a χ^2 goodness-of-fit test applied to observed and expected numbers of each sex. χ^2 values and probability-levels (P) are tabulated for each strain.

The results show that in the case of the *A*, *C57BR/cd* and the *LAC* albino strains, significantly more females than males were produced whereas the *CE* strain produced more males than females. Other strains showed a more normal sex ratio. No correlation is evident between survival of the whole litter and sex ratio.

Table 1

Strain	1 No. of pairs	2 No. of litters Total	3 100 per cent survival	4 Per cent	5 Total No. of mice	6 No. of ♂	7 No. of ♀	χ^2	P
A	57	333	184	55	775	345	430	9·32	0·01–0·001
C57BR/cd	65	346	242	70	1,413	638	775	12·90	0·01–0·001
LAC Albino*†	104	515	316	61·3	2,170	1,028	1,142	5·988	~ 0·02
CE	57	232	178	76·7	1,369	730	639	6·048	0·01–0·02
Balb/*C*	87	499	388	77·8	2,029	999	1,030	0·04736	0·95–0·05
C3H/He	67	378	211	55·8	1,176	591	585	0·00306	0·95–0·05
LAC Grey*	144	819	689	84·1	5,397	2,765	2,632	3·328	0·95–0·05
DBA/1	49	247	161	65·1	665	318	347	3·3218	0·95–0·05
A2G	70	401	284	70·8	1,528	734	794	2·356	0·95–0·05
CBA	46	223	126	56·5	796	397	399	0·005024	0·95–0·05
C57BL	45	155	94	60·6	445	231	214	0·6494	0·95–0·05

* Non-inbred strains. † Strain discontinued 1959.

Margaret J. Cook

Laboratory Animals Centre,
Medical Research Council Laboratories,
Carshalton, Surrey.

Augustin Vlček*

Centrum Laboratorních Zvířat,
Konárovice,
Kolín, Czechoslovakia.

---

* World Health Organization Fellow 1959.

[1] Bartlett, D. G., Horne, N., Kettle, J. L., and Oliver, P. S., *J. Anim. Tech. Assoc.*, **10**, 116 (1959).

Methæmoglobin in Chronic Copper Poisoning of Sheep

After protracted periods of high copper-intake some species of farm animals accumulate large excesses of copper in the liver, and in certain circumstances this copper can be suddenly released into the blood and the clinical entity known as chronic copper poisoning develops[1–4]. Sheep are particularly susceptible, and the sequence of clinical symptoms is generally: anorexia, hæmolysis of red cells, hæmoglobinuria, anæmia, jaundice and death. Although the build-up of copper in the liver may take place over a period of weeks or months, the 'hæmolytic crisis' is an acute illness and death usually occurs within 2–3 days.

Although hæmolysis and hæmoglobinuria are constant features[5,6], the occurrence of methæmoglobin does not appear to have been recorded. In outbreaks of chronic copper poisoning[3,7], it has been observed that blood samples from affected animals are often chocolate brown in colour, and spectroscopic examination showed the presence of methæmoglobin. Estimations of methæmoglobin and total hæmoglobin by the method of Evelyn and Malloy[8] have been carried out on a number of cases and typical results from three affected sheep are set out in Table 1, with normal levels for comparison.

Table 1. TOTAL HÆMOGLOBINS, METHÆMOGLOBIN AND COPPER-LEVELS IN WHOLE BLOOD IN CHRONIC COPPER POISONING

	Blood copper (μgm./100 ml.)	Total hæmoglobins (gm./100 ml.)	Methæmoglobin (gm./100 ml.)
Case 1	1,210	11·5	3·5
Case 2	1,480	8·8	3·6
Case 3	1,000	15·5	3·8
Normal sheep	170	10 to 12	0·1

Copper-levels in blood were extremely high and methæmoglobin constituted 25 per cent to 35 per cent of the total hæmoglobin present. The methæmoglobinæmia was mainly intracorpuscular, but since hæmolysis was taking place, hæmoglobin and methæmoglobin were being released into the plasma, the proportions being approximately the same as occurring in whole blood, for example, plasma total hæmoglobins 2·2 gm. per 100 ml.; methæmoglobin 0·8 gm. per 100 ml. (36 per cent).

The pathological significance of these findings is being investigated.

J. R. TODD
R. H. THOMPSON

Veterinary Research Division,
Ministry of Agriculture for Northern Ireland,
Belfast.

[1] Bisset, J., *Brit. Vet. J.*, **90**, 405 (1934).

[2] Gordon, W. A. M., and Luke, D., *Vet. Rec.*, **69**, 37 (1957).

[3] Todd, J. R., and Gracey, J. F., *Vet. Rec.*, **71**, 145 (1959).

[4] Shand, A., and Lewis, Gwyneth, *Vet. Rec.*, **69**, 618 (1957).

[5] Garner, R. J., *Veterinary Toxicology* (Baillière, Tindall and Cox, 1957).

[6] Underwood, E. J., *Trace Elements in Human and Animal Nutrition* (Academic Press, Inc., 1956).

[7] Gracey, J. F., and Todd, J. R., *Brit. Vet. J.*, **116**, 405 (1960).

[8] Evelyn, K. A., and Malloy, H. T., *J. Biol. Chem.*, **126**, 665 (1938).

Release of Heparin from the Mast Cells of the Rat

MAST cells are an important source of tissue heparin and of histamine[1,2]. Histamine is known to be released following disruption of mast cells[3] and in the dog there is an associated release of heparin[4]. In other animals it has not been possible to detect liberation of heparin coincident with release of histamine.

The liberation of heparin from rat mast cells has been investigated *in vitro* following disruption of the mast cells. Cells were obtained from the peritoneal cavity of the rat by a method already described[3]. Mast cells were separated from other peritoneal cavity cells by centrifugation for 30 min. at 0° C. over a column of bovine albumin of 1·109 specific gravity. The mast cells passed through the albumin column and were collected at the bottom of the centrifuge tube. They were washed in physiological saline solution and suspended in saline solution in a concentration of approximately 0·5 million mast cells per ml. of solution. The mast cells in 1 ml. of suspension were disrupted by the following methods: (1) alternately freezing and thawing three times; (2) mechanical disruption in a piston and cylinder type homogenizer; (3) addition of compound 48/80 to give a final concentration of 2×10^{-6} mgm./ml.; (4) osmotic disruption with distilled water; (5) disruption by contact with an antigen–antibody mixture as already described[3]. This mixture consisted of 1 vol. of a 25 per cent suspension of human red cells in physiological saline solution and 5 vol. fresh rat antiserum at 37° C. and *p*H 7. After disruption of the mast cells, the preparations were centrifuged and the supernatant solutions assayed for antithrombin activity by the method of Monkhouse and Jaques[5].

The only supernatant solution of the disrupted mast cells found to have any antithrombin activity was the preparation that had been frozen and thawed three times. Activity equivalent to approximately 3·5 I.U. of heparin was released into the supernatant solution for each million mast cells frozen and thawed. Antithrombin activity remained uniform throughout the supernatant after centrifugation for 1 hr. at 28,000*g* in a 'Spinco' ultra-centrifuge. A residual small amount of activity, less than the equivalent of 0·5 I.U. heparin per million mast cells, was found in the deposit of the disrupted cells.

If the deposits of the other four solutions were digested with trypsin at *p*H 8·0 and then neutralized, the solutions showed antithrombin activity equivalent to approximately 4 I.U. heparin per million mast cells. This value agreed with the antithrombin content of untreated mast cells. As with heparin, the activity was readily neutralized by small amounts of protamine sulphate.

Microscope examination of stained smears of the disrupted mast cells revealed disruption of the granules only in the preparations which had been frozen and thawed.

It may be concluded that the antithrombin present in rat mast cells is not released into the surrounding solution following disruption of these cells *in vitro* unless the granules also are disrupted. This is in contrast to the results with histamine, which is known to be released from disrupted rat mast cells even though the granules remain intact.

Compound 40/80 used in these experiments was kindly made available by Burroughs Wellcome and Company (Aust.), Ltd.

G. T. ARCHER

New South Wales Red Cross Blood Transfusion Service,
1 York Street, Sydney.

[1] West, G. B., Foundation Symposium on Histamine, 14 (Churchill, London, 1956).

[2] Riley, J. F., *The Mast Cells*, 72 (Livingstone, London, 1959).

[3] Archer, G. T., *Aust. J. Exp. Biol. Med. Sci.*, **37**, 383 (1959).

[4] Rocha e Silva, M., Scroggie, A. Evelyn, Fidlar, E., and Jaques, L. B., *Proc. Soc. Exp. Biol. Med.*, **64**, 141 (1947).

[5] Monkhouse, F. C., and Jaques, L. B., *J. Lab. Clin. Med.*, **36**, 782 (1950).

Mast Cells in Photosensitivity Reactions

IN recent work[1] it was found that cells obtained by washing out the rat's peritoneal cavity with saline could be rendered photosensitive. Exposure to light for a period too brief to affect leucocytes and serosal cells caused the granules of sensitized mast cells to lose their basophilic staining properties, after which they became refractory to the action of the histamine-releaser, compound 48/80. Mast cells reacted in this way even after being separated from the other cells by differential centrifugation.

In order to determine whether histamine had actually been liberated when mast cells lost their basophilic staining reaction, the cells from six rats were obtained by washing out their peritoneal cavities by the method I have described[2]. The washings were centrifuged and the deposit was re-suspended in the fluid which ran down the sides of the tubes. The cell suspension was then divided into three parts.

To two groups hæmatoporphyrin was added to an eventual concentration of 1 part in 20,000 and to the third group was added an equivalent volume of physiological saline without hæmatoporphyrin. One preparation containing hæmatoporphyrin was kept in the dark while the other two were exposed to bright sunlight for 2 min. in watch glasses. The suspending fluid was separated from each by centrifugation and the cells were re-suspended in saline containing compound 48/80 to a concentration of 1 part in 20,000.

After 2 min. the preparations were again centrifuged. The two supernatants from each and the cellular deposits were then all tested for histamine according to the method of Lowry *et al.*[3]. It was found that hæmatoporphyrin did not cause release of histamine in either the preparation exposed to light or in the one kept in the dark. Nor was there any release from the preparation exposed to light without hæmatoporphyrin. The addition of compound 48/80 caused the release of histamine from cells of the two control preparations, but in the case of the cells which had been exposed to light in the hæmatoporphyrin solution, all the histamine remained within the cells. Addition of a drop of 0·5 per cent aqueous azur II solution to a drop of cell suspension confirmed the loss of basophilia by mast cells exposed to light in the presence of hæmatoporphyrin.

When exposure to light of the sensitized cells had not been adequate for complete abolition of basophilia, any granules which retained their staining power were extruded on the addition of compound 48/80.

These features seem to indicate that release of histamine from mast cells depends in some way on the presence of basophilia. The loss of basophilia, however, does not indicate death of mast cells. Exposure to a constant source of light from a slide projector showed that 1 part of hæmatoporphyrin in 20,000 removed mast cell basophilia within 1 min., whereas death of mast cells, which was recognizable by staining of their nuclei by the non-vital stain azur II, occurred only after exposure for 15 min.

Eosin and erythrosin were tested against the rat's peritoneal cells and they, too, caused mast cells to yield their basophilic staining properties when exposed to light, and, as usual, the cells thereby became refractory to compound 48/80.

Apart from these substances, which are active only on exposure to light, there are other substances such as chlorpromazine and promethazine which cause loss of mast cell basophilia in the dark with enhancement of this property on exposure to light. Each of these is a histamine-releaser causing swelling and disruption of mast cells in dilutions up to approximately 1 part in 20,000 parts of saline. In these concentrations they do not affect the basophilia of mast cells, but in dilutions between 1 : 20,000 and 1 : 40,000, they cause mast cells to lose their basophilic staining properties and on exposure to bright sunlight for 2 min. the range is extended to 1 : 80,000, even to 1 : 160,000 in occasional animals.

In addition to the reactions of photosensitivity, heating to 50° C. for 40 sec. causes mast cells to lose their basophilia and become refractory to the action of compound 48/80. Reserpine also affects mast cells in this way, dilutions of 1 : 6,000 in physiological saline causing loss of basophilia in 2 min.. Higher dilutions take longer to accomplish this effect and dilutions between 1 : 12,000 and 1 : 24,000 require about 45 min. Exposure to light has only a small enhancing effect.

The next step in this investigation is to determine the nature of the basophilic substance of mast cell granules and its relationship to 5-hydroxytryptamine.

V. J. McGovern

Fairfax Institute of Pathology,
Royal Prince Alfred Hospital,
Camperdown,
New South Wales.

[1] McGovern, V. J., *A.M.A. Arch. Derm.*, 83, 94 (1961).
[2] Archer, G. T., *Nature*, 182, 726 (1958).
[3] Lowry, O. H., Graham, H. T., Harris, I. B., Priebat, M. K., Marks, A. R., and Bregman, R. V., *J. Pharm. Exp. Ther.*, 112, 116 (1954).

Failure of Nucleotides to diffuse freely into the Embryo of *Pteridium aquilinum*

In the ferns the embryo develops within the gametophyte and is nutritionally dependent on it until the first root and leaf emerge. In the homosporous leptosporangiate ferns the period of complete nutritional dependence is at least ten days and is usually longer. Despite the fact that the young embryo is in intimate physical contact with the gametophyte during this period, the results of experiments described here suggest that there may, nevertheless, be incomplete metabolic union between the two generations.

Gametophytes of *Pteridium aquilinum* containing young embryos were placed on media containing tritiated thymidine and tritiated uridine. The experimental technique has already been described[1], and the gametophytes were fixed four days after the feeding with the labelled nucleotide.

The autoradiographs show that although radioactivity is concentrated around the embryo there is little if any in the embryo itself. This is particularly clear with the gametophyte fed with tritiated thymidine (Fig. 1*A*), a nucleotide which would enter only the deoxyribonucleic acid. The gametophytic nuclei adjacent to the embryo are heavily labelled and appear black in the autoradiograph, but those of the embryo, despite the fact that nuclear divisions are occurring, are entirely unlabelled. The few grains scattered over the embryo can be put down wholly to background radioactivity. These results are in striking contrast with those of earlier experiments[1] in which it was shown that tritiated thymidine was able freely to enter developing and unfertilized eggs. In the gametophyte fed with tritiated uridine, which would enter only the ribonucleic acid, the cytoplasm of the cells around the embryo is clearly much more heavily labelled than that of the embryo (Fig. 1*B*). The frequency of the grains above the embryo is about four times the background, and about a quarter of that of the grains above the cytoplasm of the cells not adjacent to the embryo. Above the cells around the embryo, which have copious cytoplasm and every appearance of metabolic activity, the grains are too dense to count. Thus it appears that only a small proportion of the radioactivity entering the gametophyte with the uridine reaches the embryo.

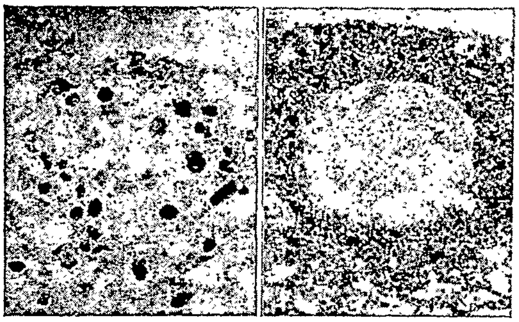


Fig. 1. Autoradiographs of embryos of *Pteridium aquilinum* after feeding the gametophyte with tritiated nucleotides. *A*, 32-celled embryo, approximately 10 days old at time of fixing. Gametophyte fed with tritiated thymidine. *B*, Larger embryo, probably about 15 days old at time of fixing. Gametophyte fed with tritiated uridine. (× 200)

These results indicate that at least two nucleotides cross the boundary between gametophyte and sporophyte with difficulty or not at all. On general principles of permeability, it would be expected that other molecules of similar or larger size would also fail to cross this boundary. In view of the clear-cut result with thymidine, it may be that the appearance of some radioactivity in the embryo when labelled uridine is fed results not from its entering the embryo directly but from catabolism of some of the uridine in the metabolically active cells adjacent to the embryo, and the passage into the embryo of simpler derived molecules, such as orotic or even aspartic acid. There is a strong possibility, therefore, that the sporophytic embryo draws only simpler molecules from the gametophyte, and that the sporophyte has much metabolic autonomy *ab initio*. This would explain why Jayasekera and I[2] were able to grow normal sporophytes from very young embryos of *Thelypteris*, surrounded by only small amounts of gametophytic tissue, placed on a simple mineral-agar medium.

That the cytoplasm of the fertilized egg comes away cleanly from the wall of the archegonium on fixation, whereas that of the unfertilized does not, suggests that the change in the bounding layer of the cytoplasm leading to the separation of the gametophytic and sporophytic metabolisms occurs on fertilization. Possibly the alteration in the surface of the egg which is believed to prevent polyspermy also results in a permanent alteration in its permeability to large molecules.

P. R. Bell

Botany Department,
University College,
London, W.C.1.

---

[1] Bell, P. R., *Proc. Roy. Soc.*, B, 153, 421 (1960).

[2] Jayasekera, R. D. E., and Bell, P. R., *Planta*, 54, 1 (1959).

Sexual Differentiation in *Hydra* in Relation to Population Density

Recent reports by Loomis and by Loomis and Lenhoff[1] of controlled induction of sexual differentiation in *Hydra littoralis* have stimulated considerable interest in this process. One of the observations reported is that hydras differentiate sexually in crowded cultures but remain asexual when uncrowded unless the cultures stagnate. This observation is of particular interest because mass cultures of the clone of *Hydra littoralis* used by Daniel and Park and by Park[2] remained continuously asexual for as long as three years. Although culture conditions were considerably different from those described by Loomis, population density and degree of stagnation were apparently comparable.

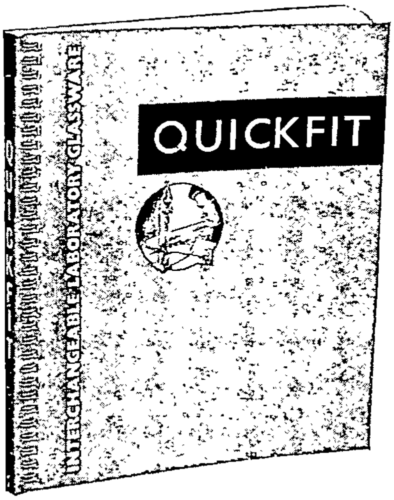
The Daniel–Park clone of hydras is no longer in existence. However, the present mass cultures in this Laboratory, derived from a single male of Loomis' stock, and maintained for two years according to the methods described by Loomis and Lenhoff, have exhibited cycles of sexuality in both sparse and crowded cultures[3]. It therefore seemed desirable to examine more critically the relation between population density and sexual differentiation.

Hydras were cultured in Loomis' bicarbonate–versene-tap water medium (BVT) at 24° ± 2° C. Daily except Sunday the cultures were fed on brine shrimp larvæ in excess for $\frac{1}{2}$ hr., rinsed with tap water and re-covered with BVT medium. Concurrently the numbers of hydras with and without gonads were recorded. Two sets of observations were made, one comparing the percentage of hydras with gonads in mass cultures of differing population densities, and the other dealing with the occurrence of gonads in isolated individual hydras.

In the mass population comparisons, a group of randomly chosen hydras in a container giving a population density of 2·5 hydras per ml. of medium served as a control. The culture was maintained at 100 by daily random thinning for 217 days. It was compared with a culture of 100 asexual hydras in a container such that the population density was originally 0·67 hydra per ml. of medium. This experimental culture was followed through the following four successive population régimes: (1) Allowed to increase to 400 individuals (attained on day 11) and thereafter maintained at 400 (density 2·67 individuals per ml.) by daily random thinning for 34 days; (2) immediate repetition of (1), 400 individuals being reattained on day 65, and the population being at 2·67 individuals per ml. for 24 additional days (total days from set up of culture, 89 days); (3) culture then reduced to 100 individuals (density 0·67 individual per ml.) and maintained at this level for 92 additional days; (4) all but 20 males with gonads discarded (density 0·13 individual per ml.) and these individual hydras maintained, all buds being discarded as soon as dropped for 52 additional days (total 233 days). The records for this experiment show that although there were transitory drops in percentage of gonad-bearing hydras in the experimental culture each time its population density was reduced, the two cultures were strikingly similar in the time sequence of sexuality and asexuality. Both cultures concurrently went through several periods, one of nearly 30 days, in which not a single individual had gonads, and both also went concurrently through several periods when 35–80 per cent of the hydras bore gonads.

In the second set of observations, 24 male hydras with gonads were transferred from the stock into individual culture dishes of a size such that the population density was 0·1 hydra per ml. of medium. Each hydra was fed, washed and re-covered with medium, as with the mass cultures, and newly dropped buds were removed within 24 hr. The hydras were kept on this régime for 86–180 days.

All the hydras became asexual after 4–13 days of isolation. However, all again became sexual within an additional 2–23 days. Thereafter, several more



Something new from "QUICKFIT"

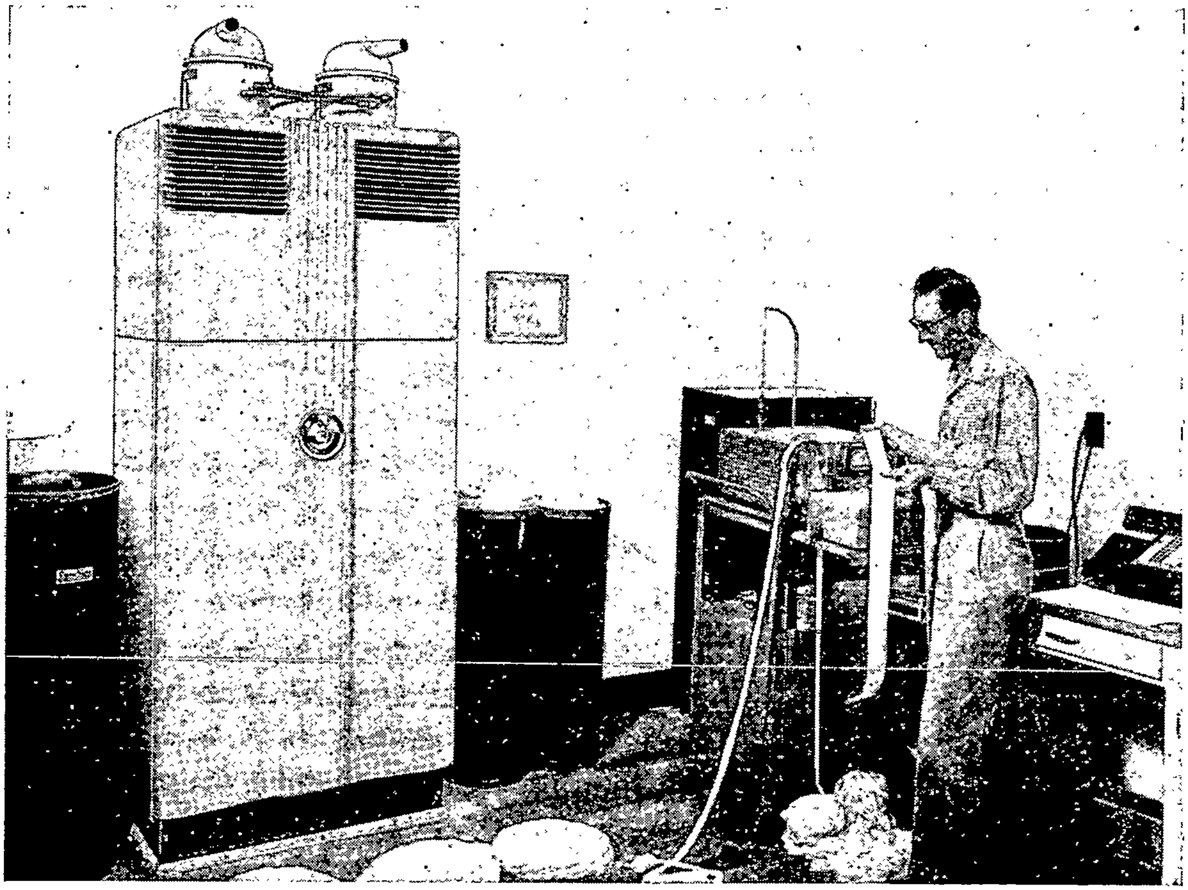
Write for your copy of this new "Quickfit" catalogue

This fully illustrated
new "Quickfit"
catalogue describes the entire
range of "Quickfit"
Interchangeable Laboratory Glassware
and includes
many new items.

QUICKFIT & QUARTZ Ltd
Dept. Z.AQ, "Quickfit" Works,
Heart of STONE, Staffordshire.
Telephone: STONE 481

CARLYLE

LABORATORY CONDITIONERS



give you 'PRECISION CONTROL' of temperature and humidity

No one knows better than you that varying air temperatures and humidity can affect the properties of materials, and the accuracy of instruments and experimental results. These problems can be overcome by the installation of a Carlyle Laboratory Air Conditioner. This completely self-contained unit—specially designed for laboratory work—will ensure year round control of temperature and humidity to within ±1°F and ±1½% R.H. The Carlyle Unit is the result of years of specialised experience in all phases of atmosphere control involving application of air conditioning and refrigeration to industrial plants and processes.

Capacities from 29,000 to 80,000 Btu/hr

Larger capacities are also available on special order. We shall be glad to co-operate with organisations in recommending the unit most suitable for their purpose.

CARLYLE

CARLYLE AIR CONDITIONING & REFRIGERATION LTD

1 King Street, St. James's, London S.W.1 Tel: WHItehall 5356

asexual–sexual cycles of varying lengths occurred. Extreme variability in the length of sexual and asexual periods was in fact an outstanding characteristic. Asexual periods varied in length from one to 97 days; sexual periods varied from one to 103 days.

In summary, although we cannot at present explain the alternating periods of sexuality and asexuality which occur in mass and isolation cultures and at population densities ranging from 0·1 to 2·7 individuals per ml., it is clear that neither crowding nor stagnation is necessary for sexual differentiation to occur.

HELEN D. PARK
CHRISTYNA MECCA*
ANNE ORTMEYER

Laboratory of Physical Biology,
National Institute of Arthritis and Metabolic Diseases,
National Institutes of Health,
Bethesda 14, Maryland.

* Present address: National Institute of Dental Research, National Institutes of Health, Bethesda 14, Maryland.

[1] Loomis, W. F., *Science*, 126, 735 (1957); *Sci. Amer.*, 200, 145 (1959); in *Cell, Organism and Millieu*, edit. by Rudnick (Ronald Press Co., New York, 1959). Loomis, W. F., and Lenhoff, H. M., *J. Exp. Zool.*, 132, 555 (1956).

[2] Daniel, George E., and Park, Helen D., *J. Cell. and Comp. Physiol.*, 38, 417 (1951); 42, 359 (1953); *Amer. J. Roent., Rad. Ther. and Nuc. Med.*, 72, 857 (1954). Park, Helen D., *Biol. Bull.*, 111, 240 (1956); *Physiol. Zool.*, 31, 188 (1958).

[3] Park, Helen D., *Anat. Rec.*, 134, 623 (1959).

The Number of Testis Follicles in Miridae

THE male reproductive system of bugs has been investigated in detail for a handful of species[1] or, in a range of species, quantitative data presented on the testis follicles[2]; but the dominant family in temperate climates, Miridae, has scarcely been examined—probably because of the difficulty specific determination presents to non-specialists.

This communication summarizes details of counts made of testis follicles in 90 of the 199 mirids listed as British[3]. Where the follicles were small, convoluted or otherwise obscured aceto-orcein staining, as in routine bug cytology, was used to aid visualization[4]. The seven species seen by Woodward[2] were re-examined.

The non-British Cylapinae and Palaucorinae—the latter of dubious status and monotypic—make up, with the six sub-families listed in Table 1, the family Miridae, a family the limits of which are not in dispute. Kullenberg believed that, as a rule, mirids have seven follicles in each testis, but this is contradicted by my work. From Table 1 it is seen that follicle numbers range from one to eight, while all sub-families in which five or more species have been examined exhibit a marked modal number, namely: Phylinae 3, Dicyphinae 1, Orthotylinae 2 and Mirinae 7. Such distinction between the groups was unexpected: there are no cases within the Arthropoda, so far as is known, in which this apparently fundamental structure, one in which it is difficult to envisage selective advantage in any of the variants, exhibits such division into compartments at sub-family level.

Table 1. TESTIS FOLLICLE NUMBER IN 90 BRITISH MIRIDS

Sub-family	Species	Follicles
Bryocorinae	*Monalocoris filicis*	2
Deraeocorinae	*Alloeotomus gothicus*	3–4 variable
	Deraeocoris lutescens	2
	D. olivaceus	7
	D. ruber	8
Phylinae	23 species	3
	Oncotylus viridiflavus	4–5 variable
Dicyphinae	5 species	1
Orthotylinae	20 species	2
	Cyrtorhinus caricis	1
	Orthocephalus coriaceus	4?
Mirinae	30 species	7
	Notostira elongata	3
	Leptopterna dolabrata	8
	L. ferrugata	8
	Stenotus binotatus	8

Discussion of the bearing of these facts on the phylogeny of Miridae must await a re-assessment of all the structures now investigated in the family: male and female genitalia[5], chromosome numbers[4], ovariole number[6] and eggs[7], in addition to the traditional 'characters' of the systematist (arolia, pseudarolia, wing venation, pronotal structure, etc.). The results do, however, suggest: that Dicyphinae warrants sub-family status, as has been suggested elsewhere on other grounds[4]; that intraspecific variation—which is accompanied by unilateral variation—is a rare phenomenon, only noted in two of the 90 species examined; that in Orthotylinae the position of certain Halticini, here represented by *Orthocephalus coriaceus*, requires investigation, as suggested on other grounds[5]; that *Cyrtorhinus* may possibly be wrongly placed at present; that it is doubtful whether *Deraeocoris lutescens* on one hand and *D. olivaceus* and *D. ruber* on the other are congeneric; that a follicle number of eight occurs only in groups in which seven is perhaps the usual number.

The diverse results obtained from the four deraeocorines, and that for the single bryocorine, mean little at present; both groups are essentially tropical. The clearest case of wide divergence from a well-defined sub-family pattern is that of *Notostira elongata*, a bug the systematic position of which has never been questioned.

The full list of species will be published elsewhere.

DENNIS LESTON

44 Abbey Road,
London, N.W.8.

[1] Bonhag, P. F., and Wick, J. R., *J. Morph.*, 93, 177 (1953). Davis, N. T., *Ann. Ent. Soc. Amer.*, 49, 466 (1956). Kullenberg, B., *Zool. Bidr., Uppsala*, 23, 1 (1946).

[2] Pendergrast, J. G., *Ent. Mon. Mag.*, 92, 275 (1956). Woodward, T. E., *Ent. Mon. Mag.*, 86, 82 (1950).

[3] Southwood, T. R. E., and Leston, D., *Land and Water Bugs of the British Isles* (London, 1959).

[4] Leston, D., *Chromosoma*, 8, 609 (1957).

[5] Kelton, L. A., *Canad. Ent.*, Supp. 11, 1 (1959). Slater, J. A., *Iowa State Coll. J. Sci.*, 25, 1 (1950).

[6] Miyamoto, S., *Sieboldia, Fukuoka*, 2, 69 (1957).

[7] Southwood, T. R. E., *Trans. Roy. Ent. Soc. Lond.*, 108, 163 (1956).

Evolution of the Sweet Potato (*Ipomoea batatas* (L.) Lam.)

RECENT work on meiosis[1,2] has given considerable support to the suggestion of King and Bamford[3], based on mitotic investigations, that the sweet potato is an allopolyploid, $2n = 90$, resulting from hybridization and natural doubling of the chromosome number in $F1$. The incidence of secondary associations of bivalent chromosomes at metaphase has been interpreted by Ting *et al.*[2] as indicating that the sweet potato, derived from somewhat related species, is a 'relatively recent species'.

While this interpretation may be of general validity in sexually reproducing plants, its unqualified application in this case may be challenged in respect of the known mode of reproduction of the sweet potato. The plant is propagated vegetatively throughout the areas of its cultivation. In present research under a grant from the Rockefeller Foundation, field collections have been made in Peru, Bolivia, Ecuador, Colombia, as well as in the Pacific Islands, Polynesia and Melanesia. In no case was purposeful propagation by seed recorded either in practice or by local tradition. In Peru and Colombia seeds were collected from cultivated crops, and although informants were aware of the occurrence of these in both coastal and marginal jungle areas, no evidence of their use was found. No wild prototypes of the sweet potato have been found, so that it seems likely that the method of propagation may have existed since very early times, possibly since the plant's domestication. In discussing the likelihood of a South American domestication, Sauer[4] places the sweet potato in "a culture complex that operated by vegetal means of reproduction . . . not by seed production".

The conservative breeding system of the plant, accentuated by the selective activity of man, thus retards the rate of evolution of this species. While chromosome behaviour in meiosis does indicate that in this respect the species has not reached maturity, this behaviour cannot be used as a time-device to signify the age of the species. It is conceivable that chromosome multivalents could have been perpetuated for as long as man has cultivated the plant.

Ames[5] has delineated a sequence of agricultural development that passed through successive horticultural and agricultural phases, an early stage in the former being the use of vegetative reproduction of plants. If this sequence is accepted, the history of some present-day sweet potato clones may have anteceded that of grains in America.

D. E. Yen

Vegetable Station,
Crop Research Division,
Department of Scientific and Industrial Research,
Otahuhu, New Zealand.

---

[1] Ting, Y. C., Kehr, A. E., and Miller, J. C., *Amer. Nat.*, **91**, 858 (1957).

[2] Ting, Y. C., and Kehr, A. E., *J. Hered.*, **44**, 5 (1953).

[3] King, J. R., and Bamford, R., *J. Hered.*, **28**, 279 (1937).

[4] Sauer, C. O., in *Handbook of South American Indians*, **6**, edit. by Steward, J. H. (Smithsonian Institution Bull. 143, Washington, 1950).

[5] Ames, O., *Economic Annuals and Human Cultures* (Bot. Museum, Harvard University, Mass., 1939).

Growth of Fern Embryos in Sterile Culture

In recent years, advances in the field of embryo culture have made possible the successful growth of a number of plant species which would not otherwise have been propagated[1]. These cultural experiments have also enriched considerably our understanding of the physiological and morphological aspects of early plant development[2,3]. Even though considerable information is available from this work on the cultural requirements of embryos, up to the present time it has not been possible to maintain very young, globular plant embryos (30–85μ) *in vitro*[4].

For the most part, experiments have been conducted utilizing embryos from both gymnosperms and angiosperms, and relatively little consideration has been given to embryos of the ferns and mosses. One of the major reasons for neglect of these plants in embryological experiments has been technical and manipulative difficulties of approaching their embryos, for they are formed and develop in the venter of the archegonium surrounded by prothallial tissue. That these difficulties are not insurmountable can be seen from the work initiated at the Harvard Laboratories several years ago by Ward and Wetmore[5], and more recently elsewhere[6,7]. Although Ward and Wetmore, utilizing surgical techniques on the fern *Phlebodium* (*Polypodium*) *aureum* J. Sm., were able partially to free the embryo from the surrounding tissue, they were unable to isolate it completely as it appeared to be attached to the wall of the venter.

In the course of work on the life-history and embryology of the fern *Todea barbara* (L.) Moore[8], it was found that the embryo was not demonstrably adherent to the venter in at least the younger stages of embryo formation. It could be removed easily from the surrounding prothallial tissue without apparent damage. The ease with which quantities of embryos of all ages, 4–20 days following fertilization, could be excised prompted the initiation of work designed to determine whether fern embryos could be grown to maturity in sterile culture when completely removed from the parent prothallus.

Sexually mature prothalli previously grown under sterile conditions on a simple nutrient medium[9] were immersed in sterile water for 3 hr. in order to facilitate fertilization. After the water was removed, the prothalli were watched for growth of embryos which were characteristically numerous. Those utilized in this work were removed aseptically from the prothalli according to the procedure outlined in a previous report[10]. Cultural experiments were conducted, employing a liquid medium consisting of Knudson's solution of half-strength to which was added 10 gm. of sucrose, 10 mgm. ferric citrate, and 1 ml. of trace element solution per litre of nutrient solution[11]. The trace element solution was modified by the addition of 25 mgm. cobaltous chloride per litre. In this basic medium, isolated twenty-day-old embryos (240–260μ) possessing stem and root meristems were grown to advanced sporelings in one month. When later planted in the soil, these young plants give every assurance of becoming adult plants, capable of fruiting. Slightly younger embryos, isolated 17 days after fertilization (160μ), showing an absorbing foot but no other obvious signs of organization, required the addition of 10 per cent autoclaved coconut milk to achieve continuing growth and development. Succeeding experiments have demonstrated that the requirements of coconut milk for 17-day-old embryos cannot be replaced by any known concentrations of varied amino-acids, but a mixture of 50 mgm./l. each of sorbitol and inositol, components of the 'neutral' fraction of coconut milk[12], will do so. Very young, unicellular embryos isolated 4–5 days after fertilization (35–55μ), before the first division of the embryo, can also be maintained in the simple basic medium when supplied with 3 per cent sucrose and 50 mgm./l. of sorbitol and inositol. The first few divisions in the post-fertilized egg stage of young cultured embryos are formed in essentially the same planes as would ordinarily be the case with such young embryos growing in their natural environment of the prothallial-contained archegonium. Succeeding divisions, however, appear to be oriented haphazardly, giving rise to embryos which, even after one month in culture, displayed little evidence of the usual pattern of development. Instead, they develop into irregular, asymmetrical, non-uniform, thalloid plants which do not become vascular, do not develop leaves and roots, but do remain associated closely with the nutritive medium, much as do prothalli. Whether these prothalloid embryos can be altered so as to develop into the usual erect, vascular leaf- and root-bearing *Todea* fern plants is part of the work now being continued.

The results reported here demonstrate that embryos of at least one vascular plant, the fern *Todea barbara*, can be removed from their enclosing archegonial venters and maintained successfully outside the plant at all stages of their development on an artificial nutrient medium. If they are removed after they have become compact, spherical aggregations of cells which have already established nutritional contact with the adjacent prothallial tissue by an absorbing foot, and have already acquired an initial leaf and a root primordium (20 days or older), they will grow into fern plants *in vitro* on a simple medium of mineral salt with a low content of sugar. They are also capable apparently of synthesizing their own hormonal and other organic needs. If they are removed when slightly younger (17 days or less) when they have already become somewhat spherical organisms, each with an absorbing foot but no appendages as yet, and are grown on the medium mentioned but supplemented by coconut milk, they develop into the usual embryos characteristic for *Todea*. Appropriate concentrations of sorbitol and inositol in place of coconut milk produce comparable embryos in approximately the same time. If, however, very young embryos are removed 5–6 days after fertilization, but before the first cell-division occurs, and if they are cultured on either of the agar-enriched nutrient media just mentioned, the embryos develop, but not into spherical organisms. Rather, they become prothalloid in their flattish, extending form until they are spreading, irregular, often branching entities—no two look alike—in close and intimate contact with the nutritive medium. They have not as yet acquired appendages, nor apical meristems nor vascular tissue.

This work suggests that in the early stages of its development the embryo depends on an external source for formative substances, the prothallus. A suggestion from which to start further work might appropriately indicate that the necessary enzymes have not yet been synthesized in very young embryos. But of equal importance, and too often neglected, is the influence of environment on these young embryos. Even when seeming to be nourished adequately, the freed embryo resembles much more the derivative growth of the freely germinating spore—the prothallus. When it develops in the containing archegonial venter, with its turgid cells, the young embryo follows a much more regularized pattern. In this environment, it develops into a spherical entity which ultimately—certainly by the seventeenth day—establishes direct, broad, nutritional contact with its neighbouring prothallial cells. When it is isolated, it clearly acquires the ability to synthesize its own biochemical needs, presumably including its own enzyme systems.

We are also forced to believe that in the venter the planes of cell division in the young embryo are so oriented as to produce a spherical organism. This end-product is clearly not produced in early, excised embryos. If mitotic spindles are formed in specific orientations in the cell under restraints, or pressures, then one could understand better early shapes and organizations of embryos. Nutrition alone does not seem an adequate explanation.

Techniques of embryo isolation and culture provide the possibility of conducting investigations into the various physiological and morphogenetic mechanisms which control the growth and development of plants. The correlations between free and contained development and the orientation of mitotic spindles with consequent cell-plate and cell-wall formation and their relations to shapes of early embryos can now be subjected to electron microscope work and possible subsequent interpretation. Moreover, the accumulating evidence from single-cell cultures must be taken into consideration.

AUGUSTUS E. DEMAGGIO
College of Pharmacy,
Rutgers,
The State University,
Newark, New Jersey.

RALPH H. WETMORE
Biological Laboratories,
Harvard University,
Cambridge, Mass.

[1] Rappaport, J., *Bot. Rev.*, **20**, 201 (1954).

[2] van Overbeek, J., *Cold Spring Harbor Symp. Quant. Biol.*, **10**, 379 (1942).

[3] Wardlaw, C. W., *Embryogenesis in Plants* (Methuen Co., London, 1955).

[4] Rijven, A. H. G. C., *Acta Bot. Neerl.*, **2**, 1957 (1952).

[5] Ward, M., and Wetmore, R. H., *Amer. J. Bot.*, **41**, 428 (1954).

[6] Bell, P. R., *J. Linn. Soc. Bot.*, **56**, 188 (1959).

[7] Jayasekera, R. D. E., and Bell, P. R., *Planta*, **54**, 1 (1959).

[8] DeMaggio, A. E., doctoral dissertation (Harvard University, 1960).

[9] Steeves, T. A., Sussex, I. M., and Partanen, C. R., *Amer. J. Bot.*, **42**, 232 (1955).

[10] DeMaggio, A. E., *Phytomorphology* (in the press).

[11] Nitsch, J. P., *Amer. J. Bot.*, **38**, 141 (1951).

[12] Shantz, E. M., Pollard, J. K., and Steward, F. C., *Plant Physiol.* (Supp.), **33**, 16 (1958).

Physiological Significance of Surface Wax on Leaves

A STUDY of the surface wax occurring on the leaves of New Zealand and Spanish white clovers (*Trifolium repens*) has been made with the electron microscope. Pre-shadowed carbon replicas were prepared in a manner similar to that used by Bradley[1], and it was found that the wax had a structure characteristic for each species and was extruded from the cuticle only while a leaf was growing. This work was later extended to include Montgomery, Broadleaf and Moroccan red clovers. During the investigation it was noted that wax was removed by weathering, for example, contact between leaves and between leaf and ground in windy conditions, and that the weathered appearance could be simulated by brushing the leaves with a camel-hair brush. In both cases the wax was removed from the crown of the dome-shaped cuticle overlying the epidermal cells. Fig. 1 shows an adaxial surface of New Zealand white clover before brushing and Fig. 2 after brushing.

To supplement this observation, in particular to provide a ready measure of the quantity of wax, the contact angle of water droplets on the leaves was measured with a microprojector[2]. Removal of wax by brushing or weathering always lessened the angle of contact.

The contribution of wax to the water balance of the plant was assessed by measuring the transpiration-rate of excised leaves using the technique described by Hygen[3]. Pairs of leaves of the same age and size were brought to full turgidity, and losses of weight recorded under fixed conditions. Fig. 3 shows a semi-logarithmic plot of weights against time, typical of brushed and unbrushed leaves. By extending the weighings over a number of hours it was found that

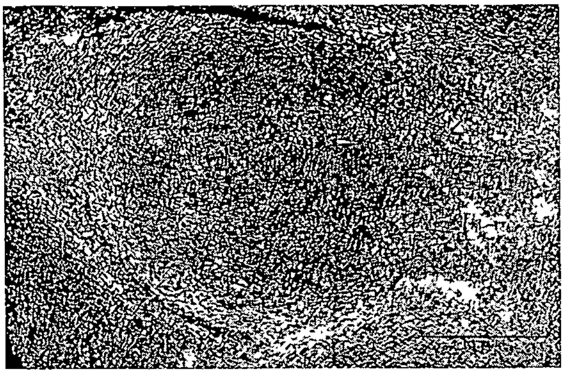


Fig. 1. Surface structure over a single epidermal cell showing undamaged waxy coating. Direct print from a micrograph

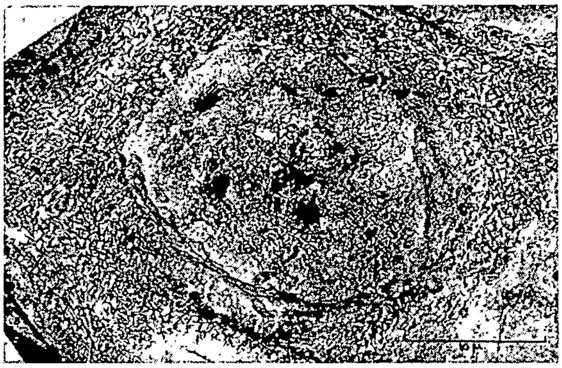


Fig. 2. Leaf surface of similar area to Fig. 1, showing removal of wax particles from dome

the increased transpiration of the brushed leaves was due to greater loss during the cuticular phase, that is, principally after the stomata had closed.

It is suggested here that when growth is interrupted by adverse conditions, the wax that is removed by weathering is not replaced quickly enough and the plant is additionally stressed through having an unusually high cuticular leaf transpiration. This is borne out by observations in the field.

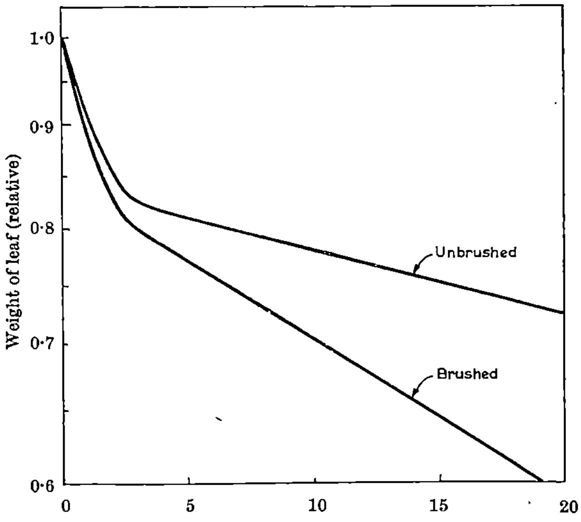


Fig. 3. Loss in weight of leaves of Moroccan red clover, brushed and unbrushed, versus time

Some evidence was also obtained suggesting that the presence and form of wax on leaf surfaces are related to frost hardiness.

D. M. Hall
R. L. Jones

Dominion Physical Laboratory,
Lower Hutt, New Zealand.

[1] Juniper, B. E., and Bradley, D. E., *J. Ultrastructure Res.*, **2**, 16 (1958).

[2] Fogg, G. E., *Proc. Roy. Soc.*, B, **134**, 503 (1947).

[3] Hygen, G., *Physiologia Plantarum*, **4**, 57 (1951).

VIROLOGY

Unusual Base Composition of the Ribonucleic Acid of Wild Cucumber Mosaic Virus

At the present time the small viruses containing ribonucleic acid (RNA-containing viruses) seem to be useful test objects for discovering the relationship between the amino-acid sequence of proteins and the structure of the RNA which is presumed to code that sequence. It is believed that the RNA of the virus specifies the virus coat protein. In addition, it perhaps carries the code for other proteins and/or plays some structural part. We wish to point out that the RNA from wild cucumber mosaic virus has an unusual base composition, that relatively small pieces of it are obtainable and that these attributes may be useful in the solution of the coding problem.

Wild cucumber mosaic virus has a molecular weight of about 7 million and an RNA content of about 2·4 million[1]. Plants infected with the virus produce two virus-like macromolecular particles (designated T_a and T_b) in addition to the virus. Various physical and chemical tests[1,2] have shown that these particles and the virus are similar (or identical) in so far as their protein portion is concerned, but that they differ in their content of RNA. $T_a (M = 4 \times 10^6)$ contains no RNA, T_b $(M = 4{\cdot}4 \times 10^6)$ contains about $0{\cdot}4 \times 10^6$ molecular weight units of RNA. The relative proportion of nucleotide residues in the RNA from the virus and from the T_b particle are similar but perhaps not identical. Both have an unusually high proportion of cytosine (see Table 1), comparable to the high proportion found in turnip yellow mosaic virus[3].

The fact that the composition of the RNA of T_b is unusual and at the same time similar to that of the virus RNA suggests that it has virus-like nucleotide sequences. In any event, its composition is different from that of ribosomes from small sugar pumpkin (see Table 1), the host on which wild cucumber mosaic virus was grown. However, the RNA of T_b cannot be identical with the virus RNA since the latter is a packet about six times as large. Possibly the virus RNA has similar or identical sub-units, one of which is present in each T_b particle. Alternatively, the individual T_b particles may contain different portions, but similar quantities, of the virus RNA. Thus only on the average would the T_b base composition be similar to that of the viral RNA.

Even though proteins other than the protein of the virus coat may be coded by the RNA of wild cucumber mosaic virus, it might be anticipated that the unusual base composition of the latter would be reflected in an unusual amino-acid composition for the protein of the coat. Evidently this is not so. The amino-acid composition is recognizably—but not startlingly—different from that

Table 1. MOLAR BASE COMPOSITION OF RNA IN WILD CUCUMBER MOSAIC VIRUS AND IN THE T_b PARTICLE OF THE VIRUS
By way of comparison, we show also the base composition of the RNA in ribosomes of uninfected sugar pumpkin (the host on which wild cucumber mosaic virus was grown) and also the base composition of the RNA in tobacco mosaic virus

	Adenine	Guanine	Cytosine	Uracil
Wild cucumber mosaic virus*	18·1	16·5	39·8	25·6
	18·5	15·3	40·2	26·0
„ „ T_b*	19·5	20·3	35·3	24·9
	18·9	17·8	37·8	25·5
Ribosomes of sugar pumpkin*	23·7	32·8	23·8	19·7
	23·4	33·8	23·4	19·4
Tobacco mosaic virus	30·0	25·0	18·5	26·5

* Duplicate experiments by the method of Smith and Markham (ref. 4).

Table 2. AMINO-ACID COMPOSITION OF THE PROTEIN OF WILD CUCUMBER MOSAIC VIRUS, AND FOR COMPARISON THE AMINO-ACID COMPOSITION OF TOBACCO MOSAIC VIRUS

	Wild cucumber mosaic virus*	Tobacco mosaic virus¶
Alanine	5·3	5·6
Ammonia	2·0	1·8§
Arginine	3·8	9·7
Aspartic acid and asparagine	7·8	11·7
Cysteine (or half-cystine)	0·9	0·6
Glutamic acid and glutamine	6·6	11·7
Glycine	2·6	1·9
Histidine	2·0	0·0
*iso*leucine	7·2	5·1
Leucine	13·0	7·7
Lysine	5·8	1·4
Methionine	0·7	0·0
Phenylalanine	6·0	6·7
Proline	9·2	4·4
Serine	9·9†	7·9
Threonine	5·8†	9·1
Tryptophan	1·7‡	3·2
Tyrosine	3·1	3·7
Valine	6·6	7·8

* Analysed by means of the method of Spackman, Stein and Moore (ref. 6); hydrolysis of the protein was carried out for 24 hr. at 110°.
† Corrected for an assumed loss of 6 per cent in the case of serine and 11 per cent in the case of threonine.
‡ Determined by the method of Goodwin and Morton (ref. 7).
§ Calculated from the amino-acid composition given by Wittmann (ref. 8).
¶ From Ramachandran (ref. 9).

of the coat protein of tobacco mosaic virus, the RNA of which has quite a low proportion of cytosine (18 per cent, see Table 1). It will be seen from Table 2 that of the 15 amino-acids present in greatest abundance in wild cucumber mosaic virus and tobacco mosaic virus, only in two instances, arginine and proline, does the relative abundance differ by more than a factor of two. The situation is not greatly different if the comparison is made instead with bushy stunt virus, which also has a relatively low content of cytosine (22 per cent, ref. 3).

It might be interpolated at this point that, especially in the case of bushy stunt virus, the amount of protein codable by the virus RNA is not large. Coding theory considerations suggest that at least three nucleotide residues would be required to code each amino-acid[5]. Very likely the codes found in Nature would be less efficient than this theoretical value. Thus from a knowledge of the nucleic acid content and the molecular weight of the protein sub-unit[10] of bushy stunt virus, it is apparent that less than 1,600 amino-acids can be coded, about 600 of which are required for the bushy stunt virus sub-unit. For tobacco mosaic virus the corresponding numbers are 2,100 and 150. By a similar computation it can be shown that less than 2,500 amino-acids are codable by the RNA of wild cucumber mosaic virus, about 200 of which are required by the protein sub-unit (the molecular weight of which is about 20,000, (ref. 1)). The RNA from wild cucumber mosaic virus T_b could code no more than 450 amino-acids.

If all the nucleotide residues in the RNA's of wild cucumber mosaic virus, tobacco mosaic virus and bushy stunt virus participate directly in coding, then codes in which one particular sequence of nucleotides determines a particular amino-acid would seem to be unlikely. In order for such a code to hold, a 40 per cent cytosine content in wild cucumber mosaic virus RNA and an 18 per cent content in tobacco mosaic virus RNA would require a very distinctive difference in the relative abundance of the amino-acids coded in the two cases. On the average, those amino-acids in the specification of which cytosine participates would have to be more than twice as abundant in the case of wild cucumber mosaic virus. If the frequently discussed 4-symbol, comma-less, non-overlapping code[11] holds, twelve amino-acids would have to have this high average abundance. Since such differences are not evident in the coat proteins, even larger differences would be required in any other proteins that conceivably are coded.

A more plausible alternative is that a considerable fraction of the nucleotide residues plays a part different from that of participating in direct specification of particular amino-acids. Perhaps they serve as spacers or as structural elements.

This work was supported by the U.S. Public Health Service and by the Research Committee of the University of Wisconsin Graduate School.

H. YAMAZAKI
PAUL KAESBERG

Department of Biochemistry,
University of Wisconsin,
Madison, Wisconsin.

[1] Yamazaki, H., thesis, University of Wisconsin (1960).
[2] Sinclair, J. B., Geil, P. H., and Kaesberg, P., *Phytopath.*, **47**, 372 (1957).
[3] Markham, R., in *The Viruses*, **2**, 55 (Academic Press, 1959).
[4] Smith, J. D., and Markham, R., *Biochem. J.*, **46**, 509 (1950).
[5] Ycas, M., in *Symposium on Information Theory in Biology*, 91 (Pergamon Press, 1958).
[6] Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).
[7] Goodwin, T. W., and Morton, R. A., *Biochem. J.*, **40**, 628 (1946).
[8] Wittmann, H. G., and Braunitzer, G., *Virology*, **9**, 726 (1959).
[9] Ramachandran, L. K., *Virology*, **5**, 244 (1958).
[10] Hersh, R. T., and Schachman, H. K., *Virology*, **6**, 234 (1958).
[11] Crick, R. H. C., Griffith, J. S., and Orgel, L. E., *Proc. U.S. Nat. Acad. Sci.*, **43**, 416 (1957).

Mechanism of Recovery from Viral Infection in the Chick Embryo

THE chick embryo lacks many of the mechanisms which are thought to be required for recovery from viral infections. Production of antibody and delayed hypersensitivity (as measured by ability to reject foreign tissue) do not occur in the chick embryo of any age[1,2], whereas the interferon mechanism begins to function in the embryo at about eight days and increases thereafter[3]. If interferon plays a major part in recovery from viral infection[4,5], then a correlation would be expected between the lethality of viral infections and absence of the interferon mechanism in young embryos. Decreasing mortality with increasing age of embryos has been reported with a number of viruses[6,7]. However, the cells of these variously aged embryos yield equal amounts of virus[3,8]. The present work was undertaken to determing whether the appearance of the interferon mechanism during development of the chick embryo is parallel to increased ability to recover from viral infection.

Groups of ten chick embryos, aged 4, 7, 10, and 13 days, were infected with each of four different

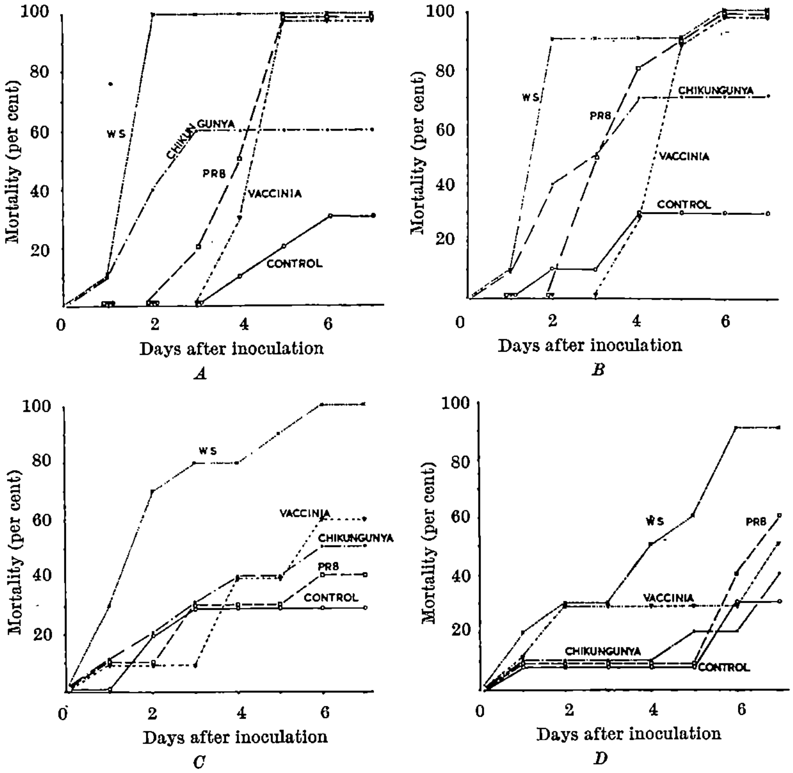


Fig. 1. Mortality following injection of four viruses into chick embryos of different ages. Eggs: *A*, 4-day; *B*, 7-day; *C*, 10-day; *D*, 13-day

viruses. Inoculations of 0·05 ml. containing 10^4–10^5 infectious doses of vaccinia, Chikungunya (Arbor virus group *A*), or influenza (*WS* and *PR* 8 strains) viruses were made into the yolk sac. Controls were injected with broth diluent. The eggs were then candled daily to determine viability of the embryos. Eggs containing embryos, which were non-viable on candling, were opened to confirm death and all embryonated eggs which survived for seven days were opened to confirm viability.

As shown by the graphs in Fig. 1 the mortality-rate of embryos decreased as the age of embryos increased. The lethality among 4- and 7-day embryos, infected with vaccinia, Chikungunya or *PR*8 viruses, was high, whereas the mortality among 10- and 13-day embryos tended to be close to that of the controls. The more virulent *WS* virus caused early deaths in young embryos, but delayed deaths in older embryos.

Fig. 2 summarizes the effect of the age of the chick embryo on its *in vitro* sensitivity to the antiviral action of interferon and on its ability to survive infection with these four viruses. Between the seventh and tenth days there was a rapid development of sensitivity to the antiviral action of interferon and an equally rapid increase in the ability to recover from viral infections. The close correlation of enhanced recovery of embryos with development of the interferon mechanism is consistent with previous findings which related recovery from viral infection of mice to production of interferon[4,9].

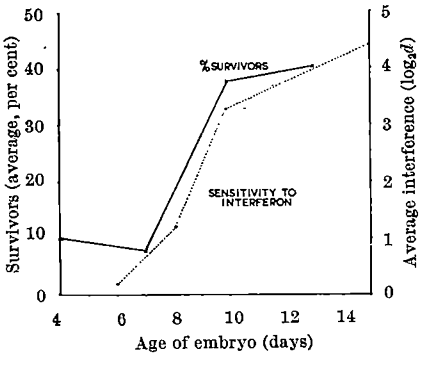


Fig. 2. Effect of age of chick embryo on its ability to survive infection and on its sensitivity to the antiviral action of interferon *in vitro*. Survival in each group was determined by averaging the percentage of embryos surviving 7 days after infection with the four viruses (Fig. 1). Sensitivity to the antiviral action of interferon was determined from the results of a previous study (ref. 3) by averaging the sensitivity of each age group at three doses of interferon

Evidence indicating that antibody is not essential for recovery from many viral infections has been reviewed[10]. In the present work survival of embryos was enhanced in the presence of a functioning interferon mechanism[3] and in the absence of antibody formation or delayed hypersensitivity[1,2], suggesting that interferon alone may be an essential factor in recovery from many viral infections.

Samuel Baron*
Alick Isaacs

National Institute for
Medical Research,
Mill Hill, London, N.W.7.

---

* United States Public Health Service.

[1] Trnka, Z., and Riha, I., *Nature*, **183**, 546 (1959).
[2] Simonsen, M., *Acta Path. Microbiol. Scand.*, **40**, 480 (1957).
[3] Isaacs, A., and Baron, S., *Lancet*, ii, 946 (1960).
[4] Isaacs, A., and Hitchcock, G., *Lancet*, ii, 69 (1960).
[5] Hitchcock, G., and Isaacs, A., *Brit. Med. J.*, ii, 1268 (1960).
[6] Beveridge, W. I. B., and Burnet, F. M., *The Cultivation of Viruses and Rickettsiae in the Chick Embryo* (H.M. Stationery Office, London, 1946).
[7] Fox, J. P., and Laemmert, jun., H. W., *Amer. J. Hyg.*, **46**, 21 (1947).
[8] White, D. O., *Virology*, **9**, 680 (1959).
[9] Hitchcock, G., and Porterfield, J. S., *Virology*, **13**, 363 (1961).
[10] Friedman, R. M., and Baron, S., *J. Immunol.* (in the press).

GENETICS

Basis of the Diploid-like Meiotic Behaviour of Polyploid Cotton

The cultivated cottons (*Gossypium barbadense* and *G. hirsutum*) are allotetraploids derived from two geographically isolated diploid species; one, an Asiatic species, donating the A genome and the other, an American species, donating the D genome. The chromosomes of the diploid progenitors differ in length, those of the A genome species being longer than those of the D genome species. Furthermore, this size difference is maintained in the polyploid, in which chromosomes of two size-categories can be recognized. A histogram (Fig. 1) of the lengths of the

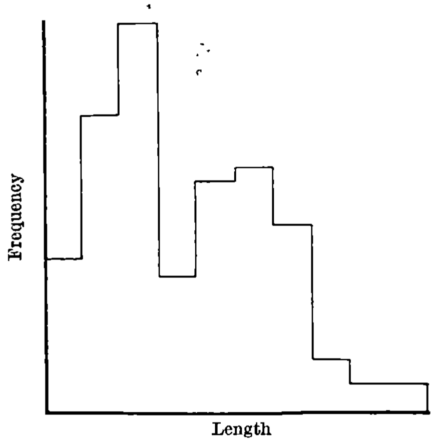


Fig. 1. Chromosome lengths in seven plants of *G. barbadense*

somatic chromosomes from root-tips of seven plants of *G. barbadense* shows a bimodal distribution of the chromosome lengths and also indicates that there is a small overlap between the two groups.

Several workers[1] have employed this differential in chromosome length to categorize meiotic configurations in interspecific hybrids. The difference in size can also be used to recognize the univalent chromosomes seen at meiosis in haploids of the tetraploid species. Chiasma formation occurs infrequently in these haploids, but the univalents often associate in pairs as though synapsis had occurred but no chiasmata had been formed. These associations can be classified into three types depending solely on the lengths of the chromosomes involved; first a long chromosome associated with another long (abbreviated to *LL*), secondly a long chromosome with a short (*LS*) and finally a short with a short (*SS*).

If the chromosomes were associating with each other randomly, and since there are equal numbers of long and short chromosomes, then the numbers of *LL*, *LS* and *SS* associations should be in the ratio 1 : 2 : 1. Table 1 shows the totals of each class of association from 124 cells that were analysed from five haploids of *G. barbadense*. These figures, when tested against the hypothesis of random association, differ significantly ($P < 0{\cdot}001$, $n = 2$) from the expectation; the *LS* associations being considerably in excess. Also it can be shown that since the frequencies of the *LL* and *SS* groups are approximately equal, then the inflation of the *LS* class cannot be due to the consistent misclassification of long for short chromosomes or vice versa.

Table 1. ASSOCIATION OF LONG AND SHORT CHROMOSOMES AT MEIOSIS IN HAPLOIDS OF *G. barbadense*

Association	*LL*	*LS*	*SS*	Total
Observed	65	202	62	329
Expected (1 : 2 : 1)	82·25	164·5	82·25	329

$\chi^2 = 17{\cdot}15$. $n = 2$. $P < 0{\cdot}001$

Since it is plausible that the A and D genome diploids diverged from a common archetype and since duplicate loci have been demonstrated frequently in the allotetraploids, the chromosome association observed in these haploids probably results from a basic similarity of the two genomic sets. In the tetraploid, however, the chromosomes only form bivalents, so that if chromosomes of the different genomes are as similar as these results would indicate, it is probable that there is a genetic system of diploidization in cotton, analogous to that in wheat, organizing the meiotic behaviour so that only homologous and not homœologous chromosomes can pair.

The identification of the chromosome, and the particular chromosome arm, involved in the cytological diploidization of wheat is only possible because of the complete range of aneuploids available in that species[3]. In the absence of a similar range of aneuploids in cotton a precise identification of a genetically controlled mechanism cannot be made. However, by a consideration of certain hybrid combinations, further corroborating evidence can be adduced indicating the existence of genetic diploidization in cotton. The pertinent data are given in Table 2.

Table 2. ANALYSES OF MEIOSIS IN THE A AND D GENOME COMBINATIONS IN *Gossypium*

Hybrid or haploid	Genome	Chromosome pairing	Author
Triploid	A × AD	13 I, 9 II and 2 IV	Gerstel (ref. 5)
Triploid	D × AD	13.00 I, 12.15 II, 0.51 III and 0.04 IV	Endrizzi (ref. 1)
Diploid	A × D	9.6 I, 7.8 II, 0.2 III and 0.05 IV	Skovsted (ref. 1)
Haploid	(AD)	26 I	Kimber

The chromosomes of the A genome diploids pair with the long chromosomes of the AD allotetraploids in hybrids, leaving 13 short univalents. Similarly, the chromosomes of D genome diploids pair with the short chromosomes of the AD allotetraploids and there are 13 long univalents (Table 2). In this way the cytological equivalence of the diploids to their respective genomes in the tetraploids was shown. A similar, and considerable, cytological equivalence between the two diploids is also shown by the high mean synapsis observed in the AD hybrid *G. arboreum* × *G. thurberi*.

These relationships can be expressed diagrammatically (Fig. 2) when it is immediately apparent that an incongruity exists. If the A genome of the diploid is equivalent to the A genome of the tetraploid, and the D genome of the diploid similarly equivalent to the D genome of the tetraploid, and simultaneously the D diploid is equivalent to the A diploid, then the AD tetraploid should have a cytological behaviour characteristic of an autotetraploid. Since the tetraploid cottons are regular bivalent formers, it is most reasonable to suppose that a genetical change has

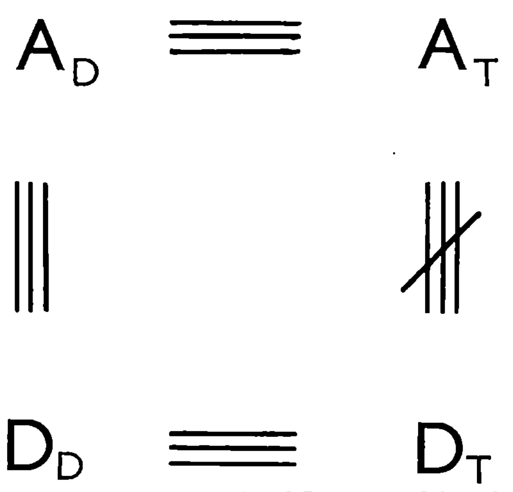


Fig. 2. Equivalence of the A and D genomes of *Gossypium*. The subscripts indicate diploid (*D*) and tetraploid (*T*)

occurred, since the inception of the polyploid, such that pairing is restricted to homologous chromosomes only.

This method of recognizing the genetical control of the cytologically diploid behaviour of allopolyploids can be applied to *Nicotiana tabacum*. Again it is easy to demonstrate the equivalence of the diploid genomes both to their respective genomes in the allopolyploid and also to each other, while the polyhaploid of *N. tabacum* shows almost no pairing.

The effective isolation of genomes in allopolyploids, when the same genomes can pair in diploid hybrids, provides a generalized method for recognizing the genetical control of the diploid-like behaviour of the allopolyploids. Theoretically, the method is applicable to any allopolyploid, but it is difficult in practice to accumulate sufficient data to make a complete cycle of comparisons. Because of the generalized nature of the method, it lacks the precision of the situation in wheat where a single chromosome arm can be held responsible for the effect. However, the method is applicable to all cases where the parents and a haploid of the allopolyploid are known and therefore can be used, even when a complete range of aneuploids is not available.

It is not possible in cotton, at the present, to obtain information on the mode of action of the genetical diploidization, and it would be unwise to assume, *a priori*, that the functioning is identical to that in wheat. However, with the development of a monosomic series in cotton more precise information may be obtained.

Other allopolyploid species may also have become diploidized by genetical processes and not by the slow accumulation of many small structural changes in the chromosomes. The efficiency of a single-step change, if the diploidization is governed by a single gene, to regular meiotic behaviour presents an attractive hypothesis. This hypothesis is conceptually simpler than that required to explain the vague and randomized processes of the addition of small chromosomal changes to already existing alterations. The acceptance of the idea that allopolyploids other than wheat have become diploidized by genetical processes does not mean that some species may not have a regular chromosome behaviour that is governed solely by differential affinity[4]. However, it is worth while considering that, for an allopolyploid to be formed, first of all an F_1 hybrid must be made. The occurrence in Nature of an F_1 hybrid implies some similarity of the two parental species. If the species are so similar that they are able to hybridize, then their chromosomes will rarely be so dissimilar that pairing is entirely autosyndetic in the derived allopolyploid.

The impact of genetical diploidization on a raw allopolyploid needs little stressing. A single-step change that could confer regular chromosomal pairing and a resultant rise in fertility would have such an evolutionary potential that it would become rapidly selected to fixation.

G. KIMBER

Plant Breeding Institute,
Maris Lane,
Trumpington, Cambridge.

[1] Endrizzi, J. E., *J. Hered.*, **48**, 221 (1957). Iyengar, N. K., *Ind. J. Agric. Sci.*, **14**, 142 (1944). Brown, M. S., and Menzel, M. Y., *Genetics*, **37**, 242 (1952). Brown, M. S., *Genetics*, **39** (Abs.), 962 (1952). Skovsted, A., *J. Genet.*, **34**, 97 (1937).

[2] Riley, R., and Chapman, V., *Nature*, **182**, 713 (1958). Riley, R., *Heredity* (in the press).

[3] Riley, R., Chapman, V., and Kimber, G., *Nature*, **183**, 1244 (1959).

[4] Darlington, C. D., *Recent Advances in Cytology* (1937).

[5] Gerstel, D. U., *Evolution*, 7, 234 (1953).

STATISTICS

A Combinatorial Assignment Problem

IN an establishment, there are $v = nk$ officers, r departments and k types of jobs in each department. Each officer has to be assigned a job in each department such that in any department there are equal numbers of officers in different jobs and any two officers have common jobs in exactly λ departments.

It may be seen that the combinatorial problem is the same as that of a resolvable balanced incomplete block design for $v = nk$ varieties, r replications and k plots per block[1]. We have to identify varieties with officers, replications with departments, and blocks in a replication with jobs in a department. We have the obvious identity $\lambda(v-1) = r(k-1)$.

An important case is when $n = k$. The question arises : What is the smallest value of λ for which the problem admits a solution for any given number k ? We thus have a classification of numbers by this minimum λ.

When k is a prime or a prime power it is known that $(k-1)$ mutually orthogonal Latin (Euler) squares exist[2,3]. Let the cells of a $k \times k$ square be taken as officers, the rows as different jobs in the first department, the columns as different jobs in the second department and the letters of the ith Latin square as different jobs in the $(i + 2)$th department. This provides an assignment of jobs to the officers such that any two officers have a common job only in one department (by virtue of the orthogonal property of the Latin squares). Thus in this case $\lambda = 1$, and therefore all primes and prime powers are of type 1.

For $k = 6$, which does not admit even two orthogonal squares, the solution is impossible with $\lambda = 1$. I have been able to find a solution with the next number $\lambda = 2$. Represent the 36 officers by pairs of residues (x, y) mod $(5, 7)$ and an invariant number I. Consider the arrangement in two departments.

Job	Department 1	Department 2
1	01, 06, 12, 15, 23, 24	01, 06, 32, 35, 13, 14
2	11, 16, 22, 25, 33, 34	11, 16, 42, 45, 23, 24
3	21, 26, 32, 35, 43, 44	21, 26, 02, 05, 33, 34
4	31, 36, 42, 45, 03, 04	31, 36, 12, 15, 43, 44
5	41, 46, 02, 05, 13, 14	41, 46, 22, 25, 03, 04
6	00, 10, 20, 30, 40, I	00, 10, 20, 30, 40, I

Adding unity to the second member of each pair (x, y) in the arrangement for any one of the two departments and reducing to mod 7, and keeping I unchanged yields the arrangement for another department. This operation applied to the arrangements for the first two departments leads to the arrangements for two other departments, which by the same operation provides two other arrangements and so on. We thus derive the arrangements for all the fourteen departments. It is not known whether the problem can be solved for any k, with $\lambda = 2$. The next number to investigate is 10, which is unlikely to have 9 mutually orthogonal squares. An arrangement with $\lambda = 4$ is, however, possible. In any event, the open problem is that when a number k is not a prime or a prime power, can we say that it belongs to at least type 2 ?

The combinatorial problem of assignments is thus a general formulation of a wider variety of problems than the problem of orthogonal Latin squares.

C. RADHAKRISHNA RAO

Indian Statistical Institute,
Calcutta-35.

[1] Yates, F., *Ann. Eugen.*, **7**, 121 (1936).

[2] Stevens, W. L., *Ann. Eugen.*, **9**, 82 (1939).

[3] Bose, R, C., *Ann. Eugen.*, **9**, 353 (1939).

FORTHCOMING EVENTS

(Meeting marked with an asterisk is open to the public)*

Wednesday, July 5

Society for Endocrinology (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Sir Charles Dodds, F.R.S.: "Rime and Reason in Endocrinology".*

Wednesday, July 5—Friday, July 7

European Organization for Research on Fluorine and Dental Caries Prevention (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2—Eighth Congress.

Thursday, July 6

Borough Polytechnic (at Borough Road, London, S.E.1) at 10 a.m.—Symposium on "Internal Stresses in Electrolytically Produced Coatings and their Influence on the Properties of the Basis Metals".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Research Fellow (Senior or Junior) in the Agricultural Biochemistry Division of the Department of Biological Chemistry, for research on the immunology of the pituitary gonadotrophic hormones—The Secretary, The University, Aberdeen (July 7).

Lecturer (graduate in agricultural chemistry, physics or geology) in Soil Physics in the Department of Agricultural Sciences at the School of Agriculture, Sutton Bonington—The Registrar, The University, Nottingham (July 8).

Research Assistant (graduate in agricultural chemistry, biochemistry or chemistry) to Prof. E. G. Hallsworth at the School of Agriculture, Sutton Bonington, for work on the nutrition and biochemistry of the Leguminosae—The Registrar, The University, Nottingham (July 8).

Senior Lecturer (male, British, with an appropriate honours degree) in Applied Mechanics at R.N. College, Greenwich, London, S.E.10, for duties which include lecturing and conducting laboratory work to degree standard and postgraduate teaching—The Director (P), Education Department, Admiralty, London, S.W.1 (July 10).

University Lecturer in Mathematical Statistics—The Secretary of the Appointments Committee of the Faculty of Mathematics, The Arts School, Bene't Street, Cambridge (July 12).

Chair of Mathematics—The Registrar, University College of Sussex, Stanmer House, Stanmer, Brighton (July 15).

Extra-Mural Tutor in Social Anthropology or related fields—The Registrar, University College, Singleton Park, Swansea (July 15).

Vokes Research Fellow (preferably with a special interest in fluids or elastic theory) in the Department of Mechanical Engineering—The Secretary and Registrar, The University, Southampton (July 15).

Head (with high qualifications in botany or zoology and experience in teaching to university degree level and in research) of the Department of Botany and Zoology (Grade V)—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (July 17).

Postdoctoral Fellow in Organic Chemistry, to carry out research in the carotenoid field in collaboration with Prof. B. C. L. Weedon—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (July 17).

Research Fellow or Assistant Lecturer in Mathematics—The Secretary, Royal College of Science and Technology, Glasgow (July 17).

Research Associate (graduate in mathematics or physics), to perform theoretical work on semiconductors, as a member of a small group in the Department of Applied Mathematics—Prof. P. T. Landsberg, Department of Applied Mathematics, University College, Cardiff (July 20).

Lecturer (physicist, chemist, or glass technologist, preferably with some industrial experience) in the Department of Glass Technology—The Registrar, The University, Sheffield (July 22).

Chemist (with a degree in chemistry or equivalent qualification, and preferably experience in pesticide analysis) in the Plant Protective Chemistry Section, to assist in studies of pesticides in fruit culture—The Secretary, East Malling Research Station, Maidstone, Kent (July 24).

Senior Lecturer or Lecturer (preferably with previous research experience (theoretical or experimental) in upper atmospheric physics and in palæomagnetism or an allied subject) in Physics; a Lecturer in Geography; and a Lecturer in Mathematics at the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 26).

Proctor Memorial Fellow (science or technology graduate with postgraduate research experience) in the Department of Leather Industries, for research on a subject pertaining to the leather or allied industries—The Registrar, The University, Leeds 2 (July 28).

Chair of Theoretical Physics; and Senior Lecturers and Lecturers in Anatomy, Biochemistry, Chemistry, Engineering, Ethology and Vertebrate Palæontology, Mathematics, Physics, and Philosophy at Monash University, Clayton, Victoria, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, July 31).

Lecturer or Senior Lecturer in Zoology at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 31).

Research Fellows and Students in the Physics Department, to work in the surface physics group—The Secretary and Registrar, The University, Southampton (August 1).

Senior Lecturer or Lecturer in Parasitology; and a Senior Lecturer or Lecturer (with a special interest in hæmatology or clinical chemistry) in Pathology at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 12).

Reader in Veterinary Medicine at the Royal Veterinary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 22).

Chair of Agricultural Botany in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 23).

Chair of Soil Science in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 23).

Agricultural Officer (male, aged 25–45, with a degree in agriculture or a related subject, and experience in tropical agriculture) in Gambia, for general agricultural extension work—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/12/03.

Assistant Lecturer (preferably with a knowledge of elementary virology and/or micology, and some teaching experience) in Microbiology in the Department of Health Education—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

Demonstrator in Physics—The Registrar, The University, Nottingham.

Lecturer (graduate at present engaged in industry, research or teaching) in the Department of Electrical Engineering, West Ham College of Technology, to teach either telecommunications or measurements to full-time students preparing for the University of London Internal B.Sc.(Eng.) Degree in Electrical Engineering—Chief Education Officer, Education Department, 95 The Grove, Stratford, London, E.15.

Lecturer (Grade I) in Theoretical Physics; a Lecturer (Grade I) in Physics; and a Lecturer (Grade II) in Physics—The Registrar (Room 22, O.R.B.), The University, Reading.

Lecturer (graduate of a British university, teaching experience, and preferably qualified to undertake some teaching of biology) in Physiology in the Department of Health Education—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

Mineralogist (preferably under 35, with a good honours degree in geology, with emphasis on mineralogy and preferably experience of spectrograph and X-ray apparatus) with the Government of Tanganyika, to be engaged in the identification of minerals—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. C1/BCD105/8/04.

Research Assistants (2), for work with Prof. R. M. Barrer on the physical and hydrothermal chemistry of soil-forming minerals—Prof. R. M. Barrer, F.R.S., Chemistry Department, Imperial College of Science and Technology, London, S.W.7.

Research Students in the Department of Chemical Engineering, Mechanical Engineering, and one other Department—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 17/G.

Senior Lecturer (graduate of a British University) in Biochemistry—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

British Association for Commercial and Industrial Education. New Routes to Further Education: Papers presented at the B.A.C.I.E. Spring Conference, Senate House, University of London, 19 April, 1961. Pp. ii+31. (London: British Association for Commercial and Industrial Education, 1961.) [144

The Cape Asbestos Company, Ltd. Asbestos The Raw Material. Pp. 32. (London: The Cape Asbestos Company, Ltd., 1961.) [144

Bulletin of the British Museum (Natural History). Entomology. Vol. 10, No. 7: A Catalogue of the Types and other Specimens in the British Museum (Natural History) of the Genus *Zygaena Fabricius*, Lepidoptera: Zygaenidae. By W. G. Tremewan. Pp. 239–314+plates 50–64. 40*s*. Vol. 10, No. 8: A Taxonomic Study of some Indo-Australian Drepanidae. By Allan Watson. Pp. 315–348+plates 65–66. 15*s*. Zoology. Vol. 7, No. 5: The Taxonomy and Identification of Pipits (genus *Anthus*). By B. P. Hall. Pp. 243–289+plates 56–61. 21*s*. (London: British Museum (Natural History), 1961.) [144

Department of Scientific and Industrial Research; and Fire Offices' Committee (Joint Fire Research Organization). Statistical Analysis of Reports of Fires Attended by Fire Brigades in the United Kingdom during 1959. Prepared by the Joint Fire Research Organization for the Home Office, the Scottish Home Department; and the Ministry of Home Affairs, Northern Ireland. Pp. ii+93. (Boreham Wood, Herts: Fire Research Station, 1961.) [144

Murphy Chemical Company Limited. Murphy Fruit Grower's Booklet, 1961. Pp. 124. (Wheathampstead: Murphy Chemical Co., Ltd., 1961.) [144

Entropy and the Unity of Knowledge. By Prof. P. T. Landsberg. (An inaugural lecture delivered at University College, Cardiff, 29th November, 1960.) Pp. 27. (Cardiff: University of Wales Press, 1961.) 3*s*. 6*d*. [174

Department of Scientific and Industrial Research: Geological Survey and Museum. British Regional Geology. Scotland: The Tertiary Volcanic Districts. By Dr. J. E. Richey, with revision by Dr. A. G. Macgregor and Dr. F. W. Anderson. Pp. viii+120+9 plates. (London: H.M. Stationery Office, 1961.) 6*s*. net. [174

The Murphy Chemical Company, Ltd. Murphy Nurseryman's Booklet, 1961. Pp. i+132. (Wheathampstead: Murphy Chemical Company, Ltd., 1961.) [174

Wool Industries Research Association. Report of the Director of Research for 1960. Pp. 39. (Publication No. 215.) (Torridon, Headingley, Leeds: Wool Industries Research Association, 1961.) [184

Building Research Station Digest (Second Series), No. 9: Dry-lined Interiors to Dwellings. Pp. 4. (London: H.M. Stationery Office, 1961.) *4d.* [184

Plant Breeding Institute, Cambridge. Annual Report, 1959–60. Pp. viii+72. (Trumpington, Cambridge: Plant Breeding Institute, 1961.) *5s. 6d.* [184

Friends of the Lake District. A Road Policy for the Lake District. Pp. 31+4 plates. Electricity in Borrowdale. Pp. 3. (Ulverston: Friends of the Lake District, 1961.) [194

The Animal Health Trust. Tenth Report, 1957–1960. Pp. 100. (London: The Animal Health Trust, 1961.) [194

University College of Swansea. Department of Chemistry—Illustrated and Descriptive Booklet. Pp. 34. (Swansea: University College, 1961.) [194

Institution of Chemical Engineers. Annual Report of the Council for the year ending 31 December, 1960. Pp. 17. (London: Institution of Chemical Engineers, 1961.) [204

Library Association. Special Subject List No. 34: Solar Energy Technology, 1954–1959. Compiled by T. Brimelow. Pp. 32. (London: Library Association, 1961.) *3s. 6d.* [214

Journal of Helminthology. Supplement in Honour of R. T. Leiper on the occasion of his 80th Birthday, 1961. Pp. xxii+229. (London: London School of Hygiene and Tropical Medicine, 1961.) *30s.* [214

Annual Report of the West African Rice Research Station, 1958. Pp. 31. (London: Crown Agents for Oversea Governments and Administrations, 1959.) *2s.* [214

Cosmic Rays and the Exploration of Space. By Prof. Harry Elliot. (Inaugural Lecture, 6 December, 1960.) Pp. 12+4 plates. (London: Imperial College of Science and Technology, 1961. Distributed by the Modern Book Co., Ltd., 19 Praed Street, W.2.) *5s.* [254

City of Sheffield. Annual Reports of the City Museum for the two years ending 31st March, 1960. Pp. 20+4 plates. (Sheffield: City Museum, 1961.) [254

The Red Deer Commission. Report for the period 1st October, 1959, to 31st December, 1960. Pp. 22. (London: H.M. Stationery Office, 1961.) *1s. 3d.* net. [254

Scientific Proceedings of the Royal Dublin Society. Series A, Vol. 1, No. 5 (November 18, 1960): The Gelatinous Polysaccharide of *Furcellaria fastigiata*. By M. J. Clancy, K. Walsh, T. Dillon and P. S. O'Colla. Pp. 197–204. *2s.* Series A, Vol. 1, No. 6 (March 6, 1961): Namurian Shales near Buttevant, North Co. Cork. By M. E. Philcox. Pp. 205–209. *1s. 6d.* Series B, Vol. 1, No. 3 (February 24, 1961): Seasonal Variations in the Activity of Cereal Root Eelworm (*Heterodera major*, O. Schmidt, 1950). By J. J. Duggan. Pp. 21–24. *1s.* (Dublin: Royal Dublin Society, 1960 and 1961.) [254

The Cambridge Expedition to British Honduras, 1959–60. General Report. Pp. 32+4 plates. (Cambridge: Cambridge Expedition to British Honduras, 1960.) [254

Other Countries

Republica de Nicaragua, C.A. Ministerio de Economia; Instituto de Fomento Nacional; y Organizacion de las Naciones Unidas para la Agricultura y Alimentacion. Estudios Ecologicos, Volumen No. 1 (1959): Estudios Ecologicas para el Aprovechamiento de la Tierra en Nicaragua. (Ecological Land Use Surveys in Nicaragua.) By Dr. B. W. Taylor. Pp. xv+338. (Managua, D. N.: Ministerio de Economia; Instituto de Fomento Nacional; y Organizacion de las Naciones Unidas para la Agricultura y Alimentacion, 1959.) [174

Government of Bombay. Annual Report of the Haffkine Institute for the year 1959. Pp. ii+74. (Bombay: Director, Government Printing and Stationery; London: High Commissioner for India, 1960.) Rs. 0.60; *1s.* [184

United States Department of the Interior: Geological Survey. Water-Supply Paper 1534: Progress Report on Wells Penetrating Artesian Aquifers in South Dakota. By R. W. Davis, C. F. Dyer and J. E. Powell. Pp. iv+100+9 plates. (Washington, D.C.: Government Printing Office, 1961.) [184

Bulletin of the American Museum of Natural History. Vol. 122, Article 1: Geographic Variation in Some Reproductive Characteristics of Diurnal Squirrels. By Joseph Curtis Moore. Pp. 1–32. (New York: American Museum of Natural History, 1961.) 1 dollar. [184

Food and Agriculture Organization of the United Nations. World Food Problems, No. 3: Fish—The Great Potential Food Supply. By D. B. Finn. Pp. iii+47. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1960.) *2s. 6d.*; 0.50 dollars. [194

Canada: Ministry of Agriculture. Board of Grain Commissioners for Canada, Winnipeg. Grain Research Laboratory—1960 Report. Pp. vi+82. (Ottawa: Queen's Printer, 1961.) [194

Proceedings of the California Academy of Sciences, Fourth Series. Vol. 30, No. 2: West American Species of the Bivalved Gastropod Genus *Berthelinia*. By A. Myra Keen and Allyn G. Smith. Pp. 47–66+1 plate. (San Francisco: California Academy of Sciences, 1961.) [194

Commonwealth Scientific and Industrial Research Organization, Melbourne. C.S.I.R.O. Divisions and Sections. Pp. 19. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1961.) [214

Carnegie Institution of Washington. Annual Report of the Director of the Department of Terrestrial Magnetism, 1959–1960. (Reprinted from *Carnegie Institution of Washington Year Book* 59, for the year July 1, 1959–June 30, 1960.) Pp. ii+191–291+10 plates. (Washington, D.C.: Carnegie Institution of Washington, 1960.) [214

Institut Royal des Sciences Naturelles de Belgique. Mémoires. Deuxième Série, Fasc. 64: Les Conacea Fossiles du Cénozoique Etranger: Des Collections de l'Institut Royal des Sciences Naturelles de Belgique. Par Maxime Gilbert. Pp. 132. (Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1960.) [214

Government of India: Ministry of Health. Report of the Central Drugs Laboratory from 1st April, 1957, to 31st March, 1958. By Dr. H. K. Banerjee. Pp. i+53. (Calcutta: Ministry of Health, Central Drugs Laboratory, 1960.) Rs. 7.25 n.p.: *11s. 6d.* [254

Commonwealth Scientific and Industrial Research Organization. Melbourne. Land Research Series. No. 4: The Lands and Pastoral Resources of the North Kimberley Area, W.A. By N. H. Speck, Janice Bradley, M. Lazarides, R. A. Patterson, R. O. Slatyer, G. A. Stewart and C. R. Twidale. Pp. 109+7 plates. No. 5: Pasture Lands of the Northern Territory, Australia. By R. A. Perry. Pp. 55+10 plates. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1960.) [254

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 42 (February, 1961): Longevity of American Presidents. Morbidity and Mortality from Heart Disease. Accidents: a Major Killer. Aspects of the Homicide Problem. Pp. 12. (New York: Metropolitan Life Insurance Company, 1961.) [254

Kungl. Sjöfartsstyrelsen, Stockholm. Ergebnisse der Beobachtungen des Magnetischen Observatoriums zu Lovö (Stockholm) im Jahre, 1957. Von Folke Eleman and Kjill Borg. Pp. 30. (Stockholm: Kungl. Sjöfartsstyrelsen, 1960.) [254

Smithsonian Institution. United States National Museum Bulletin No. 220: Type Specimens of Reptiles and Amphibians in the U.S. National Museum. By Doris M. Cochran. Pp. xv+291. (Washington, D.C.: Government Printing Office, 1961.) 1.25 dollars. [254

Det Kongelige Departement for Industro og Hadverk. Norsk Polarinstitutt. Skrifter Nr. 120: The Place-names of Jan Mayen. By Anders K. Orvin. Pp. 72+2 maps. (Oslo: Norsk Polarinstitutt, 1960. Distributed by the Oslo University Press.) [254

Occasional Papers of the California Academy of Sciences. No. 26: Descriptions of a New Species of Snake of the Colubrid Genus *Gyalopion* from the State of Zacatecas, Mexico. By Alan E. Leviton and Benjamin H. Banta. Pp. 4. (San Francisco: California Academy of Sciences, 1961.) [254

Population Reference Bureau, Inc. Population Bulletin, Vol. 17, No. 2: Latin America—a Decade of Decision. Pp. 17–40. (Washington, D.C.: Population Reference Bureau, Inc., 1961.) 50 cents. [254

Transactions of the American Philosophical Society, New Series. Vol. 51, Part 1: The Tobacco Adventure to Russia: Enterprise, Politics, and Diplomacy in the Quest for a Northern Market for English Colonial Tobacco, 1676–1722. By Jacob M. Price. Pp. 120. (Philadelphia: American Philosophical Society, 1961.) 2.75 dollars. [254

National Academy of Sciences—National Research Council. Nuclear Science Series. NAS–NS 3020: The Radiochemistry of the Rare Earths, Scandium, Yttrium, and Actonium. By P. C. Stevenson and W. E. Nervik. Pp. x+282. 3 dollars. NAS–NA 3021: The Radiochemistry of Technetium. By Edward Anders. Pp. v+50. 50 cents. NAS–NS 3023: The Radiochemistry of Tin. By W. E. Nervik. Pp. ix+68. 75 cents. NAS–NS 3025: The Radiochemistry of the Rare Gases. By Floyd F. Momyer, Jr. Pp. v+55. 75 cents. (Washington, D.C.: Office of Technical Services, Department of Commerce, 1960 and 1961.) [254

United States Department of the Interior: Geological Survey. Professional Paper No. 339: Tertiary and Quaternary Gastropoda of Okinawa. By F. Stearns MacNeil. Pp. iv+148+21 plates. 2.50 dollars. Professional Paper No. 374–A: Upper Eocene and Oligocene Larger Foraminifera from Viti, Fiji. By W. Storrs Cole. Pp. iii+7+3 plates. Professional Paper 374–B: Joints in Precambrian Rocks, Central City—Idaho Springs Area, Colorado. By J. E. Harrison and R. H. Moench. Pp. iii+14. 20 cents. (Washington, D.C.: Government Printing Office, 1960 and 1961.) [254

Bulletin of the National Speleological Society, Vol. 22, Part 1: Origin of Limestone Caves: a Symposium, with Discussion. Edited by George W. Moore. Pp. 84. (Alexandria, Virginia: The National Speleological Society, 1960.) 2 dollars. [15

Journal of Atherosclerosis Research, Vol. 1, No. 1 (January-February, 1961). Pp. 92. Published bi-monthly in six issues per volume of approx. 550 pages. Subscription price: £6 *5s. 0d.* or 17.50 dollars or D. fl. 66.50 per year (post free). (Amsterdam: Elsevier Publishing Company, 1961.) [15

General Electric Company, Philadelphia. 1960 Annual Report. Pp. 34. Space Facts: a Handbook of Basic and Advanced Space Data for Scientists and Engineers. Pp. 64. (Philadelphia: General Electric Company, 1961.) [15

United States Department of Commerce: Coast and Geodetic Survey. Technical Bulletin No. 15: Transformation of Rectangular Space Coordinates. By Erwin Schmid. Pp. 13. (Washington, D.C.: Government Printer, 1961.) 15 cents. [15

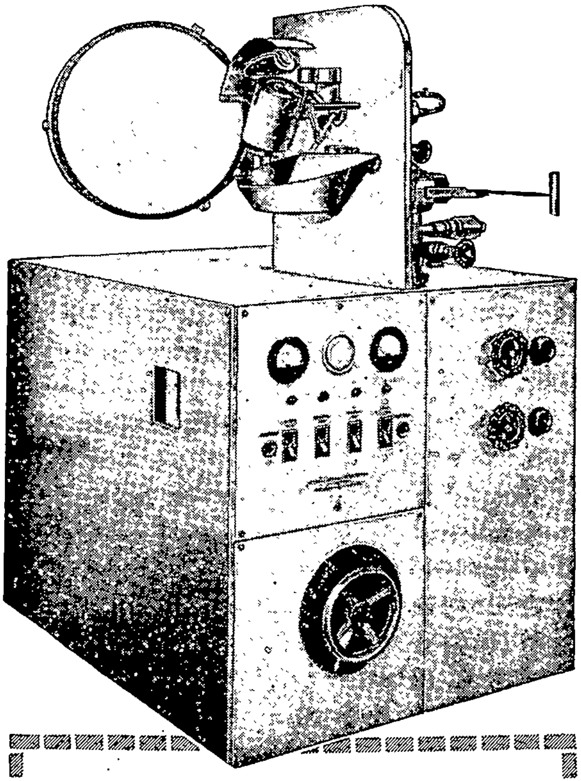
Somaliland Protectorate: Geological Survey. Report No. 3: Report on the Geology of the Las Khoreh-Elayu Area, Erigavo District. (Quarter Degree Sheets Nos. 5 and 6. D.O.S. 1 : 125,000.) By J. E. G. W. Greenwood. With an Appendix on the Cassiterite Deposits of the Dalan Area by J. A. B. Stewart. Pp. viii+36+4 plates. (Hargeisa: Stationery Office; London: Crown Agents for Oversea Governments and Administrations, 1960.) Shs. 20. [15

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £8/8/-, payable in advance,
postage paid to any part of the world

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office



WILD BARFIELD NRC

Laboratory Vacuum Furnace

Model 2904

2 lb. to 8 lb. capacity

(FOR OPERATION UP TO 2,000°C)

Made in Britain to the designs of the National Research Corporation of Mass., U.S.A. who have built more high vacuum furnaces now in successful operation than any other firm in the world. The Model 2904 Vacuum Furnace is a self-contained unit for high-temperature operation at high vacuum or under controlled atmosphere. It is heated by a tungsten resistance heating element with molybdenum heat shields in a water-cooled stainless steel sleeve.

This permits:

- Close control of temperature
- Good heat distribution
- Long life
- No outgassing or contamination from refractories

FOR MELTING • POURING • SINTERING ALLOYING • HEAT-TREATING • ANNEALING

Vacuum Arc and Vacuum Induction Furnaces
Vacuum Analysis Equipment • Vacuum Diffusion Pumps

STANDARD EQUIPMENT

- High vacuum pumping system with Wild-Barfield-NRC oil diffusion pump and Gas Ballast mechanical pump.
- Metal cabinet base, overall dimensions 35" x 43" x 34" high.
- Pyrex bell jar, 18" diameter x 12" long, protected with a wire mesh guard, or optionally a stainless steel bell jar.
- Water-cooled basin to shield the bell jar and to catch spilled metal.
- Feed-through for thermocouple wires or heater leads for special work.
- 15 kW. power supply, controlled with a variable auto-transformer. Power is supplied to the split tungsten sleeve through water-cooled coaxial leads.

* NRC *is the trade-mark of the National Research Corporation, registered in the United States Patent Office.*

WILD BARFIELD

ELECTRIC FURNACES
FOR *ALL* HEAT-TREATMENT PURPOSES

WILD-BARFIELD ELECTRIC FURNACES LIMITED

ELECFURN WORKS, OTTERSPOOL WAY, WATFORD BY-PASS, WATFORD, HERTS. TELEPHONE: WATFORD 26091 (8 LINES)

NRCS

APPOINTMENTS VACANT

SOUTH AFRICAN COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH

NATIONAL BUILDING RESEARCH INSTITUTE

SOIL MECHANICS AND FOUNDATION ENGINEERING DIVISION

HEAD OF DIVISION

Applications are invited for the post of Head of the Soil Mechanics Division of the National Building Research Institute, Pretoria.

Applicants should have a University degree or equivalent qualification and experience in the field of Soil Mechanics research and application. Details of past publications should accompany all applications, together with evidence of proven research ability.

The Head of the Division is required to lead a team of about twelve persons, giving guidance to several specific research projects. He must also encourage the application of research findings within the building and construction industry in South Africa and maintain close liaison with the industry and co-ordinate the Division's work with other research bodies in the country particularly in regard to work of a fundamental nature.

The present research programme includes the following projects:

- (i) studies of expansive clays and the development of economical solutions to the problem of founding structures on these clays;
- (ii) research on the stability of slopes of waste dumps, particularly sand and ash dumps;
- (iii) the application of air photo interpretation to foundation soil mapping for township development;
- (iv) studies of thermo-dynamics aspects of the swelling and strength properties of soils; and
- (v) studies of the engineering properties of particular types of weathered soil profiles.

Work with industry involving deep drilling and undisturbed sampling and specialized testing of a research nature is also undertaken.

Appointment will be made according to qualification and experience in the salary range £1,620 to £2,400 per annum.

Further information on service conditions may be obtained from:

South African Scientific Liaison Office,
Africa House, Kingsway,
LONDON, W.C.2,
England.

Applications should reach the Director, National Building Research Institute, P.O. Box 395, Pretoria, South Africa, from whom further information can be obtained, not later than August 31, 1961.

UNIVERSITY OF NOTTINGHAM

LECTURER IN SOIL PHYSICS

Applications are invited from graduates in agricultural chemistry, physics or geology for appointment as Lecturer in Soil Physics, in the Department of Agricultural Sciences at the School of Agriculture, Sutton Bonington. The successful candidate will be required to specialize in the field of either (a) X-ray spectrophotometry or (b) particle transport in fluids. Applications will also be considered for appointment initially at the Assistant Lecturer level from candidates recently graduated or graduating this summer in physics. Salary scales: Lecturer, £1,050 by £50 to £1,400 by £75 to £1,850; Assistant Lecturer, £800 by £50 to £950. Initial salary according to qualifications and experience, with membership of Universities Superannuation Scheme and children's allowance.

Conditions of appointment and form of application, to be returned by July 8, from the Registrar.

UNIVERSITY OF MANCHESTER

Applications are invited for the post of Lecturer in Engineering (Mechanical). Preference will be given to candidates with a major interest in applied mechanics, especially theory of machines, stress analysis and properties of materials, or applied thermodynamics. An interest in mechanical design would be an advantage. University regulations permit teaching staff to read for the degree of M.Sc. and Ph.D. The salary scale £1,050 to £1,850 per annum. Membership of F.S.S.U. and children's allowance scheme. Initial salary according to qualifications and experience.

Applications should be sent not later than July 8, 1961, to the Registrar, the University, Manchester 13, from whom further particulars and forms of application may be obtained. Applications by airmail letter, in the first instance, will be considered from overseas candidates.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

RESEARCH PHYSICIST

The Organization's **Division of Textile Physics, Ryde, N.S.W.**, invites applications for a position as Physicist from honours graduates in Science majoring in Physics (or equivalent qualifications) with at least three years' post-graduate research experience.

The organization is undertaking an investigation into the physical, chemical and physiological bases for the commonly accepted view that wool possesses natural advantages over other fibres in respect of warmth and comfort. This is important to the interest of wool use and it is hoped thereby to exploit the advantages found for wool and to find means of rectifying any disadvantages found.

The Physicist, who will be required to spend a substantial amount of his time at the School of Public Health and Tropical Medicine, University of Sydney, will carry out investigations into the role of the physical properties of cloth in determining the comfort, warmth and health aspects of clothing and such coverings as blankets. He will work in co-operation with the Environmental Health Section which is concerned both with Moist Tropic and Antarctic Conditions and is having a laboratory with temperature and humidity controllable over a wide range, and a wind tunnel added to existing facilities. It is expected that the physicist will occupy an increasingly important position in the textile field as a consultant in the design of clothing and coverings for special purposes.

Salary: Dependent on qualifications and experience within the ranges: £A.1,510—£A.2,265 per annum or £A.2,395—£A.2,720 per annum.

Salary for a woman will be £A.180 per annum less than corresponding rates for men.

Promotion within C.S.I.R.O. is by merit and may ultimately go beyond the upper limit of the scale within which the original appointment is made.

Fares paid. Further particulars of conditions, etc., are supplied on application to:

Mr. E. J. Drake, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No. 465/139), should be addressed by July 29, 1961.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

PLANT PHYSIOLOGIST

The Organization's **Irrigation Research Station, Griffith, N.S.W.**, invites applications for a position as Plant Physiologist from Honours graduates in Science or Agriculture (or equivalent qualifications), with post-graduate research experience in Plant Physiology or Agronomy.

A wide range of subjects connected with problems in irrigation are studied at the Station and a plant physiologist with special interests in plant growth is required to study the response of plants and fruit to irrigation practice. Modern laboratories with extensive research facilities are available, including constant temperature rooms and an isotope laboratory and constant environment cabinets are in process of development.

Housing may be available at an economic rent.

Salary: Dependent on qualifications and experience within the range: £A.1,510 to £A.2,265 per annum.

Salary for a woman will be £A.180 per annum less than corresponding rates for men.

Promotion within C.S.I.R.O. is by merit and may ultimately go beyond the upper limit of the scale within which the original appointment is made.

Fares paid. Further particulars supplied on application to:

Mr. E. J. Drake, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No. 500/136), should be addressed by July 29, 1961.

UNIVERSITY LOVANIUM OF LEOPOLDVILLE

Applications are invited from candidates with doctor's degree for appointment as Lecturer in Chemistry (lecturing in French language) to teach up to master's degree. Duties to commence in October, 1961. Salary scale, including overseas pay, £2,500 to £2,900 per annum. Free housing, medical care and insurance. Post is pensionable. Free passage, once each way for each tour of service for person appointed and wife; either passage allowance or maintenance allowance for children under 18. Tours of service: nine months.

Write for further information (with curriculum vitae), to Rector L. Gillon, University Lovanium, B.P. 121, Leopoldville XI, Republique du Congo.

UNIVERSITY LOVANIUM OF LEOPOLDVILLE

Applications are invited from candidates with doctor's degree for appointment as Lecturer in Mathematics (lecturing in French language) to teach up to master's degree. Duties to commence in October, 1961. Salary scale, including overseas pay, £2,500 to £2,900 per annum. Free housing, medical care and insurance. Post is pensionable. Free passage, once each way for each tour of service for person appointed and wife; either passage allowance or maintenance allowance for children under 18. Tours of service: nine months.

Write for further information (with curriculum vitae), to Rector L. Gillon, University Lovanium, B.P. 121, Leopoldville XI, Republique du Congo.

INSTITUTE OF RADIO
LIBR.

CRISIS IN MATHEMATICAL EDUCATION IN BRITAIN

THERE is a strong possibility that mathematical education, as we have known it in Great Britain and in this century, will come to an end within twenty years, a possibility of incalculable consequences for the life of the nation as a whole. This warning, so often sounded by teachers of mathematics during the past decade and so often ignored, was given again with even greater urgency by Dr. Bryan Thwaites, the new professor of theoretical mechanics in the University of Southampton, in an inaugural lecture on May 23. With industry demanding and attracting more mathematicians, university teaching posts are left unfilled, and school teaching posts are unfilled or filled by those who have little interest in or knowledge of mathematics ; thus there is a shortage of pupils having enough mathematical equipment to enable them to proceed to university or technical college training in science or technology. Hence, in the near future there will be a grave shortage of engineers and technologists, at a time when our export trade and our economic well-being will be more than ever dependent on the skill and availability of such people. Nothing less than measures on the largest scale, prosecuted with great vigour and determination, have any chance of restoring the position ; such measures must be undertaken, not in some vague future, not in the next few years, but within the next few months. Even so, the schools would not feel any significant benefit before 1965 or 1966, and the universities and technical colleges not much before 1968 or 1970. While precise statistics are difficult to obtain, Thwaites's rough estimate serves to indicate the magnitude of the crisis. In the universities, the present staffing deficiency is equal to one year's Ph.D. output, and maintenance of staff at the present level requires double that output. In the grammar schools, the deficiency is equal to three years output of honours graduates, and maintenance requires again a doubling of output. This estimate takes no account of the needs of the newly founded or promised universities, of the technical colleges and of the secondary modern schools.

What measures does Prof. Thwaites propose ? First he suggests that the mathematicians should set their own house in order. There must be a greater sense of unity among all types of mathematician, with the formation of a professional institute to which all would naturally belong ; he regrets that the Mathematical Association does not have a standing comparable with that of other recognized professional bodies. Then the dichotomy between teaching and research must be bridged. It should not be axiomatic that the only career for a first-class honours student is in research ; the appointment and promotion of a lecturer should not depend solely on his success in getting his name into print. Here Prof. Thwaites perhaps fails to do justice to the Mathematical Association ; it has never claimed or wished to be concerned with anything except the promotion of good methods of teaching mathematics. The London Mathematical Society caters for the research worker, and Prof. Thwaites might have reinforced his argument by deploring the recent tendency for a mathematician to join either the Mathematical Association or the London Mathematical Society but not both. Further, the Mathematical Association has made a significant contribution towards easing the crisis by establishing a diploma in mathematics; by passing this, those teachers who, with no specialized mathematical training, are being drafted into teaching mathematics, may equip themselves with at least the basic knowledge which they will require.

Prof. Thwaites then looks at the outside aspect of the problem, and divides it into two parts : the problem of recovery, and the problem of maintenance. For recovery, demands for mathematicians made by other professions must be voluntarily and severely contracted ; salary scales for teachers must be interpreted with great flexibility ; interchange between the school and the university on one hand, and industrial, commercial and Government establishments on the other, should be widespread, on a part-time or full-time basis. This programme must have national acceptance, and must therefore be presented as an urgent national necessity by a fully organized publicity campaign. For maintenance, we must double the mathematical entry over the whole of Britain at the earliest possible moment. Since we have not enough teachers to cope with the present entry, this would appear to be an impossible demand. But Prof. Thwaites thinks that much could be done if the syllabus for school mathematics could be reformed, if the universities revised their standards of entry, and if more undergraduates could be encouraged to take the unduly disparaged general degree in which mathematics would be read in conjunction with other subjects. Here resolute leadership from the centre is essential.

Already, this lecture has excited much comment. Questions have been asked in Parliament (see p. 127 of this issue). In the *New Scientist* of June 1, Prof. C. A. Coulson and Dr. J. M. Hammersley have directed attention to this bottleneck in British science and technology. They note the success of the National Science Foundation of the United States, set up by the Federal Government in 1950, to provide courses for the further training of practising teachers, and to establish groups charged with the revision of the school curriculum and with writing of new text-books. In particular, they stress the value of the summer institutes, which provide genuine refresher and training courses lasting 6 or 8 weeks. Letters from leading mathematicians to *The Times* have welcomed some of Prof. Thwaites's proposals, have been severely critical of others, and have suggested alternatives; but none has impugned his statement of the magnitude and urgency of this problem. There is fairly general agreement that an effort must be made to force the country to understand that a shortage of mathematics teachers is not a small, sectional matter, but the symptom of a coming national catastrophe, that, in fact, we may already have passed the point of no return. Although various bodies have seen the danger and have issued warnings, none of them has so far been able to command the attention of the nation as a whole.

If the danger is great—and no one has yet suggested that it is not—then the responsibility for meeting it lies not only on the teaching and research associations, but also on the University Grants Committee, the Ministry of Education and the Ministry for Science. Action at Cabinet-level may be needed; but a fact-finding committee would be useless. There is no time for a minute inquiry into minor statistical detail, and no salvation in a report that the problem is insoluble and so may safely be left to solve itself. A small strong committee, taking for its motto Florence Nightingale's words, "Reports are not self-executive", might yet retrieve the situation.

WAVE PROPAGATION AND GROUP VELOCITY

Wave Propagation and Group Velocity
By Léon Brillouin. (Pure and Applied Physics: a Series of Monographs, Vol. 8.) Pp. xi+154. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 6 dollars.

IT is difficult to see what purpose this book is meant to serve. Ostensibly it is to make available certain original papers which are not easily accessible because they were published in Germany in 1914. In his foreword, Dr. Brillouin says: "All these modern developments made it advisable to assemble here a systematic presentation of the original papers, which are rather difficult to find nowadays. It is hoped that the present book will be helpful to many readers and save them time and trouble, especially the trouble of recomputing and rediscovering many important features of the general theory". And again in the preface: "I discussed the solution in great detail and gave a complete answer. . . . These problems have come again into the foreground in connection with radio signals and radar. Reflection in the Heaviside layer requires a real knowledge of all these definitions (of group velocity)". But the "modern developments" referred to are in fact very old history now. Of more than sixty references given to the literature only seven are later than 1930, and of these only three are really relevant. The latest reference to propagation in the Heaviside layer is 1931.

One gets the impression that in Dr. Brillouin's view "les jeunes" have much to learn and little to teach concerning the propagation of waves, and that if they will just take the trouble to read this little book they will find all that is important about the subject. This cannot be accepted. The papers are badly written, and they do not by any means give the "complete answer" even to the limited problem with which they deal.

This is not to say that the book lacks interest. The subject is in fact a fascinating one. When a nearly monochromatic light wave with a sharp front falls on a transparent medium the disturbance that travels through the medium consists of three parts: a first forerunner, travelling with the same speed as light *in vacuo*; a second forerunner; and then the main signal, travelling with a velocity which is, except near regions of anomalous dispersion, the group velocity.

The forerunners have a very small amplitude in comparison with the main signal. The first forerunner has a very high frequency. If the incident signal rises from zero to a steady sine wave suddenly, then the frequency of the first forerunner is at first much higher than the frequency of the light, and then gets progressively less, the amplitude meanwhile increasing. But if the incident signal is not so idealized, this rapidly oscillating aspect of the first forerunner is blurred. The second forerunners are of lower frequency, comparable with the signal frequency and their onset travels with a velocity characteristic of the medium (and less than the velocity of light *in vacuo*). They then increase rapidly in amplitude as the main signal arrives, and merge with it.

These deductions from the electromagnetic theory of matter show that no signal travels faster than vacuum light velocity. The first forerunners travel with exactly that velocity. There is, of course, no mystery here. The medium is supposed to consist of electrical particles which on average are at rest before the signal arrives. When it comes, the particles take a little while to get moving, and so to modify the primary field, which, of course, is travelling with vacuum light velocity in the empty space between the electrical constituents of matter.

The book looks at this problem from several slightly different points of view, bringing forward some interesting considerations, in particular the idea of velocity of energy propagation, as distinct from signal velocity, and group velocity. These three velocities are for all practical purposes the same except in regions of anomalous dispersion, where strong absorption of the signal confuses the picture. Unfortunately, much of the value of this discussion is marred by the repetitive nature of the chapters. Had the subject been treated in a unified manner, instead of a 're-hash' of several published papers, the book could have been shorter and very much clearer. There is a deal of inelegant mathematical detail, particularly in Chapter 3, which one presumes to have been Dr. Brillouin's doctor's thesis under Sommerfeld. By contrast, Chapter 2, which is a survey of the subject by Sommerfeld himself, is a masterpiece of clarity and style. It is a pity that Dr. Brillouin did not rewrite the rest of the book nearer to this model. The final chapter is an odd one, having really very little to do with the rest of the book. It deals in a rather cursory manner with wave-guide propagation and looks strangely out of place.

The translation into English from the German and French of the originals is inept. The figures are difficult to follow because the labelling of different curves is not clearly distinguished in the legends. There are many misprints.

M. H. L. PRYCE

THE CYCLOL REVIVED

Chemical Aspects of the Structure of Small Peptides
An Introduction. By Dr. Dorothy Wrinch. Pp. viii+194. (Copenhagen: Ejnar Munksgaard, 1960.) 3.50 dollars.

REVIEWING the chemistry of the proteins in 1937, D. Jordan-Lloyd wrote "the chemistry of the proteins is a mystery no longer", and this enthusiastic overstatement was not out of touch with much informed opinion at that time. The prevailing optimism of the late 'thirties concerning our understanding of protein chemistry was, to a large extent, based on two newly postulated general hypotheses; these were the Bergmann–Niemann periodicity hypothesis and Dr. D. Wrinch's cyclol hypothesis. Although neither hypothesis was ever completely accepted, the former, indeed, being later conclusively disproved, both were of crucial significance in the development of protein chemistry, leading as they did to a renewed interest in the subject and to much important experimental work; for this reason their proponents will always occupy an honoured position in the history of protein chemistry. Very recently, something like the Bergmann–Niemann hypothesis, in much modified form, has been revived by Prof. Šorm, while, in the present book, Dr. Wrinch modifies the cyclol hypothesis and extends it to small peptides.

It is most unlikely that the views now advanced by Dr. Wrinch will command widespread acceptance; the reason for this is fundamentally semantic. Dr. Wrinch starts from the undoubted fact that in certain compounds containing amino-acid residues, such as the ergot alkaloids and the aspartyl-lysine 'dipeptide' from bacitracin *A*, these residues are not combined through simple amide linkages. She then argues that the existence of such compounds "means the repudiation of the peptide hypothesis, for if a hypothesis is subject to unpredictable exceptions, no predictions from it are reliable", and proceeds to a detailed examination of all the possible ways in which amino-acid residues might be combined, arriving finally at a generalized structural theory in which the 'cyclol' and 'amide' structures figure as special cases. Such an approach is, however, mathematical rather than chemical and will not be accepted by organic chemists. For an organic chemist, a peptide is defined as a compound made up of amino-acid residues joined head-to-tail by amide linkages, and the exceptional compounds cited by Dr. Wrinch are, quite simply, not peptides; Dr. Wrinch, on the other hand, appears to define a peptide as a compound made up of amino-acid residues, without restriction as to the manner in which these residues are joined together. In her earlier writings, Dr. Wrinch posed the question "Are proteins peptides?" and gave a possible answer to this meaningful question; now, however, she asks "Are peptides peptides?" and most organic chemists will find this a meaningless question.

In spite of these objections to her main thesis, Dr. Wrinch's new study is both significant and valuable. It is certainly possible, and some think probable, that the active centres of enzymes and other biologically active proteins involve some chemical modification of the simple peptide chain; Dr. Wrinch's systematic classification of such modifications will be of real value in stimulating further thought on this important topic. All organic chemists and biochemists interested in biologically active proteins should read this little book for, although they will probably disagree with its conclusions, they will derive from it much stimulation.

H. N. Rydon

OPERATIONS RESEARCH AND SYSTEMS ENGINEERING

Operations Research and Systems Engineering
By Charles D. Flagle, William H. Huggins and Robert H. Roy. Pp. x+889. (Baltimore, Md.: The Johns Hopkins Press; London: Oxford University Press, 1960.) 116*s*. net.

THIS book consists of a collection of the lectures given in the annual two-week course for management at the Johns Hopkins University, under the same title as the book. The lectures came from various academic departments and also the Applied Physics Laboratory and the Operations Research Office. They will be useful to the student entering on a career in operations research or industrial engineering, and also to directors, managers, and men of business generally who want to know what operations research is about, how it works, and what are its methods and achievements.

There are three parts, the first dealing with philosophical, historical, social and psychological aspects of the subjects. Some of the lectures, considering their length, are rather thinly spread with ideas of significance or importance; and it would surely be possible to say as much with fewer words. However, the lecture by Ellis A. Johnson, on "Operations Research in the World Crisis in Science and Technology", is a most important exposition and assessment of some of the great global forces which are shaping the future of mankind. The second part (more than half of the book) deals with methods and includes accounts of models, statistics, computers inventory, programming, queueing, simulation, design of experiments, information theory, system dynamics, and feed-back and stability. It must be stated that while each lecture is informative the accounts are only introductory. For example, the chapter on queueing theory gives an admirable account of what the subject is about and what it can do, but it does not go very much into mathematical methods. The third part, possibly the most valuable section of the book, is concerned with case studies. Three of these are industrial and concern the functioning of a hospital, a newspaper firm, and a telephone company. For the last-named it is the cost and value of reports which are studied. All three present thorough investigations and reveal the complex structure of practical operations research problems.

The subjects of operations research and systems engineering have developed rapidly in recent years, and the book under review demonstrates conclusively that what has been developed is not just a fashion but something much more permanent. The new way of looking at the operation of the total system, consisting of men and machines, has come to stay—at least in a society concerned with the reduction of waste, the improvement of efficiency, and progress towards a generally higher standard of living. To anyone concerned with this new point of view the book may be thoroughly recommended.

L. S. Goddard

COCKROACH ECOLOGY

The Biotic Associations of Cockroaches

By Louis M. Roth and Edwin R. Willis. (Smithsonian Miscellaneous Collections, Vol. 141, whole volume). Pp. vi+470+36 plates. (Publication 4422.) (Washington, D.C.: Smithsonian Institution, 1960.) n.p.

During the past decade the researches of Drs. Roth and Willis have contributed very greatly to our knowledge of the biology and behaviour of cockroaches, particularly those of economic importance. Not only have they published the results of much painstaking original work, but they have also brought together in the form of comprehensive compilations the fruits of the work of hundreds of other workers in this field. The present work is a compilation of this kind and its bulk is an indication of the thoroughness with which the authors have undertaken their task.

As the title of the book suggests, the authors are primarily concerned with the animals and plants associated with cockroaches; but, as a chapter of some eighty pages is devoted to the physical environment of these insects, the compilation may fairly be said to cover the whole subject of cockroach ecology. In classifying the cockroach's associates the authors begin, commendably, by defining their use of the words symbiosis, mutualism, commensalism, parasitism, and predatism. They then set out a comprehensive biological classification with page references to the account of each category in the text.

After a brief chapter on mutualism, most of the remaining plants and animals associated with cockroaches are dealt with group by group; they are classified down to the level of family and are then listed alphabetically under the generic name. The cockroaches associated with each organism are listed as natural or experimental hosts, vectors, or prey. Then follows a most useful check-list of the naturally occurring symbiotic associates of cockroaches arranged alphabetically under the cockroaches concerned, with page references to the preceding classified section of the book. The remaining few short chapters deal with such topics as commensal and predatory cockroaches, associations among cockroaches, and the biological control of cockroaches.

The bibliography covers more than eighty pages and comprises some fifteen hundred entries; it must include almost every publication having any bearing on cockroach ecology that has appeared up to the end of 1959, and certainly represents the first attempt to bring together the vast literature on this subject.

There are a few text-figures, mostly of Protozoa, and thirty-six half-tone plates showing some excellent photographs of living cockroaches and some of the more important organisms associated with them. These plates include photographs of both sexes of all the major cockroach pests and are a most valuable supplement to the book.

This compilation has achieved a very high standard and is beyond criticism in all important respects. There are, however, a few minor instances of inconsistency: English common names, for example, are given for many but not all organisms that have them, and dates—in addition to authors—are cited for the specific names of helminths, but not for those of any other group (these dates serve no useful purpose in a book of this type and might well have been universally omitted). In the Contents the page references from p. 308 onwards are all out by two pages.

It may be said in conclusion that this book can be unreservedly recommended to any worker on cockroach biology, and particularly to those concerned with the economic importance of these insects. Together with the two previous monographs by the same authors on the reproduction of cockroaches (1954) and their medical and veterinary importance (1957), it forms a most worthy contribution to the literature of cockroaches. DAVID R. RAGGE

TESTING TEXTILES

Handbook of Textile Testing and Quality Control

By Prof. Elliot B. Grover and Prof. D. S. Hamby. Pp. vi+614. (New York: Textile Book Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 132*s*.

The control techniques used in laboratories in the textile industries have increased enormously in range in recent years and become more widely and intensively applied. Despite this, text-books on the subject are much as they were ten years ago, with most students having to rely on the *British Standards Handbook* No. 11, which is not a textbook about principles but a series of sets of instructions commended for doing certain tests chosen for their reproducibility. The appearance of Grover and Hamby's book is therefore especially welcome in that it does describe up-to-date instrumental techniques in regular use in the United States.

More than a hundred pages are given to statistical methods, vital in interpreting test results on textile materials and also in the theory of yarn structure. Another hundred pages are devoted to tests on cotton fibres for length, strength, fineness and maturity and other properties. Considerable space is given to yarn tests, including the most up-to-date tests for irregularity and strength. Methods of test for fabric receive less attention, though a chapter on the evaluation of fabric defects is valuable.

The book is thus concerned with physical testing only—methods of fibre identification, or chemical analysis of blends, or estimation of damage or degree of polymerization are not mentioned, nor, oddly enough, are the highly developed methods of test for the abrasion resistance, crease resistance and water repellency of fabrics. The book is strongly oriented towards cotton, and neither wool nor synthetic fibres receive much attention, although much has been published on test methods appropriate to them.

The treatment of the selected subject-matter is thorough but elementary, perhaps too elementary in some parts. An honest attempt has been made to present some difficult concepts in simple terms—as in the chapters on statistical methods and yarn evenness—but parts of the book are taken up with instructions for operating particular instruments that would have been better left to the makers' handbooks. Whether the tests measure the right parameters, their limits of accuracy, the reproducibility of tests made on different instruments or by different operators, their relative convenience—these are matters that might have been given more space in a book on quality control. The bibliographies at the end of each chapter are generally unsatisfactory and should be thoroughly overhauled in any reprinting—some have no bibliography at all, sometimes an author is listed but with no journal or date, sometimes a reference made to unpublished work appears alone, and practically all the lists are absurdly short. The manner of presentation leads one to speculate about the kind of reader envisaged, and to the conclusion that the American executives, technical personnel and students, for whom this is written, are no better trained in science than their English counterparts. W. J. Onions

A CATALOGUE OF THE ULTRA-VIOLET

Organic Electronic Spectral Data

Vol. 1: 1946–1952. Edited by Mortimer J. Kamlet. Pp. xiv+1208. 215*s*. Vol. 2: 1953–1955. Edited by Herbert E. Ungnade. Pp. x+919. 132*s*. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.)

THE object of this work is to present all useful published data on the ultra-violet and visible absorptivity of organic compounds. The criterion of usefulness is adequacy to define the molar absorptivity for at least one stated wave-length of maximum absorption. The solvent used, or *p*H of an aqueous solvent, is also stated, if known, though the concentration is not; and literature references are given for all entries. Compounds are arranged as a molecular formula index, and the number comprised must be between twenty and thirty thousand, for many of which more than one set of data is supplied. Gas-phase measurements are included.

Critical treatment of this material, difficult in view of its great bulk, becomes nearly impossible because of the chaos of modes in which the data have originally been expressed. The editors give candid warning that, despite their best endeavours, a proportion of the figures may thus be out by a factor of ten, or of the molecular weight, or both, and that the reference to the original paper should be followed up in any case of doubt. Within this limitation, absorptions are here uniformly expressed as molar absorptivity, log ε, where ε has the value $\log(I_0/I)/cb$, c being in cm. and b in moles/l. The latter expression is now adopted as the definition of 'extinction coefficient' preferred by the Optical Data Commission of the International Union of Pure and Applied Chemistry. A statement of only two significant figures of the mantissa, however, can be appreciably less precise than the measurement it reports. Thus a figure of 4·23, signifying the range 4·225–4·234, would cover (for 0·001 per cent of a solute of molecular weight 300) measured extinctions from 0·559 to 0·571. For quantitative purposes, significance in the third figure is desirable and attainable, and it seems matter for some regret that, in cases where such precision has been recorded, it must be lost in the editorial reduction to two figures.

The search for material in more than seventy journals appears to have been extremely thorough. Other sources—to judge by the omission of, for example, amodiaquine (*British Pharmaceutical Codex*, 1954), chlorpheniramine (*U.S. Pharmacopoeia*, 1955) and the chlorosalicylanilides (*American Medical Association's Standards for New and Non-official Remedies*, 1955)—have not been drawn on.

The choice between systematic and coined names is not always made consistently; for example, "Antazoline" is used, but not "chloroquine" (*U.S. Pharmacopoeia*, 1955, and *Chemical Abstracts Index*, 1954), though the latter is an example where the coined name would make for easier reference among the seven entries under its molecular formula.

The numerals in the formulæ of Volume 2 are smaller but sharper than in Volume 1; a combination of the best qualities of both would seem ideal. That a work of such enormous scope should realize everyone's ideal in every respect, however, is scarcely conceivable; and the measure achieved here, and promised in the further volumes by which the project will be brought up to date, must claim the gratitude and respect of all users of data of this kind.

E. G. Kellett

EUROPEAN PARASITES OF MAN

Parasitologie für Ärzte

Von Prof. Otto Jírovec. Unter Mitarbeit von Dr. J. Jíra, Dr. E. Kmetý, Dr. J. Kramář, Dr. M. Petrů und Dr. B. Rosicky. Pp. xi+684. (Jena: Gustav Fischer Verlag, 1960.) 93.65 D.M.

THERE are, Dr. Jírovec says in his preface, several good books on medical parasitology; but most of these deal more especially with parasites found in man in tropical and subtropical countries and few concentrate on species found in European climates. This book, however, gives more of its space to these, the tropical and subtropical species being relatively briefly described. The emphasis throughout is on bionomics, epidemiology, diagnosis and therapy, less space being given to the clinical signs of parasitic disease.

The chief author, Dr. Jírovec, has written the general account of parasitism (Part 1), the description of parasitological techniques (Part 2) and the sections on the Protozoa (Part 3) and helminthology (Part 4). The section on the Protozoa occupies almost half the book and reflects, in considerable detail, the work of Dr. Jírovec and his colleagues, and many will be glad to have the accounts here given of parasites such as *Pneumocystis*, *Toxoplasma* and *Trichomonas vaginalis*, which are less extensively discussed in other text-books. The section on the arthropods (Part 5), on parts of which Dr. Rosický collaborated, is on the whole less evenly covered. The section on the leptospiroses was written by Dr. Kmetý, the account of *Toxoplasma* by Dr. Jírovec in collaboration with Dr. Jíra and the sections on the rickettsias, *Trichomonas vaginalis* and *Pneumocystis* were revised by Prof. Vaněk, Prof. Peter and Dr. Syrůček respectively. Other collaborators were Dr. Kramář on the Diptera and Dr. Petrů on paragonimiasis and ascariasis of the lung.

The book, which is written in clear and simple German, fully maintains the high standard of production of the house of Gustav Fischer. The 285 illustrations are all good and many of them, supplied by the author or his colleagues, are not available in other books. They include useful photographs of helminth eggs and of parasites in the host's tissues, and some of these were taken through the phase-contrast microscope. There are several illustrations in colour, and, although most of these will be helpful to the reader, some of them raise the question, also raised by coloured illustrations in other text-books, whether colour, especially when it depends on the staining techniques employed, is worth its high cost. The reproduction of all the illustrations is, no doubt, improved by the use of glazed paper throughout the book, but this also increases the weight of the volume. The bibliography is scarcely adequate for so comprehensive a book; it might have been better to have given to it the eight pages taken up by the list of equipment needed for a parasitological laboratory and the notes on the transport of diagnostic material. The book, nevertheless, strikes out a new line in the balance of its material, and anyone who has to deal with parasites encountered in European climates will find it a useful addition to his library. G. LAPAGE

EFFECTS OF SMOKING

Smoking

Its Influence on the Individual and Its Role in Social Medicine. By C. Van Proosdy. Pp. viii+319. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1960.) 57*s*. 6*d*.

EVER since its introduction into Europe at the end of the fifteenth century, tobacco-smoking has met with periodic opposition from temporal and ecclesiastical authorities, and other self-appointed guardians of public health and morals. Much of the criticism of the use of tobacco has been ill-founded and based on ignorance or prejudice, but in recent years a considerable body of scientific evidence has accumulated which indicates that tobacco-smoking may have deleterious effects on the smoker's health. The relationship between cigarette-smoking and lung cancer is now well established, and there is evidence that cigarette-smoking may also be connected with some forms of heart disease and certain other diseases. Most smokers would probably argue that smoking has beneficial effects as well, and it obviously has very strong attractions. These appear to be associated with the nicotine content of the smoke, and psychological and social factors may also be important.

In his book, Dr. Van Proosdy has brought together a great deal of the available evidence relating to the medical, psychological and social aspects of smoking. This is a very wide field with an extensive literature, and the herculean nature of Dr. Van Proosdy's labours is indicated by the bibliography, which occupies forty pages of the book. The pleasures of smoking are not entirely disregarded, but the emphasis is on its dangers and drawbacks, and nearly half the book is devoted to a discussion of "The Damage done by Tobacco to the Human Organism". This reviews the epidemiological and clinical evidence and also, where available, the experimental evidence, relating to the influence of smoking on the development of cancer of the lung, larynx and mouth, on other diseases of the respiratory tract, on diseases of the heart and blood vessels and on the digestive organs, nervous system and sexual organs. Other chapters are concerned with the psychological and pharmacological aspects of smoking and the use of tobacco as a socio-psychological phenomenon. Techniques used for investigating the effects of smoking and possible methods of making smoking less harmful are also discussed.

The coverage appears to be fairly complete, at least in the field with which I myself am most familiar, and moderately well up to date with references to results obtained up to and during the year 1959. The large amount of information given in a relatively small book makes for succinctness; the book is to be recommended as a useful work of reference on the medical, psychological and social aspects of smoking. W. CARRUTHERS

FROM BUTTONS TO BULLION

Two Hundred Precious Metal Years

A History of the Sheffield Smelting Company Limited, 1760–1960. By Ronald E. Wilson. Pp. xxii+316+54 illustrations. (London: Ernest Benn, Ltd., 1960.) 63*s*. net.

IN these days of large corporations, it is rare to find a sizeable firm where a family has maintained a big interest for 200 years. The author of this book is a great-great-great-grandson of John Read, the founder of the Sheffield Smelting Co., which has been for a long time one of the most important precious-metal smelting firms in the country. He is thus in a unique position to survey the development of the firm which reflects, in some measure, the growth of Sheffield as a metallurgical centre. While this book will interest particularly those people familiar with the Sheffield scene, it provides the general reader with an accurate account of the early days of precious-metal smelting in Hallamshire. At first, the prosperity of the infant firm rested in the skill with which gold and silver were recovered from jewellers' waste or sweep. As the Sheffield plate trade developed in the latter half of the eighteenth century Read's business flourished and already by 1783 he was selling more than 50,400 ounces of silver a year in Sheffield. In the early days the business contacts of the firm already ranged from the Royal Mint to the scores of button makers in Birmingham and Sheffield.

By the end of the century, the firm was smelting waste from many parts of the country and from Europe. There is ample evidence that dealings in precious metals were never without risk, for much depended on the skill of the assayer and the accuracy of the sample, not to mention the reliability of the Company's agents, which varied from time to time. Add to this the habit of gold and silver prices to change unpredictably, and the occasional imposition of precious-metal taxes, and one has all the necessary ingredients for an exciting commercial life. Consequently, in the nineteenth century, the fortunes of the Company waxed and waned, and disaster threatened from time to time. However, it is a measure of the resilience of the family that the firm survived to become the leading precious-metal smelters in Britain towards the end of the nineteenth century. The part of the book dealing with the eighteenth and nineteenth centuries is likely to be of greatest interest because of the many side-lights both on the social life and industrial development of the times.

While the twentieth century led to continued expansion and broadening of activities, the actions of Governments could turn fair wind into foul, as when the U.S. Treasury ceased to support the silver market in 1935. The author is perhaps more reticent about progress after the Second World War, but it is clear that since then there has been great development and that the Sheffield Smelting Co. commands a very important position among the refiners and fabricators of precious metals.

This book is an interesting historical survey against an ever-changing social and economic background, with sufficient technological information for the reader to appreciate the development of this rather exotic industry from humble beginnings.

R. W. K. Honeycombe

THE RETRIEVAL OF PUBLISHED INFORMATION

Retrieval Guide to Thermophysical Properties Research Literature

Vol. 1. Edited by Y. S. Touloukian. Book 1, Part A: Guide to Substance Classification and Numerical Codes. Book 1, Part B: Dictionary of Synonyms and Trade Names. Book 1, Part C: Directory of Substances. Pp. xii+1352. Book 2: Classified Search Index. Pp. vii+169. Book 3: Master Bibliography; Author Index. Pp. ix+372. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Co., Ltd., 1960.) £46 10*s*. 0*d*.

THIS massive work constitutes the first fruits of one particular plan to master the enormous wealth of knowledge which is the outcome of scientific research. The general problem is well known. As laboratories are multiplied and scientists grow in numbers, any use of the information they obtain is liable to become increasingly patchy, because it is becoming increasingly difficult to locate every mention of a subject in published scientific literature. Often much of the information available has, in fact, not been used because its existence was not known. In quite a few cases there is wasteful duplication of effort in planning research to obtain scientific information that is already adequately available, for the purpose in view, in published literature.

The Thermo-Physical Properties Research Centre, Purdue University, has been working since 1957 to overcome the present limitations to the use of acquired knowledge in a strictly defined and fairly narrow field of thermophysical properties of matter. These aim to be those of importance primarily to engineers, and these three books of Volume 1 are the first publication from this Centre. They deal with 10,000 references concerning the thermal conductivity, specific heat, viscosity, emissivity and related optical properties, diffusion coefficient, and Prandtl number of a long list of materials. By the time Volume 3 is published, the aim is to include linear and cubical coefficients of thermal expansion surface tension, vapour pressure and *P.V.T.* data, a group of derived thermodynamic parameters, latent heats of fusion, critical and phase transformation constants, thermo-electric constants, and electrical conductivity.

One of the main goals of this *Retrieval Guide* is to reach the ultimate in comprehensiveness of coverage of information about these thermophysical properties, part of which may never be found by merely consulting the abstracting journals. To reach this goal, a high level of financial expenditure and intellectual effort has obviously been involved. An interesting feature is that unlike other collections of similar data, this group of books develops its own method of coding substances, which needs to be mastered carefully to use the interlocking method of coding properties and of the 10,000 literature references given in Book 3 of Volume 1.

It need scarcely be stated that effective retrieval systems are becoming of increasing importance for applied and even for pure science. Before deciding just how good the present system will prove to be, one would wish to proceed experimentally, and to expose one or two working librarians to the use of this volume by comparison with other methods of retrieving desired information from scientific literature.

Sampling suggests that the present publication is likely to prove most useful and to repay handsomely its initial cost for central libraries that deal with many inquiries. For smaller libraries, appropriate, if clumsier, books of tables of physical properties are likely to remain more acceptable for some time to come, even though they suffer from the grave defect of lack of comprehensiveness. The standing of the Research Centre is a warrant for the continuation of this series in further volumes. Their production is of high quality, and the series should prove itself to be increasingly indispensable as more volumes increase the range and dates of publication of the information covered. A. R. Ubbelohde

DICTIONARY OF THE FUNGI

Ainsworth and Bisby's Dictionary of the Fungi

Fifth edition by Dr. G. C. Ainsworth. Pp. viii+547. (Kew, Surrey: Commonwealth Mycological Institute, 1961.) 30*s*.

AINSWORTH and Bisby's *Dictionary of the Fungi* has been regarded as a major work of scholarship since the publication of the first edition in 1943. It has earned for itself a place on the bookshelves of all interested in the study of fungi, and so the appearance of the fifth edition is warmly welcomed by mycologists and plant pathologists alike.

The new edition resembles closely the previous one. In the dictionary proper, which occupies 440 pages, the information is organized under headings arranged alphabetically on the lines of Willis's *Dictionary of the Flowering Plants and Ferns*. More than 5,000 corrections and alterations have been made to the text of the fourth edition, and much new material has been added. The inclusion of 500 more generic names than in the fourth edition ensures that most genera included in the *Index of Fungi* up to the end of 1960 are to be found in the *Dictionary*. The taxonomic position of each genus is indicated, together with the number of species in a genus and their distribution. Full attention is paid to synonyms. Excellent short accounts are provided of the chief families, orders and classes of Fungi and of bacteria and lichens. Definitions are given of many terms used in mycology and plant pathology, while the general articles on such subjects as collection and preservation of fungi, the genetics, nutrition, and phylogeny of fungi are valuable. Throughout the text there are numerous references to appropriate literature.

Following the alphabetical dictionary, 55 pages are devoted to listing the genera of fungi by classes, orders and sometimes by families. A revised key to the families of fungi by G. W. Martin occupies 21 pages. A new set of drawings illustrating fungal structure and terminology is provided.

The *Dictionary of Fungi* may be regarded as a mine of carefully compiled and well-presented information, being undoubtedly one of the landmarks of mycology. The new edition is good value at the price of thirty shillings. John Colhoun

The Major Achievements of Science

By A. E. E. McKenzie. Vol. 1. Pp. xvi+368+24 plates. 30*s*. net. Vol. 2: Selections from the Literature. Pp. xii+195. 17*s*. 6*d*. net. (Cambridge: At the University Press, 1960.)

IN the first of these two volumes the author attempts to describe, in twenty-four chapters, the progress made in various branches of science during the past four centuries. The first chapter introduces the subject with an outline of the development of science and technology from the Stone Age to the Renaissance; and four chapters, appropriately placed, give some account of the state of philosophy in each century from the seventeenth to the present, showing how the changes in outlook are related to advances in science. Of the remaining chapters, more than half are devoted to the physical sciences; chemistry occupies two chapters, geology one, and some aspects of biology, including medicine, five. Neither botany nor zoology is mentioned in the index. Chapter twenty-four is philosophical rather than historical.

This work is intended primarily as an introduction to science for students of the humanities, either in the sixth form at school, or at universities, and is an expression of the belief of its author that such an introduction is best effected by the historical approach. In furtherance of this object, the second volume consists largely of selected extracts from scientific literature that are historically significant, and it supplements, chapter by chapter, the text of volume 1. Again some philosophy is included. Most of the extracts are quite short, and have been chosen so that they offer no technical difficulties to the non-scientific reader. All are in English, many of the translations having been supplied by the author, Mr. A. E. E. McKenzie. As seems usual in modern works on the history of science, and is perhaps inevitable in the 'space age', there is a bias towards the physical rather than the biological aspects of science. With this limitation, and provided it is realized that, though covering a wide field, the work is not comprehensive, it can certainly be recommended as likely to serve its purpose. The book is well produced, and contains numerous attractive illustrations.

J. M. EYLES

Principles of Human Genetics

By Curt Stern. Second edition. Pp. x+753. (San Francisco and London: W. H. Freeman and Company, 1960.) 68*s*.

PRINCIPLES OF HUMAN GENETICS possesses all the attributes of the perfect text-book: a wealth of accurate, up-to-date, well-illustrated information evenly covering every aspect of the subject presented in a logical, intelligible and interesting way. The form of the first edition is retained in the second, but the book has been extensively rewritten to include the enormous advances that have been made during the past twelve years. These have particularly necessitated considerable revision of the sections dealing with linkage, sex determination, biochemical genetics, mutation and the role of selection, and completely new chapters are now included on polymorphism and the genetic hazards of radiation. True to its title the book is primarily concerned with principles and all the detail is presented in exemplifying these. Particularly meritorious is the way that Stern has managed to explain simply the basis to the mathematical and statistical procedures that are necessary in human genetics.

The recent studies of human populations have spectacularly elucidated many general genetic principles. This is, no doubt, why Stern's consideration of population genetics seems especially brilliant, but it would be unjust to single out any one topic for particular praise if by doing so it is implied that others are not as well treated as they could be. The only adverse criticism that might reasonably be made is that polygenic inheritance is not considered so comprehensively as the other topics, for no systematic attention is given to the statistical methods of analysing quantitative variation such as have been used by S. B. Holt in her rigorous study of dermatoglyphic patterns. However, in all, it is confidently predicted that *Principles of Human Genetics* will long be indispensable to those who study human biology, and many will be permanently indebted to the author of this magnificent book.

G. AINSWORTH HARRISON

Ovum Humanum

Growth, Maturation, Nourishment, Fertilization and Early Development. By Prof. Landrum B. Shettles. Pp. viii+78 (64 figures). (New York: Hafner Publishing Company, Inc., 1960.) 55*s*.

INTEREST in the development of the mammalian embryology dates from the dawn of medical science, and there was much speculation as to the origin of embryos. It was not, however, until 1827 that Von Baer discovered the mammalian ovum. More than one hundred years passed before a human ovum was recovered from the uterine tube by Allen *et al.* (1930). Once the mammalian ovum had been recognized the science of mammalian embryology was established on a sound basis.

The author of the present atlas is to be congratulated for giving us a pictorial presentation in sixty-four superb photomicrographs of the development, growth, maturation, ovulation, fertilization and cleavage of the human ovum.

In the captions of different illustrations the following topics, among many, are discussed and illustrated: the enzymatic action of the tubal mucosa on the corona radiata cells, the movements of the vitellus within the zona pellucida, the presence of two primary oocytes within a single follicle and its possible bearing in twinning, and the nourishment of the ovum by the cells of the corona radiata.

The book makes a valuable contribution to our knowledge of the development of the human ovum, and it can be recommended without reserve.

W. J. HAMILTON

Mammals of the British Isles

By Philip Street. Pp. 185+32 plates. (London: Robert Hale, Ltd., 1961.) 21*s*. net.

THIS is an exceptionally lucidly written book, which will appeal to a wide public. It will provide easy and enjoyable reading for anyone wishing to improve his knowledge of the huge subject of mammals. It may, in some ways, be too straightforward for the specialist, yet even he will appreciate the interesting and well-organized treatment of fundamentals, and the fact that much of the information is based on recent research.

There are thirteen chapters to the book, an index, and fifty-six excellent photographs of mammals.

Although these are not colour plates, they are carefully produced pictures, all large enough to be detailed and revealing.

The first chapter is a general introduction to mammals, covering their evolution, and the distinctive features of all the Orders. The eight Orders found in Britain are dealt with in detail, including both wild species and alien species bred in captivity. From this chapter alone, an enormous amount can be learnt about mammals in general.

Chapters 2–12 deal, by groups, with the different species of mammals in Britain, including domestic animals, including domestic pets. Among a host of interesting information, there are accounts of origins, and of present-day efforts to preserve species that are gradually becoming extinct. The final chapter is on breeding animals for fur.

This would be an excellent book for a senior school library, or for any layman's shelf.

B. J. G. MAITLAND

A Biology of Crustacea

By Dr. J. Green. (Aspects of Zoology Series.) Pp. xv+180. (London: H. F. and G. Witherby, Ltd., 1961.) 30*s*. net.

IF the severe limitations of length are accepted, then this is an excellent book. It deals mainly with the biology of the intact animal, and it is said that the aim "is to provide a general background of biological knowledge for all who may encounter the Crustacea"; however, a background of biological knowledge is required to read this book. Although a good attempt is made to keep technicalities to a minimum, a fair number of terms are introduced without explanation.

Most chapters are over-condensed and, unfortunately, the first suffers most. This chapter endeavours to give a picture of the origin and radiation of the entire class, introducing, what, to the non-specialist, must be a bewildering array of names from sub-class to species. The account of radiation is confused by details of classification and it is suggested that the book would be much improved if the appendix (a systematic classification of the genera mentioned in the text) included a short list of the diagnostic characters of each group. Other chapters deal with feeding, growth, behaviour, blood, distribution etc., and the species used to exemplify the subjects are well chosen and from a wide geographical range. A considerable proportion of the subject-matter is taken from recent works. As might be expected, Dr. Green has drawn largely from studies of the Branchiopoda, but this certainly does not detract from the value of the book or invalidate the generalizations made. Typographical errors are few and errors of fact less.

J. A. ALLEN

Biochemistry of Steroids

By Erich Heftmann and Erich Mosettig. (Reinhold Organic Chemistry and Biochemistry Textbook Series.) Pp. xi+231. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1960.) 55*s*. net.

THE authors acknowledge the help of 28 well-known colleagues who reviewed part or all of the manuscript. It is not surprising, therefore, that this book is both well written and remarkably free from errors. Although the text occupies only 169 pages, the most important features of the biochemistry (with relevant chemical and physiological background where necessary) of cholesterol and related sterols, tetracyclic triterpenes, vitamin D, sapogenins, steroidal alkaloids, cardiac glycosides, bile acids, progesterone, corticosteroids, androgens and œstrogens are presented in a concise, readable style. At the end of the text, 774 references to recent books and reviews are appended; the headings and sub-headings to this bibliography simplify the search for more detailed accounts of selected topics. In addition, there is an extensive subject-index, which enables structural formulæ in the text to be found rapidly. Moreover, reference numbers in the bibliographic section are quoted in the subject index under the various entries, thereby facilitating a search of the literature. Honours students and those research workers who wish to acquire a working knowledge of an important and rapidly developing field with the minimum of reading will find this book very valuable. Unfortunately, the price suggests that their access to it is likely to be through the medium of a library.

D. T. ELMORE

Plant Viruses

By Dr. Kenneth M. Smith. (Methuen's Monographs on Biological Subjects.) Third edition. Pp. xii+209+8 plates. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1960.) 16*s*. 6*d*. net.

AT a time when virus research is so thriving, it is regrettable that plant viruses are so little studied in British universities. A book intended for "the student who has no previous knowledge of plant viruses" may encourage such study and is therefore very much to be welcomed, especially when its author is so experienced in plant-virus work as Dr. Kenneth Smith. But although the new edition of this little book contains more than twice as many pages as the old, one feels that it is still too small to do justice to the subject, which has grown immensely in recent years. No doubt, to limit the book's size, the author has left out many of the new lines of work which have developed in his "fourth period" in the history of virus study, starting in 1935. Thus, for example, the chemical nature of plant viruses is scarcely referred to, although the fact that all those yet sufficiently adequately studied have proved to be ribonucleoproteins is basic to any attempt to understand their stability, serological properties, multiplication, and methods of purification. Then again, the student, like myself, may not understand the techniques of X-ray crystallography in detail, but he should know more of what X-ray studies have contributed to knowledge of the structure of virus particles.

In the first part of the book are described symptomatology, modes of dissemination, electron microscopy and effects on physiology of host plants; all students of biology should read the chapter on the relation of plant viruses to their vectors. The chapters on practical methods in plant virology which form the second part cannot fail to benefit students. There is, however, little to indicate which of the methods described are of greatest practical use and which are the most reliable. Some, such as neutralization of infectivity, and anaphylactic shock, are unfashionable; some others, such as certain of the diagnostic colour tests, are unreliable.

In short, although there is plenty of good information for the student in this book, the spice of modern work might have been a little more liberally applied.

B. D. HARRISON

THE NATIONAL PHYSICAL LABORATORY

OPEN DAYS

MORE than 4,000 people visited the National Physical Laboratory during May 10–11, when items of the work of all the scientific divisions were on display. In addition to the usual number of invitations sent to industry, public and national boards and Government Departments, this year saw a greater number of visitors from the universities and colleges of advanced technology.

A new feature of the open days was the lectures given by Dr. H. Barrell on "Industrial Measurement Standards", and Dr. J. A. Pople on "Research in the Basic Physics Division", in the new lecture theatre of Glazebrook Hall.

The work of the Laboratory falls into three main categories, and examples from each of these were included in the programme for the two open days. The Laboratory has the national responsibility for establishing and maintaining the standards and units on which all measurements ultimately depend. Secondly, the Laboratory carries out basic research in the scientific disciplines covered by its nine divisions and publishes the results. Thirdly, the Laboratory assists industry by undertaking sponsored research, making tests and calibrations for fixed fees, and giving advice on problems that come within its scope.

It is impossible to describe, or even mention, all the items on display—more than 170 in all. But the three functions of the Laboratory can adequately be illustrated by a selection of exhibits covering each.

Standards

In October 1960 the eleventh General Conference of Weights and Measures adopted the metre defined in terms of the wave-length of the orange line of krypton-86. This marks the attainment of an objective towards which the National Physical Laboratory has been working since 1923. The use of metal bars as fundamental standards of length has been eliminated and another step taken towards the complete use of natural phenomena as fundamental standards in place of arbitrary material standards.

The Standards Division of the Laboratory celebrated this culmination of nearly 200 years of development of the metric unit of length by an exhibition of the "History of the Metre". Exhibits were shown beginning with the original Paris end-standard used by La Condamine in the measurement of the length of a degree at the equator and ending with the International Prototype Metre of 1875. The second group of exhibits showed some of Michelson's work, which estimated the possibility of measuring lengths of about 1 metre accurately in terms of wave-lengths of light emitted by a cadmium lamp.

The third group of exhibits showed developments since 1945 leading to the use of the orange line of krypton-86, the wave-length of which should be reproducible to an accuracy of one part in a hundred million. Recent work at the Laboratory, however, has been carried out with an accuracy approaching one in a thousand million.

The definition of the metre in terms of the orange line of krypton-86 maintains the previously accepted value of the wave-length of the cadmium red line in terms of the International Prototype Metre. (1 metre is 1 650 763·73 wave-lengths of the orange line of krypton-86.)

Industrial firms are always under pressure to meet increasing demands for greater precision in the manufacture of their products. They can be assisted in this by an increase in speed and accuracy in the calibration of standards used in engineering measurement. An exhibit was shown of recent work in the Standards Division which is effecting such improvements in the calibration of slip-gauges and angle standards. An automatic comparator is being developed in which slip-gauges are compared with reference standards by a pneumatic calliper. The calliper, automatically adjusted by a servo-system to accommodate different sizes, receives the gauges from a mechanical feed. Any known errors of the standard gauges are injected into the measuring system, so that the errors of the gauges being calibrated are shown directly on the machine. It is hoped that in the final model the results will be printed automatically. Visitors were informed that the machine should ultimately possess an accuracy of ± 1μ in. (± 0·025 μm.), and will, therefore, be able to undertake work which is at present carried out by the much slower interference techniques.

A second exhibit showed the results of an investigation into some of the factors which influence the accuracy with which angle standards in the form of polygons can be calibrated. Photo-electric auto-collimators were used for the experimental observations. An eight-sided steel polygon had been calibrated at three different establishments under normal industrial inspection-room conditions and it was found that some of the limitations imposed by commercially available equipment could be partially removed by improvements in its design and handling.

The Light Division staged a demonstration based on the illumination of prints of paintings and coloured fabrics, showing the latest developments in its work on colour-rendering or the ability of a light to give a correct presentation of colour values. This work has been made necessary by the general adoption of fluorescent and other discharge lamps, the light from which can depart considerably from the 'black-body' type of spectral distribution to which the eye is naturally adapted. A general theory of the tolerance of the eye to such departures, worked out in the Laboratory, has now been confirmed on a practical scale at the National Gallery and the Victoria and Albert Museum with the aid of numerous observers, and it will soon be possible to lay down rules for defining the effectiveness of colour rendering in lamp and lighting specifications.

Basic Research

The preliminary work on solids at extremely high pressures demonstrated last year by the Basic Physics Division has now been extended, and some very interesting results were shown. The apparatus demonstrated last year builds up pressures of the order of 100,000 atmospheres and creates, at the same time, working temperatures ranging from −200° to +2,000° C. The high pressures are obtained by squeezing, with four anvils, the focus of a tetrahedral pressure cell of ¾-in. edge-length. High temperatures are obtained by heating electrically an inner cell containing the sample; low temperatures by circulating a cryogenic liquid around the anvils. Since the latter also serve as electrical contacts, the apparatus is suitable for making electrical conductivity measurements. These have been made on inter-metallic semi-conductor compounds. For example, indium antimonide has been found to change abruptly from the semi-conducting to the metallic state at 30,000 atmospheres and room temperature, a phenomenon probably due to the melting of the compound.

In addition to studying the behaviour of known materials, ultra-high pressure techniques may be used for the synthesis of new ones. The simultaneous application of high pressures and temperatures sometimes causes a re-arrangement of atoms in the sample under pressure. When normal conditions are restored, this arrangement remains 'frozen in', thus forming a new material. The Division has synthesized, in this way, coesite from quartz and diamond from graphite, the conditions for the latter transition being 80,000 atmospheres and 2,000° C. with nickel as a catalyst. Although diamond was first synthesized in the United States six years ago, this is the first time equipment with this capability has been set up in the United Kingdom.

The same Division displayed a technique for identifying new species of radicals in irradiated materials by utilizing spectron-spin resonance. Identification is by interpretation of the hyperfine coupling pattern. Emphasis is being laid on measurement of typical coupling constants in simple cases as a means of predicting the pattern to be expected for any particular chemical species. Work has been done on γ-irradiated amino-*iso*butyric acid; evidence has been obtained for CO_2^- in irradiated sodium formate.

The Metallurgy Division demonstrated how the use of the modern electron microscope has provided a means of studying the processes that occur during the ageing of iron. It had been thought for some time that the increase in strength of alloy steels above that of pure iron was due to fine dispersion of precipitate particles; but the nature of this strengthening process could not be investigated in its entirety until the advent of the electron microscope, since the particles were below the resolution of the optical microscope (about 1/100,000 in.). Particles less than 1/100 of this size can now be examined *in situ* in the metal. The specimen to be studied is thinned by electro-polishing until it is about 1/100,000 in. thick; it is then transparent to the electron illumination of the 100-kV. electron microscope. The internal structure can now be observed, both the precipitates and the lattice defects or dislocations being seen. It is the movement of these dislocations that causes plastic flow in metals, and where they can move easily, as in a pure metal, the metal is soft. It is thought that precipitate particles strengthen the metal by acting as obstacles to dislocations. The Division expects to be able to study the details of the interaction and to extend its investigations to commercial steels where the additional elements present can affect the precipitation processes.

The Ship Hydrodynamics Laboratory at Feltham displayed some new experiments to determine the effects of entrained water for typical arrangements of ship control surfaces. In the experiments a ship

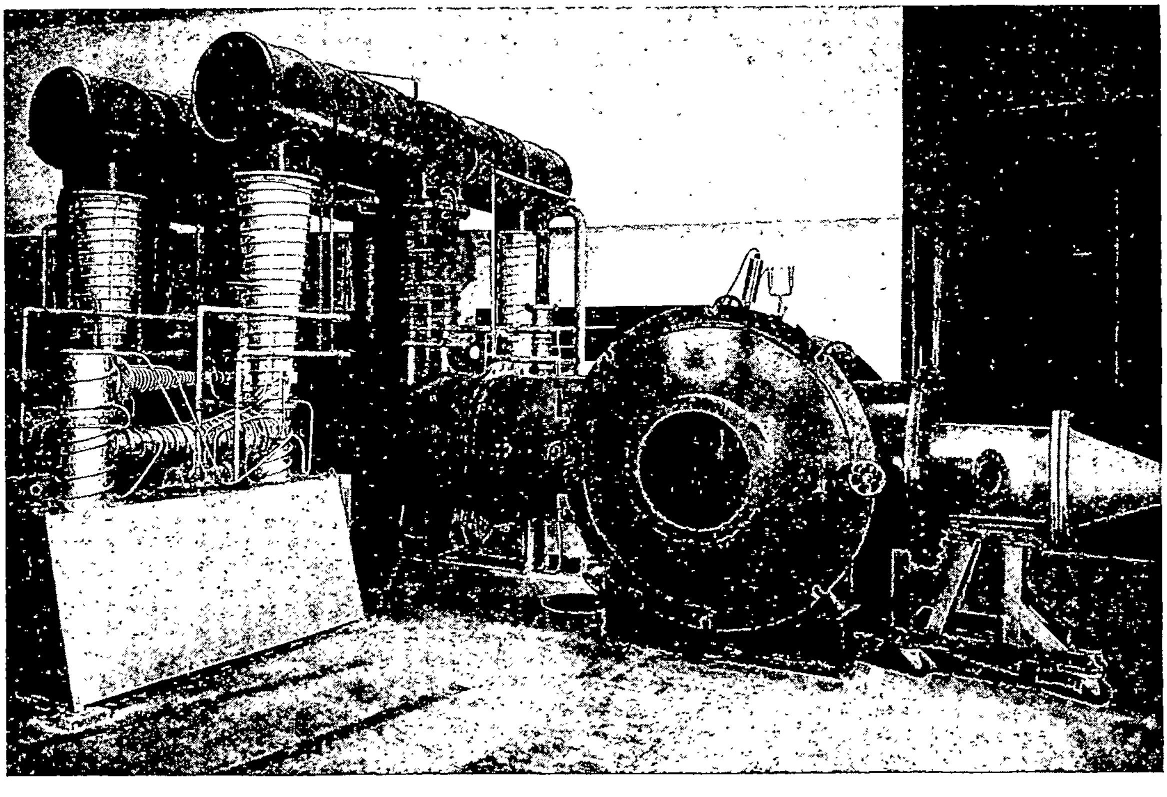


Fig. 1. New low-density tunnel of the Aerodynamics Division. Stagnation pressures, 0·0001–5 atmo.; Mach number range, 0–10
(*Crown copyright reserved*)

rudder is represented by a vertical, rigid plate capable of rotating about a vertical axis and of lateral deflexion by bending this axis. This is then vibrated in either or both of its torsional and flexural modes and measurements made to distinguish between four sets of pairs of natural frequencies, with the assembly in air and in water, uncoupled and coupled. From these measurements, the Division will be able to calculate the hydrodynamic masses and moments of inertia for conditions of restraint corresponding to those in which ship control surfaces operate.

In the Aerodynamics Division, new hypersonic shock facilities were demonstrated, including a low-density wind-tunnel and plasma jet (Fig. 1), a 'hot-shot' wind-tunnel and a magnetically driven shock tube.

Some initial experiments were shown on the noise generated by air jets. The object of the work is to investigate the basic relationship between jet turbulence and noise.

Test and Research Work for Industry

The amount of work for industry carried out by the Aerodynamics Division is steadily growing. An interesting exhibit this year was that showing the aerodynamic stability investigation for London's new micro-wave tower, which has to be sufficiently high (500 ft.) to enable transmission and reception of micro-wave signals without interruption from other tall buildings. Periodic forces set up by vortex shedding tend to cause tall slender structures of circular section to oscillate in a bending motion when exposed to a steady wind. The exceptional dimensions of the new tower and its location make considerations of aerodynamic oscillations an important factor in its design. The design engineers therefore needed a wind-tunnel investigation. The scale-model, which was more than 8 ft. high, was tested in a 13 ft. × 9 ft. tunnel; its shape, elastic stiffness and mass distribution simulated those of the final tower, so that the oscillatory behaviour of the tower could be predicted from that of the model.

The sugar industry and manufacturers of products of biochemical interest should derive great satisfaction from the recording spectropolarimeter exhibited by the Light Division. The measurement of optical rotatory dispersion through the visible and ultra-violet regions of the spectrum has applications in the analysis of sugar mixtures and other optically active compounds. Up to the present time, this technique has not been appreciated fully by industry, because of the lack of an instrument which would give accurate results over a wide range of the spectrum. The new instrument demonstrated allows light transmission at the shorter wave-lengths; its range is 600–220 mμ, but this can be extended to 200 mμ by using prisms with better ultra-violet transmission. This facility would be of great value to those wishing to study biological compounds, since many of these are expected to have informative rotatory dispersion curves in the wave-length region of 200–260 mμ.

The merging of the Test House with the Applied Physics Division in 1958 made possible simplification and rationalization of thermometer testing. The display this year demonstrated improvements which will help to meet the increasing demands of industry for the testing of a greater quantity of instruments over wide temperature-ranges and with higher accuracy. Improvements include trunking of services, centralization of marking operations, mechanization of the production of ice-shavings and modernization of the testing baths to ensure uniformity of temperature and simultaneous testing of similar instruments. One of the chief advantages of the new layout is that it will link routine calibrations more closely with the final standard of reference, which is a platinum resistance thermometer as specified in the International Practical Scale of Temperature of 1948.

The Standards Division has provided the civil engineering industry with a new instrument which was demonstrated for the first time this year. It will be known as the 'NPL Microwave Mekometer' and rapidly measures long distances, with an accuracy of a few thousandths of a centimetre, by utilizing an elliptically polarized light-beam modulated at 9300 Mc./s. The distance of a reflector from the modulator is determined in terms of the wave-length of the polarization modulation. The device should be usable in full sunshine, since it is insensitive to background illumination.

The control of complicated industrial processes by trial-and-error methods is becoming accepted by industry. The Autonomics Division demonstrated analogue equipment for the study of adaptive control systems. Two items were shown, a simultaneous three-parameter controller and high-speed analogue computing equipment.

The Mathematics Division invited visitors to discuss with members of its staff the contents of the revised edition of its manual on modern computing methods which was on display, and also any computational problems of interest to them.

J. R. ILLINGWORTH

DENSITY OF THE UPPER ATMOSPHERE DETERMINED FROM SATELLITE ORBITS, AND ITS VARIATION DURING 1957–61

By D. G. KING-HELE and MRS. D. M. C. WALKER
Royal Aircraft Establishment, Farnborough

IN the past few years a consistent and satisfactory picture of atmospheric density at heights between 180 and 700 km. has been obtained from the rate of contraction of satellite orbits[1–5]. It has been found that the main variations in density are linked with: (1) solar activity and (2) changes between day and night. In this article (which is based on ref. 6, where fuller details are given) an improved method of calculating air density is applied to the orbits of 29 satellites to obtain a picture of the air density at heights of 180–700 km., which shows the general decrease in density as sunspot activity declined between 1957 and 1961, and the large day-to-night variation at heights greater than 400 km. The influence of latitude on density is also examined.

Appearing in July

Metallurgy and Fuels

Progress in Nuclear Energy, Series V, Volume 3

Edited by H. M. FINNISTON and J. P. HOWE

Papers contained in this issue include Advances in the Physical Metallurgy of Uranium and its Alloys—H. H. CHISWIK; Irradiation induced Phase Changes in Uranium Base Alloys—M. L. BLEIBERG; The Corrosion of Plutonium and Uranium—J. T. WABER; Sources of Fuel Element Instability—A. H. WILLIS; The Assessment and Testing of Fuel Elements—H. K. HARDY and H. LAWTON. £5 5*s. net* ($15.00)

Mechanism of Action of Steroid Hormones

C. A. VILLEE and L. L. ENGEL, *Harvard Medical School*

This volume contains the proceedings of a recently held conference where the attention of participants was concentrated on the chemical and physical events which may occur at the molecular level when a steroid hormone affects some living tissue. Also dealt with at the conference were theories regarding mechanism of action of all the steroid hormones—androgens, estrogens, corticoids and progestins. 70*s. net* ($10.00)

Treatment and Disposal of Radioactive Wastes

C. B. AMPHLETT, *Chemistry Division, A.E.R.E. Harwell*

After describing the sources, characteristics, and hazards of radioactive wastes, particularly in relation to atomic energy programmes, the underlying principles of active waste treatment and disposal are discussed under the different types of waste involved. 70*s. net* ($12.00)

Report of the Commission on Enzymes

of the International Union of Biochemistry 1961

The terms of reference of the Enzyme Commission were as follows—"To consider the classification and nomenclature of enzymes and coenzymes, their units of activity and standard methods of assay, together with the symbols used in the description of enzyme kinetics"—and this volume includes chapters on Enzyme Units; Symbols of Enzyme Kinetics; The Nomenclature of Coenzymes; The Classification and Nomenclature of Cytochromes; The Classification and Nomenclature of Enzymes; The Terminology of Enzyme Formation; General Conclusions; Summary of Recommendations with 5 useful appendices including a valuable Enzyme List and Index. 50*s. net* ($7.50)

Functions of a Complex Variable

and Some of their Applications—Volume II

B. A. FUCHS and V. I. LEVIN

This book deals with the analytic theory of differential equations, the Laplace transformation and its applications, and Hurwitz's problem for polynomials. In each chapter the reader will find a large number of worked examples which are intended to serve not only as illustrations of the theoretical conclusions, but also as models for solving independently any similar problems encountered. 50*s. net* ($7.50)

A TECHNICAL WRITING SERVICE FOR INDUSTRY

Do you need the services of professional technical writers, highly competent illustrators and compilers of modern, effective technical literature? A technical writing service is available to industrial companies and research organizations to prepare, illustrate, print and disseminate instruction manuals, parts lists, user handbooks, sales brochures, internal reports, etc.

The Technical Writing Division is staffed with experienced and highly qualified technical writers, engineers and illustrators and at their disposal are all the very extensive resources and connections of the Pergamon Press.

For further information please write to:

The Manager, Pergamon Press Technical Writing Division,
4 & 5 Fitzroy Square, London, W.1

PERGAMON PRESS

OXFORD LONDON NEW YORK PARIS

Headington Hill Hall, Oxford. 4 *&* 5 *Fitzroy Sq., London, W.*1. 122 *East* 55*th St., New York* 22, *N.Y.*

Back Numbers of Periodicals Required

BIOCHEMICAL JOURNAL
Vols. 8, 21, 36, 39.

CHEMICAL SOCIETY (LONDON) JOURNAL
Years 1871, 1872, 1874, 1875/Transactions, 1876–1881, 1901/TPI of Abstracts ; 1903.

CHEMISCHE BERICHTE
Vols. 11, 12, 64–74.

JOURNAL OF EXPERIMENTAL BIOLOGY
Vols. 1–19.

JOURNAL OF PATHOLOGY AND BACTERIOLOGY
Vols. 54 No. 1 ; 56 No. 3 ; 57–59 ; 62 ; 73 ; 74.

JOURNAL OF PHARMACY AND PHARMACOLOGY
Vols. 1–5.

JOURNAL OF PHYSIOLOGY
Vols. 51–56 ; 69–71 ; No. 3, 99, No. 1 ; 121–126 ; 130 ; 131.

MINERALOGICAL MAGAZINE
Vols. 1–31.

NATURE
Vols. 74, 77, 86, 93, 99–101, 145–147, 150, 151.

ROYAL SOCIETY PROCEEDINGS, SERIES A
Vols. 1–3, 9, 10, 15, 51, 90–106, 109, 111, 122, 186–190, 193, 194, 196–199, 231.

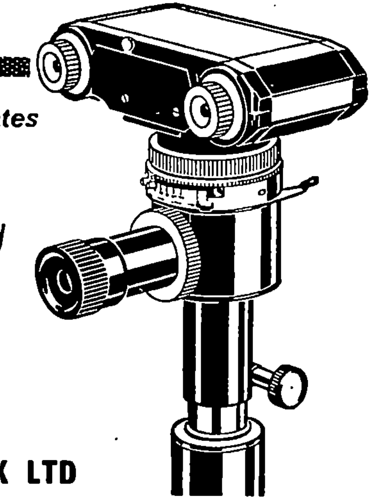
YOUR HOLDINGS OF DUPLICATES OR JOURNALS NO LONGER WANTED

We will gladly pay highest market prices for your duplicates, and if needed will send one of our own librarians to collate, pack and despatch them. Should you have any journals for disposal please telephone or write—

I. R. MAXWELL and Co. Ltd.
Headington Hill Hall, Oxford
Tel. 64881. Cables : LEXICOM. Telex 83177.

Offices also at :
103, Fifth Avenue, New York 3, Tel. Chelsea 2–2100.
Kaiserstrasse 75, Frankfurt am Main. Tel. 33.74.61.
24 rue des Ecoles, Paris Ve. Tel. ODEon 30–00.

ATTACHMENT CAMERA for PHOTOMICROGRAPHY



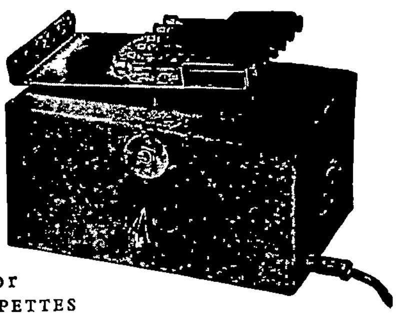
35 mm. or plates

* **Versatility**
* **Speed**
* **Quality**

R. & J. BECK LTD
69/71 MORTIMER ST · LONDON W.1
Pre-eminent for more than a century

FOUNDED IN 1869

"CRISTA" PIPETTE SHAKER
with
Interchangeable Heads



For
PIPETTES
KAHN TUBES
BOTTLES

Details on request

HAWKSLEY & SONS LIMITED
17 NEW CAVENDISH ST. LONDON WI
WELBECK 8557

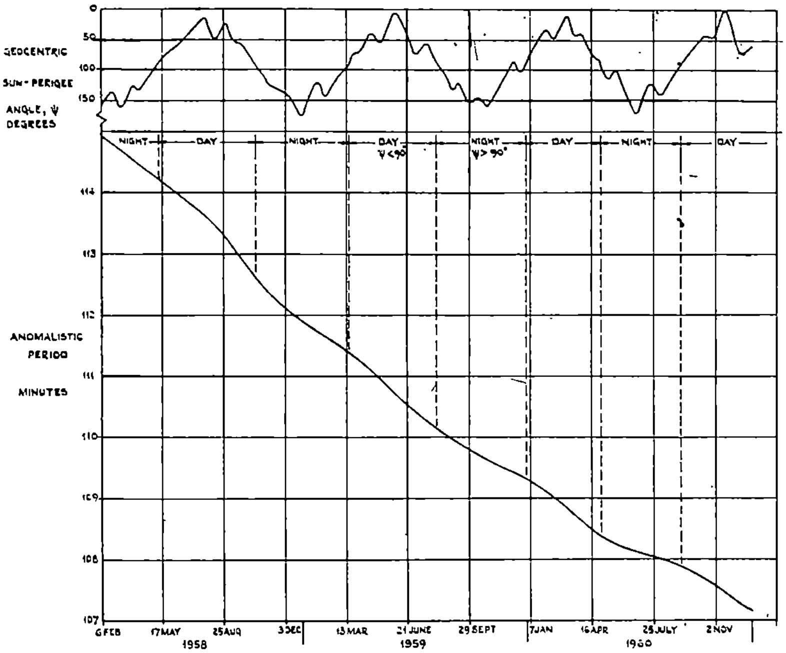


Fig. 1. Orbital period and geocentric Sun-perigee angle for *Explorer I*, 1958α

Method of Analysis

The air drag acting on a satellite rises to a maximum where it comes closest to the Earth, at the perigee, and is very small by comparison at heights more than about 100 km. above perigee. Therefore the integrated effect of air drag, which decelerates the satellite in the neighbourhood of perigee and hence causes the orbital period T to decrease, is a measure of the air density at some mean height within a band extending upwards for roughly 100 km. above perigee: and the best height at which to evaluate density is a little above that of perigee. If the air density ρ at heights above that of perigee is assumed to vary exponentially with height y above the Earth's surface, so that:

$$\rho = \rho_p \exp\left(-\frac{y - y_p}{H}\right) \qquad (1)$$

where suffix p denotes perigee and H is taken as constant, the optimum height for evaluating air density is found[6] to be $\frac{1}{2}H$ above perigee, for orbits with eccentricity e between 0·02 and 0·2. The appropriate value of H varies with height and with time, and furthermore is not known exactly. Let H^* be the best estimate of H, subject to maximum errors of perhaps 30 per cent. Then it can be shown[6,7] that the air density ρ_A at a height $\frac{1}{2}H^*$ above perigee is given, with error less than 3 per cent if H^* differs from H by a factor of less than 1·5, by the equation:

$$\rho_A = -\frac{0{\cdot}158\,\dot{T}}{\delta}\sqrt{\frac{e}{aH^*}}\left\{1 - 2e + \frac{5}{2}e^2 - \frac{H^*}{8ae}\left(1 - 10e + \frac{7H^*}{16ae}\right) + \frac{\varepsilon}{e}\sin^2 i \cos 2\omega\right\} \qquad (2)$$

if $0{\cdot}02 < e < 0{\cdot}2$. In this equation, $\dot{T}$ is the rate of change of the orbital period of the satellite, a is the semi-major axis of the orbit, ε is the ellipticity of the atmosphere (which may be taken as equal to the Earth's ellipticity, 0·00335), i is the inclination of the orbit to the equator and ω is the argument of perigee.

The quantity δ in equation (2) is given by:

$$\delta = \frac{FSC_D}{m} \qquad (3)$$

where F is a factor allowing for rotation of the atmosphere (usually lying between 0·9 and 1), S is the mean cross-sectional area of the satellite perpendicular to its direction of motion, C_D is a drag coefficient, taken[8] as 2·2 for most satellites, and m is the mass of the satellite. In calculating S, it is assumed that the satellites have rotated about an axis of maximum moment of inertia, and S is taken as the mean of the cross-sectional areas for the two extreme modes of motion, which for a satellite with large length/diameter ratio are: (1) rotating like an aeroplane propeller, and (2) tumbling end-over-end. The estimated resulting error (s.d.) in δ is[6] between 5 and 10 per cent. These assumptions are the same as in ref. 1, except that the equation for ρ_A has been improved.

If $0 < e < 0{\cdot}02$, equation (2) becomes inaccurate, and it is found best to evaluate the density ρ_B at a height $\frac{1}{4}H^*$ above perigee height. ρ_B is given[6,7] by:

$$\rho_B = -\frac{\dot{T}}{3\pi a\delta}\cdot\frac{\exp(z + c\cos 2\omega - 0{\cdot}25)}{I_0 + 2eI_1 + cI_2\cos 2\omega + \frac{1}{4}c^2 I_0} \qquad (4)$$

where $z = ae/H^*$, $c = \dfrac{\varepsilon a}{2H^*}\sin^2 i$, and I_n is the Bessel function of the first kind and imaginary argument, of order n and argument z. This equation is much less satisfactory than (2), having a maximum error not exceeding 9 per cent if H^* differs from H by a factor of less than 1·2. Fortunately, however, only 4 of the 92 values of density given in this article depend on equation (4).

Variations in Air Density

If the air density at a given height were constant from day to day, the orbital period of a satellite would decrease in a smooth and predictable manner. In practice, the rate of decrease is found to be erratic, and the irregular variations in air density thus revealed have proved to be linked with solar disturbances[9–14], the density being highest when the Sun is most active. In 1958, at sunspot maximum, the short-term fluctuations in density about its mean value from this cause sometimes amounted to ± 20 per cent at a height of 200 km. and ± 40 per cent at 700 km. This correlation also implies that there will be a long-term variation in density during the course of a sunspot cycle, a topic to be discussed in more detail later.

The variation in density between night and day is small at heights between 200 and 300 km., the density being about 10 per cent higher by day[3,10,15]. But above 300 km., this day-to-night variation is much

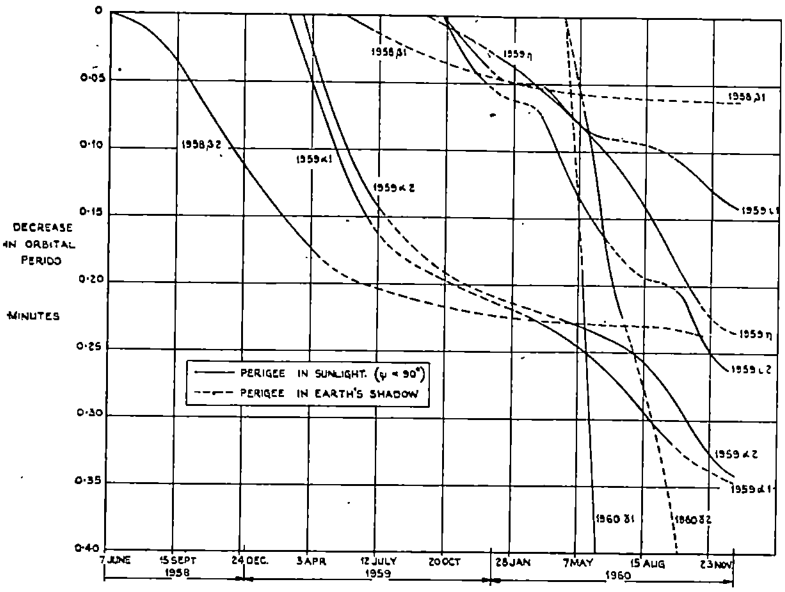


Fig. 2. Decrease in orbital period for satellites with perigee heights of 280–700 km.

greater, and is the dominant feature of the atmosphere. At a height of 600 km. the density is 5–10 times greater at its day-time maximum (attained in the afternoon) than at its night-time minimum (attained between midnight and dawn)[1-3] and consequently the temperature is several hundred degrees higher by day.

Changes in density between day and night have been clearly revealed by satellites, because their perigees move only slowly relative to the Sun: it takes at least two months and sometimes several years for perigee to accomplish a full day-to-night cycle. If therefore the orbital period of a long-lived satellite is plotted against time, the slope of the curve, $\dot{T}$, can be regarded as proportional to the air density ρ_A, since the quantities a, e, H^* and δ in equation (2) undergo little change. So day-to-night variations in density are at once apparent from the slope of the curve.

Fig. 1, which gives the orbital period of *Explorer* 1 (1958α, perigee height 350 km.), shows clearly that the slope is greatest by day and least by night. The quantity plotted at the top of Fig. 1 is the angle $\psi = SCP$ subtended at the Earth's centre C by the Sun S and the perigee point P: if ψ is greater than 90°, the point on the Earth's surface vertically beneath perigee is in darkness, and ψ equal to 90° is taken as forming a convenient boundary between 'day' and 'night'.

For orbits which are inclined to the equator at angles less than about 40°, the perigee moves eastward relative to the Sun, like a point on the Earth's surface, and experiences the normal progress from morning to evening conditions. *Explorer* 1, with inclination 33°, is in this category, and Fig. 1 thus shows that the slope of the period-versus-time curve (and hence the air density) increases only slowly during the morning, reaches a maximum some 2–3 hr. after noon, and then begins to decrease appreciably just before dusk, reaching a minimum between midnight and dawn.

The change in density between day and night has now been exhibited by nine other satellites with perigee heights between 280 km. and 700 km., and the decreases in their orbital periods are shown in Fig. 2: all the curves tend to be steeper by day (full lines) than by night (broken lines). Four of the curves, for *Explorer* 7 and its rocket (1959ι 1 and 2) and *Transit* 1*B* and rocket (1960γ 2 and 1), refer to orbits inclined at about 50° to the equator, for which the perigee moves westward relative to the Sun, going from evening to morning. The maximum slopes of these curves should therefore be attained soon after the perigee emerges into the evening sunlight. This expectation is confirmed by Fig. 2, most clearly for 1959ι2, while the slopes of the other curves, which refer to *Vanguards* 1, 2 and 3, increase much more slowly when the perigee enters the (morning) sunlight.

The decrease in density between 1958 and 1960 can also be recognized from Fig. 1. For if the density had been constant during these years, the 'average' slope of the curve in Fig. 1, with the oscillations smoothed out, would have tended to increase. In fact, it plainly decreases, thus reflecting a substantial decrease in density.

Influence of Latitude

The perigee latitude of a satellite usually varies widely, and since the value of $\dot{T}$ depends primarily on the density of the air near perigee, any strong and systematic variation with latitude would show itself in the period-versus-time curve. So far there has been no obvious sign of any such variation for the many satellites with inclinations less than 50°. For latitudes greater than 50°, evidence is so far available only for heights between 200 and 300 km., the best information being provided by *Sputnik* 3, for which the perigee moved slowly in the course of the life of the satellite from 50° N. to 65° S. and then back again to a point north of the equator. Fig. 3 shows the density of the air at a height of 235 km. as found from the orbit of Sputnik 3 by a method described in detail in ref. 6 : the error (s.d.) in the values is estimated as 7 per cent. The full line gives the calculated values: but, since some points refer to darkness and others to sunlight, a better comparison is obtained by correcting the values to conditions corresponding to the maximum day-time value of density, as shown

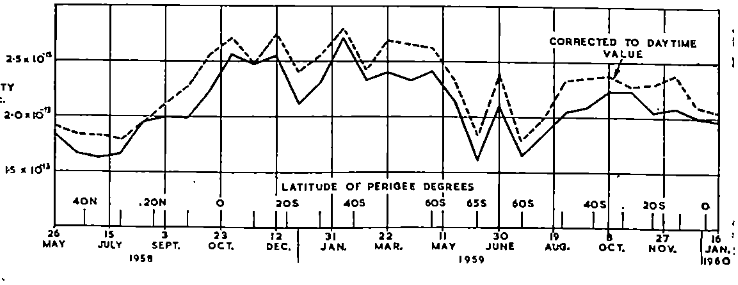


Fig. 3. Air density at height of 235 km., as given by *Sputnik* 3

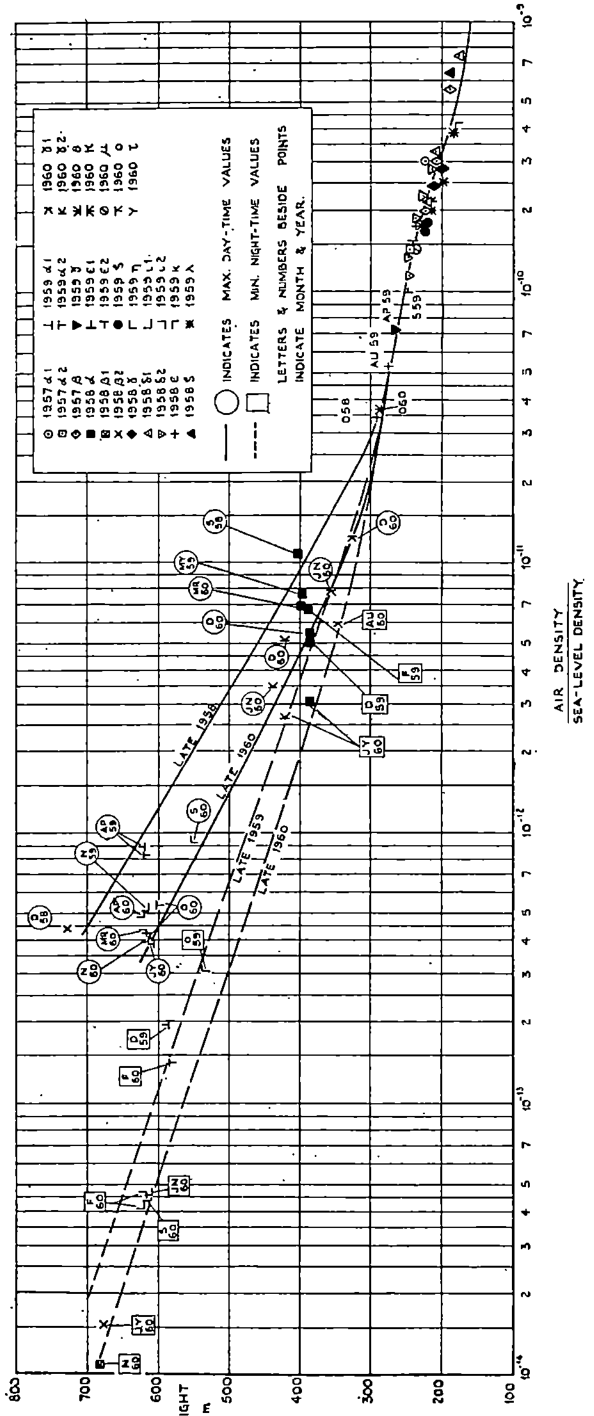


Fig. 4. Upper-atmosphere density obtained from the orbits of 29 satellites launched before January 1961

by the broken line. Because of the spread of drag about the perigee, the values of density can be regarded as referring to a band of latitude between 15° and 40° wide—the band being narrowest when perigee is at 65° S. and widest at the end when eccentricity is lowest and perigee near the equator. We can conclude from Fig. 3 that the day-time density at a height of 235 km. did not depart from its average value by a factor of more than 1·25, and most of this variation could well be ascribed to the influence of solar activity. Investigations of *Discoverer* satellites[6,15–17], with perigee latitudes up to 84°, have also revealed no sign of any significant variation of density with latitude.

Density of the Air during the Years 1957–61

In order to arrive at a general picture of the density of the upper air during the past four years, it is necessary to make due allowance for the well-established variations already discussed—the changes due to solar activity and between day and night. It is also advisable to utilize every possible satellite, so as to minimize the unknown individual bias associated with each.

Both these policies have been followed here. The curves of density-versus-height are divided according to date, that is, the particular year of the sunspot cycle, and a distinction is drawn between day- and night-time values. Also, a scrutiny was made of 66 satellites with the view of obtaining values of density: more than half these had to be rejected either because their weights and dimensions were unknown or because their orbits have not changed sufficiently. There remained 29 satellites, from which values of air density could be obtained, ranging from *Sputnik* 1 (1957α) to *Discoverer* 19 (1960τ).

The results are presented in Fig. 4, the density being expressed as a fraction of its sea-level value, taken as 0·00123 gm./c.c. The various satellites are distinguished by different symbols, and for heights above 300 km. each of the calculated points is labelled to show its date and whether it refers to day or night. The error (s.d.) in the majority of values of density, due to errors in δ and the orbital data, is estimated as about 15 per cent.

At heights less than 280 km. the points in Fig. 4 are closely packed, and a single curve has been drawn through them. Replotting of the points on a larger scale shows that they can be separated according to date, and that the density of the air at heights between 180 and 250 km. decreased by about 20 per cent between mid-1958 and mid-1960, so that the mean curve in Fig. 4 can be taken as referring to mid-1959. When, on the larger-scale diagram, curves appropriate to mid-1958 and mid-1960 were drawn, 27 of the 30 points at heights between 180 and 250 km. differed from the curve appropriate to their date by factors of less than 1·2. Thus the scatter among the points, which are quite independent, is rather less than would be expected from the estimated standard deviation of 15 per cent and, since the values refer to a wide variety of latitudes, Sun-perigee angles, seasons, etc., they confirm that there is little variation of density with any of these parameters at heights between 180 and 250 km.

At heights above 300 km. the curve in Fig. 4 divides into four parts: the two full lines indicate the maximum day-time density at the end of 1958 and of 1960; the two broken lines show the minimum night-time density at the end of 1959 and of 1960. Once again the points, although independent, fit into a consistent pattern. The curves indicate that the maximum day-time density decreased between late 1958 and late 1960 by a factor of about 2·0 at a height of 400 km. and about 2·7 at 600 km. The minimum night-time density decreased between late 1959 and late 1960 by a factor of about 1·8 at 400

Table 1. Values of Air Density and H given by Fig. 4

Height (km.)	Density (gm./c.c.)				H (km.)			
180	$6\cdot2 \times 10^{-13}$				35			
190	$4\cdot8 \times 10^{-13}$				46			
200	$3\cdot9 \times 10^{-13}$				53			
210	$3\cdot3 \times 10^{-13}$				58			
220	$2\cdot8 \times 10^{-13}$				55			
230	$2\cdot3 \times 10^{-13}$				51			
240	$1\cdot8 \times 10^{-13}$				39			
250	$1\cdot4 \times 10^{-13}$				32			
260	$9\cdot8 \times 10^{-14}$				32			
270	$7\cdot0 \times 10^{-14}$				34			
	Day		Night		Day		Night	
	Late 1958	Late 1960	Late 1959	Late 1960	Late 1958	Late 1960	Late 1959	Late 1960
300	$3\cdot6 \times 10^{-14}$	$2\cdot5 \times 10^{-14}$	$2\cdot8 \times 10^{-14}$	$2\cdot3 \times 10^{-14}$	70	46	46	39
350	$2\cdot0 \times 10^{-14}$	$1\cdot1 \times 10^{-14}$	$1\cdot1 \times 10^{-14}$	$6\cdot2 \times 10^{-15}$	94	74	57	47
400	$1\cdot2 \times 10^{-14}$	$5\cdot7 \times 10^{-15}$	$4\cdot5 \times 10^{-15}$	$2\cdot4 \times 10^{-15}$	96	81	58	52
450	$6\cdot9 \times 10^{-15}$	$3\cdot1 \times 10^{-15}$	$1\cdot9 \times 10^{-15}$	$9\cdot4 \times 10^{-16}$	97	84		53
500	$4\cdot1 \times 10^{-15}$	$1\cdot7 \times 10^{-15}$	$8\cdot1 \times 10^{-16}$	$3\cdot9 \times 10^{-16}$	98	86		
550	$2\cdot4 \times 10^{-15}$	$9\cdot6 \times 10^{-16}$	$3\cdot3 \times 10^{-16}$	$1\cdot6 \times 10^{-16}$	99	88		
600	$1\cdot5 \times 10^{-15}$	$5\cdot5 \times 10^{-16}$	$1\cdot4 \times 10^{-16}$	$6\cdot6 \times 10^{-17}$	100	90		
650	$9\cdot1 \times 10^{-16}$		$5\cdot7 \times 10^{-17}$	$2\cdot6 \times 10^{-17}$	101	92		
700	$5\cdot5 \times 10^{-16}$		$2\cdot3 \times 10^{-17}$		102	94	↓	↓

km. and about 2·1 at 600 km. In late 1960 the maximum day-time density exceeded the minimum night-time density by a factor which increased from 1·1 at 300 km. height to 2·4 at 400 km., 4·3 at 500 km. and 8·3 at 600 km.

Numerical values of air density at the heights covered by Fig. 4 are listed in Table 1, together with values of the quantity H defined in equation (1). The individual values of H are obtained from the local slope of the density-versus-height curve and are therefore sensitive to the form of the curve in Fig. 4, with a possible maximum error of up to perhaps 30 per cent. But the average values of H over a height-range of, say, 100 km. should be much more accurate than this.

The air temperature can be determined[18] from H and the molecular weight M. Unfortunately, M is not known exactly at these heights. If, as is customary, M is assumed to fall from 25 at a height of 200 km. to 16 at a height of 500 km., and then to be constant up to 700 km., the following temperatures are obtained: at heights between 180 and 280 km. the average temperature is about 1,100° K.; between 400 and 600 km. it is, by day, 1,600° K. in 1958, decreasing to 1,450° K. in 1960, and by night about 900° K. in 1959–60. The error (s.d.) in these figures is probably about 15 per cent. At heights above 400 km. the temperature shows a tendency to be constant, in good accord with the theories of Nicolet[25].

The values of density given in Fig. 4 agree well with two independent determinations, from the expansion of a sodium cloud, and from manometers in *Sputnik* 3. The sodium vapour diffusion method[19] gave a density of $(6\cdot7 \pm 2) \times 10^{-15}$ gm./c.c. at a height of 430 km. in September 1958 at a time near sunrise. The corresponding value from Table 1, extrapolated and therefore less accurate than the tabulated values, is $(6 \pm 1\cdot5) \times 10^{-15}$ gm./c.c. The results[20] from the manometers in *Sputnik* 3, for example $8\cdot7 \times 10^{-14}$ gm./c.c. at 260 km., also agree well with Table 1.

Evidence on the density at heights up to 1,600 km. has now become available, from the orbit of *Echo* 1. At these heights, however, the validity of the method and the accuracy of the results are more questionable, particularly because of uncertainty about the drag coefficient and the effects of charged drag. For *Echo* 1, too, the effect of solar radiation pressure must be taken into account. The contribution of radiation pressure to $\dot{T}$, to be subtracted from $\dot{T}$, is given[21] by:

$$\dot{T}_s = 3Fh\left(\frac{T}{2\pi a}\right)^2$$

where F is the radiation force per unit mass acting on the satellite (to be taken negative) and h is the increase in the distance of the satellite from the Sun between entering and leaving the Earth's shadow. The values of air density obtained from *Echo* by various authors[22–24] are somewhat scattered: the values they derive, for maximum day-time conditions in 1960 at 1,500 km. height, lie between 10^{-18} and 5×10^{-18} gm./c.c.

We are grateful to the Smithsonian Astrophysical Observatory and the U.S. National Space Surveillance Control Center: many of the results given in this article are derived from the orbital data issued with their satellite predictions.

[1] King-Hele, D. G., and Walker, D. M. C., *Nature*, **186**, 928 (1960).

[2] Jacchia, L. G., *J. Geophys. Res.*, **65**, 2775 (1960).

[3] Priester, W., Martin, H. A., and Kramp, K., *Nature*, **188**, 200 (1960).

[4] Kallmann, H. K., *J. Geophys. Res.*, **64**, 615 (1959).

[5] Paetzold, H. K., and Zschörner, H., paper presented at Second Intern. Space Science Symp., Florence (April 1961).

[6] King-Hele, D. G., and Walker, D. M. C., paper presented at Second Intern. Space Science Symp., Florence (April 1961).

[7] King-Hele, D. G., Cook, G. E., and Walker, D. M. C., Ministry of Aviation Report, 1960 (to be published).

[8] Cook, G. E., Ministry of Aviation A.R.C. Current Paper *C.P.* 523 (H.M.S.O., London, 1960).

[9] Nonweiler, T. R., *Nature*, **182**, 468 (1958).

[10] Jacchia, L. G., *Nature*, **183**, 526 (1959).

[11] King-Hele, D. G., and Walker, D. M. C., *Nature*, **183**, 527 (1959).

[12] Priester, W., *Naturwiss.*, **46**, 197 (1959).

[13] Jacchia, L. G., *Nature*, **183**, 1662 (1959).

[14] Jacchia, L. G., paper presented at Second Intern. Space Science Symp., Florence (April 1961).

[15] King-Hele, D. G., and Walker, D. M. C., *Nature*, **185**, 727 (1960).

[16] Landberg, S. H., *A.R.S. J.*, **31**, 155 (1961).

[17] Groves, G. V., *Proc. Roy. Soc.* (to be published).

[18] King-Hele, D. G., Chap. 1 in *Progress in the Astronautical Sciences*, 1 (North Holland Pub. Co., 1961) (to be published).

[19] Shklovskii, I. S., and Kurt, V. G., *Iskuss. Sputniki Zemli*, **3**, 66 (1959).

[20] Michnevich, V. V., Danilin, B. S., Repnev, A. I., and Sokolov, V. A., *Iskuss. Sputniki Zemli*, **3**, 84 (1959).

[21] Cook, G. E., Ministry of Aviation Report, 1961, *Geophys. J.* (to be published).

[22] Römer, M., Bonn Observatory Report No. 37 (1961).

[23] Jastrow, R., and Bryant, R., *J. Geophys. Res.*, **65**, 3512 (1960).

[24] Shapiro, I. I., and Jones, H. M., *Science*, **132**, 1484 (1960).

[25] Nicolet, M., *Planet. Space Sci.*, **5**, 1 (1961).

LIMNOLOGICAL TRENDS IN LAKE KARIBA

By D. HARDING

Joint Fisheries Research Organization, Samfya Laboratory, Northern Rhodesia

ON December 2, 1958, the great dam across the Kariba Gorge was closed and Lake Kariba began to fill. The importance of hydrological study to the future Lake had been appreciated before this event, however, and water analyses, etc., had been collected from the Zambezi River in the future dam basin since 1956[1]. This work was done by the Joint Fisheries Research Organization from its base at Samfya, and it was even at that time hoped that at least one permanent scientist would be appointed to do research work at Kariba on a full-time basis. Up to the present, however, this has not been possible owing to the lack of the necessary funds, though the importance of hydrobiological research at Kariba has been stressed by various authors[2]. Sufficient data have nevertheless been obtained by periodic visits to the Lake to form a good general picture of the trends from the time of closure, though unfortunate gaps in our records have occurred from time to time. But further work is essential to make the most of Kariba, not only as a producer of hydro-electric power but also as potentially one of the most important fishing industry centres in Central Africa. Similar schemes, also, are in various stages of progress in other parts of Africa, and Lake Kariba, which at high water will cover 1,087,000 acres and be about 175 miles long by 50 miles wide, offers a unique opportunity for research in a variety of fields, which will be of value to all concerned with the lakes so formed by these projects.

The observations from this fifth largest body of water in Africa, and largest man-made lake in the world, which are presented here, were mainly collected at a station about a mile from the dam site in Kariba Gorge, between February and December 1960. The depth of water here at a lake-level of 1,500 ft. above mean sea-level (as against an eventual height of 1,580 ft. above mean sea-level) was a little more than 70 metres. Other records have been taken elsewhere on the Lake and are mentioned briefly. As the Lake filled in 1959, the few records of temperature distribution with depth which were taken at the wall of the dam indicated that no permanent stratification had taken place by the time high-water mark for the year had been reached in July. The next available record is for November of that year, and this showed a distinct thermocline at approximately 10 m. In February 1960, this thermocline was still well established at 12 m.[3] and records of oxygen distribution in depth at several widely separated stations on the Lake indicated that only the epilimnion above the discontinuity layer contained oxygen (Fig. 1). Below this level there was no trace of oxygen even in shallow sheltered areas, although in the middle of the shallow western basin the Lake was 50 per cent saturated with oxygen down to the bottom at 15 m. As the year progressed into the cold season the epilimnion cooled and the thermocline moved gradually deeper under the stress of winds at the surface of the Lake. On May 26, 1960, the thermocline was still well established at 30 m.; the water above was homothermal at 25·3° C., and dissolved oxygen was again limited to this layer. Stratification broke down on July 6, 1960, and the Lake 'overturned', the water being homothermal at 23·4° C. Unfortunately, no oxygen records were taken at this period, but on June 28 the epilimnion was approximately 35 per cent saturated down to 36 m., when the temperature difference between surface and bottom water was less than 1° C. and the thermocline just detectable at 40 m. Shortly after the period of overturn, on August 23, when the Lake was homothermal at 22·2° C., records of dissolved oxygen showed a gradual decline from 65 per cent saturation at 4 m. to 35 per cent saturation at 51 m., and a gradual increase to 45 per cent saturation at 70 m. Similar conditions prevailed at the end of September. Warming began in late August, and by November 1, 1960, temperature conditions were similar to those of November 1959, a thermocline being well established at 12·5 m., and on December 2, 1960, dissolved oxygen was again confined to the epilimnion, which was approximately 17·5 m. deep.

Isotherms for the nine months between February and November illustrate the regime of temperature variation in Lake Kariba during 1960 (Fig. 2). A study of these isotherms indicates the presence of a layer of colder water in the deeper part of the Lake in October, which may be attributed to a source other than by direct mixing with the surface water in this area. The station is sited over the old Zambezi River channel in the gorge and is the deepest part of the Lake. Thus one would expect colder water from as far afield as the inflowing Zambezi to maintain its identity in the submerged Zambezi River channel and eventually accumulate in the deepest part of the Lake in Kariba Gorge. This

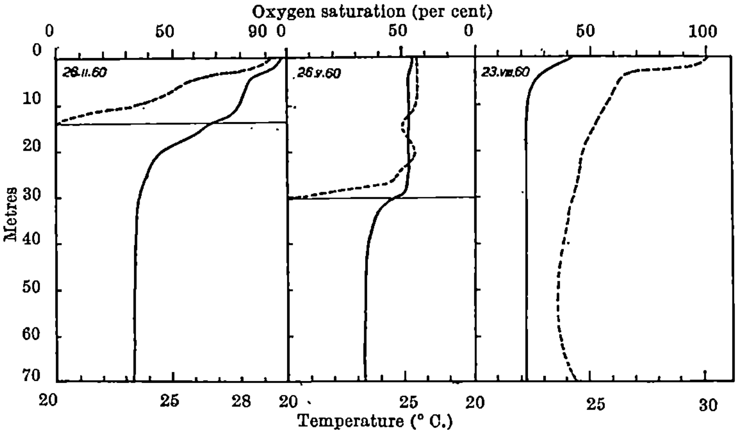


Fig. 1. Oxygen saturation (broken line) and temperature at three dates in Lake Kariba

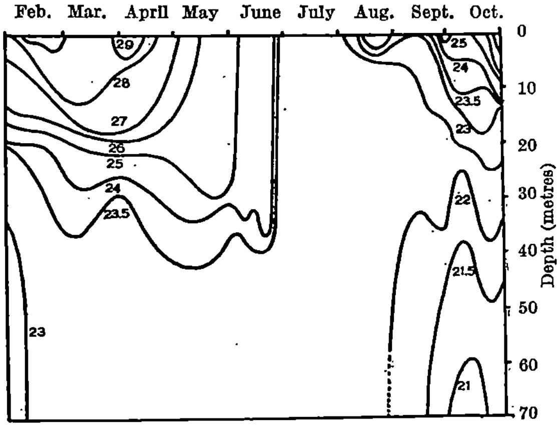


Fig. 2. Isotherms in Lake Kariba, 1960

water also differed from the overlying water mass in its higher dissolved oxygen content (Fig. 1), which tends to support this theory.

No mention of Kariba would be complete without reference to the water fern *Salvinia auriculata*, which has covered between 150 and 200 square miles of the surface of the Lake during the past two years. Apart from the mechanical effects of choking backwaters and river estuaries, and blocking access routes to cleared fishing pitches, harbour sites, and indeed the main Zambezi River channel itself, a number of side effects, such as that on the dissolved oxygen content of the Lake water, have been observed during recent work in the affected areas. Long-established patches of the fern, in sheltered backwaters and river estuaries, have prevented exchange taking place with the atmosphere, resulting in deoxygenation of the water lying beneath the floating mat of closely packed plants. The more mobile patches of *Salvinia* have no such lasting effects on the water beneath, and in the main Zambezi inflow and inflowing river estuaries, oxygen values are relatively high beneath the mat (Table 1). The effects of water movements in the open Lake also tend to maintain a circulation beneath patches of *Salvinia* bordering on the open water. Thus a transect beneath a stable patch, which has been immobilized among semi-submerged trees in the Songwe estuary, showed an oxygen gradient decreasing from a saturation value of 71 per cent in the open Lake to 37 per cent approximately two miles into the weed, then increasing once again to 69 per cent in an area of open water a mile farther on near the shore.

Table 1. Dissolved Oxygen in Lake Kariba, 1960

	Percentage saturated*	
	February	April
River Zambezi at Devil's Gorge	140	—
River Zambezi near River Masumo confluence beneath *Salvinia* mat	74	78
River Songwe estuary beneath *Salvinia* mat	62	6
River Songwe estuary 8 m. beneath *Salvinia* mat	69	2
Open lake near River Chezia estuary	93	79
Open lake at edge of *Salvinia* mat	93	66
Beneath marginal *Salvinia* mat	67	39
Beneath marginal *Salvinia* mat at 5 m.	43	24

* Oxygen saturation values corrected for an altitude of 1,500 ft. above sea-level.

Table 2. Chemical Analyses of Surface Water from Lake Kariba*

	October samples		
	1958	1959	1960
Total solids dried at 180° C.	103	74	69
Loss of weight on ignition	25	15	9
Specific conductivity at 20° C. ($\times 10^4$)	1·21	1·00	0·93
Dissolved salines deduced from conductivity	69	58	52
Total hardness as $CaCO_3$	49	45	42
Chloride	3	2	1
Sulphate	3	2·5	None
Nitrate nitrogen	None	None	None
Nitrite nitrogen	None	None	None
Ammoniacal nitrogen	0·06	0·05	0·06
Albuminoid nitrogen	0·26	0·21	0·13
Bicarbonate	72	66	62
Calcium	14	14	13
Magnesium	3·6	3·0	2·0
Total iron	None	None	None
Sodium	8	7	5
Potassium	None	None	None
Silica (SiO_2)	10	8	11
*p*H	7·3	7·2	7·2
Oxygen-absorbed test, 4 hr. at 27° C.	1·2	1·8	1·5
Main combinations:			
Calcium carbonate†	35	33	32
Magnesium carbonate†	12	10	8
Sodium carbonate†	11	10	10
Sodium sulphate	4	4	0
Sodium chloride	5	3	2
Total of above	67	60	52
Plus silica (SiO_2)	10	8	11‡

* These analyses were carried out by the Government Analyst in Salisbury, Southern Rhodesia.

† Present as bicarbonate.

‡ Results in milligrams per litre.

The implications of reduced oxygen tensions either by thermal stratification or by mats of *Salvinia auriculata* are obviously important to the fishery in Lake Kariba. On one hand, the process of stagnation of water below the thermocline, followed by an overturn, may be of general benefit to the Lake fishery, in so far as the nutrient salts released to the hypolimnion under anaerobic conditions as a result of the decomposition of drowned vegetation are made available for the growth of certain elements of the plankton and ultimately to the fish. On the other hand, the extensive oxygen-poor hypolimnion will prevent the development of the profundal fish fauna, and *seiche* movements could result in killing juveniles and breeding fish, should deep water devoid of oxygen reach the surface near the margins of the Lake. The extensive *Salvinia* mats may also exclude fish from their breeding grounds or, by deoxygenating water close in shore, result in deaths of juvenile fish. Furthermore, the position of *Salvinia auriculata* is such that salts leached from the surrounding land during a succession of annual inundations will be immediately available to the plant and this may well be the reason for the 'explosive' growth of the fern.

Conditions in Lake Kariba during 1960 simulate the cycle of events which occur in the surface layers of the deep tropical lakes Nyasa and Tanganyika. Stratification occurs during the hot season, accompanied by a depletion of oxygen below the thermocline. This is followed by a breakdown of stratification during the cold season, which results in a complete overturn in Lake Kariba; but because of their great depths and in some cases a second thermocline, only the upper levels of Lakes Nyasa[4] and Tanganyika[5] are affected, the abyssal water remains isolated and reduced, and there is never a complete overturn. Furthermore, even when Lake Kariba is full, almost a third of its total volume will be replaced each year from the inflowing rivers. Thus large quantities of nutrients will be available for biological production from two sources, the inflows and the bottom deposits, and for this reason the Lake may be extremely productive. Chemical analyses (Table 2) show there has been an overall decrease in the total concentration of dissolved solids since 1958, which may be accounted for by remarkable plankton blooms, *Salvinia auriculata* and the expansion of the fish population during the past two years.

Limnological research at Kariba during 1961 will include such specific studies on the abundance of phytoplankton, the hydrology and chemistry, the ecology and abundance of fishes and the autecology and control of *Salvinia auriculata*, and will continue throughout the period of filling, which is expected to be completed in 1963/64, until the Lake enters its permanent phase of development.

---

[1] Jackson, P. B. N., *Kariba Studies: Ichthyology, the Fish of the Middle Zambezi* (Manchester University Press, 1961).

[2] Edney, E. B., *S. Afr. J. Sci.*, **55**, 255 (1959). Balinsky, B. I., and James, G. V., *S. Afr. J. Sci.*, **56**, 101 (1960). Jackson, P. B. N., *The New Scientist*, **7**, 877 (1960). Crowcroft, P., *ibid.*, **8**, 933 (1960).

[3] Jackson, P. B. N., *Proc. First Fed. Sci. Congress, Salisbury* (1960).

[4] Beauchamp, R. S. A., *J. Ecol.*, **41**, 226 (1953). Harding, D., in Jackson *et al.*, *A Report on a Fisheries Survey of Lake Nyasa* (Govt. Printer, Nyasaland) (in the press).

[5] Beauchamp, R. S. A., *Intern. Rev. Hydrobiol.*, **39**, 316 (1939). Dubois, J. Th., *Hydrobiologia*, **10**, 215 (1958).

OBITUARIES

Prof. J. H. Andrew

THE death on May 5 of Prof. John Harold Andrew, at the age of seventy-four at Ruthin Castle Hospital in North Wales, was no surprise to his friends; he had been in ill health for a number of years, and Mrs. Andrew, to whom he was devoted, had died in January. Andrew retired from the chair of metallurgy in the University of Sheffield in September 1950, having been advised medically to take this step two years before the end of his statutory period of office; he was made emeritus professor. For reasons of health and because they loved the country, he and Mrs. Andrew retired to Llandudno.

A native of Ashton-under-Lyne, he was educated at Bickering House School, Birkdale, and the University of Manchester, where he graduated in chemistry in 1907 and was awarded the degree of D.Sc. in 1915. In 1910 he was appointed a Research Fellow and demonstrator at Manchester, where he was a Dalton Scholar. After six years as head of the Metallurgical Research Department of Sir W. G. Armstrong Whitworth and Co., Ltd., he was, in 1920, appointed professor of metallurgy at the Royal Technical College, Glasgow, succeeding the late Dr. C. H. Desch. With an established reputation in the field of metallurgy, he again succeeded Desch as professor of metallurgy and dean of the Faculty of Metallurgy at Sheffield in 1932.

Andrew was an original member of the Institute of Metals, was elected a member of the Iron and Steel Institute in 1911 and was a founder Fellow of the Institution of Metallurgists. He was a prominent member of the Heterogeneity of Steel Ingots, Alloy Steel Research and Steel Castings Research Committees of the Iron and Steel Institute, all which have now been incorporated in the committees of the British Iron and Steel Research Association.

During his thirty years as a professor at Glasgow and at Sheffield he published a very large volume of research covering extensive fields of interest. As a director of research, his fertility of mind, his unorthodox points of view and his continuing encouragement were a source of inspiration to many postgraduate students. The number of joint papers he published bears testimony to his great sense of loyalty and co-operation.

Andrew was not only well known for his research work. His administrative ability and great talent for organization brought about a striking development of the Departments of Metallurgy at Glasgow and at Sheffield. In recognition of his work in metallurgy, he was awarded in 1949 the Bessemer Gold Medal of the Iron and Steel Institute, an honour which he richly deserved. Shortly after his retirement in 1950, a group of Sheffield steel firms established the J. H. Andrew Senior Research Fellowship tenable in his old department, where a laboratory is also named after him.

To his staff, from the most senior to the humblest laboratory assistant, he was a friend who, with his sympathetic understanding and lively sense of humour, made difficulties seem trivial.

Prof. and Mrs. Andrew leave friends in Glasgow and Sheffield in all walks of life who will not forget their unfailing kindness, courtesy and generous hospitality. It was the hope that some of the happiness they had given should have been theirs in retirement; unfortunately, their retirement was sadly marred by continued ill-health. W. R. MADDOCKS

Dr. H. Horn af Rantzien

ON September 15, 1960, Dr. Henning Horn af Rantzien was killed at Montmorency, France, by a stone which slipped while, in the company of his wife and a French friend and colleague, he was examining a quarry for fossil plant remains.

Henning Horn graduated at Stockholm and, from 1945 on, held research positions in the Botany and Palæobotany Departments at the Riksmuseum, Stockholm, and later in the Geology Department of the University of Stockholm, where he taught historical geology. During 1948–51, he was an editor of *Svensk Botanisk Tidskrift*. At the time of his death, he was pursuing a long-term research programme on the taxonomy and phylogeny of the Charophyta, a work to which he had devoted most of his time during the past ten years and which had carried him, at the age of thirty-eight, to a leading position in the field of micropalæontology.

Those who knew him only as a highly specialized palæobotanist may be surprised to learn that a great part of his early writings were on recent vascular plants. He paid particular attention to the taxonomy and distribution of *Phleum*, *Pleurospermum*, and the West African and Latin American species of *Mayaca*, *Najas*, and *Triglochin*. Simultaneously, he revised the American *Nitellae* and thoroughly reviewed the literature available on all American Charophyta. In 1951, following extensive field-work, he published an ecological–geographical study of aquatic and amphibious plant associations (including bryophytes and charophytes) of the shallow lakes and temporary pools of the peculiar 'alvar' limestone barrens of the island of Öland, in the Baltic. It was in the course of this work that his interest in the charophytes became definitively established.

This broad approach did not shatter his conviction that only on a firm morphological basis can useful work be done on plant systematics and distribution. Therefore, when extending his attention to the fossil Charophyta, he ventured on a detailed description of the fructifications of modern, Cenozoic and Mesozoic forms. In doing this work he developed his own techniques for preserving and examining these minute objects. The newly recognized value of such fructifications as layer markers over a wide range of geological strata, from Upper Silurian to late Tertiary, lends special importance to the results he achieved. It is sad to reflect that this voluminous pioneer work was but the first step on a road which he was not to complete.

Privately, Henning Horn had a gift for finding pleasure in all manifestations of living Nature and in the most diverse fields of learning. He took particular interest in history and archæology, and in the life-history of land vertebrates (he was an expert herpetologist). A shyness which he never quite overcame usually caused him to avoid gatherings of more than a few people, a fact much to be regretted, for his versatile mind, coupled with a great sincerity as soon as basic facts were under discussion, made him an impressive and uncommonly stimulating partner in discussions with friends within and outside his own scientific domains. He is survived by his wife and five children.

C. EDELSTAM

NEWS and VIEWS

Royal Institution Fullerian Professorship of Physiology: Prof. R. J. Harrison

THE Managers of the Royal Institution have elected Prof. R. J. Harrison to be Fullerian professor of physiology, Royal Institution, in succession to Prof. J. Z. Young. Prof. Harrison is University professor of anatomy in the London Hospital Medical College, a post he has held since 1954. He was educated at Oundle School and Gonville and Caius College, Cambridge, and received his medical training at St. Bartholomew's Hospital Medical College. He has held appointments in the University of Glasgow and Charing Cross Hospital Medical School, and is the author of *Anatomical Terms* and *Man the Peculiar Animal*, in addition to numerous papers on embryology and anatomy. He is especially known for his research on the physiology of diving seals. The Fullerian professorship of physiology was endowed in 1834 by the gift of John Fuller, an early generous benefactor of the Royal Institution. P. M. Roget was the first holder of this office. Richard Owen, Thomas Huxley and Sir Charles Sherrington are among the other previous Fullerian professors. The appointment is normally for a period of three years.

Research on the Effects of Radioactivity in the Sea: Prof. Ilmo Hela

PROF. ILMO HELA, at present director of the Institute of Marine Research, Finland, has been appointed chief scientist in charge of the programme of research on the effects of radioactivity in the sea, to be conducted in Monaco under a trilateral agreement between the International Atomic Energy Agency, the Government of the Principality and the Institute of Oceanography. Prof. Hela is expected to take up his new appointment at the beginning of October. Prof. Hela was educated in the Universities of Helsinki, Königsberg and Hamburg. He received his Ph.D. in oceanography from the University of Helsinki in 1945; earlier he had studied physics and meteorology as well. After several years research work at the Institute of Marine Research in Finland, he joined the Marine Laboratory of the University of Miami as associate professor of oceanography and meteorology in 1951. In 1955 he returned to the Institute of Marine Research as director and professor. The programme of research in Monaco, which Prof. Hela will direct, has three major objectives. First, it will be aimed at acquiring knowledge about the movement of water and marine organisms and the deposition of organic and inorganic matter. Secondly, there will be a special study of the distribution in marine organisms of radioactive materials already existing or that may be introduced into various locations. Finally, investigations will be carried out on the effects of radioactive materials at various concentration-levels on the marine ecology. The research will be conducted at the Oceanographic Museum in Monaco, but the laboratories and working facilities of the scientific centre of the Government of the Principality, including a wide variety of electronic and monitoring equipment, will also be available.

Mathematics at Leeds: Prof. J. W. Craggs

DR. J. W. CRAGGS, reader in applied mathematics in the Newcastle division of the University of Durham, has been appointed to the newly created chair of mathematics in the University of Leeds. Dr. Craggs graduated with first class honours in mathematics at the University of Manchester and afterwards obtained the degree of doctor of philosophy both at Manchester and Cambridge. At Cambridge he held a senior studentship awarded by the Commissioners for the Royal Exhibition of 1851. Dr. Craggs has had teaching appointments at the Military College of Science, at the University of Manchester, University College, Dundee, and at King's College, Newcastle upon Tyne, University of Durham. He has been at Newcastle since 1951 and was later appointed to a senior lectureship and then to a readership. Dr. Craggs's research interests have ranged over a wide field. His first work was concerned with potential and diffusion problems and with three-dimensional elastostatics. He then moved on to compressible fluid-dynamics and more recently has made important contributions to problems of wave-propagation in elastic and plastic bodies.

The Leopoldina German Academy of Sciences: Elections

THE Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated: *Physics*, Prof. Helmut Hönl (Freiburg-Br.). *Chemistry*, Prof. T. R. Seshadri (Delhi). *Biology*, Sir Ronald A. Fisher (Cambridge). *Agriculture*, Prof. Walter Fuchs (Göttingen). *Anatomy*, Prof. Joachim Hermann Scharf (Halle). *Physiology*, Sir John C. Eccles (Canberra). *Pathology*, Prof. A. von Albertini (Zürich); Prof. Ernst Letterer (Tübingen). *Biochemistry*, Prof. Marcel Florkin (Liège); Prof. Otto Westphal (Freiburg-Br.); Prof. Edgar Lederer (Paris). *Hygiene*, Prof. Walter Liese (Berlin); Prof. Josef Tomcsik (Basle). *Stomatology*, Prof. Richard Trauner (Graz).

Scientific Societies and the Rating and Valuation Bill

At the Committee stage of the Rating and Valuation Bill in the House of Lords on June 22, Lord Brabazon of Tara, speaking as President of the Royal Institution, moved an amendment removing from the Bill the repeal of the Scientific Societies Act, 1843. Emphasizing the national importance of the scientific work which the Royal Institution had done during the past 160 years, and was still doing, he said that under the Bill the Royal Institution, which received no State funds of any kind, would be called on to find a further £3,000 a year, and scientific societies altogether would have to find £128,000 a year. Lord Brabazon indicated his willingness to withdraw the amendment if the Treasury could give any assurance that such a sum would be made available for the purpose to the Joint Committee of the Royal Society and the British Academy, and did in fact do so after hearing Lord Hailsham's reply and on the understanding that his point about the Royal Institution's Laboratory would be borne in mind on Clause 9 on the Report stage. Lord Hailsham fully agreed as to the national importance of the scientific work of the Royal Institution, mentioning also its library, but did not agree either that the Royal Institution should be treated separately or that the anomalies of the Scientific Societies Act should be perpetuated. Confirming all that he had said earlier as to the value of the work of the scientific societies and the needs of the societies, Lord Hailsham maintained that the correct course was to examine sympathetically any hardship resulting from the Act in the context of their needs.

The International Council of Scientific Unions, 1961

The Year Book of the International Council of Scientific Unions, 1961, includes, besides the calendar of arrangements for 1961, the usual details of the membership of the Executive Board, the National and Scientific Members of the Council, of the Special Committees, Joint Commission and Executive Bodies of the Permanent Services of the International Council (Pp. 110. The Hague: International Council of Scientific Unions, Paleis Noordeinde, 1961). There are also the statutes and bye-laws of the Union rules for Special Committees and their charter. The organization of the Commissions of the several Unions, the relations of the International Council with the specialized agencies of the United Nations, the alphabetical list of members of the Bureau, Executive Board, Special Committees, Joint Commission, and other organs of the Council and of officers of the Scientific Unions add further to the value of a most useful and handy work of reference, which well displays the manifold ways in which scientific work is interlocked at the international level.

Scientific Research in the U.S.S.R.

At a recent conference in Moscow attended by two thousand Soviet scientists, impressive figures were given of the Soviet research effort. Government spending on science in 1950 was 874 million (old) roubles, but by 1960 this had increased to 3,260 millions and during 1961 it is expected to reach 3,800 millions. Last year there were more than 354,000 scientific workers, about 11,000 of whom had a doctor's degree and 100,000 had a master's degree. There were 3,800 scientific establishments, including 1,500 research institutes. Academician Keldysh, president of the U.S.S.R. Academy of Sciences, outlined a new national research plan. He proposed that such a plan should be in three parts: the first to deal with economic problems at the experimental stage; the second for long-range projects aimed at solving already known problems; and the third to investigate the laws of Nature to open up new fields of progress. He described as "Task No. 1" the total electrification of the entire country. In that connexion the problem of finding new sources of power was of vital importance.

Oil Consumption in the United Kingdom during 1960

Following precedent, the Petroleum Information Bureau (on behalf of the U.K. Petroleum Industry Advisory Committee) has published recently statistics relative to oil consumption in Great Britain for 1960. (*U.K. Petroleum Industry Statistics relating to Consumption and Refinery Production 1959 and 1960*. Pp. 8. London: Petroleum Information Bureau, 1961.) The trend continues upwards, there being a 16·9 per cent increase in the total consumption of oil compared with 1959, reaching more than 42·5 million tons (excluding bunkers for ships engaged in foreign trade). Of the major oil products, fuel oil registered the greatest increase by more than 25 per cent over 1959, reaching a total of 17,437,805 tons. Gas/diesel oil deliveries were up by 13 per cent. Motor spirit consumption accounted for 7,625,184 tons, an increase of 7 per cent over the 1959 total. Diesel-engined road vehicle fuel (*DERV*) consumption rose by 13·5 per cent, a pointer to the growing demand for this commodity. As might also be expected, there was a remarkable uprise in the amount of chemical feedstock delivered, totalling 1,562,060 tons compared with 1,067,265 tons in 1959, representing a 46·4 per cent increment. This record reflects the magnitude of present-day commercial activities concerned with 'petro-chemicals' as distinct from traditional petroleum products, and at the present state of evolution of this now vital branch of the industry a commensurate increase in these 'by-products' may be anticipated confidently for 1961. There was in the year under review an increase in demand for bitumen, much of which was due to extension of the road-construction programme in so far as asphalt surfacing is concerned.

Shortage of Scientific Teachers in the United Kingdom

In answer to a question in the House of Commons on June 22, Sir David Eccles gave the number of vacancies at March 31, 1960, for teachers of mathematics in the technical colleges as 64 in an authorized establishment of 1,464; for physics the corresponding figures are 61 and 893, and for chemistry, 55 and 994. About 1,500 full-time women teachers employed in maintained grammar schools had degrees which included mathematics. Surveys made last year by the Incorporated Association of Headmasters and the

Association of Headmistresses showed that about 100 grammar, including maintained, independent and direct grant, schools had unfilled vacancies for teachers of mathematics. In addition, about 370 schools had posts which were not filled with teachers having satisfactory qualifications. About 225 graduates with at least a pass-level degree standard in mathematics out of nearly 3,100 students were at present taking the teacher's diploma in university departments of education. Of the 62 general training colleges for women, only 45 had full-time lecturers in mathematics and 14 had part-time lecturers. Five such colleges and 8 mixed colleges provided specialist courses in chemistry and physics; 6 colleges and 11 mixed colleges provided main courses with full-time lecturers in chemistry or physics; 51 women's colleges and 21 mixed colleges had no full-time lecturers in chemistry or physics.

Replying to further questions in the House of Commons on June 29 about the supply of teachers of science and mathematics, the Minister of Education, Sir David Eccles, said he believed that the universities now realized the importance of the problem. He had asked local education authorities last November to take the initiative in providing local courses for serving teachers in those subjects, such as mathematics, in which there was a shortage of well-qualified teachers. Institutes of education had been asked to collaborate closely and he would ask university extra-mural departments to consider if they could help. Industry was already lending many teachers of science and mathematics to the technical colleges. Research into methods of teaching mathematics in primary schools was being undertaken by the National Foundation for Educational Research, and close attention to this was also being given by the inspectorate, training colleges and university departments of education, teachers and local education authorities. Many local short courses for teachers being arranged resulted in setting up of study groups for informal research. Much experimental work was being carried out, and Sir David agreed to take a special interest in seeing that the results of these experiments were diffused throughout the teaching service.

Trade in European Birds for Caging

In the autumn of 1960 information was received from the chairman of the Belgian National Section of the International Council for Bird Preservation, Monsieur E. Kesteloot, of the considerable export of live wild birds to England, particularly goldfinches and serins. As outlined in the British Section's annual report for 1960 (Pp. 38+4 plates. London: International Council for Bird Preservation, British Section, c/o British Museum (Natural History), 1961. 3*s*.), these birds are exported both direct and also through Belgium from other countries. Though in Great Britain goldfinches and other birds may not be sold alive unless they are close-ringed specimens bred in captivity, there is reason to believe that this law is being evaded. In 1956–57 the Royal Society for the Protection of Birds successfully prosecuted three different dealers who had imported hawfinches and other birds from Belgium and forced close-rings on their legs. The British Section has set up a subcommittee, consisting of Guy Mountfort (chairman), Miss P. Barclay-Smith, P. J. Conder, R. H. Spencer and R. S. R. Fitter as honorary secretary, to make recommendations which will control this unfortunate practice. A request has also been made for the subject to be discussed by the Conference of the European Section of the International Council for Bird Preservation which is being held in Norway.

Science in Space

The publication of a report by the National Academy of Sciences—National Research Council, entitled *Science in Space* (Washington, D.C.: National Academy of Sciences—National Research Council, 1961), which consists of nine chapters (each published as a separate volume), has now been completed with the issue of the four remaining chapters (Chapters 1, 6, 8, 9). The report is divided as follows: Chapter 1, Dimensions and Problems: a general review; Chapter 2, The Nature of Gravitation; Chapter 3, The Earth; Chapter 4, The Moon; Chapter 5, The Planets; Chapter 6, The Sun; Chapter 7, Physics of Fields and Energetic Particles in Space; Chapter 8, Galactic and Extragalactic Astronomy, and Chapter 9, The Biological Sciences and Space Research. The aim of this report is to interest scientists working in the fields covered by the nine chapters of the report in the possibilities offered by space research. As such the report is of value since the major problems in each of the above fields are concisely discussed with reference to the relevance of research in space for their solution. In this connexion each chapter contains a short factual section relating these problems with our present knowledge of the respective field of study and a short bibliography of some of the more important recent references. However, since the background is surveyed so concisely and the outstanding problems are presented with such clarity and with the minimum of technicalities, the report could well serve as an introduction to some of the problems confronting astronomy and physics at the present time. In particular it could influence the young research worker in the choice of a fruitful field study. Each chapter (costing one dollar) can be obtained from the Publications Office of the National Academy of Sciences, 2101 Constitution Avenue N.W., Washington 25, D.C., but it is hoped to produce the report, revised and expanded, in book form published by the McGraw-Hill Book Co., Inc., New York.

British Museum (Natural History) : Recent Acquisitions

Among the more important recent acquisitions of the British Museum (Natural History) is the unique collection of British Microlepidoptera belonging to the late Mr. L. T. Ford. This collection, of upwards of 40,000 specimens, is stated to be the best of its kind in existence, its unique merit being the high proportion of material which was reared by Mr. Ford himself. Most species are represented by long series such as are indispensable for modern systematic research, and all are provided with full and accurate data. It forms the basis of Ford's published work.

Museum of Applied Science of Victoria

The report of activities of the Museum of Applied Science of Victoria for the year ended June 30, 1960, records progress in the construction of a new wing adjoining the Museum and also in the establishment of the Radiocarbon Dating Laboratory. (Pp. 23. Melbourne: Museum of Applied Science of Victoria, 1961.) The persistent difficulties with the latter work have now been overcome and the complicated equipment has functioned satisfactorily and age determinations of check specimens have been made. Several new major displays, including those illustrative of economic minerals and metals, economic botany, and the deterioration of timbers, have been set up. It should be noted that as from January 23 the name of this Museum has been changed to the Institute of Applied Science of Victoria.

Swimming Ability of Reptiles

SYSTEMATIC experiments have been carried out by R. R. Tercafs to test the swimming ability of non-aquatic reptiles (Bulletins de la Société Royale de Zoologie d'Anvers. No. 20. Pp. 19 (6 planches). Anvers: Société Royale de Zoologie d'Anvers, 1961). Among snakes, the following species have been studied: *Vipera verus, Boa canina, Thamnophis sauritus, Coronella austriaca, Chlorosoma schottii, Vipera ammodites* and *Eryx johnii.* The first two species cannot co-ordinate their movements, nor keep their heads out of water; the remaining species can swim very well, by movements similar to those they make on the ground. Some lizards (*Trachydosaurus rugosus, Tarentol mauritanica, Chamaeleo dilepis* and *Anguis fragilis*) have the same behaviour: they go forward in water by walking (thus using the same movements as on the ground). On the other hand, other species (*Lacerta viridis, Tiliqua scinoides, Lacerta muralis, Crocodilurus lacertinus, Iguana iguana* and *Tropidurus torquatus*) change their usual movements and swim in the same way as aquatic Caudata: they keep their members close to the body and go forward by waving with body and tail. The adaptation to swimming seems to be more ethological than morphological; the inability to swim of some reptiles might be explained by the lack of nervous co-ordination in response to stimuli in water; in the same way integration of these stimuli could explain the adaptation to swimming of some reptiles. Two adaptive stages exist, co-ordinated movements like the movements on the ground and swimming movements, identical with those of aquatic Caudata.

Reactions of Salt Solutions and Calcium Chloride on Mortar and Concrete

THE December issue of the *Australian Journal of Science*, 2, No. 4, December 1960) contains two significant communications to concrete specialists; one by J. A. Roberts and H. E. Vivian on "The Widening of Expansion Cracks in Mortar by Salt Solutions", the other by M. Lawrence and H. E. Vivian on "The Action of Calcium Chloride on Mortar and Concrete". In the former paper a new mechanism is described by which pre-existing cracks in mortar are enlarged and expanded by salt solutions, for example calcium nitrate and sodium silicate. Action of salt solutions tends to accelerate disintegration of concrete by opening of fine cracks therein, especially where the concrete is exposed to weathering conditions, for example roads. A conclusion is reached that concrete road-surfacings may be damaged by application of such salts as calcium chloride for the purpose of removing ice. It may be that certain aggregates may be similarly affected by salt reaction. In the second paper cited, it is shown that severe reaction, causing disruption of mortar and concrete, occurs when strong calcium chloride solution comes into contact with them. The disruption is similar to that caused by sulphate attack, but in this case it is considered to be due to formation of chloroaluminate, derived from the 'susceptible' compound in cement paste, tricalcium aluminate hydrate. It is anticipated that control of severe sulphate or chloride attack may develop from further work by employing "some method of rapidly modifying the susceptible hydration products of tricalcium aluminate and tetra-calcium alumino-ferrite to ensure that no reactable material is available after the cement sets".

U.S. National Science Foundation: Office of Antarctic Programs

AN Office of Antarctic Programs has been established within the National Science Foundation. This new Office replaces the Antarctic Research Program, formerly a part of the Foundation's Office of Special International Programs. Dr. Thomas O. Jones has been appointed head of the new Office. Dr. Albert P. Crary has been appointed chief scientist and will also serve as science adviser on Antarctica to the director, Dr. Alan T. Waterman. The Office of Antarctica Programs is responsible for the United States Antarctic Research Program, which is the name used in the field to include all U.S. scientific work carried out in Antarctica. Scientists from colleges, universities and Government agencies, working under sponsorship of the National Science Foundation, carry out research in biology, geodesy and cartography, geology, glaciology, meteorology, oceanography, upper atmosphere physics and seismology.

U.K. Courses in Nuclear Energy, 1961–63

A CATALOGUE entitled *Nuclear Studies* has been compiled by the Science Department of the British Council. This lists courses in pure and applied sciences concerning the use and development of nuclear energy which may be undertaken in the United Kingdom. These courses are full-time, and are generally of one week or more duration. The information provided by the catalogue includes notes on each of the courses and the institution at which it takes place; also the fee and the qualifications required for participation. Further information can be obtained from the Science Department, The British Council, 65 Davies Street, London, W.1.

Eleanor Roosevelt International Cancer Fellowships

THE International Union against Cancer, by means of funds made available by the Eleanor Roosevelt Cancer Foundation, will award annually fellowships for research on cancer. These are senior post-doctoral awards designed for the support of persons who, as full-time members of the staff of universities, teaching hospitals, research laboratories or other institutions, have demonstrated interest and ability or promise as independent investigators in the field of research on basic cancer, and who wish to broaden their experience by a period of study in another country. The duration of the fellowships ordinarily will be one year, but this period may be extended or shortened in special circumstances. The stipend will be based on the current salary of the applicant and the salary of persons of comparable qualifications in the place where the applicant expects to study. Application forms can be obtained from the International Union against Cancer, P.O. Box 400, Geneva 2.

Nutrition and Metabolic Defects

THE Nutrition Society is organizing a symposium on "Nutrition and Metabolic Defects", to be held at the Laboratory of Physiology, Oxford, on July 15. The programme will include the following subjects: historical aspects of inborn errors of metabolism, by Dr. H. M. Sinclair (Oxford); clinical aspects of inborn metabolic defects, by Dr. Philip Evans (London); nutrition in relation to phenylketonuria, by Dr. L. I. Woolf (Oxford); the metabolism of copper and Wilson's disease, by Prof. J. N. Cumings (London); chronic magnesium deficiency, by Dr. Paul Fourman (Cardiff); the possible role of dietary factors in the ætiology and pathogenesis of sprue, cœliac disease and idiopathic steatorrhœa, by Prof. A. C. Frazer (Birmingham). Further information can be obtained from Miss D. F. Hollingsworth, c/o Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, S.W.1.

Royal Statistical Society

At the annual general meeting of the Royal Statistical Society, Prof. M. G. Kendall was re-elected president for the ensuing year. Dr. B. Benjamin, Dr. P. Armitage and Mr. B. P. Emmett were re-elected honorary secretaries, and Mr. C. T. Saunders honorary treasurer. Prof. David R. Cox, professor of statistics in the University of London (Birkbeck College), was awarded the Society's Guy Medal in silver.

Royal Society and Nuffield Foundation Commonwealth Bursaries

Awards under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme have been made as follows:

Mr. L. G. Campbell, of the Imperial College of Tropical Agriculture, Trinidad, to enable him to study aspects of drainage and irrigation in Ontario and Alberta during July and August, 1961.

Dr. Rosalie F. Ewer, research associate, Rhodes University, to assist her to study fossil material of the early archosaur reptile *Euparkeria* at University College, London, for four months from September 1961.

Dr. K. A. Kermack, lecturer in zoology, University College, London, to enable him to examine in museums in South Africa specimens of reptiles which were the ancestors of the Mammalia and to make collections from the beds of Uppermost Triassic age in South Africa, from October 1961 for five months.

Dr. D. F. McMichael, curator of molluscs, the Australian Museum, Sydney, to assist him to spend about four months from October 1961 at the British Museum (Natural History) studying the collections of molluscs with special reference to the type specimens of Australian and New Guinea land and freshwater molluscs housed there.

Dr. W. Macnae, lecturer in zoology, University of the Witwatersrand, to enable him to spend 6–8 months from December 1961 based on the University of Queensland and studying the ecology of the *Cymodocea* fields and mangrove swamps on shores within the Great Barrier Reef and in northern Australia.

Dr. J. N. Phillips, of the Commonwealth Scientific and Industrial Research Organization (Australia) Division of Plant Industry, Canberra, to enable him to extend his physico-chemical studies on plant and animal pigments at Imperial College of Science and Technology, London, for about eleven months from December 1961.

Dr. Muriel L. Tomlinson, university demonstrator in organic chemistry, Oxford, to enable her to study polydentate compounds at the University of Sydney for about three months early in 1962.

Prof. D. Walker, professor of physics, Victoria University of Wellington, to enable him to make a study of experimental research work at present of interest to workers with low-energy Van de Graaff accelerators (or similar machines), and to study the working of the National Institute for Nuclear Science in Britain, for eight months from June 1962.

Dr. H. B. S. Womersley, reader in botany, University of Adelaide, to assist him with maintenance costs at Vancouver for three months from April 1962 while making ecological comparisons of the intertidal biology (especially marine algae) of southern Australia and the western North American coast.

Announcements

Sir Howard Florey, president of the Royal Society and professor of pathology in the University of Oxford, and Dr. Owen H. Wangensteen, chairman of the Department of Surgery, University of Minnesota, and formerly president of the American College of Surgeons, have been elected Honorary Fellows of the Royal College of Surgeons of England.

Mr. G. C. Tootill (Royal Aircraft Establishment, Farnborough) has been appointed chairman of a special committee to consider international standards work on information technology by the International Federation of Information Processing Societies.

An international conference on elementary particles will be held during September 14–20 at Aix-en-Provence, France. Further information can be obtained from the Scientific Conference Secretariat (E. W. D. Steel), CERN, Geneva, 23, Switzerland.

The Technical Section of the British Paper and Board Makers' Association is organizing a conference on "The Formation and Structure of Paper", to be held at the Dyson Perrins Laboratory, Oxford, during September 25–29. Further information can be obtained from the Technical Section, British Paper and Board Makers' Association, St. Winifred's, Welcomes Road, Kenley, Surrey.

Under the auspices of a committee appointed by the Conchological Society of Great Britain and Ireland and the Malacological Society of London, the first European Malacological Congress is to be held in London during September 17–21, 1962. The object of the Congress is to discuss all aspects of European Malacology and the formation of a European Malacological Union. The Congress is open to all interested in Malacology, and further information can be obtained from the honorary secretary, Rev. H. E. J. Biggs, 19 Siward Road, Bromley, Kent.

The Institute of Physics and the Physical Society and the British Radio Spectroscopy Group are jointly holding a two-day conference on "Radio Spectroscopy of Solids" in the Department of Physics, University College of North Wales, Bangor, during September 21–22. The Institute and Society's Guthrie Lecture will be presented during the conference by Dr. D. Shoenberg, who will speak on "The de Haas–van Alphen Effect and the Electronic Structure of Metals". Further information can be obtained from the Administration Assistant, The Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

Errata. In the review of Prof. Fraenkel's *Abstract Set Theory* (*Nature*, June 17, p. 1047) the publisher was given as Elsevier Publishing Company, of Amsterdam. This should have read North-Holland Publishing Company, Amsterdam.

In the report on the Arthur D. Little Research Institute (*Nature*, 190, 1068; 1961), five lines from the end, for 'acid' read 'non-acid'.

SHORTAGE OF MATHEMATICS TEACHERS IN BRITAIN

A QUESTION was asked by Lord Boothby in the House of Lords as to whether, having regard to the essential part played by school mathematics in the training of scientists and technologists, and in view of the present shortage of more than 3,000 mathematical teachers in British schools and the fact that replacements were not matching retirements, the Government now proposes to take action to avert the incipient disintegration of technological training in Britain.

In replying on June 7, Lord Hailsham said that the Government has already taken action to meet the serious shortage of mathematics teachers in the schools, which it recognizes to be a matter of serious concern. He did not accept Lord Boothby's conclusion. The grave shortage of mathematics graduates able and willing to teach has been made known to the university authorities, and consultations are taking place with them about how the problem could best be tackled. The Minister of Education has also taken measures to improve the supply of non-graduate teachers of this subject.

Lord Boothby then referred to action taken by the National Science Foundation in the United States, of which full details had been given to Lord Hailsham, and to the recommendations of the Mathematics Conference at Southampton last April; but Lord Hailsham insisted that the core of the problem is the provision of graduates and that this is the province of the universities. As regards non-graduate teachers, the provision of a three-year as distinct from a two-year course has enabled the Minister of Education to raise the standard in teacher-training colleges. Lord Hailsham promised to note Lord Boothby's suggestion about better co-ordination between the Ministry of Education, his own Ministry, the University Grants Committee and the universities. He believes that there are difficulties in Baroness Summerskill's suggestion of a special financial premium for those teaching science and mathematics, and in reply to Lord Alexander of Hillsborough said that the anxiety of parents in relation to the present teaching of mathematics is not well founded. One factor in the shortage is the whole range of new opportunities now open to mathematics graduates. What is really wanted is an increase in the total output of graduates, and he is not satisfied we have as yet exhausted the potentialities of mathematical ability. He said that personally he is not satisfied that the methods of teaching mathematics could not be improved, so as to attract some who had hitherto been repelled. He thought that the shortage of mathematics teachers is likely to become, and is already, very much worse than the general shortage of teachers.

In a written answer in the House of Commons on June 13, the Minister of Education said that as a result of his Department's request to training colleges to give greater prominence to mathematics, the annual number of students reading mathematics as a main subject has risen from 770 in 1955–56 to 1,650 in 1960–61. The three-year course should greatly help to raise academic standards, and the colleges should then be sending forward to the primary and secondary schools more and better teachers of mathematics. He expected the numbers to rise further with the expansion of the colleges. The colleges are also providing one-year courses for substantial numbers of serving teachers, and various other shorter courses are also available for serving teachers and members of mathematics staffs in the training colleges. Advanced courses in the technical colleges leading to a degree or diploma in technology are also being expanded. The main problem is the shortage of mathematics graduates, particularly those able and willing to teach. Mathematicians in schools and universities had been actively discussing the question and canvassing possible solutions. He said he has made sure that the gravity of the shortage and the need for action are known to the university authorities. His Department has been working for some months with the Teachers' Advisory Council, and the universities have been approached through the Vice-Chancellors' Committee. It is proposed to hold a conference at an early date under university auspices to examine the evidence collected and consider possible action, and Sir David promised to bear in mind the suggestion of a White Paper.

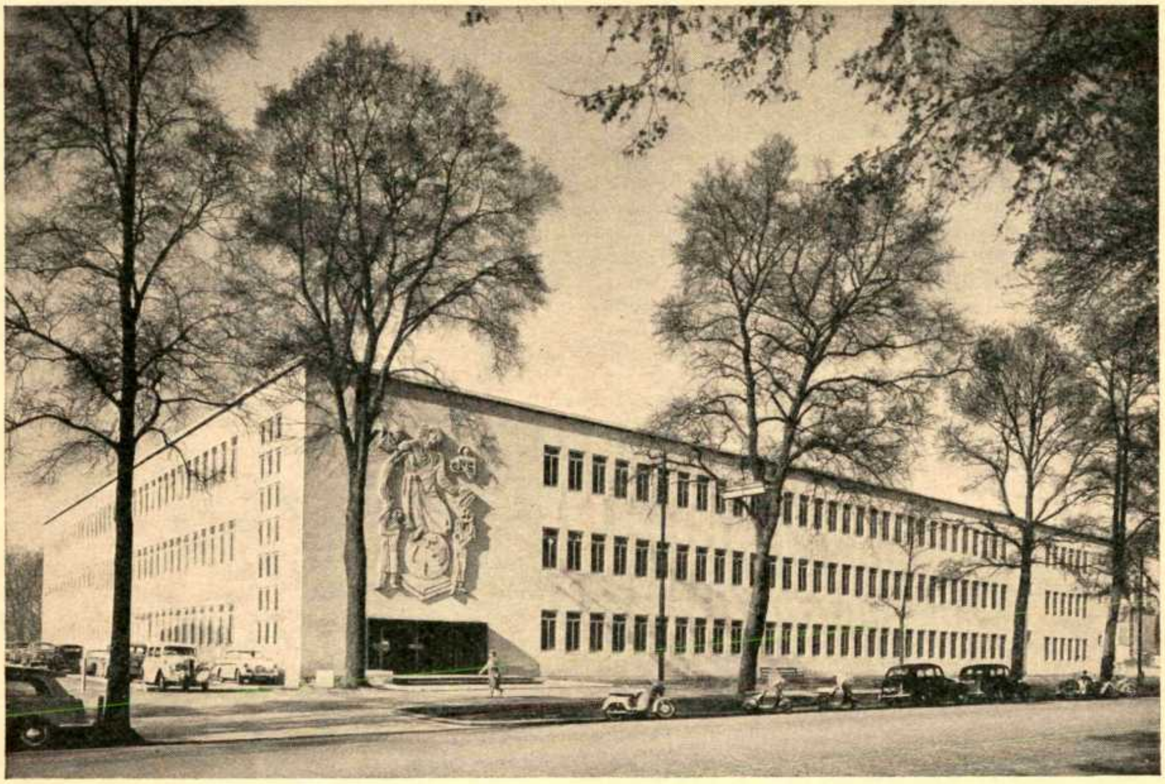
It might be noted here that in the House of Commons on June 8, in replying to a question about difficulties anticipated in recruiting scientific staff for the universities expansion programme, Sir Edward Boyle said that the increase in recent years of the number of Department of Scientific and Industrial Research postgraduate training grants—from 979 in 1958 to 1,259 in October 1960—would contribute materially to the supply of potential teachers. Besides considering representations about a further salary revision, the University Grants Committee is at present giving particular attention to the provision of adequate facilities for university staff, both for teaching and research (see also p. 103 of this issue).

THE WELSH COLLEGE OF ADVANCED TECHNOLOGY

By Dr. A. HARVEY
Principal of the College

AN additional building for the Welsh College of Advanced Technology, situated in Cathays Park, Cardiff, was formally opened by H.R.H. The Duke of Edinburgh on June 2, the ceremony forming part of the Welsh programme for Commonwealth Technical Training Week. The building re-houses the three Departments of Chemistry and Biology, Navigation and Pharmacy. It also provides much improved amenities for the College as a whole, including a refectory, great hall, senior common room and a self-contained Students' Union suite.

The architect was Sir Percy Thomas, who was also (in conjunction with Mr. Ivor Bishop) responsible for the main building of the College, which was com-



[Photo: Hylton Warner

Fig. 1. Extension of the Welsh College of Advanced Technology

pleted in 1916. The college buildings are about 100 yards apart, being separated by the Temple of Peace, for which Sir Percy was also the architect (1938). The main contractors were Messrs. J. Gerrard and Sons, Ltd., of Swinton, the cost of the building being £626,724, and that of the furniture £66,600. The equipment, most of which is now installed, is costing approximately £150,000.

The structure is steel-framed and the exterior faced with Portland stone, so as to harmonize with the other buildings in the Civic Centre. The floors were made from pre-cast concrete units and have a variety of finishes, depending on the use to which a particular room is put. It is worth noting that the use of battleship lino in the corridors has largely eliminated the noise problem often encountered. All the usual services, including compressed air, steam and vacuum, are available, housed either in ducts or above the ceilings, and the layout of the services is such that any non-laboratory type of room can, when necessary, be converted into a laboratory. A goods lift is available for heavy or bulky supplies, and there are small electrically operated hoists in the science departments. Thus bulk supplies are received on the ground-floor and distributed from there, as required, to store rooms on the upper floors.

The Department of Chemistry and Biology occupies the three floors comprising the east side of the building, and its accommodation includes ten main laboratories with ancillary balance and preparation rooms. There are four research laboratories and two lecture rooms. The specialized provisions worth noting are for polymer chemistry and technology (two laboratories, a flame-proof room and workshop for the preparation and testing of plastics and rubber products) and for work with radioisotopes (laboratory, counting room, dispensary and an underground store).

Before the War this Department was well known as a research centre and during the late 1930's eight students obtained the Ph.D. degree, four the M.Sc. degree. Now, after a rather lengthy break, research is developing once more. Work in progress at present includes a series of studies in polymer chemistry and technology dealing with rubber vulcanization, rubber mastication, molecular shapes of polypropylene glycols and viscoelastic properties of epoxide resins. Other work includes a theoretical study of counter-current extraction, electrolytic dissociation, uptake of fall-out products by lower plants and studies of vitamin C deficiency.

Because of the importance attached to the education and training of industrial chemists, special attention has been paid to instrumental analysis. A wide range of instruments, including ultra-violet and infra-red recording spectrophotometers, is housed in an instrument room, equally available for instruction and research. In addition to this a laboratory has been equipped for the ultimate analysis of organic compounds.

The Welsh School of Pharmacy occupies the whole of the three floors forming the south side of the building. It thus has available some 21,000 sq. ft. of accommodation which includes sixteen teaching laboratories, eight research laboratories, together with nineteen other rooms (lecture rooms, stores, preparation rooms, etc.). The largest lecture theatre will seat 150 and has been so sited that it can be used, in conjunction with an adjacent committee room and cloakroom, as a self-contained suite available for professional bodies and societies.

This Department is responsible for the education and training of pharmacists who may enter hospitals, industry or retail work. The College is affiliated, in respect of this work, to the University of Wales, so that students can take either the B.Pharm.(Wales) or the pharmaceutical chemist qualifying course. The Department has been designed for an annual intake of 64 students for a three-year course, but this could be increased to 96 without much difficulty.

The Department is an active research centre and is recognized by the University as a place for research for M.Pharm., while application has been made for recognition for Ph.D. There have now been seven M.Pharm. awards to members of the College.

Research at present in progress in the Department includes: physico-chemical studies on phosphatides; kinetics of the bromination of phenolic aldehydes; synthetic studies on rotenoids; synthesis of 2 : 2 dimethylchromenes which may have physiological activity; synthesis of apomorphine derivatives; synthesis of sulphones as antibacterials; properties of liquorice and its constituents, with particular reference to its value as a pharmaceutical excipient; control and prediction of particle size in ball milling; conductance of emulsions; effect of moisture on porcelain dental fillings; chromatographic techniques applied to crude vegetable drugs; pharmacology and biochemistry of substituted glutarimides; cotton dust and other vegetable dusts in relation to byssinosis; macrocyclic compounds related to steroid hormones; action of bases on chloroform; transformation of glycyrrhetic acid; changes in drug sensitivity in states of avitaminosis.

The Navigation Department occupies the bulk of the two upper floors above the Students' Union. Apart from a small amount of part-time work for yachtsmen, its function is the preparation of serving merchant navy officers for Ministry of Transport Certificates of Competency. It is exceptionally well equipped, as befits the only Navigation Department in a College of Advanced Technology. One special feature is an instrument room, which houses all the aids, electronic or otherwise, which an officer might find on the bridge of a modern merchant ship. A radar simulator (costing £12,000) is now being installed, and this will supplement the training given in the College Radar School at Cardiff docks. Another interesting feature is an extremely compact demonstration set-up, where twenty students can sit around the working bench with not one of them more than 6 ft. from the demonstration.

The communal facilities in this new building include a refectory, kitchen, etc. Above this, and extending through the two upper floors, is the Great Hall. Measuring 80 ft. × 60 ft., this hall, with its gallery, can provide seating for nearly 800. It has a large stage, fully equipped, with ancillary stores and storage space, and two dressing-rooms. A large window at the back of the gallery enables the classroom behind it to be used as a control room for radio and television programmes. Access to the building is arranged in such a way that the great hall can be used without interfering with the rest of the building. The meetings of the British Association for the Advancement of Science, 1960, saw the first use of the Hall, and a number of exhibitions have already been held in it, including one staged by the United Kingdom Atomic Energy Authority.

Finally, reference must be made to the Students' Union. This is a self-contained suite of rooms of some 5,000 sq. ft. and the accommodation includes two lounges, two recreation rooms, a council room, chapel, a music room, a photographic room, together with various offices for the conduct of Union business. Although the Union has always been a very live body, the improvement in facilities has already resulted in a marked development of student activities.

GAS-PHASE REACTIONS OF THE HALOGENS AND THEIR COMPOUNDS

An informal discussion on "Mechanisms of the Gas-Phase Reactions of the Halogens and their Compounds" was held on March 28 at University College, Cardiff, under the auspices of the Faraday Society. About seventy members and guests were present. The chairman of the first session, Prof. A. G. Evans (Cardiff), welcomed the visitors to Cardiff and introduced the foreign guests. Dr. P. G. Ashmore (Cambridge) then reported further investigations in his detailed study of the pyrolysis of nitrosyl chloride. He showed that the addition of nitrogen dioxide to nitrosyl chloride leads to a fast but rapidly inhibited decomposition. The catalysis was explained by postulating the intermediate formation of nitryl chloride, that is :

$$NOCl + NO_2 \xrightarrow{12} NO_2Cl + NO$$

$$NO_2Cl + M \xrightarrow{14} NO_2 + Cl + M$$

$$NO_2Cl + NOCl \xrightarrow{15} NO_2 + NO + Cl_2$$

The auto-inhibition was explained by reaction of the product nitric oxide with nitryl chloride :

$$NO + NO_2Cl \xrightarrow{13} NO_2 + NOCl$$

Estimates were given for k_{14} with $M = NO_2$ and N_2 and new values had been obtained for k_3 in the reaction :

$$NOCl + M \xrightarrow{3} NO + Cl + M$$

with $M = NOCl$, NO, N_2 and H_2. One surprising feature of these last results was the very small differences found for the activation energies and pre-exponential factors when M was varied.

The second paper by Prof. R. G. W. Norrish (Cambridge) gave an account of the flash photolysis of nitrosyl chloride in the presence of a large excess of inert gas to ensure isothermal conditions. Many absorption bands were produced in the region 2100–4000 Å. The conditions of the experiments were such that the light causing photolysis was mainly in the region below 2700 Å. Since the N—Cl bond in NOCl has a dissociation energy of approximately 38 kcal., this corresponds to an excess energy of 70–110 kcal. (at the limit of transmission of quartz). The absorption bands in the spectrum that follows the flash have been assigned to transitions from the ground-state of NO ($X^2\pi$) to excited states $A^2\varepsilon$, $B^2\varepsilon$, $C^2\pi$ and $D^2\varepsilon$, and it was demonstrated that all vibrational levels of the ground-state up to $v'' = 10$ were populated.

Norrish was able to show that at least 50 kcal. of the excess energy of the flash appears as vibrational energy in the nitric oxide molecule, and suggested that this occurs in the primary process, namely:

$$NOCl + h\nu \rightarrow NO^* + Cl$$

Various other possibilities were discounted on energetic or theoretical grounds.

Finally, he suggested that such a process may have a fairly wide generality, and could explain the production of many of the highly vibrationally excited molecules produced in flash photolysis.

Dr. J. H. Thomas (Cardiff) then discussed the gas-phase reactions of methyl chloride, methylene chloride and chloroform with nitrogen dioxide. He showed that the reactions were autocatalytic in nature, and led to complex oxidation products. Two of the products, hydrogen chloride and nitrosyl chloride, had marked catalytic effects when added to the initial reactants, as did the addition of chlorine. He suggested that the autocatalysis resulted from the production of chlorine atoms by reactions such as:

$$HCl + NO_2 \rightarrow Cl + HNO_2 \quad (1)$$

and

$$NOCl \rightarrow NO + Cl \quad (2)$$

followed by hydrogen abstraction by the chlorine atoms, for example:

$$CH_3Cl + Cl \rightarrow CH_2Cl + HCl \quad (3)$$

The final products were explained by a mechanism involving further reactions of the radicals produced in (3). The reactions of the three chloromethanes were compared, and it was explained that carbon tetrachloride was unreactive until much higher temperatures were reached.

In the second session, with Prof. R. G. W. Norrish in the chair, two papers on aspects of the photochlorination of chlorinated ethylenes were presented by Prof. F. S. Dainton (Leeds) and Prof. P. Goldfinger (Brussels). These reactions all involve chains initiated by chlorine atoms via :

$$A + Cl \underset{2}{\overset{1}{\rightleftharpoons}} ACl$$

where A is an olefine. The radical ACl finishes in the main as ACl$_2$ and various chain-termination steps may occur. Both groups of workers used experiments with intermittent light to find the Arrhenius parameters for the various chain steps.

Dainton has studied the olefines $C_2H_nCl_{4-n}$, $n = 0$–4, with the exception of vinylidene chloride. Reaction (1) is very exothermic for these olefines, hence the ACl radical should have considerable energy and may be regarded as an excited molecule ACl*. The Arrhenius parameters for reaction (2) should then be quite different from those for the decomposition of a normal ACl radical. This was found to be so for A = vinyl chloride, where $E_2 = 0{\cdot}7$ kcal. mole^{-1} compared with $E_2 = 22{\cdot}0$ kcal. mole^{-1} from pyrolysis studies.

Goldfinger has studied trichloroethylene to find out if reaction (1) above gives the symmetric ($\dot{C}Cl_2.CCl_2H$) or asymmetric ($CCl_3.\dot{C}HCl$) radical. The method used involved the photochlorination of trichloroethylene in the presence of either sym. or asym. tetrachloroethane (since hydrogen abstraction by chlorine atoms from the last two substances will give the sym. and asym. radicals referred to above). An analysis of the kinetics of the reactions of these mixtures showed that reaction (1) gives the sym. radical, that is :

$$CHCl = CCl_2 + Cl \rightarrow CHCl_2 - \dot{C}Cl_2$$

The activation energy for the reverse of this reaction was found to be 19 kcal. mole^{-1}, which is much larger than the value of 6·6 kcal. mole^{-1} found by Dainton for the same reaction. Goldfinger was unable to find evidence of the formation of excited ACl radicals.

After lunch, Prof. E. C. Kooyman (Leyden) was chairman and the first speaker was Dr. E. Whittle (Cardiff), who described the photosensitized brominations of methanol, ethanol, formaldehyde and acetaldehyde. The main purpose of these investigations was to obtain information about the strengths of certain of the bonds in the molecules. The products of the reaction were identified and explained in terms of chain reactions involving bromine atoms. Dr. Whittle discussed in detail the photobromination of methanol and its kinetics under varying conditions, which resulted in different chain termination processes. The reaction $Br + CH_3OH \underset{2}{\overset{1}{\rightleftharpoons}} CH_2OH + HBr$ was found to have an activation energy E_1 = 6 kcal. mole^{-1}. This gives $\Delta H_1 \leqslant 4$ kcal. mole^{-1} and hence $D(H{-}CH_2OH) \leqslant 92$ kcal. mole^{-1}. The CH bond in methanol is thus considerably weaker than in methane, for which $D(H{-}CH_3) = 102$ kcal. mole^{-1}.

Dr. J. M. Tedder (Sheffield) then discussed the fluorination, chlorination and bromination of the butanes and normal and secondary butyl halides. For unsubstituted butanes the activation energy for abstraction of hydrogen atoms by different halogen atoms are of the order : fluorination 100 cal. mole^{-1}, chlorination 1,000 cal. mole^{-1}, bromination 10,000 cal. mole^{-1}. In all cases the activation energies decrease for different hydrogen atoms in the order tertiary < secondary < primary.

The introduction of substituent halogen atoms into butane has a considerable effect on the rates of halogenation of sites adjacent to the substituent. The previously accepted assertion that a substituent halogen atom directs attack by chlorine atoms away from itself was shown to be incorrect, for example, in the chlorination of 2-chlorobutane the dichlorobutane formed in the largest amount was 2.2-dichlorobutane. For brominations similar effects were found, but in the fluorination of 2 halobutanes only slight attack occurs at the 2- position. The experimental results were interpreted in terms of the Evans and Polanyi picture of the transition state.

The final session with Prof. F. S. Dainton in the chair was opened by Prof. Kooyman, who described the results of a study of the products of the chlorination and bromination of aromatic materials in the gas phase. Chlorinations were carried out at temperatures between 300°–450° C. and brominations between 400°–500° C. Typical reactions were between bromine or chlorine and diphenyl ether, fluorobenzene, chlorobenzene and benzonitrile. The striking feature of all these reactions is that substitution occurs with major products being the meta-isomers. This is in complete contradiction in all cases to liquid-phase halogenations where *ortho/para* substitution predominates.

Kooyman said that a radical mechanism alone could not readily explain the specificity of the results obtained and put forward the novel suggestion that the reactions occurred through an ionic intermediate, that is :

$$X_2 \rightarrow 2X$$
$$X + \mathrm{ArH} \rightarrow [\mathrm{ArH}^+][X^-]$$

A proton is then lost from the position with highest positive charge, that is, the *meta* position :

$$[\mathrm{ArH}^+][X^-] \rightarrow \mathrm{Ar} + \mathrm{H}X$$
$$\mathrm{Ar} + X_2 \rightarrow \mathrm{Ar}X + X$$

This mechanism provoked a lively discussion, and the outcome of Prof. Kooyman's present work on the kinetics of these reactions is awaited with interest.

Finally, Dr. B. A. Thrush (Cambridge) described spectroscopic studies of bromine atom recombination. The studies employed the technique of flash photolysis to study the recombination of bromine atoms in excess argon to determine whether the bromine molecules formed in the recombination are produced in an electronically excited state or with excess vibrational energy. No evidence for the former was found but absorption spectra obtained during the recombination showed an appreciable increase in the population of the lower excited vibrational levels of the ground-state, notably $v'' = 1,2,3,4$ and 5.

The distribution of this energy corresponded to temperatures much higher than those to be expected from the amount of energy absorbed from the flash.

The papers presented stimulated a lively discussion throughout and thus ensured the success of the meeting.

J. H. Thomas
E. Whittle

AUTHOR AND PUBLISHER

PROBLEMS arising in connexion with the publication of scientific books were discussed at a recent meeting of the Scientific Publications Council held at the Ciba Foundation, London. Prof. G. W. Harris was in the chair.

Mr. David Dickens (Pitman Medical Publishing Co.) said that the publication of scientific books is not a lucrative business. Scientific books are generally written for the specialist, and consequently the market for them is small. A publisher of scientific text-books must maintain a staff of highly qualified editors and sub-editors, generally with science degrees, who work together with the authors to get the manuscripts into shape for publication. This is often a long process involving heavy investment: one book alone might cost £4,500 to produce and may take many years to bring back the outlay. The publisher contributes, as indicated by his 'imprint', prestige, knowledge of the field and 'know-how'. He has the same interest as the author in getting a book published as quickly as he can.

Ownership of Copyright

Mr. Dickens stated that the copyright is the author's as soon as he puts pen to paper. The publisher is generally better able to deal with selling the rights than the author, and he should do this in the author's name. Difficulties may arise if a book is written by joint authors who later decide to part. Mr. D. M. Neale (Oxford University Press) believed that it is often more convenient for the publisher to hold the copyright in order to protect the author's interests, although this is not necessarily a good general rule. His firm has been taking legal action to stop a book being pirated in Hong Kong: it could not have done this if the copyright were in the author's name, since the author was in Europe doing research. The cost of fighting an action of this kind is enormous and is all borne by the firm. When an institute publishes a series of volumes, the copyright is sometimes held by the institute: otherwise the Oxford University Press prefers to keep the copyright and return to the author whatever they may get from sale of the rights. Difficulties can arise if a publisher holding the copyright is taken over by another firm: the author has generally no defence against that. Miss M. E. Barber (Society of Authors) said that some publishers never take the copyright, but others do claim it, often in the first clause of the contract. The copyright includes translation, broadcasting, television, film, drama, digests and paperback editions. The Society of Authors takes the view that the publisher does not need the copyright: he should negotiate the rights as the author's agent. Scientists often regard writing as a side-line, and they do not take the trouble they should over contracts: publishers therefore treat scientists in a way in which they would not treat other authors. Only if piracy were rife enough to be an overriding consideration would it be justified for the publisher to hold the copyright.

Mr. A. L. Bacharach (Nutrition Society) said that the author of a paper in a scientific journal is in a different position from the same author when writing a text-book. The copyright of a scientific paper is owned by the author, unless he has specifically assigned it to a body, such as a learned society: but recently it has been suggested that the copyright of a paper belongs to the employer of the author. Dr. E. A. Moelwyn-Hughes (Cambridge) said that some countries still take no notice of copyright. When in the U.S.S.R. he had discovered by chance that 20,000 copies of a book by him had been distributed there, but he had received no royalties from the Russians. He had heard that there was also a Japanese edition, from which he also had no royalties. It is generally understood that an author can quote material from another publication so long as he gives the source and reference, but some publishers object to this. What is the legal position ? Miss Barber replied that quoting from another publication is often done by scientists, but it is an infringement of copyright to quote "a substantial part" of a copyright work without permission. Even summarizing a publication of another author or publishing an abstract is an infringement of copyright if done without permission.

Contractual Relationships

Prof. G. V. R. Born (London) said he knew of scientists who had experienced difficulties with their publishers. How can scientists be warned about publishing difficulties ? Contracts often contain an arbitration clause which may delay decision in disputes; would it be better for authors if there was no such clause? Can a publisher be asked for an audited account? Miss Barber said that an author is entitled to detailed royalty statements and that, if he had good reason to doubt their accuracy, he had a common law right to inspect the publishers' books of account, though this was a little cumbersome to enforce. The name of an unreliable firm cannot be published openly or broadcast; but a name can be omitted from a list of recommended firms. Mr. Bacharach said that the good publisher presents no difficulty: contracts are necessary, because there are bad publishers who try to manipulate an agreement to the author's disadvantage. Sometimes contracts contain a clause giving the publisher the right to call on the author to prepare a new edition; they should include also a clause requiring the publisher to let the author make such revisions as he may wish to a new issue. This is particularly desirable in scientific broadcasting; the B.B.C. has agreed that there should be no repeat after six months without giving the author a chance to revise.

Dr. Moelwyn-Hughes referred to an eminent scientist who had been cheated by a publisher. Something needs to be done in the scientific world to combat the recent development of mushroom firms that are financially slick and trade on the business incompetence of the scientist. Dr. D. Richter (Carshalton) said that British publishers have built up a high reputation in the past, so that authors have come to trust their publishers as they would their doctors or solicitors, but recently publishers of a different type, interested mainly in quick profits, have entered the scientific field. There have been instances when the publisher has sold rights without informing the author, when inaccurate royalty statements have been given and when scientists have received no royalties at all from sales of their books. Unfortunately the reliable old-established firms tend to be slow and do not always appreciate the special requirements of the scientist. The mushroom firms are exploiting the good faith of the scientific writer and giving British publishers a bad name. Prof. G. W. Harris said there is a need especially for the younger scientists to be informed of the situation.

D. Richter

SOUTH-EASTERN UNION OF SCIENTIFIC SOCIETIES ANNUAL CONGRESS

At the invitation of the Haslemere Natural History Society and the management committee of the Educational Museum, the South-eastern Union of Scientific Societies held its sixty-sixth annual congress at Haslemere during May 26–28, under the presidency of Prof. S. W. Wooldridge. The Council's choice of the theme for the congress of "The Changing Countryside" proved successful, allowing the lecturers full freedom in the presentation of their subjects and of focusing attention on the need for preservation and the desirability of intelligent management in Nature conservation. The delegates were welcomed by Dr. Rolston, chairman of the management committee of the Museum.

The young naturalists' evening included a brains trust with questions answered by a panel of experts, under the chairmanship of Maxwell Knight, followed by the screening of a film "Journey into Spring", introductory to the visit to Selborne.

The presidential address was given by Prof. S. W. Wooldridge, whose subject was "The Land and Man in the Western Weald". He commenced by emphasizing that, although the land existed before man, it was not an environment until man entered on it and reacted to it. The notion that the Weald is a denuded simple anticline has existed for some 150 years, from the time of John Fairey, who also introduced the use of the term 'denudation'. His observations of the area were made from the top of a stage-coach in the days of leisurely travel. It is now known that between the south coast and the Thames valley there are six anticlinal crests, somewhat resembling corrugated iron in form. For convenience of understanding, Prof. Wooldridge defined the country west of Stane Street as the Western Weald, wherein the Hythe Beds suddenly expand to form a major feature. It was important to remember that at one time the Chalk stood 2,000–2,500 ft. above the present land-level near Haslemere. Dealing with the influence on subsoil and soil types, it was pointed out that the intermediate and loamy soils are not derived from one particular formation, but have different soil parents. Our ancestors chose the Upper Greensand at the base of the chalk as good growing land, the best arable ground. It should be remembered that the dry valleys were not dry even in Romano-British times. Prof. Wooldridge next dealt with the succession of occupants. During Mesolithic times, about 6,000 B.C., when the climate was wetter than at present, the area on the Surrey–Sussex border was occupied. Evidence of man's later activities, during Neolithic times, had been found in the ditches at the Trundles. The following early Bronze Age had the same type of life and culture, and it was not until the late Bronze Age that the real cultivation of the land began. There is evidence of industry and trade during the Iron Age, 500 B.C.; the so-called hill forts are really the beginnings of cities on the hills. A later stage was the creation of the village communities, about A.D. 600, located by the early Saxon place-names, often those of pagan deities. The last stage is indicated by the distribution of the Domesday vils, the parish boundaries showing a remarkably even pattern, especially along the edge of the Tertiary beds.

The zoological section was addressed by John Sankey, on "Invertebrate Life in South-east England". He has for 14 years been fighting a campaign for greater investigation of the lesser-known invertebrate animals. The butterflies and beetles had been studied well enough, but such groups as centipedes and spiders need far more attention, especially observational work on their life-history and ecology.

Unless this can be done now, the changing countryside will eliminate them before they can be studied. Foam, muck and dirt are polluting many of their habitats. Specimens of the lesser-known invertebrates do not make a great display, but the need is for the collection of information rather than of specimens. Information is especially required of the habits of the fresh-water sponge, flat worms, millipedes, spiders and molluscs; the life-cycles of many are not known. The only locality in the south-east of England for the raft spider is at Frensham bogs, and it would be a great pity if the area is drained, causing its extinction. Mr. Sankey emphasized the necessity for a true understanding of conservation, not by leaving areas alone, but by proper management by people who know what they are doing.

The address to the botanical section was given by J. E. Lousley on "The Changing Flora of South-east England". He said that conditions are changing constantly and rapidly, but all changes are not necessarily bad; things need not always remain as they were when one was young. In a progressive world one must be progressive, and it is well to remember that the quarries and sandpits excavated by our grandfathers are now some of our best habitats. There was considerable upheaval by the building of railways, but there has been good recolonization of the railway embankments. The canals, many of which are now abandoned, are rich in aquatic plants, some of which had been introduced by the barges, and the filled-in canals provide habitats for many species. The greatest losses have been through building extensions; that around London has destroyed many habitats, while drainage has reduced the number of boggy areas. The indiscriminate dumping of rubbish is deplorable; the example at Mitcham Common was to be avoided. Changes are to be seen in the village ponds (no longer required for animals), which have now become the receptacles for rubbish. A further undesirable change is brought about by the use of the ground by the Army for tank training, and the carelessness of the public is a constant threat, by fire, on heathlands. Great changes in the vegetation have been brought about by the reduction of grazing and the deplorable elimination of the rabbit, resulting in the vast increase of scrub. It is now necessary for a conservation corps to be constantly clearing the scrub to maintain the survival of the interesting plant life.

The general interest of the public has increased, particularly in the British flora, and this aids the propaganda for the need of preservation and conservation.

Miss Phyllis Bond addressed the delegates on "Birds in a Changing World". The change in south-east England was swifter than in most areas, owing to its proximity to London, increasing greatly since the beginning of the century. The spread of residential building may increase the bird population, gardens providing a variety of habitats, but the occupants are the so-called common birds and the need is to provide suitable habitats for the rarer species in danger of elimination. One of the hazards of progress is that a large part of the resident population is not indigenous and is always tidying up, thus removing the habitats necessary for some birds. Nature and man have from the earliest times moulded the shape of the countryside, and man's effort is often not to its advantage. Miss Bond regretted the passing of the rabbit; the change in the nature of the herbage has been to the disadvantage of many birds, depriving them of their usual nesting sites and reducing the insect population, which provided their food. She deplored the practice of spraying road-side verges and hedgerows with poisonous compounds, especially between the months of April and August. The shrubs and herbage which were the breeding places of many birds are either killed or starved; there is a chain of deterimental reaction the ultimate result of which cannot be perceived. Poisoned seed is also responsible for much injury, for not only are birds and animals killed by directly taking the seed, but also their food of worms and other invertebrates carries the poison and thus adds to the casualties.

The attitude of the public towards birds has changed for the good; the shooting of birds and the collection of eggs are now regarded as belonging to past generations.

Dr. J. F. D. Frazer, conservation officer for England of the Nature Conservancy, chose as the title of his address, "Problems of Managing Nature Reserves". The Royal Charter granted to the Nature Conservancy included a clause enabling it to acquire and manage nature reserves. Land left alone becomes a wilderness so the principles of management have to be studied and applied. First, the type of nature reserve desired has to be determined, and the drawing up of a management plan is the work of two or three years. The past history of the area has to be studied, its geology, the arrival of man, up to its present state. The climate and existing species have to be studied; it has to be decided what is wished to be done and how it is going to be achieved. In some cases the public can be allowed access without harm being done, but when sand dunes are the subject the public must be kept off. An area may need to be managed correctly, as at Blean Wood, Kent, for the protection of one butterfly or as at Wye for the preservation of an orchid population. The desirability of introducing 'foreign' animals and plants or the re-introduction of those that have become extinct has to be discussed and the good relations with the owner of adjacent property encouraged.

Dr. Frazer showed slides of areas taken from the same point yearly during 1954–60 showing clearly the evil results of the extermination of the rabbit, which controlled the growth of ragwort, gorse and the scrub. Without grazing, the only way to reproduce the former condition is by cutting, and a corps of conservationists is required to work on this, under the direction of a conservation officer, who determines the areas to be cleared, the amount necessary and the regularity of the period of clearance to ensure success. Favourable weather conditions enabled the full programme of excursions to be carried out. The archæologists visited Selborne Priory and church, and a general natural history party after visiting 'The Wakes' at Selborne continued to Nore Hill for the Chalk flora. Botanists and zoologists went to the Juniper Hall Field Centre, Box Hill, and the surrounding countryside for the Chalk flora and invertebrates; particular interest was shown in the trap-door spiders at their only known locality in south-east England.

The excellent arrangements for the congress were undertaken by a local committee with John Clegg as secretary.

The sixty-seventh annual congress of the Union will be held at Colchester during June next year, and it is hoped that the meeting will provide an opportunity to stress the importance of the natural sciences in the curriculum of the new university to be established there.

F. J. Epps

PARASITE BIOLOGY

THE third spring meeting of the Parasitology Group of the Institute of Biology was held at Wills Hall, Bristol, during April 11–13. The meetings were well attended, and a number of visitors from overseas were present. There can be no doubt that this annual meeting held at a provincial centre has become established firmly in the activities of the Group. An important item in the programme was the address by an invited speaker. This item was introduced last year at Liverpool when Prof. T. H. Davey took as his subject "Parasites as Enemies and Allies". It proved a very useful focus of interest, and this year Prof. C. Read was invited to speak. He gave as his title "The Host-Parasite Interface: Molecular Basis of Parasitism". Prof. Read is professor of biology in the Rice Institute, Houston, Texas, and is at present on sabbatical leave working at the Molteno Institute, Cambridge. He began by discussing parasitism as part of a broad definition of 'symbiosis' considered as a function of the intensity of the field of association established between organisms, and of the internal and external resistances surmounted in its establishment. The parasite comes to depend on the host for the maintenance of homeostasis, and the regulation of homeostasis of a parasite intimately involves a region in space and time which can be designated 'the host-parasite interface'. The host, however, is not a passive environment, and interaction plays an important part in this concept of parasitism. The primary exchanges at the interface are chemical, and Prof. Read proceeded to discuss examples of these obtained from work with which he has been associated in the United States. He concluded by suggesting how host-parasite specificity and pathology might be discussed in terms of chemical exchanges.

The general sessions were devoted to a series of 19 short papers, each being followed by a period of discussion. This type of meeting is useful because it gives an indication of the fields in which the main research is taking place and provides an opportunity for discussion of its progress and the conclusions reached.

The growing interest in marine parasitology was reflected in two papers by Z. Kabata (Marine Laboratory, Aberdeen) and H. H. Williams (University College of Wales, Cardiff). Dr. Williams had examined a number of elasmobranchs caught in British fishing areas and recovered the tetraphyllidean cestodes which they contained. A high degree of host-specificity exists in this group, and, although there are still many unidentified species, a very interesting discussion was given of the physiological, ecological and morphological factors which contribute towards restricting a species to a particular host. Dr. Kabata based his discussion on the parasitological examination of some 500 whiting and haddock and presented some data relating the age of the fish to the parasitic fauna which it supports.

Temperature and climatic conditions have an important influence on the level of helminthic infestation in domestic animals. H. D. Crofton (Bristol) has devised a technique for accurately observing the rate of development of nematode eggs and larvæ from sheep. The data, which he has obtained on the limits of temperature tolerance of different species and the effects of temperature on the rate of development, provided the basis for a useful discussion on the ecology of the parasites in relation to domestic stock. The importance of temperature and moisture-levels on different stages in the life-cycle of *Fasciola hepatica* was discussed by C. B. Ollerenshaw (Central Veterinary Laboratory, Weybridge), with particular reference to the incidence of fascioliasis in Anglesey. Dr. Ollerenshaw was able to correlate the incidence of the disease in this area, which was particularly high during 1958, with meteorological data obtained during the preceding year.

The significance of the trypanosomatid kinetoplast figured in the discussions by three speakers. J. R. Baker (London School of Hygiene and Tropical Medicine) and K. Vickerman (University College, London), in a joint paper, showed a series of electron micrographs of insect and vertebrate trypanosomatids, and the possible functions of the kinetoplast were reviewed with particular reference to kinetoplastic and akinetoplastic strains and to the life-history of the species studied. When trypanosomes (*T. gambiense* and *T. rhodesiense*) are exposed to dilute solutions of acriflavine they become photosensitive and are rapidly killed by light. The kinetoplast of the sensitized trypanosome fluoresces intensely in ultra-violet light, but experiments by P. J. Walker (National Institute for Medical Research, Mill Hill) have indicated that this organelle is not the only site of the photosensitivity.

The role of arthropods as vectors of parasitic infections was discussed in the course of three papers. D. S. Bertram and R. G. Bird (London School of Hygiene and Tropical Medicine) described the subcellular morphology of *Aedes aegypti* with the aid of a series of electromicrographs. This study was particularly concerned with the transmission of Semliki Forest virus, and the presence of virus particles within the cells was correlated with the infectivity of the insects. These workers also suggested that the complexity of the membranes surrounding the anopheline ovary reduces the possibility of the transmission of infection through the mosquito egg. In ticks, however, the membrane structure is simpler, and transovarian transmission of parasitic infections therefore can take place more easily. Transmission in this way of *Babesia divergens* by larvæ of *Ixodes ricinus* following the feeding of adult ticks on infected calves had been observed by Davies and Joyner in the course of a series of transmission experiments carried out at the Central Veterinary Laboratory, Weybridge. The feeding mechanism by which *Ixodes ricinus* obtains infected blood was the subject of an investigation by E. Sutton (King's College, London). Histological preparations were shown to illustrate the digestion of the host tissue which takes place around the inserted mouth-parts. Spectrophotometric measurements of the hæmoglobin content of individual ticks indicate that ticks feed on varying amounts of non-blood and blood tissue.

Parasites in relation to natural fauna were also discussed. A. M. Dunn (Veterinary School, Glasgow) has recorded the intestinal helminth parasites of the three species of wild deer on Rum in the Inner Hebrides. This paper was notable for some very beautiful illustrations of the habitats. The rigour of a littorine habitat was apparent in the discussion by B. L. James (Aberystwyth) of the development and life-history of *Parvatrema homoeotecnum* n.sp., a gymnophalline trematode which parasitizes the gastropod mollusc, *Littorina saxatilis* and oyster catchers. This parasite shows several specialized and primitive features. The perch and pike in Llyn Tejid in Merionethshire are the hosts of a pseudophyllidean cestode, *Triaenophorus nodulosus*. By taking regular nettings, J. C. Chubb (Liverpool) was able to obtain data relating the incidence and development of the parasite to the growth of the fish. Also following this theme of seasonal incidence, but with quite a different parasite, W. N. Beesley (Weybridge) discussed the development and migration of *Hypoderma bovis* and *H. lineatum* in domestic cattle.

Work during recent years has shown that parasitology is a fertile field for the application of new laboratory techniques. Thus, cytochemical techniques were used to characterize the functions of the male accessory and reproductive glands of *Argas persicus* (R. J. Tatchell, Cambridge), and the pseudosuckers and holdfast organ of the trematode *Diplostomum phoxini* (D. L. Lee, Cambridge). The nutritional requirements of the latter have been the subject of *in vitro* cultural studies by C. A. Hopkins and M. O. Williams (Glasgow). In the field of immunology, agar diffusion techniques have been applied to such widely differing subjects as *Eimeria* infections in fowls (M. E. Rose and P. L. Long, Houghton) and schistosomes in monkeys (S. R. Smithers, National Institute for Medical Research). Despite the application of new techniques, parasitology still occasionally requires systematic descriptions, an example of which was the paper by S. T. Fernando (Cambridge) dealing with the developmental stages of *Mecistocirrus digitatus*, a nematode found in the abomasum of cattle in Ceylon, India and the Far East.

The proceedings of this meeting will not be published in detail. The spring meeting in 1962 is to be held in Cambridge during April 3–5.

L. P. Joyner

WATER RESOURCES IN WALES

LARGELY as a result of the water supply difficulties of 1955 and 1957, the Minister of Housing and Local Government (who is also Minister for Welsh Affairs) appointed in February 1958 a committee to advise him on the use and conservation of water resources in Wales. The Committee has already expressed interim views in February 1959 on the Milford Haven barrage scheme (which it favoured), and has recently published its first full *Report on the Water Resources of Wales**.

This report is a notable advance forward in our knowledge of the Water Resources of Great Britain and in their proper employment. The Minister indicated to the Committee that the most urgent problem was to assess the water resources in relation to foreseeable demand, and three guiding questions were posed: (*a*) What are the water resources ? (*b*) What is the exploitable surplus ? and (*c*) How can such surplus best be employed ?

The Committee consisted of persons from different parts of Wales with interests in hydrology but not themselves engineers or technical experts. The report, as a result, considers not only the hydrological survey of Wales carried out by the Ministry's engineers in 1957 but also social, economic and cultural aspects of the problems encountered. The hydrological survey (not generally available) gives detailed estimates of the maximum supply of water which could be obtained from Wales on the assumption that hydrological considerations are the sole governing factor. The Committee, however, examined more than 180 suggested reservoir sites in the light of (*a*) geological structure, (*b*) agricultural interests, (*c*) existing communities, (*d*) amenities, and (*e*) cost. The sites were then placed in one of four categories: '*A*' sites, to which no objection could be seen; '*B*' sites, where a moderate degree of objection was foreseen; '*C*' sites, development of which could only be envisaged if the need for water in the country as a whole were to become over-riding; and lastly, sites rejected as completely unsuitable.

As the Committee also considered the various resources in relationship to conventional impounding reservoirs, regulating reservoirs, estuarine reservoirs and direct abstraction, it will be immediately apparent that there is no clear-cut and definite answer to the first question: "What are the water resources of Wales ?". Appendix 8 of the report, however, sets out the various possibilities: these, on the Committee's definition, range from a minimum of 1,195 million gallons a day to a maximum of 2,895 million gallons a day.

These resources are next considered in relationship to the demand for water. Here the Committee has not only considered the present situation but also has looked ahead into the 'foreseeable future'. This has been taken, despite the hazards of extrapolation, as a period of 30 years. For this purpose Wales is divided into five geographical zones—south-west, north-west, north-east, south-east and central. In only one of these zones, the south-east, has the Committee found serious prospective deficiency of water: in all the others there is not only an adequate amount of available water but also an exploitable surplus. Ample allowance has been made in these calculations for a continued expansion in the Welsh economy and for increased demands for water. The Committee estimates that, taken as a whole, the Principality has an exploitable surplus of resources over demand of between 235 and 800 million gallons a day according to the system of development adopted (this is seen in perspective when it is recollected that public undertakings in England and Wales at present supply some 2,400 million gallons a day).

The Committee suggests that that part of the surplus which can be most easily used to supply areas in England which are short of water is to be found in the upper basins of the Severn, Wye and Dee (the 'central zone'). This is an area of heavy rainfall and very low population with limited local demands on the water resources. The report stresses that this should be achieved by means of regulating reservoirs such as that proposed in the Tryweryn scheme. This type of reservoir regulates the flow of water down the river, and water is then abstracted from the lower reaches: there is no pipe-line as with the impounding reservoir.

---

* Welsh Advisory Water Committee. *Report on the Water Resources of Wales*. Pp. iv+93. (Cmnd. 1231.) (London: H.M.S.O., 1961.) 5*s*. net.

The Committee raises an important point in the suggestion that rural areas where large reservoirs have been sited have not always received adequate economic returns. They believe a case exists for a system whereby material benefit should be received by such areas in addition to the extra revenue available in the form of rate income. Adequate compensation for displaced residents and disrupted farm-economies would also ease the transition from traditional types of land use to water storage. A realistic approach on these lines would help to create a better understanding between the city and town dwellers and the inhabitants of the rural catchment areas.

The report is full of valuable statistical data, and provides for the first time an indication, in the light of economic, social and cultural considerations, of the hydrological situation within the Principality in relationship to present and future needs of water supply. A clear and concise geographical account is also given of each of the five zones demarcated (although the authors of the report have somewhat oddly listed the physical geography as 'geography'; actually the whole of each of the respective chapters falls within the scope of a geographical consideration). As a work of reference and as a guide for future policy decisions the report will be invaluable.

Water in Wales may also raise from time to time 'political' problems regarding the 'export' to England. This aspect is also considered by the Committee. It believes that, in a period when water will become relatively more scarce, Wales will have many advantages for water-seeking firms and for agricultural irrigation. While noting that public opinion in Wales would not take kindly to the idea of a wholesale export of water without reference to long-term needs within Wales, the Committee is able to conclude that Wales has such an abundant rainfall that there is, and will continue to be, a large exploitable and 'exportable' surplus of water if the resources were properly developed by the construction of suitably sited regulating reservoirs. W. G. V. BALCHIN

CHEMICAL COMPOSITION OF PRECIPITABLE WATER VAPOUR OVER THE UNITED STATES

By DRS. HENRY M. PAPÉE and ALBERTO MONTEFINALE

Centre for Aerosol Nucleation, National Research Council of Italy, Via Vettore 4 (Monte Sacro), Rome

DATA on annual average amounts of precipitable water vapour in the atmosphere over several stations of the United States and up to 525 m.b. have recently been published by Reitan[1], and it appeared to be of interest to us to attempt a correlation between these data, Junge's rain-water content analyses[2,3] and the total precipitation values. It was thought, in particular, that further evidence of a connexion between precipitation and such occurrences as may induce changes and activation[4] in aerosols could be obtained in this way. Since about half the stations selected by Reitan for his work coincide with points which were used by Junge, there appeared to be a good possibility of gaining further insight into natural nucleation phenomena.

If the precipitable water vapour π is taken as a potential measure of the water stored in the atmosphere, and other parameters are maintained constant, an increment of precipitation R with increase of π will depend on an effective value of π itself, and therefore on the difference:

$$\alpha\pi - \beta R$$

α and β are constant for the purpose of this article (see later); more generally α is a number which depends on temperature, while β has the dimensions of time.

Therefore:

$$\frac{\mathrm{d}R}{\mathrm{d}\pi} = K(\alpha\pi - \beta R) \tag{1}$$

where K is a constant.

When $\pi = 0$, $R = 0$; equation (1) integrates to:

$$R = \frac{\alpha}{\beta}\left\{\pi - \frac{1}{K\beta}\left[1 - \exp(-K\beta\pi)\right]\right\} \tag{1a}$$

The derivative of (1a) with respect to π can also be written:

$$\frac{\mathrm{d}R}{\mathrm{d}\pi} = \frac{\alpha}{\beta}\left\{1 - \exp(-K\beta\pi)\right\} \tag{1b}$$

Thus, it is significant that for very high precipitable water vapour contents of the atmosphere (1b) approaches α/β, while it is near to zero for very small values of π.

Clearly, the 'tendency of π to precipitate' will depend considerably on temperature. If, however, differences in vertical temperature gradients from station to station are assumed to be such as not to have much influence on the data at hand, it becomes readily possible to correct π for these effects. Rather than make corrections on the basis of the $\pi - T$ empirical relationship and the 'potential temperature' formula, we used an empirical latitude correction. A plot of π against geographical latitude for lower stations (Fig. 1) gives an approximately straight-line relationship, on the basis of which Reitan's data of precipitable water vapour were all reduced to a latitude of 35° N. by means of multiplication by the corresponding values of $(\pi_{35°}) : (\pi_{\text{lat.}})$ which were obtained from the straight line derived by the method of least squares. This procedure results in a plot given in Fig. 2, where the corrected points are seen to fall into a type of pattern which might be expected to be in agreement with equation (1a).

When the exponential in (1*a*) is expanded in series, the expression becomes:

$$R = \alpha\left(\frac{K\pi^2}{2!} - \frac{K^2\beta\pi^3}{3!} + \ldots\right) \tag{2}$$

if *K* is now assumed to depend simply on the quantity of an active chemical species in the atmosphere, and if that quantity were taken, for the purpose of this communication, to be proportional to the concentration *Ch* of the species concerned in the water that dissolves it, multiplied by the volume of precipitation itself:

$$K = aChR$$

substitution into (2) gives:

$$\pi^{-2}(Ch)^{-1} = \alpha\left(\frac{a}{2} - \beta\frac{a^2}{6}(ChR\pi) + \ldots\right) \tag{2a}$$

Similarly, if the ratio Na : Cl (ref. 5) is assumed to influence *K* so that:

$$K = a\frac{\text{Na}}{\text{Cl}}$$

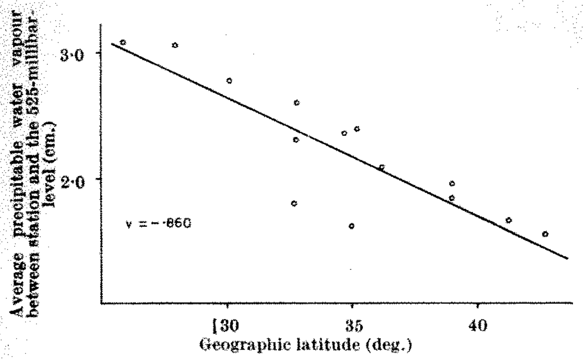


Fig. 1. United States stations; altitude 0–580 ft.

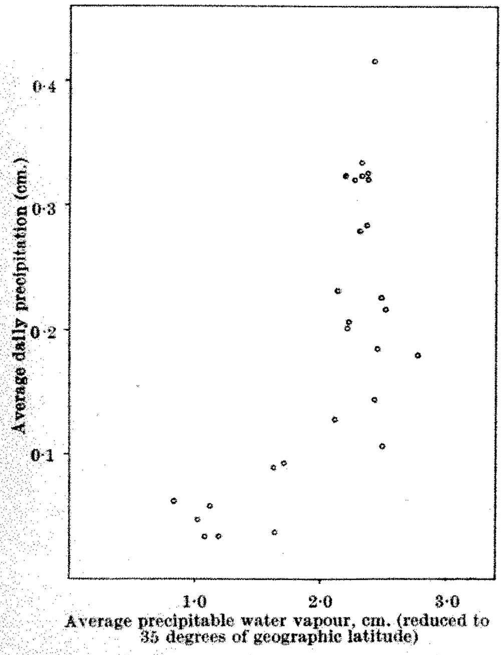


Fig. 2

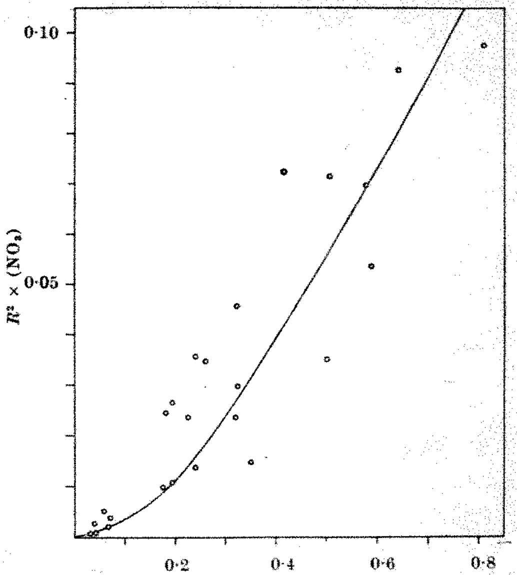


Fig. 3

then equation (2) becomes:

$$\frac{R}{\frac{\text{Na}}{\text{Cl}}\pi^2} = \alpha\left(\frac{a}{2} - \beta\frac{a^2}{6}\frac{\text{Na}}{\text{Cl}}\pi + \ldots\right) \tag{2b}$$

By means of (2*a*), (2*b*) correlation tests: $(\pi^{-2}(Ch)^{-1}) - ChR\pi$ can be carried out; linear correlation coefficients were computed for the following:

$$\text{NO}_3;\ \text{Na};\ \text{K};\ \text{SO}_4;\ \text{Cl};\ \text{NH}_4;\ \text{Ca};\ \frac{\text{Na}^2}{\text{Cl}};\ \frac{(\text{Na}+\text{K})^2}{\text{Cl}};\ \text{Na}\times\text{NO}_3;\ \frac{\text{Na}}{\text{Cl}};\ \frac{\text{Na}+\text{K}}{\text{Cl}}$$

(having expressed *Ch* in gram-equivalents × 10^5 l.$^{-1}$, π and *R* in cm. and cm. × day^{-1} respectively, and using equation (2*b*) for the last two functions listed).

The following values are worthy of note:

Ch	NO_3	$\frac{\text{Na}}{\text{Cl}}$	$\frac{\text{Na}+\text{K}}{\text{Cl}}$	SO_4	NH_4	Ca
r	−0·77	−0·57	−0·60	−0·51	−0·58	−0·46

All others gave $0 > r > -0{\cdot}4$; calculation of the correlation coefficient between $R\pi^{-2}$ and π (equation 1), where the 'salt effect' was neglected, yields $r = 0{\cdot}21$.

As can be seen, NO_3 is the chemical species that fits equation (2*a*) most closely. Equation (1*a*) can be written in this case, after substitution:

$$R^2\text{NO}_3 = \frac{\alpha}{\beta}\left\{\pi' - \frac{1}{a\beta}\left(1 - \exp(-a\beta\pi')\right)\right\} \tag{3}$$

where

$$\pi' = \text{NO}_3R\pi$$

$a\beta$ can be obtained in first approximation from the linear regression parameters of a plot of $\pi^{-2}\text{NO}_3^{-1}$ versus $\text{NO}_3R\pi$ (equation 2*a*) since its value is:

$$\frac{3\ \text{gradient}}{\text{intercept}} = 3{\cdot}6 \pm 0{\cdot}5$$

Using this value, α/β can be computed with good approximation from equation (3); we find:

$$\frac{\alpha}{\bar{\beta}} = 0{\cdot}209 \pm 0{\cdot}015$$

This now enables a check of $\alpha\beta$ to be carried out; the method of consecutive adjustments, using the *S.D.* of equation (3) as a monitor, yields a value of $\alpha\beta$ in agreement with that obtained previously.

Fig. 3 illustrates a plot according to equation (3) and the calculated line. The standard deviation of this function is found to be:

$$S.D._{R^2NO_3} = 0{\cdot}013$$

whereas, introducing the constants computed into equation (1) we calculate the value of:

$$S.D._R = 0{\cdot}10$$

We are now in the process of extending the computations based on the use of nitrate to the four seasons during 1955–60. While the results obtained will be the subject of a future publication, it can now be stated that the same correlation appears to exist throughout, and that consistency appears to be higher during the warmer seasons than in the cold ones.

We are indebted to Prof. V. Caglioti for his help and encouragement, and to Prof. G. Fea for the interest he has shown in our work.

[1] Reitan, C. H., *Bull. Amer. Meteor. Soc.*, **2**, 79 (1960).
[2] Junge, C. E., and Werby, R. T., *J. Meteor.*, **15**, 417 (1958).
[3] Junge, C. E., *Trans. Amer. Geophys. Union*, **39**, 241 (1958).
[4] Papée, H. M., and Smith, A. C., *Geof. Pura ed Appl.*, **44**, 216 (1959).
[5] Papée, H. M., and Montefinale, A., *Nature*, **187**, 1103 (1960).

CRYSTALLIZATION IN WOOL KERATIN

By Dr. ALLAN SKERTCHLY

Textile Physics Research Laboratory, Department of Textile Industries, University of Leeds

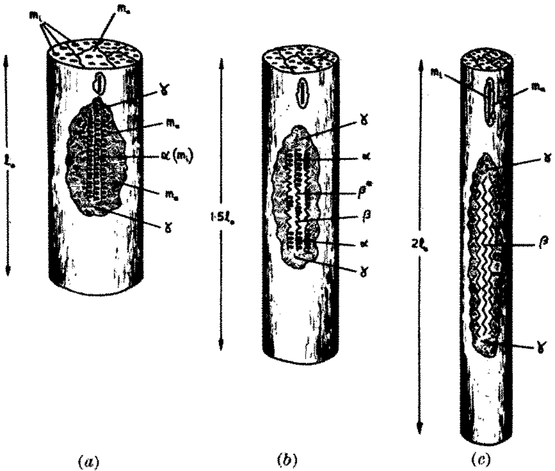
WITH the advent of rapid scanning X-ray diffractometry[1,2] it has become possible to investigate, more fully, certain aspects of crystallization in the fibrous proteins. In particular, it is now apparent that crystallization in wool keratin may be a fruitful field of study. One of the simplest systems to investigate is that of the keratin–water complex, both under external stress free, and tensile stress deformation, conditions. The degree of hydration of the keratin component is intimately related to the type of crystallization observed, and some aspects of this relationship are described here.

Present-day hypotheses[3,4] tend to support the view that unstrained wool keratin consists of 'crystalline' microfibrils embedded in an 'amorphous', or at least somewhat disordered, 'matrix'. Evidence from electron microscopy[5] suggests that the microfibril/matrix areal ratio is near unity, and data from infrared spectroscopy[6] are compatible with the idea that the most readily deuterium exchangeable fraction is an 'amorphous' region, and most probably the matrix.

The lateral dimensions of the microfibrils are approximately the minimum required[7] in order to secure a 'crystalline' X-ray reflexion. For this reason, and because the matrix is 'disordered', it is reasonable to ascribe the origin of the wide-angle X-ray diffraction pattern to interference from within the microfibrils. Before proceeding, it is necessary to distinguish between the configuration of a crystallographic component of structure and the configuration of the fibre as a whole. Thus, in the present case, the maximum amount of material available for the crystalline phase is 50 per cent, and it is normally assumed that an appreciable fraction of the molecules in the microfibrils are in the helical configuration which gives rise to the characteristic reflexions. However, we may also say, loosely, that the fibres are in the α-form, by which we simply mean that chains in the α-configuration are dominant in the crystalline regions.

Fig. 1*a* shows in outline the gross details of the generally accepted model of structure for wool keratin fibres in the 'dry' state. If the system is hydrated, for example, soaked in water at room temperature (18° C.) for a reasonable period, it is observed: (1) that the equatorial spacing about 9·8 Å. is little altered[8,9]; (2) (from the results of this investigation) that there is little change (less than 5 per cent) in the radially 'integrated' scattered intensity of the 9·8 Å. reflexion. These results and those of the deuterium exchange reaction[6] suggest that few water or deuterium oxide molecules penetrate into the helical interstices. Thus, under conditions of zero external stress the crystallographic configuration of both dry and hydrated keratin remains unaltered, at least to within the limitations of detectability of the present detecting system.

With the application of an external stress of sufficient magnitude to cause an appreciable strain, an entirely new and more complicated system is developed. The nature of the end-products of the reaction depend, in an as yet ill-defined manner, on



(*a*) (*b*) (*c*)

m_i, microfibrils; m_a, matrix; l_o, original length

Fig. 1. (*a*) Model of unstretched wool keratin. Microfibrils, α-transformable helices; γ, invariant fraction. Matrix, an initially disordered region of high water accessibility. (*b*) Wool stretched 50 per cent and dried. β-crystalline phase derived from α. β*, a mixed α- and β-crystallite. Matrix, becoming strained and more ordered. (*c*) Wool stretched 100 per cent and dried. β-Crystalline phase derived from all the formerly ordered α-material. Matrix, attaining a further degree of order

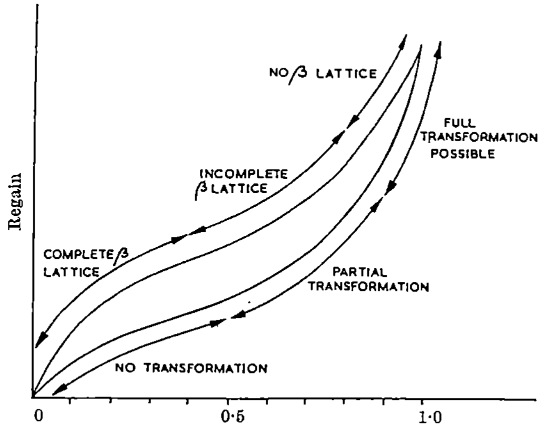


Fig. 2. The relationship of partial vapour pressure to the extent of crystallization in the β-form for wool keratin stretched up to 50 per cent. The absorption curve represents the extent of transformation of α-helices into β-crystallites, for stretching at the vapour pressures shown, after the fibres have been dried in the extended condition at 105° C. The desorption curve shows the extent of β-crystallization during lowering of the vapour pressure, after the fibres have been stretched wet to 50 per cent extension

the initial vapour pressure under which the fibres are stretched, and on the external conditions into which the fibres are finally brought.

In this connexion it is of value to consider the reaction products which may be obtained under two important limiting sets of conditions: (1) when a stress is applied to the fibres under a number of different partial water vapour pressures, and the then extended fibres are dried at 105° C. (conditions where there is a maximum likelihood of β-crystallites being formed); (2) when fibres are stretched wet and then the extent of formation of β-crystallites for progressively reduced vapour pressures is observed. In all cases the fibres were extended, either up to the maximum possible (determined by their initial conditions), or to an arbitrary and convenient limit of 50 per cent. The extent of crystallization was determined by a technique, depending on the magnitude of the radially integrated reflexion intensities, which has been reported elsewhere[10].

The results of one such set of crystallization experiments are summarized in Fig. 2. It will be seen that there is a strong correlation between the increase in the initial moisture content (regain) and the predisposition of the system to form β-crystallites after stretching and drying. Thus, it seems likely that an intermediate hydrate is a necessary precursor to an observable crystalline β-phase. The upper curve of Fig. 2, the desorption curve, confirms this view, for it is found that crystallization does not in fact occur until after the relative water vapour pressure falls below a certain value (0·8), and thereafter continues to increase until a limiting value, which represents the optimum equilibrium concentration of material in the ordered crystalline β-form, is attained.

In these experiments it was not found possible to obtain any evidence for the existence of crystalline β-keratin unless the vapour pressure of the extended material was reduced below that at which the extension took place. This result is in conflict with the interpretation of some observations reported earlier by Bendit[11].

The crystallization at 50 per cent extension of the mixed system of α- and β-keratin affords a striking illustration of the effectiveness of the rapid scanning technique, and some sequences from the X-ray diffractometer records are, therefore, reproduced in Fig. 3. These show the reappearance of the crystalline α- and β-reflexions as the solvent vapour pressure is reduced and indicate clearly the nature of the phenomenon taking place.

In earlier work[12] it has been established that at intermediate extensions the dehydrated equilibrium crystalline configuration contains a mixed crystallite which does not contribute to either the α- or the β-crystalline X-ray diffraction pattern. This has been designated β* (ref. 13). However, the evidence available now suggests that hydrated and extended material may consist of unchanged α-chains, hydrated α-chains, hydrated β*-material, and hydrated β-material. Further, it is observed[14] that the limiting reversible extension of the system is near 100 per cent. However, unconstrained α-helices would be expected to extend by at least 120 per cent[14], and measurements on the meridional reflexions confirm this[15]. Any interpretation of the changes in the equilibrium relative intensities of the α- and β-crystalline reflexions indicates that a smooth and continuous transition from the crystalline α- to β-phase occurs. These facts, taken together, suggest that there is an inextensible component of the microfibril in series with the helical α-crystalline phase. The work of Middlebrook[16] has suggested that cystine-rich regions alternate with cystine-poor regions along the fibre

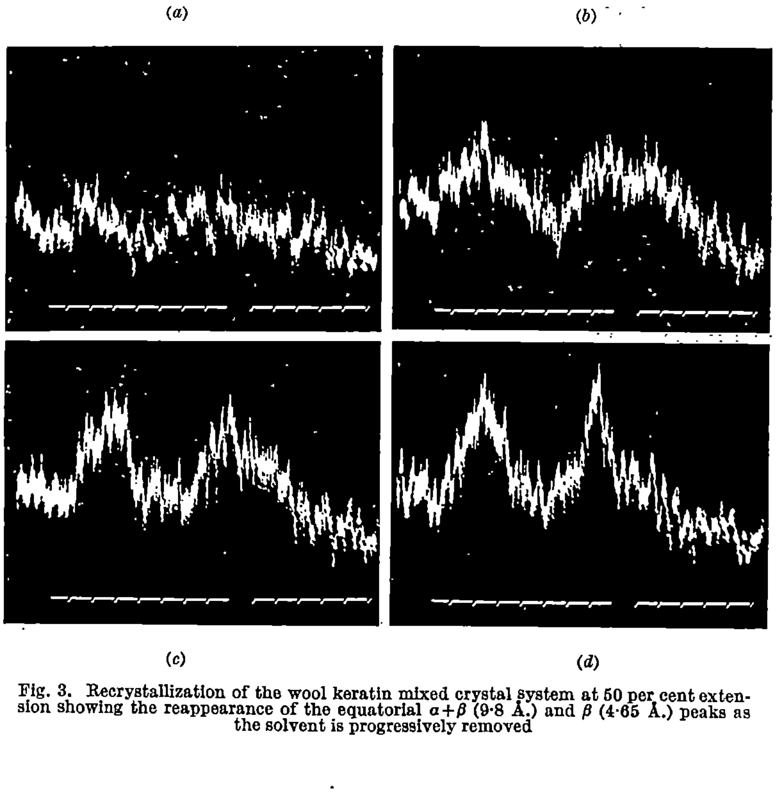


Fig. 3. Recrystallization of the wool keratin mixed crystal system at 50 per cent extension showing the reappearance of the equatorial α+β (9·8 Å.) and β (4·65 Å.) peaks as the solvent is progressively removed

axis and a component of higher sulphur content, γ-keratose[17], has been isolated. Thus, it is proposed to label the inextensible phase γ-keratose. Since this phase is not sufficiently well ordered to contribute to the discrete X-ray reflexions and is likely to be closely linked, it is suggested that hydration will have little effect on it. Because of this, it will be taken to form a largely unalterable component of the microfibrils. This analysis also leads to the value of 40 per cent for the 'crystallinity' of the fibre as a whole, a figure which compares well with those obtained by other workers and in particular 44 per cent by Hailwood and Horrobin[18].

Earlier interpretations[15] also suggest that it is only at extensions near 100 per cent that all the α-crystalline phase is transformed into the β-crystalline phase.

Thus the two extensions of 0 and 100 per cent form limiting cases for relatively simple keratin–water complexes. At any intermediate extension the possibility of several more components being present suggests itself. Table 1, therefore, indicates in outline the sequence of phases that may be involved in the α–β transformation, and underlines, too, the complexity of the situation in what was once thought[19] to be a simple polymorphic transformation. Next it is desirable to return to Fig. 1 and to contemplate the nature of the interaction between the matrix and the microfibrils.

Table 1. SEQUENCE OF PHASES INVOLVED IN THE α–β TRANSFORMATION IN WOOL KERATIN

Condition of fibres	Phase states		Phase designation
	Microfibrils	Matrix	
Dry under no external stress	$\alpha + \gamma$	Dehydrated	α
Wet under no external stress	$\alpha + \gamma$	Hydrated	α_H
Wet under stress extended 50 per cent	$\alpha + \alpha_H + \beta^*_H + \beta_H + \gamma$	Hydrated	Mixed M_H hydrate
Dry under stress extended 50 per cent	$\alpha + \beta^* + \beta + \gamma$	Dehydrated	Mixed dehydrate M
Wet under stress extended 100 per cent	$\beta_H + \gamma$	Hydrated	β_H
Dry under stress extended 100 per cent	$\beta + \gamma$	Dehydrated	β

Since the transformation from crystalline α to crystalline β material is uniform, there is no evidence to suggest that marked gross crystallographic differences exist along the transformable microfibrillar component. On the other hand, evidence from the analysis of the stress-strain curves[14,19] leads us to expect three distinct phases which may affect the extensometric characteristics. Successively, the fibre becomes more difficult to transform, but since initially Young's modulus of the matrix may be supposed to be small[3], the hindering of the transformation by the matrix is also initially small.

It is, however, reasonable to suppose that on extension the initially disordered matrix becomes more ordered and also contributes increasingly to the resistance to deformation, while the stress contribution of the crystalline material follows a uniform course depending on the difference in energy between the α- and β-forms.

The recent work of Crewther and Dowling[20] has reaffirmed that there exists a high degree of interaction between the microfibrils and the matrix under suitable conditions, but there does not seem to be any direct evidence to suggest, as has Feughelman[21], that the microfibrils themselves have a periodic sequence of X (more easily extensible) and Y (less easily extensible) zones. All that can be definitely asserted, in the absence of the crystallographic transformation data, is that the system (microfibril–matrix), acting as a whole, produces the observed supercontraction and stress-strain discontinuities. The view to be taken here, however, is that as the crystallographic transformation in the microfibrils is reasonably uniform throughout the entire range of extensions, it is the matrix, with differing degrees of deformability, that is the source of complexities in the other observable physical parameters. The contribution of the microfibrils to the developed stress over the whole transformation-range may thus be derived from an extrapolation of the wet stress at low extensions, assuming the simple uniform transformation of the crystallites. On the basis of this assumption, it is possible to calculate a value for the difference in free energy between the α-phase and the β_H-phase using the relationship $\Delta F^0 = \lambda\frac{ARf}{k}$ (ref. 14). This equation holds for the condition $[\alpha] = [\beta]$, which is obtained near 50 per cent extension.

If we assume a single NH . . . O = C hydrogen bond in a Pauling and Corey type α-helix[22] to be the fundamental flow unit, we find, for hexagonal packing, the following values for certain of the required parameters:

A, the cross-sectional area $\simeq (5{\cdot}8)^2 \times 3{\cdot}46 \times 3{\cdot}7^{-1} \times 10^{-16}$ cm.^{2}; λ, the translation distance of a single segment of chain, $\simeq 6{\cdot}35 \times 10^{-8}$ cm.; $R = 1{\cdot}99$ calories/mole deg.; $k = 1{\cdot}375 \times 10^{-16}$ erg/deg.

The remaining parameter, f, the equilibrium stress, is rather difficult to establish accurately, owing to long-term stress relaxation. However, it might reasonably be argued that in so far as the reversible crystallographic transformation is concerned, equilibrium is, in fact, reached in a relatively short time (within the duration of the experiment). Stress relaxation continuing after this time may be due to a significant change in keratin structure not within the purview of present considerations. Such possibilities have been confirmed recently by Woods[14] and Kubu *et al.*, who have shown that the first (rapid) stage of relaxation involves hydrogen bonds only, while long-term relaxation depends to a large extent on primary (particularly disulphide) bond fission. If Crewther and Dowling's ideas[20] are substantiated, the matrix is, in fact, rich in sulphur in comparison with the transforming section of the microfibrils. Their suggestions thus provide confirmatory evidence for the keratin model outlined above. As an approximation, the extrapolated value of stress adopted by Peters and Woods[14] from Woods's earlier data may be chosen. This yields an extrapolated stress of $\simeq 8 \times 10^8$ dynes/cm.^{2} at $\simeq$ 50 per cent extension in water at about 30° C. Since the microfibrils are in effect taking all the stress at low extensions, the effective loading is approximately twice this value, namely, 16×10^8 dynes/cm.^{2}.

This leads to a free-energy difference of $\simeq 4{\cdot}5$ kcal./mole, which may be compared with a figure derived by Ruoff *et al.*[23] of 4·7 kcal./mole, and thus further confirms the adequacy of the molecular model involved.

Further work on elucidating the transformation mechanism, and the factors involved in crystallization in wool keratin is proceeding.

I am grateful for the assistance of Prof. J. B. Speakman, H. J. Woods and the Wool Textile Research Council.

[1] Skertchly, A., *Nature*, **180**, 802 (1957).

[2] Skertchly, A., *J. Sci. Instrum.*, **37**, 6 (1960.

[3] Feughelman, M., *Text. Res. J.*, **29**, 223 (1959).

[4] Andrews, M. W., and Sikorski, J., *Proc. Conf. Electron Microscopy Stockholm* (1956).

[5] Rogers, G. E., *J. Ultrastructure Res.*, **2**, 309 (1959).

[6] Fraser, R. D. B., and MacRae, T. P., *J. Chem. Phys.*, **28**, 1120 (1958).

[7] Klug, H. P., and Alexander, L. E., *X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials* (Chapman and Hall, London, 1954).

[8] Astbury, W. T., and Lomax, R., *J. Chem. Soc.*, 846 (1935).

[9] Fraser, R. D. B., and MacRae, T. P., *Nature*, **179**, 732 (1957).

[10] Skertchly, A., *J. Text. Inst.*, **51**, *T*528 (1960).

[11] Bendit, E. G., *Nature*, **179**, 535 (1957).

[12] Skertchly, A., and Woods, H. J., *J. Text. Inst.*, **51**, *T*517 (1960).

[13] Skertchly, A., *Nature*, **181**, 639 (1958).

[14] Woods, H. J., in Meredith, R. (edit.), *The Mechanical Properties of Textile Materials* (North Holland Pub. Co., Amsterdam, 1956).

[15] Astbury, W. T., and Street, R., *Phil. Trans. Roy. Soc.*, A, **230**, 75 (1931).

[16] Middlebrook, W. R., *Biochim. Biophys. Acta*, **7**, 547 (1951).

[17] Corfield, M. C., Robson, A., and Skinner, B., *Biochem. J.*, **68**, 348 (1958).

[18] Hailwood, A. J., and Horrobin, S., *Trans. Farad. Soc.*, **42**, B, 84 (1946).

[19] Astbury, W. T., and Woods, H. J., *Phil. Trans. Roy. Soc.*, A, **232**. 333 (1933).

[20] Crewther, W. G., and Dowling, L. M., *J. Text. Inst.*, **51**, *T*775 (1960).

[21] Feughelman, M., *J. Text. Inst.*, **51**, *T*589 (1960).

[22] Pauling, L., and Corey, R. B., *Nature*, **171**, 59 (1953).

[23] Ruoff, A. L., and Eyring, H., *Proc. Intern. Wool Textile Res. Conf.*, Australia (1955).

PEAK-SHIFT TECHNIQUE IN GAS-LIQUID CHROMATOGRAPHY: TRIMETHYLSILYL ETHER DERIVATIVES OF ALCOHOLS

By STANLEY H. LANGER* and PETER PANTAGES

U.S. Bureau of Mines, Pittsburgh 13, Pa.

STABLE solid crystalline derivatives with melting points in the range of 50°–300° C. have been of greatest interest for classical qualitative organic chemical analysis and identification. Recently, the growth of the analytical application of mass spectrometry[1,2] and particularly gas chromatography[3–11] has stimulated considerably greater interest in liquid derivatives of organic compounds with their increased volatility, reduced adsorption on solid surfaces and enhanced separation factors. Despite this, with the exception of several peak subtraction techniques and the hydrogenation of unsaturated materials[12], identification and analysis via the 'peak shift' resulting from conversion of volatile materials to volatile derivatives has received only limited attention. Some advantages, as well as complications, can be illustrated by consideration of a hydrogenated Fischer–Tropsch fraction, b.p. 110°–115° C., after hydrogenation obtained from a synthesis using iron nitride catalyst[13].

The principal components of the Fischer–Tropsch fraction were octane, 2-methyloctane, nonane, 1-butanol, 2-methylbutanol, and 1-pentanol. Treatment of the mixture with hexamethyldisilazane was used to convert the alcohols to their trimethylsilyl derivative[3]. Some retention volume data for three liquid phases are shown in Table 1. The trimethylsilyl ethers will emerge from the column before the parent alcohols on the polar polyesteramide 'Flexol 8*N*8' liquid phase (because of hydrogen bonding, the alcohols tend to be retained) and after the parent alcohols on Dow Corning 'Silicone 550' (solubility increased as solubility parameters are more nearly equal[14]). Thus, peak shift should be a definite aid to identification after treatment with a selective reagent such as hexamethyldisilazane. Furthermore, the volatile derivative provides access to another gas chromatographic parameter for identification similar to that obtained from chromatography of one compound on two liquid phases[15,16]. That is, a plot of log retention volume of parent compound versus log retention volume of derivative is linear for a homologous series. A plot of such values for Dow Corning 'Silicone 550' is shown in Fig. 1. The 2-methyl and normal alcohols apparently fall on different lines. The linearity of plots such as that shown in Fig. 1 is to be expected when the logarithm of retention volumes of both the parent compounds and derivatives are linear functions of the number of carbon atoms in the chain.

Difficulties are encountered when members of different classes occur in the same mixture[16] except when there is a large disparity in amounts present. Thus, the retention volume of 1-butyl trimethylsilyl ether coincides with that of octane on silicone oil and on 'Flexol 8*N*8', while the trimethylsilyl derivative of pentanol tends to emerge close to nonane. However, the disappearance of the alcohol peak is definite and the increase in the hydrocarbon peak, if there is a hydrocarbon present, is often apparent. The parent compound of interest may also be trapped and reacted with hexamethyldisilazane for definite identification. Other workers[10] at the U.S. Bureau of Mines have performed identifications similar to this by comparing retention volumes of sulphur-

Table 1. CORRECTED RETENTION VOLUMES* ON VARIOUS LIQUID PHASES AT 97·5°

	D.C. 'Silicone 550'		'Flexol 8*N*8'†		Pentatriacontane ‖	
Octane	68·8		108·0		184·8	
2-Methyloctane	—		—		351·0	
Nonane	140 §		227·0 §		402 §	
2-Methyl-1-propanol	27·6	48‡	123		—	
2-Methyl-1-butanol	63·0	100·4‡	—		—	210‡
2-Methyl-1-pentanol	128·8	180·5‡	—		—	
1-Propanol	17·8	35·6‡	72·4	64·7‡	—	65·8‡
1-Butanol	38·3	68·5‡	162	108·5‡	—	133‡
1-Pentanol	80·6	135‡	352	216·3‡	—	280‡
1-Hexanol	167	270·3‡	—	—	—	—
1-Heptanol	338	550‡	—	—	—	—

* In ml./gm. at column temperature (V_R of air = 0) and atmospheric pressure.

† Product of Union Carbide Chemical Co., 2,2′(2-ethylhexamido)-diethyl-di(2-ethylhexoate).

‡ For trimethylsilyl ether of indicated alcohol.

§ Calculated from other hydrocarbon retention data.

‖ From hydrogenation of 17-pentatriacontene (Armour and Co., Chicago, Illinois).

---

* Present address: American Cyanamid Co. Central Research Department, Stamford, Connecticut.

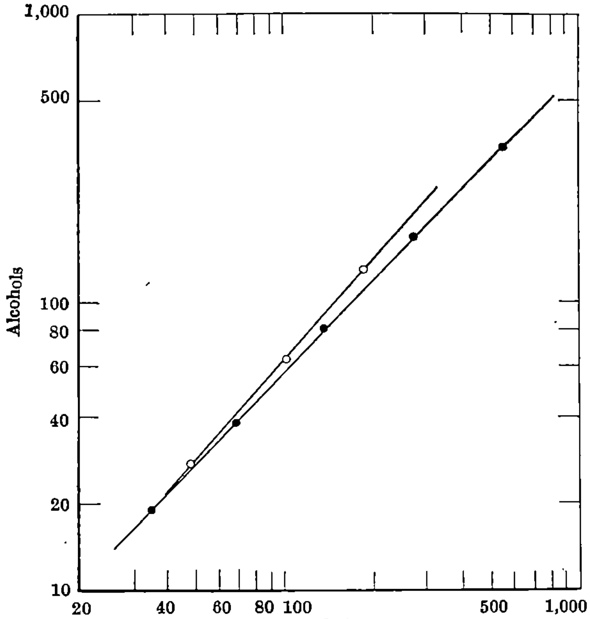


Fig. 1. Corrected retention volumes, ml./gm. on Dow Corning 'Silicone 550' at 97·5° C. Alcohols versus their trimethylsilyl derivatives (log log scale)
●, *n*-alcohols; ○, 2-methyl alcohols

containing materials and derived hydrocarbons after desulphurization.

Chromatograms illustrating the behaviour of the Fischer–Tropsch fraction on pentatriacontane before and after treatment with hexamethyldisilazane are shown in Fig. 2. From curve B, it is seen that the alcohols are not resolved and are eluted early (9–16 min.) from the non-polar hydrocarbon liquid phase so that the hydrocarbon portion of the chromatogram is simplified. The shifting of the alcohols after treatment with hexamethyldisilazane, as shown in curve *A*, is in itself diagnostic; in addition to identification, analysis of the alcohols as well as hydrocarbons is now possible on the non-polar liquid phase by calibration against one of the hydrocarbons or addition of an 'internal standard' which may be a hydrocarbon or a hydroxyl-containing material. The peak at 6–9 min. is due to excess hexamethyldisilazane and some hexamethyldisiloxane due to reaction with water[6]. The reaction with, and removal of, water by hexamethyldisilazane to form hexamethyldisiloxane is an additional advantage of this reagent. Peaks at about 21, 41, and 45 min. are due to octane, 2-methyloctane and nonane. Identity of the alcohols is indicated in Fig. 2.

Experience with the trimethylsilyl ethers as derivatives for alcohols indicates that additional development of convenient reagents for functional groups which react quantitatively to give volatile

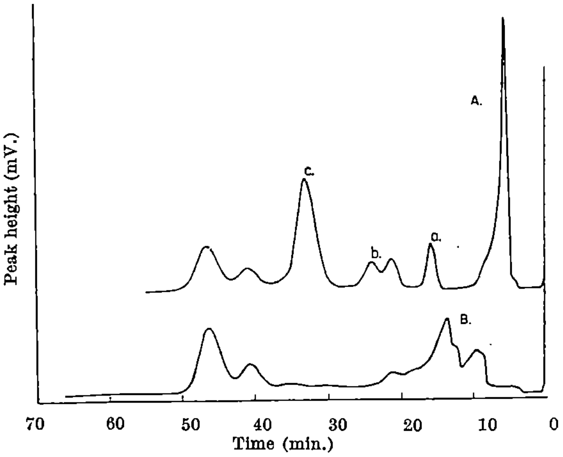


Fig. 2. 'Peak shift' after treatment of Fischer–Tropsch product with hexamethyldisilazane. *a*, Trimethylsilyl ether of 1-butanol; *b*, trimethylsilyl ether of 2-methyl-1-butanol; *c*, trimethylsilyl ether of 1-pentanol. B.P., 110–115° C.; flow, 40 c.c./min.; ΔP, 415 mm.; pentatriacontane column; *T*, 97·8° C.

derivatives should increase the utility of gas chromatography for organic analysis appreciably. It is desirable also that such reagents be specific and that the retention volumes of derivatives obtained be considerably different from those of present compounds.

[1] *Advances in Mass Spectrometry*. Proc. Joint Conf. by Inst. Petroleum Hydrocarbon Res. Group and ASTM Comm. *E*-14, edit. by Waldron, J. (Pergamon Press, London 1959).

[2] Sharkey, jun., A. G., Friedel, R. A., and Langer, S. H., *Anal. Chem.* **29**, 770 (1957). Langer, S. H., Friedel, R. A., Wender, I., and Sharkey, jun., A. G., *ibid.*, **30**, 1353 (1958).

[3] Cropper, F. R., and Heywood, A., *Nature*, **172**, 1101 (1953). James, A. T., and Martin, A. J. P., *Biochem. J.*, **63**, 144 (1956). Beerthuis, R. K., and Recourt, J. H., *Nature*, **186**, 372 (1960). Orr, C. H., and Callen, J. E., *Ann. N.Y. Acad. Sci.*, **72**, 649 (1959). And other references on fatty acid esters. Cf. *Gas Chromatography Abst.*, edit. by Knapman, C. E. H. (Butterworths, London, 1958, 1959).

[4] Carruthers, W., Johnstone, R. A. W., and Plimmer, J. R., *Chem. and Indust.*, **11**, 331 (1958).

[5] Langer, S. H., Pantages, P., and Wender, I., *Chem. and Indust.*, 1664 (1958).

[6] McInnes, A. G., Ball, D. H., Cooper, F. P., and Bishop, C. T., *J. Chromatog.*, **1**, 556 (1958).

[7] Chang, C. D., and Hass, H. B., *J. Org. Chem.*, **23**, 773 (1958), Hedgley, E. J., and Overend, W. G., *Chem. and Indust.*, 378 (1960).

[8] Hunter, I. R., Dimick, K. P., and Corse, J. V., *Chem. and Indust.*, 294 (1956).

[9] Bayer, E., *Gas Chromatography*, edit. by Desty, D. H., 333 (Butterworths, London, 1958).

[10] Thompson, C. J., Coleman, C. J., Ward, C. C., and Rall, H. T.. *Anal. Chem.*, **32**, 424 (1960).

[11] Drawert, F., and Kupfer, G., *Angew. Chem.*, **72**, 33 (1960).

[12] Nelson, K. H., Hines, W. J., Grimes, M. D., and Smith, D. E., *Anal. Chem.*, **32**, 1110 (1960).

[13] Anderson, R. B., Feldman, J., and Storch, H. H., *Indust. and Eng. Chem.*, **44**, 2418 (1952). Schlesinger, M. D., Benson, H. E., Murphy, E. M., and Storch, H. H., *ibid.*, **46**, 1322 (1954).

[14] Hildebrand, J. H., and Scott, R. L., *Solubility of Non-Electrolytes*, third ed. (Reinhold Publishing Co., New York, 1950).

[15] James, A. T., and Martin, A. J. P., *J. App. Chem.*, **6**, 105 (1956).

[16] Lewis, J. S., Patton, H. W., and Kaye, W. I., *Anal. Chem.*, **28**, 1370 (1956).

MANNOSYL D-GLUCOSAMINE HYDROCHLORIDE ISOMERS

N-Mannosyl D-Glucosamine Hydrochloride

When a D-glucosamine hydrochloride-D-mannose mixture (each 5 per cent w/v in water) was applied to Whatman No. 3 paper, heated for 15 min. at 100° and thereafter submitted to ionophoresis in 0·2 *M* acetate buffer *p*H 5 at 8 V./cm. for 4 hr. a new component, now identified as *N*-mannosyl D-glucosamine hydrochloride, was detected. This novel compound ($M_{\text{glucosamine}}$ 0·56) gave positive reactions with ninhydrin[1], the Elson–Morgan reagents[2], aniline hydrogen phthalate[3], alkaline silver nitrate[4] and alkaline triphenyltetrazolium chloride[4], but not with naphthoresorcinol[2]. In order to isolate larger quantities of *N*-mannosyl D-glucosamine hydrochloride it was necessary to carry out the reaction described above using the sugar mixture (50 gm.) freeze-dried on to cellulose powder (200 gm.). Batches of the products (4–6 gm.) were then eluted with water, freeze-dried, dissolved in 0·3 *N* hydrochloric acid and applied to a column of 'Zeokarb 225' [H^+] (length, 53 cm.; diam., 8 cm.). Elution with 0·3 *N* hydrochloric acid and analysis of the 50 ml. fractions collected showed separations between D-mannose (tubes 20–30; optically active), 5-hydroxymethyl 2-furaldehyde[5] (tubes 86–96 ; absorption at 284 mμ), *N*-mannosyl D-glucosamine hydrochloride (tubes 96–106; determined by Elson–Morgan[6] and ninhydrin[7] reagents) and unreacted D-glucosamine (tubes 140–175; optically active, Elson–Morgan and ninhydrin-positive). The 5-hydroxymethyl 2-furaldehyde was characterized by its ultra-violet absorption spectrum ($\lambda_{\max.}$ 284 mμ), chromatographic behaviour, partition coefficients between two different solvent systems (0·3 *N* hydrochloric acid–chloroform and 0·3 *N* hydrochloric acid–ether) and isolation of its 2 : 4-dinitrophenylhydrazone (m.p. and mixed m.p. 200–1°). The solution containing *N*-mannosyl D-glucosamine was neutralized by extraction with a chloroform solution of di-*n*-octylmethylamine[8] and freeze-dried (yield 50–100 mgm.).

N-mannosyl D-glucosamine hydrochloride showed no ultra-violet absorption and was virtually unchanged in 0·3 *N* hydrochloric acid at room temperature during two weeks. Complete hydrolysis of *N*-mannosyl D-glucosamine hydrochloride (300 mgm.) occurred when it was heated in water (30 ml.) at 100° for 12 hr. and was accompanied by extensive browning. The products were separated on the ion-exchange column and characterized as D-mannose, 40 mgm. (isolated as its *p*-nitroanilide m.p. and mixed m.p. 208°), a neutral sugar present in much smaller amount (separated from mannose by paper chromatography; probably glucose from its R_F value and the ionophoretic mobility of its borate complex), 5-hydroxymethyl 2-furaldehyde (isolated as its 2 : 4-dinitrophenylhydrazone m.p. 201°) and D-glucosamine hydrochloride (88 mgm. obtained crystalline with $[\alpha]_D + 86°$ (init.) → + 69° (equil.) (*c*, 1·69 in water) and further characterized as its *N*-carbobenzoxy derivative, m.p. and mixed m.p. 211°–213° (*d*)). When *N*-mannosyl D-glucosamine hydrochloride was left in sterile water at room temperature it was almost completely hydrolysed in 30 days to glucosamine hydrochloride and mannose. No ultra-violet absorption attributable to 5-hydroxymethyl 2-furaldehyde was detectable. Direct determination of the mannose (assayed with orcinol–sulphuric acid[9]; chromogen identical with that of authentic mannose) and glucosamine hydrochloride (Elson–Morgan reagents[6]; chromogen $\lambda_{\max.}$ 530 mμ) content of *N*-mannosyl D-glucosamine hydrochloride revealed a ratio by weight of glucosamine hydrochloride to mannose of 98 : 95, confirming that it contained equimolar proportions of the two sugars. The instability of the new compound in aqueous solution precludes the possibility of an *O*-glycosyl linkage. When the condensation was effected between ^{14}C-mannose and glucosamine hydrochloride it was found that the 5-hydroxymethyl 2-furaldehyde obtained both from the reaction mixture and the degradation of the *N*-mannosyl-D-glucosamine was radioactive as indeed was the *N*-mannosyl D-glucosamine hydrochloride. Hence the 5-hydroxymethyl 2-furaldehyde originates from the mannose moiety as would be expected for a glycosylamine. *N*-mannosyl D-glucosamine hydrochloride gave negative results in the methylene blue, 2 : 6-dichlorophenol–indophenol and Seliwanoff tests for Amadori compounds[10,11], and its infra-red spectrum did not contain a peak at 1,720 cm.$^{-1}$ due to a carbonyl group such as has been observed in the infra-red spectra of certain Amadori compounds[12].

Indirect evidence in support of the glycosylamine structure accrues from a study of the formation of compounds analogous to *N*-mannosyl D-glucosamine hydrochloride by heating several other mixtures of amino-sugars with reducing sugars and their derivatives. Since no analogous compound was produced from *N*-acetylglucosamine and a reducing sugar or from glucosamine hydrochloride and non-reducing sugars (mannitol, α-methylmannoside, 1-deoxy D-glucose, sorbitol, dulcitol and α-methylglucoside) it appeared that such reactions were a consequence of reaction between the reducing group of a sugar (aldose, not a ketose) and the amino group of a second compound. Thus mannose formed a condensation compound not only with D-glucosamine and D-galactosamine hydrochlorides but also with the hydrochlorides of D-glucosaminol and 2-aminoglycerol. Another necessary condition appears to be that the C_2 position of the reducing sugar should not be substituted. Thus while condensation compounds were formed from 3-*O*-methyl and 3 : 4-di-*O*-methyl D-glucose and 3 : 4-di-*O*-methyl D-mannose when heated with D-glucosamine hydrochloride, no such compounds were detected with 2 : 3-di-, 2 : 3 : 6-tri- and 2 : 3 : 4 : 6-tetra-*O*-methyl D-glucose. Steric hindrance is the most likely cause of this phenomenon.

One of us (K. M.) is grateful to Glaxo, Ltd., for the award of a scholarship.

S. A. Barker
K. Murray
M. Stacey

6-O-Mannosyl D-Glucosamine Hydrochloride

Since the work reported in the previous article, we have found that under more vigorous conditions (3 hr. at 120°) disaccharides of the *O*-glycosyl type are also formed. The isolation and characterization of one of these, 6-*O*-mannosyl D-glucosamine hydrochloride, is the subject of the present communication.

After heat treatment, the resultant products were extracted with water and boiled for 12 hr. to hydrolyse the *N*-mannosyl D-glucosamine hydrochloride present since this had a similar elution position to the oxygen-linked disaccharides on 'Zeokarb 225' (H^+, 200–400 mesh, 4½ per cent cross-linking). Subsequent fractionation on the resin yielded the residual disaccharides in two major fractions *A* (250 mgm.) and *B* (100 mgm.), being the products from 10 gm. of reaction mixture. *B* was homogeneous ($R_{\text{glucosamine}}$ 0·35) on a paper chromatogram irrigated with the organic phase of butan-1-ol, ethanol and water (4 : 1 : 5) while *A* contained two components ($R_{\text{glucosamine}}$ 0·35 and 0·47). *B* reacted with ninhydrin[1], the Elson–Morgan reagents[2], alkaline silver nitrate[4], aniline hydrogen phthalate[3], alkaline triphenyltetrazolium chloride[4], but not naphthoresorcinol[2]. It had $[\alpha]_D^{20} + 55°$ (*c*, 1·0 in water) and a mobility 0·7 that of glucosamine when separated by paper electrophoresis in 0·2 *M* acetate buffer *p*H 5.

Complete hydrolysis of *B* with 2 *N* hydrochloric acid for 3 hr. at 100° yielded equimolar amounts of mannose and glucosamine hydrochloride. These results suggested that *B* was an *O*-mannosyl glucosamine hydrochloride. *N*-acetylation[13] of *B* (250 mgm.) yielded a product (250 mgm.; $R_{\text{glucosamine}}$ 0·6) the chromogen of which exhibited 26 per cent of the absorption at 590 mμ of an equal weight of *N*-acetylglucosamine in the Morgan–Elson determination[14]. Reduction of *N*-acetyl *B* (200 mgm.) with sodium borohydride (200 mgm.) in water (20 ml.) at room temperature for 5 hr. yielded a crystalline *O*-mannosyl 2-acetamido 2-deoxy glucitol (43 mgm.; from methanol–acetone), m.p. 202°–203°; $[\alpha]_D$ − 50·6° (*c*, 1·0 in water). Found: C, 43·5; H, 6·9; N, 3·6. Calc. for $C_{14}H_{27}O_{11}N$: C, 43·6; H, 7·1; N, 3·6 per cent. Hydrolysis with 2 *N* hydrochloric acid for 3 hr. at 100° gave D-mannose and D-glucosaminol, establishing that the glucosamine-moiety formed the reducing portion of the original disaccharide. On periodate oxidation, the *O*-mannosyl 2-acetamido 2-deoxy glucitol consumed 3·9 moles of periodate and liberated 1·8 moles of formic acid per mole of alcohol. No formaldehyde was liberated. Reduction of the periodate oxidized disaccharide alcohol followed by hydrolysis with 0·5 *N* hydrochloric acid at 100° for 1 hr. yielded *inter alia* 2-amino 2-deoxy glyceritol. Hence the disaccharide alcohol was 6-*O*-D-mannopyranosyl 2-acetamido 2-deoxy D-glucitol. Application of Hudson's rules in the manner used by Stavely and Fried[15] to assess the configuration of the mannosyl residue in mannosido–streptomycin gives a value (*A*) of *c*. − 18,000 for the contribution of carbon atom one of the mannose residue to the molecular rotation. The negative value of *A* (cf. A_{OMe} = 14,230) indicates a β-linkage in the disaccharide alcohol. The rotation of the 6-*O*-mannosyl glucosamine hydrochloride, that is, + 55°, compares favourably with the rotations of related 6-*O*-β-D-mannosyl disaccharide derivatives[16].

The disaccharide probably arises from a condensation between 1 : 6-anhydro-D-mannopyranose, formed during the pyrolysis, and D-glucosamine hydrochloride. Indeed, a component with the same R_F value as the β-anhydro sugar has been detected in the pyrolysis mixture, which is not unexpected since pyrolysis is used in the preparation of the anhydro sugar from ivory nut mannan[17]. It is noteworthy that a heated mixture (1 : 1) of this anhydro sugar and D-glucosamine hydrochloride gave a 20 per cent greater yield (by weight and by Elson–Morgan reaction[6]) of *O*-mannosyl glucosamine type disaccharides than a heated D-mannose D-glucosamine hydrochloride mixture. Furthermore, L-rhamnose (6-deoxy-L-mannose), which cannot form a 1,6-anhydro derivative, did not yield Elson–Morgan-positive[6] disaccharides.

We are indebted to Dr. N. K. Richtmyer for a sample of 1 : 6-anhydro β-D-mannopyranose and one of us (D. B. E. S.) thanks the University of Birmingham for a scholarship.

S. A. Barker
K. Murray
M. Stacey
D. B. E. Stroud

Chemistry Department,
University, Birmingham 15.

[1] Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).

[2] Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).

[3] Partridge, S. M., *Nature*, **164**, 443 (1949).

[4] Trevelyan, W. E., Proctor, D. P., and Harrison, J. S., *Nature*, **166**, 444 (1950).

[5] Barker, S. A., Murray, K., and Stacey, M., *Nature*, **186**, 469 (1960).

[6] Svennerholm, L., *Acta Soc. Med. Upsal.*, **61**, 287 (1956).

[7] Yemm, E. W., and Cocking, E. C., *Analyst*, **80**, 209 (1955).

[8] Lester-Smith, E., and Page, J. E., *J. Soc. Chem. Indust.* (Trans.), **67**, 48 (1948).

[9] Svennerholm, L., *J. Neurochem.*, 1, 42 (1956).

[10] Hodge, J. E., *J. Agric. Food Chem.*, **1**, 928 (1953).

[11] Anet, E. F. L. J., *Austral. J. Chem.*, **10**, 193 (1957).

[12] Micheel, F., and Frowein, A., *Chem. Ber.*, **90**, 1599 (1957); **92**, 304 (1959).

[13] Roseman, S., and Ludowieg, J., *J. Amer. Chem. Soc.*, **76**, 301 (1954).

[14] Aminoff, D., Morgan, W. T. J., and Watkins, W. M., *Biochem. J.*, **51**, 379 (1952).

[15] Stavely, H. E., and Fried, J., *J. Amer. Chem. Soc.*, **71**, 135 (1949).

[16] Talley, E. A., Reynolds, D. D., and Evans, W. L., *J. Amer. Chem. Soc.*, **65**, 575 (1943).

[17] Knauf, A. E., Hann, R. M., and Hudson, C. S., *J. Amer. Chem. Soc.*, **63**, 1447 (1941).

COUPLING OF PHOSPHORYLATION TO ELECTRON AND HYDROGEN TRANSFER BY A CHEMI-OSMOTIC TYPE OF MECHANISM

By Dr. PETER MITCHELL

Chemical Biology Unit, Zoology Department, University of Edinburgh

At present, the orthodox view of the coupling of phosphorylation to electron and hydrogen transfer in oxidative and photosynthetic phosphorylation stems from knowledge of substrate-level phosphorylation[1,2]. It is based, consequently, on the idea that water is expelled spontaneously between two chemical groups, *A* and *B*, by the formation of a strong bond (of low hydrolysis energy), and that subsequent or simultaneous oxidation or reduction of *A–B* to *A–B** can result in a weakening of the bond, popularly written $A \sim B^*$, so that adenosine triphosphate (ATP) can be synthesized by coupling the opening of $A \sim B^*$ to the closing of the 'high-energy bond' between adenosine diphosphate (ADP) and phosphorus through group transfer systems of appropriate substrate and oxido-reduction-carrier specificities[3–5]. There are a number of facts about the systems catalysing oxidative and photosynthetic phosphorylation that are generally acknowledged to be difficult to reconcile with this orthodox (chemical) view of the mechanism of coupling. For example: (*a*) The hypothetical 'high-energy' intermediates (for example, reduced diphosphopyridine nucleotide (DPNH) ∼ ?, reduced flavin adenine dinucleotide (FADH) ~ ?, cytochrome ~ ?) are elusive to identification[4,6]. (*b*) It is not clear why phosphorylation should be so closely associated with membranous structures[7–9]. It has sometimes been assumed that digitonin-treated mitochondrial 'particles' can couple oxidation to phosphorylation without membranes[10]; but it is doubtful whether this assumption can be justified, for such 'particles' give only poor respiratory control and contain much lipid[11], which may well exist as a leaky membrane[12]. (*c*) Coupling may vary with the stress, causing variation of respiratory control without corresponding variation in phosphorus/oxygen quotient[4,13]—a phenomenon difficult to explain in terms of molecular stoichiometry. (*d*) Hydrolysis of external ATP by mitochondria causes reduction of internal DPN, accentuated by the oxidation of succinate[14]. There is disagreement as to what complex assumptions offer the better explanation[15]. (*e*) Uncoupling can be caused at all three hypothetical oxido-reduction sites in mitochondria by agents that do not share an identifiable specific chemical characteristic[4] (for example, dinitrophenol, dicoumarol, salicylate, azide). (*f*) Unexplained swelling and shrinkage phenomena accompany the activity of the phosphorylation systems[7,9a,11,16].

Structural features have been invoked as the causes of the departure of the phosphorylation systems from 'ideal' behaviour[4,14,17]; but, as Green has pointed out in a far-sighted paper[18], although the structural features have been recognized as playing an important part in the catalytic activity of multi-enzyme systems, they have so far been treated rather conservatively. The structural (or supramolecular) features have, in fact, generally been regarded only as modifiers of the basic chemical type of coupling process outlined above. The general conception of enzyme-catalysed group translocation that we have been developing in my laboratory for some years[19] offers a more radical approach to the problem; but this has not, so far, been made use of by those working in the field of oxidative and photosynthetic phosphorylation.

The purpose of this article is to suggest that in view of the difficulties confronting the orthodox chemical conception of coupling in oxidative and photosynthetic phosphorylation, one might now profitably consider the basic requirements and potentialities of a type of mechanism that is based directly on the group translocation conception. This type of mechanism differs fundamentally from the orthodox one in that it depends absolutely on a supramolecular organization of the enzyme systems concerned. Such supramolecularly organized systems can exhibit what I have called chemi-osmotic coupling[19c,20], because the driving force on a given chemical reaction can be due to the spatially directed channelling of the diffusion of a chemical component or group along a pathway specified in space by the physical organization of the system[20]. We shall consider chemi-osmotic coupling between the so-called ATPases on one hand and the electron and hydrogen transfer chain on the other: mediated by the translocation of electrons and the elements of water across the membrane of mitochondria, chloroplast grana, and chromatophores.

The first basic feature of the chemiosmotic coupling conception is a membrane-located reversible 'ATPase' system[20b] shown very diagrammatically in Fig. 1. This system, which may include lipids and other components as well as proteins, is assumed to be anisotropic so that the active centre region (indicated by the dotted circle) is accessible to OH' ions but not H^+ ions from the left (inside organelle), and to H^+ ions but not OH' ions from the right (cytoplasm side of organelle). The active centre, like that of phosphokinases in general, is assumed to be relatively inaccessible to water as water. To illustrate the hydrolytic activity of the system, the OH' ion is depicted as diffusing from left to right and finally combining at the active centre region with the terminal phosphorylium (P^+) derived from the ATP (ADP—O′—P^+) giving inorganic phosphate (POH) and ADP having a terminal ionized oxygen (ADP—O′), assuming the right-hand phase to be at about *p*H 7 and at electrical neutrality. The elegant conception of the transfer of phosphoryl as phosphorylium ion is due to Lipmann[21]. The system, of course, catalyses hydrolysis equilibrium, and the reverse or phosphokinetic activity of the 'ATPase' is indicated by the single barbs on the arrows of Fig. 1. In the case of the phosphokinetic activity, the OH' ion is depicted as passing down a free-energy gradient towards the left from an inorganic phosphate group (POH) that passes to the active centre region from the right. The phosphorylium ion (P^+), created by the withdrawal of the OH', is attacked by the negative atom ($-R'$) in the active centre region, and the phosphorylium ion is then donated to the terminal oxygen of ADP—O′ to give ATP. The chemi-osmotic coupling hypothesis depends thermodynamically on the fact that in such an anisotropic ATPase system, the electrochemical activity of the water at the active centre ($[H_2O]_c$), which determines the poise of the hydrolysis equilibrium in the ATP/ADP system, would be given, not by the product of $[H^+] \times [OH']$ in the aqueous phases L or R, but by the product $[H^+]_R \times [OH']_L$ (where [] stands for electrochemical activity, and R and L for right- and left-hand phases respectively). The ratio of the electrochemical activity [ATP]/[ADP] (including all ionic forms) can be raised, consequently, and the ATPase activity can be reversed to give an ADP phosphokinase activity proportional to the lowering of $[H_2O]_c$, in accordance with the mass-action law for hydrolysis equilibrium, written to include the elements of water as follows:

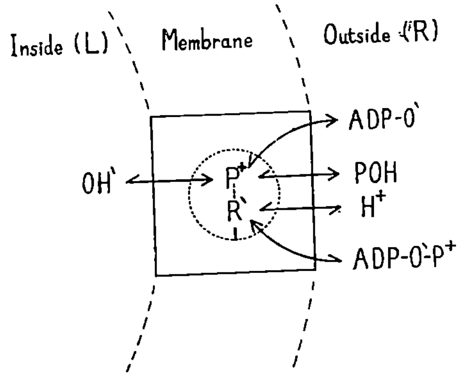


Fig. 1. Anisotropic reversible 'ATPase' system located in an ion-impermeable membrane between aqueous phases *L* and *R*

$$\frac{[ATP]}{[ADP]} = \frac{[P]}{K_1[H_2O]_c} \qquad (1)$$

The electrochemical activity of a component in a certain place defines absolutely the escaping tendency of the particles of the component due both to the chemical and to the electrical pressure to which the particles are subject in that place at equilibrium. Since

$$K_2 = \frac{[OH']_L \times [H^+]_L}{[H_2O]_L} \tag{2}$$

and K_2 is independent of the medium because we are using electrochemical activities, we can describe the electrochemical activity of the water at the active centre of the 'ATPase' system as follows:

$$[H_2O]_c = [H_2O]_{aq.} \times \frac{[H^+]_R}{[H^+]_L} \tag{3}$$

where $[H_2O]_{aq.}$ stands for the electrochemical activity of water in the aqueous physiological media of phases L or R, and is equivalent to about 55·5 M water.

By the definition of the electrochemical activity:

$$\frac{[H^+]_L}{[H^+]_R} = 10^{pH_{R-L}} \times 10^{(mV._{L-R})\frac{F}{2303\,RT}} \tag{4}$$

where pH_{R-L} is the pH of phase R minus that of phase L; $mV._{L-R}$ is the membrane potential in millivolts, positive in phase L; R is the gas constant; F is the faraday; and the factor $\frac{F}{2303\,RT}$ is approximately 1/60. It can be seen from equation (4) that the ratio $[H^+]_L/[H^+]_R$ is multiplied by a factor of 10 for each pH unit more negative on the left, relative to the right, and for each 60 mV. membrane potential, positive on the left. Equations 1 and 3 show that the ratio [ATP]/[ADP] at equilibrium is determined by $[H^+]_L/[H^+]_R$ as follows:

$$\frac{[ATP]}{[ADP]} = \frac{[P]}{K_1[H_2O]_{aq.}} \times \frac{[H^+]_L}{[H^+]_R} \tag{5a}$$

When the right-hand phase (representing the cytoplasm) is the region of the zero or reference potential, the electrochemical activity ratio of total ATP to ADP will be nearly the same as the corresponding concentration ratio, and [P] will correspond approximately to the inorganic phosphate concentration. Thermodynamic data[22] show that at pH 7 and at physiological temperatures the 'hydrolysis constant' as usually defined or the product $K_1[H_2O]_{aq.}$ is approximately 10^5; and when [P] is at the physiological level of 10^{-2} M, equation (5a) can be written:

$$\frac{[ATP]}{[ADP]} \simeq 10^{-7} \frac{[H^+]_L}{[H^+]_R} \tag{5b}$$

Thus, the [ATP]/[ADP] equilibrium can be poised centrally through the 'anisotropic 'ATPase' by making the ratio $[H^+]_L/[H^+]_R$ about 10^7. Equation 3 shows that this could be done, for example, by poising the left side 2 pH units below and 300 mV. above the right side. Such is the basic thermodynamic conception of the mechanism of reversal of the 'ATPase' activity. In kinetic terms, the reversal of the 'ATPase' activity can be understood by regarding the electrochemical activity gradient of hydrogen and hydroxyl ions across the active centre region of the 'ATPase' as the cause of the donation of the phosphorylium ion to the negative acceptor atom ($-R'$) by the simultaneous withdrawal of an OH' ion from the inorganic phosphate down a steep gradient to the left and of an H^+ ion from $-R$H to the right to ionize $-R$H or to prevent H^+ from competing with phosphorylium ion for the acceptor $-R'$. Dehydration is accomplished by using the high $[H^+]$ region as a sink for OH' and the high $[OH']$ region as a sink for H^+. It should be understood that the phosphorylation of ADP can be strictly described as dehydration only when the standard state is less than pH 6 (see Lipmann[21]). Since, however, the hydrogen atom of the terminal hydroxyl group of ADP, involved in phosphorylation, is dissociated reversibly as an H^+ ion, when the standard state is taken as pH 7, the phosphorylation process is mainly that of dehydroxylation. From the kinetic point of view, the fundamental processes involved are dehydroxylation + deprotonation, or dehydroxylation. It is relevant to note that phosphorylation is not directly caused by raising $[H^+]$, but is due to depression of $[OH']$. For this reason, the hydrogen ion depicted in phase R of Fig. 1 (and correspondingly in Figs. 2 and 3) is shown as equilibrating with the active centre region of the 'ATPase' system, but not as being withdrawn stoichiometrically in relation to the withdrawal of OH'.

The second basic feature of the chemi-osmotic coupling conception is the electron and hydrogen translocation system, which is assumed to create the gradient of electrochemical activity of H^+ and OH'. Unlike the conception of the anisotropic 'ATPase', the idea of the anisotropic o/r system is not new, but stems from the work of Lund[23], and Stiehler and Flexner[24], and was first stated explicitly by Lundegårdh[25] more than 20 years ago. Lundegårdh's idea was more exactly defined in relation to ion transport by Davies and Ogston[26], and was elaborated by Conway[27]. It has been excellently reviewed by Robertson[28].

Fig. 2 illustrates how the electron transfer can affect the ratio $[H^+]_L/[H^+]_R$. The electron translocation and 'ATPase' systems are depicted as being placed in opposition in a charge-impermeable membrane. The hydrogen ions generated on the left and the hydroxyl ions generated on the right by the electron translocation system dehydrate ADP and inorganic phosphate (now simply denoted by P) to form ATP by withdrawing hydroxyl ions to the left and hydrogen ions to the right through specific translocation paths in the 'ATPase' system as described here. Conversely, of course, the effect of the back pressure of ATP hydrolysis is to force the o/r system towards reduction on the left (inside the

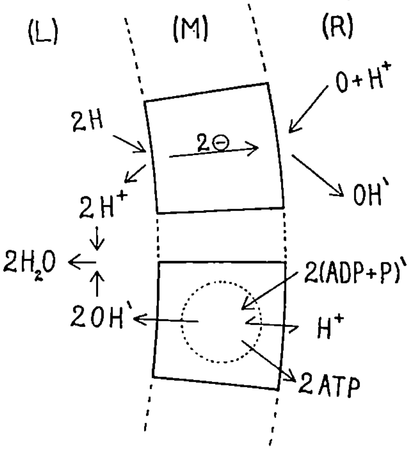


Fig. 2. Electron transport system (above) and reversible 'ATPase' system (below) chemiosmotically coupled in a charge-impermeable membrane (M) enclosing aqueous phase L in aqueous phase R

organelle). Note that the stoichiometry is 2 ATP per O, as in succinate oxidation by mitochondria. As shown in this simple diagram, the dehydrating force driving the phosphokinetic activity of the 'ATPase' system would be due largely to the chemical potential differential of the H^+ and OH' ions across the membrane, which would have to show as a *p*H difference of some 7 units across the membrane, acid on the left when the [ATP]/[ADP] equilibrium was poised centrally (see equations (5*b*) and (4)). We assume, however, that exchange diffusion carriers, as defined by Ussing[29], are present in the membrane and that they will allow strictly coupled one-to-one exchange of H^+ against K^+ or of OH' against Cl', for example. The *p*H differential would thus tend to be reduced to a relatively small figure and would be equivalently replaced by a membrane potential as described by equation (4).

When the oxido-reduction and phosphorylation systems are in chemi-osmotic equilibrium, one ATP molecule will be produced per electron translocated across the membrane. The relationship between the *o*/*r* potential span (ΔE) of the electron and hydrogen translocation system and the poise of the [ATP]/[ADP] equilibrium will be given as follows:

$$\frac{[\mathrm{ATP}]}{[\mathrm{ADP}]} = \frac{[\mathrm{P}]}{K_1[\mathrm{H_2O}]_{\mathrm{aq.}}} \times 10^{\frac{\Delta E.F}{2303\,RT}} \qquad (6a)$$

or approximately:

$$\frac{[\mathrm{ATP}]}{[\mathrm{ADP}]} = \frac{[\mathrm{P}]}{10^5} \times 10^{\Delta E/60} \qquad (6b)$$

ΔE being in millivolts. It should be understood that ΔE, as defined here, is equivalent to the free-energy change in the electron-translocating system per electron translocated. Assuming that ATP synthesis were occurring when the [ATP]/[ADP] ratio was poised centrally at *p*H 7 and in the presence of 10^{-2} *M* inorganic phosphate, the *o*/*r* span, ΔE, would have to be about 420 mV. This, of course, being the equilibrium potential, represents the minimum *o*/*r* span of the electron and hydrogen translocation system required to drive ATP synthesis under the conditions specified above. The span between the succinate–fumarate couple and oxygen at 76 mm. mercury pressure is about 750 mV.—well above the minimum ΔE of 420 mV.

In practice, the mitochondrial or chloroplast membrane across which the chemi-osmotic coupling may be organized would allow a certain amount of ion leakage, and the translocation paths for H^+ and OH', connecting the internal and external phases (*L* and *R*) with the active centre region of the 'ATPase', would not be expected to have absolute specificity for H^+ and OH' respectively. Consequently, equations (6*a*) and (6*b*) would represent the practical state of affairs in the most tightly coupled systems. On 'loosening' the membrane system, or if 'uncoupling' were effected by catalysing the equilibration of H^+ and charge across the lipid of the membrane with reagents like dinitrophenol or salicylate, the relationship between the poise of the [ATP]/[ADP] ratio and ΔE in the steady state would be described by the inequality:

$$\frac{[\mathrm{P}]}{K_1[\mathrm{H_2O}]_{\mathrm{aq.}}} \leqslant \frac{[\mathrm{ATP}]}{[\mathrm{ADP}]} \leqslant \frac{[\mathrm{P}]}{K_1[\mathrm{H_2O}]_{\mathrm{aq.}}} \times 10^{\frac{\Delta E.F}{2303\,RT}} \quad (6c)$$

The outer terms of equation (6*c*) represent the extreme values of the [ATP]/[ADP] ratio from complete uncoupling on the left to complete coupling on the right.

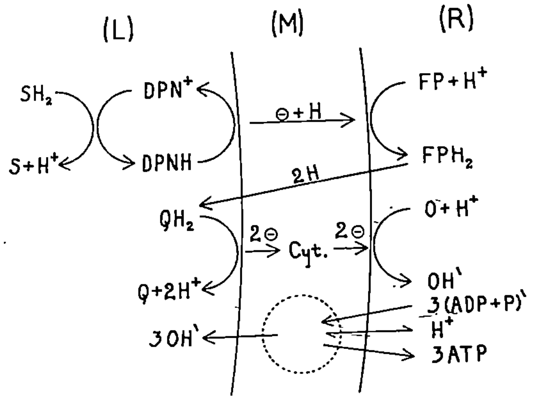


Fig. 3. Diagram of chemi-osmotic system for coupling phosphorylation to the oxidation of substrate (SH_2) through DPN, *FP* (tentatively identified with flavoprotein), *Q* (tentatively identified with a quinone) and the cytochromes (*Cyt.*). The other conventions are as in Figs. 1 and 2

Fig. 3 shows, in principle, a rather fuller description of oxidative phosphorylation, in which I have included the *o*/*r* components *FP* and *Q*, tentatively identified with flavoprotein and quinol–quinone systems respectively. The main aim is to illustrate how a stoichiometry of 3 ATP per O can readily be obtained for substrate (SH_2) oxidation through DPN, by the obligatory transport of one (net) hydrogen atom inwards per O, owing to the spatial arrangement of the electron and hydrogen transfer chain and the zero, one, or two hydrogen-transfer characteristics of the carriers involved. In this system, the span of both parts of the *o*/*r* chain across the membrane would have to be poised against the same ratio $[H^+]_L/[H^+]_R$ at equilibrium, according to equation (5*a*). Using the conventions of Dixon[2], the $-\Delta F$ value for the DPN/DPNH couple at about *p*H 5 would be some 3,000 cal. and the $-\Delta F$ for *FP* (corresponding to an E'_0 (*p*H 7) of -60 mV.) (ref. 30) would be about 17,000 cal., giving a span of 14,000 cal.; equivalent to a ΔE of 600 mV. Assuming the $-\Delta F$ of the *Q* system to be about 24,000 cal., corresponding to an E'_0 (*p*H 7) of $+100$ mV., as in the ubiquinone system[31], the span from the *Q* system to oxygen at 76 mm. mercury pressure would be equivalent to a ΔE of about 750–100 = 650 mV. The tendency of the two *o*/*r* values to drift together and the exact magnitude of the composite *o*/*r* potential would, of course, depend on many factors that it would be premature to consider here. It will suffice to point out at present that the value of 600–650 mV. for ΔE is appropriately above the required minimum of 420 mV. and that the proposed system is thus in accord with the thermodynamic facts.

The above basic chemi-osmotic conception can be applied to photosynthetic phosphorylation with the difference that the electron and hydrogen translocation are seen as being driven, not by the affinity of oxygen for the hydrogen atoms and electrons, but by the energy of the absorbed photons, according to the type of mechanism described by Calvin[9b]. It can readily be shown that in a chemi-osmotically coupled system for non-cyclic photophosphorylation, the photon-activated movement of 2 electrons and 2 hydrogen atoms outwards through the membrane of the grana would produce one O_2 and 2 ATP molecules. Similarly, in non-cyclic photophosphorylation, the skew of $[H^+]_L/[H^+]_R$, and thus the synthesis of ATP, could be caused by the photon-activated passage of equal numbers of hydrogen atoms and electrons in opposite directions across the membrane of the grana or chromatophores.

The facts that were listed at the beginning of this article as being difficult to reconcile with the orthodox chemical conception of the mechanism of coupling phosphorylation to electron and hydrogen transfer can now be reconsidered in relation to the chemi-osmotic coupling hypothesis: (*a*) The elusive character of the 'energy-rich' intermediates of the orthodox chemical coupling hypothesis would be explained by the fact that these intermediates do not exist. (*b*) According to the chemi-osmotic coupling hypothesis, the differential of the electrochemical activity of the hydrogen and hydroxyl ions across the membrane, generated by electron transport, causes the specific translocation of hydroxyl and hydrogen ions from the active centre of the so-called ATPase system, thus effectively dehydrating ADP + P. The charge-impermeable membrane would therefore be an absolute requirement for tight coupling. (*c*) Coupling would be expected to vary with the extent of leakiness or strain in the membrane, determined, of course, by the osmotic and electrical stress. (*d*) The internal components of mitochondria such as DPN would tend to be reduced by the high electrochemical activity of H^+ caused by hydrolysis of external ATP, which would withdraw OH' ions from the inside in competition with the electron-transport system. This effect would be accentuated by oxidation of succinate (which would raise the internal value of $[H^+]$) but not, of course, by substrates such as acetoacetate that directly oxidize DPNH. (*e*) Uncoupling would be caused by lipid-soluble reagents, such as DNP, salicylate, azide, and ammonia, catalysing equilibration of H^+ or OH' and charge across the membrane. (*f*) According to the chemi-osmotic type of hypothesis, the coupling of phosphorylation to electron and hydrogen translocation would cause considerable electrical and mechanical stress in the membrane across which coupling was effected. Complex swelling and shrinkage effects would therefore be expected to accompany the activity of the system.

It is evident that the basic features of the chemi-osmotic coupling conception described here and elsewhere[20] are in accord with much of the circumstantial evidence at present available from studies of oxidative and photosynthetic phosphorylation. This simple hypothesis also has the merit that it represents the result of carrying to its logical conclusion the present trend towards recognizing the equivalent status of supramolecular and molecular features in the channelling of chemical processes in living organisms[18]. Further experimental support for the chemi-osmotic coupling conception may best be sought by attempting to characterize separately each of the three hypothetical basic elements of which the system is thought to be built: (1) the anisotropic 'ATPase' system which I have defined above; (2) the anisotropic *o/r* system of the type originally defined by Lundegårdh; (3) the specific charge-impermeable membrane in which the systems 1 and 2 are supposed to be orientated in opposition. Work along these three lines is proceeding in my laboratory.

In the exact sciences, cause and effect are no more than events linked in sequence. Biochemists now generally accept the idea that metabolism is the cause of membrane transport. The underlying thesis of the hypothesis put forward here is that if the processes that we call metabolism and transport represent events in a sequence, not only can metabolism be the cause of transport, but also transport can be the cause of metabolism. Thus, we might be inclined to recognize that transport and metabolism, as usually understood by biochemists, may be conceived advantageously as different aspects of one and the same process of vectorial metabolism[20a,32].

I am indebted to the Nuffield Foundation for grants in aid of this work. It is also a pleasure to thank Dr. G. D. Greville, Prof. D. Keilin, Dr. R. Hill, Dr. Jennifer Moyle, Sir Rudolph Peters, and other colleagues for very helpful discussion and criticism.

[1] Lipmann, F., *Adv. Enzymol.*, **1**, 99 (1941); *Currents in Biochemical Research*, edit. by Green, D. E., 137 (Intersci. Pub., Inc., New York, 1946).

[2] Dixon, M., *Multienzyme Systems* (Cambridge Univ. Press, 1949).

[3] Slater, E. C., *Nature*, **172**, 975 (1953). Chance, B., and Williams, G. R., *Adv. Enzymol.*, **17**, 65 (1956). Myers, D. K., and Slater, E. C., *Nature*, **179**, 363 (1957). Dawkins, M. J. R., Judah, J. D., and Rees, K. R., *ibid.*, **182**, 875 (1958).

[4] Slater, E. C., and Hülsmann, W. C.; Chance, B.; Lehninger, A. L., Wadkins, C. L., and Remmert, LeM. F., in *Ciba Found. Symp. Regulation Metabolism*, edit. by Wolstenholme, G. E. W., and O'Connor, C. M., 58, 91 and 130, and associated discussion (Churchill, Ltd., London, 1959).

[5] Arnon, D. I., *Nature*, **184**, 10 (1959). Hill, R., and Bendall, F., *ibid.*, **186**, 136 (1960).

[6] Slater, E. C., in *Biological Structure and Function*, First IUB/IUBS Joint Symp., Stockholm, September 1960, edit. by Goodwin, T. W. (Academic Press, Inc., New York, in the press).

[7] Slater, E. C., *Symp. Soc. Exp. Biol.*, **10**, 110 (1957).

[8] Zeigler, D. M., Linnane, A. W., and Green, D. E., *Biochim. Biophys. Acta*, **28**, 524 (1958).

[9] (*a*) Lehninger, A. L., in *Biophysical Science: A Study Program*, edit. by Oncley, J. L., *et al.*, 136 (John Wiley and Sons, Inc., New York, 1959). (*b*) Calvin, M., *ibid.*, 147. (*c*) Vatter, A. E., and Wolfe, R. S., *J. Bacteriol.*, **75**, 480 (1958).

[10] Cooper, C., and Lehninger, A. L., *J. Biol. Chem.*, **219**, 489 (1956).

[11] Lehninger, A. L., *et al.*, *Science*, **128**, 450 (1958).

[12] Lehninger, A. L. (personal communication, 1960).

[13] Hoch, F. L., and Lipmann, F., *Proc. U.S. Nat. Acad. Sci.*, **40**, 909 (1954). Lipmann, F., in *Enzymes: Units of Biological Structure and Function*, edit. by Gaebler, O. H., 444 (Academic Press, Inc., New York, 1956).

[14] Chance, B., *Nature*, **189**, 719 (1961).

[15] Krebs, H. A., Hopkins Memorial Lecture, March 1961, *Biochem. J.* (in the press).

[16] Ernster, L., and Lindberg, O., *Ann. Rev. Physiol.*, **20**, 13 (1958). Beechey, R. B., and Holton, F. A., *Biochem. J.*, **73**, 29 P (1959). Lehninger, A. L., *Ann. N.Y. Acad. Sci.*, **86**, 484 (1960). Emmelot, P., *et al.*, *Nature*, **186**, 556 (1960). Emmelot, P., *ibid.*, **188**, 1197 (1960).

[17] Green, D. E., *Adv. Enzymol.*, **21**, 73 (1959). Lehninger, A. L., in *Biological Structure and Function*, First IUB/IUBS Joint Symp., Stockholm, September 1960, edit. by Goodwin, T. W. (Academic Press, Inc., New York, in the press).

[18] Green, D. E., *Symp. Soc. Exp. Biol.*, **10**, 30 (1957).

[19] (*a*) Mitchell, P., *Symp. Soc. Exp. Biol.*, **8**, 254 (1954); (*b*) *Nature*, **180**, 134 (1957); (*c*) in *Structure and Function of Subcellular Components*, Sixteenth Symp. Biochem. Soc., February 1957, edit. by Crook, E. M., 73 (Cambridge Univ. Press, 1959). (*d*) Mitchell, P., and Moyle, J., *Nature*, **182**, 372 (1958); (*e*) *Proc. Roy. Phys. Soc., Edinburgh*, **27**, 61 (1958).

[20] (*a*) Mitchell, P., in *Biological Structure and Function*, First IUB/IUBS Joint Symp., Stockholm, September 1960, edit. by Goodwin, T. W. (Academic Press, Inc., New York, in the press); (*b*) *Biochem. J.*, **79**, 23 P (1961).

[21] Lipmann, F., in *Molecular Biology*, edit. by Nachmansohn, D., 37 (Academic Press, Inc., New York, 1960).

[22] Atkinson, M. R., Johnson, E., and Morton, R. K., *Nature*, **184**, 1925 (1959).

[23] Lund, E. J., *J. Exp. Zool.*, **51**, 327 (1928).

[24] Stiehler, R. D., and Flexner, L. B., *J. Biol. Chem.*, **126**, 603 (1938).

[25] Lundegårdh, H., *Lantbr. Hogsk. Ann.*, **8**, 233 (1940).

[26] Davies, R. E., and Ogston, A. G., *Biochem. J.*, **46**, 324 (1950).

[27] Conway, E. J., *Internat. Rev. Cytol.*, **2**, 419 (1953).

[28] Robertson, R. N., *Biol. Rev.*, **35**, 231 (1960).

[29] Ussing, H. H., *Nature*, **160**, 262 (1947); *Physiol. Rev.*, **29**, 127 (1949).

[30] Kuhn, R., and Boulanger, P., *Ber.*, **69**, 1557 (1936).

[31] Morton, R. A., *Nature*, **182**, 1764 (1958).

[32] Mitchell, P., in *Membrane Transport and Metabolism*, Symposium, Prague, August 1960, edit, by Kleinzeller, A., and Kotyk, A. (Academic Press, Inc., New York, in the press).

TWO SEROLOGICALLY ACTIVE TRISACCHARIDES ISOLATED FROM HUMAN BLOOD-GROUP *A* SUBSTANCE

By I. A. F. LISTER CHEESE and PROF. W. T. J. MORGAN, C.B.E., F.R.S.

Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1

IT is recognized that the serological characters of human blood-group specific mucopolysaccharides are due to relatively simple carbohydrate structures[1]. A direct means of studying these characters in detail consists in the examination of hydrolysis fragments which retain a measure of the serological activity of the intact substance.

Côté and Morgan[2] reported the isolation of five disaccharides from group *A* substance and showed that one of them, believed to be *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose, was serologically active and inhibited the agglutination of *A* cells both by human *iso*-agglutinins and by an *A* specific reagent prepared from lima bean. They concluded that the disaccharide had a structure more closely associated with *A* specificity than any that had then been identified. This work has been extended and two trisaccharides showing *A* specificity have been isolated and are now described.

Blood-group *A* substance (22 gm.) was heated as a 2 per cent w/v solution in 0·1 *N* hydrochloric acid at 100° for 30 min. The solution was cooled and thoroughly dialysed to recover material of low molecular weight. This procedure was repeated on the indiffusible material, subsequent hydrolysis periods being 0·5 hr., 1 hr. and 2 hr. After removal of acid by passage through 'Deacidite *E*' (carbonate form) the diffusible material was broadly fractionated on a charcoal–'Celite' column into monosaccharides, disaccharides and higher oligosaccharides.

Higher oligosaccharides (1·5–2 gm.) were fractionated further on a charcoal–'Celite' column (70 cm. × 3·6 cm.) by gradient elution with aqueous ethanol (30 per cent v/v → 5 l.). Fractions of about 40 ml. were collected and examined by chromatography. All fractions were found to contain mixtures of sugars, and one group only (fractions 106–118) has so far been resolved. These fractions contained little of substances other than the two major trisaccharides now to be described. The material (230 mgm.) was fractionated by paper chromatography (solvent *A*, butanol–pyridine–water, 3 : 1 : 1, Whatman 3 *MM* paper) to yield trisaccharide I (28 mgm.) and trisaccharide II (40 mgm.). Further purification was carried out by eluting these materials from charcoal columns.

Trisaccharide I $[\alpha]_D + 147°$ was homogeneous by the criteria of paper chromatography (R_{lactose}: 0·97, in solvent *A*, Whatman 3 *MM* paper) and paper ionophoresis (M_G: 0·39, in borate buffer, *p*H 10). With benzidine-trichloroacetic acid[3] spray reagent it gave a yellow-brown colour similar to that given by *O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine. It reacted only feebly with the Ehrlich reagents of Partridge[4]. Complete acid hydrolysis yielded galactose, galactosamine and glucosamine, which were identified chromatographically. Determination of the sugars by Wilson's method[5] showed them to be present in equimolecular amounts. A preparation of enzyme from *Trichomonas foetus*[6] which destroyed the serological activity of *A* substance hydrolysed the trisaccharide to galactose and the two *N*-acetylated amino-sugars, but at an intermediate stage in the hydrolysis it was possible to detect a Morgan–Elson-negative saccharide indistinguishable chromatographically from *O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine, which had also been identified by Côté and Morgan[2] as a constituent unit in *A* substance.

Oxidation of the trisaccharide (0·06 m*M*) after reduction with sodium borohydride was carried out in 0·4 m*M* periodate, *p*H 3–5 at room temperature. Selective oxidation of the alcohol terminal takes place under these conditions[7]. The release of formaldehyde—1·00 mole per 590 gm. sugar—was complete within 10 min. A similar rapid and complete liberation of 1 mole proportion of formaldehyde was observed in the oxidation of reduced *O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine.

The trisaccharide was partially hydrolysed with *N* sulphuric acid at 100° for 36 min. and the hydrolysate examined by paper chromatography (solvent *A*). Among the products, which included the three constituent sugars and a little unchanged trisaccharide, there were found two reducing substances indistinguishable from *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose and *O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine. It is concluded that trisaccharide I is most probably *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-*O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine.

In serological tests the trisaccharide was found to inhibit not only the agglutination of *A* cells by the common anti-*A* reagents, but also the lysis of sheep cells by rabbit anti-*A* immune serum and complement. *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose alone of the disaccharides tested showed the same properties (Table 1).

Trisaccharide II $[\alpha]_D + 136°$ was homogeneous by chromatographic criteria (R_{lactose}: 1·10, in solvent *A*, Whatman 3 *MM* paper; M_G: 0·39, in borate buffer, *p*H 10). Both the benzidine-trichloroacetic acid spray reagent, with which it gave a dark

Table 1. AMOUNTS OF *A* SUBSTANCE AND SUGARS GIVING INHIBITION OF (*a*) HUMAN *ISO*-AGGLUTININS AND (*b*) RABBIT ANTI-*A* SHEEP CELL HÆMOLYSINS

Substance	Minimum amount of substance giving inhibition (μgm./0·1 ml.)	
	a	*b*
Human *A* substance	0·04	0·01
N-acetylgalactosamine	> 1,000	> 1,000
α-Methyl-*N*-acetylgalactosaminide	500	500
O-α-*N*-acetylgalactosaminoyl-(1→3)-galactose	60	250
O-α-galactosyl-(1→3)-galactose	> 1,000	> 1,000
O-β-galactosyl-(1→3)-*N*-acetylglucosamine	> 1,000	> 1,000
O-β-galactosyl-(1→4)-*N*-acetylglucosamine	> 1,000	> 1,000
Trisaccharide I	60	15
Trisaccharide II	60	30

greenish-brown colour, and the Ehrlich reagents, provided ready means of detecting the substance. The component sugars, identified and determined as for trisaccharide I, were galactose, galactosamine and glucosamine in equimolecular amounts. Hydrolysis with the enzyme preparation obtained from *T. foetus* gave galactose and the two *N*-acetylated aminosugars.

Trisaccharide II, partially hydrolysed with *N* acid and examined as described for trisaccharide I, gave the same three constituent sugars and *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose. In place of *O*-β-galactosyl-(1 → 4)-*N*-acetylglucosamine there was found a Morgan–Elson-positive spot corresponding to *O*-β-galactosyl-(1 → 3)-*N*-acetylglucosamine.

Treatment of the substance with 0·05 *N* sodium carbonate at 18–20° for 30 min. yielded, apart from some unchanged trisaccharide, several 'direct' Ehrlich chromogenic substances and a reducing substance indistinguishable in both chromatography and ionophoresis from *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose. The lability of the trisaccharide to alkali and the high alkalinity of the borohydride reagent renders unsatisfactory the determination of formaldehyde liberated after reduction and selective oxidation with periodate.

Trisaccharide II also possessed the capacity to inhibit a number of anti-*A* reagents, and it is noteworthy that in tests for inhibition of sheep cell hæmolysis, it was rather less active (Table 1) than trisaccharide I. Trisaccharide II is believed to be *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-*O*-β-galactosyl-(1 → 3)-*N*-acetylglucosamine.

The long-known and unique association of group *A* antigens with both *iso*-agglutinins and heterophile (Forssman) antibodies[8] seems to depend on the similar specificities of these antibodies. It seems probable that they correspond to different but overlapping parts of the same carbohydrate structure, the common part being represented by *O*-α-*N*-acetylgalactosaminoyl-(1 → 3)-galactose. This relationship will be discussed in more detail elsewhere. The association of each of the trisaccharides with these two serologically different reagents strengthens the belief that they arise from specific determinant groups in the macromolecule rather than from latent structures which are not directly concerned with specificity.

We wish to thank the Nuffield Trust for financial support. One of us (I. A. F. L. C.) thanks the Lister Institute for a studentship.

[1] Morgan, W. T. J., *Proc. Roy. Soc.*, B, **151**, 308 (1960). Kabat, E. A., *Blood Group Substances* (Academic Press, New York, 1956).

[2] Côté, R. H., and Morgan, W. T. J., *Nature*, **178**, 1171 (1956).

[3] Bacon, J. S. D., and Edelman, J., *Biochem. J.*, **48**, 114 (1951).

[4] Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).

[5] Wilson, C. M., *Indust. Eng. Chem.* (Anal.), **31**, 1199 (1959).

[6] Watkins, W. M., *Biochem. J.*, **71**, 261 (1959). Tyler, H. M., and Watkins, W. M. (unpublished work).

[7] Clancy, M. J., and Whelan, W. J., *Chem. and Indust.*, 673 (1959); and unpublished work.

[8] Schiff, F., and Adelsberger, L., *Z. Immun. Forsch.*, **40**, 335 (1924).

THE FEULGEN-STAINED SOMATIC CELL NUCLEUS AS A HOMOGENEOUSLY ABSORBING OBJECT

By DR. SIMON IVERSEN

Cancer Research Department, Royal Beatson Memorial Hospital, Glasgow

IT is generally accepted that a somatic cell nucleus doubles its genetic material between two consecutive divisions, that the deoxyribonucleic acid (DNA) content of the nucleus is identical with, or at least proportionate to, the nuclear amount of genetic material; further, that the Feulgen reaction is specific for the nuclear DNA, and finally, that the intensity of the Feulgen stain is proportionate to the amount of DNA. So by measuring the intensity, E, of the Feulgen stain of a nucleus and multiplying E with the squared nuclear diameter, D^2, it is imagined that a direct measure of mass is obtained. This is seemingly corroborated by the finding by numerous investigators that the ED^2 values from haploid, diploid, tetraploid and octoploid nuclei show modal distributions with modes at (approximately) 1 ×, 2 ×, 4 × and 8 × ED^2. In this article it will be shown that ED^2 is not a direct measure of mass.

The interpretation of ED^2 as a direct measure of mass would be correct if the nuclei were homogeneously absorbing objects, that is, objects in which the absorbing particles are completely randomly distributed, non-interacting, and retaining their size and shape. For such a system the absorbance, E, is a linear function of the number of absorbing particles, C, the path-length, L, and the molecular absorbancy index ε, which latter can be omitted when dealing with relative amounts of the same compounds. As the concentration is mass per volume (M/V) and as the path-length may be taken as being equal to the diameter, the absorbance of a homogeneously absorbing sphere will be:

$$E = \frac{MD}{1{\cdot}33\pi r^3} = 1{\cdot}92\ MD^{-2} \qquad (1)$$

So by plotting E against D^{-2} a straight line starting in origin and with slope of 1·92 M should be obtained. As a paradigm of the straight line course, Alfert's[1] data on rat liver nuclei have been plotted this way in Fig. 1. From Table 1, where the results of regression analyses of different investigators' published data are given, it is seen that in seven out of nine cases the numerical value of the constant a deviates significantly from zero. This indicates that the relationship between E and D^{-2} most frequently is:

$$E_m = a + bD^{-2} \qquad (2)$$

If the nuclei were homogeneously absorbing objects the occurrence of the constant a would mean that the measured absorbance, E_m, is the sum of the absorbance of the compound in question, E_r, and a constant non-specific absorbance, thus:

$$E_m = E_r + a \qquad (3)$$

Table 1

	Cell type	No.	$y = a + bx^{-2}$	$P\{t_a\}$	
Alfert* *et al.*	Diploid (liver)	30	$E = -0\cdot026 + 15\cdot67\,D^{-2}$	0·0025 – 0·001	(3*a*)
	Tetraploid (liver)	30	$E = 0\cdot042 + 27\cdot36\,D^{-2}$	$\leqslant$ 0·0005	(3*b*)
	Octoploid (liver)	20	$E = 0\cdot014 + 54\cdot17\,D^{-2}$	0·25 – 0·1	(3*c*)
	Thyroid (control)	28	$E = -0\cdot019 + 15\cdot14\,D^{-2}$	0·10 – 0·05	(3*d*)
	Thyroid (experimental)	30	$E = 0\cdot237 + 10\cdot35\,D^{-2}$	$\leqslant$ 0·0005	(3*e*)
Pasteels* and Bullough	Untreated epithelial nuclei	30	$E = 0\cdot120 + 11\cdot86\,D^{-2}$	$\leqslant$ 0·0005	(3*f*)
	Treated epithelial nuclei	41	$E = 0\cdot092 + 15\cdot93\,D^{-2}$	$\leqslant$ 0·0005	(3*g*)
Swift	Neuron	10	$E = 0\cdot014 + 15\cdot21\,D^{-2}$	0·05 – 0·025	(3*h*)
	Liver	10	$E = 0\cdot163 + 9\cdot35\,D^{-2}$	$\leqslant$ 0·0005	(3*i*)

* Dr. Alfert and Dr. Pasteels have kindly sent me their original data.

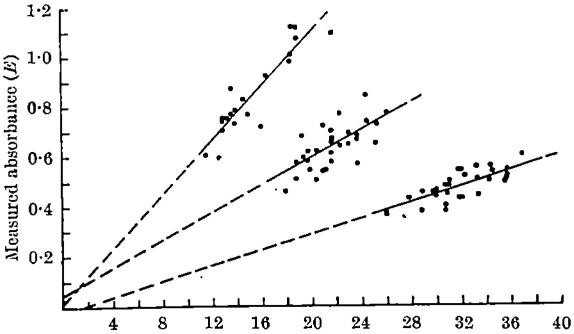


Fig. 1. Alfert's (1955) data for rat liver nuclei

The mass of the compound in question will therefore be:

$$E_r D^2 = (E_m - a)D^2 \tag{4}$$

and from equation (2):

$$(E_m - a)D^2 = (a + bD^{-2} - a)D^2 = b \tag{4a}$$

The mass remains constant, as is also obvious in the case of equation (1), where:

$$E_m D^2 = E_r\,D^2 = 1\cdot92\,M \tag{5}$$

However, some investigators have claimed that their photometric data show synthesis to occur. Here it will be demonstrated that the reason for these claims seems to be that the mass has been calculated as $E_m D^2$ instead of $E_r D^2$.

Thus Swift[7] claims to have demonstrated that synthesis occurs in embryonic mouse liver nuclei. The majority of his data are given as histograms showing the number of nuclei of a given nuclear 'mass'. He also gives, however, the raw data of E and D for 10 measurements on embryonic mouse liver, which presumably are representative for the rest of the measurements on this tissue. From Fig. 2 where E is plotted against D^{-2} it is seen that the data conform to a straight line course with a significant y-intercept (equation 3*i*). If the mass in this case is calculated as $E_m D^2$ and plotted against D the resulting curve, shown in Fig. 3, might be thought to show that the mass increased with increasing diameter, that is, that synthesis does take place. The correctly calculated mass would, however, be $E_r D^2 = b$. The mass remains constant, indicated in Fig. 3 by line B, and no synthesis occurs.

Walker and Yates[8] measured the light absorption in the ultra-violet and visible regions of living and Feulgen-stained nuclei of chick heart tissue cultures at known times after mitosis. The total amount of absorbing matter, which for the sake of convenience here will be called 'mass', was calculated as $E_m D_T{}^2$, where D_T is the nuclear diameter at time T after mitosis. It should be emphasized that a given nucleus was measured at only one time after mitosis, the same nucleus was not measured at known different times after mitosis, from which it follows that the additional information about the intermitotic age of the nuclei does not make these measurements different in any way from measurements on nuclei of unknown intermitotic age. In both cases the relation between

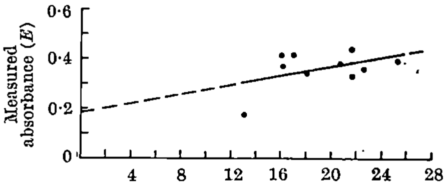


Fig. 2. Swift's (1950) data for embryonic mouse liver nuclei

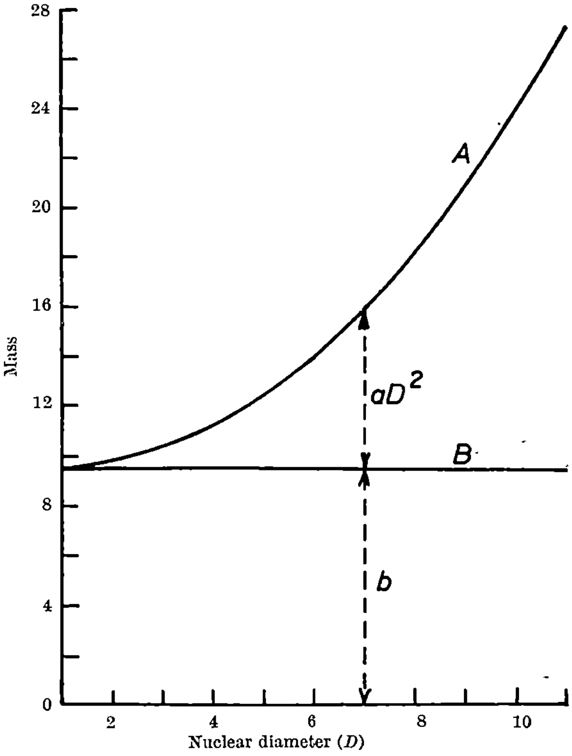


Fig. 3

E and D^{-2} is completely independent of time, inhomogeneity of the population and non-random selection.

Walker and Yates plot mass against time after mitosis. Obviously the course of such curves must be determined by the way in which the nuclear diameter alters with time. If we assume in conformity with the vast majority of data on growth[2] that the increase in geometrical dimensions (= 'growth') of a nucleus is an exponential function of time, or, in other words, that the diameter is increasing in time with a rate which is proportional to the diameter, we have:

$$D_T \sim e^{KT} \qquad (6)$$

Thus plotting E_mD^2 (= 'mass') against time would be equivalent to plotting E_mD^2 against $\ln D_T$. In Figs. 4 and 5, $\ln E_mD^2$ is plotted against time, the experimental points being read off Walker and Yates's graphs of E_mD^2 against T for the total ultra-violet absorption and for the Feulgen-stained material, respectively. As is seen from these figures, the regression lines which have the equations:

$$\ln E_mD^2 = 1{\cdot}903 + 0{\cdot}035\,T \text{ for Fig. 4}$$

and

$$\ln E_mD^2 = 1{\cdot}393 + 0{\cdot}077\,T \text{ for Fig. 5}$$

fit the data well.

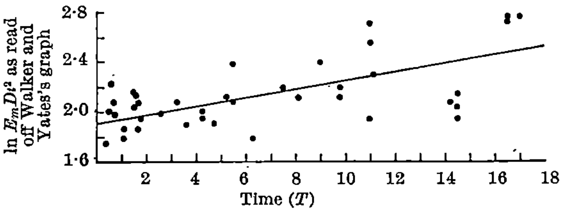


Fig. 4. Walker and Yates's (1952) data for the total Feulgen stain intensity

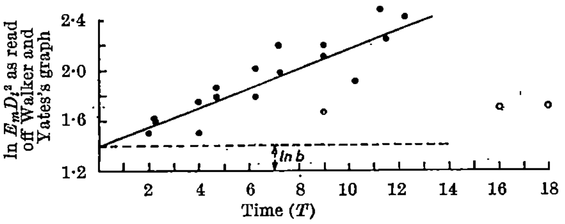


Fig. 5. Walker and Yates's (1952) data for the total ultra-violet absorbance

A plot of $\ln E_mD^2$ against $2KT$ ($\simeq \ln D_T{}^2$) would also result in a straight line, but having half the slope of the lines in Figs. 4 and 5. Consequently, a plot of E_mD^2 against D^2 would result in curves which were upwards concave and having y-intercepts of 6·69 and 4·01, respectively. The correctly calculated 'mass' would therefore be:

$$E_mD^2 = (a + bD^{-2} - a)D^2 = b \qquad (7)$$

where $D^2 \sim e^{2KT}$, giving constant masses of 6·69 and 4·01 respectively. It should be pointed out that even if the nuclear growth were not exponential in time, but was another function of time, a linear transformation of such function would lead to similar results.

The claims of having demonstrated, on the basis of photometric data, that synthesis takes place during interphase cannot therefore be said to be proved. If homogeneity is assumed and if the data conform to equations (1) or (2) an increase of M is indeed an impossibility.

This is also evident from the following argument: if the chromosome number ($\sim$ 'mass') doubles during interphase, a tetraploid nucleus at the beginning of the interphase would have the same mass as a diploid nucleus at the end of the interphase. We would therefore expect, if the nuclei were homogeneously absorbing objects, that the absorbancy of nuclei of different ploidy, when plotted against D^{-2}, would all fall on the same straight line. But as seen from Fig. 1, and from Table 1, straight lines with definitely different slopes are obtained, giving different absorbances for nuclei of identical geometrical dimensions and (presumably) mass.

It could then be argued that the doubling of the nuclear genetic material takes place when the nuclei are in a phase which is not represented in the population of measured nuclei, that is, during the mitotic process. The nuclear interphase mass (= Feulgen stain intensity) would remain constant and if the absorbing particles were randomly distributed, E would be linear with D^{-2}, as observed. There are, however, two reasons for not accepting this interpretation. The first is that chemical estimation of DNA (ref. 5) shows the synthesis to occur during the interphase ; and the other is that in fixed nuclei the Feulgen-stained material (and nucleoli) is distributed in a peripheral zone[3,4], the peripheral edge of which cannot be distinguished from the nuclear membrane. Therefore, calculation of nuclear mass from photometric data is not possible until such parameters as thickness, possible concentration gradient of the stained particles in this zone, and possible orientation of the absorbing particles in this zone can be estimated.

Further, if the population of measured nuclei is representative of the true population of interphase nuclei, an assumption which is tacitly granted, and if the mitoses in the parent tissue do not occur synchronously, but randomly, and if all the nuclei follow the same basic growth equation, then the probability of including any given nucleus of any given interphase age in the measured population is constant and identical for all the nuclei irrespective of the type of the underlying kinetic equation. If ED^2 were a direct measure of mass we should not expect, therefore, to observe a modal distribution of these values.

We must conclude, therefore, that ED^2 is not a direct measure of mass and the seemingly good agreement between these values and chromosome numbers must therefore be due to some other cause.

I wish to thank Dr. P. R. Peacock for valuable discussion and for reading and criticizing the manuscript.

This investigation was supported by a full-time grant from the British Empire Cancer Campaign.

[1] Alfert, M., Bern, H. A., and Kahn, R. H., *Acta Anatomica*, **23/24**, 185 (1955).
[2] Brody, S., *Bioenergetics and Growth* (New York, 1945).
[3] De Smul, A., and LeComte, C., *Arch. Biol.*, Paris, **64**, 259 (1953).
[4] Iversen, S., *Acta Anatomica*, **41**, 160 (1960).
[5] Newton, A. A., and Wildy, P., *Exp. Cell. Res.*, **16**, 624 (1959).
[6] Pasteels, J., and Bullough, W. S., *Arch. Biol.*, Paris, **64**, 271 (1953).
[7] Swift, H., *Physiol. Zool.*, **23**, 169 (1950).
[8] Walker, P. M. B., and Yates, Helen B., *Proc. Roy. Soc.*, B, **140**, 274 (1952).

REGENERATION OF ELEMENTS OF DONOR ORIGIN IN ORTHOTOPICALLY GRAFTED SKIN FOLLOWING THE HOMOGRAFT RESPONSE

By Dr. J. K. DINEEN and Dr. ALEKSANDER SZENBERG

Walter and Eliza Hall Institute of Medical Research, Melbourne

THE work reported here arose out of attempts to induce immunological tolerance of orthotopic skin grafts in adult inbred mice by the administration of 6-mercaptopurine (6-MP). Groups of recipient mice were treated with 0·25, 0·50, 1·0, 1·5 and 2·0 mgm. of 6-MP daily for 7–10 days before grafting, and for several days thereafter. In order to provide a more generalized antigenic stimulus, several groups were also injected intraperitoneally with isolated spleen cells of the graft-donor strain during treatment with 6-MP. However, the mean survival-time for *C57B*1 homografts on *C3H* mice was only prolonged by 3 days by these procedures and this effect was only observed in recipients in which gross lymphoid involution had been produced by toxic doses of 6-MP. The survival of *C3H* skin grafted on to *C57B*1 mice was not prolonged. The results of these experiments are in agreement with the findings of Meeker *et al.*[1]. They failed to observe prolongation of homograft survival in mice treated with 6-MP. Similar experiments in which grafts were exchanged between the closely related *AKR*, and *C3H* mice treated with 6-MP, gave the same negative result. In the course of this work, we observed in a number of the mice which had been pre-sensitized with spleen cells that homografts survived indefinitely if the removal of the plaster protective bandages was delayed until 16–17 days after grafting. This indefinite survival occurred in mice that had received 6-MP and in others which had not received the drug. The present article is concerned with this phenomenon of the apparent survival of skin homografts in specifically immunized mice.

In a typical experiment six *C3H* adult mice were each injected intraperitoneally three times during a 10-day interval with 20×10^6 *C57B*1 nucleated spleen cells and grafted with *C57B*1 skin by Medawar's technique 5 days after the third course of injections. A control group of 6 *C3H* adult male mice which were not treated received *C57B*1 grafts at the same time as the injected group. The protective bandages were removed from both control and treated animals 16 days after grafting. The grafts on the treated animals, though non-pigmented and showing no hair growth, were retained without gross change in appearance for an indefinite period. The grafts on the control animals had a similar appearance when the bandages were removed, but rapidly dried out on exposure to the air, and were afterwards completely rejected. The surviving grafts were retained for several months, although macroscopic pigmentation and normal hair growth did not occur. Similar results have been observed when skin homotransplants have been transferred to specifically treated animals in other strain-combinations (*C3H* and *C57B*1 grafts on appropriately immunized *AKR* mice). Attempts to extend this phenomenon to inter-species graft survival (rat skin on mouse, mouse skin transplanted to rats) have failed.

Although the response observed in *C3H* mice pretreated by injection of cells differs sharply from the response observed in untreated animals at 16 days this difference was not so apparent when the plaster bandage was retained intact for a further 4–5 days. A proportion of homografts remained intact on control recipients when the protective bandages were removed at 20 days. The possible explanation of this unexpected finding was sought by histological examination of the development of the homograft reaction in pretreated and untreated mice. Histological sections of grafts were prepared at 2-day intervals following the orthotopic transfer of *C57B*1 skin to treated and untreated *C3H* recipients. Two distinct kinds of cellular infiltration were observed in sections obtained from both treated and control animals. The more superficial band, predominantly polymorphonuclear, developed immediately below the epithelial layers of the grafted tissues and, concurrently, a deeper band of infiltrating polymorphs and lymphocytes demarcated the graft tissue from the panniculus carnosus of the host. The development of the destructive sub-epithelial infiltrate was an acute event in both treated and control mice. The only obvious difference in response in the two groups of animals was that the sub-epithelial accumulation of polymorphs was most intense in the treated group of mice at 4–6 days, while this response was delayed for 8–10 days in the control animals. The demarcating inflammatory response at the deeper level also developed at 4–6 days in treated animals, and at 8–10 days in the controls, but afterwards followed a more chronic course in both groups. By about the third week after grafting, the demarcating zone between graft and graft bed was characterized by a mononuclear infiltrate containing primitive reticulum cells, lymphocytes and plasma cells and fibroblastic elements. Few polymorphs were then evident in this zone. With the subsidence of the acute polymorph response, the graft epithelium began a process of regeneration which was essentially complete in 10 days; that is, 14–16 days after grafting in the treated group, and correspondingly later in that proportion of the control animals in which regeneration occurred, that is, at 18–20 days after grafting.

Perhaps the most significant feature of these findings is that the sub-epithelial infiltration of polymorphs did not recur on the regeneration of the epithelium in either group. This failure of the host to react against the newly established epithelium has usually been interpreted as indicating that the regeneration is by cellular elements wholly derived from the host. There are difficulties in accepting this view, and we have therefore considered three

Table 1. TRANSFER OF REGENERATED TISSUES

Strain of skin donor	Recipient strain and No.*	Time of transfer of regenerating graft and strain of recipient	Strain of donor of challenge graft	Histological appearance of challenge graft at 6 days—classification of immunological response
*C*57*B*1	10 × *C*3*H*	*C*3*H*—16 days	*C*57*B*1	*Epithelial layers*—some areas of old necrotic epithelium remaining; 1–4 layers of regenerating epithelial cells showing mitotic activity. *Sub-epithelial infiltrate*—single lymphocytes and/or polymorphonuclear debris. *Demarcating infiltrate*—intense lymphocytic and fibroblastic with polymorphs mainly in and around the vessels. *Classification*—second set homograft response.
*C*57*B*1	6 × *C*3*H*	*C*57*B*1—17 days	*C*3*H*	As above.
*C*57*B*1	6 × *C*3*H*	*AKR*—16 days	*C*57*B*1	As above.
*C*57*B*1	4 × *C*3*H*	*AKR*—16 days	*C*3*H*	*Epithelial layers*—large areas of destroyed epithelium remaining; 1–2 layers of regenerating epithelium showing mitotic activity in remainder of grafts. *Sub-epithelial infiltrates*—as above. *Demarcating infiltrate*—as above. *Classification*—second set homograft response.
*C*57*B*1	4 × *AKR*	*AKR*—18 days	*C*57*B*1	*Epithelial layers*—epithelium completely destroyed; no regeneration observed. *Sub-epithelial infiltrate*—intense polymorphonuclear infiltration. *Demarcating infiltrate*—moderate polymorphonuclear, lymphocytic and fibroblastic; some plasma cells. *Classification*—second set homograft response.

* All recipient animals were injected intraperitoneally with 20 × 10^6 nucleated spleen cells of the skin donor strain three times during the 10-day period before grafting.

other possibilities. The failure of response might also be due to: (*a*) a change in the general immunological status of the host; (*b*) a change in the local immunological status of the host; or (*c*) failure of regenerating donor tissue to attain antigenic competence either by 'adaption' or by deletion of antigenic determinants followed by a process of local selection for survival. A change in the general immunological status of the host is excluded by the observation that a second set graft reaction is invoked in these animals by a second graft with the skin of the donor (*C*57*B*1) strain. The possibility that the graft has not regained antigenic competence of donor strain specificity can be experimentally tested. Transfer of the regenerated tissue to normal mice of the recipient (*C*3*H*) strain invokes a first set homograft reaction as judged both macroscopically and microscopically. It has not been possible to include as a control an equivalent area from a *C*3*H* mouse in which regenerating epithelium of known host origin has replaced the normal skin, but the validity of our observation is supported by the fact that mice having rejected the graft of regenerated tissue were afterwards able to reject a further *C*57 graft with a typical second set, accelerated reaction (Table 1).

This is *prima facie* evidence that a regenerating element in the transferred graft has donor (*C*57*B*1) specificity, and can sensitize the new recipient to give a second set response. It is quite unlikely that either sub-cellular antigenic material or a small proportion of surviving, but inert, epithelial cells of *C*57*B*1 provenance, protected in some way among regenerating host cells, could be responsible for this sensitization. The possibility that presensitization of the recipient mice might be 'adoptively'[2] acquired by the coincidental transfer of isologous, immunologically competent cells present in the regenerated tissue could be tested by transfer in a homologous system where the transferred lymphoid cells would not be expected to survive. Thus *C*57*B*1 grafts surviving on *C*3*H* mice have been transferred at 16 days to normal *AKR* mice (Table 1). A subsequent *C*57*B*1 graft on these animals stimulated a characteristic second-set graft response, while the appropriate control-*C*3*H* skin grafted on to normal *AKR* failed to induce a second-set graft reaction when these animals were grafted with *C*57*B*1 tissue later.

Confirmatory evidence that homograft survival in treated mice is related to regeneration of donor elements was obtained by X-irradiation of the donor tissue at a level (1,000 r.) which might be expected to suppress mitotic activity. The irradiation was done as soon as possible after removal of the skin from the donor, the graft being applied to the recipient immediately after irradiation. Despite delay in removal of the protective bandages for 16 days, such grafts were rejected, and this observation is in accord with the hypothesis that regeneration of some elements of donor origin are essential for the graft survival. If regeneration of the graft were wholly due to cellular elements derived from the host then it would be unlikely that moderate X-irradiation of the donor tissue would inhibit regeneration.

There is no doubt that host cells do invade the primary graft. Histologically there is evidence for the entry of connective-tissue elements, and when a surviving graft was transferred to a normal recipient of the donor *C*57*B*1 strain it was destroyed by a typical homograft response. The results we have outlined may be summarized as providing evidence that, in the *C*57*B*1 or *C*3*H* system, donor cells persist in the homograft for much longer than is now believed, and that if the area is adequately protected, they may regenerate and persist indefinitely despite the recurrence of a homograft reaction. It is much easier to demonstrate this survival if the animals have been pretreated by an injection of the donor type spleen cells, and there are obvious resemblances to the 'enhancement' phenomenon[3,4]. If this is the case, a similar type of homograft survival might be promoted by passive administration of serum from mice which have been hyperimmunized with donor type cells. Accordingly, a group of 16 adult male

C3H mice were grafted with *C57B*1 skin. On the sixth day after grafting, concurrent with the onset of the cellular reaction in the first-set graft response, 10 mice were each injected intraperitoneally with 0·2 ml. of serum obtained from *C3H* mice which were hyperimmunized by the intraperitoneal injection of *C57B*1 spleen cells at weekly intervals over a period of 6 weeks. Thereafter the grafted mice received intraperitoneal injections of 0·2 ml. of the hyperimmune serum on alternate days until the twentieth day after grafting. The remaining 6 control animals were injected with normal *C3H* serum using the same procedure of route, and dosage, with which the test group was treated with hyperimmune serum. The plaster bandages were removed from both groups of mice at 16 days after grafting, and survival of the grafts in the group injected with hyperimmune serum was both macroscopically and microscopically similar to the homograft survival observed in the actively immunized animals described here. The grafts were destroyed in the control group injected with normal *C3H* serum. This is only a preliminary result but it points strongly towards an enhancement effect. Following Billingham, Brent and Medawar[4], we regard this as due to antibody providing an afferent block in the flow of antigenic material (or information) from the implanting donor cells to the immunologically competent lymphoid centres of the host.

The conclusion to be drawn from our experiments may not necessarily be applicable to any other system than the one we have used, but there is equally nothing to show that they are not of general validity. With this reservation we can formulate them as follows:

(1) A proportion of cells of donor origin survive the homograft response and, provided adequate physical protection is given to the graft, many may survive indefinitely. The cells concerned are not, however, sufficient to conserve the donor material as a functioning tissue; they constitute at most a local chimera containing also a proportion of host cells.

(2) The difference between first and second set reactions is essentially in their timing; second set reactions are initiated and reach their acme 4–6 days earlier than primary ones.

(3) Accepting the orthodox interpretation that homograft rejection is mediated by the sequence; movement of antigenic material from the graft to lymphoid tissue; induction and proliferation of appropriate immunologically competent cells; passage of such cells to the graft site; destructive interaction between immunologically competent cells of the host and the donor cells in the graft, we should add a further component to the sequence, namely, production of circulating antibody which, by intervening between foreign antigenic determinant and receptors on immunologically competent cells at either afferent or effector stages, can diminish or annul the immunological rejection of the homograft.

(4) The intensity and completeness of the homograft response is therefore highly dependent on conditions in the system which modify the degree of physical protection of the graft and on the relative timing and intensity of the cellular and humoral aspects of the immune response.

We are indebted to Sir Macfarlane Burnet for his criticism during the course of this work.

[1] Meeker, W. R., Condie, R. M., Good, R. A., and Varco, R. L., *Ann. N.Y. Acad. Sci.*, **87**, 203 (1960).

[2] Billingham, R. E., Brent, L., and Medawar, P. B., *Proc. Roy. Soc.*, *B*, **143**, 58 (1954).

[3] Kaliss, N., and Snell, G. D., *Cancer Res.*, **II**, 122 (1951).

[4] Billingham, R. E., Brent, L., and Medawar, P. B., *Transplantation Bull.*, **3**, 84 (1956).

A NEMIN ASSAY PROCEDURE

By E. J. WINKLER, SHIMPEI KUYAMA and Prof. DAVID PRAMER

Department of Agricultural Microbiology, Rutgers, The State University, New Brunswick

PRAMER and Stoll[1] provided unequivocal evidence that trap formation by the predacious fungus *Arthrobotrys conoides* is induced by a metabolic product of the nematode *Neoaplectana glaseri* and proposed 'nemin' as the name for the substance or group of substances that cause morphogenesis in nematode-trapping fungi. In order to study the chemical nature of nemin and to proceed with isolation and identification of the compound, it was necessary to have a nemin assay procedure. Therefore, the influence of various factors on growth and trap formation by *A. conoides* was determined, and the results were used as a basis for the nemin assay agar and the method described here.

The fungus used as assay organism was isolated from decomposing leaves and identified as *A. conoides*. It is representative of a group of nematode-trapping fungi in which short lateral branches of the mycelium anastomose with similar branches or with the parent hyphæ to form eventually a three-dimensional network of loops. The hyphæ that comprise the loops are covered with a sticky secretion, and nematodes contacting the network are captured by entanglement in and adhesion to the mycelium[2]. *A. conoides* developed readily on a variety of laboratory media, but stock cultures were maintained by periodic transfer on maizemeal-extract agar. Pure cultures of the fungus produced some traps spontaneously, but they were few in number, and no difficulty was encountered in differentiating between control plates on which traps were produced without provocation and treated plates on which trap formation was induced by nemin.

The assay agar was prepared by dissolving 17 gm. of dehydrated maizemeal-extract agar (Difco Laboratories, Inc.) in 1 l. of *M*/45 Sorenson's phosphate buffer at *p*H 5·8. The liquid was brought to the boil and when the agar dissolved 10 p.p.m. of novobiocin and 20 p.p.m. of bromcresol purple were added. The antibiotic prevented bacterial contamination of assay plates. The buffer maintained the reaction of the assay agar at *p*H 5·5–5·8 and thereby kept spontaneous trap formation to a minimum. Bromcresol purple provided visual evidence of any change in the

Table 1. Nemin Activity of Various Blood Sera and Nematode Extracts

Preparation assayed	Highest dilution causing trap formation	Dry weight of preparation (mgm./ml.)	Nemin (units/mgm.)
(1) Sterile pooled normal serum (Colorado Serum Co., Denver, Colorado)			
Cow	1/1,000	86	11·6
Horse	1/2,000	93	21·5
Pig	1/2,000	87	23·0
Sheep	1/1,000	82	12·2
(2) Bovine serum (Hyland Laboratories, Yonkers, New York)			
Liquid	1/500	58	8·6
Dried*	1/1,000	85	11·8
Ultrafiltrate	1/100	15	6·7
Dialysed	1/100	72	1·4
(3) Water extracts of nematodes			
Panagrellus redivivus	1/1,000	5	200·0
Pig ascarids	1/1,000	27	37·0
(4) Culture broth of *Neoaplectana glaseri*†	1/100–1/1,000	37	2·7–27·0

* Reconstituted in sterile distilled water saturated with CO_2.

† Obtained from Dr. N. R. Stoll, of the Rockefeller Institute, New York.

reaction of the medium caused by material incorporated into the agar for assay or by growth of the fungus. The antibiotic, buffer, and *p*H indicator at the concentrations employed had no influence on growth or trap formation by *A. conoides*. The complete assay agar was sterilized by autoclaving.

Solutions for assay were sterilized by filtration through membranes or sintered glass and diluted in sterile water blanks. One-ml. quantities thereof were added to separate Petri dishes and mixed thoroughly with 9 ml. of the assay agar poured at approximately 45° C. When the agar had solidified, the centre of the surface of each assay plate was inoculated with a plug having a diameter of 4 mm. and cut with a cork borer from those portions of a maizemeal-extract agar plate on which the growth of *A. conoides* was 1–2 days old. All assays were made in triplicate. Results were obtained by microscopic examination (× 100) of the mycelium that developed during 48 hr. storage at 28° C. Observation was neither tedious nor prolonged since the amount of growth made by the assay organism was relatively scant and traps were easily distinguished.

The total solids content of preparations assayed was determined by drying an appropriate quantity at 105° C. for approximately 18 hr. Nemin activity was measured in terms of dilution units/mgm. The nemin unit was the reciprocal of the highest dilution of the preparation that caused the fungus to form traps. If a preparation had a total solids content of 10 mgm. and was active at a dilution of 1 : 1,000 it contained 1,000 dilution units/10 mgm. or 100 units/mgm.

The assay procedure as described was used to determine the nemin activity of various animal sera, aqueous extracts of two nematodes, and culture broths of the nematode *N. glaseri*. It is apparent from the results summarized in Table 1 that water extracts of the free-living nematode *Panagrellus redivivus* had the greatest nemin activity. Sera derived from the cow, horse, pig, and sheep had comparable activity, and dried bovine serum did not differ greatly in nemin content from normal serum. The nemin activity of an ultrafiltrate of bovine serum was similar to whole serum, indicating that the size of the nemin molecule is not too great to pass through 'Cellophane'. This was verified by dialysing bovine serum against distilled water for 2 days. The whole serum had a nemin activity of 8·6 units/mgm., but after dialysis it decreased to 1·4 units/mgm. The loss of nemin from blood serum during dialysis supports the statement of Pramer and Stoll[1] that nemin is not a protein or polysaccharide of high molecular weight.

Since chemical fractionation and isolation work frequently yield small quantities of material, a microassay method was developed and tested. The same assay agar was employed and 10-ml. quantities were added to sterile Petri dishes. When the agar had solidified the plates were inoculated as described above and incubated at 28° C. for 5 days. Holes (5 mm. diameter) were punched from the centre of absorbent paper disks 13 mm. in diameter and 5 disks were placed equally distant from each other around the circumference of the agar surface in each plate. The disks were located such that the mycelium exposed through the centre holes was 1–2 days old. The preparations assayed were diluted serially in water and 0·1 ml. aliquots of the solutions added directly to the fungus mycelium visible through the holes in the centre of the absorbent disks. All tests were made in triplicate and the plates returned to the incubator for 36 hr. The treated areas were then examined microscopically for traps and nemin activity measured as dilution units/mgm. The micromethod was found suitable for locating nemin on paper strip chromatograms. For this purpose the strips were cut into 2-cm. lengths and these were substituted for the absorbent disks.

The micromethod requires 0·1 rather than 1·0 ml. of solution for assay. Moreover, 5 determinations instead of 1 are made per plate and results are read after 36 rather than 48 hr. Repeated tests have shown that both assay procedures yield comparable results. The nature, quantity of material, and time available for assay dictate which of the two methods is the more suitable. A dilution assay such as that developed for the estimation of nemin is limited in its sensitivity since measurements can be made only between concentrations of the dilution series tested. Precision can be increased by using more narrow increments in a dilution series, but this necessitates an increased expenditure of time and effort. In the nemin assay the preparations to be tested were sterilized. Although this is not a requirement of the micromethod, results are more reproducible and clearly defined when aseptic technique is employed. In spite of these disadvantages which are inherent in all serial dilution assays the procedure remains a useful and important means of measuring the activity of biologically active compounds[3]. Repeated tests have shown that the results obtained with the nemin assay are reliable and reproducible and the method as developed is suitable for the control of fractionation studies for the chemical isolation and identification of nemin. For this purpose pig ascarids are being used. These worms are numerous, individually large, and obtainable in quantity from slaughter houses. The results listed in Table 1 show that water extracts of pig ascarids have greater nemin activity than animal sera and culture broths of *N. glaseri*.

This investigation was supported in part by National Science Foundation research grants *G*5949 and *G*9749 and the Rockefeller Foundation.

[1] Pramer, D., and Stoll, N. R., *Science*, **129**, 966 (1959).

[2] Drechsler, C., *Mycologia*, **29**, 447 (1937).

[3] Grove, D. C., and Randall, W. A., *Assay Methods of Antibiotics* (Medical Encyclopedia, Inc., New York, 1955).

LETTERS TO THE EDITORS

RADIOPHYSICS

Group and Phase Velocities of a Travelling Disturbance in the *F* Region of the Ionosphere

A PUZZLING feature of the reported association[1] between travelling disturbances in the *F* region and the occurrence of sporadic *E* is that travelling disturbances move with a velocity of about 10 km./min. (ref. 2) whereas sporadic *E* patches have only about half this velocity[3]. However, the travelling disturbance velocity was measured using widely spaced stations (~ 1,000 km.) whereas that of the sporadic *E* patches was found using relatively close stations (~30 km.). Therefore whereas it is the group velocity of the travelling disturbances which has been measured, the sporadic *E* patches may be associated with a particular phase of the isoionic contours in the travelling disturbance and may thus appear to move with the phase velocity of the travelling disturbances.

To test this hypothesis, three methods have been used for measuring directly the phase velocity of a travelling disturbance, making use of existing records. The ionograms taken at one station have been reduced to give isoionic curves plotted against time. These curves are then used in the following ways:

(1) The radius of curvature of the isoionic contours in space may be estimated from the observed splitting of the echo. For example, when the second echo is split, but the first is not, the radius of curvature is between once and twice the height of the contour. When the radius of curvature at any time is known, the phase velocity may be estimated.

(2) The second echo virtual path is often less than twice the virtual path of the first echo, because of the different paths followed. If the isoionic contours are assumed to be concentric circles over short distances, it is possible to calculate the difference in terms of known quantities and the phase velocity.

(3) The wave-fronts of travelling disturbances are believed to be approximately along the Earth's magnetic field[3,4]. If this is assumed to be correct, a further estimate of phase velocity may be made using the isoionic curves.

One set of two-minute records taken at Brisbane has been found suitable for analysis. The group velocity of the disturbance was found from ionograms at this and other stations to be 10 km./min. All three methods gave a phase velocity between 4 and 6 km./min., confirming the hypothesis.

The difference in group and phase velocities may also explain the short apparent life-time (~30 min.) of phase-path irregularities[5] and the long life-time of disturbances on ionograms at spaced stations (~5 hr.). The form of the phase path irregularities changes rapidly due to the large dispersion.

'Satellite' traces on ionograms are due to sloping isoionic contours which are observed to move with a velocity of ~4 km./min. (ref. 6). Because of the method used for measuring it, this velocity is evidently the phase velocity. Thus the one obstacle to the identification of the cause of satellite traces as travelling disturbances, that is, their different velocities, is removed.

The travelling disturbances discussed here are those which cause gross distortions of ionogram records. Small disturbances on $h' : t$ records have been shown to move with speeds of nearly the same magnitude[7], but differing in direction by about 20°. This may be due to the influence of the phase velocity, which may have an east–west component not appearing in the group velocity which is nearly due north.

The reported dispersion does not agree with that given by Hines[8] for internal gravity waves which he regards as the cause of travelling disturbances.

Further details of the experimental method will be presented elsewhere.

This work has been carried out as part of the programme sponsored by the Radio Research Board of the Commonwealth Scientific and Industrial Research Organization.

L. H. HEISLER
J. D. WHITEHEAD

Radio Research Board,
School of Electrical Engineering,
University of Sydney.

[1] Heisler, L. H., and Whitehead, J. D., *J. Geophys. Res.*, 65, 2767 (1960).
[2] Heisler, L. H., *Austral. J. Phys.*, 11, 79 (1958).
[3] Munro, G. H., and Heisler, L. H., *Austral J. Phys.*, 9, 359 (1956).
[4] Bowman, G. G., *Planet. Space Sci.*, 2, 133 (1960).
[5] Gusev, V. D., Mirkotan, S. F., Berezin, I. B., and Kiyanovsky, M. P., *Some Ionospheric Results obtained during the I.G.Y.*, edit. by Benyon, W. J. G., 304 (Elsevier Pub. Co., 1960).
[6] McNicol, R. W. E., Webster, H. C., and Bowman, G. G., *Austral. J. Phys.*, 9, 247 (1956).
[7] Munro, G. H., *Austral. J. Phys.*, 11, 91 (1958).
[8] Hines, C. O., *Canad. J. Phys.*, 38, 1441 (1960).

OCEANOGRAPHY

Evidence of an Eastward Equatorial Undercurrent in the Atlantic from Measurements of Current Shear

RECENTLY there has been speculation as to the existence of an equatorial countercurrent in the Atlantic Ocean similar to the Cromwell Current[1,2] in the Pacific. G. Neumann[3] has cited evidence for such a current from early observations (1911) of anomalous drift currents and from a re-analysis of dynamic heights from oceanographical data along the equator. In the first two weeks of March, 1961, during an extensive bathymetry survey of the mid-Atlantic Ridge along the equator, I was able to make a number of measurements of horizontal current shear in the upper 100 m. surface water. The shears obtained at different points over an area extending from 19° W. to 10° W. longitude and from the equator to 3° S. latitude do indicate the presence of an eastward flowing equatorial undercurrent. The measurements were made from the R.V. *Chain* during the first part of a three-month cruise to study oceanographical problems associated with the Romanche Trench and the equatorial mid-Atlantic Ridge.

The current shear was measured by a method suggested to me by W. S. Richardson of this Institution. Two pitotmeters were mounted at the top and bottom, respectively, of the 600-ft. towed chain which carried thermistor elements for the continuous temperature profiling system[4]. When the ship was under

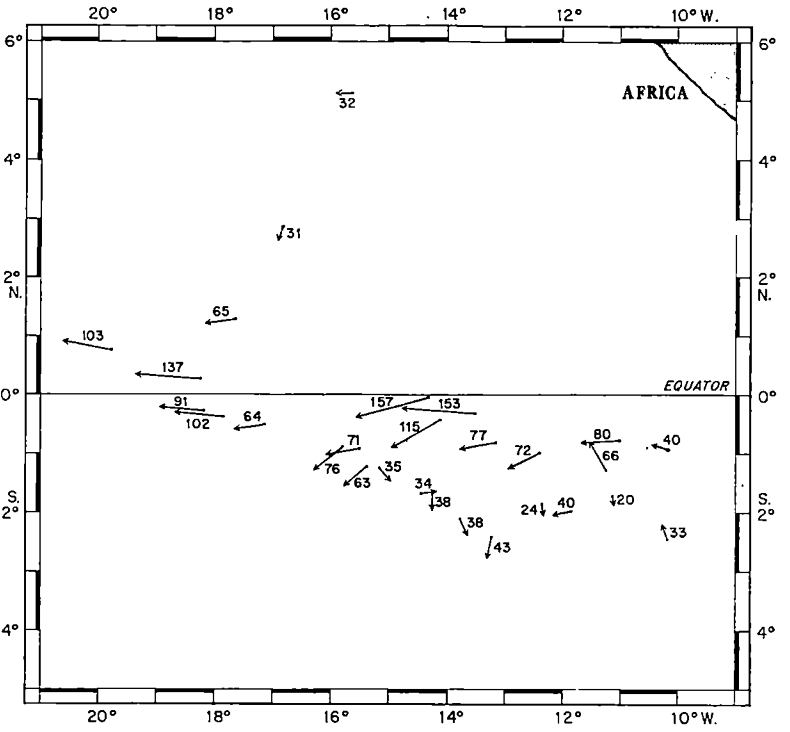


Fig. 1. Horizontal current shear [$\vec{V}$ (15 m.) − $\vec{V}$ (105 m.)] measured near the equator during March 4–21, 1961 (shear magnitude in cm./sec. indicated beside each vector)

way at a normal speed of 11 knots the shallow pitotmeter was at a depth of 15 m. and the deep pitotmeter at a depth of 105 m. The water speed past each pitotmeter was determined electrically from a differential pressure transducer driven by the pitot. By manoeuvring the ship at constant speed (11 knots) around a box course I could measure the vector difference between the current velocity at the pitotmeter and the drift velocity (wind drift and surface current) of the ship at the surface. By subtracting the result of one pitotmeter from that of the other I could determine the vector difference or shear between the current velocities at the two pitotmeters.

The preliminary results of shear measurements are shown on the chart in the accompanying Fig. 1. Each vector represents a measurement and gives the magnitude and direction of the difference: $\left[\vec{V}\text{ (15 m.)}-\vec{V}\text{ (105 m.)}\right]$, where $\vec{V}$ (15 m.) is the current velocity at a depth of 15 m. and $\vec{V}$ (105 m.) is the current velocity at 105 m. The magnitude of the shear in cm./sec. is indicated beside each vector. Clearly apparent in Fig. 1 is the unusually strong westerly shear of the 15-m. water over the 105-m. water along the equator. This shear was as high as 150 cm./sec. in two places. The shears shown give no information, of course, about absolute current velocities. However, determinations of the set of the ship while under way and while drifting indicate a current velocity at the surface of 40–60 cm./sec. to the westward at the equator. Because the current at 15 m. was about the same as that at the surface it is clear from the shears shown that the undercurrent at the 105-m.-level is toward the east along the equator and at some points as high as 100 cm./sec. The presence of this undercurrent was indicated very clearly during the lowering of a plankton net prior to a tow. When the net reached a depth of about 50 m. the wire angle changed drastically and stood out from the ship towards the east.

The shears shown in Fig. 1 indicate that the southern boundary of the undercurrent in these longitudes lies somewhere between 1° and 2° south latitude. The few measurements north of the equator show very little concerning the northern boundary of the undercurrent, but suggest that the current is more or less symmetrical about the equator. The thermal structure of the water, which was recorded continuously throughout the survey to a depth of about 110 m., underwent an interesting transition whenever the ship crossed the boundary of the current. South of the current most of the total drop in temperature from 28° C. at the surface to 15° C. at 110 m. was concentrated within a very strong negative gradient at a depth of about 50 m. This gradient is the well-known equatorial discontinuity layer. Along the equator, however, the drop in temperature was distributed evenly from the surface to 110 m. with an approximately constant negative gradient.

This work was carried out under contract with the Office of Naval Research (*Nonr*-1367).

Measurements of the undercurrent are continuing aboard the R.V. *Chain* in this area at the time of writing. Detailed measurements of the undercurrents velocity profile are being made using current meters and current drogues by C. Parker.

ARTHUR D. VOORHIS

Woods Hole Oceanographic Institution, Mass.

[1] Cromwell, T., Montgomery, R. B., and Stroup, E. D., *Science*, **119**, 648 (1954).

[2] Knauss, J. A., *Deep-Sea Res.*, **6**, 265 (1960).

[3] Neumann, G., *Deep-Sea Res.*, **6**, 328 (1960).

[4] Hubbard, C. J., and Richardson, W. S., Woods Hole Oceanographic Institution Ref. No. 59-16 (1959).

GEOLOGY

Ultrabasic Liquids

THERE is a considerable body of geological evidence suggesting that, in a submarine environment, extrusion of silicate liquids with a composition comparable to peridotite occurs. Later, this material is hydrated to form serpentine. In part, this evidence comes from repeated association of serpentines with marine sediments such as greywackes and cherts, and repeated association with volcanics including pillow lavas. Further, the ultramafics of this environment show features to be expected from rocks which have flowed. An excellent description of such a situation is provided by Bailey and McCallien[1]. Hess[2] has summarized some of the data from field evidence suggesting that many ultrabasic rocks were formed by intrusion of liquids. But laboratory investigation has consistently heaped difficulties on an extrusive explanation of such rocks. Known surface lava temperatures do not exceed 1,200° C. while the melting temperatures of the anhydrous equivalents of serpentine are of the order of 1,700° C. Clearly there are difficulties in moving such materials to the surface of the Earth, or even in forming liquids at reasonable depths in the crust. The following statement from Hess[2] bears repeating : "For most of this century a magnificent argument has gone on between field geologists who have worked on the peridotites of alpine mountains and laboratory investigators of their chemistry (particularly Bowen). The former stoutly maintains that the evidence indicates that they were intruded in a fluid state, as magmas ; and the latter equally forcefully has proved to his satisfaction that such magmas are not possible. . . . At the other extreme we find Bailey and McCallien suggesting that the serpentines of Turkey are submarine lava flows."

Recent work has shown that submarine volcanic activity is widespread but the consequences of this have received insufficient attention. Under moderate pressures of water, the melting points of rocks and minerals are normally depressed several hundred degrees. When a lava is extruded at the surface, it cannot contain volatiles at pressures much in excess of atmospheric pressure. When the same material is extruded in a submarine environment, volatiles can be retained at pressures corresponding to the water pressure of the given depth. It follows that submarine lavas will occur at much lower temperatures than their surface equivalents. If silicate liquids corresponding to compositions with high melting points are to be expected as extrusives, the most likely environment will be that of the deep oceans.

With these considerations in mind, a series of preliminary experiments on the melting points of serpentine compositions have been conducted under water pressures of 500–1,000 atm., corresponding to the deeper parts of the oceans. The material chosen was a typical serpentine from a typical 'flow' environment in New Zealand. Hydrothermal treatment indicated that above 500° C., the serpentine was replaced by talc and forsterite. At higher temperatures, forsterite, enstatite and minor chromite were dominant. Between 900–1,200° C., pyroxene and olivine were fine-grained, but near 1,300° C. the first suggestions of melting were indicated. By 1,400° C. it was clear that most of the material was liquid during the experiment. Residual olivine was coarse-grained; glass was present together with masses of feathery growths of olivine or pyroxene. This latter growth habit is typical of that in greatly supersaturated environments and probably took place during quenching, a matter of a few seconds. Samples also suggested that considerable flow of material had occurred.

It thus appears that serpentine lava could flow at temperatures of about 100° C. above observed surface lava temperatures, in a submarine environment. In our opinion, such temperatures cannot be considered beyond the range of geological possibility. At greater depths, intrusion of partially melted ultramafic materials should be possible at even lower temperatures. Our quenching results also indicate that well-quenched margins should be rare.

It has been noted frequently that ultrabasic rocks seldom show thermal effects at their contacts, which might be anticipated from their melting temperatures (for a summary, see Turner and Verhoogen[3]). In the case of submarine flows, no contact metamorphism would be anticipated. But even in deeper intrusions it must be noted that the demand for water imposed by the serpentine reaction in chilled marginal phases may cause flow of water into the cooling magma and an abnormally low heating effect at contacts. This situation is in direct opposition to that with granitic rocks where internal water requirements are small, and transfer of water out from the magma may heat contact rocks.

We wish to acknowledge support of this work by the National Science Foundation and the Petroleum Research Fund, American Chemical Society.

R. H. CLARK
W. S. FYFE

Victoria University of Wellington,
New Zealand, and
University of California,
Berkeley.

---

[1] Bailey, E. B., and McCallien, W. J., *Trans. Roy. Soc. Edin.*, 62, Part 2, 403 (1953).

[2] Hess, H. H., *Geol. Soc. Amer. Special Paper*, 62, 391 (1955).

[3] Turner, F. J., and Verhoogen, J., *Igneous and Metamorphic Petrology* (McGraw-Hill, New York, 1960).

Preliminary Age-Determinations in the Lizard Area

A PETROLOGICAL re-examination of the Lizard area by one of us (D. H. G.) has led to an attempt to date the igneous and metamorphic assemblages using the potassium–argon method.

Four muscovite samples from the Old Lizard Head series and two samples of biotite from the Kennack gneiss have been measured, using the total volume technique. Determinations of potassium oxide were made using a flame photometer. Results are shown in Table 1. Overall errors are estimated at ± 4 per cent. On geological evidence, the relevant sequence of events in the Lizard is as follows :

Oldest: (1) Regional metamorphism of (*a*) Old Lizard Head series, (*b*) Landewednack hornblende schists, accompanied by the intrusion of the Lizard peridotite. (2) Localized intrusion of gabbro. (3) Intrusion of a basic dyke swarm and related sills, intruded in a stress field approximately at right angles to that operating during the regional metamorphism. (4) Minor intrusions of Kennack granite and injection gneiss. (5) Thrusting of the Lizard block over the Palæozoic sequence to the north.

The age of event (5) is probably Upper Devonian or Lower Carboniferous, but events (1) have been assigned to ages from Archaean[1] to Upper Devonian or Lower Carboniferous[2].

Ages given in Table 1 are not consistent with the established sequence of geological events. Retrograde effects in the biotite–hornblende injection gneiss of the Kennack gneiss are absent or restricted to slight sericitization of feldspar. The specimens of Old Lizard Head series are muscovite–chlorite schists in which the chlorite probably has replaced biotite, and in which cordierite has altered to secondary products. Plagioclase is fresh or shows slight sericitization. Muscovite itself appears quite fresh.

The interpretation placed on these results is that the date given for the Kennack gneiss is a true one, indicating a Caledonian[3] age for the acid intrusion. That obtained for the Old Lizard Head series probably reflects loss of argon caused by slight retro-

Table 1. Results of Potassium-Argon Dating

Material	K_2O (per cent) Average of 5 determinations	Volume of argon (mm.^{3}) / Weight of sample (gm.)	Atmospheric contamination present	δV_R	δt	Age in millions of years
96388 (coarse). Old Lizard Head Series. Pen Olver	9·03	0·1206	2·7	0·4	3·25	357
96388 (fine)	9·01	0·117	1·6	0·2	3·21	352
96391 (fine). Old Lizard Head Series, nr. Life-boat station, Polperro	9·11	0·130	8·4	0·4	3·27	359
96391 (coarse)	9·17	0·121	2·0	0·2	3·20	355
						Mean = 356
96392 Kennack Gneiss, Kennack Sands	6·97	0·1188	14·1	0·5	3·58	397
96393 Kennack Gneiss, Polgwidden	5·98	0·0874	3·8	0·6	3·47	384
						Mean = 391

$\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$. $\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$.
δV_R = Percentage standard deviation in volume of radiogenic argon due to uncertainty in isotope ratios of specimen.
δt = Error in age (in millions of years) due to a 1 per cent error in proportion of K_2O or volume of radiogenic argon.

grade metamorphism during the late (? Hercynian) thrusting.

In order to check the figures yielded by the Old Lizard Head series, determinations are to be made on hornblendes from the Landewednack hornblende schist and from hornblende granulites in the metamorphic aureole of the peridotite.

J. A. Miller

Department of Geodesy and
Geophysics,
Cambridge.

D. H. Green

Department of Mineralogy
and Petrology,
Cambridge.

---

[1] Flett, J. S., and Hill, J. B., *The Geology of the Lizard and Meneage*, second ed., Mem. Geol. Surv. (1946).

[2] Hendriks, E. M. L., *Geol. Mag.*, **96**, No. 3, 253 (1959).

[3] Holmes, A., *Trans. Edin. Geol. Soc.*, **17**, Part 3, 183 (1959).

CRYSTALLOGRAPHY

Epitaxial Growth of Crystals on One-Degree Orientated Substrates

During the oxidation of one-degree orientated metals, an unexpected variation in the dispositions of oxide crystallites was observed which appears to be similar to epitaxial growth[1] of crystals on single crystal substrate. In this process, the growing crystals (overgrowths) aligned themselves in a certain manner depending on the arrangement of atoms or ions of the substrate and more correctly on the potential energy configuration at or near the surface layers. Consequently a definite orientation relationship is established between their crystal axes, and the overgrowths are said to develop a two-degree orientation such that two axes of theirs are parallel to two of the substrate. This is more often expressed by a plane and axis of each. Single crystal substrates thus greatly influence the orientation of the overgrowths at least in the initial stage of their growth[2]. On different faces of the substrate, the deposit orientation would normally be different, provided the growth condition is not too drastic or temperature not too high. The same orientation may, however, be induced on different faces of the substrate, but is again determined by the substrate structure. Numerous illustrations of such epitaxial growth of crystals are cited in the literature (see Pashley[3]).

On polycrystalline and amorphous substrates, this type of orientated overgrowths is not observed, simply because there is no regular order in the disposition of crystallites of the substrates in the former and no regular periodicity in the atomic arrangement in the latter. In one-degree orientated substrates, however, there is some partial order in distribution of crystallites in the sense that they have a common and parallel axial direction (or plane), known as orientation axis (or plane); but otherwise they are all randomly disposed. No result is yet published which suggests the influence of such substrate on the growing crystals. In view of my observation mentioned earlier, orientated metal substrates were oxidized slowly in air and the nature of the oxide crystals studied by electron diffraction.

Nickel electro-deposits of {100}, {110}, {210} and {10$\bar{1}$0} + {211} orientations (one-degree) and cobalt of {110} were prepared by electro-deposition[4]. These were then heated in air in an electric furnace to a temperature of about 320° C. for about an hour and the surface layers examined by electron diffraction both before and after oxidation in the usual way[3,4].

Table 1

Substrate orientation (1-*d*)*	Oxide orientation (1-*d*)*
Ni {100}	NiO {100}
Ni {110}	NiO {110}
Ni {210}	NiO {210}
Ni (10$\bar{1}$0) + {211}	NiO {111}
Ni {Poly}	NiO
Co {110}	Co_3O_4
Co {110} †	CoO {110}

* 1-*d*: one-degree orientation.
† At about 10^{-2} mm. mercury pressure of air.

Nickel metal was oxidized to nickel monoxide (NiO) only, whereas cobalt to cobalto-cobaltic oxide (CO_3O_4) in air, but to cobaltous oxide (CoO) only at low pressure ($\simeq 10^{-2}$ mm. mercury) of air. The surface layers on nickel substrates consisted not only of orientated polycrystalline monoxide but also of unreacted metal. Nickel monoxide (NiO) crystallites developed different orientations depending on the nature of initial substrate orientation (Table 1). But under the same conditions, polycrystalline nickel was oxidized to unorientated nickel monoxide. On further heating, however, entire surface layers, whether of orientated or unorientated nickel, consisted of polycrystalline nickel monoxide only. Further oxidation would lead to nickel monoxide, having preferred orientation determined entirely by the oxidation conditions similar to cuprous and cupric oxides on copper[5]. For better orientation the oxidation should be carried out at mild conditions. On cobalt {110} surface under milder conditions of oxidation at low pressure, again {110} orientated cobaltous oxide was observed.

These results clearly show that the overgrowth orientations can also be influenced considerably by substrates orientated one degree, at least in the initial stage of growths, and the process closely resembles the epitaxial growth on single crystals.

A. Goswami

National Chemical Laboratory,
Poona, 8.

[1] Royer L., *Bull. Soc. Franc., Min.*, 51, 7 (1928).

[2] Finch, G. I., Wilman, H., and Yang, L., *Disc. Farad. Soc.*, A, 141, 398 (1947).

[3] Pashley, D. W., *Phil. Mag.* Supp., 5, 173 (1956).

[4] Banerjee, B. C., and Goswami, A., *J. Electrochem. Soc.*, 106, 20 (1959).

[5] Goswami, A., and Trehan, Y. N., *Trans. Farad. Soc.*, 52, 358 (1956); 54, 1703 (1958).

GEOCHEMISTRY

Fossil Chlorins in a Triassic Sediment

Spectroscopic evidence for the presence of green chlorophyll-derivatives in ancient sediments and crude oils has been reported by Treibs[1] and by Blumer[2]. We have now succeeded in isolating and characterizing the green pigments responsible for the absorption band at about 640 mμ in the extracts of the porphyrin-rich Triassic oil-shale from Serpiano (Switzerland) (Fig. 1*A*). The pigments are isolated by first removing from the extracts the asphaltenes by iso-octane precipitation and extracting the ether solution of the pigment-concentrate with 6 *N* hydrochloric acid. Subsequent partitioning between ether and hydrochloric acid of increasing concentration removes the stronger bases deoxophylleryrthroetioporphyrin (DPEP) and meso-etioporphyrin (MEP). The final purification of the green pigment is then accomplished by repetitive chromatography over partially deactivated alumina (Brockman III). The spectrum of the pure pigment (Fig. 1*B*) is characteristic of the chlorin (dihydroporphin) configuration, and the partial resolution of the band at 490 mμ in addition to the presence of a small band at 612 mμ is indicative of a meso-chlorin, a chlorophyll-derivative in which the vinyl-group in the 2-position has been hydrogenated to an ethyl-group[3]. The high chromatographic mobility of this pigment on alumina and on paper (R_F = 0·75 on Whatman No. 3 paper, carbon tetrachloride–iso-octane developer, 7 : 3, see ref. 4) eliminates the possibility of the presence of acid-, ester- and other polar groups like —COH, —CO, —OH.

The pigment, which is thus characterized as a meso-etiochlorin, can be converted to the corresponding porphin by dehydrogenation with excess chloranil in boiling xylene. The reaction product (Fig. 1*C*) exhibits a typical porphyrin spectrum, intermediate between those of DPEP and MEP and identical with that of the DPEP/MEP-mixture present as uncomplexed pigments in the same sediment. This dehydrogenation product can be resolved by chromatography over alumina into fractions with the etio-spectrum of MEP and phyllo-spectrum of DPEP. Paper chromatography of these fractions confirms the spectroscopic evidence, the R_F-values agreeing within ±0·01 unit with those for pure DPEP and MEP.

For additional structural confirmation, the unknown dihydroderivative of DPEP was synthesized by hydrogenating with Raney-nickel a DPEP concentrate isolated from the oil shale. The spectrum of the hydrogenation product is identical to that of the fossil chlorin. A small difference in the R_F-values (0·68 for the synthetic compound, compared with 0·75 for the natural chlorin) is explained by the presence of a larger fraction of the more rapidly moving dihydro-MEP in the natural pigment.

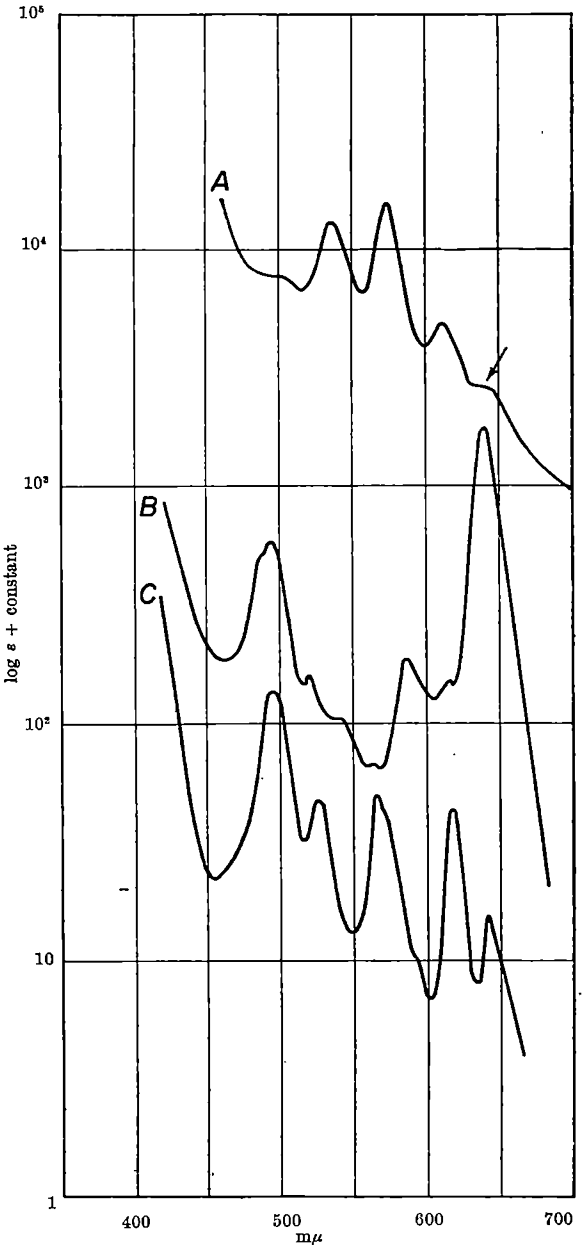


Fig. 1. Spectra. *A*, Benzene–methanol extract of Serpiano oil-shale; *B*, natural dihydro-DPEP containing some dihydro-MEP; *C*, porphyrin mixture obtained by dehydrogenation; the small peak at 641 mμ is a trace of residual chlorin

Thus, the fossil chlorin is characterized as a mixture of dihydro-DPEP (deoxo-meso-etio-pyro-pheophorbide *a*) with some dihydro-MEP (etiochlorin III). The etiochlorin must have been formed by intensive reduction from hæmin, protoporphyrin, mesoporphyrin and meso-etioporphyrin being intermediates. The chlorin structure of dihydro-DPEP is also more likely to be of secondary than of primary origin. The vinyl-group of the chlorophyll-pigments disappears early in geological history, probably by isomerization of the vinyl-chlorins to meso-porphyrins.

Any meso-chlorins that might be formed at this stage would be re-oxidized to meso-porphyrins because of their extremely low redox-potential. Only much later, after complete reduction of all carbonyl-groups, can the porphyrin-system be partially reduced with formation of the fossil chlorins.

A more detailed report of this work is being prepared for publication elsewhere. This investigation has been supported by a research contract with the United States Office of Naval Research (Contract *NONR*-2196 (00)) and the grant of a summer fellowship to one of us (G. O.).

Max Blumer
Gilbert Omenn

Woods Hole Oceanographic Institution,
Woods Hole,
Mass.

[1] Treibs, A., *Ann.*, **517**, 172 (1935); *Angew. Chem.*, **49**, 682 (1936).
[2] Blumer, M., *Helv.*, **33**, 1627 (1950).
[3] Stern, A., and Pruckner, F., *Z. phys. Chem.*, A, **180**, 321 (1937).
[4] Blumer, M., *Anal. Chem.*, **28**, 1640 (1956).

PHYSICS

Iron-55 from Nuclear Detonations

It is well known that the large numbers of neutrons released during nuclear bomb detonations (~0·5 kgm. neutrons per megaton explosion)[1] can induce high levels of radioactivities in the hardware and associated structures of the apparatus. Of particular interest is iron-55, an X-ray emitter with a half-life of 2·6 years, formed by (n, $2n$) and (n, γ) reactions on iron-56 and iron-54, respectively.

The site of high specific activity of iron-55 should be in the marine environment where stable iron exists in very low concentrations (~10 μgm./l. sea water). Radioactive iron falling on continental surfaces will be diluted readily in the large amounts of iron already existing there.

We thought it desirable to ascertain the present content of iron-55 in the oceans. Although this can be accomplished by measuring the vertical depth profiles of iron-55 contents at a number of marine locations, a more expeditious approach is the isolation of the iron activity from organisms that concentrate this element from the oceanic environment. The tuna fish which inhabit both the deep and shallow waters of the seas seemed especially suitable for this purpose since they may be considered to sample rather large areas because of their extensive migratory habits.

Ten kilograms of livers and hearts of tuna fish collected from the Pacific Ocean were digested with nitric and perchloric acids. The residue was dissolved in 8 *N* hydrochloric acid and the iron extracted with isopropyl ether. Further purification was achieved by fixing the iron on an anion exchange resin. The amount of elemental iron recovered was 2·5 gm.

The sample was assayed in a proportional counter shielded by 8 in. of iron and with an appropriate anti-coincidence arrangement. The purity of the activity was determined by comparing the spectrum of emitted X-rays with that of a standard iron-55 source. The specific activity of the samples, in February 1961, was found to be 82,000 d.p.m./gm. of iron. This value may be compared with those of other artificial and natural isotopes in the marine environment: strontium-90, 10 d.p.m./gm. strontium; cæsium-137, 500,000 d.p.m./gm. cæsium; and uranium-238, 700,000 d.p.m./gm. uranium. Since iron is generally the most abundant heavy metal in the marine biosphere, the activity-level of iron-55 is probably the highest. It should be noted that it is not great enough to constitute a health hazard.

An approximation of the iron-55 inventory in the Pacific may be obtained from the observed average specific activity of the oceanic iron. Assuming a concentration of 10^{-5} gm. iron/l. of sea water and an average oceanic depth of 4,000 m. (that is, 400 l./cm.^{2}), the iron-55 inventory in the Pacific should be of the order of $82,000 \times 10^{-5} \times 400$ or 330 d.p.m./cm.^{2} of oceanic area. Most of this activity probably still resides in the upper water inasmuch as the marine biosphere tends to promote an accumulation of iron in the oceanic mixed layer[2].

An attempt was made to find out whether any significant fraction of the iron-55 activity had already settled to the sea floor. The upper 1 cm. of sediment layer was taken from two cores of total cross-sectional area of 40 cm.^{2}, raised in the Pacific (28° 58′ N.; 117° 28′ W.; 3,600 m. depth) in April 1961. The sample was leached with 6 *N* hydrochloric acid and the iron in solution was extracted and purified as described above. The iron-55 activity in the entire sample was very small ($\leq$ 3 d.p.m.), implying that only an insignificant fraction of the activity had settled to the ocean bottom during the past few years.

In order to obtain a measure of atmospheric fall-out to the oceans, rain from San Diego was assayed for iron-55 activity. It yielded a value of 0·8 d.p.m./l. in February 1961. This indicates that the present contribution of atmospheric fall-out is small compared with the total inventory in the ocean. One metre of rainfall introduces only about 0·08 d.p.m. of iron-55 annually per cm.^{2} of oceanic area.

Acknowledgment is made to the Inter-American Tropical Tuna Commission for aid in obtaining the fish-livers. This work was sponsored by the Division of Biology and Medicine, U.S. Atomic Energy Commission.

Rama*
Minoru Koide
Edward D. Goldberg†

Department of Earth Sciences,
University of California,
San Diego.

* On leave from the Tata Institute of Fundamental Research, Bombay.

† Present address: Institute of Physics, University of Bern, Switzerland.

[1] Libby, W. F., *Science*, **123**, 657 (1956).
[2] Lewis, G. S., and Goldberg, E. D., *J. Marine Res.*, **13**, 183 (1954).

Quenching of Cation Vacancies in Doped Crystals of Sodium Chloride

As is well known, the thermodynamic defects developed by alkali halide crystals at high temperatures are predominantly of the Schottky type. The number of cation and anion vacancies in equilibrium with the crystal, at temperatures near the melting point, for example, may be as large as 10^{-4} of the total number of atoms. Attempts have, therefore, been made to quench these vacancies by very rapid cooling from these high temperatures, but without success, judging from the very similar values obtained for the electrical conductivities of the quenched and the annealed specimens[1]. (From the melt, however, some of the defects can be quenched.) This, however, is not surprising, since the relaxation times corresponding to the migration of vacancies, leading either to formation of pairs or to their exit from the crystal, remain small, even at temperatures several hundred degrees below the melting point.

Cation vacancies can also be introduced, as is well known, by suitably doping the crystal. For example, by doping sodium chloride with cadmium chloride, it is easy to see that at sufficiently high temperatures the number of cation vacancies produced by the doping will be just the number of Cd^{++} ions held in solution. We find experimentally that such vacancies, unlike the natural Schottky ones, can be quenched effectively. There are many advantages, as we shall see presently, in working with such quenched crystals, since the number of cation holes is then known definitely, and can be made fairly large. It may be mentioned incidentally that though extensive work has been done on annealed specimens of doped crystals, by Maurer *et al.*[1], quenched specimens do not seem to have been studied.

In the present experiments large clear crystals of sodium chloride containing known molar concentrations c of cadmium chloride, ranging from 2×10^{-5} to 7×10^{-4}, were grown from the melt in the usual manner. They were annealed at about 1,050° K., which is 27° below the melting point of the pure crystal, and then suddenly dropped into liquid nitrogen and thus allowed to cool rapidly. Although many of the crystals developed cracks, it was possible to obtain specimens free from cracks that were large enough for electrical conductivity measurements. Fig. 1 gives a plot of the conductivity σ of the quenched crystals at four different temperatures up to about 720° K. These temperatures were low enough to leave the quenching undisturbed. The observed small residual conductivity of the 'pure' crystal at these temperatures was corrected for. It corresponded to a doping concentration of cadmium chloride of about $4 \cdot 6 \times 10^{-6}$. It will be seen that they plot into straight lines, naturally passing through the origin. In Fig. 2 are plotted the values of $\log(\sigma T/c)$ against $1/T$, from which it will be seen that the values for all the concentrations plot on the same straight line. One may draw the following conclusions from this: (1) that even at the highest temperature of measurement, namely, 720° K., the number of cation vacancies produced by quenching remains undisturbed, and is therefore independent of the temperature of measurement; (2) that this number is proportional to the concentration c up to the highest concentration studied, namely, 7×10^{-4}, and must, therefore, be the number obtaining at the high temperature, and hence the same as the number of Cd^{++} ions doped into the lattice; in other words, while all the cation vacancies produced by the dope at 1,050° K. have been frozen intact, almost none of the natural cation vacancies of the Schottky type, the number of which also is considerable at this high temperature, has been frozen; (3) since the relaxation time corresponding to the migration of a cation hole to the neighbourhood of Cd^{++} is small, that the observed proportionality of the quenched number of holes to the concentration of cadmium points to a low value for the energy of pairing of a cation vacancy with a Cd^{++} ion.

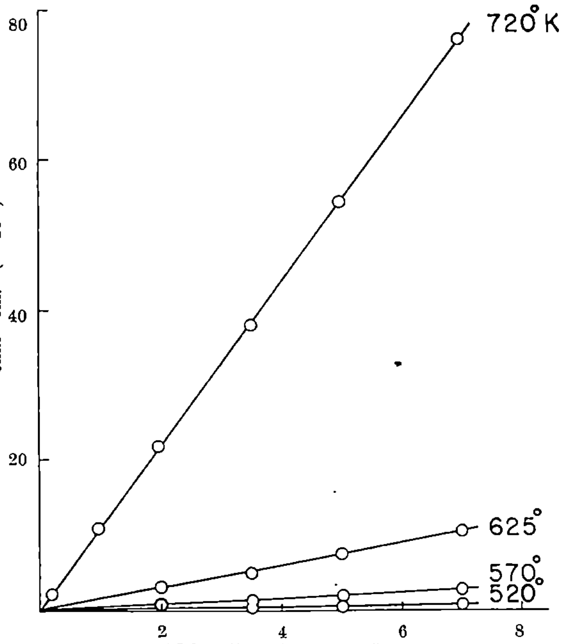


Fig. 1

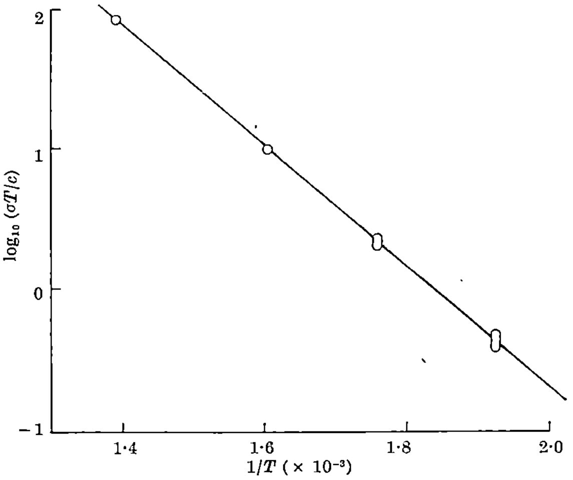


Fig. 2

Using the well-known expression for the conductivity, namely, $\sigma = ne\mu$, where $\mu = (eva^2/kT)$, $\exp(-U/kT)$ is the mobility of the cation vacancy, and $U = U_0 - \alpha T$ is the activation energy corresponding to the mobility, one obtains from the slope of the straight line in Fig. 2, $U_0 = 0 \cdot 85$ eV., and from the intercept on the y-axis the value $\exp(\alpha/k) = 460$, which is large, but of the right order of magnitude. This value of α would correspond to a temperature coefficient of U given by $-(1/U_0)\mathrm{d}U/\mathrm{d}T = 6 \times 10^{-4}$.

On annealing the crystal by very slow cooling from high temperatures, by about 15° in a day, the effective number of cation holes drops. This cannot be attributed to the formation of pairs of cation holes with Cd^{++} ions, since this process has the same small relaxation time as for the pairing of the Schottky vacancies, and would therefore have occurred even without the annealing, if the energy of combination had been large enough.

One is forced to the conclusion that the reduction in the number of cation holes by annealing must be due to the elimination of these holes, and of the same number of Cd^{++} ions, by a much slower process, probably by precipitation as cadmium chloride. This accounts for the possibility of quenching these cation vacancies, but not the Schottky vacancies. Such precipitation may be merely a consequence of the decrease in the solubility of cadmium chloride with decrease of temperature. The development of turbidity by these crystals on annealing lends support to such a precipitation.

K. S. Krishnan
S. C. Jain

National Physical Laboratory,
New Delhi.

---

[1] For a detailed review of the work, see Lidiard, A. B., *Handbuch der Physik*, **20**, 246 (Berlin, 1957).

Ultrasonic Velocity in Aqueous Solutions of Some Electrolytes

While carrying out investigations in a number of aqueous solutions of electrolytes, we have observed the interesting feature of a decrease of ultrasonic velocity with increase of concentration in the case of aqueous solutions of calcium iodide, zinc bromide, silver nitrate and cerous acetate, contrary to the normal type of behaviour exhibited in general by electrolytes. Velocity measurements in the above solutions of electrolytes at 30° C. were carried out using a fixed-path, variable frequency interferometer[1], at a frequency of 1·5 Mc./sec. The accuracy of the measurements is ± 1 m./sec. The variation of density with concentration in all the above cases is found to be linear.

The variation of ultrasonic velocity with concentration is shown in Fig. 1. In all the electrolytes investigated, zinc bromide, calcium iodide and silver nitrate exhibit a decrease in velocity of 88, 43 and 40 m./sec., respectively, for a concentration of 3 moles/litre. In spite of the peculiar behaviour in the variation of velocity with concentration, it is found that the adiabatic compressibility shows a regular decrease with increase of concentration in all these electrolytes. All other properties, like molar sound velocity and apparent molal compressibility, also showed a regular linear variation with concentration except in the case of cerous acetate, for which the variation of apparent molal compressibility with concentration is irregular.

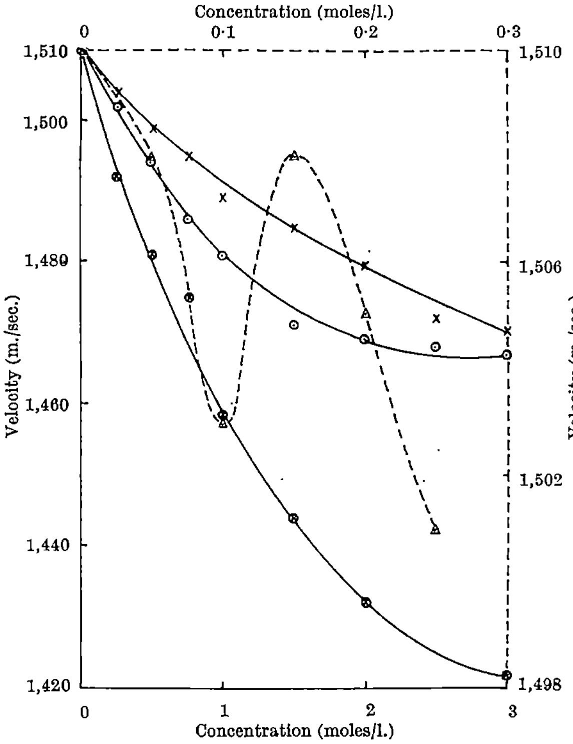


Fig. 1. Variation of ultrasonic velocity with concentration in aqueous solutions of electrolytes. ⊙, Calcium iodide; ×, silver nitrate; ⊗, zinc bromide; △, cerous acetate

It has been reported by earlier investigators[2-4] that electrolytes with heavy radicals or those having complex ions exhibit a decrease in velocity with increase in concentration. The decrease of ultrasonic velocity in the case of zinc bromide can be explained as due to the formation of complex ions, and that in calcium iodide as due to the heavy acid radical. But in the case of silver nitrate which does not form any complexes in aqueous solutions this result is unusual as similar salts like barium nitrate[5], with heavier metallic radicals, show an increase of velocity with concentration. In the case of cerous acetate the irregular variation of velocity with concentration is interesting.

M. G. Seshagiri Rao
B. Ramachandra Rao

Ultrasonic Laboratories,
Physics Department,
Andhra University,
Waltair.

---

[1] Rao, K. S., and Rao, B. R., *J. Sci. Ind. Res.*, **16**, B, 483 (1957).

[2] Barthel, R., *J. Acous. Soc. Amer.*, **260**, 227 (1954).

[3] Balachandran, C. G., *Nature*, **187**, 136 (1960).

[4] Murty, M. S., and Murty, Bh. K., *J. Sci. Ind. Res.*, **17**, B, 216 (1958).

[5] Krishnamurty, Bh., *J. Sci. Ind. Res.*, **9**, B, 215 (1950).

RADIATION CHEMISTRY

Photochemical Production of Semiconducting High Polymers

Polymers may be rendered semiconducting by heating to form graphitic structures[1]. Such non-specific treatment results in a drastic deterioration of physical properties of the polymer and a change in form of the samples. We have found that ultra-violet light irradiation of chlorinated high polymers transforms them into semiconductors. This treatment has advantages over the pyrolysis method in that: (*a*) by selective choice of ultra-violet wave-lengths specific chemical reactions are produced; (*b*) the reactions may be confined to specified areas. In fact, we have found that at the border of irradiated regions unique *p–n* junctions are produced.

In our experiments we irradiated films about 12µ thick of a commercially available saran which is a copolymer of vinylidene chloride (85 per cent) and vinyl chloride (15 per cent). All additives present were removed by soaking in acetone and then in ethanol so that no carbonyl groups are observed in the infra-red spectrum. For exclusively far ultra-violet irradiation we employed a 15-W. mercury resonance lamp (General Electrical Germicidal) which produces mainly 254 mµ. For near ultra-violet irradiation we used a 100-W. intermediate pressure lamp (Hanovia *SH*) in conjunction with a Corning 9–53 glass filter which removes all wave-lengths below 300 mµ, and for mixed irradiation this lamp was used without a filter.

Irradiation with far ultra-violet produces materials the spectra of which are given in Fig. 1. After subtracting out the background (curve *A*) the curves exhibit a broad maximum at about 285 mµ. The shape of the curve and the position of the maximum are maintained

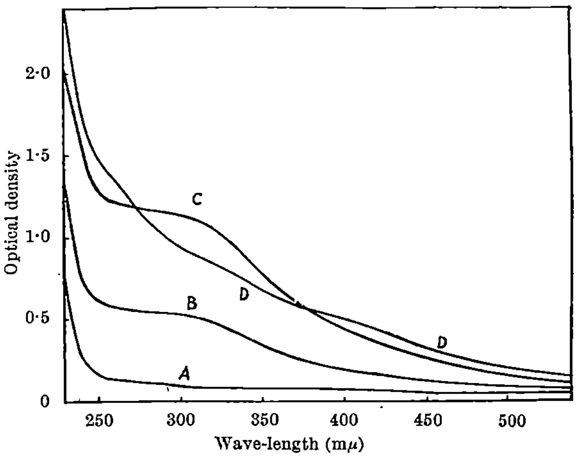


Fig. 1. Spectra of irradiated saran. *A*, original; *B*, far ultra-violet irradiated 2 hr.; *C*, far ultra-violet irradiated 4 hr.; *D*, sample *C* irradiated with near ultra-violet 1 hr.

as the irradiation is continued. This shows that distinct species are being produced in greater and greater amounts, but their relative concentrations are fixed. This is contrary to the generally adopted view[2] that increased irradiation of chlorinated polymers results in progressively more long-chain conjugation. The maximum at 285 mμ suggests the presence of a chlorinated compound containing three conjugated double bonds. It is possible that this is a ring compound formed between neighbouring chains. In fact, irradiation of saran by far ultra-violet is accompanied by cross-linking just as is the case with other high polymers[3], and the gel fraction (determined by insolubility in dioxane) is proportional to time of irradiation. Heating at 100° C. for a few hours of previously irradiated sample results in both an increase in absorption and an increase in cross-linking, both effects being enhanced the greater was the time of previous irradiation. Unirradiated samples heated in this manner show no cross-linking and no change in absorption. The absorption maximum at 285 mμ is proportional to the time of irradiation even up to large optical densities. It is evident that the sensitizer for this reaction is present in large concentration and since the original sample has a low absorption at 254 mμ this strongly suggests that the carbon–chlorine bond is the site of the photochemical reaction.

The tail of the absorption spectrum extends into the visible region and accounts for the brownish appearance of the irradiated film. Irradiated film exhibits a yellow-orange fluorescence (phosphorescence at liquid nitrogen temperature) under excitation with light up to about 400 mμ. Near ultra-violet light irradiation results in destruction of the species associated with the 285 mμ maximum by a photoxidation reaction and causes an increase in absorption below 240 mμ and above 390 mμ (curve *D*). Samples not previously irradiated with far ultra-violet are unaffected by near ultra-violet irradiation.

Far ultra-violet irradiation produces stable free radicals as shown by electron spin resonance spectra, and the spectral curves are symmetrical and structureless. For sample *B* of Fig. 1 there are 10^{15} spins/gm. The intensity of the signal is proportional to the time of irradiation and heating at 100° C. destroys the signal. Samples first irradiated with far ultra-violet and then with near ultra-violet show an unsymmetrical magnetic resonance absorption band. The free-radical species produced by far ultra-violet is preserved and the next species produced by near ultra-violet is superposed on the spectrum. Obviously the free radicals are not associated with the 285 mμ peak.

Samples irradiated with far ultra-violet show a considerable decrease in electrical resistance from that of the original saran sample (resistivity 10^{15} ohm cm.). Thus sample *C* of Fig. 1 has a thousand-fold smaller resistance than sample *A*. Subsequent irradiation with near ultra-violet influences the resistivity only slightly. The irradiated samples are photoconducting when excited by light below 300 mμ, and the photocurrent is proportional to the intensity of exciting light. Momentary illumination with the intermediate pressure lamp lowers the resistance to a hundredth that in the dark. Additional near ultra-violet irradiation increases the photoconductivity still further, while heating at 100° C. of previously irradiated samples lowers markedly the photoconductivity. It appears, therefore, that photoconductivity is associated with trapped unpaired electrons.

Interesting electrical effects are produced by irradiating two adjacent areas with the unfiltered intermediate pressure lamp for different lengths of time. For example, a portion of the film is irradiated for 5 min. and the whole sample is irradiated for 10 min. so that a sharp boundary is produced. Dusting with a mixture of charged powders (sulphur and red lead) reveals that negative charges lie mainly along a line in the less-irradiated region close to the boundary while positive charges lie mainly along a line close by in the more-irradiated region. The lines of opposite charge are separated by a thin neutral zone. This *p*–*n* junction exhibits rectification properties when illuminated with far ultra-violet, but does not do so in the dark. The presence of the neutral zone is also revealed photometrically. The samples show a colourless non-fluorescent line between adjoining areas, suggesting that a photochemical product migrates to the less-irradiated portion and reverses the effects of prior irradiation. An oxidation-reduction process might be the basis of this effect.

GERALD OSTER
GISELA K. OSTER
MARIAN KRYSZEWSKI

Polytechnic Institute of Brooklyn,
Brooklyn 1, New York.

[1] For reviews, see Berlin, A. A., *Khim. Tekh. Polimerov*, Nos. 7–8 (1960). Oster, G., Oster, G. K., and Kryszewski, M. (to be published).

[2] Boyer, R. F., *J. Phys. Colloid Chem.*, **51**, 80 (1947). Matheson, L. A., and Boyer, R. F., *Indust. Eng. Chem.*, **44**, 867 (1952).

[3] Oster, G., Oster, G. K., and Moroson, H., *J. Polymer Sci.*, **34**, 671 (1959).

CHEMISTRY

Effect of Chloride Concentration on the Aqueous Corrosion of a Magnesium Alloy

AT each nuclear power station of the Central Electricity Generating Board, the spent-fuel elements will be stored under water for about one hundred days, by which time their radioactivity will have decayed sufficiently to permit them to be removed for reprocessing of the uranium fuel. The water in each cooling pond will be maintained at about *p*H 11·5 by adding caustic alkali to minimize corrosion of the magnesium alloy can (*A*12). Although corrosion should be slight, it is still, however, important, since the corrosion product is radioactive due to the presence of active isotopes developed under irradiation from impurities in the magnesium alloy. In addition, accelerated corrosion can be expected on about 50 per cent of the cans in the pond due to carbon deposited on their surface from the carbon dioxide coolant during reactor operation. This type of accelerated corrosion is very sensitive to the presence of chloride ion in the water; the critical level for rapid attack has been estimated from corrosion tests as less than 10 p.p.m.[1]. Since all the power stations are sited near or on the coast, and most have uncovered cooling ponds, the possibility of enhanced corrosion, due to chloride pick-up, is very real, and an electro-chemical assessment of the effect of chloride and carbon on magnesium alloy corrosion is in progress.

The experimental method involves potentiostatic polarization runs from −2·0 V. cathodic to + 2·0 V. anodic versus a saturated calomel electrode, at a rate of 0·17 V./min., the current and potential values being plotted simultaneously on an X–Y recorder.

Fig. 1 compares the result obtained using an annealed and pickled magnesium alloy specimen with that obtained from a specimen having a carbon deposit. The runs were carried out in a synthetic pond water solution containing 200 p.p.m. sodium hydroxide and 530 p.p.m. sodium carbonate.

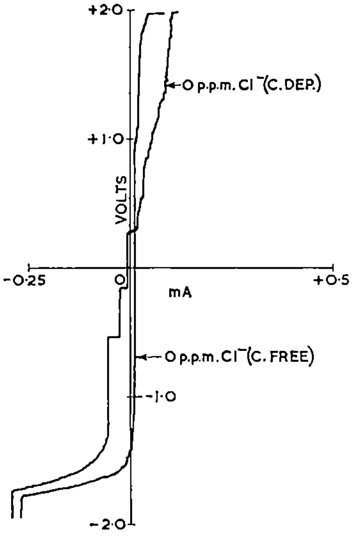


Fig. 1. *A*12 alloy. Effect of carbon-deposition

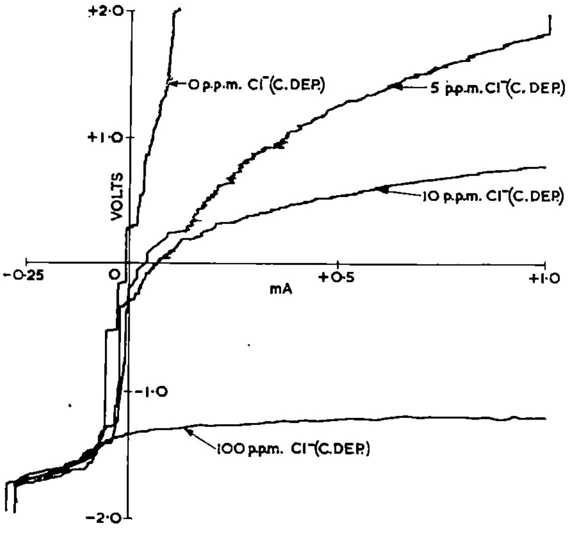


Fig. 2. *A*12 alloy with carbon-deposition. Effect of chloride-level

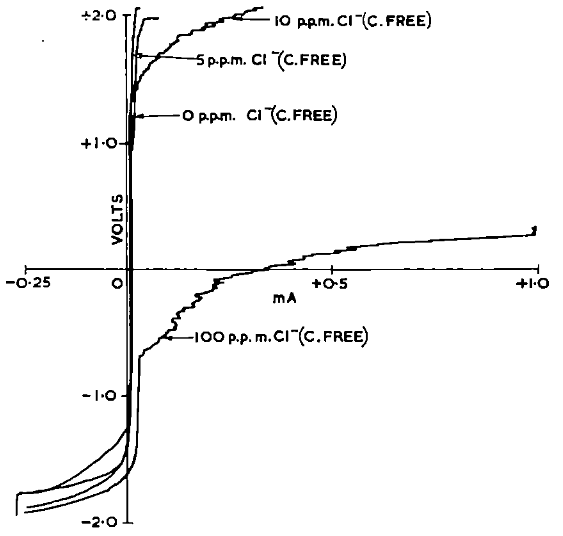


Fig. 3. *A*12 alloy annealed and pickled. Effect of chloride-level

In Fig. 2, results are shown for a number of specimens with carbon deposition, in the same solution but at various levels of added chloride. These may be compared with the corresponding effect of chloride concentration on runs with annealed and pickled specimens in Fig. 3.

From these results it may be seen that while chloride exerts an effect on the behaviour of annealed and pickled magnesium alloy specimens at levels of 10–15 p.p.m. and above, the corresponding level for specimens with a carbon deposit is much lower, and is about 4 p.p.m.

We are grateful to Dr. J. S. Forrest, director of the Central Electricity Research Laboratories, Leatherhead, for permission to submit this communication.

D. de G. Jones
H. G. Masterson

Physical Chemistry Section,
Central Electricity Research Laboratories,
Leatherhead.

---

[1] Masterson, H. G., and Harrison, J. T., *Fuel Element Storage at the Nuclear Power Stations of the Central Electricity Generating Board*, First Intern. Congr. Metallic Corrosion, London (1961).

A New Inorganic Reaction: Nitric Acid–Hydrazoic Acid

Reaction of trivalent nitrogen with the azide ion, usually called the 'nitrite-azide reaction', is generally known and has been a subject of recent detailed investigations[1–4]. This azide reaction has been considered to be specific for trivalent nitrogen even in strongly acidic media, and the fact that certain reactions of pentavalent nitrogen (for example, oxidation processes of nitric acid, lead chamber process) are stopped by the addition of azide ions has been accepted as a proof that they proceed through the trivalent nitrogen[5].

Now it has been found that pentavalent nitrogen compounds react with hydrazoic acid in strongly acidic solutions. The reaction was studied polarographically, and the reaction products were analysed by means of gas chromatography. The experiments were performed with sodium nitrate and sodium azide added to the strongly acidic solution. The course of the reaction can be followed polarographically either by means of the nitronium ion waves which appear in 15 *M* and more concentrated sulphuric acid[6], or by means of the wave of hydrazoic acid which is well developed in 8 *M* and more concentrated sulphuric acid solutions[7]. The stoichiometry of the reactants is nitric acid : hydrazoic acid = 1 : 2 and the products are a gaseous mixture of nitrogen, nitrous oxide and in some cases nitric oxide. The rate of the reaction is influenced markedly by the acidity of the reaction medium. When using sulphuric acid the following regions can be distinguished: with more than 13 *M* sulphuric acid the reaction proceeds so quickly that its time-course in the solution cannot be followed polarographically. In Fig. 1 it is seen that after addition of nitrate to the azide solution in sulphuric acid the azide wave with $E_{1/2} = 0{\cdot}7$ V. disappears and a new wave is formed with $E_{1/2} = -0{\cdot}9$ V. against a saturated mercurous sulphate electrode in 17 *M* sulphuric acid. This new wave is independent of time (cf. Fig. 1, curves 2 and 3) and can be easily removed by passing nitrogen through the solution (cf. curves 4 and 5). By further addition of nitrate, two waves are obtained which correspond to the depolarization effects of nitrites in the same medium[6] (Fig. 2). Below 13 *M* sulphuric acid the homogeneous kinetics of removing the hydrazoic acid from the solution can be followed after addition of nitrate (Fig. 3). The wave of hydrazoic acid is somewhat distorted after the addition of nitrate. This is due to the indistinct depolarization effects of nitrates in this medium which are manifested by shifting the hydrogen discharge towards the positive potentials and which are superimposed to the hydrazoic acid wave.

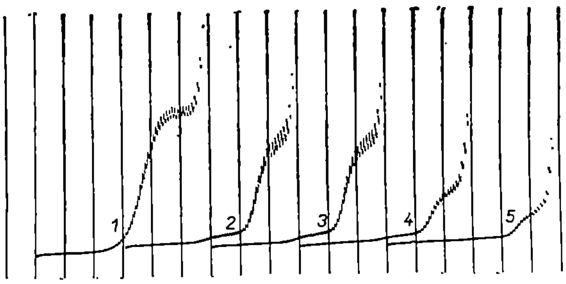


Fig. 1. Course of the hydrazoic acid–nitric acid reaction at acidities higher than 13 *M* sulphuric acid. 1, 10 ml. 14·5 *M* H_2SO_4 + 0·4 ml. 0·1 *M* NaN_3; 2, + 0·2 ml. 0·1 *M* $NaNO_3$; 3, curve 2 after standing for 10 min.; 4, nitrogen passed for 30 sec.; 5, nitrogen passed for another 1 min. *S* = 1 : 40; saturated mercurous sulphate electrode; from 0·0 V.; 200 mV./absc.

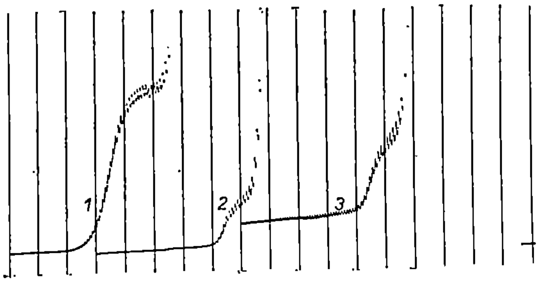


Fig. 2. Influence of nitrate on the product of hydrazoic acid–nitric acid reaction at acidities higher than 13 *M* sulphuric acid. 1, 10 ml. 14·5 *M* H_2SO_4 + 0·4 ml. 0·1 *M* NaN_3; 2, + 0·2 ml. 0·1 *M* $NaNO_3$; 3, + 0·1 ml. 0·1 *M* $NaNO_3$. *S* = 1 : 40; saturated mercurous sulphate electrode; from 0·0 V.; 200 mV./absc.

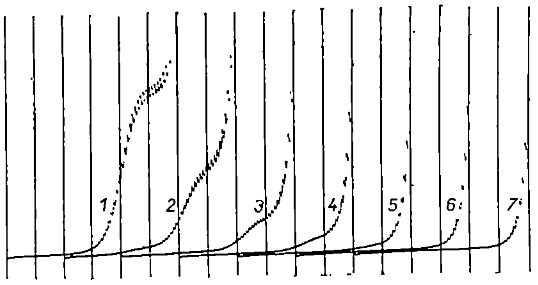


Fig. 3. Course of the hydrazoic acid–nitric acid reaction at acidities lower than 13 *M* sulphuric acid. 1, 10 ml. 13·0 *M* H_2SO_4 + 0·4 *M* NaN_3; 2–7, after addition of 0·2 ml. 0·1 *M* $NaNO_3$, the curves recorded at 3-min. intervals. *S* = 1 : 40; saturated mercurous sulphate electrode; from 0·0 V.; 200 mV./absc.

From the experimental results it follows that the reaction proceeds in three steps :

$$HN_3 + NO_2^+ \xrightarrow{k_1} 2NO + N_2 + H^+ \qquad (1)$$

$$2NO + NO_2^+ + 2H^+ \xrightarrow{k_2} 3NO^+ + H_2O \qquad (2)$$

$$3 \times (NO^+ + HN_3 \xrightarrow{k_3} N_2O + N_2 + H^+) \qquad (3)$$

$$4HN_3 + 2NO_2^+ \longrightarrow 4N_2 + 3N_2O + H_2O + 2H^+ \qquad (4)$$

Reaction (3), which is the known 'nitrite-azide' reaction, proceeds very rapidly even at low acidities and its rate increases with the acidity of the medium[1–4]. Hence it follows that in the strong acid medium which was used in the present experiments the rate of this reaction exceeds by far the rate of reactions (1) and (2). At acidities lower than 13 *M* sulphuric acid the rate-determining step is reaction (1), which is followed by rapid reactions (2) and (3) leading to the overall reaction (4). In the region above 13 *M* sulphuric acid the reaction (1) proceeds very quickly, $k_1 > k_2$, and the scheme is more complicated. From the 2 moles of nitronium ions (taking the stoichiometry according to (4) for $4HN_3 + 2NO_2^+$ . . .) more than one mole can be consumed in the reaction (1) so that less than 1 mole of nitronium ions is left for reaction (2), and consequently a surplus of nitric oxide is formed which cannot be consumed in reaction (2). This is shown in Fig. 1, where the wave with $E_{1/2} = -0{\cdot}9$ V. corresponds to nitric oxide (as mentioned above the wave can be easily removed by bubbling nitrogen through the solution or by adding the nitrate, which leads to the formation of species of trivalent nitrogen according to (2) (Fig. 2). These conclusions in both concentration regions of sulphuric acid were confirmed quantitatively by gas-chromatographic analysis of the reaction products.

The full report of this work including further experiments will be published in *Collection of Czechoslovak Chemical Communications.*

J. Mašek

Polarographic Institute,
Czechoslovak Academy of Sciences,
Prague.

[1] Seel, F., and Schwaebel, D., *Z. anorg. allg. Chem.*, **274**, 169 (1953).
[2] Seel, F., Wölfle, R., and Zwarg, G., *Z. Naturforsch.*, **13**, *b*, 136 (1958).
[3] Stedman, G., *J. Chem. Soc.*, 2943, 2949 (1959); 1702 (1960).
[4] Bunton, C. A., and Stedman, G., *J. Chem. Soc.*, 3466 (1959).
[5] Seel, F., *Angew. Chem.*, **68**, 272 (1956).
[6] Mašek, J., *Advances in Polarography*, 1, 340 (Pergamon Press, London, 1960).
[7] Mašek, J., *Coll. Czechoslov. Chem. Comm.*, **25**, 3137 (1960).

Spin Resonance and Electrical Conductivity of Indole-Iodine

Indole and iodine form a black complex that exhibits a strong electron spin resonance signal[1]. The interpretation that has been given is that indole, if complexed with a small acceptor such as iodine, may act as an especially strong electron donor due to the negative formal charge that exists on carbon 2 and 3.

A number of other iodine complexes also show strong spin resonance signals[2,3]. They also have a rather high electrical conductivity, at least for organic compounds. For the perylene and pyrene complexes, the spin resonance signal appears to be due to charge carriers[2]. On the other hand, this appears not to be true for the carotene complex[3]. Results presented here indicate that this is also not true for the indole-iodine complex, since there appears to be no correlation between the dependence on temperature of the spin resonance and that of the conductivity.

Spectra were taken on Varian *V*-4500 equipment modified to exhibit second derivatives[4]. The *R-F* power-level was approximately 3 mW., at which point the sample exhibited no saturation.

The spin resonance signal is slightly asymmetrical at low temperatures. It has a *g*-value of 2·0036 ± 0·0006. The line-width changes with temperature as indicated in Fig. 1. Since second derivatives were recorded, the number of free electrons was taken as proportional to $(\Delta H)^3 yT$, where y is the peak ordinate of the spectrum and T is the absolute temperature. The factor T is introduced to correct for the differences in distribution between the upper and lower spin states at different temperatures. The absolute number of unpaired spins was estimated by comparing the signal with a known amount of diphenylpicrylhydrazyl (DPPH).

Temperature was varied by running a stream of nitrogen at various temperatures past the sample. A thermocouple was inserted 1 in. from the sample and the assumption was made that the thermocouple measured the sample temperature when repeated runs showed no variation when taken over at least a 10-min. interval. In addition, equilibrium was checked by taking points on both increasing and decreasing temperature runs. The results (Fig. 2) indicate a lowering of the number of spins as the temperature is lowered.

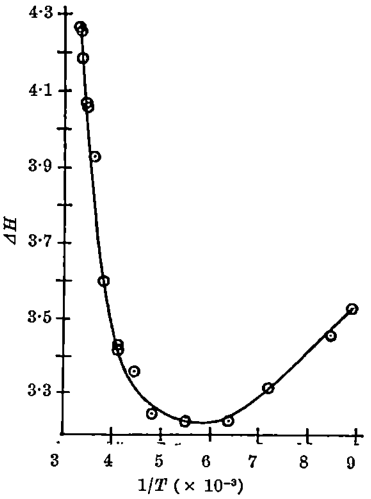


Fig. 1. Line-width in oersteds versus inverse temperature in inverse degrees K. The line-width is taken as the separation of the points of maximum slope of the absorption

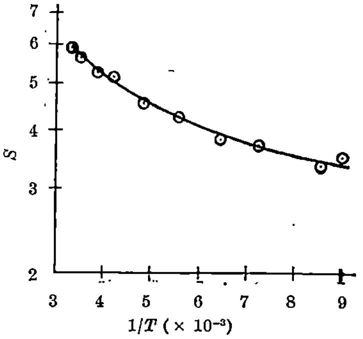


Fig. 2. Number of spins in 10^{19} spins/gm. versus inverse temperature

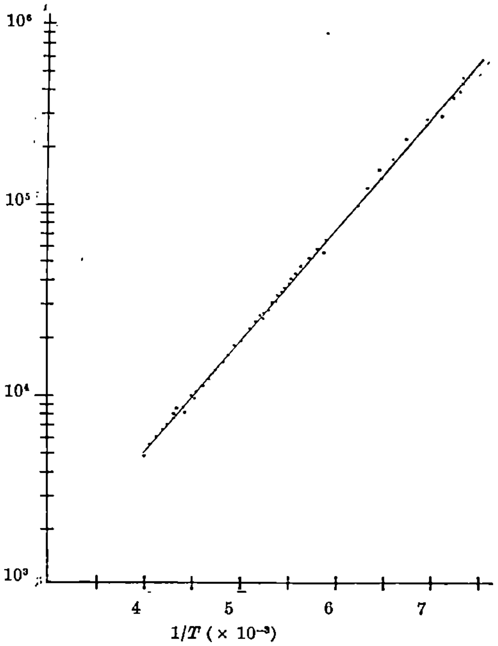


Fig. 3. Resistivity in ohm.–cm. versus inverse temperature

The electrical conductivity was measured on a sample that had been compressed between two brass screws that fit tightly into a 'Pyrex' tube of internal diameter 3·5 mm. Compression was applied and continued until changes in the electrical conductivity could be accounted for solely on the basis of changes in length of the sample. The brass screws served as electrodes, and the resistance was measured by a standard bridge arrangement. Results are shown in Fig. 3 and yield an activation energy of 0·11 eV.

A four-electrode method was also used and gave the same activation energy provided that the current density was sufficiently high. It was noted that below a current density of approximately 2×10^{-2} μamp.-mm.$^{-2}$ the resistivity was non-ohmic and followed a $V \sim i^{0 \cdot 8}$ law.

It may be seen that no correlation appears between the spin resonance characteristics and those of the electrical conductivity. The spin resonance signal thus does not appear to be due to charge carriers. However, no interpretation of the signal is evident at present.

This work was supported by a grant from the Commonwealth Fund, grant No. *H*-2042(*C*3) of the National Heart Institute, a grant from the National Science Foundation, and grant No. 1 from the Quartermaster Research and Engineering Command, U.S. Army (Natick, Massachusetts). The work was done while one of us (B. S.) was a visiting investigator at the Institute for Muscle Research.

B. SMALLER*
I. ISENBERG
S. L. BAIRD, JUN.

Institute for Muscle Research,
Marine Biological Laboratory,
Woods Hole, Massachusetts.

---

*Argonne National Laboratory, Solid State Science Division, Argonne, Illinois.

[1] Szent-Györgyi, A., and Isenberg, I., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1334 (1960).

[2] Kommandeur, J., and Hall, F. R., *Bull. Amer. Phys. Soc.*, **6**, 421 (1959). Singer, L. S., and Kommandeur, J., *ibid.*, **6**, 421 (1959).

[3] Huggins, C. M., and LeBlanc, jun., O. H., *Nature*, **186**, 552 (1960).

[4] Isenberg, I., Szent-Györgyi, A., and Baird, S. L., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1307 (1960). Smaller, B., and Yasaitis, E. L., *Rev. Sci. Instr.*, **24**, 991 (1953).

Dependence on Temperature of the Distribution of Cations in Oxide Spinels

THE fact that the cations in the typical spinel A_2BO_4 can be distributed in the so-called normal manner $A_2{}^{\mathrm{VI}}B^{\mathrm{IV}}O_4$, in the inverse manner $(AB)^{\mathrm{VI}}A^{\mathrm{IV}}O_4$, or 'randomly' with *A* and *B* ions statistically distributed over the sites, has long been used to characterize spinel structure phases. Verwey and Heilman[1], Gorter[2] and Romeign[3] have presented many data and lists on the 'type' of a particular spinel. Recent crystal field theory has also attempted to explain the position of a cation in oxide spinels[4]. Certain phases, for example $NiAl_2O_4$, have been characterized as partially inverse or even quantitatively as '85 per cent inverse', the cation distribution being $(Ni_{0\cdot15}Al_{0\cdot85})^{\mathrm{IV}}(Ni_{0\cdot85}Al_{1\cdot15})^{\mathrm{VI}}O_4$. There is in addition, of course, possible ordering on the octahedral and tetrahedral sites in phases such as $LiAl_5O_8$.

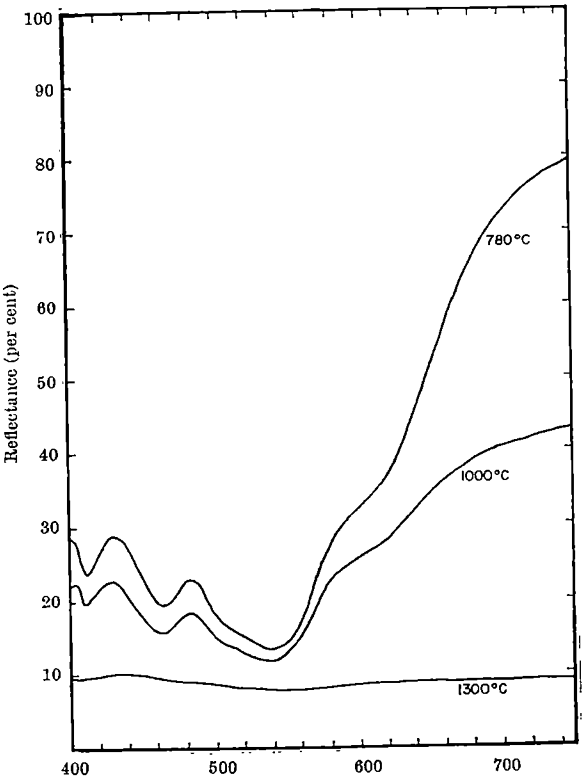


Fig. 1. Reflectance spectra of Co_2GeO_4 (with respect to MgO) samples. 780° C. sample prepared at 1,000 bars, then heated to 1,170° and 1,300° C.

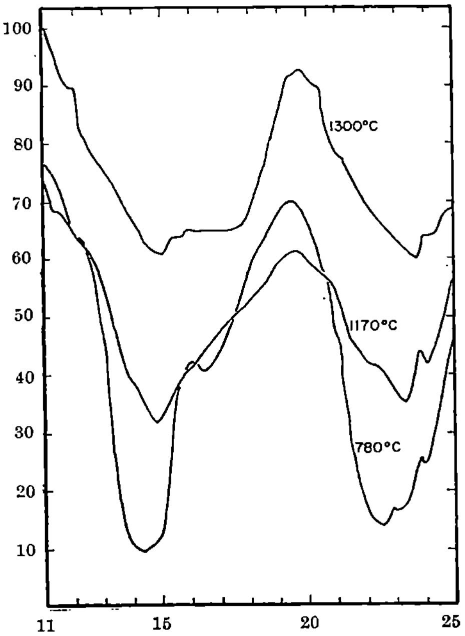


Fig. 2. Infra-red absorption patterns of Co_2GeO_4 samples after different heat treatments

It is the purpose of this communication to direct attention to the fact that in an extensive series of experiments it has been shown that in the majority of spinels, including very common ones, the cation distribution is a function of the temperature and pressure of formation. The following phases have been prepared and equilibrated in the temperature-range 400–1,800° C. and pressure-range 1–100,000 atm.:

Ni_2GeO_4, Co_2GeO_4, $NiAl_2O_4$, $MgAl_2O_4$, Zn_2TiO_4, Co_2TiO_4, 'Ni_2TiO_4', $CdGa_2O_4$, $ZnGa_2O_4$, $NiCa_2O_4$, $LiAl_5O_8$, etc.

The change of the cation distribution has been characterized by the correlation of four properties: unit cell dimensions, X-ray intensities, reflectance spectra in the visible region and infra-red absorption spectra from 11–25μ. Figs. 1 and 2 compare the lowest temperature, high-pressure form of Co_2GeO_4 with the same sample at higher temperatures at 1 atm. There is a very substantial change from a nearly perfect inverse state at low temperature to a substantially normal state at high temperature. The actual assignments of different infra-red absorption bands to various modes involving cation-anion dipoles with the cations changing co-ordination has been shown to be a complex but useful problem[5]. Substantial changes were found for each of the phases mentioned.

Mutual crystalline solubilities of many spinels have also been studied as a means of showing the cation distribution. Different degrees of deviation from ideality at different temperatures (for the same system) indicate the relation between the temperature and cation distribution in spinels. The mutual crystalline solubility relations between Ni_2TiO_4–Zn_2TiO_4 can likewise be explained by the temperature dependence of the degree of 'normalcy' of the 'Ni_2TiO_4', which does not form a compound at all. At higher and higher temperatures more and more nickel enters the tetrahedral sites, permitting 'nearly pure' Ni_2TiO_4 to be prepared in normal structure.

There is still some question as to whether or not all these reactions are reversible and whether we have true second-order phase changes over a wide temperature-range, with the degree of order determined by the temperature.

It is clear from these results that no spinel can be adequately characterized by reference to a table since the temperature and pressure of its formation determine (either stably or metastably) the cation distribution. The results also point to the necessity of presenting temperature-dependent functions for each ion as an essential refinement in crystal field approaches to the problem[4].

This work forms part of the studies in crystal chemistry supported by the U.S. Army Signal Corps under Contract No. *DA*–36–039–*SC*–85311.

Ranajit K. Datta
Rustum Roy

College of Mineral Industries,
The Pennsylvania State University,
University Park,
Pennsylvania.

[1] Verwey, E. J. W., and Heilman, E. L., *J. Chem. Phys.*, **15**, 174 (1947).
[2] Gorter, E. W., *Philips Res. Rep.*, **9**, 295, 321, 403 (1954).
[3] Romeign, F. C., *Philips Res. Rep.*, **8**, 304 (1953).
[4] Miller, A., *J. App. Phys.*, **30**, 24*S* (Supp.) (1959).
[5] Stubican V., and Roy, Rustum, *Z. Krist.* (in the press).

Reaction of Ethane with a Clean Rhodium Film

I have observed that when ethane at low pressure ($1 \cdot 2 \times 10^{-2}$ torr) comes in contact with a 27° C. rhodium film which was prepared in ultra-high vacuum (1×10^{-9} torr), it will decompose to yield gaseous methane and an adsorbed residue with a composition of $CH_{1 \cdot 5}$. Others[1,2] have observed this cracking reaction to occur at temperatures above 160° C. on metal surfaces of unknown cleanliness.

The rhodium film was prepared in ultra-high vacuum by pulse evaporating[3] a small coil of 0·005 in. diameter, 99·99 per cent pure rhodium wire in the centre of a 4 in. diameter spherical 'Pyrex' glass reaction vessel which had been baked at 450° C. for 18 hr. The reaction vessel was held at 100° C. during the film deposition. The average film thickness, measured at the completion of an experiment by X-ray emission spectroscopy, was found to be about 30 Å. in most cases. The volume of the system was about 900 c.c.

After the film was deposited, the reaction vessel was isolated from the pumping system by closing a metal valve (Granville-Phillips Co., Pullman, Washington). Five minutes were allowed for the 0·090-in. diameter filament leads to cool below 100° C. Mass spectrometrically pure ethane was admitted through a glass break-seal. The pressure in the reaction vessel was followed by a thermocouple gauge which was calibrated against a McLeod gauge for each gas present and for mixtures thereof. At various intervals, the reaction was quenched by freezing out the ethane in a cold finger with liquid nitrogen and the residual pressure measured.

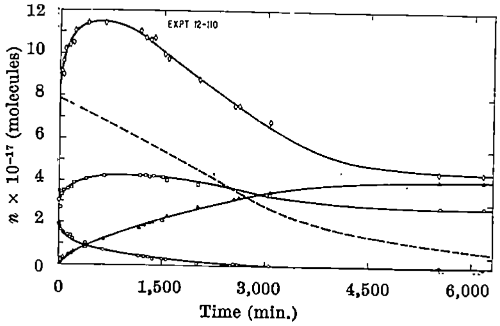


Fig. 1. Rate of decomposition of ethane on a 27° C. rhodium surface and the rate of product formation. ○, $n_{C_2H_6}$; △, n_{CH_4}; □, n_C; ◇, n_H. – – –, H/C (surface)

When the liquid nitrogen non-condensable pressure attained a constant value, it was assumed that the reaction had ceased. At this point the gases in the reaction flask were removed and analysed with a mass spectrometer. In all cases only methane was found.

These experiments were also carried out at a film temperature of 100° C. The films used at 100° C. were deposited on a 100° C. glass substrate.

A blank experiment was performed in which ethane was admitted to a baked-out reaction vessel at 100° C. There was no decomposition after 47 contact hr.

The results from a typical experiment carried out on a rhodium film with an average thickness of 30 Å. at 27° C. are shown in Fig. 1. The number of carbon atoms and hydrogen atoms present on the film at time *t* were calculated from the initial amount of ethane and the amount of ethane and methane present at time *t* as determined from the thermocouple gauge readings.

Plotting the log of the ethane pressure against time yielded a straight line. Thus, the disappearance of ethane obeys the first-order rate equation:

$$dn_{C_2H_6}/dt = -kn_{C_2H_6}$$

with an initial rate constant of $2 \cdot 13 \times 10^{-3}$ min.$^{-1}$. The rate of disappearance of ethane on a rhodium film at 100° C. also obeyed a first-order equation with a rate constant of about 0·5 min.$^{-1}$. The temperature dependence of the initial rate constant gives an Arrhenius activation energy of 16 ± 1 kcal./mole for the disappearance of ethane. The (H/C) surface ratio is about 1·5 at the end of the reaction for both film temperatures. When deuterium was added, the residue desorbed from the rhodium film as deuterated methane, indicating the presence of only monocarbon fragments on the surface.

The addition of oxygen to a clean rhodium film at 100° C. was found to inhibit the subsequent decomposition of ethane. The rate of disappearance of ethane was decreased by a factor of ten over that for a clean film at 100° C.

These experiments show that ethane will crack on a clean rhodium surface at room temperature. The rate of reaction is a sensitive function of both the surface temperature and surface cleanliness.

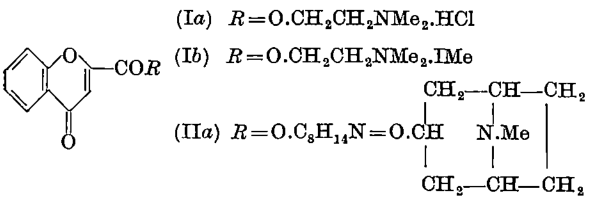
Richard W. Roberts

General Electric Research Laboratory,
Schenectady, New York.

[1] Cimino, A., Boudart, M., and Taylor, H., *J. Phys. Chem.*, **58**, 796 (1954).
[2] Kemball, C., and Taylor, H. S., *J. Amer. Chem. Soc.*, **70**, 345 (1948).
[3] Hickmott, T. W., and Ehrlich, G., *J. Phys. Chem. Solids*, **5**, 47 (1958).

Some New Esters of Chromone-2-Carboxylic Acid

WITH the view of further pharmacological work by one of us (K. V.-B.), it was decided to synthesize certain esters of chromone-2-carboxylic acid, not previously described[1–4]. The following compounds were obtained: dimethylaminoethyl-chromone-2-carboxylate hydrochloride (I*a*), the methiodide of the base (I*b*); tropyl-chromone-2-carboxylate (II*a*), its hydrochloride (II*b*), methobromide (II*c*) and methiodide (II*d*):


(I*a*) $R = O.CH_2CH_2NMe_2.HCl$

(I*b*) $R = O.CH_2CH_2NMe_2.IMe$

(II*a*) $R = O.C_8H_{14}N = O.CH(CH_2{-}CH{-}CH_2)(CH_2{-}CH{-}CH_2)N.Me$

(II*b*) $R = O.C_8H_{14}N.HCl$; (II*c*) $R = O.C_8H_{14}N.BrMe$;

(II*d*) $R = O.C_8H_{14}N.IMe$; (III*a*) $R = OH$; (III*b*) $R = Cl$

Chromone-2-carboxylic acid (III*a*), white needles from alcohol–water, melting point 256–259° (decomposes) (lit. (1), m.p. 256–263° (decomp.)), and its chloride (III*b*), white needles from petroleum ether (50–65°), m.p. 105–106° (lit. (3), m.p. 105·5–106·5°) (found: Cl, 16·6. Calc. for $C_{10}H_5O_3Cl$: Cl, 17·0) were synthesized following the technique of Zagorevskii *et al.*[3].

(I*a*) was obtained by treating 1·5 moles of (III*b*) with 1 mole of 2-dimethylaminoethanol, for 4·5 hr. in toluene by refluxing; white needles from methanol, m.p. 202–204° (decomp.); R_F: *n*-butanol/acetic acid/water (6 : 1 : 2; v/v) (Solvent *A*), 0·35; *n*-butanol/2 *N*-hydrochloric acid (1 : 1; v/v; upper phase) (Solvent *B*), 0·70 (found: C, 56·4; H, 5·5; Cl, 12·0; N, 4·8. $C_{14}H_{15}NO_4.HCl$ requires C, 56·5; H, 5·4; Cl, 11·9; N, 4·7).

(I*b*) was obtained by treating 1·5 moles of (III*b*) with 1 mole of choline chloride, for 8 hr. in toluene by refluxing. The crude methochloride was transformed into methiodide by adding sodium iodide in methanol; white plates from methanol, m.p. 224–226° (decomp.); R_F: Solvent *A*, 0·59 and *B*, 0·56 (found: C, 44·4; H, 4·7; I, 31·0; N, 3·4. $C_{15}H_{18}INO_4$ requires C, 44·7; H, 4·5; I, 31·5; N, 3·5).

(II*a*) was obtained by treating 1·5 moles of (III*b*) with 1 mole of tropine in pyridine, left overnight at room temperature, poured into dilute potassium carbonate and extracted with chloroform; white prisms from petroleum ether (50–55°), m.p. 107–109°; R_F: Solvent *A*, 0·68 and *B*, 0·72 (found: C, 68·8; H, 6·0; N, 4·4; $C_{18}H_{19}NO_4$ requires C, 69·0; H, 6·1; N, 4·5).

(II*b*) was obtained by bubbling dry hydrogen chloride into a solution of (II*a*) in ether; white needles from methanol-ether, m.p. 247–251° (decomp.); R_F: Solvent *A*, 0·68 and *B*, 0·73 (found: C, 58·8; H, 6·2; Cl, 9·4; N, 4·0. $C_{18}H_{19}NO_4.HCl.H_2O$ requires C, 58·8; H, 6·0; Cl, 9·6; N, 3·8).

(II*c*) was obtained from (II*a*) dissolved in acetone, by adding methyl bromide at room temperature; white plates from methanol-ether, m.p. 270–271° (decomp.); R_F: Solvent *A*, 0·60 and *B*, 0·60 (found: C, 53·6; H, 5·8; Br, 19·0; N, 3·4. $C_{19}H_{22}BrNO_4.H_2O$ requires C, 53·5; H, 5·7; Br, 18·7; N, 3·3).

(II*d*) was obtained from (II*a*) in a similar way to (II*c*), but using methyl iodide; white plates from aqueous ethanol, m.p. 276–277° (decomp.); R_F: Solvent *A*, 0·60 and *B*, 0·60 (found: C, 49·8; H, 5·0; I, 27·7; N, 3·0. $C_{19}H_{22}INO_4$ requires C, 50·1; H, 4·9; I, 27·9; N, 3·1).

The melting points were determined on a Kofler hot stage (Leitz, *M*350). The chromatograms were run on Whatman No. 1 paper by ascending technique, at 24°. The spots were developed with Draggendorff's reagent[5].

We are grateful to Prof. Mario R. Falco for advice and encouragement during this research.

Note added in proof: We have now obtained the original work of Zagorevskii *et al.* (*J. Gen. Chem. U.S.S.R.*, 29, 2267; 1959) in which the ester (I*a*) is described. This compound is not mentioned in *Chem. Abs.*, 54, 11007 (1960).

JUAN X. DE VRIES
Organic Chemistry Laboratory,
Facultad de Quimica.

KEMPIS VIDAL-BERETERVIDE
Pharmacology Department,
Facultad de Medicina,
Montevideo, Uruguay.

[1] Schmutz, J., Lauener, H., Hirt, R., and Sanz, M., *Helv. Chim. Acta*, 34, 767 (1951).

[2] Vejdelek, Z. J., Trcka, V., Chyba, O., and Chybova, H., *Chem. Listy*, 47, 575 (1953); *Chem. Abst.*, 49, 306 (1955).

[3] Zagorevskii, V. A., Zykov, D. A., and Pronina, L. P., *Zhur. Obshchei Khim.*, 29, 1026 (1959).

[4] Zagorevskii, V. A., Zykov, D. A., and Vinokurov, V. G., *Zhur. Obshchei Khim.*, 29, 2302 (1959).

[5] Cramer, F., *Papierchromatographie*, fourth ed., 164 (Verlag Chemie, Weinheim, 1958).

BIOCHEMISTRY

Structure of Feather Keratin

FEATHER keratin is the outstanding protein fibre structure that still lacks an interpretation generally acceptable in any detail. It has long been known[1] that its X-ray diffraction diagram is of a β-type with the strong indication that the residue-length is only about 3$\frac{1}{10}$ Å. instead of the 3⅓ Å. and 3½ Å. found in β-keratin and silk fibroin, and it has corpuscular properties besides[2], especially as demonstrated in the paper by Bear and Rugo[3], supported also by physico-chemical studies and the finding that the unit is probably a cyclic polypeptide[4]. In Bear and Rugo's scheme the structure is based on a net of corpuscular units the nodes of which are at approximately (0, 0), (0, ½), (½, ¼), and (½, ¾), the plane of the net containing both the main-chain and side-chain directions. Continuing from this point and associated observations by the same authors, it is the purpose of this communication to state what we believe to be a very strong case for the structure of the corpuscles themselves. The principal steps in the argument may be summarized as follows :

(1) There are three polypeptide β-configurations ('pleated sheets')[5] with interchain CO . . . NH hydrogen bonds that are linear (see, for example, ref. 6 for our own style of models illustrating this), and in these the amino-acid residue-lengths are approximately 3½ Å., 3⅓ Å., and 3$\frac{1}{10}$ Å., respectively. In the first two the chains are anti-parallel and parallel dyad screws, respectively, and they satisfy the requirements of silk fibroin and β-keratin. In the third the chains are 'parallel polar'; that is, they run in the same direction but are not screws, each chain having all its CO groups on one side and all its NH groups on the other side. It is a fair hypothesis that

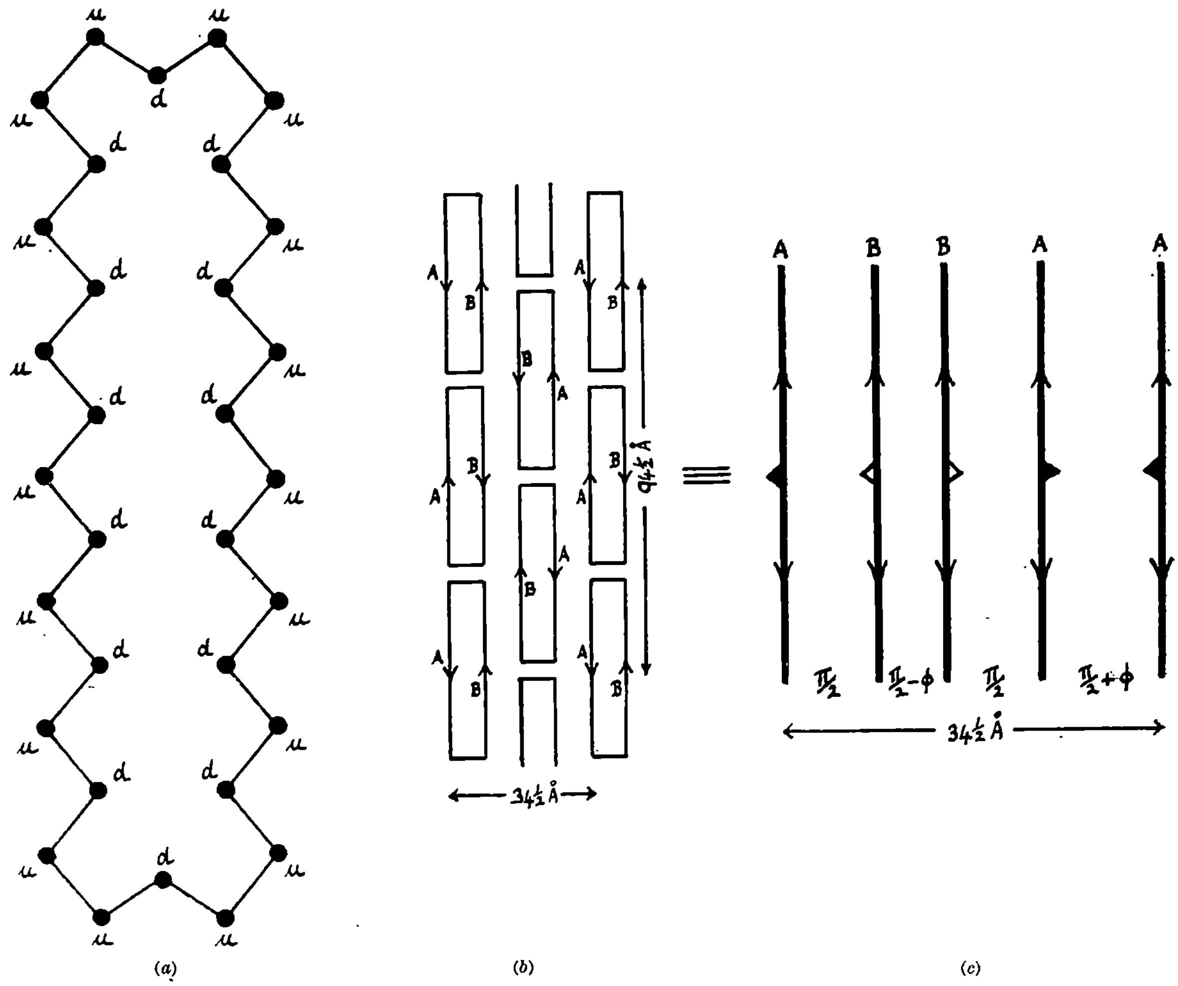


Fig. 1. Diagrammatic representation of (*a*) the proposed 32-residue loop-corpuscle, α-carbons being indicated by black dots and the bonds to β-carbons by *u* (up) and *d* (down); (*b*) the arrangement of polypeptide chains in conformity with the net pattern of Bear and Rugo; and (*c*) the equivalent scheme of chains for the purpose of deriving the equatorial intensities

this third arrangement, with its residue-length of about $3\frac{1}{10}$ Å., is the basis of the third known natural β-structure, that of feather keratin[6,7].

(2) The inference receives immediate strong support from the striking contrast between the meridional reflexions of fibroin and β-keratin on one hand and of feather keratin on the other. In relation to the axial sub-unit of a pair of consecutive residues, the former show no 1st order but a strong 2nd order (and also a 6th), while the latter shows a strong 1st order but no 2nd order (and no 6th either).

(3) Feather keratin contains a high proportion of proline residues (about one-tenth)[8], and these fit readily into the parallel-polar configuration[9], but not without strain into the other two β-configurations. We suggest that it is indeed the proline residues which impress the parallel-polar configuration on the feather keratin structure.

(4) Apparently the only other chain-configuration which has a residue-length of about 3·1 Å. and which can also incorporate proline without strain is the trigonal helix that is the basis of collagen[9]. A collagen-type structure for feather keratin has in fact been advocated by Ramachandran[10], but in our view it is quite ruled out by the infra-red data. Collagen has a unique, 'diagnostic' NH-stretching frequency at 3,330 cm.$^{-1}$ as compared with the 3,300 cm.$^{-1}$ found with the other fibrous proteins including feather keratin, and its other CO and NH frequencies are also above the range found with accepted β-structures and feather keratin[11].

(5) In conformity with Bear and Rugo's Fig. 6, we propose the loop-corpuscle structure represented diagrammatically, and most briefly for the present, by Fig. 1, for which we have constructed satisfactory scale-models both of our own and of the Courtaulds type. There are 32 residues per loop, and two features to be specially noted are: (1) that, looking transversely, the β-carbons on opposite sides are in register as well as the α-carbons; (2) that the CO groups all point upwards along one side but downwards along the opposite side, thus cancelling out the polar imbalance and any bending tendency that might otherwise accrue.

(6) Bear and Rugo's net in any event accounts most plausibly for the dominant meridional reflexion at 23·6 Å., and now our own calculations show also that, on the basis of Fig. 1*a* and the chemical-analytical[8] average residue-weight of 98·5, the reflexion near 6·2 Å., corresponding to the 'first order' of the characteristic residue-pair, should be about 150 times as strong as the 'second and sixth orders' near 3·1 Å. and 1·03 Å., respectively. (The observed intensities are strong : 0 : 0.)

(7) The equatorial reflexions are the first four orders of about 34½ Å., plus a fuzzy reflexion apparently corresponding to the CO . . . NH linkage transverse to the loop. The former change with the water-content and various swelling pre-treatments[3], and they have still to be worked out in detail; but preliminary calculations based on effectively four chains of appropriately different weights and separations, as in Fig. 1*c*, are already in sufficiently good agreement to indicate that this is probably the correct approach.

(8) Bear and Rugo's requirement of a plane-net structure, and the vagueness of the CO . . . NH linkage, are explained at once by our models, which show that continued regular superposition of loops will in general be obstructed by the proline residues. With one special arrangement of the latter, though, correct 'sandwich-pairs' are possible, but nothing thicker, suggesting a limiting corpuscle-weight of perhaps about 6,400 as compared with Woodin's[4] physico-chemical estimate of about 10,000.

(9) Finally, the calculated density of our structure is 1·32, compared with the observed value of 1·27 (ref. 12). The difference is in the right direction for a high polymer and is quite unobjectionably small.

Two other proposed structures for feather keratin are those of Krimm and Schor[13] and of Fraser and MacRae[14]; but we believe that the one outlined here offers a better interpretation of the experimental facts, as we hope to show more fully in a later publication.

W. T. ASTBURY
E. BEIGHTON

Department of Biomolecular Structure,
University of Leeds.

[1] Astbury, W. T., and Marwick, T. C., *Nature*, **130**, 309 (1932).

[2] Astbury, W. T., and Lomax, R., *Nature*, **133**, 795 (1934). Ward, W. H., High, L. M., and Lundgren, H. P., *J. Polymer Res.*, **1**, 22 (1946).

[3] Bear, R. S., and Rugo, H. J., *Ann. N.Y. Acad. Sci.*, **53**, 627 (1951).

[4] Woodin, A. M., *Biochem. J.*, **57**, 99 (1954); **63**, 576 (1956).

[5] Pauling, L., and Corey, R. B., *Proc. U.S. Nat. Acad. Sci.*, **37**, 251 (1951); **37**, 729 (1951); **39**, 255 (1953).

[6] Astbury, W. T., *J. Text. Inst.*, **51**, P, 515 (1960).

[7] Astbury, W. T., *Ann. Ital. Derm. Sif.*, Anno 15 (1960).

[8] Schroeder, W. A., and Kay, L. M., *J. Amer. Chem. Soc.*, **77**, 3901 (1955).

[9] Astbury, W. T., Ninth Procter Memorial Lecture, *J. Soc. Leather Trades' Chem.* (in the press).

[10] Ramachandran, G. N., *Acta Cryst.*, **10**, 816, (1957); *Recent Advances in Gelatin and Glue Research*, 32 (Pergamon Press, 1958).

[11] Fraser, R. D. B., *Faraday Soc. Disc. on Spectroscopy*, 398 (1950). Ambrose, E. J., and Elliott, A., *Proc. Roy. Soc.*, A, **206**, 214 (1951). Bradbury, E. M., Burge, R. E., Randall, J. T., and Wilkinson, G. R., *Disc. Farad. Soc.*, No. 25, 173 (1958). Parker, K. D. (personal communication).

[12] Fraser, R. D. B., and MacRae, T. P., *Text. Res. J.*, **27**, 384 (1952)

[13] Krimm, S., and Schor, R., *J. Phys. Chem.*, **24**, 922 (1956).

[14] Fraser, R. D. B., and MacRae, T. P., *J. Mol. Biol.*, **1**, 387 (1959).

Simultaneous Determination of Dextran and Glucose in Serum

IN the course of an investigation on the mechanism of 'anaphylactoid' reaction in rats, the need was felt for an accurate and simple procedure for the simultaneous determination of dextran and glucose in small amounts of serum. For this purpose the following method was developed. It utilizes the *o*-toluidin – glucose coupling reaction[1], producing a green colour suitable for colorimetric measurement. Dextran is precipitated from the serum by the *o*-toluidin – acetic acid reagent followed by hydrolysis and the colour reaction. Serum glucose is directly determined from the supernatant layer of the dextran precipitate.

Blow 0·1 ml. serum, containing 25–400 μgm. dextran, into 10-ml. glass-stoppered test-tubes containing 4 ml. of *o*-toluidin reagent (6 per cent *o*-toluidin in glacial acetic acid prepared immediately before use). Put the tubes in a refrigerator at 4° C. for 30 min. Centrifuge (3,500 r.p.m.) for 30 min. Decant the supernatant carefully into another test-tube. Place the latter in a boiling water-bath for exactly 8 min. Cool and read the optical density in a spectrophotometer or filter photometer at or near 625 mμ. A standard glucose solution is treated in the same way as serum.

The tubes containing the precipitated dextran are dried by placing them in an inverted position on a towel. 0·2 ml. of 4 *N* sulphuric acid is added and the precipitate dissolved by shaking. The tubes are loosely stoppered with glass stoppers (secured by a rubber band) and placed in a boiling water-bath for 75 min. After cooling, 8 ml. of *o*-toluidin reagent is added and the contents mixed. After boiling for exactly 8 min. the colour intensity is determined as above. A standard solution of dextran or glucose is carried through the entire procedure. In the latter case the result is multiplied by 0·9 (ref. 2), since 100 mgm. dextran is equivalent to 111 mgm. glucose.

The efficiency of dextran precipitation by the reagent was checked as follows: 0·1 ml. portions of 200, 400, 600, 800 mgm. dextran/100 ml. solutions were pipetted into 4 ml. of 6 per cent *o*-toluidin in acetic acid. After cooling and centrifugation, 20 ml. of 96 per cent alcohol was added to 2 ml. of the supernatant to precipitate the part of the dextran possibly remaining soluble in the reagent. The tubes were kept in the refrigerator overnight and, after centrifugation and decantation of supernatant, an anthrone reaction[3] was carried out with the residue. Sixteen determinations were made, optical densities being read in a Beckman *DU* spectrophotometer. In the range of 200–600 mgm./100 ml., the supernatant contained no anthrone-positive material which could be precipitated with alcohol. The supernatant contained only in the case of the 800 mgm. dextran/100 ml. an average of 1·37 per cent of total dextran.

The adequacy of the hydrolysis of dextran as used by Dimler *et al.*[2] was also investigated. 10, 50, 75, 100, 150 and 200 mgm. dextran/100 ml. solutions in 4 *N* sulphuric acid were kept in a water-bath at 100° C. for 75 min. After cooling, 8 ml. of 96 per cent alcohol were added to 0·8 ml. of hydrolysate, the tubes were kept at 4° C. overnight, centrifuged, the residue was washed once with 96 per cent alcohol and the anthrone reaction carried out. Six triplicate determinations were carried out. Traces of anthrone-positive material were found in 3 tubes, but not in the parallel tubes ;

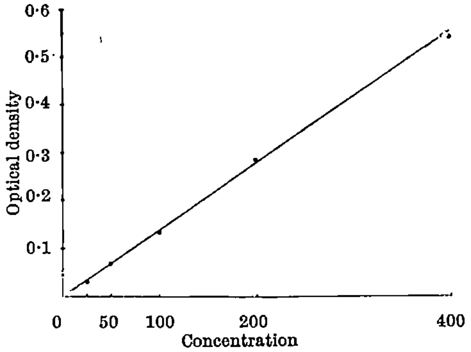


Fig. 1. Colour reactions of dextran solutions in water after hydrolysis. Abscissa: concentration of solutions. Ordinate: optical density determined at 625 mμ in 1-cm. cells in the Beckman *DU* spectrophotometer. Each point is the average of three determinations

therefore extraneous carbohydrate contamination was assumed in these cases.

In Fig. 1 is shown the colour reaction of dextran solutions in water after hydrolysis. There is good agreement with Beer's law within the concentration-range 25–400 mgm./100 ml. Dilution of serum is recommended for higher concentrations.

The coefficient of variation (per cent standard deviation) obtained by 20 parallel determinations on rat serum was 2·7 per cent for glucose at a level of 194 mgm./100 ml. and 2·5 per cent for dextran at a level of 184 mgm./100 ml.

We are indebted to Glaxo Laboratories, Ltd., for a gift of 'Clinical Dextran' used in this work. Dr. G. Szepessy, of the Central Research Laboratory of this University, kindly determined the optical densities.

A. Rédei
S. Nagy

Institute of Pathophysiology,
Medical University of Szeged.

[1] Hultman, E., *Nature*, **183**, 108 (1959).

[2] Dimler, R. J., Davis, H. A., Gill, G. J., and Rist, C. E., *Anal. Chem.*, **26**, 1142 (1954).

[3] Földes, J., *Kisérl. Orvostud.*, **11**, 1 (1959).

Emulsifying Capacity of Blood Sera

In view of the suspected link between fat metabolism and atherosclerotic disease, the state and behaviour of the emulsified fats in the blood serum have been the object of numerous investigations. Thus, for example, stabilized emulsions of lipoproteins, of coconut oil, and of olive oil have been added to sera in order to demonstrate the clearing reaction of heparin[1]. It seems, however, that the ability of different sera to emulsify various fats has never been investigated systematically.

In order to test the emulsifying ability of different sera, a simple, reproducible turbidimetric method of measurement has been developed. Briefly, the serum is shaken under controlled conditions with 0·5 per cent olive oil. The optical extinction rises to a maximum with time and then begins to fall; at 37° C. the maximum extinction is reached in about 20 min. The maximum extinction value we termed the 'emulsifying capacity' of the serum. The dispersion of 0·5 per cent olive oil in 0·005 per cent 'Lissapol' (run parallel with the experiments) served as control.

The emulsifying ability of a given serum has been found to be reproducible to within about ± 2 per cent.

Microscopic investigation showed that greater turbidity corresponds to a smaller average oil-droplet diameter.

After the reproducibility of the results was proved beyond doubt, studies have been initiated of the influence of various factors on the maximum turbidity obtained. The following is a summary of the results:

(1) There is an optimum shaking rate of about 200 beats/min. for the development of high turbidity emulsions.

(2) At oil concentrations between 0·1 and 1 per cent the maximum turbidity has been found to be proportional to the logarithm of the oil concentration.

(3) The emulsifying ability of sera from different animals has been found to vary both from animal to animal, as well as from species to species. Thus, sheep and cattle sera show higher emulsifying ability than pig sera; this variation in emulsifying capacity is well outside the variations found with sera from individual animals of the same species.

(4) The type of oil used determines, to a large extent, the maximum turbidity. In general, oils yielding high-turbidity emulsions have high acetyl and iodine numbers, and low viscosity. It must be stressed, however, that these differences are substantially smaller when the experiments are conducted at 37° C., although some differences still persist; thus, coconut oil yields substantially coarser emulsions both at room temperature and at 37° C.

(5) The addition of surface-active compounds exerts a strong influence on the maximum turbidity obtained. I found that in the presence of small concentrations (5×10^{-4} *M*) of surfactive acids (for example, oleic acid) the emulsification decreases, while the addition of the same amount of surfactive bases (for example, long-chain amines) or of neutral surfactants (for example, cetyl alcohol) increases the maximum turbidity. The effect of the surfactant is the same, whether it is added to the serum or to the oil phase.

(6) Diluting the serum up to ten-fold with isotonic phosphate buffer (*p*H 7·2) increases the maximum obtainable turbidity; further dilution results in a decrease of the maximum turbidity. The reasons for this particular behaviour remain to be investigated; they could be attributed tentatively to either changes in the protein surface and/or to viscosity changes.

Examination of some of the physico-chemical properties of the resulting emulsions have shown that:

(1) The electrophoretic mobilities of the oil droplets seemed unchanged by the nature of the surfactant added, a result which warrants further investigation.

(2) The interfacial surface tension is only slightly lowered in the presence of surface-active compounds; the degree of emulsification obtained seems unrelated to the interfacial surface tension.

Clinical investigations are at present being carried out in the Lidcombe State Hospital, Sydney. Although it is too early at present to draw conclusions from the results so far obtained, it is already evident that there are individual variations in the emulsifying abilities of human sera; sera obtained at different times from one and the same person can also show marked variations. Thus, the emulsifying ability obtained with the serum of a given individual invariably decreases after the ingestion of fats. Interesting effects have been observed on intravenous injection of heparin; they will form the subject of a separate communication, as will the details of the work presented here.

B. Breyer

Section of Agricultural Chemistry,
Department of Agriculture,
University of Sydney,
New South Wales.

[1] Shore, B., Nichols, A. V., and Freeman, N. K., *Proc. Soc. Exp. Biol., New York*, **83**, 216 (1953). Brown, R. K., Boyle, E., and Anfinsen, C. B., *J. Biol. Chem.*, **204**, 423 (1953). Meng, H. C., Hollett, C., and Cole, W. E., *Amer. J. Physiol.*, **179**, 314 (1954).

Hydrolysis of Fatty Acid Esters of Riboflavin by Pancreatic Lipase

Fatty acid esters of riboflavin, fat-soluble riboflavin derivatives, have been synthesized in our laboratory to widen the application of riboflavin to pharmaceutical and nutritional fields[1–3]. As a fundamental problem of this application, the possibility of enzymic hydrolysis of these esters to riboflavin and fatty acids has to be investigated first. This communication deals briefly with their hydrolysis *in vitro* by pancreatic lipase.

Fatty acid esters were synthesized by esterification of riboflavin with fatty acid chloride, and riboflavin 2′, 3′, 4′, 5′-tetrabutyrate, tetracaprate, and tetrapalmitate were obtained as reported before[1-3]. Pancreatic lipase was supplied by the Nutritional Biochemical Corporation. The activity of the enzyme preparation was checked by using tributyrin and 'Tween 20' as substrate. The reaction mixture consisted of 0·5 ml. of 4 mmole/l. fatty acid ester of riboflavin suspended in 1 per cent polyvinyl alcohol (degree of polymerization: 500), 0·5 ml. of enzyme solution (20 mgm. protein/ml.) and 1·0 ml. of McIlvaine buffer (*p*H 7·3).

The reaction mixture was incubated at 37° C. for 2 hr. with gentle shaking. After incubation, the test solution was analysed by paper chromatography using an upper layer of *n*-butanol/acetic acid/water (4 : 1 : 5, v/v) as a mobile phase. After 10 hr. development at 20° C., the paper was removed and allowed to dry at room temperature in the dark. The paper strip was examined under ultra-violet light, and flavins were detected by their yellow fluorescence.

When riboflavin tetrabutyrate was incubated with pancreatic lipase, yellow fluorescent spots were observed on the paper at R_F values of 0·3, 0·5, 0·6, 0·7 and 0·97. Substances at R_F value 0·3 and 0·97 were identified with riboflavin and riboflavin tetrabutyrate respectively. Substances at R_F values 0·5, 0·6 and 0·7 were supposed to be the intermediate compounds from riboflavin tetrabutyrate to riboflavin. The riboflavin part was eluted with hot water, and the other yellow fluorescent spots were eluted with 98 per cent ethyl alcohol. The amount of flavin in both eluates was measured by the lumiflavin fluorescence method[4]. The enzyme activity was expressed by the amount of free riboflavin split off from fatty acid ester of riboflavin.

Riboflavin tetracaprate and tetrapalmitate were also examined in the same way, and hydrolyses of these substances by pancreatic lipase were compared with each other. The results are shown in Table 1. Among fatty acid esters of riboflavin tested, tetrabutyrate was found to be most easily hydrolysed, followed by tetracaprate and tetrapalmitate. In the case of tetrabutyrate, optimum *p*H and temperature of the enzyme were found at 7·3 and 37° C. respectively.

Table 1. COMPARISON OF HYDROLYSIS OF FATTY ACID ESTERS OF RIBOFLAVIN BY PANCREATIC LIPASE

Substrate	Rate of hydrolysis (per cent)
Riboflavin tetrabutyrate	45·4
Riboflavin tetracaprate	3·4
Riboflavin tetrapalmitate	1·2

Reaction mixture: 0·5 ml. of 4 mmole/l. substrate suspended in 1 per cent polyvinyl alcohol, 0·5 ml. of the enzyme (20 mgm. protein/ml.) and 1·0 ml. of McIlvaine buffer, *p*H 7·3. Incubation: 37° C., 2 hr.

Fatty acids liberated from these esters by the enzyme were checked. For example, in the case of tetrabutyrate, liberated fatty acid in the incubated reaction mixture was extracted with ethyl ether, concentrated, and then analysed by paper chromatography followed by coloration with bromocresol purple[5]. A single spot corresponding to butyric acid was detected.

From these results it was clear that these fatty acid esters of riboflavin could be hydrolysed to riboflavin and the corresponding fatty acid by the enzyme action of pancreatic lipase. Considering also the fact that these esters are effective as riboflavin by the test of animal growth[6], it is considered that these esters may be converted to riboflavin and then act as a vitamin.

It may be noticed that pancreatic lipase can also attack these newly synthesized esters besides substrates hitherto known.

KUNIO YAGI
YOSHIKO YAMAMOTO
JUN OKUDA

Department of Biochemistry,
School of Medicine,
Nagoya University, Nagoya.

[1] Yagi, K., Okuda, J., and Dmitrovskii, A. A., *J. Biochem.*, **48**, 621 (1960).

[2] Yagi, K., Okuda, J., Dmitrovskii, A. A., and Honda, R., *Vitamins* (Japan), **21**, 217 (1960).

[3] Yagi, K., Okuda, J., and Matsubara, T., *Vitamins* (Japan), **21**, 525 (1960).

[4] Yagi, K., *J. Biochem.*, **43**, 635 (1956).

[5] Reid, R. L., and Lederer, M., *Biochem. J.*, **50**, 60 (1951).

[6] Yagi, K., and Okuda, J., *Vitamins* (Japan) (in the press).

Neutral 17-Ketosteroids in Porcine Urine

IN searching for an experimental animal which, in regard to its steroid metabolism, would be suitable for an investigation into the mechanism of action of certain synthetic steroids, particularly 19-norsteroids, we decided to use the domestic pig. A few preliminary experiments were made, during which we determined, among other things, the concentration of Zimmermann chromogens in the urine of male and female pigs and in the urine of castrated animals, while the influence of chorionic gonadotrophin and adrenocorticotrophic hormone on the excretion of Zimmermann chromogens in adult male pigs was also determined. After these preliminary experiments we started to isolate and identify the substances which produce the Zimmermann reaction.

For this purpose, 5,760 ml. urine from an adult male animal was extracted by the method of Dingemanse *et al.*[1]. Two extracts were obtained:

(*a*) An extract obtained by heating the unacidified urine to about 80° C., during 4 hr., while simultaneously extracting with benzene.

(*b*) An extract obtained by adjusting the urinary residue resulting from treatment of urine as in (*a*) to *p*H 1, after which it was heated to about 80° C., for 4 hr., while simultaneously extracting with benzene.

The former extract, which contained about 660 mgm. 'dehydroepiandrosterone equivalents', was purified by the Girard method, by chromatography over aluminium oxide and over cellulose powder. In this way we obtained four different Zimmermann positive fractions, two of which have so far been completely purified and identified. The first fraction to be isolated was 3-chloro-androst-5-ene-17-one, which was identified as follows:

(1) By paper chromatography (system propylene glycol: methanol/heptane).

(2) By determination of the melting-point (148–152° C.) and the mixed melting-point with authentic 3-chloro-androst-5-ene-17-one (with the aid of the Eimer and Amend apparatus, uncorrected).

(3) By establishing that no acetate is obtained at acetylation.

(4) By infra-red analysis: the infra-red spectrum was completely identical with that of authentic 3-chloro-androst-5-ene-17-one.

(5) By elementary analysis, which yielded the following results: C = 74·68 per cent, calculated for $C_{19}H_{27}OCl$: 74·39 per cent; H = 8·69, calculated for $C_{19}H_{27}OCl$: 8·81; Cl = 11·35, calculated for $C_{19}H_{27}OCl$: 11·58. The extract of 5,760 ml. urine was estimated to contain more than 300 mgm. 3-chloroandrost-5-ene-17-one.

The second 17-ketosteroid found to be present in urine of male pigs is dehydroepiandrosterone, which was identified as follows:

(1) By paper chromatography (system propylene glycol: methanol/heptane).

(2) By determination of the melting-point (138–142° C.) and the mixed melting-point with authentic dehydroepiandrosterone.

(3) By acetylation, which yielded an acetate which was, as judged by its infra-red spectrogram, melting-point and mixed melting-point, identical with authentic dehydroepiandrosterone acetate.

(4) By infra-red spectrometry: the infra-red spectrum was completely identical with that of authentic dehydroepiandrosterone.

(5) The substance produces the reaction with phosphomolybdic acid which, carried out under certain conditions, is specific of unsaturated steroids.

A total quantity of 77·3 mgm. pure dehydroepiandrosterone was obtained from 5,760 ml. urine.

The urinary extract obtained at *p*H 7 in addition contains 1–2 other 17-ketosteroids oxygenated on C_{11}.

The extract obtained at *p*H 1 has so far been examined only with the aid of paper chromatography. It contains several Zimmermann chromogens. The chromogen present in the highest concentration has an R_F value with paper chromatography = R_F dehydroepiandrosterone, or epiandrosterone, respectively (system propylene glycol: methanol/heptane).

We are indebted to N.V. Organon, Oss, Netherlands, from whom we received authentic dehydroepiandrosterone and authentic dehydroepiandrosterone acetate.

We received a quantity of authentic 3-chloro-androst-5-ene-17-one from the Steroid Reference Collection, London, through the courtesy of Prof. W. Klyne. The infra-red spectra were registered under the supervision of Dr. K. v. Lier, of the National Institute of Public Health, Utrecht. We thank Mr. P. Hubers, of the Organic-Chemical Laboratory of the Municipal University of Amsterdam, for carrying out analytical work.

L. G. Huis in 't Veld
B. Louwerens
W. Reilingh

National Institute of Public Health,
Utrecht, Netherlands.

[1] Dingemanse, E., Huis in 't Veld, L. G., and de Laat, B. M., *J. Clin. Endocrinol.*, **6**, 535 (1946).

Oxytocinase Activity of Plasma of Pregnant Women

It has been shown[1] that plasma or serum from pregnant women has the power of inactivating oxytocin when incubated with the hormone. This power is thought to be dependent on an enzyme, called oxytocinase. The enzyme inactivates both natural and synthetic oxytocin[2–5]. The rate at which plasma inactivates oxytocin increases with the age of pregnancy[1,4,6–9]. Maximum values are said to be reached around the 36th week of pregnancy, after which they remain at the same level until delivery. After parturition the oxytocinase activity decreases progressively, becoming very low in about one month.

Most of the work on oxytocinase has been performed with biological assays with oxytocin as substrate.

According to Tuppy *et al.*[10], oxytocinase is an aminopeptidase with the power of splitting the peptide-bond between tyrosine and cystine in the oxytocin molecule. With regard to this mode of action, Tuppy *et al.* synthesized a substrate, L-cystine-di-β-naphthylamide, with partly the same molecular configuration as oxytocin and worked out a chemical method for the assay of the oxytocinase activity with this synthetic substrate[11].

The results reported by Tuppy *et al.* with their chemical method are different from those obtained by other authors using biological methods. It must, however, be pointed out that even the values reported by different authors using biological assays are indeed different; the activity at the end of pregnancy is reported to be either 1,000 times or 10 times higher in comparison with the activity at the beginning of pregnancy[9,12]. According to Tuppy *et al.* the oxytocinase activity at the end is about 28 times higher in comparison with the values in the non-pregnant state. These authors could not confirm the results, outlined in the introduction, that maximum values are reached around the 36th week of pregnancy with no increase thereafter. Their values show a steady increase up to the time of delivery. These differences in the values reported probably depend on experimental error.

The present preliminary report deals with the results obtained in assays of oxytocinase activity in pregnant women with both the chemical and a biological method in parallel.

A total of about sixty healthy pregnant women were examined, plasma being the enzyme source and syntocinon and L-cystine-di-β-naphthylamide respectively the substrates. The enzyme reaction occurred under carefully standardized conditions, and the determination of the remaining oxytocic activity was made on rat uterus preparations using a four-point

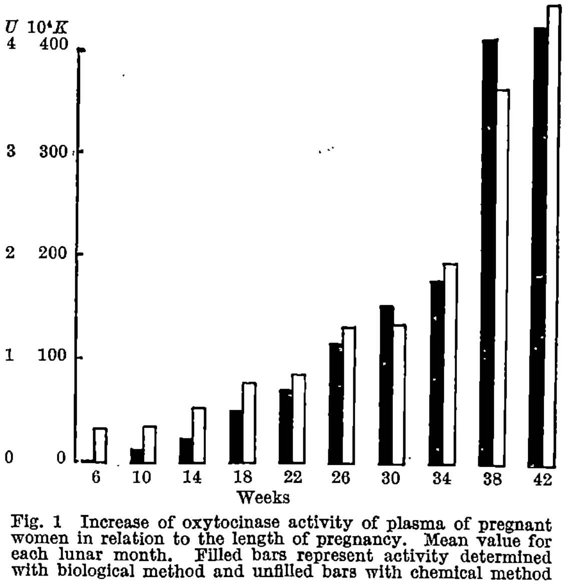


Fig. 1 Increase of oxytocinase activity of plasma of pregnant women in relation to the length of pregnancy. Mean value for each lunar month. Filled bars represent activity determined with biological method and unfilled bars with chemical method

assay with statistical treatment according to Gaddum[13], and Borth *et al.*[14]. Enzyme activity was expressed as $10^4 \times K$; $K =$ the velocity constant according to the formula $K_1 = \frac{1}{t} \ln \frac{a}{a-x}$. In using Tuppy's chemical method I have expressed the enzyme activity as units (U) of Tuppy, 1 mgm. liberated β-naphthylamine per 100 ml. plasma per hr. adopted as 1 U. All estimations were performed in duplicate.

Fig. 1 shows the increase of plasma oxytocinase activity with the length of pregnancy, the activity in each case being determined with biological and chemical method in parallel.

As may be seen, the values obtained with the two methods show on the average the same increase of oxytocinase activity in relation to the length of pregnancy. The results make it probable that the two methods determine identical enzyme activities; this has been questioned earlier. The activity also shows a steady increase up to the time of delivery, which is in agreement with the results of Tuppy *et al.*[11].

Work is now in progress in order to secure these preliminary results and investigate the enzyme activity in abnormal conditions of pregnancy. Attempts to concentrate and purify the enzyme are also in progress.

I have not had the opportunity of investigating closer the kinetics of the enzyme reaction. It may be that the reaction is of zero order; but the values of K_0 according to the formula $K_0 = \frac{x}{t}$ differ little from the values of K_1 and the increase of enzyme activity, expressed as K_0, is the same as that expressed as K_1. The kinetics of the reaction are being investigated.

STIG E. J. MELANDER

Department of Obstetrics and Gynaecology,
University of Uppsala.

[1] Semm, K., *Das Wehenproblem mit besonderer Berücksichtigung des Oxytocin-Oxytocinasehaushaltes* (Ferdinand Enke Verlag, Stuttgart, 1960).

[2] Beller, F. K., and Göbelsmann, U., *Klin. Wschr.*, 36, 1005 (1958).

[3] Caldeyro-Barcia, R., and Poseiro, J. J., *Ann. N.Y. Acad. Sci.*, 75, 813 (1958).

[4] Dicker, S. E., and Whyley, G. A., *J. Obstet. Gynaec. Brit. Emp.*, 66, 605 (1959).

[5] Semm, K., *Naturwiss.*, 44, 424 (1957).

[6] Mendez-Bauer, C., and Carballo, M. A., *Proc. Fourth Panamer. Congr. Endocrin.*, 293 (1957).

[7] Mendez-Bauer, C., and Carballo, M. A., *Proc. Second Uruguayan Congr. Obstet. Gynaec.*, 2, 31 (1957).

[8] Page, E. W., *Amer. J. Obstet. Gynec.*, 52, 1014 (1946).

[9] Page, E. W., *Science*, 105, 292 (1947).

[10] Tuppy, H., and Nesvadba, H., *Mh. Chem.*, 88, 977 (1957).

[11] Tuppy, H., Müller-Hartburg, W., and Nesvadba, H., *Arch. Gynäk.*, 191, 442 (1959).

[12] Caldeyro-Barcia, R., *Acta Endocrinol.* (*Kbh.*), Supp. 50, 41 (1960).

[13] Gaddum, J. H., *Pharmacol. Rev.*, 5, 87 (1953).

[14] Borth, R., Diczfalusy, E., and Heinrichs, H. D., *Arch. Gynäk.*, 188, 497 (1957).

Inhibition of Chemi-luminescence of Luminol by means of Oximes

IT is known that oximes with widely different chemical structures are effective reactivators of cholinesterase inhibited by organo-phosphorous compounds (esters of phosphoric or thiophoric acid, insecticides, nerve gases)[1]. The mechanism of this reaction is considered to be due to the chemical interaction of oximes with the free or bound ester of phosphoric acid, which is hydrolysed by the oxime[2], and is not available for the inhibition of the cholinesterase. It is known that organo-phosphorous compounds catalyse the luminol reaction causing an intensive chemi-luminescence of luminol (3-aminophthalylhydrazide) in alkaline medium in the presence of sodium perborate as oxygen donor[3].

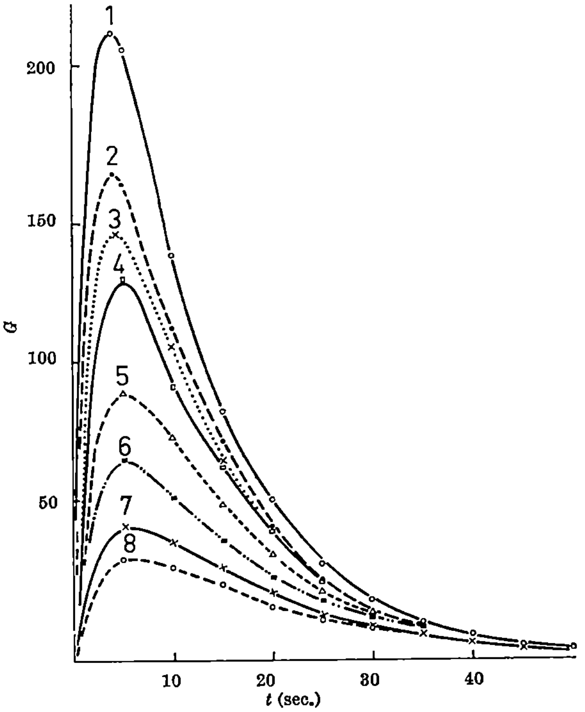


Fig. 1

In view of this evidence it was felt that the oximes will inhibit the luminol reaction catalysed by organophosphorous compounds or other suitable catalyst, that is, hæmoproteins or other complexes with iron. This type of inhibition would proceed with a mechanism analogous to the reactivation of cholinesterase as mentioned here.

Quantitative investigations of the chemi-luminescence of luminol have proved that a large number of oximes inhibits the luminol reaction catalysed by organo-phosphorous compounds or complexes of iron. The inhibition caused a significant decrease of the chemi-luminescence. The results of a series of experiments are presented (Fig. 1). G represents the relative intensity of chemi-luminescence in relation to the reaction time t. Curve 1 records the results obtained

Table 1

Hæmoglobin (gm. per cent) / Inhibitor	2×10^{-5}	4×10^{-5}	1×10^{-4}
	$i_{1/2}\,M$		
2-Hydroxyiminopropane	$5{\cdot}90 \times 10^{-2}$		$6{\cdot}10 \times 10^{-2}$
4-Hydroxyimino-methyl-N-methyl-pyridinium iodide	$1{\cdot}34 \times 10^{-2}$		$1{\cdot}54 \times 10^{-2}$
2-Hydroxyiminobutanone-(3)	$1{\cdot}11 \times 10^{-2}$	$1{\cdot}25 \times 10^{-2}$	$1{\cdot}40 \times 10^{-2}$
2-Hydroxyimino-methyl-N-methyl-pyridinium iodide	$2{\cdot}25 \times 10^{-3}$	$2{\cdot}50 \times 10^{-3}$	$3{\cdot}25 \times 10^{-3}$
N,N′-Tetramethylene-*bis*(4-hydroxyimino-methylpyridium bromide)	$6{\cdot}90 \times 10^{-4}$	$7{\cdot}10 \times 10^{-4}$	$7{\cdot}80 \times 10^{-4}$

without oxime, while curves 2–8 illustrate the effect of increasing concentrations of pyridine-4-aldoxime methiodide. In all these experiments the luminol reaction was catalysed by hæmoglobin. The results clearly demonstrate that the maximal intensities of chemi-luminescence as well as the total amount of light (area under each curve) were reduced when increasing amounts of oxime were used.

Essentially the same results were obtained when other oximes (inhibitors) and other catalysts of luminol reaction were applied. Table 1 shows molar concentrations of various oximes which decrease the chemi-luminescence to a value of 50 per cent at various concentrations of hæmoglobin. It is evident that oximes inhibit the luminol reaction effectively.

These findings present not only an additional proof for the mechanism of reactivation of cholinesterase with oximes, but also the method described in this communication provides a means for a simple evaluation of oximes as reactivators of cholinesterase.

Further details of these and other experiments on related subjects will be published elsewhere.

K. Weber
J. Matković
D. Fleš

Yugoslav Academy of Sciences and Arts,
Institute for Medical Research
(incorporating the Institute of Industrial Hygiene),
Zagreb.

---

[1] Davies, D. R., and Greeb, A. L., *Brit. J. Industr. Med.*, **16**, 128 (1959). Holmstedt, B., *Pharmacol. Rev.*, **11**, 567 (1959).

[2] Green, A. L., and Sasille, B., *J. Chem. Soc.*, 3887 (1956).

[3] Goldenson, J., *Anal. Chem.*, **29**, 877 (1957).

Inactivation and Reactivation of Chymotrypsin

In previous work on the immediate effects of acylation of trypsin it was shown[1] that the enzyme can be obtained in a fully inactive form by acylation with various anhydrides at *p*H 8–9, and that partial instantaneous reactivation can be effected by treatment with alkaline hydroxylamine[2].

In an extension of this work it was found that chymotrypsin behaves similarly to trypsin on acylation at higher *p*H values, complete inactivation occurring at *p*H 8. Even though a reactivation was observed on incubation, it was almost negligible in the course of 2 hr. The acylations were carried out using an automatic titration unit (Polarad Electronics Corp.), at various constant *p*H values. In Table 1 are recorded the percentages of inactivation and reactivation in terms of proteolytic activity obtained on acetylation at 0–5° C. at different *p*H values, whereby 60 equiv. of acetic anhydride were used per mole of enzyme. The activity was assayed at *p*H 7·8, using the usual hæmoglobin substrate. Any inactivation rapidly reversed at that *p*H [3,4] could accordingly not have been observed.

Table 1. Inactivation of Chymotrypsin at Different *p*H Values

Time (min.)	Activity (per cent) 5	6	7	8
0	100	100	100	100
8	65	56	31	0
20	64	58	35	0
120	67	60	38	0

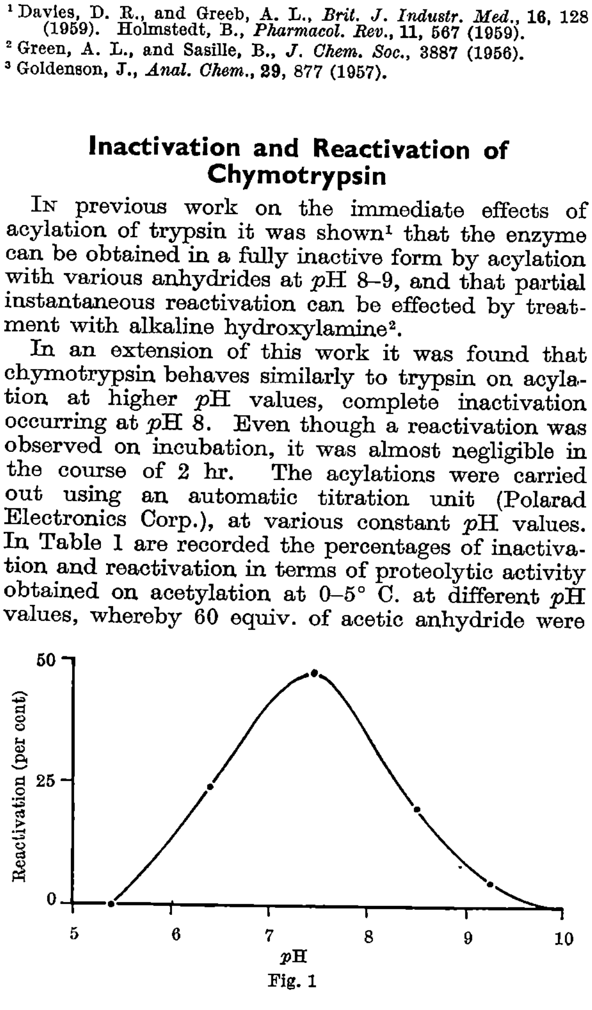


Fig. 1

Butyric and propionic anhydrides gave similar results. As observed in the case of trypsin, citraconic anhydride gave rise to an inactivation amounting to only about 50 per cent.

Even though the spontaneous reactivation of acetylchymotrypsin is much slower than in the case of acetyltrypsin, an instantaneous reactivation was obtained on treatment with hydroxylamine. In Fig. 1 is shown the extent of reactivation recorded at various *p*H values on treatment of the inactivated chymotrypsin for a period of 8 min. with 4 equiv. of hydroxylamine per equivalent of anhydride added. It can be seen that maximum reactivation takes place in the region of *p*H 7·4–7·8. The reactivation bears a striking resemblance to the reaction of inactive acetyltrypsin with hydroxylamine[2] except that the *p*H optimum of reactivation is shifted to lower *p*H values by about one *p*H unit.

The inactive monoacetyl chymotrypsin obtained by other investigators[3,4] was reactive with hydroxylamine in the region of *p*H 5–6. In reaction with nitrophenylacetate there is also a great similarity between trypsin and chymotrypsin and the monoacetyl enzymes obtained were more stable at *p*H 5–6, easily undergoing hydrolysis at *p*H 7–8. However, in the case of the inactive acetyl derivatives obtained at *p*H 8, it was found that they were more stable at *p*H 7–8 than at *p*H 5–6. The hypothesis[2] that there are two susceptible sites in the active centre of trypsin, acetylated at *p*H 5 and *p*H 8 respectively, appears to be supported in the case of chymotrypsin. The small deviations in the *p*H optima of reactivation with hydroxylamine could be attributed to secondary differences between the two enzymes.

This work was supported by the U.S. Atomic Energy Commission, the National Science Foundation and the U.S. Public Health Service.

T. Therattil Antony*

Laboratory of Organic Chemistry
and Enzymology,
Fordham University,
New York 58.

---

* Permanent address: Department of Chemistry, University of Delhi.

[1] Antony, Teresa Therattil, Bier, M., and Nord, F. F., *Arch. Biochem. Biophys.*, **87**, 150 (1960).

[2] Antony, Teresa Therattil, Bier M., and Nord, F. F., *Nature*, **189**, 809 (1961).

[3] Balls, A. K., and Wood, H. N., *J. Biol. Chem.*, **219**, 245 (1956).

[4] Dixon, G. H., and Neurath, H., *J. Biol. Chem.*, **225**, 1049 (1957).

Activity of Glutamic Acid Decarboxylase in Insect Nerve Tissue

As compared with the nerve tissue of mammals, that of insects is known to be particularly rich in acetylcholinesterase[1] and in choline-acetylase[2]. On the other hand, nothing has been reported so far about the occurrence in insects of another enzyme which, in mammals, is characteristic of nerve tissue, namely, glutamic acid decarboxylase. Its reaction product, γ-aminobutyric acid, has been recently found in the free state in bee nerve tissue, in amounts of the same order, on the basis of fresh weight, as in mammalian nerve tissue (Carta, S., and Frontali, N., unpublished work).

Homogenates of worker honeybee brains (including the optic lobes) have been used for determinations of glutamic acid decarboxylase by the manometric technique[3], and compared with mouse brain homogenates. The average values thus obtained were 690 µl. carbon dioxide/hr./gm. of fresh tissue for the mouse and 1,250 for the bee.

Further work on the characteristics of the glutamic acid decarboxylase of bee nerve tissue was made difficult by the large amounts of material necessary for each determination, even when Warburg microvessels were used (at least 0·1 gm. tissue, which is approximately 80 brains). For this reason a different method of assay of glutamic acid decarboxylase was worked out and found suitable for activity determinations on as little as 2–4 mgm. of tissue. It was based on the use of uniformly labelled glutamic acid-^{14}C as substrate, and on the measurement of the radioactivity of the γ-aminobutyric acid produced.

Preliminary experiments showed (1) that labelled glutamic acid, incubated with a bee brain homogenate under the same conditions as the Warburg determination and subjected to two-dimensional paper chromatography for amino-acids, gave rise to one new radioactive spot, having the R_F values of γ-aminobutyric acid ; (2) that this spot, eluted and co-chromatographed with authentic inert γ-aminobutyric acid, could be superimposed perfectly on the resulting ninhydrin spot.

The general procedure was as follows, each determination being run in duplicate. Small test-tubes (30 mm. × 6 mm.) were pre-cooled in ground ice with 20 µl. of the reaction mixture, which was prepared in such a way as to give a final concentration of 0·0005 *M* pyridoxal phosphate and 0·025 *M* L-glutamic acid ; each test-tube contained 0·2 µc. of labelled L-glutamic acid (from the Radiochemical Centre, Amersham, England). Next, 20 µl. of bee brain homogenate 1 : 5 in 0·1 *M* phosphate buffer *p*H 6·4 were added. The test-tubes were closed with rubber stoppers, and by means of two injection needles (inlet and outlet) through the stopper, pure nitrogen was passed through them for 2 min.; the needles were quickly removed at the end of the gassing period. At zero time the test-tubes were immersed in a water-bath at 38°, except for one which was immediately deproteinized to serve as a control. After incubation for 20 min., the test-tubes were chilled on ice, opened and immediately deproteinized with 100 µl. of 99 per cent ethanol. After centrifugation, measured aliquots of the supernatant fluids were subjected to one-dimensional ascending paper chromatography in phenol/water/concentrated ammonia (77·5 : 21·5 : 1). The radioactivity was measured by the quantitative paper radiochromatographic technique described by Beloff-Chain *et al.*[4]. The results obtained by this method were found to agree with those obtained by the manometric technique.

The above method was then applied to the study of some properties of the glutamic acid decarboxylase of bee brain. Enzyme activity, as a function of coenzyme concentration and of time, was examined. The results of these experiments showed that (1), the optimal concentration of pyridoxal phosphate is between 4 × 10^{-4} and 10^{-3} *M*, (2) the time/activity curve is almost linear during the first 20 min.

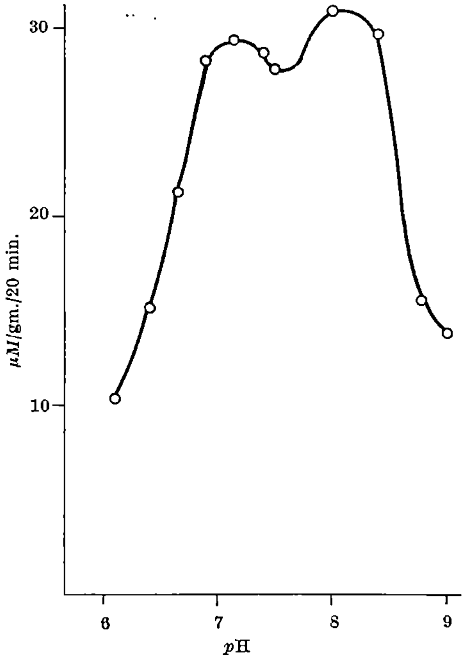


Fig. 1. Production of γ-aminobutyric acid from glutamic acid as a function of *p*H

Enzyme activity as a function of *p*H was then investigated. A homogenate of bee brain in 0·1 *M* potassium chloride was incubated with the usual reaction mixture and with a phosphate-borate buffer[5] ranging from *p*H 6 to 9. The amounts of γ-aminobutyric acid formed were determined in the usual way and plotted against the *p*H. The resulting curve showed two peaks of maximum γ-aminobutyric acid production at *p*H values around 7·2 and 8·0 respectively (Fig. 1). The second peak could correspond to an alternative route of γ-aminobutyric acid production, in which glutamic acid decarboxylase is not involved. If this can be shown to be true, the method described in this communication should be used as a measure of glutamic acid decarboxylase activity only between certain limits of *p*H.

N. Frontali

Istituto Superiore di Sanità,
Rome.

---

[1] Metcalf, R. L., and March, R. B., *J. Econ. Entomol.*, **43**, 670 (1950).
[2] Smallman, B. N., *J. Physiol.*, **132**, 343 (1956).
[3] Roberts, E., Younger, F., and Fränkel, S., *J. Biol. Chem.*, **191**, 277 (1951).
[4] Beloff-Chain, A., Catanzaro, R., Chain, E. B., Masi, I., Pocchiari, F., and Rossi, C., *Proc. Roy. Soc.*, B, **143**, 481 (1955).
[5] Kolthoff, J. M., and Vleeschhouwer, J. J., *Biochem. Z.*, **189**, 191 (1927).

Enzymic Conversion of Tetrahydrofolic Acid to Folinic Acid by Plant Protein

ENZYMIC synthesis of folinic acid (CF) has been investigated in a variety of cells of both bacterial and animal origin[1–7].

The purpose of this communication is to report the isolation and partial characterization of a crude protein fraction from a plant source demonstrating 'transformylase' activity in converting tetrahydrofolic acid to folinic acid.

Folic acid was obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio. Formylfolic acid (FF) was prepared by the method of Shive *et al.*[8]. Identity was verified by the method of Weislogel *et al.*[9]. Tetrahydrofolic acid (THF) was prepared by an adaptation of the method of O'Dell *et al.*[10]. Folinic acid-SF was supplied through the courtesy of Eli Lily Co., Indianapolis, Indiana. Mustard leaves were obtained locally.

Turnip or mustard leaves (2·5 kgm.) were homogenized in a Waring blendor in a v/v solution of acetone chilled with dry ice and phosphate buffer (0·05 M) at *p*H 7·2. The homogenate was collected by filtration; acetone was removed by vacuum distillation, and the residue was extracted with 500 ml. phosphate buffer at *p*H 7·2.

The volume was reduced to 250 ml. by pervaporation and the debris which accumulated was removed by centrifugation. Protein was precipitated by adding ammonium sulphate (42 gm./100 ml. volume).

Further purification of the fraction was accomplished by suspending the protein precipitate in a minimum of buffer, clarifying the suspension by centrifugation, and by discarding the small amount of insoluble precipitate. The centrifugate was reprecipitated with ammonium sulphate at the same saturation-level as used in the precipitation of the crude fraction. The precipitate was collected by centrifugation, dissolved in buffer, and dialysed against potassium phosphate buffer until no precipitate occurred on the addition of 0·1 M barium chloride to 5 ml. of the buffer bath. The protein was dissolved in phosphate buffer to give 63·20 μgm. in 0·2 ml. of solution. Protein determination was by the method of Lowrey[4].

The biosynthesis media contained the following substances: tetrahydrofolic acid, 2 μgm.; glucose, 8 μM; ascorbic acid, 4 μM; sodium formate, 20 μM; $MgSO_4.7H_2O$, 1 μM; diphosphopyridine nucleotide, 0·05 μM; adenosine triphosphate, 0·05 μM; protein fraction, 0·1 mgm.; buffer (potassium phosphate *p*H 7·0), 1·0 ml. in a total volume of 4·0 ml.

After 4 hr. incubation at 30° C., the biosynthesis samples were clarified by centrifugation, the *p*H adjusted to 7, and assayed for folinic acid activity with *P. cerevisiae* (*ATCC* 8081) in a manner described previously[2]. Samples containing no protein, as well as samples containing only protein, gave no stimulation of growth of *P. cerevisiae*, when assayed under identical conditions as the biosynthesis samples.

Substrate specificity for this enzyme was estimated by using formyl-THF, THF, and folic acid. THF gave the best conversion to folinic acid, although, in each case, the yield of folinic acid was low, due, perhaps, in part to substrate instability, unacceptability of D-forms of the stereoisomers, and the impure condition of the enzyme. In determinations of the effects of *p*H, and temperature, 2 μgm. of THF per 5 ml. of biosynthesis medium were employed, and the amount of protein was reduced from 0·1 mgm. to 0·03 mgm.

Because of the observed influence of both temperature and *p*H (Table 1), as well as the absence of activity of folinic acid, when no protein was present, it can be said that the conversion of THF to folinic acid by this plant protein is enzymic.

These results show the normally observed inactivation of enzyme activity at extremes of, as well as the optimum, activity within the mid-range of *p*H and temperature, which compares favourably with observations of effects of *p*H and temperature on similar enzymic conversions of folic acid derivatives to folinic acid in both bacteria and animals.

Table 1. EFFECT OF *p*H AND TEMPERATURE ON THE ENZYMIC CONVERSION OF TETRAHYDROFOLIC ACID TO FOLINIC ACID

*p*H	Conversion (per cent)*	Temperature (° C.)	Conversion (per cent)†
4·0	0	10	0
6·0	12	25	8
8·0	12	37	16
10·0	2	90	0

* Percentage conversion of tetrahydrofolic acid to folinic acid per mgm. of crude protein at 30° C. Incubation time, 4 hr. Assays for folinic acid activity were for 18 hr. at 37° C.

† *p*H 7.

Further purification of the active fraction is necessary in order to make a thorough study of the properties of this enzyme.

This work was supported in part by a National Cancer Institute grant No. *C*1552 (*C*8).

E. H. BYERS
J. STEWART
T. J. BOND

Department of Chemistry,
Baylor University, Waco, Texas.

[1] Broquist, H. P., and Kohler, A. R., *J. Biol. Chem.*, **202**, 59 (1953).
[2] Bond, T. J., *Science*, **117**, 563 (1953).
[3] Heisler, C. R., and Schweigert, B. S., *Fed. Proc.*, **14**, 436 (1955).
[4] Miller, Steve, and Bond, T. J., *J. Bacteriol.*, **78**, 488 (1959).
[5] Colon, Julio I., *J. Bacteriol.*, **79**, 741 (1960).
[6] Donaldson, Kenneth O., and Keresztesy, John C., *Federation Proc.*, **18**, 853 (1959).
[7] Couch, J. R., and Reid, B. L., *Fed. Proc.*, **14**, 197 (1955).
[8] Shive, William, Bardos, T. J., Bond, T. J., and Rogers, L. L., *J. Amer. Chem. Soc.*, **72**, 2817 (1950).
[9] Weislogel, Orville, and Bond, T. J., *Arch. Biochem. Biophys.*, **89**, 221 (1960).
[10] O'Dell, B. L., Vandenbelt, J. M., Bloom, E. S., and Pfiffner, J. J., *J. Amer. Chem. Soc.*, **69**, 250 (1947).

Regulation of Pyrogallol Oxidations by Alcohols and Sugars

PREVIOUSLY, it has been shown that the catalytic activity of Fe(III) or peroxidase can be enhanced by a variety of colloidal substances including deoxyribonucleic acid[1], celluloses[2], and starch[3]. Other investigations[4–6] have demonstrated the macromolecular activation or modification of a number of oxidative or hydrolytic systems. Such observations direct attention to the role of environmental factors other than those usually encountered *in vitro* in biochemical reactions.

Although investigations of this type are not common, they possess potentially great significance as demonstrations of the regulatory effects to be expected when reactions take place within the colloidal system of the cell. Such investigations also stimulate interest in still other kinds of model environments which might possess biological counterparts. We may consider, for example, the properties of the solvent medium wherein reactions occur, such as dielectric constant and viscosity. Using alcohols and polyhydric substances such as glycerol and sugars as solvent-modifying solutes, it has been found that the autoxidation and enzymic oxidation of pyrogallol can indeed be regulated by solvent properties.

In these experiments, the oxidation of 0·05 M pyrogallol at 25° C. in 0·01 M phosphate buffer, *p*H 5·6, was followed photometrically at 425 mμ. In the higher alcohol concentrations, buffer salts were omitted.

It is known that the autoxidation-rate declines in pure alcohols with increasing length of carbon chain[7],

Table 1. Effects of Alcohols on Autoxidation of Pyrogallol
(*a*) Simple alcohols

Concentration (*M*)	Methanol	Ethanol	*n*-Propanol	Glycerol
0*	100	100	100	100
0·01	—	198	350	226
0·1	175	200	330	253
1·0	152	233	330	253
5·0	101	110	153	150
10·0	—	64	14	33

(*b*) Sugars

	Sucrose	Glucose
0†	100	100
0·01	88	88
0·1	119	94
0·5	123	—
1·0	105	100
2·0	32	65
5·0	—	16

*, In water, the control absorbance at 425 mμ after 24 hr. is 0·152; †, in water, the control absorbance at 425 mμ after 50 hr. is 0·450.

but the enhancement of oxidation by dilute aqueous alcohols was unexpected (Table 1*a*). Several alcohols at 0·01–5 *M* cause pronounced acceleration and at concentrations in excess of 5 *M* pronounced inhibition.

Glycerol acts like the monohydric alcohols; however, other polyhydric substances, sucrose and glucose (Table 1*b*), affect only the inhibition of autoxidation when supplied at concentrations greater than 1 *M*. On the basis of their molar concentrations, the sugars are more inhibitory than the simple alcohols. At 5 *M*, the simpler alcohols are inactive or stimulatory whereas both sucrose and glucose inhibit autoxidation at the 2 *M* concentration.

The theoretical and mechanistic bases for these effects will be discussed elsewhere, and it will suffice here to mention several possibilities in outline: (1) Enhancement by dilute ethanol and *n*-propanol may involve participation in radical chain transfer reactions. (2) Inhibition by more concentrated alcohols may involve reduced ionization of pyrogallol in media of much lower dielectric constant than water. Inhibition by ethanol and *n*-propanol specifically may also involve their radical chain-stopping (anti-oxidant) activities. (3) Enhancement by methanol and glycerol does not now lend itself to explanation. (4) Inhibition by the sugars and glycerol may involve the retarding effect of viscous media. The non-inhibitory concentrations give solutions of 5 centipoise or less, whereas the viscosities of inhibitory solutions range upwards from 50 centipoise.

Some of the effects described are not restricted to homogeneous autoxidation systems, but may be extended to enzymic peroxidations as well. Using the peroxidase system described in previous work[3], we have found that methanol at concentrations of 5 *M* or less accelerates pyrogallol oxidations appreciably (Table 2).

Table 2. Effects of Methanol on Enzymic Peroxidation of Pyrogallol

Methanol *M*	Yield of purpurogallin (mμmoles/l.) at 0·5	1·0	1·5	2·0	2·5	3·0 min.
0	11·3	23·7	33·8	38·8	44·0	48·1
2·5	8·1	19·4	34·4	47·5	62·5	66·0
3·75	5·6	14·4	27·5	39·4	53·8	62·5
5·0	4·1	9·1	17·2	28·1	40·6	53·1
7·5	2·8	5·6	9·4	13·8	18·8	25·0
10·0	1·9	4·7	7·8	11·0	14·7	18·5
12·5	1·0	1·3	2·0	2·8	3·5	4·4

At concentrations of 0·5 *M*, sugar solutions inhibited enzymic oxidation by 15–20 per cent, but at 5 *M* the inhibition was increased to about 50 per cent. Hence, methanol and the sugars exert similar effects on the slow reaction between pyrogallol and oxygen and the fast catalytic reaction between pyrogallol and hydrogen peroxide. The biological significance of these results lies mainly in the principle illustrated, namely, that modifications in aqueous reaction media brought about by the presence of various simple hydroxylic substances can influence the rates of oxidative reactions.

Such effects could give rise to differences in rate among cells or parts of cells without requiring corresponding differences in catalyst or reactant concentrations.

This work was supported by Public Health Service grant *C*–2730, National Institutes of Health, to the University of Rochester.

B. Z. Siegel
4 Orchard Parkway,
White Plains,

S. M. Siegel
Union Carbide Research Institute,
White Plains, New York.

N. S. Goodman
Department of Biology,
Brandeis University,
Waltham, Massachusetts.

[1] Siegel, S., and Siegel, B. Z., *Nature*, **179**, 421 (1957).
[2] Goodman, N. S., and Siegel, S., *Nature*, **184**, 53 (1959).
[3] Siegel, B. Z., and Siegel, S., *Nature*, **186**, 391 (1960).
[4] Keilin, David, and Hartree, E. F., *Biochem. J.*, **44**, 205 (1949).
[5] Siegel, S., *Sub-Cellular Particles* (edit. by Hayashi, T., Amer. Physiol. Soc., Washington, 1959).
[6] Siegel, S., Abstracts, First Intern. Congress of Histochem. and Cytochem., *Annales D'Histochimie*, 5 (Supplement) 45 (1960).
[7] Siegel, S., Porto, F., and Frost, P., *Arch. Biochem. Biophys.*, **82**, 330 (1959).

PHYSIOLOGY

Importance of Magnesium in the Control of Plasma Calcium in the Calf

It has been known for many years[1] that when calves are fed for an abnormally long time on milk only they usually, sooner or later, become hypomagnesæmic. In my experiments, four out of sixteen calves fed to about five months of age or more on milk without supplementary vitamin D showed neither hypomagnesæmia nor hypocalcæmia while the remainder became hypomagnesæmic. Of the latter, all but one showed a fall in plasma calcium more or less closely correlated with the fall in plasma magnesium. An example is shown in Fig. 1 and other examples have been reported previously[2]. My work has shown fairly clearly that the hypomagnesæmia is due to magnesium deficiency resulting from a decrease in the ability of the calves to absorb dietary magnesium as they get older[3], but the reason for the hypocalcæmia is less clear.

I have found that the efficiency of calcium utilization as well as that of magnesium decreases as the calves get older and that addition of a large amount of vitamin D (70,000 I.U./day) to the diet both improves calcium utilization and rapidly restores plasma calcium to normal[2]. Smaller amounts of vitamin D (300–7,000 I.U./day) have also been found to be beneficial, but less so than the larger amount[2].

Table 1

Age (weeks)	Diet	Magnesium intake (gm./day)	Calcium intake (gm./day)	Calcium net absorption (per cent of intake ± S.E.)	Calcium excretion in urine (per cent of intake ± S.E.)	Plasma magnesium (mgm./100 ml.) mean and range	Plasma calcium (mgm./100 ml.) mean and range
16½–18½	Milk	0·69	7·1	61 ± 6	0·35 ± 0·09	1·35 (0·91–2·08)	8·5 (7·4–10·6)
19½–21½	Milk + $MgCl_2$	4·0	7·1	42 ± 11	1·44 ± 0·74	2·34 (1·94–2·54)	10·8 (9·4–12·7)

Mean results from 8-day balance experiments with 4 calves kept out of direct sunlight and previously fed only on milk (4·4–6·15 l./day with supplementary iron, copper and manganese) throughout their lives. Immediately after the first balance period, the magnesium chloride supplement was added to the diet and subcutaneous magnesium sulphate injections (1 gm. magnesium/day) were given for 7 days. The second balance period was started 4–6 days after the injections ceased. General procedure and analysis was carried out as described previously (ref. 2).

Now milk, particularly winter milk which is what the calves here received, contains little vitamin D (about 10 I.U./l.); but, although it is clear that the vitamin D level must be low for the hypocalcæmia to occur, there are a number of facts which suggest that vitamin D-deficiency is not its sole cause. First, calves on vitamin D-deficient but otherwise normal diets do not readily show hypocalcæmia, even though they may suffer bone changes and show clinical signs of rickets[4]. Secondly, deficiency of vitamin D cannot explain the correlation between falls in plasma magnesium and plasma calcium; plasma magnesium and magnesium absorption are not appreciably influenced by vitamin D (ref. 2). Finally, I have now found that a restoration of plasma calcium to nearly normal levels occurred in four milk-fed hypomagnesæmic and hypocalcæmic calves when the plasma magnesium was restored by giving oral and parenteral supplements of magnesium even though no supplementary vitamin D was given. An example of the effect is shown in Fig. 1. With different animals an increased amount of magnesium in the diet has been variously reported as improving[5], inhibiting[6] and having no marked effect on[7] net calcium absorption. To examine the possibility of an improvement in calcium utilization accompanying magnesium supplementation in the above calves, calcium balances were carried out just before daily magnesium supplementation was started and again about ten days after it was started. Mean results are shown in Table 1.

Although the results do not prove that magnesium supplementation rather than a simple age effect caused the decrease in net calcium absorption, it is nevertheless true that an increase in plasma calcium occurred in all these calves in the face of decreased net absorption of dietary calcium and, as a mean effect, increased urine excretion of calcium (in some calves urine calcium increased markedly, in others it remained unchanged). It seems very probable, therefore, that the homeostatic mechanism for calcium, presumably that controlling the bone/extracellular fluid equilibrium, is sensitive, under certain conditions, to the magnesium status of the calf as reflected by the level of plasma magnesium. I have no evidence as to whether magnesium exerts its effect directly on the bone/extracellular fluid equilibrium or otherwise, but bearing in mind that, in the young calf, the plasma magnesium-level reflects the magnesium content of the bone[8], it can be seen that the idea of a direct effect is in accordance with the *in vitro* findings of W. F. Neuman and his co-workers[9] that the presence of certain foreign ions, including magnesium, increases the effective solubility of bone hydroxy-apatite crystals.

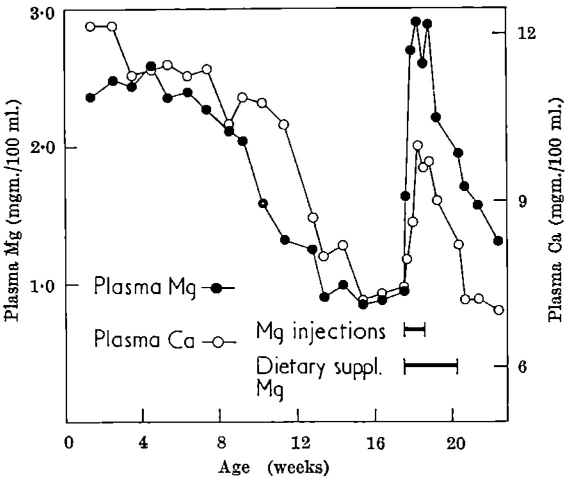


Fig. 1. Calf fed only on milk (4·4–6·15 l./day with supplementary iron, copper and manganese) and kept out of direct sunlight. Dietary supplement of 28 gm. $MgCl_2.6H_2O$/day and daily subcutaneous injections of 10 gm. $MgSO_4.7H_2O$ given as shown. During injection period blood samples were taken 24 hr. after an injection

Analogous considerations might account for the frequent occurrence of hypocalcæmia in adult bovines developing clinical hypomagnesæmia.

Although some tendency for low plasma calcium to be associated with low plasma magnesium has occasionally been observed by other workers with milk-fed calves (particularly by Parr[10], who often found hypocalcæmia in hypomagnesæmic calves even though they received about 430 I.U. vitamin D/day and who also found that the hypocalcæmia was alleviated by supplementary magnesium), the close correlation which I observed has not been reported previously. Its absence is often explicable by differences in vitamin D intake and might also sometimes be explained by differences in the amounts and types of foreign solid matter eaten by the calves in addition to their milk. Calves kept on fluid diets for longer than a few weeks develop a very strong desire for solid material to chew and will often eat almost anything. The calves used here were kept muzzled and were bedded on wood shavings, but they still usually succeeded in eating a small amount of these shavings.

R. H. SMITH

National Institute for Research in Dairying,
Shinfield, Reading, Berkshire.

[1] Duncan, C. W., Huffman, C. F., and Robinson, C. S., *J. Biol. Chem.*, **108**, 35 (1935).

[2] Smith, R. H., *Biochem. J.*, **70**, 201 (1958).

[3] Smith, R. H., *Biochem. J.*, **71**, 306 (1959); *Nature*, **184**, 821 (1959).

[4] Thomas, J. W., and Moore, L. A., *J. Dairy Sci.*, **34**, 916 (1951).

[5] Heaton, F. W., *Biochem. J.*, **77**, 1 *P* (1960).

[6] Alcock, N., and MacIntyre, I. I., *Biochem. J.*, **76**, 19 *P* (1960).

[7] Randoin, L., Causeret, J., Hugot, D., and Morel, G., *Bull. Soc. Chim. Biol.*, **34**, 1159 (1952).

[8] Smith, R. H., *Biochem. J.*, **71**, 609 (1959).

[9] Neuman, W. F., and Neuman, M. W., *The Chemical Dynamics of Bone Mineral*, 33 (University of Chicago Press, 1958).

[10] Parr, W. H., *Vet. Rec.*, **69**, 71 (1957).

Synaptic Transmission in Neurosecretory Cells

SINCE Scharrer's discovery[1], many types of neurosecretory systems have been investigated histologically in almost all kinds of animals. However, there is no publication dealing with them in terms of electrical activities except the brief note of Potter and Loewenstein[2], in which the presence of synapses between neurosecretory cells and other nerve cells was only indirectly indicated.

The caudal neurosecretory system of the eel, *Anguilla japonica*, was found by Enami[3]. In this system the cells are fairly large and clearly seen in fresh material under a dissecting microscope. This fact enabled us to use the micro-electrode technique, and the results obtained with this method clearly showed the synaptic transmission at the cell-bodies as well as other characteristics of the nervous function.

After the caudal spinal column of approximately 20 vertebræ was isolated from the eel, the spinal cord was exposed at several vertebræ including the tail end. This preparation was fixed with 'Vaseline' on a paraffin block in a leucite chamber, and the exposed spinal cord was washed sufficiently with the teleost Ringer. Intracellular records from the cell-bodies of these neurosecretory cells showed resting membrane potentials of 50–60 mV. inside negative and overshoots of 10 mV. or more in action potentials. The duration of the action potential was 8–10 msec. at 14–17° C. and the threshold membrane depolarization was around 12–13 mV. A long lasting outward current pulse resulted in repetitive discharge of spikes, at the end of which an under-shoot of the membrane potential was clearly seen (Fig. 1). These characteristics are very similar to those of the spinal ganglion cells of the frog[4].

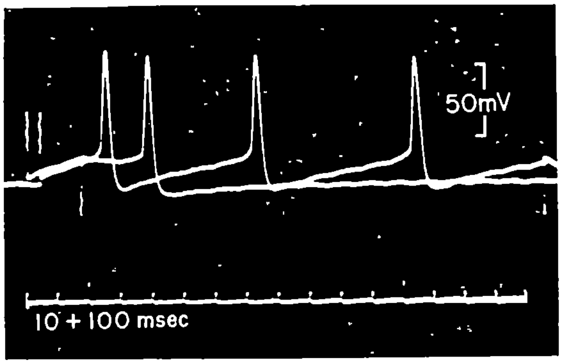


Fig. 1. Spike potentials of a cell body elicited by outward current pulses. The responses to brief and long pulses are superimposed

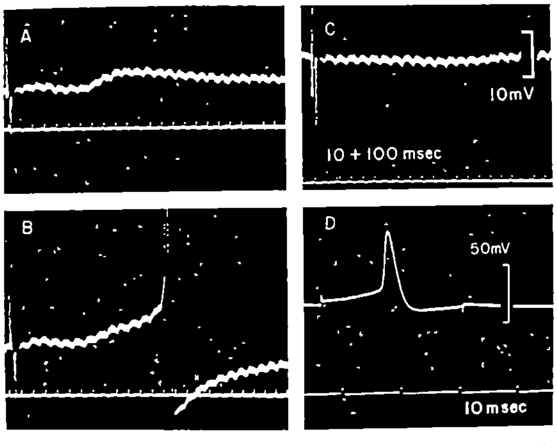


Fig. 2. An experiment showing synaptic transmission. *A*, synaptic potential evoked by a rather weak stimulation of a spinal cord at an anterior part; *B*, synaptic potential followed by a spike in the case of a stronger stimulation; *C*, after treatment of the spinal cord with potassium chloride in a region between the stimulating and recording positions. The stimulus was much stronger than in *B*; *D*, after *C*, the cell was directly stimulated by an outward current

When the spinal cord was stimulated at an anterior segment, a slow potential change was recorded from the neurosecretory cell-body after some delay (Fig. 2*A*). If the stimulus was strong enough, a spike potential was initiated at the summit of the slow potential (Fig. 2*B*). After this spinal cord was treated with potassium chloride in a region between the stimulating and recording positions, no electrical changes were evoked by a stimulus several times as strong as that which could elicit the spike potential (Fig 2*C*). Even then, this cell responded to a stimulus directly applied to the cell-body (Fig. 2*D*). Thus, the slow potential mentioned above may be safely regarded as the synaptic potential, which is also supported by the fact that the temporal summation was clearly observed. The conduction velocity of the fastest fibres in the presynoptic nerve was about 0·6 m./sec. at 15° C., but that of the neuro-secretory cell could not be measured owing to the short length of the axone, though antidromic stimulation of it was possible.

From the results of the present work it may be concluded that the neurosecretory cells have complete nervous characteristics, and that the activities of these cells are controlled by axones from higher nervous centres, though their locations are quite open for further investigations.

HIROMICHI MORITA
TAKAAKI ISHIBASHI
SATORU YAMASHITA

Department of Biology,
Faculty of Science,
Kyushu University,
Fukuoka.

[1] Scharrer, E., *Z. vergl. Physiol.*, 7, 1 (1928).

[2] Potter, D. D., and Loewenstein, W. R., *Amer. J. Physiol.*, 183, 652 (1955).

[3] Enami, M., *Gunma J, Med. Sci.*, 4, 23 (1955).

[4] Ito, M., *Jap. J. Physiol.*, 7, 297 (1957).

Changes in Blood Serotonin during Exposure to Cold

SEROTONIN is a recently discovered biogenic amine which can be extracted from brain, blood, and other organs, and which is active on a wide variety of systems when injected into the animal[1]. Gathering of knowledge concerning serotonin's physiological importance has been slowed by an inability to observe fluctuations in its concentration in brain and other tissues, in a wide variety of circumstances simulating physiological mobilization[2–4]. A single exception to this is the observation of a release of serotonin into the bowel, controlled by, and in turn modifying, peristaltic activity[5]. Also, more recently, Toh has reported rat brain serotonin to drop following exposure to cold associated with drop in body-temperature of 36–30° C. (ref. 3).

Below I present further data relating serotonin to natural mechanisms, reporting serotonin perturbations in the rat's blood during a short-term exposure to cold, not associated with change in body temperature. 200-gm. female rats were taken from an atmosphere varying from 75° to 80° F. and placed on a metal surface in a cold room maintained at 36–38° F. Animals of exps. 1–3 were injected with 50 mgm./kgm. sodium heparin intraperitoneally to facilitate blood collection, 30–40 min. before the beginning of exposure to cold. At specified intervals, of 15 sec.–20 min. duration, they were decapitated, and bled into cold siliconized test-tubes containing 1 drop of heparin. Adapting a method of Weissbach[6], 0·5 c.c. rat blood was pipetted into 7 c.c. of cold distilled water and lysed, and 2·5 c.c. of a 5 per cent zinc sulphate solution was added with shaking. The tubes were then neutralized with the appropriate quantity of sodium hydroxide, shaken again and centrifuged. The supernatants were treated in one of three ways: they were analysed for 5-hydroxyindole content by direct fluorimetry[6], or extracted and analysed specifically for 5-hydroxytryptamine (serotonin) by combination of the zinc sulphate protein precipitation[6] with the Bogdanski extraction procedure[7], one very close to Weissbach's extraction method, or, they were split, and both direct and extraction analyses carried out on the same sample. The fluorimetric readings of samples and recoveries were performed on a Farrand spectrophotofluorimeter. Samples were found to have emission and absorption spectra identical with processed serotonin.

Table 1. EFFECT OF COLD EXPOSURE ON WHOLE BLOOD SEROTONIN

Exp.	No. of samples	Duration cold exposure	Whole-blood γ/c.c. 5-hydroxyindole		*P*
			Direct method	Extraction method (serotonin)	
1 Rat (heparin)	5	0 min.	0·94 ± 0·24	0·43 ± 0·09	< 0·01
	4	5–7 ,,	1·77 ± 0·20	1·08 ± 0·22	
	4	9–11 ,,	1·63 ± 0·33	1·08 ± 0·17	
	4	20 ,,	1·44 ± 0·50	0·72 ± 0·23	
2 Rat (heparin)	16	0 min.	0·95 ± 0·21	—	< 0·001
	18	15–75 sec.	1·39 ± 0·24	—	
	8	2 min.	1·40 ± 0·34	—	
	8	4 ,,	1·09 ± 0·26	—	
	5	6 ,,	1·01 ± 0·12	—	
	5	11 ,,	0·94 ± 0·28	—	
3 Rat (heparin)	3	0 min.	1·95 ± 0·67	1·77 ± 0·88	—
4 Rat (no heparin)	10	0 min.	—	1·52 ± 0·46	< 0·01
	10	5–9 ,,	—	2·20 ± 0·49	
5 Rabbit	7	0 min.		3·29 ± 0·86	—
Human	7	0 ,,	—	0·12 ± 0·06	
Rat	8	0 ,,	—	1·41 ± 0·29	

The results are described in Table 1. Exp. 1 depicts increases in blood 5-hydroxyindole and serotonin that are approximately equivalent, about 0·7γ serotonin/c.c. whole blood. This elevation begins falling away between the 11th and 20th minute. Exp. 2 examines the same phenomenon by the direct method only. In this experiment the rise and fall of blood 5-hydroxyindole follows a more rapid time course. Exps. 3 and 4, performed a month later, demonstrate an equivalent cold-induced serotonin elevation in animals not injected with heparin. In these experiments, the normal values are seen to be somewhat high in comparison with the values obtained in other experiments for the rat[8,9]. In contrast, as shown in Exp. 5, human and rabbit bloods analysed simultaneously with rat samples gave serotonin titres similar to those obtained by other methods[6,8–11]. No other laboratories report whole-blood analyses for the rat, all blood serotonin values for rats reported to date being derived by calculation from analysis of platelets or platelet-rich plasma[6,8–11]. Thus the somewhat higher normal values we observe remain to be explained: they may reflect a seasonal variation, an inefficiency of methods for gathering platelets in the rat, or, possibly, the existence of a non-platelet source of blood serotonin in this species.

In contrast to Toh's observations[3], it is important to note that the alterations we have observed could not have been provoked by a non-specific lowering of body temperature. Rectal temperatures were not significantly altered in rats exposed to cold for 30 min.

The question of the source of the increase in blood serotonin and the relation of this observation to a local and general increased serotonin metabolism are under investigation at present. We speculate that the perturbations in serotonin concentration observed indicate serotonin participation in cardiovascular and/or metabolic adjustments to cold.

Note added in proof. In recent experiments employing blood drawn by cardiac puncture a cold-induced increase in plasma serotonin was not observed.

This research was supported by U.S. Public Health grant *A*–4064 *Met.*

PAUL GORDON*

Department of Pharmacology,
Northwestern University,
Medical School,
Chicago, Illinois.

---

* Present address: Department of Pharmacology, The Chicago Medical School, Chicago, Illinois.

[1] Erspamer, V., *Pharm. Rev.*, **6**, 425 (1954).
[2] Towne, J. C., and Sherman, J. O., *Proc. Soc. Exp. Biol. Med.*, **103**, 721 (1960).
[3] Toh, C. C., *J. Physiol.*, **151**, 410 (1960).
[4] Towne, J. C. (personal communication).
[5] Lee, C. Y., *J. Physiol.*, **152**, 405 (1960).
[6] Weissbach, H., Waalkes, T. P., and Udenfriend, S., *J. Biol. Chem.*, **230**, 865 (1958).
[7] Bogdanski, D. F., Pletscher, A., Brodie, B. B., and Udenfriend, S., *J. Pharm. Exp. Ther.*, **117**, 82 (1956).
[8] Erspamer, V., *J. Physiol.*, **133**, 1 (1956).
[9] Humphrey, J. H., and Jaques, R., *J. Physiol.*, **124**, 305 (1954).
[10] Udenfriend, S., and Weissbach, H., *Fed. Proc.*, **13**, 412 (1954).
[11] Erspamer, V., and Faustini, R., *Naturwiss.*, **40**, 317 (1953).

Effect of Sodium Fluoride on Calcium Metabolism of Human Beings

ALTHOUGH bones of human subjects become abnormally dense after several years of industrial exposure to fluorides[1,2], no work on the effect of fluoride on calcium balance of human beings has been carried out. Roholm[1] and others[2] have shown that the severity of skeletal changes is correlated approximately with the extent and duration of exposure to fluoride: moderate exposure resulting in increased density of otherwise grossly normal bone, but prolonged heavy exposure resulting in marked changes, including periosteal bone-formation and calcification of ligaments and tendons. How such complex skeletal effects are brought about is unknown; however, the microscopic appearance of depressed or absent osteoclastic activity in bones of moderately affected human beings[1] suggests that relatively low doses of fluoride may somehow depress resorption of bone.

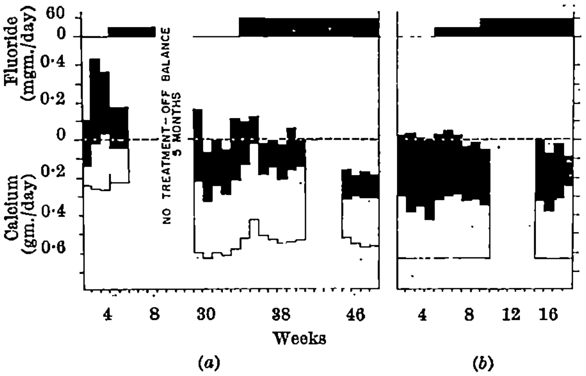


Fig. 1. Calcium balances of a patient with widespread and active Paget's disease (*a*) and of a patient with inactive post-menopausal osteoporosis (*b*). Data are plotted after Albright with intake down and urinary above fæcal calcium

Because of the demonstrated skeletal effect of fluoride in human beings, and because of the relatively benign nature, even of advanced fluorosis[1], it is possible that fluoride could be of use in treatment of diseases where skeletal mass is reduced, the rate of bone resorption accelerated, or both. This work was carried out to determine whether or not metabolic effects, particularly retention of calcium, could be observed during treatment of human beings with sodium fluoride. Six patients with osteoporosis and one with Paget's disease were given carefully measured diets which contained approximately constant amounts of calcium, phosphorus and nitrogen. After a preliminary period of at least one week, stools and urine were collected and balance measurements carried out. Control observations were made, usually for three weeks, after which the patients were given sodium fluoride orally in divided doses (all other treatments remaining unchanged). In early work small doses of fluoride were used and treatment was given for only a few weeks. As it was found that the medication was well tolerated, and that effects observed were seen only after several weeks, the dose was increased to 60 mgm. fluoride a day and duration of treatment extended to 14 or more weeks.

Changes in balance of calcium (the analysis[3] for calcium was unaffected by addition of sodium fluoride) of a subject with widespread and progressive Paget's disease are illustrated by Fig. 1*a*. The decreased rate of excretion of calcium in urine and the calcium retention were most marked late in the investigation, and did not appear to have become maximal during the period of observation. Fig. 1*b* shows similar but less-striking results from study of a post-menopausal patient with minimal X-ray evidence of osteoporosis. Other subjects with osteoporosis also responded by positive change in calcium balance and decreased rate of excretion of calcium in urine. (In one, with osteoporosis secondary to treatment of rheumatoid arthritis with prednisone, urinary calcium excretion fell from 204–240 mgm. per day to 24–68 mgm. per day from the twelfth to the fifteenth week of treatment. This was accompanied by increase of about 80 mgm. a day of fæcal calcium; the intake remaining constant.)

Other significant changes were, in several osteoporotic patients, increased alkaline phosphatase activity in plasma, from normal pre-treatment values to 16–38 King–Armstrong units per 100 ml., decreased rate of excretion of citrate and accelerated excretion of acid mucopolysaccharides in urine. No significant alteration occurred, during treatment of any patient, of concentration in plasma of calcium, phosphorus or citrate. No change in X-ray appearance of the skeleton or abnormality of hæmatological, renal or hepatic function could be measured. The only evidence of possible untoward effect was that, during the period of treatment, two of the seven subjects developed sub-acromial bursitis.

Although these results do not show whether or not fluoride will be useful in treatment of human subjects, they do demonstrate profound effects of fluoride ion on calcium metabolism of patients with several skeletal diseases.

This work was supported in part by a grant (*A*-3347) from the National Institutes of Health, U.S. Public Health Service.

CLAYTON RICH*
JOHN ENSINCK†

The Rockefeller Institute,
New York.

---

* Present address: Veterans Administration Hospital, Seattle, Washington.

† Present address: University of Washington School of Medicine, Seattle, Washington.

[1] Roholm, K., *Fluorine Intoxication* (H. K. Lewis Co., Ltd., London, 1937).

[2] Greenwood, D. A., *Physiol. Rev.*, **20**, 582 (1940).

[3] Rich, C., *J. Clin. Endocrinol. and Metabol.*, **20**, 147 (1960).

PHARMACOLOGY

Effect of Serotonin on Absorption and Distribution of Calcium-45 in the Rat

CERTAIN pharmacological effects of serotonin might be related to its action on the permeability of cell membranes to various ions. It has been shown that serotonin inhibits the pigment effusion, sodium uptake and potassium efflux from slices of red beetroots *in vitro*[1]. The loss of potassium from erythrocytes in cold storage is reduced by serotonin[2]. An increase of the efflux of sodium and potassium from the isolated skin of a frog was also shown in the presence of serotonin[3]. This latter effect was not considered to be the result of non-specific damage of the cell membranes, because the permeability to Evans blue or sucrose was not affected by serotonin[3]. It is, however, well known that serotonin exerts a marked effect on the capillary permeability in rats[4–6]. Following a different approach, Woolley has proposed that there is an interaction between serotonin and calcium in isolated organs on the basis that serotonin no longer stimulates the uterus in the presence of a calcium-chelating agent[7]. This finding was interpreted as an effect of serotonin in facilitating the penetration of calcium into the cells[8]. On the other hand, calcium seems to be an important factor for serotonin release during the antigen–antibody reaction[9,10].

These results prompted us to investigate a possible role of serotonin in the absorption and distribution of calcium. Experiments have been carried out *in vitro* on the everted intestinal sac of the rat, according to the method of Wilson and Wiseman[11]. Serotonin (5-hydroxytryptamine creatinine sulphate) at a concentration of 20 μgm./ml. increased the absorption of calcium-45 (after 15, 30 or 60 min. of incubation), while acetylcholine (20 μgm./ml.) had no effect. Creatinine sulphate (20 μgm./ml.) was also ineffective. Serotonin retained its effect when the everted intestinal sac was incubated at 5° C. Some of the results are summarized in Table 1.

Table 1. EFFECT OF SEROTONIN AND ACETYLCHOLINE ON CALCIUM-45 ABSORPTION FROM THE EVERTED INTESTINAL SAC AFTER 30 MIN. OF INCUBATION

Substance added	Per cent of calcium-45 absorbed*	Significance (P)
None	$4 \cdot 14 \pm 0 \cdot 09$	—
Serotonin 10	$5 \cdot 49 \pm 0 \cdot 16$	$< 0 \cdot 01$
Acetylcholine 10	$3 \cdot 45 \pm 0 \cdot 06$	$= 0 \cdot 01$
None	$4 \cdot 26 \pm 0 \cdot 11$	—
Serotonin 20	$8 \cdot 05 \pm 0 \cdot 13$	$< 0 \cdot 01$
Acetylcholine 20	$3 \cdot 67 \pm 0 \cdot 10$	$> 0 \cdot 05$

*Each value is the mean of 8 determinations.

The everted intestinal sac (10 cm.) was suspended in 5 ml. of medium containing: NaCl 0·9 per cent (100), KCl 1·15 per cent (4), KH_2PO_4 2·11 per cent (1), buffer phosphate at *p*H 7·4 (10), glucose 0·3 per cent in the final solution. 1 ml. of the same medium was introduced inside the sac. $^{45}CaCl_2$ was added to the outside medium at the dose of 0·4 µc./ml. (specific activity 2·5 µc./mgm. calcium). The flasks were incubated in O_2 at 37° C. in a Warburg apparatus at 96 oscillations/min.

The values represent the percentage of the added radioactivity recovered 'inside' the sac.

The effect of serotonin does not seem to be related to the contraction of the intestine, because acetylcholine at the same concentration is ineffective. Serotonin also increased the penetration of calcium into human cancer cells (strain *Af*(*H.Ep*2))[12] cultivated *in vitro*, as shown in this laboratory by autoradiography (A. Leonardi and R. Vertua, unpublished observations).

Further experiments were carried out to see if the serotonin effect also occurs *in vivo*. Male Sprague Dawley rats, 150 gm. weight, were fasted for 6 hr. and then given both serotonin and calcium, simultaneously, by various routes. The animals were killed at different intervals of time afterwards.

Samples of liver (3 gm.), brain (1 gm.), washed small intestine (2·5 gm.) and blood (1 ml.) were combusted for 12 hr. at 200° C. and for an additional 24 hr. at 700° C.[13]. The ashes were dissolved in 2 *N* hydrochloric acid and mixed with 6·45 mgm. of calcium chloride as carrier. Ammonium oxalate, 4 per cent, was added in excess and then neutralized with ammonia, 25 per cent. When calcium oxalate was completely precipitated (after 4 hr.) it was centrifuged and washed several times with ammonia, 2 per cent, and distilled water. The sample was then plated and the radioactivity was measured using a thin mica end-window Geiger counter.

Table 2. EFFECT OF SEROTONIN ON DISTRIBUTION OF CALCIUM-45

Serotonin mgm./kgm.	hr. between calcium uptake and killing	c.p.m./mgm. calcium			
		Blood	Small intestine	Liver	Brain
—	2	1,023	1,878	1,362	321
25 oral	2	1,230	3,529	1,935	376
—	4	1,153	2,075	703	326
25 oral	4	1,213	2,397	1,120	344
—	1	2,240	9,432	1,519	191
10 i.p.	1	3,824	30,114	1,948	212
—	2	1,572	3,848	1,143	260
10 i.p.	2	2,974	17,816	3,349	390
—	4	1,405	2,730	1,170	378
10 i.p.	4	1,822	5,227	1,903	380

$^{45}CaCl_2$ (specific activity 2·5 µc./mgm. calcium) was given by oral route at the dose of 150 µc./kgm.

It is evident from Table 2 that serotonin increases the absorption of calcium from the intestinal tract. In other experiments not reported in detail, the distribution of calcium-45 after intravenous administration was not greatly affected by serotonin. For this reason, it is likely that the increase of calcium in liver, when calcium was given orally, should probably be related to the increased absorption of calcium from the intestine.

The pharmacological implications of these observations are now under investigation.

S. GARATTINI
E. GROSSI
P. PAOLETTI
R. PAOLETTI
M. POGGI

Institute of Pharmacology,
Medical School, University,
Milan.

[1] Pickles, V. R., and Sutcliffe, J. F., *Biochim. Biophys. Acta*, **17**, 244 (1955).

[2] Pickles, V. R., *J. Physiol.*, **134**, 484 (1956).

[3] Pickles, V. R., *J. Physiol.*, **138**, 495 (1957).

[4] Sparrow, E. M., and Wilhelm, D. L., *J. Physiol.*, **137**, 51 (1957).

[5] Spector, W. G., and Willoughby, D. A., *J. Path. Bact.*, **74**, 57 (1957).

[6] Ungar, G., Kobrin, S., and Sezesny, B. R., *Arch. Int. Pharmacodyn.*, **123**, 71 (1959).

[7] Woolley, D. W., *Proc. U.S. Nat. Acad. Sci.*, **44**, 197 (1958).

[8] Woolley, D. W., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1202 (1958).

[9] Huidobre, H., and Valette, G., *C.R. Acad. Sci., Paris*, **250**, 1375 (1960).

[10] Humphrey, J. H., and Jaques, R., *J. Physiol.*, **128**, 9 (1955).

[11] Wilson, T. H., and Wiseman, G., *J. Physiol.*, **123**, 116 (1954).

[12] Leonardi, A., *Giorn. Ital. Chemioter*, **5**, 41 (1958).

[13] Cremer, H. D., and Her, W., *Radioactive Isotope in Physiologie, Diagnostik und Therapie*, 370 (edit. by H. Schwiegk, Springer Verlag, 1958).

Potentiation of Epinephrine Action

THE following experimental results have been reported by our colleagues on the potentiation of epinephrine action by various substances, clarifying part of its mechanism: (1) The potentiation of epinephrine action on vasoconstriction by certain amino-acids was attributed to a reaction in which the oxidized form of epinephrine gave rise to the oxidative de-amination of amino-acids and was reduced by the generated hydrogen[1,2]. (2) The epinephrine potentiation in the presence of histidine resulted from its combination with cupric ion in the tissues, which was considered as one of the oxidizing catalysts of epinephrine[3].

On the other hand, it is generally accepted that there exist some kinds of enzymes which attack epinephrine in living organisms. To investigate the influence of the so-called epinephrine-potentiating substances such as cocaine, ephedrine and catechol-derivatives on these enzymes, O-methyl transferase[4] especially has caught our interest. We have carried out a series of experiments to evaluate the epinephrine potentiation which is attributable to the inhibition of monoamine oxidase and O-methyl transferase by these substances. The following results have been obtained: (1) The inhibitory strength of monoamine oxidase inhibitors such as cocaine, ephedrine, procaine, marsilid and methylene blue *in vitro* did not parallel the degree of epinephrine-potentiating action upon vasoconstriction[5]. (2) Cocaine, ephedrine and procaine manifested no influence on O-methyl transferase activity. Pyrogallol, gallic acid, catechol, some catechol-derivatives and flavonoids proved to be the substrates of O-methyl transferase, and these substances, when co-existing with epinephrine, competitively inhibited the metanephrine formation (Table 1). (3) The epinephrine potentiation by hydroquinone[6] was observed to have no relation to the change in the activity of O-methyl transferase, but it seemed more likely that the reduction of Fe^{+++}-protein followed by the increment of Fe^{++}-protein, a probable constituent of epinephrine receptor[7,8], was the main cause.

Table 1. INHIBITION OF VARIOUS SUBSTANCES ON METANEPHRINE FORMATION

Substance	Dose (μmole)	Metanephrine formed (μmole)
Control	—	$4 \cdot 7 \times 10^{-2}$
Cocaine	2·0	$4 \cdot 8 \times 10^{-2}$
Ephedrine	2·0	$4 \cdot 2 \times 10^{-3}$
Procaine	2·0	$4 \cdot 8 \times 10^{-2}$
Catechol	0·5	$1 \cdot 3 \times 10^{-2}$
Protocatechu aldehyde	0·5	0
Protocatechuic acid	0·5	$0 \cdot 7 \times 10^{-2}$
3,4-dihydroxy phenylacetic acid	0·5	$2 \cdot 1 \times 10^{-2}$
Hydroquinone*	0·5	$4 \cdot 9 \times 10^{-2}$
Gentisic acid*	0·5	$4 \cdot 9 \times 10^{-2}$
Pyrogallol	0·5	0
Gallic acid	0·5	0
Phloroglucin	0·5	$4 \cdot 2 \times 10^{-2}$
Rutin	0·5	0
Quercetin	0·5	0

Each substance was added to the following mixture and incubated at 37·5° C. for 30 min. under anaerobic conditions: epinephrine, 0·5 μmole; magnesium chloride, 20 μmole; *S*-adenosylmethionine, 0·5 μmole; *tris*-buffer, 100 μmole; crude enzyme (the soluble supernatant fraction obtained from rat liver prepared by Axelrod's method (ref. 4)), 0·6 ml. and water to make a final volume of 2·0 ml. Metanephrine formed was estimated by Pisano's method. Each value is the mean of three experiments. The standard deviation was within 10 per cent.

* Gentisic acid and hydroquinone proved to be the substrates of O-methyl transferase in this reaction mixture.

Table 2. INHIBITION OF THE UPTAKE OF EPINEPHRINE BY COCAINE, EPHEDRINE AND PROCAINE

	Dose (final concentration, *M*)	Epinephrine uptake (μgm./mgm. of dry weight)
Control (0° C.)	—	0·35
Control (37·5° C.)	—	3·30
Ephedrine	10^{-3}	0·48
Cocaine	10^{-3}	0·51
	10^{-4}	0·73
	4×10^{-5}	0·99
	4×10^{-6}	1·99
	4×10^{-7}	2·64
Procaine	4×10^{-5}	2·79
	4×10^{-6}	3·06
	4×10^{-7}	3·10

Platelets were suspended in 2·5 ml. of the phosphate saline containing 200 μgm. of epinephrine and different concentrations of each substance. The mixture was incubated at 37·5° C. for 90 min. Values given represent the mean of three experiments. The standard deviation was within 10 per cent.

Recently, problems on the uptake of catecholamines by some tissues and release from them have become of interest to us. It is a matter of course that the mechanism of epinephrine potentiation should also be analysed from this point of view. Accordingly, we have tried a model experiment on the uptake and release of epinephrine using rabbit blood platelets, and obtained the following result: The uptake of epinephrine by rabbit blood platelets was inhibited by cocaine, ephedrine and procaine (Table 2), and their inhibitory strength was parallel with their potentiation degree of epinephrine action.

From our experimental results mentioned here, we have classified the mechanism of the epinephrine potentiation by various substances as follows:

(1) Competitive inhibition on the enzymes, especially *O*-methyl transferase in inactivating epinephrine. (Pyrogallol, gallic acid, catechol, some catechol-derivatives, and flavonoids.)

(2) Inhibition of the uptake of epinephrine by blood platelets, blood cells and other tissues. (Cocaine, ephedrine and procaine.)

(3) Chelation with cupric ion that is a catalytic agent of the oxidation of the phenol ring of epinephrine. (Histidine, alanine, glycine, methionine and phenylalanine.)

(4) Acceleration of 'oxidation-reduction' turn-over of epinephrine. (Certain amino-acids and ascorbic acid.)

(5) Reduction of Fe^{+++}-protein to Fe^{++}-protein, a probable constituent of epinephrine receptor. (Hydroquinone, cysteine, ascorbic acid and glutathione.)

We wish to express our sincere thanks to Prof. T. Suzuki, Kyoto University, for his donation of S-adenosyl methionine.

R. IMAIZUMI
Y. HASHIMOTO
M. OKA

Department of Pharmacology,
Osaka University,
Medical School, Osaka.

[1] Yamamoto, I., *Osaka Daigaku Igaku Zassi*, 2 (8), 15 (1948).
[2] Imaizumi, R., and Yoshida, H., *Med. J. Osaka Univ.*, 4, (I), 115 (1953).
[3] Matsumoto, H., *Osaka Daigaku Igaku Zassi*, 10, 349 (1958).
[4] Axelrod, J., and Tomchick, R., *J. Biol. Chem.*, 233, 702 (1958).
[5] Imaizumi, R., *et al.*, *Jap. J. Pharmacol.*, 8, 87 (1959).
[6] Yoshida, H., and Noda, T., *Osaku Daigaku Igaku Zassi*, 6 (3), 193 (1954).
[7] Ito, M., *Osaka Daigaku Igaku Zassi*, 9, 555 (1957).
[8] Unoki, A., *Osaka Daigaku Igaku Zassi*, 11, 1547 (1959).

HÆMATOLOGY

Action of Influenza Viruses, Receptor-destroying Enzyme and Proteases on Blood Group Agglutinogen M^g

THE ability of influenza viruses and receptor-destroying enzyme from *Vibrio cholerae* to inactivate human erythrocyte agglutinogens *M* and *N* was first noted in this Laboratory[1] and afterwards independently in Finland[2]. Lu^a and Lu^b agglutinogens are also inactivated by influenza viruses but not by the receptor-destroying enzyme[3]. None of 17 additional human blood group agglutinogens—including *S* and *s* which are considered to be products of genes very closely linked to the *M* and *N* genes—was destroyed by these agents[1,3]. On the other hand, the inactivation of a number of erythrocyte agglutinogens including *M* and *N* by papain, trypsin and other enzymes has been known for a considerable time[4–6].

Recently a new blood-group factor, M^g, was described by Allen, Corcoran, Kenton and Breare[7] and thought to be the product of a gene allelomorphous to the *M* and *N* genes. It was of interest, therefore, to investigate the effect of enzymically active agents on this 'new' erythrocyte agglutinogen.

B, MM^g and *O*, NM^g erythrocytes (Paul and John Gilfeather respectively; cf. ref. 7) and serum (lot No. SM^g-50) containing saline agglutinating anti-M^g antibodies of a titre of 1 : 8 were obtained with the co-operation of Dr. F. H. Allen, jun., from the Blood Grouping Laboratory, Boston. A saline-agglutinating anti-M^g serum with a titre of 1 : 1–1 : 2, found in this Hospital's blood bank, was also used. The erythrocytes were washed with 0·85 per cent saline and treated with purified influenza viruses (Melbourne and Swine S_{15} of type *A* and Lee of type *B*), with the receptor-destroying enzyme from *Vibrio cholerae*, papain and trypsin as previously described[1,8] and also with three times the amount of virus used in earlier experiments. Physiological saline buffered with 0·005 *M* veronal to *p*H 7·4 and containing a trace of calcium chloride was substituted for the phosphate buffer used earlier.

Table 1. Effect of Influenza Viruses and Receptior-Destroying Enzyme on Human Erythrocyte Agglutinogens (Gilfeather)

Serum		Agglutinogen tested for	Erythrocytes	Serum unabsorbed: Native	Serum unabsorbed: Neuraminidase-treated	Serum absorbed with neuraminidase-treated erythrocytes: Native	Serum absorbed with neuraminidase-treated erythrocytes: Neuraminidase-treated
				Agglutination of erythrocytes*			
anti-*M*	Human (*J.C.*)	*M*	Paul Gilfeather *B*, MM^g	8	4–8	8	<1
	Human (*SJM*–1)	*M*		4–8	1	4	<1
anti-M^g	Human (SM^g–50)	M^g		4–8	16	<1	<1
anti-*B*	Human (*P.W.*)	*B*		64	128	<1	<1
anti-*N*	Human (Ar. donated by Dr. H. Gershowitz)	*N*	John Gilfeather *O*, NM^g	32	16	16–32	<1
	Vicia graminea (*J.C.* 60)	*N*		16	<1	32	<1
anti-M^g	Human (SM^g–50)	M^g		8	32	<1	<1
	Human (2111/61)	M^g		1–2	4–8		
anti-*H*(*O*)	Eel (*E.D.*60)	*O*		128	512	2	8

* Expressed as reciprocal of serum dilution.

The results with influenza viruses and the receptor-destroying enzyme were similar and are listed in Table 1. These results show clearly that treatment of Gilfeather erythrocytes with influenza viruses or the receptor-destroying enzyme inactivates *M* and *N* but not M^g (columns 3 and 4, Table 1). The *B* and *H*(*O*) antigens of these erythrocytes, serving as controls, are not inactivated by this treatment. Lack of inactivation of the M^g, *B* and *O*(*H*) agglutinogens is demonstrated by the increased titres of the respective agglutinins (columns 3 and 4, Table 1) against neuraminidase-treated erythrocytes as compared to the titres against native erythrocytes. The increase in agglutinin titre against non-inactivated agglutinogens appears to be due to the additive effect of the titre against the native receptor plus *T*-agglutination against the *T* antigen unmasked by neuraminidase (cf. ref. 1). The *T* agglutinin alone, however, is not sufficiently strong to prevent the demonstration of the inactivation of the *M* and *N* factors as the agglutinin titre does not rise but decreases (column 4, Table 1). To eliminate the interfering action of *T* agglutinins, erythrocytes treated with the same enzymically active agent were used for both absorption of sera and titration of agglutinins. A comparison of the data in the third and fifth columns of Table 1 shows that absorption with neuraminidase-treated erythrocytes removes anti-M^g, anti-*B* and anti-*H*(*O*) agglutinins but not anti-*M* and anti-*N* agglutinins. The anti-*M* and anti-*N* titres against untreated erythrocytes are the same before and after absorption. This indicates inactivation of *M* and *N* agglutinogens of the neuraminidase-treated red cells, as does also a comparison of the last two columns of Table 1, where the titres are listed after removal of anti-*T* agglutinins. Trypsin and papain, however, completely inactivate the M^g receptor. Untreated *M* and *N* erythrocytes removed their respective antibodies in one absorption with one volume of packed erythrocytes.

There was no demonstrable change of the agglutinogens under investigation when they were incubated with either uninoculated allantoic fluid, boiled influenza viruses or autoclaved enzymes.

B, MM^g and *O*, NM^g erythrocytes were agglutinated by influenza viruses as were other human erythrocytes and also became inagglutinable by the virus to which they had been exposed extensively.

The lack of inactivation of agglutinogen M^g by influenza viruses and the receptor-destroying enzyme, in contrast to that of antigens *M* and *N*, is unexpected in view of the assumed close serological and genetical relation of blood group receptors *M*, *N* and M^g (ref. 7). It recalls, however, similar observations for the *ABO* blood-group system where certain enzyme preparations will inactivate all three blood group antigens while others will affect only one. It is believed that influenza viruses possess only one enzyme, neuraminidase[9]. Based on the activity of influenza viruses, it has been assumed that derivatives of neuraminic acid (sialic acids) are closely involved in the specificity of blood group agglutinogens *M* and *N*[1,2,10,11]. While this assumption is reasonable, it is obvious from the data presented here that if M^g is indeed an allele to *M* and *N*, it is unlikely that identical sialic acids in identical linkages are involved in the specificities of the products of the allelomorphic genes *M*, M^g and *N*. Influenza virus enzyme may thus be a more sensitive tool than papain and trypsin in the investigation of fine structural differences of products of blood-group genes of the *MNS* system.

This work was supported by grant *E*-2191 of the U.S. National Institutes of Health.

Georg F. Springer*
Karlheinz Stalder†

Immunochemistry Section,
William Pepper Laboratory and
Department of Medical Microbiology,
University of Pennsylvania,
Philadelphia.

* Established Investigator of the American Heart Association.
† On leave from the Max Planck Society, Göttingen.

[1] Springer, G. F., and Ansell, N. J., *Proc. U.S. Nat. Acad. Sci.*, **44**, 182 (1958).
[2] Makela, O., and Cantell, K., *Ann. Med. Exp. et Biol. Fenn.*, **36**, 366 (1958).
[3] Springer, G. F., and Ansell, N. J., *Proc. Seventh Congr. Intern. Soc. Blood Transf.*, 697 (S. Karger, Basle and New York, 1959).
[4] Morton, J. A., and Pickles, M. M., *J. Clin. Path.*, **4**, 189 (1951).
[5] Watkins, W. M., and Morgan, W. T. J., *Brit. J. Exp. Pathol.*, **35**, 181 (1954).
[6] Howe, C., MacLennan, J. D., Mandl, I., and Kabat, E. A., *J. Bacteriol.*, **74**, 356 (1957).
[7] Allen, jun., F. H., Corcoran, P. E., Kenton, H. B., and Breare, N., *Vox Sang.*, **3**, 81 (1958).
[8] Springer, G. F., and Rapaport, M. J., *Proc. Soc. Exp. Biol. and Med.*, **96**, 103 (1957).
[9] Gottschalk, A., *The Chemistry and Biology of Sialic Acids*, 99 (Cambridge University Press, 1960).
[10] Klenk, E., and Uhlenbruck, G., *Z. Physiol. Chem.*, **319**, 151 (1960).
[11] Baranowski, T., Lisowska, E., Morawiecki, A., Romanowska, E., and Strozecka, K., *Arch. Immunol. terap. Doswiad.*, **7**, 15 (1959).

The Diego Factor in a Puerto Rican Family: a Case of Anti-Diego$_a$ in Monozygotic Twins

The Diego blood factor exists in many South American people having a substantial Indian admixture[1]. The following family is the first reported from Puerto Rico.

Mrs. P. F. M., a Puerto Rican woman, age thirty-seven, residing in New York City, delivered premature, monozygotic, female twins, with positive direct Coombs tests. She had four full-term spontaneous deliveries by her first husband (Di^{a-}), and two early abortions and one miscarriage before the twins, by her second husband (Di^{a+}). On close observation for seven weeks neither twin developed any signs or symptoms of hæmolytic disease of the new-born other than minimal hepato-splenomegaly.

Serological investigations revealed the father to be group O, R_1R_1, Di^{a+}, both twins to be group A, Di^{a+}, R_1R_2, the mother, group A_2, R_2r and Di^{a-}. The hæmolytic antibody formed by iso-immunization was anti-Diego, detected by an indirect Coombs test with a titre 1 : 32. Investigation of the father's family revealed the Diego factor in his aunt, paternal grandmother, and great uncle, all natives of Puerto Rico and exhibiting Indian features. The Di^{a+} cases were typed for ABO, D, C, c, E, e, V, $MNSs$, P, K, k, Fy^a, Le^a, Le^b, Jk^a, Jk^b, Lu^a, Lu^b, Js^a, Yt^a, Kp^b, Wr^a, Vel, Good, Santos, Gerbich, without adding new information pertaining to the independent segregation of Di^a gene.

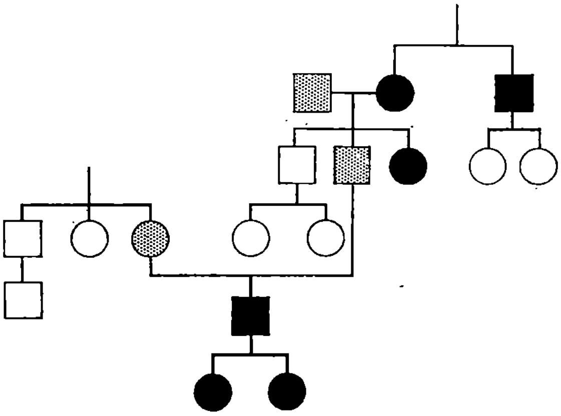


Fig. 1. The anti-Diego (Di^a) factor in four generations of a Puerto Rican family. Black, Di^a-positive; shaded, not tested, deceased; open, Di^a-negative

This anti-Diego is the first reported which caused no symptoms in the infants who provoked immunization. The prematurity of these twins should have heightened their susceptibility to hæmolytic disease. Perhaps several near-term gestations are necessary to stimulate formation of a clinically apparent antibody, a state analogous to Rh disease in non-transfused patients.

The original case (Diego), a Venezuelan child, who died of hæmolytic disease of the new-born, was fourth in birth order with two older Di^{a+} siblings[2]. The second case (W.O.J.) was third in birth order with non-fatal hæmolytic disease (Levine, P., personal communication). The third case was fifth in birth order (Mart) and died from severe erythroblastosis fœtalis[3].

Table 1

	Mother	Twins	Father
Group	A_2	A	O
Rh D	+	+	+
C	−	+	+
E	+	+	−
d	+	−	−
c	+	+	−
e	+	+	+
	cDE/cde	*DCeDcE*	*DCe/DCe*
WR^a	−	−	−
Kp^a	−	−	−
Kp^b	+	+	+
He	−	−	−
Di^a	−	+	+

The true hazard of sensitization to Di^a is not known. Layrisse stated that in 50 cases of Di^a incompatible matings no antibodies were formed in an unspecified number of gestations[4].

Among Chinese with a 2·5 per cent incidence of Di^a, hæmolytic disease is very rare. Clinically, anti-Diego has not yet proved to be of great significance. Nevertheless, the present case demonstrates that undetected antibodies to Di^a will be missed if not sought for. Our rule of an antiglobulin test on premature infants revealed iso-immunization. Among populations with a high incidence of the Diego factor, it remains a potential cause of fœtal wastage.

HUBERT J. VAN PEENEN*
JOHN SCUDDER
JAMES A. JACK†
ERIKA AWER

Departments of Pathology and Surgery,
College of Physicians and Surgeons,
Columbia University,
and
The Blood Bank,
Presbyterian Hospital in the City of New York.

---

* Present address: Veterans Administration Hospital, Oakland, California.

† Knickerbocker Foundation, Inc., New York.

[1] Layrisse, M., *Amer. J. Phys. Anthropol.*, **16**, 173 (1958).

[2] Levine, P., and Robinson, E. A., *Blood*, **12**, 448 (1957).

[3] Tatarsky, J., Stroup, M., Levine, P., and Ernoehazy, W. S., *Vox Sang.*, **4** (2), 152 (1959).

[4] Layrisse, M., and Arends, T., *Blood*, **12**, 115 (1957).

PATHOLOGY

Effects of Carrageenin on Serum Lipids and Atherosclerosis in Rabbits

IN recent years considerable interest has developed regarding an important role of aortic mucopolysaccharides with advancing age. On the other hand, a number of reports have dealt with the activities of heparin and other sulphated polysaccharides to clear visible lipæmia and also to retard arteriosclerotic process in arterial walls.

The object of this communication is to report a hitherto undescribed effect of carrageenin, a natural sulphated polysaccharide, obtained from seaweeds, in suppressing hyperlipæmia and atherosclerosis in cholesterol-fed rabbits. Carrageenin obtained from *Chondrus ocellatus* contained 11·86 per cent sulphur. It showed a specific optical rotation of +28°. The experiment was performed as follows :

A number of male albino rabbits were all fed a daily diet consisting of bean-curd-mash and cholesterol at the rate of 100 mgm. per kgm. body-weight during the course of 18 weeks. The animals were divided into two groups : (1) cholesterol-fed plus carrageenin-injected group consisted of 21 rabbits ; (2) a control group of 19 rabbits fed cholesterol only. The animals of the treated group received individually a daily intravenous injection of 5 mgm. of carrageenin per kgm. body-weight. At 2-week intervals, serum lipids such as total lipid[1], total and esterified cholesterol[2], phospholipid[3], β/α lipoprotein ratio[4] and turbidity of the serum were measured. At the end of the experiment, all rabbits were killed, and the atheromatous lesions in the aorta were examined macroscopically and microscopically.

The results of the experiment revealed that carrageenin possesses suppressing effects on the rise of

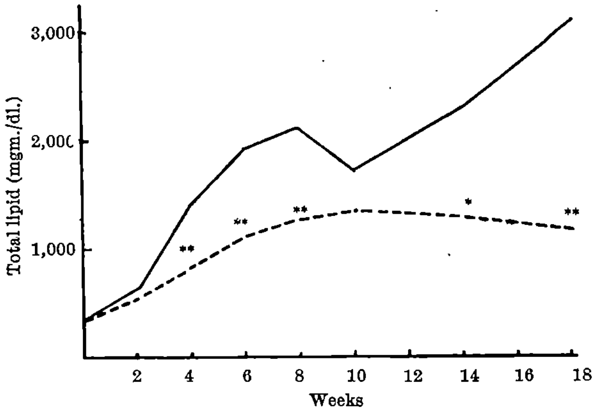


Fig. 1. Mean serum total lipid in cholesterol-fed plus carrageenin-injected group and in cholesterol-fed control group. – – –, Carrageenin; ——, control. *, significant; **, highly significant

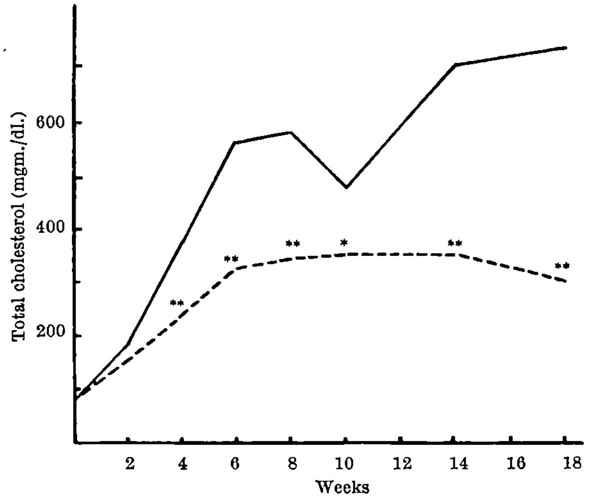


Fig. 2. Effect of carrageenin on serum total cholesterol. – – –, carrageenin; ——, control. *, significant; **, highly significant

serum lipids in cholesterol-fed rabbits. Figs. 1 and 2 show that the mean serum total lipid and total cholesterol-levels in the treated group showed less increase compared with the control after 4 weeks following the beginning of injection of carrageenin, and significant differences between the two groups were noticed during the experiment except for the early period. In addition, remarkable differences of the mean levels in cholesterol and phospholipid ratio, β/α lipoprotein ratio and serum turbidity in both the groups were noticed. Lipæmia-clearing activity was shown following the administration of carrageenin. However, carrageenin failed to prove its anticoagulant activity, at least at the dosage of 5 mgm. per kgm. body-weight, according to the Lee and White method.

The visual grading of severity of atheromatous lesions, as determined by their distribution, size and number, was shown to be almost parallel to the concentration of the serum lipids. The microscopic findings, according to several stainings, supported the result in the gross findings that the grade of severity of atheromatous lesions was much less in the carrageenin-treated rabbits than in the controls.

T. Mori[5] reported that carrageenin consists of 30–40 per cent D-galactose and 20–30 per cent sulphate. The finding[6] that the subcutaneous injection of carrageenin into animals stimulated the formation of new connective tissue will support the presumption that carrageenin plays a part in promoting the repair of atheromatous lesions, when an adequate supply of it is provided.

Grateful acknowledgment is made to Prof. Y. Oshima for his kind advice while carrying out this investigation.

KATSUMI MURATA

Department of Physical Therapy and Internal Medicine,
School of Medicine,
University of Tokyo.

---

[1] Huerga, J., Yesinick, C., and Popper, H., *Amer. J. Clin. Pathol.*, **23**, 1163 (1953).

[2] Sperry, W. M., and Webb, M., *J. Biol. Chem.*, **187**, 97 (1950).

[3] Allen, R. J. L., *Biochem. J.*, **34**, 858 (1940).

[4] Swahn, B., *Scand. J. Clin. Lab. Invest.*, **5**, suppl. 9 (1953).

[5] Mori, T., *Adv. Carbohydrate Chem.*, **8**, 315 (1953).

[6] Jackson, D. S., *Biochem. J.*, **65**, 277 (1957).

Immunofluorescence of Pleuropneumonia-like Organisms isolated from Tissue Cell Cultures

It has been established that continuous tissue cell cultures are commonly contaminated with pleuropneumonia-like organisms and/or *L*-forms of bacteria[1-5]. These results have encouraged investigators to search for reasonably simple methods for the detection of these agents, especially in relation to studies for their elimination. Of methods proposed for their detection, cultivation and isolation have proved most successful[6]. With the advent of the fluorescent antibody technique as a rapid method for the identification of micro-organisms, it seemed to us that this procedure might be useful as a means of detecting contamination of pleuropneumonia-like organisms in tissue cell cultures. The feasibility of this technique is presented here.

Strain *HEp* 2, isolated from the human epidermoid carcinoma of skin (Fjelde) cell line, was propagated in primary rabbit kidney cells for the preparation of antigen for antibody production in New Zealand white rabbits. The antiserum was conjugated with fluorescein *iso*thiocyanate in the manner described by Marshall *et al.*[7]. Lissamine rhodamine *RB* 200 was conjugated to normal bovine serum albumin and added to the labelled antiserum as a counterstain[8].

Contaminated continuous cell cultures and infected primary rabbit kidney cells grown on cover-slips in Leighton tubes were fixed in buffered formalin, washed, and then stained with the fluorescein-conjugated antiserum for 1 hr. at 37° C. in a wet chamber.

The strains of pleuropneumonia-like organisms, isolated from the cell lines mentioned here, were also grown on membrane disks (Millipore Filter Corporation, Bedford, Massachusetts) placed on PPLO agar medium[6]. Impressions were made by transferring colonies from disks to microscopic slides, air drying, and staining them for 15 min. at room temperature. The stained preparations were examined with a Zeiss fluorescence microscope, using the Osram *HBO* 200 light source, the *BG*-12 exciter filter, and the *GG*4-*OG*4 barrier filter.

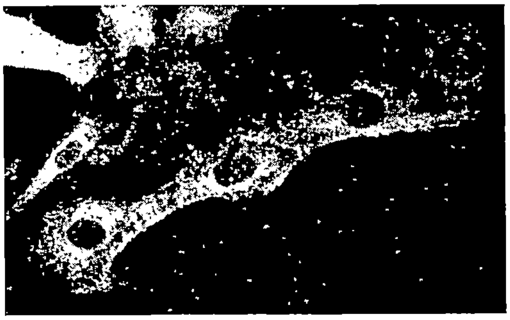
Fluorescein-conjugated antiserum reacted specifically with the homologous pleuropneumonia strain (Fig. 1) when tested with the original contaminated cell lines, infected primary cells, and colonial impressions. Strains isolated from five other continuous cell cultures maintained regularly in both of our laboratories reacted in the same manner. These cultures were HeLa—human carcinoma of cervix (Gey); HA—human amnion (Fogh and Lund); HI—human embryonic intestine (Henle); KB—human carcinoma of nasopharynx (Eagle); and conjunctiva—human adult (Chang). Non-infected primary tissue cell cultures previously found by cultural methods to be free of pleuropneumonia-like organisms gave no staining reactions. These preliminary findings suggest that tissue culture strains of these organisms may have similar antigenic structures. Since the organisms were isolated from tissue cultures used in different laboratories, the chance of contamination from a common source could probably be ruled out. On the other hand, it may be that tissue cell cultures favour the selection and development of the same or related strains of the organism.

Heterologous human urethral strain 07 (Dr. Paul Smith, Philadelphia) and strain Campo (Dr. Louis Dienes, Boston) did not react with the *HEp* 2 tissue culture antiserum. This indicates that there are differences between the antigenic structures of these strains. The reaction and non-reaction of colonial impressions of *HEp* 2 and 07, tested with the antiserum simultaneously on the same slide, are shown in Fig. 2.

In order to establish the specificity of the fluorescein-conjugated antiserum of the organisms, 24 bacterial and mycotic organisms were tested. None reacted. Included were strains from the genera *Salmonella, Shigella, Proteus, Staphylococcus, Streptococcus, Diplococcus, Corynebacterium, Pseudomonas, Serratia, Escherichia, Aerobacter,* and *Cryptococcus.*

Antisera to vaccinia, adenovirus type 7, herpes simplex, and infectious canine hepatitis viruses prepared in rabbits and conjugated with fluorescein *iso*thiocyanate did not react with the six continuous cell cultures contaminated with the organism.

(a)



(b)

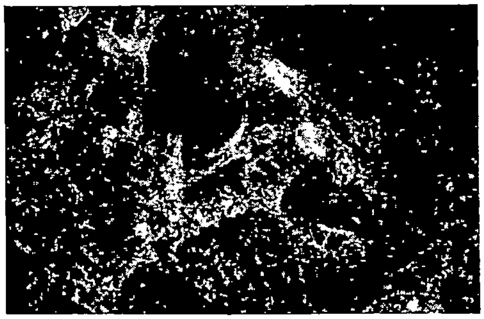


Fig. 1. Monkey (*a*) and rabbit (*b*) kidney tissue cells infected with pleuropneumonial strain *HEp* 2 stained with labelled homologous immune serum. (*a*, × 225; *b*, × 200)

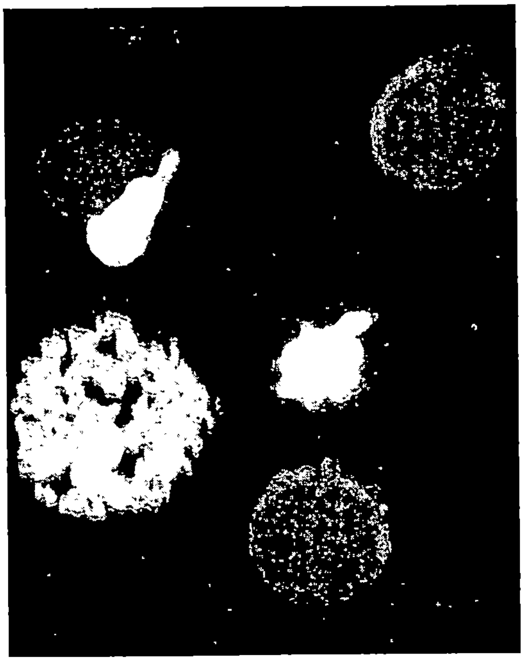


Fig. 2. Impression smears of pleuropneumonial strains *HEp* 2 and 07 stained with labelled *HEp* 2 immune serum. (× 270)

Recent reports indicate that *L*-forms of bacteria rather than pleuropneumonial organisms are the responsible contaminants of cell cultures. Workers in the United States[9] have identified a Gram-negative rod as the bacterial parent, whereas workers in Great Britain[5] have reported a *Corynebacterium.* If the organisms discussed in this communication were in fact *L*-forms of bacteria, then there is reason to suggest that a common bacterium was the source or that the *L*-forms of at least some heterologous bacteria have common antigens.

The staining pattern of fluorescence of pleuropneumonial organisms in tissue cell cultures is similar to the intracytoplasmic granulation pattern consistent with contamination by pleuropneumonial organisms. The fluorescent bodies were found concentrated along the cytoplasmic membrane, in the cytoplasma, and at times along the nuclear membrane. They were not seen within the nucleus.

W. F. MALIZIA
Armed Forces Institute of Pathology,
Washington, D.C.

M. F. BARILE
D. B. RIGGS

Laboratory of Bacterial Products,
National Institutes of Health,
Bethesda, Maryland.

[1] Robinson, L. R., Wichelhausen, R. H., and Roizman, B., *Science*, 124, 1147 (1956).
[2] Collier, L. H., *Nature*, 180, 757 (1957).
[3] Rothblat, G. H., and Morton, H. E., *Bact. Proc.*, 73 (1958).
[4] Hayflick, L., and Stinebring, W. R., *Ann. New York Acad. Sci.*, 79, 433 (1960).
[5] Macpherson, I. A., and Allner, K., *Nature*, 186, 992 (1960).
[6] Barile, M. F., Yaguchi, R., and Eveland, W. C., *Amer. J. Clin. Path.*, 30, 171 (1958).
[7] Marshall, J. D., Eveland, W. C., and Smith, C. W., *Proc. Soc. Exp. Biol. Med.*, 98, 898 (1958).
[8] Smith, C. W., Marshall, J. D., and Eveland, W. C., *Proc. Soc. Exp. Biol. Med.*, 102, 179 (1959).
[9] Holmgren, N. B., and Campbell, jun., W. E., *J. Bact.*, 79, 869 (1960).

IMMUNOLOGY

An Immunological Study of a Human Pituitary Interstitial Cell-stimulating Hormone

Several investigators[1–5] have reported recently the production of rabbit antiserum to human urinary gonadotrophins, but no similar data have appeared with respect to human pituitary gonad-stimulating substances. However, an immunochemical investigation in this Laboratory[6] has shown that a highly purified preparation of sheep pituitary interstitial cell-stimulating hormone (ICSH) is a potent antigen. The rabbit antiserum obtained in that work[6] was highly specific for the antigen and was able significantly to counteract its ability to stimulate the ventral prostate in hypophysectomized rats. By the Ouchterlony double diffusion test in agar-gel and the quantitative precipitin technique, the sheep interstitial cell-stimulating hormone was demonstrated to be homogeneous. Moreover, antiserum to sheep interstitial cell-stimulating hormone was capable of neutralizing significantly the interstitial cell-stimulating hormone activity of pituitary extracts from the rat, pig and whale, as well as of purified preparations of human pituitary interstitial cell-stimulating hormone and pregnant mare serum gonadotrophin. Injected antiserum to sheep interstitial cell-stimulating hormone was also shown to cause an inhibition of endogenous activity of the hormone in normal rats. The results of similar investigations on the antigenicity of human pituitary interstitial cell-stimulating hormone are reported here.

The antigen, human interstitial cell-stimulating hormone, was prepared from human pituitaries by the procedure previously described[7]. Two albino rabbits weighing initially 2·6–2·8 kgm. were immunized with a total dose of 8 mgm. of the purified hormone per rabbit, according to the following schedule. Each rabbit was given three injections, consisting of 1·5 mgm. of human interstitial cell-stimulating hormone in Freund's adjuvant (complete), once weekly for three consecutive weeks, was allowed to rest for ten days, and was then given a fourth injection of 1·5 mgm. of human interstitial cell-stimulating hormone. The animals were once again rested for ten days, during which time they were usually test-bled from the marginal ear vein. Finally, animals were bled by heart puncture ten days after a booster shot of 2 mgm. of human interstitial cell-stimulating hormone in saline. The pooled sera showed the presence of contaminating antibodies to normal human serum proteins and, to a very slight extent, to human growth hormone. These contaminating antibodies were selectively removed by absorption by the addition of small quantities of 1 : 10 diluted normal human serum and a dilute solution of human growth hormone. The agar-gel diffusion test was used to determine whether the absorption was complete. This absorbed serum was used in all subsequent experiments.

The presence in the absorbed antiserum of a precipitating type of antibody specific to human interstitial cell-stimulating hormone was demonstrated by means of the qualitative ring test[8], double diffusion on Ouchterlony agar-gel plates[9], immunoelectrophoresis[10], and on the basis of antihormone activity as measured by the capacity of the antiserum to neutralize the activity of the test material in hypophysectomized male rats[11,12]. The capacity of antiserum to human interstitial cell-stimulating hormone to neutralize the biological activity of human chorionic gonadotrophin (HCG) was also tested in the normal female rat.

In the qualitative precipitin ring test, by layering 0·1 ml. of absorbed antiserum under a solution of antigen, it was possible to detect 1 μgm. of human interstitial cell-stimulating hormone. The single precipitin line which human interstitial cell-stimulating hormone gave with the absorbed antiserum in both agar-gel double diffusion analysis and immunoelectrophoresis demonstrated the homogeneity of the antigen–antibody system. It was further noted that the antiserum cross-reacted with a component (interstitial cell-stimulating hormone) in a saline extract of human pituitary glands, giving a single continuous precipitin band with that formed with purified human interstitial cell-stimulating hormone in the agar-gel double diffusion experiment.

Table 1 presents results concerning the capacity of the human interstitial cell-stimulating hormone antiserum to inhibit or neutralize effectively the activity of human gonadotrophins in hypophysectomized male rats ; a total dose of 1·0 ml. of the antiserum given over a period of 4 days effectively neutralizes the biological activity of human interstitial cell-stimulating hormone and human chorionic gonadotrophin.

In normal female rats, the biological activity of human chorionic gonadotrophin was counteracted by the simultaneous administration of rabbit antiserum to human interstitial cell-stimulating hormone ; when human chorionic gonadotrophin alone was given to a group of 10 rats (21 days of age at onset), a total dose of 40 μgm. administered intraperitoneally over a period of 3 days produced an ovarian weight (mean ± standard error) of 36·2 ± 1·92 mgm., whereas with the same dose of human chorionic gonadotrophin plus a 1-ml. total dose of the antiserum the weight of the ovaries was only 15·5 ± 1·09 mgm. Furthermore, when tested by the Ouchterlony double diffusion technique, human chorionic gonadotrophin cross-reacted with rabbit antiserum to human interstitial cell-stimulating hormone. It can thus be concluded that human interstitial cell-stimulating hormone is a potent antigen and that it should be possible to develop immunochemical assay methods to determine the content of interstitial cell-stimulating hormone in human pituitary gland and in body fluids.

Table 1. Effect of Rabbit Antiserum to Human Interstitial Cell-stimulating Hormone on the Biological Activity of Gonadotrophin Preparations as assayed in Immature Hypophysectomized Male Rats

Group	No. of rats per group	Material assayed	Total dose in 4 days (μgm. + ml.)	Weight of ventral prostate (mgm.)	Statistical comparison of groups
1	9	Uninjected controls	0	7·0 ± 0·14*	
2	15	Human ICSH	8 + 0	18·1 ± 0·49	
3	7	Human ICSH + antiserum	8 + 1	7·5 ± 0·30	2, 3; $P < 0{\cdot}001$†
4	6	HCG	1 + 0	15·5 ± 1·5	
5	5	HCG + antiserum	1 + 1	10·0 ± 0·83	4, 5: $P < 0{\cdot}01$

* Mean ± standard error. † Values calculated according to Fisher's *t*-test.

This work was supported in part by a grant (*RG*-4097) from the U.S. Public Health Service of the National Institutes of Health, and a grant from the Albert D. and Mary Lasker Foundation, New York.

N. R. MOUDGAL
CHOH HAO LI

Hormone Research Laboratory,
University of California,
Berkeley.

[1] Butt, L. R., Crooke, A. C., Cunningham, F. J., and Gell, P. G. H., *J. Endocrinol.*, **20**, 9 (1960).

[2] Brody, S., and Carström, G., *Lancet*, ii, 99 (1960).

[3] Wide, L., and Gemzell, C. A., *Acta Endocrinol.*, **35**, 261 (1960).

[4] Brody, S., and Carström, G., *Nature*, **189**, 841 (1961).

[5] Lunenfeld, B., Givol, D., and Sela, M., *J. Clinical Endocrinol. Metabol.*, **21**, 478 (1961).

[6] Moudgal, N. R., and Li, C. H., *Arch. Biochem. Biophys.* (in the press).

[7] Li, C. H., Squire, P. G., and Gröschel, U., *Arch. Biochem. Biophys.*, **86**, 110 (1960).

[8] Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry* (Thomas, Springfield, Ill., 1948).

[9] Ouchterlony, O., *Arkiv. Kemi*, B, **26**, 1 (1949).

[10] Grabar, P., and Williams, C. A., *Biochim. Biophys. Acta*, **10**, 193 (1953).

[11] Greep, R. O., van Dyke, H. B., and Chow, B. F., *Proc. Soc. Exp. Biol. and Med.*, **46**, 644 (1941).

[12] Lostroh, A. J., Squire, P. G., and Li, C. H., *Endocrinol.*, **62**, 833 (1958).

Occurrence of an *Rh*-like Antigen in Red Cells of the Sheep

Landsteiner and Wiener[1] discovered that the sera of rabbits immunized by the erythrocytes of the rhesus (macacus) monkey would agglutinate those of certain human subjects irrespective of their *ABO* groups. They added, therefore, the *Rh*-antigen to the list of antigens characterizing the human red cells. Some human red cells, therefore, must be assumed to possess a receptor-substance for antibodies which rabbits or still better guinea pigs can form when immunized with rhesus erythrocytes. Later, Wiener and Peters[2] demonstrated the antigenicity of the *Rh*-receptor of human erythrocytes in man, thus establishing the principal cause for erythroblastosis in the new-born.

Apart from the *Rh*-antibody obtained by immunization of guinea pigs with rhesus erythrocytes, there are antibodies formed in women eventually immunized when bearing a fœtus possessing *Rh*-erythrocytes or possibly formed in human males and females following transfusion of blood incompatible with *Rh*.

As is known, *Rh* denotes a group of antigens distinguishable in man and the *Rh*-antigen marked *D* according to Fisher[3] is the most common in man, and human *D*-erythrocytes are also agglutinated by an anti-*Rh* guinea pig immune serum.

Therefore, two anti-*D* sera occur, one the human anti-*D* and the other an animal immune anti-*Rh* serum which can be used as anti-*D*. These sera, however, are different: the human anti-*D* serum contains only human *Rh*-antibodies, for example, anti-*D*, besides other human isohæmagglutinins of the *ABO* and other systems, while the guinea pig immunized with rhesus erythrocytes produces, besides antibodies against the rhesus *D*, those which are able to react with some receptor-substance on human erythrocytes very similar to the rhesus *D*, and when immunized with human *D*-erythrocytes the guinea pig (or rabbit) may produce besides anti-*D* also human species-specific antibodies. The latter may explain the observation[4] that animal anti-*Rh* immune serum can react with human fœtal (*Rh*-) erythrocytes. Thus, there are many differences between the animal and the human anti-*D* sera. But the anti-*D* itself appears to be different in that the rhesus *D* cannot be identical to the human *D*, since rhesus erythrocytes, while able to absorb the anti-*D* antibodies from a guinea pig anti-rhesus erythrocyte immune-serum, cannot absorb the anti-*D* from human anti-*D* serum, which itself can be absorbed easily by human *D*-erythrocytes. Even by washing three times the rhesus erythrocytes sediment which is in 1 : 1 ratio to human anti-*D* serum, we did not succeed in nine cases in decreasing the anti-*D* titre, but to one dilution step only. This failure confirms earlier observations[5] where there was no success in absorbing a guinea pig anti-rhesus erythrocytes immune serum by human *D* red blood cells.

On account of the immunizing effect, however, *D*-antigen must be present in both human and rhesus erythrocytes, but apparently in such a way masked or concealed in the stroma that its presence cannot be proved serologically, but by immunization only. Murray and Clark[6], by immunizing guinea pigs with human *dd*-erythrocytes, are believed to have succeeded in stimulating the formation of anti-*D* antibodies.

In our experiments, boiling in distilled water did not free enough *D* from rhesus erythrocytes to enable the boiled cells completely to absorb human anti-*D* antibody, but, however, to decrease its titre to one or, in some experiments, to two more dilution steps as had been possible in the experiments mentioned by using fresh rhesus red cells. Boiling red cells, however, had proved effectual in demonstrating the presence of *D* antigen in experiments with sheep erythrocytes.

When sheep red cells, suspended in distilled water (not in saline solution), are boiled, they can absorb completely anti-*D* antibodies from both anti-*D* sera from patients as well as from commercial anti-*D* test sera, while human erythrocytes, treated in the same way by boiling, were not able to absorb anti-*D* to a similar extent. A more detailed account of these experiments will be published elsewhere[7]. Here only some details may be mentioned which require further experimental research: (1) Whether the presence of the *D* antigen in the red cells of sheep bears any relation with other blood grouping characteristics in sheep is not ascertained as yet. (2) Why boiling must be done in distilled water instead of saline remains to be shown. (3) The *Rh*-antigen in sheep red cells appears to represent only that portion which is common to both *C* and *D* antigens, since the absorbing power of boiled ovine red cells, investigated so far, does not distinguish between *C* and *D* antibodies. (4) Experiments are in progress to show whether immunization of guinea pigs or rabbits with boiled red cells of the sheep will produce, besides Forssman antibodies, *Rh*-(*D*?)-antibodies.

B. URBASCHEK
K. TRAUTWEIN

Tierhygiene Institut der Universität,
Freiburg i. Br.

[1] Landsteiner, K., and Wiener, A. S., *Proc. Soc. Exp. Biol. N.Y.*, **43**, 223 (1940).

[2] Wiener, A. S., and Peters, R. H., *Ann. Int. Med.*, **13**, 2306 (1940).

[3] Fisher, R. A., *Ann. Eugen.*, **13**, 150 (1946).

[4] Fisk, R. T., and Foord, A. G., *Amer. J. Clin. Path.*, **12**, 545 (1942).

[5] Davidsohn, I., and Toharsky, B., *Amer. J. Clin. Path.*, **12**, 434 (1942).

[6] Murray, J., and Clark, E. C., *Nature*, **169**, 886 (1952).

[7] Urbaschek, B., *Z. Immunitätsforsch.*, **120**, 145, 154 (1960).

BIOLOGY

Experimental Evidence for the Mechanism of Photosynthesis

FIVE types of short-timed absorption changes (type 0, 1, 2*a*, 2*b*, 3) have been found in photosynthesis[1]. Type 0 is caused by the formation of chlorophyll-triplet states, and occurs only when conduction of energy is interrupted by altering the arrangement of chlorophyll in the chromatophores. Type 1 is caused by the formation of a chlorophyll-*a* derivative (life-time 3×10^{-5} sec.) and occurs only in the normal arrangement of chlorophyll. For details regarding types 0 and 1, see ref. 1 and especially ref. 2.

The difference spectrum of type 2 absorption change is shown in Fig. 1*a*; it appears in $\leqslant 10^{-5}$ sec. and has a life-time of $\sim 10^{-2}$ sec. Type 2 consists of two processes: type 2*a* (negative peak at ~ 420 mμ) and type 2*b* (negative peak at ~475 mμ and positive peak at ~515 mμ). At −150° C. type 2*b* does not occur; the remaining reaction is type 2*a*. From the appearance at −150° C. it follows that type 2*a* must be caused by one primary product of photosynthesis. From the dependence on wave-length at −150° C., it follows that the primary product is probably an oxidized cytochrome[3].

Type 2*b* change of absorption is caused by a reduction process[4]: $X0 \rightarrow X$. On extracting chloroplasts with petroleum ether, the absorption changes of type 2*b* no longer occur. They reappear on recondensation[3]. As plastoquinone is extracted by petroleum ether[5], $X/X0$ may be identical with plastoquinone. Since plastoquinone has no absorption at 515 and 475 mμ, the absorption changes probably take place indirectly by changing the absorption of other pigments during the redox reaction of $X/X0$ (ref. 1).

Since 1956 it has been known[6] that probably two chlorophyll pigments are involved in photosynthesis (Emerson effect): after Govindjee[6] one chlorophyll *a* (*b* ?) with an absorption maximum at ~ 670 mμ and a second one with a maximum at ~ 680 mμ or at longer wave-lengths. We have tried to find out by which of the two pigments the primary product type 2*a* is produced and what is the function of the other pigment. Exposure at ~ 670 mμ causes excitation of Chl*a*-670 and Chl*a*-680, because of the strong overlapping of the absorption bands of the two pigments. Such an excitation causes the absorption changes of type 2*a* and type 2*b*, as already shown in Fig. 1*a*. Exposure at ~ 710 mμ causes mainly excitation of Chl*a*-680. The changes in absorption thus appearing are illustrated in Fig. 1*b*. Type 2*b* is reduced strongly (dotted line), whereas the absorption changes at ~ 420 mμ (characteristic of type 2*a*) remain unchanged. Moreover, changes in absorption can be observed at ~ 400 mμ and ~ 555 mμ, which, owing to their kinetic behaviour, are to be ascribed to type 2*a* (solid line in Fig. 1*b*). These additional changes cannot be seen in Fig. 1*a*, as they are probably compensated by type 2*b*. From the dependence on wave-length of type 2*a* absorption changes (solid line in Fig. 1*b*), it follows convincingly that type 2*a* in *Chlorella* is caused at least by an oxidation of a cytochrome, probably cytochrome *f*.

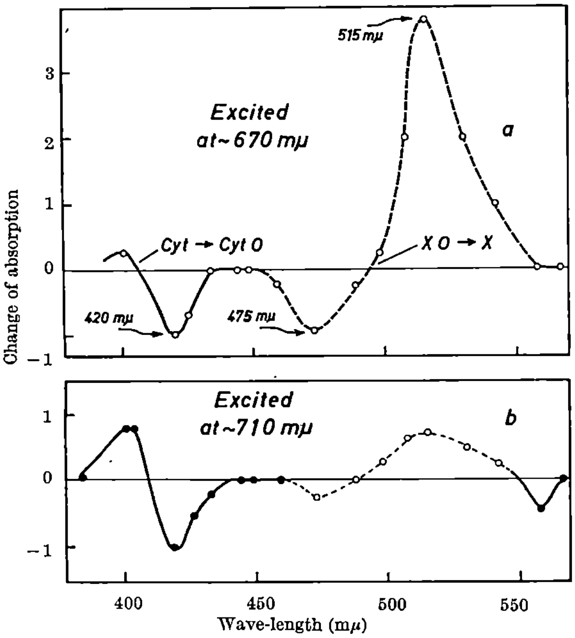


Fig. 1. Changes of absorption caused by illumination of *Chlorella* at two different wave-lengths. Temperature, 20° C.

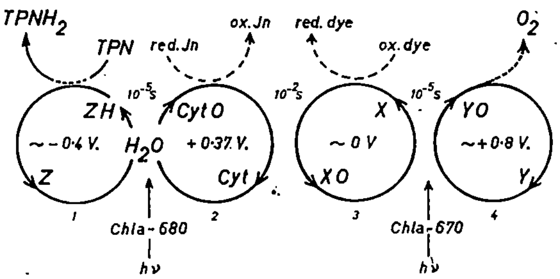


Fig. 2. Reaction pattern of photosynthesis

These results show directly that photosynthesis is triggered by two different photochemical reactions: oxidation of cytochrome by Chl*a*-680 and reduction of $X0$ by Chl*a*-670. The occurrence of two photochemical processes results in a reaction pattern of photosynthesis which differs considerably from previous conceptions. The pattern presumed is shown in Fig. 2. ZH means the reduced form of Z (addition of H or electron); $Y0$, etc., the oxidized form of Y, etc. (loss of electron). The location of water between cycle 1 and 2 is only symbolic; the splitting probably occurs by $Y^+ + 1/2\ H_2O \rightarrow Y + 1/4\ O_2 + H^+$ and $Z^- + H^+ \rightarrow ZH$. Y and Z are chemically not known. The redox potential of cytochrome *f* is + 0·37 V. (ref. 7); that of $X/X0$ has been estimated as ~ 0 V. (ref. 4). The potential of $Y/Y0$ must correspond approximately to that of OH^-/O_2 (~ + 0·8 V.). That of ZH/Z amounts to ~ − 0·4 V., as triphosphopyridine nucleotide[8] and substances with redox potentials up to − 0·4 V. can be reduced by chloroplasts[9]. Thus an energy equivalent of at least ~ 0·8 V. each must be produced by the two primary processes. Phosphorylation[10] very probably occurs by the energy released between cycles 2 and 3. With reduced triphosphopyridine nucleotide and adenosine triphosphate, carbon dioxide can be reduced to sugar via the Calvin cycle[11]. The reaction pattern is in accordance with the results already reported here and also with the following findings (20° C., *p*H ≈ 7).

(1) On excitation of Chl*a*-670 and Chl*a*-680 in *Chlorella*, the life-time τ of the changes in absorption at

Now available in English Translation

RADIOCHEMISTRY U.S.S.R.

translated from the Russian RADIOKHIMIIA
under the editorship of
A. G. MADDOCK, *Cambridge*

SELECTED CONTENTS OF VOLUME I NO. I—JUST PUBLISHED

A. I. MOSKVIN, G. V. KHALTURIN and A. D. GEL'MAN: Investigation of the complexing of trivalent americium in oxalate and ethylenediamine tetra-acetate solutions by the ion-exchange method.

V. P. SHVEDOV and A. V. STEPANOV: An electromigration method of determination of the stability constants of complex compounds of elements present in microconcentration —I. Determination of the stability constants of complex compounds of some lanthanides with the ethylenediamine tetra-acetic acid anion.

I. E. STARIK, A. D. SHEIDINA and A. I. IL'MENKOVA: The state of microquantities of radioelements in dilute solutions—VII. Study of the state of proactinium in aqueous solution by the adsorption/desorption method.

V. I. SPITSYN and V. V. GROMOV: The problem of the effect of the radioactivity of precipitates on their sorption properties.

D. M. ZIV and V. A. ISHINA: Investigation of the electrochemical deposition of bismuth from its dilute solutions, by means of radioactive indicators I.

L. K. PONOMAREVA and V. L. ZOLOTAVIN: The determination of radioactive strontium in water from open reservoirs.

E. K. GERLING and Ia. A. SHUKOLIUKOV: The composition and content of xenon isotopes in uranium minerals.

K. A. PETRZHAK, E. B. NIKOL'SKAIA, Iu. G. PETROV and E. A. SHLIAMIN: The feasibility of applying to the study of fragmentary isotopes the technique of stopping and collecting fission fragments in a gas—I. Radiochemical examination of the disintegration of fragments using their range as a criterion.

A. N. MURIN and Yu. G. PLINER: The mechanism of formation of anomalous mixed crystals.

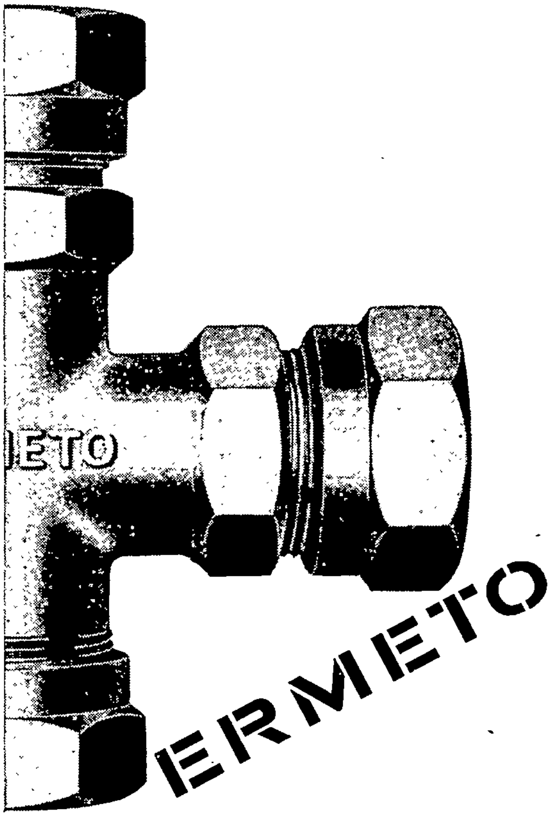
V. B. SHEVCHENKO, A. S. SOLOVKIN, I. V. SHILIN, L. M. KIRILLOV, A. V. RODIONOV and V. V. BALANDINA: The influence of the nature of the diluent on the extraction of uranyl nitrate by tributyl phosphate.

Two issues per annum. Subscription rates: £15 ($40.00) *per annum*

Send for a free inspection copy

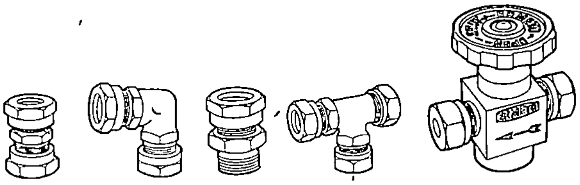
PERGAMON PRESS
OXFORD LONDON NEW YORK PARIS

Headington Hill Hall, Oxford. 4 & 5 *Fitzroy Square, London, W.*1. 122 *East 55th Street, New York* 22, *N.Y.*



The name that counts in High Pressure

Ermeto Engineers have developed the largest range of high pressure fittings in the world. The couplings are made in mild steel, stainless steel, brass, aluminium alloy and high duty bronze. High pressure valves and flexible hose assemblies are also manufactured. A large number of non-standard fittings are produced to meet customers' individual requirements. Our technical staff are always ready to provide further information. The Ermeto Catalogue is an essential reference when designing high pressure circuits. We shall be happy to forward a copy on request.



ERMETO

BRITISH ERMETO CORPORATION LTD

HARGRAVE RD · MAIDENHEAD · BERKS · Tel: MAIDENHEAD 5100

A member of the Alenco Group of Companies

INVERTED BIOLOGICAL MICROSCOPE
M2650
FOR HANGING DROP CULTURES
Cooke Troughton & Simms LTD
YORK ENGLAND

GLASS

All Glass Requirements for Scientific Instrument Manufacturers and Research Workers

- SHEET & PLATE-GLASS—cut and ground to exact dimensions.
- LENSES, PRISMS & FLATS—to any standard of accuracy.
- MIRRORS—back silvered and front surface aluminised, etc.
- BLOOMING of optical components.

C. J. WHILEMS, LTD.
ILFORD OPTICAL WORKS
FOREST ROAD, BARKINGSIDE, ESSEX

Telephone : HAInault 5454-5

420 mμ and 515 mμ are the same ($\tau_{420} = \tau_{515} = 2 \times 10^{-2}$ sec.) because oxidized cytochrome and X react jointly.

(2) On excitation of mainly Chl*a*-680, τ_{420} is greatly prolonged (up to $\tau_{420} \approx 1$ sec.), as only small amounts of X are formed for the reduction of oxidized cytochrome.

(3) In the case of spinach chloroplasts extracted with water, oxidized dyes with a redox potential up to ~ 0 V. can be coupled to X (ref. 4). This is shown by the observation that τ_{515} is greatly shortened (down to $\tau_{515} \approx 10^{-3}$ sec.) as X is oxidized in addition by the dyes.

(4) Vice versa reduced dichlorophenol indophenol (red. In.) can be coupled to Cyt0. In this case τ_{420} is greatly shortened (down to $\tau_{420} \approx 10^{-3}$ sec.) as Cyt0 is reduced additionally by red. In.

(5) Preliminary experiments show that CMU (*p*-chlorophenyl-dimethylurea) uncouples cycle 4 from oxygen production. Heating to 50° C. destroys cycles 3 and 4. Heating to 60° C. destroys cycles 1 and 2. At the same temperature, type 1 chlorophyll reaction ceases[2]. Probably reaction type 1 sensitizes cycles 1 and 2.

The reaction pattern of Fig. 2, derived from experimental observations, is similar to a hypothetical one given by Hill[12].

This investigation was supported by the Deutsche Forschungsgemeinschaft.

Note added in proof. (1) In the meantime the redox potential of cycle 2 has also been estimated experimentally. This was done as in the case of cycle 3 by coupling different substances (red. In., ferrocyanide, etc.) to Cyt0. The result is a potential of $\sim +0{\cdot}4$ V. (2) Prof. H. Kuhn has directed our attention to a publication of Prof. H. Kautsky[13] in which a pattern corresponding with Fig. 2 was derived from changes of fluorescence. The principal differences between both will be discussed in a later publication.

H. T. WITT
A. MÜLLER
B. RUMBERG

Physikalisch-Chemisches Institut,
Universität Marburg/Lahn,
Germany.

[1] Witt, H. T., *Naturwiss.*, **42**, 72 (1955). Witt, H. T., and Moraw, R., *Z. phys. Chem.*, N.F., **20**, 253, 283 (1959). Witt, H. T., and Müller, A., *ibid.*, N.F., **21**, 1 (1959). Witt, H. T., Moraw, R., Müller, A., Rumberg, B., and Zieger, G., *ibid.*, N.F., **23**, 133 (1960); *Z. Elektrochem.*, **64**, 181 (1960).

[2] Zieger, G., Müller, A., and Witt, H. T., *Z. phys. Chem.* (in the press). Moraw, R., and Witt, H. T., *ibid.* (in the press).

[3] Müller, A., and Witt, H. T., *Nature*, **189**, 944 (1961).

[4] Witt, H. T., and Müller, A., *Z. phys. Chem.*, N.F., **21**, 1 (1959).

[5] Bishop, N. I., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1696 (1959).

[6] Emerson, R., Chalmers, C., Cederstrand, C., and Brody, M., *Science*, **123**, 673 (1956). Emerson, R., *ibid.*, **125**, 746 (1957). Emerson, R., Chalmers, C., and Cederstrand, C., *Proc. U.S. Nat. Acad. Sci.*, **43**, 113 (1957). Brody, S. S., *Science*, **128**, 838 (1958); *Nature*, **189**, 547 (1961). Myers, J., and French, C. S., *J. Gen. Physiol.*, **43**, 729 (1960). Emerson, R., and Rabinowitch, E,. *Plant Physiol.*, **35**, 477 (1960). Govindjee, R., and Rabinowitch, E., *Science*, **132**, 355 (1960). Govindjee, R., Thomas, B. B., and Rabinowitch, E., *Science*, **132**, 421 (1960).

[7] Davenport, H. E., and Hill, R., *Proc. Roy. Soc.*, B, **139**, 327 (1952).

[8] San Pietro, A., and Lang, H. M., *Science*, **124**, 118 (1956).

[9] Jagendorf, A. T., and Avron, M., *J. Biol. Chem.*, **231**, 277 (1958).

[10] Arnon, D. I., *Handbuch Pflanzenphysiol.*, **5/1**, 773 (1960).

[11] Bassham, J. A., and Calvin, M., *Handbuch d. Pflanzenphysiol.*, **5/1**, 884 (1960).

[12] Hill, R., and Bendall, F., *Nature*, **186**, 136 (1960).

[13] Kautsky, H., Appel, W., and Amann, H., *Biochem. Z.*, **332**, 277 (1960).

Effect of Spacing and Nitrogen-level on Floral Initiation in Cocksfoot (*Dactylis glomerata* L.)

COCKSFOOT, like many perennial herbage grasses, is a long-day plant[1], and is reported to have an obligate winter requirement for cold and short days[2] or cold alone[3], before floral initiation can occur. It has not been possible to satisfy this requirement artificially by cold or short-day treatment given to germinating seed or young seedlings, (Calder, D. M., and Cooper, J. P., unpublished data) although such treatment has been effective in *Lolium* and the winter cereals[1]. Preliminary observations suggested that spacing of plants and nitrogen supply might have an overriding influence on flowering in cocksfoot and the following experiment was planned to investigate these effects.

Two contrasting varieties, *Bc* 4542, a natural population from Israel, and *S*.143, a late-flowering variety developed at Aberystwyth, were sown outdoors on April 29, 1960. At the third-leaf stage 160 plants of each variety were transplanted into 8-in. pots containing steam-sterilized soil, 80 at the rate of 10 plants per pot and 80 at one plant per pot. These spacing treatments were further divided into high and low nitrogen-levels, the high nitrogen group having Nitro-Chalk added at the rate of 1 gm. per pot every 2 wks., while the low nitrogen-level group had no nitrogen added. There were thus four spacing/nitrogen treatments per variety, each treatment containing ten plants, replicated four times.

Table 1. FREQUENCY OF REPRODUCTIVE PLANTS IN SPACING AND NITROGEN TREATMENTS

Variety	One plant/pot High N	 Low N	Ten plants/pot High N	 Low N
Bc 4542				
Reproductive	35 (5)*	25 (3)	9 (2)	0 (0)
Vegetative	5	15	31	40
S. 143				
Reproductive	15 (4)	6 (4)	0 (0)	1 (0)
Vegetative	25	34	40	39

Variety, spacing and nitrogen differences all significant at $P < 0{\cdot}001$.
* Figure in parentheses denotes number of plants with heads initiated but not emerged.

Table 1 shows the number of plants in each treatment which produced heads by September 26 (150 days after sowing), the number which initiated inflorescences without ear emergence, and the number remaining vegetative. No further initiation would be likely to occur after this date, because of a decreasing photoperiod. Both spacing and nitrogen-level had a marked influence on floral initiation, although the Israel population produced a higher proportion of flowering plants than *S*.143 in practically all treatments. In the denser spacing with low nitrogen, only one plant out of 80 produced heads, while in the wider spacing with high nitrogen 87·5 per cent of the Israel plants and 37·5 per cent of the *S*.143 initiated heads. Although the level of nitrogen applied has not compensated for the more severe competition at the denser spacing, the spacing/nitrogen interaction is not significant, suggesting that nitrogen supplied at greater rates might overcome the effects of plant density.

Little work has been done on the effects of plant spacing on floral development in herbage grasses and cereals, and reports on the effects of nitrogen-level are somewhat conflicting. In winter rye, for example, Gott *et al.*[4] have shown that nitrogen has little or no effect on any stage of the flowering reaction in fully vernalized plants, but in unvernalized material a low nitrogen-level may slightly retard progress to flowering. Von Denffer[5], on the other hand, reports that nitrogen deficiency accelerates flowering in barley and wheat. Leopold[6] states that, in general, nitrogen-level may have a profound influence on stages subsequent to floral initiation, but not on initiation itself. In the present case, both spacing and nitrogen-level have had a pronounced effect on floral initiation in cocksfoot. None of the plants received any cold exposure and the photoperiod was more than 13 hr. throughout the experiment, which indicates either that wide spacing and high nitrogen-level have largely replaced the inductive requirement, or that dense spacing with low nitrogen have inhibited the photoperiodic response.

These results should have important implications for the management of cocksfoot swards for continued leaf production on one hand, or for seed production on the other. They also provide a possible explanation for the findings of Evans[7] on the effects of plant density on seed-yield in a number of herbage grasses.

It is a pleasure to acknowledge the facilities and encouragement given by the director, Prof. P. T. Thomas.

D. M. Calder*
J. P. Cooper

Welsh Plant Breeding Station,
Plas Gogerddan,
Nr. Aberystwyth.

---

* On study leave from Massey Agricultural College, University of New Zealand.

[1] Cooper, J. P., *Herb. Abstr.*, **30**, 71 (1960).
[2] Gardner, F. P., and Loomis, W. E., *Plant Physiol.*, **28**, 201 (1953).
[3] Wycherley, P. R., *Meded. LandbHoogesch. Wageningen*, **52**, 75 (1952).
[4] Gott, M. B., Gregory, F. G., and Purvis, O. N., *Ann. Bot., Lond.*, N.S., **19**, 87 (1955).
[5] Von Denffer, D., *Planta*, **31**, 418 (1940).
[6] Leopold, A. C., *Quart. Rev. Biol.*, **26**, 247 (1951).
[7] Evans, Gwilym, *Welsh Plant Breed. Sta. Ann. Rep.*, 1959, 88 (1960).

Effects of Light Intensity on Reproduction in *S*.48 Timothy (*Phleum pratense* L.)

In recent years the role of light intensity as a major factor controlling growth of herbage in the sward has been emphasized. The production of inflorescences in herbage grasses also appears to be influenced by light intensity in conditions otherwise known to favour the onset of floral initiation. Several investigators[1–3] have remarked on the reduced flowering obtained in the glasshouse in winter, when photoperiod and temperature are artificially maintained at summer-levels. Experiments in the glasshouse have shown that inflorescence production in *S*.48 timothy occurs readily in the spring and early summer, whereas floral initiation is sporadic and delayed when plants are exposed to similar photoperiods and temperatures in the autumn and winter. In an attempt to separate the effect of light intensity, which presumably exerts an indirect effect on flowering by influencing photosynthesis, from that of factors such as photoperiod, which have a direct effect on flowering, an experiment was conducted in summer to test the effect of reducing the natural light intensity on the reproduction of *S*.48 timothy.

In 1958, seed of timothy was sown on damp vermiculite on March 20. A few days after germination the seedlings were transplanted to 3-in. pots of soil maintained under 8-hr. day-lengths in an unheated glasshouse. After the production of 7–8 leaves, the plants were moved outside to soil beds and planted 5 in. apart. Three of the beds were covered with layers of muslin arranged on metal frames to give varying light transmissions; the fourth bed of plants was left uncovered to provide a control. During the first half of the experiment, integrated light readings under the different treatments were obtained with Megatron photometers; the percentage transmissions in terms of natural light decreased in the following manner: (*A*) control 100 per cent, (*B*) 60 per cent, (*C*) 51 per cent and (*D*) 33 per cent. Thermograph recordings showed reduced temperature differentials under the muslin covers compared with the control, but the mean daily temperatures did not differ substantially.

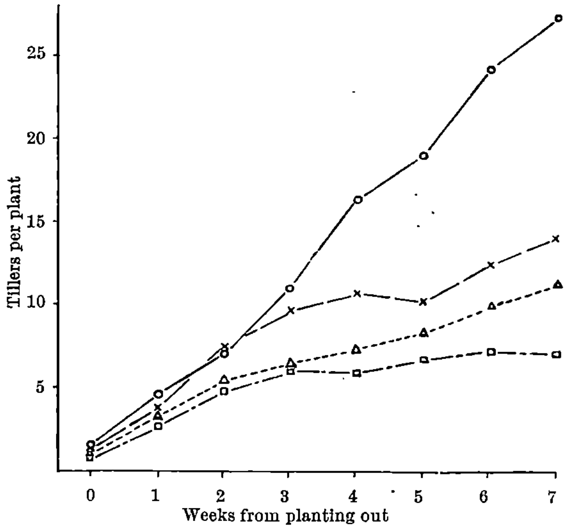


Fig. 1. Influence of light intensity on tillering in *S*.48 timothy. In full daylight, ○; in 60 per cent daylight, ×; in 51 per cent daylight, △; in 33 per cent daylight, □

The influence of light intensity on vegetative growth was assessed from tiller numbers before the ears began to emerge (Fig. 1). Light intensity had a marked effect on tiller numbers, and a significant response began to appear 2–3 weeks after the beginning of the treatments. After 7 weeks the control plants had produced nearly four times as many tillers as comparable plants in the densest shade; and this trend continued to the final harvest (Table 1). Several plants receiving only 33 per cent of the

Table 1. Effects of Light Intensity on Reproduction in *S*.48 Timothy. (Mean of 32 Plants)

Main stem	*A* 100 per cent (full daylight)	*B* 60 per cent	*C* 51 per cent	*D* 33 per cent
Mean No. days to ear-emergence	57·4 ± 0·63	58·0 ± 1·02	59·0 ± 1·05	71·8 ± 2·02
Mean leaf No. to ear-emergence	14·6 ± 0·15	13·9 ± 0·26	14·0 ± 0·23	15·5 ± 0·41
Mean ear-length (cm.)	9·6 ± 0·33	9·2 ± 0·39	8·5 ± 0·34	6·2 ± 0·45
Mean stem-length (cm.)	80·4 ± 1·87	100·1 ± 1·60	101·0 ± 1·52	90·7 ± 1·85
Percentage fertility	100	100	100	67
Whole plant				
Mean tiller No./plant	38·0 ± 2·99	28·4 ± 1·90	24·5 ± 1·86	12·4 ± 0·92
Mean ear No./plant	12·2 ± 1·01	8·4 ± 0·76	6·1 ± 0·52	1·9 ± 0·44
Percentage fertility	32·2	29·6	24·9	15·4

natural light carried only three or four living tillers when the experiment was terminated. The mortality of new tillers was very high in this degree of shading.

Ears began to emerge on the main stems, first on the control and lightly shaded plants and later on the plants in dense shade. The final results for days to energence of the ear (Table 1) showed increasingly delayed emergence which became significant when the light was reduced from 51 to 33 per cent of full daylight. To some extent, this trend was reflected in the mean leaf numbers to ear-emergence, which also increased in the densest shade, but the leaf number in full daylight was exceptionally high. However, at the lowest level of light, the delayed ear-emergence, in those plants that did flower, was accompanied by a complete suppression of reproduction in about a third of the main stems (Table 1). These shoots showed internode elongation similar to that of flowering shoots, but micro-dissections at the final harvest revealed strictly vegetative apices. Length of ear in the flowering shoots also showed the effect of light intensity. In normal light conditions, ears averaged almost 10 cm., whereas, as the shading was intensified, the length decreased progressively to approximately 6 cm. Unpublished evidence from other experiments has revealed a good linear relation between ear-length and total floret number in this variety ; thus a direct relation between light intensity and potential seed yield per shoot is indicated. The mean number of ears per plant decreased to one-sixth as the light intensity fell from full daylight to dense shade, with the result that the percentage of fertile tillers per plant was also markedly influenced.

These results show that reduced light intensity appreciably affects both vegetative and reproductive growth in *S*.48 timothy. The total daily radiation received by plants in the densest shade increased from 80 to more than 100 cal. cm.$^{-2}$ throughout the period of the experiment, an intensity frequently exceeding that found in the glasshouse during autumn and winter. Similarly, the light intensity near ground-level in developing herbage stands falls in spring to less than 20 per cent of full daylight[4,5], which would suggest that a proportion of the shoots may not receive sufficient light for inflorescence initiation. This may in part account for the elongated, sterile tillers which are observed frequently in timothy seed crops.

G. J. A. Ryle

Grassland Research Institute,
Hurley, Berkshire.

[1] Benedict, H. M., *J. Agric. Res.*, 61, 661 (1940).
[2] Cooper, J. P., *J. Brit. Grassl. Soc.*, 13, 81 (1958).
[3] Roberts, R. H., and Struckmeyer, B. E., *J. Agric. Res.*, 59, 699 (1939).
[4] Black, J. N., *Herb. Abstr.*, 27, 89 (1959).
[5] Mitchell, K. J., and Calder, D. M., *N.Z. J. Agric. Res.*, 1, 61 (1598).

Artificial Production of Buds fom the Embryos of *Cuscuta reflexa*

This communication forms part of an extensive programme on the *in vitro* culture of embryos and ovules. The observations are of interest in that the parent embryo produced additional 'adventive' embryos.

Embryos of *Cuscuta reflexa* (0·5–2·5 mm. long) were cultured on a modified White's medium containing minerals + sucrose (5 per cent) + casein hydrolysate (400 mgm./l.) + indole-3-acetic acid (1 mgm./l.).

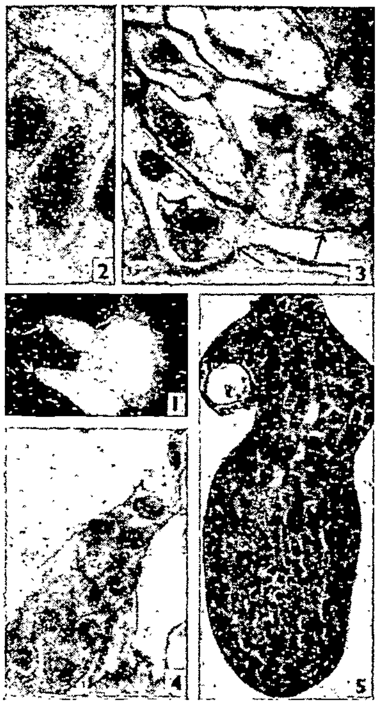


Fig. 1. Fifty-day-old culture of zygotic embryo showing adventive embryos at the radicular end (arrows); note the narrow apical end above. (× *c*. 5·3)

Figs. 2 and 3. Portions enlarged from a transection of the 'radicular' end of zygotic embryo, 40 days after inoculation, showing a dividing cell and two-celled proembryo (arrow-marked) respectively. (× *c*. 233)

Figs. 4 and 5. Further stages in the development of adventive embryos. (Fig. 4, × *c*. 170; Fig. 5, × *c*. 100)

When relatively small embryos (0·5–1·0 mm. long) were taken, they did not pass through the normal stages of development but swelled considerably and ultimately became transformed into a mass of callus. Small, whitish or yellowish-green bodies differentiated from this mass within six weeks of inoculation. They strongly resembled normal embryos. When larger embryos (1·0–2·5 mm. long) were cultured, they produced seedlings within a month. However, callus developed at certain points from the 'radicular' end, and, as in the cultures of smaller embryo, adventive embryos differentiated either from the callus or directly from the superficial cells (Fig. 1). Sometimes 10–15 such embryos were produced in a single culture.

Externally these embryos of adventive origin resembled normal embryos in possessing a narrow apical end and a broader 'radicular' end (Fig. 1). During their development, some of the epidermal cells of the original embryo became large and meristematic and underwent a transverse division (Fig. 2) to form 2-celled proembryos (Fig. 3). Further irregular divisions resulted in a globular structure looking like an embryo (Fig. 4). As in Nature[1], the embryos, instead of passing into a heart-shaped stage, showed vertical elongation (Fig. 5) and ultimately the distal end differentiated into the shoot apex.

The formation of vegetative buds has been previously reported from the callus of tobacco and carrot[2–4] and the embryos of some orchids[5]. Reinert has emphasized the close resemblance of the vegetative buds arising from the callus of carrot tissue with adventive embryos. In the present work it has been shown that in their development these adventive structures pass through many important stages fully resembling those in the normal development of the embryo.

Some of the adventive embryos proceeded to form a normal shoot, but the majority callused if left in the same medium. This callus is capable of continuous growth and produces a fresh crop of adventive embryos, thus providing an unlimited source of similar material.

P. MAHESHWARI
B. BALDEV

Department of Botany,
University of Delhi,
Delhi, 6.

[1] Johri, B. M., and Tiagi, B., *Phytomorph.*, **2**, 162 (1952).
[2] Skoog, F., and Miller, C. O., *Symp. Soc. Exp. Biol.*, **11**, 118 (1957).
[3] Steward, F. C., Mapes, Marion, and Smith, Joan, *Amer. J. Bot.*, **45**, 693 (1958).
[4] Reinert, J., *Planta*, **53**, 318 (1959).
[5] Curtis, J. T., and Nichol, M. A., *Bull. Torrey Bot. Club*, **75**, 358 (1948).

Transformation in Plants of Sodium Dimethyldithiocarbamate into other Fungitoxic Compounds

IN investigations concerning plant chemotherapy it was demonstrated that the fungicide sodium dimethyldithiocarbamate (NaDDC), having been taken up by cucumber seedlings (*Cucumis sativus* L.), has some chemotherapeutic activity against *Cladosporium cucumerinum*[1]. In work on the uptake and translocation of NaDDC by these plants I have found that dimethyldithiocarbamate could not be recovered from the tissues. Instead the presence could be demonstrated of three unknown fungitoxic compounds which have the same mode of action as NaDDC, but differ in chemical properties. These observations show that, in the plant, NaDDC is subject to certain transformations which most probably are of a biochemical nature.

I have found that the addition of plant sap to NaDDC strongly reduces its fungitoxicity. For this reason small amounts of NaDDC in plant sap ($\leqslant 20$ p.p.m.) cannot be assessed by a direct determination of the fungitoxicity of the sap. If, however, a solution of NaDDC (10 p.p.m.) in cucumber sap is chromatographed, the presence of a fungicide can be detected with an R_F value of 0·87. To this end the solvent propanol/water (85 : 15) is allowed to run down the paper for 16 hr. at 22° C. The chromatogram is developed according to Weltzien[2], by spraying with a conidial suspension and subsequent incubation. Highly satisfactory chromatograms were obtained by adding an equal volume of ethanol (96 per cent) to the sap and placing 0·03 ml. or a higher quantity of the resulting supernatant on 1 cm. of the starting line of Whatman paper No. 3 ; the strips were developed by placing them on a metal frame stringed with nylon threads and subsequent spraying with a conidial suspension of *Glomerella cingulata* in a glucose-mineral salts medium (10^7 conidia/ml.). In this medium *G. cingulata* is highly sensitive to dialkyldithiocarbamates[3]. The strips were incubated for two days in a moist glass box at 27° C.

If a chromatogram is made of NaDDC dissolved in water (Fig. 1*a*) instead of in plant sap, a fungicide is detected at the same spot (R_F 0·87). Yet, in neither of these cases does the spot contain NaDDC. Instead the presence of its copper salt, $((\mathrm{CH_3})_2\mathrm{N}\underset{\overset{\|}{\mathrm{S}}}{\mathrm{C}}\mathrm{S})_2\mathrm{Cu}$, could be demonstrated spectrophotometrically by Dr. M. J. Janssen. Apparently, under the present conditions, NaDDC forms a chelate with copper from the paper.

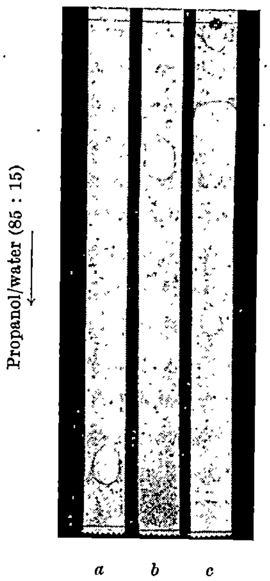


Fig. 1. Paper chromatograms of *a*, NaDDC dissolved in water (0·03 ml. of 33 p.p.m.); *b*, 1 : 1 alcoholic solution of sap from hypocotyls and cotyledons of cucumber seedlings after uptake of NaDDC by the roots (0·03 ml.); *c*, the same solution as in *b* (1·2 ml.)

Without previous chromatography the presence of small amounts of NaDDC in plant sap cannot be revealed on paper ; this suggests that the sap contains antagonists of NaDDC which are separated from the fungicide by the chromatographic procedure.

Once this method of detecting NaDDC in plant sap had been developed, I chromatographed the sap of cotyledons and hypocotyls of ten cucumber seedlings which had been standing for 2 days with their roots in 50 ml. of a 150 p.p.m. solution of NaDDC. Development of the strip, to our surprise, revealed a fungitoxic compound with an R_F value of 0·27 (Fig. 1*b*) while no fungicide could be detected with an R_F value of 0·87. If, moreover, the sap was concentrated about forty times, two other fungitoxic compounds (R_F values 0·03 and 0·58) were visible as well (Fig. 1*c*).

All three fungicidal compounds have the same mode of action as dimethyldithiocarbamate, since they could be counteracted by the addition of histidine or dibutyldithiocarbamate[4] to the nutrient medium used for spraying the strips. I therefore must assume that the three unknown compounds are derivatives of dimethyldithiocarbamic acid, which most probably have been produced by mediation of plant enzymes.

This transformation of NaDDC into other compounds which exert fungitoxic activity was also found to occur in broad bean plants (*Vicia faba* L.) and in chicory (*Cichorium intybus* L.).

It seems possible that biochemical transformation products of NaDDC in the plant are responsible for the observed chemotherapeutic effect.

I thank Dr. A. Kaars Sijpesteijn for many helpful suggestions. This work was carried out as part of the programme of the T.N.O. Research Unit for Internal Chemotherapy of Plants, at the Laboratory of Phytopathology, Wageningen, The Netherlands.

H. M. Dekhuijzen

Institute for Organic Chemistry,
T.N.O., Utrecht,
The Netherlands.

[1] Pluijgers, C. W., dissertation, Utrecht (1959).

[2] Weltzien, H. C., *Naturwiss.*, 45, 288 (1958).

[3] Sijpesteijn, A. Kaars, and Janssen, M. J., *Nature*, 182, 1313 (1958).

[4] Sijpesteijn, A. Kaars, and Janssen, M. J., Ant. v. Leeuwenhoek, *J. Microbiol. and Ser.*, 25, 422 (1959).

ENTOMOLOGY

Induced Changes in the Amounts of Material in the Neurosecretory System of the Desert Locust

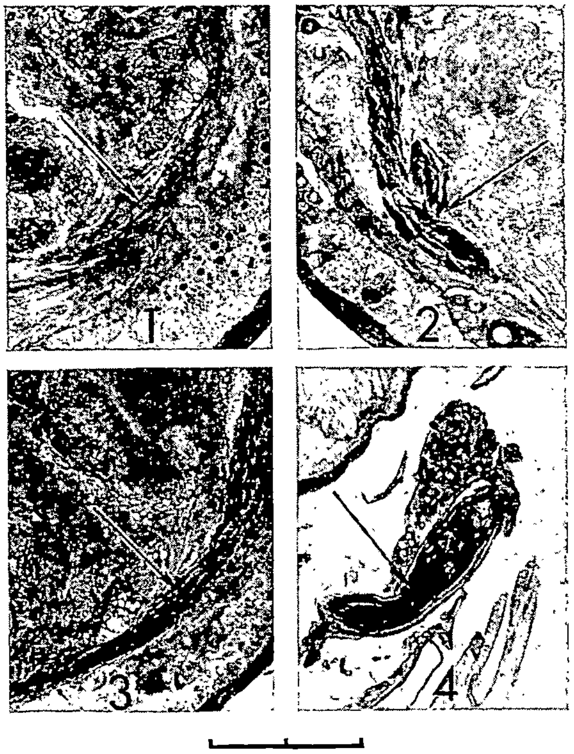
Mature adult female *Schistocerca gregaria* contain large amounts of neurosecretory material in the corpora cardiaca, the nervi corporis cardiaci I and the neurosecretory cells of the pars intercerebralis of the protocerebrum[1]. The neurosecretory systems of 2-week-old females, reared without males, similarly contain large amounts of stainable material; in these females, the terminal oocytes are largely undeveloped.

Forty-five minutes after administering high-frequency electrical shocks (10 V., 40 pulses/sec. for periods of 15 min. or longer) through the optic nerves of 2-week-old females, reared without males, the neurosecretory systems are almost devoid of stainable material (Fig. 1). Low-frequency electrical shocks (10 V., 40–75 pulses/min. for 15–30 min.) administered through the ventral nerve cord, the surface of the brain or through the optic nerves, result in the appearance of a very large excess of material along the nervi corporis cardiaci I (Fig. 2) and within the storage regions of the corpora cardiaca, compared with the operated controls (Fig. 3).

These effects of high- and low-frequency electrical stimulation are not necessarily inconsistent. It is probable that both kinds of stimulation accelerate movement of neurosecretory material along the nervi corporis cardiaci I and into the corpora cardiaca (and presumably into the blood). Low-frequency stimulation illustrates the early stages of the process; high-frequency stimulation the final stages.

Depletion of material in the neurosecretory systems of 2-week-old females, reared without males, also results when the insects are rotated in a flask for 45 min. in such a way that they are continually turned upside down and continually struggle to regain their normal orientation. Hodgson and Geldiay[2] record a diminution in amount of neurosecretory material in the corpora cardiaca of *Blaberus craniifer* following similar electrical stimulation and enforced activity.

Adult females killed during their first observed copulation reveal amounts of neurosecretory material along the nervi corporis cardiaci I (Fig. 4) and within the corpora cardiaca which are remarkably similar to the amounts along the nerves and in the glands of females after low-frequency electrical stimulation. Twelve hours after copulation, the material in the neurosecretory system is in small amount, resembling the condition following high-frequency electrical stimulation and enforced activity. It is clear that in this respect electrical stimulation and enforced activity mimic the effect of the natural stimulation of copulation.



Figs. 1–4. Photomicrographs of brain sections of *S. gregaria* after various treatments. Heads fixed in Bouin's fluid and sections stained with paraldehyde–fuchsin following permanganate oxidation. Arrows point to the nervi corporis cardiaci I in each figure; neurosecretory material appears black. 1, Nervi corporis cardiaci I in brain of female after 15 min. of high-frequency electrical shocks through the optic nerves; amount of neurosecretory material negligible. 2, Nervi corporis cardiaci I in brain of female after 15 min. of low-frequency electrical shocks through the optic nerves; very large amount of neurosecretory material present. 3, Nervi corporis cardiaci I in brain of female after electrodes had been in position in the optic nerves for 15 min., but the animal not stimulated (operated control); amount of neurosecretory material intermediate between 1 and 2. 4, Nervi corporis cardiaci I just beneath the brain; female killed *in copula*; very large amount of neurosecretory material present

Norris[3] has shown that copulation in *S. gregaria* is normally necessary for the development of eggs to their full size, preceding oviposition. Further, neurosecretory cells are necessary for egg development in this species[1]. The present observations suggest that copulation in *S. gregaria* makes available larger amounts of neurosecretory material for the full development of the eggs.

It should, therefore, be possible to simulate the effect of copulation on egg development by subjecting virgin females to electrical shocks or enforced activity. The results of electrical stimulation are inconclusive, since, although the terminal oocytes develop rapidly following the treatment, so also do the terminal oocytes of the operated controls. It is known that injury can accelerate maturation in the desert locust[3]. Enforced activity in 14-day-old females reared without males produces changes in the amount of material in the neurosecretory system similar to electrical stimulation, but the insects are not injured in the process. Fourteen 14-day-old females, reared without males, were subjected to enforced activity for 1 hr./day for 3 days. Dissected seven days later, the terminal oocytes measured 5·56 ± 0·64 mm.; 10 control females placed in the flask but not rotated possessed terminal oocytes 2·69 ± 0·57 mm. long seven days later. This result supports the hypothesis that the availability of neurosecretory material is important for egg development.

Hodgson and Geldiay[2] have suggested that the loss of adrenalin-like activity of extracts of corpora cardiaca of *Blaberus craniifer* following electrical stimulation or enforced activity should be correlated with the disappearance of neurosecretory material from the glands. It is difficult to reconcile this view with Cameron's observation[4] that the corpora cardiaca of *Periplaneta americana* yielded extracts with adrenalin-like activity, even after the nervi corporis cardiaci had been cut some time previously. The present results suggest that the release of neurosecretory material from the corpora cardiaca of *S. gregaria* has a positive effect on oocyte development; experiments in progress indicate that an adrenalin-like substance is produced from the *glandular* parts of the corpora cardiaca in *S. gregaria*.

I am grateful to the Anti-Locust Research Centre, London, for providing the locusts used in this work; and to Mr. W. Mosley for making the photomicrographs.

K. C. Highnam

Department of Zoology,
The University,
Sheffield, 10.

[1] Highnam, K. C., *Quart. J. Micro. Sci.*, 102, 27 (1960).

[2] Hodgson, E. S., and Geldiay, S., *Biol. Bull. (Woods Hole)*, 117, 275 (1959).

[3] Norris, M. J., *Anti-Locust Bull.* No. 18 (1954).

[4] Cameron, M. L., *Nature*, 172, 349 (1953).

PSYCHIATRY

Spike and Wave Discharges and Alterations of Conscious Awareness

During a study of the mental concomitants of electroencephalographic changes in human beings suffering from petit mal, it was observed that changes in level of awareness did not coincide with the onset and cessation of surface spike and wave activity.

The minimal clinical signs of petit mal attacks are usually accompanied by the most obvious and dramatic electroencephalographic evidence of surface electrical changes, yet no other evidence of pathology in the nervous system has so far been discovered. The clinical accounts of the condition include all degrees of interruptions of conscious awareness with variations in form and frequency of the spike and wave activity. Our work was restricted to patients exhibiting typical clinical petit mal, with the classical bilaterally synchronous 3/sec. spike and wave activity seen on the electroencephalogram usually lasting 3–30 sec.

Eight patients were examined during the simultaneous recording of the electroencephalogram, certain peripheral autonomic changes and the interview. In all, 1,075 petit mal attacks were clinically observed. Five cases were recorded for a total of 31¼ hr. during which 91 attacks were recorded: in addition 3 cases were studied intensively for a total of 97½ hr. during which 684 attacks were recorded, and this latter group formed the main material for this work. Particular attention was paid to alterations in conscious awareness correlating with episodes of spike and wave activity of more than 3 sec. duration. The investigation of mental state was by question and answer, so that the highest level of brain function was involved since an appreciation of meaning was necessary for a sensible answer.

The onset of spike and wave activity varied greatly as to amplitude, lateral predominance and time of recruitment to full amplitude (Fig. 1).

During period *A*, when the spike and wave activity had reached full amplitude, the patient was capable of speaking and answering questions, and during or following period *D* could similarly recall events; the cessation of speech did not coincide consistently with any change in the electrical records.

Questions given in period *C* could produce the response 'Mmmm ?' during this period, or the answer to the question during or following period *D*. Any movement by the patient during this period could be recalled after the attack.

In period *D*, when spike and wave activity had ceased, there was a variable delay before reply, but on other occasions an immediate response was forthcoming. There was often an increase in pulse-rate about 1½ sec. after the end of spike and wave activity.

In period *B*, the patients were unable to respond to questions or to recall afterwards events during it. They were capable of a variety of movements and changes in facial expression (for example, smiling), and very frequently in two patients it was noticeable that, although they often turned their heads to one side, they could still return their gaze to look at the observer as spike and wave continued even when he changed his usual position from one side of the bed to the other before or after the attack had started.

It appeared from the above that the patients could speak for 3 sec. after the onset of an attack. They could perceive speech for 1½ sec. before the end of spike and wave activity, since they could answer the question or on occasion reply 'Mmmm ?' after the end of the attack. It also appeared that the ability to speak was sometimes delayed for some seconds after the spike and wave activity had ceased. It was observed that changes in expression of the eyes occurred before and after the end of spike and wave activity.

Alterations in pulse-rate, psychogalvanic skin response and respiration rarely correlated during the incidence of spike and wave activity. In one patient changes in pulse-rate were clearly seen; occasionally there was an increase in rate which usually occurred about 1½ sec. after the end of spike and wave; but it could also occur some seconds before or after the end of the attack. Changes before the onset of the attack were inconsistent and were complicated by sinus arrhythmia.

In other circumstances it has been demonstrated that electro-physiological changes may not be coincident with changes in conscious awareness[1,2]. Bates[3] also has demonstrated that patients can talk up to 3 sec. after the onset of electroencephalographic spike

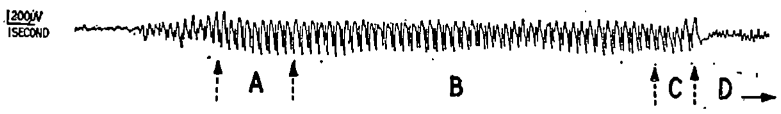


Fig. 1. Diagrammatic representation of attack.
Interval *A*, first 3 sec. of full-amplitude bilaterally synchronous spike and wave activity; *B*, duration of the attack excluding the last 1½ sec. of spike and wave that occur before any final slow wave; *C*, last 1½ sec. of spike and wave before any final slow wave; *D*, end of spike and wave and an arbitrary 10-sec. period

and wave activity; A. Jus (personal communication) using different methods has found that the cessation of spike and wave pattern does not coincide with the return to consciousness. Since the attempt in animal experiments is to simulate the clinical characteristics of human petit mal, it is important, as Jasper[4] has also urged, that a close examination be made of them. It may be worth while to study complex changes in psychological function in circumstances such as at operation when electrocorticograms and depth recordings are made.

These observations lead one to think of a common underlying process which variously affects consciousness and the cerebral electrical activity as recorded from the scalp. It has been shown that more refined techniques are necessary for the investigation and study of subtle alterations of conscious awareness. The causal connexion between spike and wave activity and alterations in consciousness deserves special investigation, since it appears that gross electroencephalographic changes are related to mental changes but that the points in time at which these events occur do not correlate.

L. Goldie[*]
J. M. Green

Institute of Psychiatry,
Maudsley Hospital,
Denmark Hill,
London, S.E.5.

[*] Supported by a Ford Foundation Grant.

[1] Goldie, L., and Green, J. M., *Nature*, **187**, 952 (1960).
[2] Goldie, L., and Green, J. M., *Nature*, **189**, 581 (1961).
[3] Bates, J., *EEG. Clin. Neurophysiol.*, **5**, 445 (1953).
[4] Jasper, H., and Droogleever-Fortuyn, J., in *Res. Pub. Assoc. Nerv. Ment. Dis.*, **26**, 272 (1947).

Distribution of Piperidine in the Brain and its Possible Significance in Behaviour

Recent work on the use of piperidine for treating psychiatric disorders[1] has prompted an extensive programme here to investigate the mechanism of action of this simple chemical. The basis for using piperidine as a psychotherapeutic agent originally developed from the discovery that the agent antagonized the central actions of N-methyl 3-piperidylglycolate and other piperidinoglycolates possessing psychotomimetic properties[2]. These findings were particularly significant because piperidine has been found in human urine[3] and mammalian brains[4]. Piperidine might be an endogenous agent serving some normal function in the regulation of behaviour; that is, perhaps the amine is an endogenous psychotropic agent.

How much piperidine actually enters the brain after the agent is administered in an effective dose would be significant. Experiments were therefore conducted in rabbits, using tritium-labelled piperidine. The material was made by reducing pyridine with tritium, using palladium as a catalyst. After repeated recrystallization of the hydrochloride salt, the final material had a specific activity of 285 μc./mgm. Adult male albino rabbits were injected intravenously with 100 mgm./kgm. of piperidine hydrochloride per kgm. body-weight. This dose produced marked muscle relaxation and sedation in rabbits. After 30 min., the rabbits were bled by carotid cannulation, and the brain was perfused with saline until all trace of jugular blood was removed. The brain was carefully removed and dissected. After being weighed in the frozen state, the brain segments were suspended in 20 volumes of 5 per cent formalin for a period of 24 hr. at room temperature, a procedure extracting over 99 per cent of the piperidine (the formalin extract was filtered and dried in an oven at 90°). Further preparation of the dried residue for counting in a liquid scintillation counter is described in ref. 5.

The greatest concentration of piperidine occurred in the caudate nucleus while other regions in the diencephalon contained much less than half the concentration of the caudate (Table 1). Within the telencephalon, the greatest concentration appeared in the anterior neocortex, and although the lowest concentration occurred in the white matter, the concentration in the corpus callosum was relatively high. Next to the caudate, the quadrigemmina contained the greatest concentration of piperidine. Distribution of piperidine into the various segments of the diencephalon and mesencephalon was fairly uniform. With the exception of the telencephalon, the distribution of piperidine between grey and white matter was surprisingly similar.

At present, it is not possible to relate the distribution of piperidine to its action on the central nervous system, although a few general remarks can be made. Preliminary findings indicate that the electrophysiological effects of the piperidinoglycolates in regions such as the reticular formation and caudate nucleus can be reversed by piperidine, which is a weak nicotinic agent. It remains to be seen whether the muscle relaxation and weakness, ataxia, and the generalized decrease in motor responsiveness produced by piperidine in rabbits can be attributed to the distribution of the drug in such regions as the anterior neocortex and caudate nucleus.

In view of the fact that piperidine is normally present in mammalian brain, the question arises as to its possible significance in normal brain function. Honegger and Honegger[4] have reported the concentration of about 0·05 μgm. of piperidine per gm. of rabbit brain, which is approximately one-tenth the concentration present when 100 mgm./kgm. of the drug is administered (Table 1). When one considers that piperidine normally may be localized in certain brain regions, and that it appears to be excreted rapidly in quite large amounts (20 mgm./day in

Table 1

Vesicle	Derivative	Counts/mgm.
Telencephalon	anterior neocortex	6·6
	posterior neocortex	5·3
	pyriform cortex	3·5
	hippocampus	3·1
	white matter	1·8
	corpus callosum	3·2
Diencephalon	corpus striatum	2·5
	caudate nucleus	10·8
	lateral thalamus	1·7
	medial thalamus	3·1
	hypothalamus	4·8
Mesencephalon	tegmentum	3·3
	foot of mid-brain	3·2
Rhombencephalon	cerebellum	3·8
	pons	3·5
	medulla	3·6
	quadrigemmina	7·5

Localization of tritium-labelled piperidine in various regions of rabbit brain. Radioactivity is expressed in terms of counts/min./mgm. wet weight tissue. Since the ratio unlabelled piperidine/tritiated piperidine = 100, and since the specific activity of the tritiated material is 285 μc./mgm., 1 μgm. of piperidine is equal to approximately 3,000 c.p.m. The total amount of piperidine in a rabbit brain weighing 8 gm. would then be approximately 5 μgm. 30 min. after injection.

human beings)[3], it is quite conceivable that the endogenous piperidine may be influencing behaviour. Von Euler[3] had speculated that, because of its nicotinic property, piperidine may be regarded as an endogenous 'synaptotropic substance'. An important problem is to determine whether piperidine is synthesized within the body or arises from some exogenous source. In plants, piperidine and other cycloalkylamines are derived from amino-acids such as lysine, ornithine and proline[6]; but there is no evidence that such biosynthetic pathways can occur in mammalian systems. The presence of piperidine and other volatile amines in the central nervous system[4] nevertheless suggests the existence of many endogenous agents capable of influencing or regulating neural function. One exogenous source of piperidine is directly through the intestinal flora which can decarboxylate lysine to cadaverine, while the latter product is de-aminated and cyclized to form piperidine.

This work was supported by grants from the Mental Health Fund, State of Illinois, and the Teagle Foundation.

We are indebted to Abbott Laboratories for preparing the tritiated piperidine.

L. G. ABOOD
F. RINALDI
VIRGINIA EAGLETON

Departments of Psychiatry and
Biochemistry,
University of Illinois College of
Medicine,
and
Abbott Laboratories,
North Chicago.

[1] Tasher, D. C., Abood, L. G., Gibbs, F. A., and Gibbs, E., *J. Neuropsychiat.*, **1**, 266 (1960).
[2] Abood, L. G., Ostfeld, A., and Biel, J. H., *Arch. int. de Pharmacodyn.*, **120**, 186 (1959).
[3] Von Euler, U. S., *Acta Pharmacol.*, **1**, 29 (1945).
[4] Honegger, C. G., and Honegger, R., *Nature*, **185**, 530 (1960).
[5] Abood, L. G., and Rinaldi, F., *Psychopharmacologia*, **1**, 117 (1959).
[6] Dawson, R. F., *Amer. Scientist*, **48**, 321 (1960).

STATISTICS

Nodal Analysis of Associated Populations

A METHOD of subdividing an associated population the individuals of which are specified by the presence or absence of a number of attributes has been described previously[1]; the process is ultimately derived from the R-techniques of factor analysis, and the groups of individuals so obtained will be called R-groups. The attributes may similarly be subdivided into Q-groups by the corresponding Q-techniques. There results a 2-way table in which every attribute-individual record is referred to one of each kind of group. The requirement is to extract from this table the most concentrated groups of records, the attribute-individual noda around which the population may be regarded as varying. The problem is essentially that of establishing coincidences between R and Q factors; even in normal factor analysis the coincidences are impaired in the formation of the correlation matrix[2,3], and in a subdivision process involving successive correlation matrices the divergence may well be considerable.

Despite many attempts, we have found no way of operating directly on the 2-way table, and have therefore devised a method which involves finding the best possible coincidences with each direction taken as reference direction in turn, followed by collation of the two sets of results so obtained. The population is first R-divided, producing a group of individuals defined by the possession of the attribute a with the highest loading on the first centroid R-axis. The same population is then Q-divided; that individual (i') which, of those possessing a, has the highest loading on the first centroid Q-axis is used to delimit the group in the other direction. The residual group is similarly delimited by the individual with the highest loading among those not possessing a. Each resulting group we call an RQ-group. The division is continued to the significance-level required, resulting in a series of R-groups, with each of which is associated its corresponding RQ-group. It will normally be desirable to set a lowest acceptable limit for the loading of i'; if no individual attains this level, RQ is set $\equiv R$. The transpose of the entire process similarly gives a series of Q-groups together with their corresponding QR-groups.

Collation consists in taking one group from each side and subjecting the pair to a logical 'and' operation, whereby only those records common to both tables are retained. The conditions for collation are obtained from the singly defined (Q- and R-) tables. If any R-individual possesses all the Q-attributes and any Q-attribute occurs in all the R-individuals, QR is collated with RQ; the result is a nodum. If any R-individual possesses all the Q-attributes, but no Q-attribute occurs in all the R-individuals, QR is collated with R; the result is an R-subnodum. The transposed situation will give a Q-subnodum. If neither condition is fulfilled, all records common to Q and R are discarded. In the unlikely, but perhaps not impossible, case where collation is permissible but the nodum vanishes, the coincidence may be treated as intersecting subnoda.

The noda are extremely concentrated, and contain all the genuinely central information at the significance-level in use. They are not, however, necessarily all the same type. All are defined in the form $(a'i')$; but a nodum may also contain a and/or i (the attributes and individuals which define the non-residual R and Q groups), and the presence of these may be regarded as conferring a rise in status on the nodum. The subnoda constitute a penumbra of diffuse groups, some at least of which would become noda were the subdivision to be carried further on one side or the other by lowering the significance-level; they are always defined as (a') or (i'), but their status can rise to $(a'\ i)$ or $(i'\ a)$.

Our existing *Pegasus* subdivision-programme limits the analysis to a 76 × 76 population matrix. As yet, we have applied the method only to ecological data, but with very promising results. Whereas our earlier R-subdivisions[4,5] gave only groupings of quadrats on species-attributes, the 2-way tables provide a double abstraction in terms of both plant and habitat: the doubly defined noda thus represent true 'vegetation-units' in the widest sense.

W. T. WILLIAMS
J. M. LAMBERT

Botany Department,
University of Southampton.

[1] Williams, W. T., and Lance, G. N., *Nature*, **182**, 1755 (1958).
[2] Slater, P., *Nature*, **181**, 1225 (1958).
[3] Cattell, R. B., *Factor Analysis*, 90 (Harper and Bros., New York, 1952).
[4] Williams, W. T., and Lambert, J. M., *J. Ecol.*, **47**, 83 (1959).
[5] Williams, W. T., and Lambert, J. M., *J. Ecol.*, **48**, 689 (1960).

FORTHCOMING EVENTS

Monday, July 10—Friday, July 14

INTERNATIONAL COMMITTEE OF DIETICIANS, and the BRITISH DIETETIC ASSOCIATION (at Church House, Westminster, London, S.W.1)—Third International Congress of Dietetics. Theme: "Tradition, Science and Practice in Dietetics".

Tuesday, July 11—Thursday, July 13

ASSOCIATION FOR THE STUDY OF ANIMAL BEHAVIOUR (in the Department of Zoology, University of Cambridge), at 10 a.m. daily—Symposium on "Orientation and Migration"; and Annual General Meeting.

Saturday, July 15—Sunday, July 16

BRITISH ACADEMY OF FORENSIC SCIENCES (at the Londoner Hotel, Welbeck Street, London, W.1)—Meeting on "Crime-Responsibility, Investigation and Treatment". (Second Annual Scientific Meeting.)

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

PRINCIPAL LECTURER (preferably with teaching and industrial experience) IN THE DEPARTMENT OF MATHEMATICS—The Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff (July 15).

LECTURER or ASSISTANT LECTURER IN MATHEMATICS at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 17).

POSTDOCTORAL FELLOW IN ORGANIC CHEMISTRY, to carry out research in the carotenoid field in collaboration with Prof. B. C. L. Weedon—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (July 17).

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER (with a first- or second-class honours degree in engineering or equivalent, and preferably an interest in stress analysis or the theory of elasticity), for research on wood machining processes and fundamental studies of the theory of cutting—Forest Products Research Laboratory, D.S.I.R., Princes Risborough, Aylesbury, Bucks, quoting Ref. FPRL 3W (July 17).

ASSISTANT BIOCHEMIST (with a degree or equivalent qualification in chemistry, and experience in, or wishing to take up clinical biochemistry)—The Secretary, Welsh National School of Medicine (University of Wales), 34 Newport Road, Cardiff (July 22).

ASSISTANT LIBRARIAN (Grade B) (with a good honours degree) in the University Library, to be Sub-Librarian for the Faculties of Medicine and Veterinary Science—The Registrar, The University, Liverpool, quoting Ref. CV/N (July 22).

RESEARCH ASSISTANT (graduate in botany or graduating this year) IN THE DEPARTMENT OF BOTANY, to assist the Professor in the establishment and maintenance of a collection of plant organ and tissue cultures and in research involving these cultures—The Registrar, University College, Singleton Park, Swansea (July 22).

RESEARCH FELLOWS and RESEARCH ASSISTANTS (with a good honours degree or equivalent in engineering, mathematics, physics or metallurgy) IN THE DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS—The Secretary and Registrar, The University, Southampton (July 22).

ASSISTANT LECTURER IN THEORETICAL PHYSICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (July 24).

RESEARCH ASSISTANT (with a good degree in either physics with geology or geology with physics) IN THE SEDIMENTARY RESEARCH LABORATORY, to assist in establishing and operating an X-ray unit, and to undertake research on the application of X-ray techniques to the mineralogy of sediments—The Bursar, The University, Reading (July 24).

LECTURER IN MECHANICAL ENGINEERING—The Registrar, The University, Sheffield (July 29).

AGRICULTURAL CHEMIST, Scientific Officer or Senior Scientific Officer grade (with an honours degree and experience in research on soils and fertilizers), at Broom's Barn Experimental Station, Barrow, West Suffolk, to work on manuring of sugar beet—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (July 31).

LECTURER or ASSISTANT LECTURER IN ORGANIC CHEMISTRY—The Registrar, The University, Manchester 13 (July 31).

LECTURER (preferably with an interest in plant ecology) IN BOTANY at the University of Natal, Pietermaritzburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, July 31).

LECTURERS or ASSISTANT LECTURERS (3) (with a recognized honours degree or equivalent qualification, and preferably with experience in teaching and/or research) IN THE DEPARTMENT OF MATHEMATICS, University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Malaya and London, July 31).

POSTGRADUATE SCHOLAR (suitably qualified in zoology or physiology) at the Gatty Marine Laboratory, St. Andrews, for work on the analysis of nervous systems of lower animals—Joint Clerk to the University Court, College Gate, St. Andrews (July 31).

SENIOR LECTURER or LECTURER (veterinary surgeon) IN VETERINARY MEDICINE, for duty at the field station near Potters Bar, Middlesex—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (July 31).

LECTURER/SENIOR LECTURER IN CLASSICAL ARCHÆOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 11).

CHAIRS (2) OF MATHEMATICS at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, August 14).

CHAIR OF PHYSIOLOGY in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 18).

READER IN ELECTRIC TRACTION at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 21).

LECTURER (with an honours degree in zoology and preferably qualified to take courses in vertebrate embryology) IN ZOOLOGY at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, August 30).

LECTURER IN PHILOSOPHY at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, August 31).

WAITE CHAIR OF ENTOMOLOGY in the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, September 15).

LECTURER or ASSISTANT LECTURER IN PSYCHOLOGY at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, September 30).

ASSISTANT (Grade B) IN THE DEPARTMENT OF MATHEMATICS—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

ASSISTANT (man or woman) TO TEACH MATHEMATICS AND SCIENCE up to, and including, "O" level G.C.E.—The Headmaster, Cheney School, Cheney Lane, Headington, Oxford.

DEMONSTRATOR IN PHYSICS—The Registrar, The University, Nottingham.

LECTURER IN BIOCHEMISTRY IN THE DEPARTMENT OF APPLIED MICROBIOLOGY AND BIOLOGY—The Secretary, Royal College of Science and Technology, Glasgow.

LECTURER (well qualified and experienced in any branch of electrical engineering or related branches of physics, and preferably an interest in materials) IN ELECTRICAL ENGINEERING—The Assistant Registrar (Science), The University, Birmingham.

LECTURER (with doctor's degree) IN CHEMISTRY, to teach up to master's degree (lecturing in French)—Rector L. Gillon, University Lovanium, B.P. 121, Leopoldville XI, Republique du Congo.

LECTURER (with doctor's degree) IN MATHEMATICS, to teach up to master's degree (lecturing in French)—Rector L. Gillon, University Lovanium, B.P. 121, Leopoldville XI, Republique du Congo.

RESEARCH ASSISTANT (with, or expecting shortly to have, a first- or good second-class honours B.Sc. or equivalent, for example, A.R.I.C.) IN THE DEPARTMENT OF CHEMISTRY, for electro-chemical studies in molten salts—The Bursar, The University, Reading.

SENIOR RESEARCH ASSISTANT (preferably with research experience in the physics of discharges or allied fields of gaseous electronics), for work on the production and utilization of augmented ion concentrations in flame plasma—Dr. F. J. Weinberg, Chemical Engineering Department, Imperial College of Science and Technology, London, S.W.7.

TECHNICAL ASSISTANT (with practical experience in pot mending, metal cleaning, developing and enlarging of film and/or archæological draughtsmanship) IN THE DEPARTMENT OF ARCHÆOLOGY AND NICHOLSON MUSEUM—The Registrar, University of Sydney, Sydney, New South Wales, Australia.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Arthur D. Little Research Institute. Annual Report for 1960. Pp. 44. (Inveresk, Midlothian: Arthur D. Little Research Institute, 1961.) [15

Department of Scientific and Industrial Research: Geological Survey and Museum. British Regional Geology—North Wales. By Dr. Bernard Smith and Dr. T. Neville George. Third edition. Completely revised by Dr. T. Neville George. Pp. vi+96+12 plates. (London: H.M. Stationery Office, 1961.) 6s. net. [15

Ciba (A.R.L.), Limited. Technical Notes, No. 219: Glued Wood Sports Car. Pp. 6. (Duxford: Ciba (A.R.L.), Limited, 1961.) [15

The Association of the British Pharmaceutical Industry. Annual Report and Year Book, 1960–61. Pp. 51. (London: The Association of the British Pharmaceutical Industry, 1961.) [15

Illustrated Newspapers, Ltd. Report of the Directors to the Thirty-fifth Annual General Meeting of Shareholders and Statement of Accounts for the year ended 30th November, 1960. Pp. 8. (London: Illustrated Newspapers, Ltd., 1961.) [15

South-West Essex Technical College and School of Art. Annual Report, Session 1959–60. Pp. 31. (Walthamstow, London: South-West Essex Technical College and School of Art, 1961.) [15

B.B.C. Record, No. 1 (New Series). (A News Letter from the British Broadcasting Corporation.) Pp. 8. (London: British Broadcasting Corporation, 1961.) [15

Department of Scientific and Industrial Research: National Physical Laboratory. Notes on Applied Science No. 16: Modern Computing Methods. Second edition, completely revised. Pp. 170. (London: H.M. Stationery Office, 1961.) 21s. net. [15

The Electrical Research Association. 40th Annual Report for the year ended 31st December, 1960. Pp. 107. (Leatherhead: The Electrical Research Association, 1961.) [15

The British Gelatine and Glue Research Association. Twelfth Annual Report for the year October 1959 to September 1960. Pp. 16. (London: The British Gelatine and Glue Research Association, 1961.) [15

Republic of Ireland. Department of Lands: Fisheries Division. Reports on the Sea and Inland Fisheries, incorporating Statistics of the Capture of Salmon, Sea Trout and Eels, and certain scientific papers relating to Fisheries. Year 1957: Pp. 99. Year 1958: Pp. 86. Year 1959: Pp. 80. (Dublin: Stationery Office.) 4*s*. 6*d*. each. [15

Bulletins of Marine Ecology (formerly *Hull Bulletins of Marine Ecology*). Edited by Sir Alister Hardy, Dr. C. E. Lucas and R. S. Glover. No. 42, Vol. 5: Continuous Plankton Records—Contributions towards a Plankton Atlas of the North-Eastern Atlantic and the North Sea. Pp. 65–111+plates 15–32. (Edinburgh: Scottish Marine Biological Association, 1961.) 15*s*. 6*d*. [15

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1030, Vol. 253 (27 April 1961): The Structure of the Vibrational-Rotational Bands of an Asymmetric Rotor. By H. C. Allen, Jr. Pp. 335–357. 7*s*. 6*d*. No. 1031, Vol. 253 (27 April, 1961): A Neutral Line Discharge Theory of the Aurora Polaris. By S.-O. Akasofu and Prof. S. Chapman. Pp. 359–406. 21*s*. No. 1032, Vol. 253 (27 April, 1961): The Behaviour of Two-Valued Response Regulators Applicable to Adiabatic Calorimeters. By A. J. B. Cruickshank. Pp. 407–458. 16*s*. 6*d*. (London: Royal Society, 1961.) [15

Technical Assistance from the United Kingdom for Overseas Development. Pp. 43. (Cmnd. 1308.) (London: H.M. Stationery Office, 1961.) 2*s*. net. [35

Mental Health Research Fund. Pamphlet No. 1: Schizophrenia. Pp. 8. (London: Mental Health Research Fund, 1961.) [35

University of Cambridge. Report of the Observatories Syndicate for the year ending 1960, September 30. Pp. 5. (Cambridge: The University, 1961.) [35

Falkland Islands Dependencies Survey. Scientific Reports, No. 24: Upper Cretaceous Decapoda and Serpulidae from James Ross Island, Graham Land. By Dr. H. W. Ball. Pp. 30+7 plates. (London: H.M. Stationery Office, 1960.) 25*s*. net. [35

Town Planning Institute. Planning Research: a Register of Research of interest to those concerned with Town and Country Planning. Pp. xvi+158. (London: The Town Planning Institute, 1961.) 18*s*. 6*d*. net. [35

Petroleum Information Bureau. U.K. Petroleum Industry Statistics relating to Consumption and Refinery Production 1959 and 1960. Pp. 8. (London: Petroleum Information Bureau, 1961.) [35

Observatory of the School of Cosmic Physics, Dublin Institute for Advanced Studies. Dunsink Observatory Publications. Vol. 1, No. 2: Cape Lyot Heliograph Results, II: The 3+ Flare of 1960 June 1, and Its Influence on the *Ha* Striation Pattern. By M. A. Ellison, Susan M. P. McKenna and J. H. Reid. Pp. 39–49. (Co. Dublin: Dunsink Observatory, 1961.) [35

Welsh Advisory Water Committee. Report on the Water Resources of Wales. Pp. iv+93. (Cmnd. 1231.) (London: H.M. Stationery Office, 1961.) 5*s*. net. [35

Ministry of Health and General Register Office. Report on Hospital In-Patient Enquiry for the year 1959. Part 1: Preliminary Tables. Pp. 16. (London: H.M. Stationery Office, 1961.) 1*s*. 6*d*. net. [35

British Museum (Natural History). A Short Guide to the Exhibition Galleries of the Natural History Museum. Eighth edition. Pp. vi+32. (London: British Museum (Natural History), 1961.) 1*s*. 6*d*. Bulletin of the British Museum (Natural History). Zoology. Vol. 7, No. 6: Free-Living Nematodes from South Africa. By William G. Inglis. Pp. 291–319. The Species of *Rhabditis* (Nematoda) found in Rotting Seaweed on British Beaches. By William G. Inglis and John W. Coles. Pp. 320–333. 14*s*. (London: British Museum (Natural History), 1961.) [55

Other Countries

Annals of the New York Academy of Sciences. Vol. 91, Article 2: Geochronology of Rock Systems. By J. Laurence Kulp and 77 other authors. Pp. 159–594. (New York: New York Academy of Sciences, 1961.) [35

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Memoir 308: Nelson Map-Area, West Half, British Columbia (82 F W½). By H. W. Little. Pp. xiii+205+5 plates. 1.75 dollars. Memoir 311: Ammonoid Faunas of the Upper Triassic Pardonet Formation, Peace River Foothills, British Columbia. By F. H. McLearn. Pp. x+118+21 plates. 2.50 dollars. Paper 61–2: Summary Account of the Cretaceous Alberta Group and Equivalent Rocks, Rocky Mountain Foothills, Alberta. By D. F. Stott. Pp. ii+34. 50 cents. Paper 61–3: The Mineral Industry of the District of Mackenzie, Northwest Territories. By W. R. A. Baragar. Pp. ii+29. 25 cents. Paper 61–4: Pollucite (Caesium) in Canada. By R. Mulligan. Pp. 4. 25 cents. (Ottawa: Queen's Printer, 1960 and 1961.) [35

National Academy of Sciences—National Research Council. Nuclear Science Series. NAS–NS 3024: The Radiochemistry of Magnesium. By A. W. Fairhall. Pp. vi+22. 50 cents. NAS–NS 3027: The Radiochemistry of Copper. By F. F. Dyer and G. W. Leddicotte. Pp. v+54. 75 cents. NAS–NS 3028: The Radiochemistry of Rhenium. By G. W. Leddicotte. Pp. v+42. 50 cents. (Washington, D.C.: Office of Technical Services, Department of Commerce, 1961.) [35

United States Department of Health and Welfare: National Library of Medicine. Russian Drug Index. Compiled by Stanley Jablonski. Revised by Dr. Robert M. Leonard. Pp. vi+103. (Washington, D.C.: Government Printing Office, 1961.) 60 cents. [35

Proceedings of the United States National Museum. Smithsonian Institution, Washington. Vol. 112, No. 3448: Stargazer Fishes from the Western North Atlantic (Family Uranoscopidae). By Frederick H. Berry and William W. Anderson. Pp. 563–586+4 plates. Vol. 113, No. 3450: *Paraconger*, a New Genus with Three New Species of Eels (Family Congridae). By Robert H. Kanazawa. Pp. 1–14+2 plates. Vol. 113, No. 3451: Revision of the Milliped Genus *Deltotaria* (Polydesmida: Xystodesmidae). By Richard L. Koffman. Pp. 15–35. Vol. 113, No. 3452: Four New Species of Pseudocyclops (Copepoda: Calanoida) from Puerto Rico. By Thomas E. Bowman and Juan G. González. Pp. 37–59. (Washington, D.C.: Government Printing Office, 1961.) [35

National Academy of Sciences—National Research Council. Publication No. 848: Effects of Inhaled Radioactive Particles: Report of the Subcommittee on Inhalation Hazards. Committee on Pathologic Effects of Atomic Radiation. Pp. vi+78. Publication No. 849: Report of the Subcommittee on Long-Term Effects of Ionizing Radiations from External Sources. Pp. v+82. Publication No. 875: Effects of Ionizing Radiation on the Human Hemapoietic System: Report of the Subcommittee on Hematologic Effects, Committee on Pathological Effects of Atomic Radiation. Pp. v+14. (Washington, D.C. National Academy of Sciences—National Research Council, 1961.) [35

Global Technology, No. 1 (April, 1961). Pp. 56. (A Digest of the World's Technical Literature.) Published monthly. Subscription rate: U.S.A. and Canada 25 dollars; elsewhere 35 dollars. Single copies 3 dollars. (Cleveland, Ohio: Trans-World Publishing Company, Euclid Avenue at E. 107th Street, 1961.) [35

The American Philosophical Society Year Book 1960. Pp. 786. (Philadelphia: American Philosophical Society, 1961.) [35

Notulae Naturae of the Academy of Natural Sciences of Philadelphia. No. 326 (March 4, 1960): A New Species of Cataspilota (Orthoptera; Mantodea; Manteidae) from West Africa. By James A G. Rehn. Pp. 5. No. 327 (March 4, 1960): An Additional Tribe of the Romaleinae (Orthoptera; Acrididae). By James A. G. Rehn and Harold J. Grant, Jr. Pp. 4. No. 328 (March 4, 1960): A New Calopsectra from Kansas (Diptera; Tendipedidae; Calopsectrini). By Selwyn S. Roback. Pp. 4. No. 329 (March 25, 1960): A New Ophichthid Eel of the Genus *Pseudomyrophis* from the Gulf of Mexico. By James E. Böhlke. Pp. 8. No. 330 (March 25, 1960): Comments on Serranoid Fishes with Disjunct Lateral Lines, with the description of a New One from the Bahamas. By James E. Böhlke. Pp. 11. No. 331 (June 17, 1960): New and Variable Diatoms. By John H. Wallace. Pp. 8. No. 332 (June 17, 1960): A New Race of *Hyloctistes subulatis* (Furnariidae) from Colombia. By Rudolphe Meyer de Schauensee. Pp. 2. No. 333 (August 19, 1960): The Neotropical Katydid Genus *Parascudderia* Brunner (Orthoptera: Tettogoniidae: Phaneropterinae). By Harold J. Grant, Jr. Pp. 11. (Philadelphia: Academy of Natural Sciences of Philadelphia, 1960.) [35

Notulae Naturae of the Academy of Natural Sciences of Philadelphia. No. 334 (August 19, 1960): Distributional Records and Notes on the Family Tanaoceridae (Orthoptera; Acridoidea). By James A. G. Rehn and Harold J. Grant, Jr. Pp. 6. No. 355 (August 19, 1960): A New Ostracod (Crustacea; Ostracoda) of the Genus *Entocythere* from Pennsylvania. By C. W. Hart, Jr. Pp. 4. No. 336 (December 9, 1960): *Prorocorypha, Eremiacris, Paropomala*—Members of the Sub-Family Acridinae (*Sensu* Rehn and Grant) (Orthoptera; Acrididae). By James A. G. Rehn and Harold J. Grant, Jr. Pp. 2. No. 337 (December 9, 1960): Two New Bahaman Species of the Clinid Fish Genus *Paraclinus*. By James E. Böhlke. Pp. 8. No. 338 (December 20, 1960): New Caledonian Non-Marine Snails collected by T. D. A. Cockerell in 1928. By Alan Solem. Pp. 9. No. 339 (December 20, 1960): The Identity of the Natricine Snake *Tropidonotus baramensis* Smith. By Edmond V. Malnate. Pp. 2. (Philadelphia: Academy of Natural Science of Philadelphia, 1960.) [35

United States Department of the Interior: Geological Survey. Bulletin 1101: Vanadium-Uranium Deposits of the Rifle Creek Area, Garfield County, Colorado. By Richard P. Fischer. With a section on mineralogy by Theodore Botinelly. Pp. iv+52+10 plates. Water-Supply Paper 1491: Geology and Ground-Water Features of the Butte Valley Region, Siskiyou County, California. By P. R. Wood. Pp. v+150+3 plates. Water-Supply Paper 1535–A: Ammoniated Thermal Waters of Lake and Colusa Counties, California. By C. E. Roberson and H. C. Whitehead. Pp. iii+11. 15 cents. (Washington, D.C.: Government Printing Office, 1960.) [35

Kungl. Skogshögskolans Skrifter. (Bulletin of the Royal School of Forestry, Stockholm.) Nr. 34: Development and Yield of Douglas Fir (*Pseudotsuga taxifolia* (Poir.) Britt.) and Sitka Spruce (*Picea sitchensis* (Bong.) Carr.) in Southern Scandinavia and on the Pacific Coast. By Sten Karlberg. Pp. 141. (Stockholm: Skogsbiblioteket Skogshögskolan, 1961.) 8 Swedish kronor. [35

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 42, (March, 1961): Growth in Hospital Utilization. Recent Population Trends in Large Cities. Incidence of Disability in 1960. Types of Fatal Heart Disease. Pp. 12. (New York: Metropolitan Life Insurance Company, 1961.) [55

Smithsonian Contributions to Astrophysics. Vol. 4, No. 3: Orbital Elements of Meteors. By Gerald S. Hawkins. Pp. ii+85–96. 15 cents. Vol. 4, No. 4: Precision Orbits of 413 Photographic Meteors. By L. G. Jacchia. Pp. ii+97–129. 25 cents. Vol. 5, No. 4: Observations of Simulated Meteors. By Richard E. McCrosky. Pp. ii+29–37. 15 cents. (Washington, D.C.: Government Printing Office, 1961.) [55

United States Department of the Interior: Fish and Wildlife Service. Fishery Bulletin No. 182: Embryological Stages in the Sea Lamprey and Effects of Temperature on Development. By George W. Piavis. Pp. iv+111–143. (Washington, D.C.: Government Printing Office, 1961.) 30 cents. [55

Il Dramma dell'Astrofisica e la sua Soluzione. Da Giuseppe Pallotti. Pp. 7. (Albino, Bergamo: Giuseppe Pallotti, 1961.) [55

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £8/8/-, payable in advance,
postage paid to any part of the world

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office

INFRA RED TRAINING COURSES

INTENSIVE COURSES IN ANALYTICAL TECHNIQUES ARE BEING ARRANGED.
IF YOU ARE INTERESTED WE SHALL BE PLEASED TO HEAR FROM YOU.

THE SCOPE OF THE COURSES IS ROUGHLY OUTLINED BELOW

Introductory talk : Operation of spectrometer, including wavelength and intensity calibration, also routine checks : Sampling techniques : Various types of cell : Suggested reading matter : KBr disc preparation : Nujol mull preparation : Use of internal standard : Filling, emptying and cleaning gas and liquid cells : Solution Work, limitations and uses : Variable-path cells : Practical examples : Gas Analysis—routine methods and uses of high resolution : Use of variable slit programming : Analytical problems : Etc. : Etc.

GRUBB PARSONS

GRUBB PARSONS : WALKERGATE : NEWCASTLE UPON TYNE 6

(Subsidiary of C. A. Parsons & Company Limited)

SIC NOS NON NOBIS

Plan Ahead

by effecting now "the young man's policy" which gives to a man under 35 immediate life assurance cover at low cost and the valuable right to effect within ten years further assurances even though his health has become impaired.

The Equitable has no shareholders and pays no commission for the introduction of business; unusually attractive terms can, therefore, be offered.

Write today for details to

The Equitable Life Assurance Society

(Founded 1762)

Head Office : 19 Coleman Street, London, E.C.2

Please send me, without obligation, details of the young man's policy.

Name..

Address...

....

Stop Watches

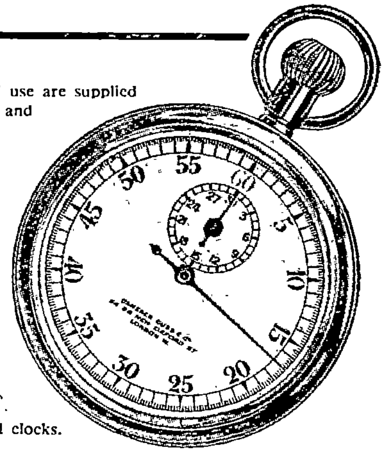
for scientific and industrial use are supplied to research establishments and industrial organizations. Enquiries are welcomed.

Sectional catalogues and leaflets available:

Watches, stop watches and chronographs.
Recording apparatus.
Clocks, domestic and industrial.
Process timers and time interval meters.
Electrically controlled stop watches.

Repair Service

Watches, stop watches and clocks.
Marine chronometers.
Instruments.



Model 01 1/5th second escapement 30 minute recorder. Model 03 1/10th second escapement 15 minute recorder.

CAMERER CUSS

Timing Division

Makers of good Clocks and Watches since 1788
at 54/56 NEW OXFORD STREET, LONDON, W.C.1
MUSeum 8968/9
Service and Repairs: MUSeum 2255. Our only other
address: 91 Kingsway, W.C.2

APPOINTMENTS VACANT

CEMENT AND CONCRETE ASSOCIATION

invites applications from

PHYSICISTS

to carry out independent research and to take an interest in a broad range of applications of physics to the building industry.

The work of the Physics Group of the Materials Research Department includes fundamental studies of the properties of matter and strength of materials, measurement of heat flow, strain gauging, X-ray diffraction analysis, and design of instruments.

Scope exists for long-term work at the fundamental level as well as on applied physics projects, and the facilities include a Sirius computer.

The publication of results and attendance at scientific meetings is encouraged. The appointment will be made in the career range of £1,000 to £2,000, with possibilities of further advancement, and the post is permanent and pensionable.

Applications to Dr. A. R. Collins, Director of Research and Technical Services, Cement and Concrete Association, Wexham Springs, Stoke Poges, Buckinghamshire.

WORKS CHEMIST. A VACANCY OCCURS at the Extraction Plant of the Pyrethrum Board of Kenya at Nakuru for a Works Chemist. The duties will be chiefly analytical control but participation in factory operations is also included. The appointment is open to male candidates, who should possess a degree in chemistry or the equivalent Higher National Certificate in chemistry would be considered where the applicant has had approved experience. Salary: £1,400 by £50 to £1,750. The contract would be initially for a tour of three or four years, and subject to renewal. Return air fares paid up to three adult passages. Flat or house, with hard furnishings, available at a rental of not more than 15 per cent of salary with a maximum of £150 per annum. Provident Fund contribution of 5 per cent of salary, plus 10 per cent contribution by the Board. Medical and hospital benefits are offered, the Board paying two-thirds of the premium. Annual local leave of eighteen days is granted, with three or four months overseas leave at the end of the tour. Nakuru is in the Kenya Highlands amidst fine scenery, at an altitude of 6,000 ft., and has a pleasant, temperate climate. The Board's factory is for the solvent extraction of pyrethrum flowers, and is now being increased to treble its original capacity (1959) as a result of the rapid expansion of the Overseas market.—Applications, giving full details of age, education, qualifications and experience, etc., should be sent to the African Pyrethrum Technical Information Centre, 216 Grand Buildings, Trafalgar Square, London, W.C.2.

THE BRITISH OXYGEN COMPANY LIMITED

A Senior position is available in the Scientific Division for a

GRADUATE

IN CHEMISTRY OR PHYSICS

with experience in paint technology, particularly emulsion paints and preferably also in thermosetting resins.

The successful candidate will be required to carry out evaluation and technical service work on resins with special regard to paint applications.

Please apply to

THE ADMINISTRATION MANAGER,
SCIENTIFIC DIVISION,
THE BRITISH OXYGEN COMPANY, LTD.,
DEER PARK ROAD, LONDON, S.W.19.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

RESEARCH STAFF

(No. 464/233) (Several Positions)

The Organization's **Division of Textile Industry, Geelong, Victoria**, invites applications for research positions from honours graduates in science, or engineering with some years post-graduate research experience.

The Division, one of three constituting the Wool Research Laboratories, is broadly concerned with development of new physical and chemical techniques in wool textile processing and provides well-equipped laboratories for fundamental studies and full scale mill equipment for application of results. The wide field of research includes absorption studies on wool, fibre friction, permanent setting, shrinkage prevention, mechanical action of machines on wool fibres, servo-control mechanisms in processing and development of new machines.

The appointees will carry out duties in the following fields:

(i) Chemical studies on modification of wool fibres. This work would involve the study of reactions with a view to developing new fabric finishes;
(ii) The development of new physical methods for separating vegetable material from wool;
(iii) Physical studies on fibre friction, and the effects of lubricants:
(iv) Investigation of new methods of yarn production;
(v) Fibre behaviour in processing using radioactive and high speed photographic techniques.

Applications and enquiries from research workers in fields not strictly related to the above are also warmly invited.

SALARY: Dependent on qualifications and experience within the ranges, £A.1,510 to £A.2,265 per annum or £A.2,395 to £A.2,720 per annum.

EXPERIMENTAL OFFICER

(No. 464/232)

The Organization's **Division of Textile Industry, Geelong, Victoria**, also invites applications for a position as Experimental Officer from graduates in Electrical Engineering, or in Science with Physics and Electronics as major subjects (or equivalent professional qualifications). Experience in design of electronic equipment is essential.

The appointee will be required to assume full responsibility for an electronics laboratory. The function of the laboratory will be to develop special electronic devices for application in research on woollen and worsted processing; to advise research staff in projects of an electrical engineering nature: and to provide servicing facilities for all electronic equipment of the Division. First-class laboratory facilities available.

SALARY: Dependent on qualifications and experience within the ranges, £A.1,620 to £A.1,950 per annum or £A.2,070 to £A.2,330 per annum.

Salary for a woman will be £A.180 per annum less than corresponding rates for men.

Promotion within C.S.I.R.O. is by merit and may ultimately go beyond the upper limit of the range within which the original appointment is made.

Fares paid. Further particulars supplied on application to:

Mr. E. J. Drake, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting relevant Appointment number) should be addressed by August 5, 1961.

UNIVERSITY COLLEGE OF THE WEST INDIES

Applications are invited for (a) Lectureship or (b) Assistant Lectureship in Zoology (Entomology). Salary scales (a) £1,050 by £50 to £1,400 by £75 to £1,850 per annum, (b) £800 by £50 to £950 per annum, plus local allowance of £250 per annum (pensionable). Entry according to qualifications and experience, but in case of Assistant Lecturers and Lecturers below merit bar consideration is given to age in fixing salary. F.S.S.U. Child allowance. Settling-in allowance. Unfurnished accommodation at rental of 10 per cent of basic salary. Up to five full passages on appointment, normal termination, and triennial study leave.

Detailed applications (six copies), naming three referees, by August 7, 1961, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

BIRKBECK COLLEGE

(UNIVERSITY OF LONDON)

Applications are invited for the post of Assistant Lecturer in Theoretical Physics (Professor of Theoretical Physics, Dr. D. J. Bohm). Salary scale, £800 by £50 to £950 plus £60 per annum London allowance.

Further particulars should be obtained from the Secretary, Birkbeck College, Malet Street, W.C.1. Closing date: July 24.

UGANDA

DEPARTMENT OF AGRICULTURE

CHEMIST

For research work on soil fertility problems connected with tropical crop rotations, manures and fertilizers. Candidates (male) must have an honours degree in agricultural chemistry or chemistry and should preferably have had at least one year's postgraduate training at an experimental station. Appointment on contract/gratuity terms for one tour of 21-27 months with emoluments in scale £1,221 to £2,448. Gratuity 25 per cent of total substantive emoluments. Free passages. Generous leave. Furnished accommodation usually available at low rental.

Apply, stating full name and giving brief particulars of qualifications and experience, to Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD/ 63/9/09/C1.

UNIVERSITY OF SHEFFIELD

Applications are invited for a post of Lecturer in Mechanical Engineering to begin duties on October 1, 1961, or as soon as possible thereafter. Initial salary according to qualifications and experience on the scale £1,050 by £50 to £1,400 by £75 to £1,850, with F.S.S.U. provision and family allowance. A grant towards removal expenses will be made.

Further particulars may be obtained from the Registrar to whom applications (four copies), should be sent by July 29, 1961.

FINANCING GOVERNMENT RESEARCH IN GREAT BRITAIN

IN the main, the report of the Council for Scientific and Industrial Research for 1960 is disappointing. The year has indeed been one of steady expansion, the pace and character of which have made it necessary, as Lord Hailsham points out in his introduction, to change the method of determining the Department's financial resources, a summary of which is given on p. 230 of this issue. The description of the change of methods is one of the most important features of the report; nevertheless, the report does not provide the information that might be expected, in view of the growing interest now being taken in the work of the Department, both in Parliament and elsewhere, on its contribution to the organization of Britain's research effort and the effective co-ordination of that effort. There is indeed a long and interesting section on the Department's relations with the universities and colleges of technology in Britain, dealing with the system of research grants and training awards; but a separate report is to be published surveying developments since 1956, emerging problems and trends in this field. Accordingly, that aspect of the Department's work is more appropriately considered when the promised report appears, just as the research associations and their work are now best discussed in the context of the separate report, *Research in Industry*.

Likewise, although no further information is given on the work of the Department's Intelligence Division, we are promised that the 1961 report of the Council will review at length the development of information services, and it is to be hoped that this report at least will supply the long overdue information on that part of the work of the Department. The Council, too, has been much concerned with the organization and maintenance of scientific representation, a function which the Department undertakes for all the Government departments concerned, and in which, in future, it may well be particularly concerned with the work of the new Department of Technical Co-operation. Here, the Council already recognizes from post-war experience that the true functions of scientific representatives grow naturally out of the local conditions in which they operate. The increasing importance of scientific questions in the political and commercial relations of the United Kingdom abroad has already made advice to ambassadors and other official representatives of the United Kingdom a major task of the scientific attachés. It is also increasingly important to promote good contacts between scientific organizations in Britain and corresponding bodies overseas, and the Government's own official scientific advisers continued to supply what is probably the most emphatic evidence of the consequences of the refusal to establish a Science Centre in London. Even now, there is little indication that official circles appreciate the extent to which the new National Lending and Reference Libraries of Science and Technology will, when fully operative, fall short of what such a Centre could have achieved in assisting scientific attachés in their task of securing an adequate supply of scientific and technical information from Britain.

The Council, however, recognizes that it is now necessary to consider extending the network of scientific representation so as to include not only countries that are scientifically and technologically advanced but also those that are at an earlier stage of development. Its decision that the time has come for a systematic assessment of the Government's needs in all areas which are not fully covered by the present network is welcome, as is the statement that the Council is also striving to ensure that the existing representatives have sufficient staff at their disposal to handle this increasing flow of important work. The Council has already embarked on a world-wide survey of the position in consultation with the Foreign Office, the Commonwealth Relations Office and the Board of Trade.

In view of what the Council itself writes in this connexion in the present report, one might have expected that the Colonial Office and the Department of Technical Co-operation would also be associated with this inquiry. Thus, the Department has been responsible since 1959 for the Tropical Products Institute, and this has led to a growing association of the Department of Scientific and Industrial Research with the needs of the dependent and emergent territories. The section of the report in which research into tropical problems is discussed refers to a steady demand from the less-developed countries for visiting experts, and they are continually looking for help in achieving a more effective utilization of their resources. The more advanced countries, on the other hand, request help with specific research problems or in finding new uses for over-produced commodities. Again, the report refers to investigations of fairly general interest, such as the possible physiological importance of tropical plants, and also the utilization of solar energy, which if it becomes economic could greatly benefit tropical and sub-tropical countries deficient in other sources of energy.

It is not, of course, the Tropical Products Institute alone which is concerned with studies of interest to the dependent territories, in the tropics and elsewhere. The Road Research Laboratory, the Building Research Station and the Forest Products Research Laboratory have long given assistance to such territories in particular fields. The Hydraulics Research Station undertakes, as part of its normal service to users, model studies and field investigations of problems arising overseas, and the territories concerned with the Station's present range of projects include Aden, Hong Kong, Iran, Mauritius, Nigeria and East and West Pakistan; while during the year a number of its staff visited both Ghana and Nigeria to advise on the need for, and possible scope of, specialist laboratories there.

These developments, however, raise more than the questions of liaison on which the present report focuses attention. They involve questions of co-ordination and allocation of resources in which the Overseas Research Council at least is concerned, and the Department of Technical Co-operation may well be in future. There is no indication in the report that this is appreciated; but the matter is one which the Minister for Science might well examine as a matter of urgency. Some of the most urgently needed research could well be in territories least able at present to finance such investigations, and there appears to be *prima facie* a danger that the Department's disposition to insist on payment by the beneficiary may result in important investigations not being initiated. A clear-cut recognition of responsibilities and allocation of resources as between the Overseas Research Council and the Council for Scientific and Industrial Research should go far to eliminate this risk.

For the rest, the report shows that the Council is manifestly seeking to ensure, by its allocation of resources, that the scientific effort of Great Britain is balanced in relation to national needs. It regards the assessment of the balance of research in the universities and colleges of technology as fundamental, because in each subject strong and effective research departments are needed to attract good staff and students and to supply the nation with well-trained research workers. It is anxious to secure balance in the Department's own research programme and many of the changes introduced in recent years are for that purpose. In future, international action is likely to contribute much to the achievement of balance, and the Council recognizes that it must be prepared to play its part in co-operative projects.

In particular, the Council re-emphasizes the importance of surveying research needs in particular subjects and industries, as has already been done, for example, by its Economic Committee in respect of the British machine tool industry, and the shipbuilding and marine engineering industries. The Committee appointed under Sir Arthur Pugsley's chairmanship to review the need for research on the fatigue of engineering structures made of metal has also reported, and the Council has accepted its report. The main recommendation is that a co-ordinated programme of research should be undertaken in specified fields relating to structural materials used under fatigue conditions, particularly materials in structural sizes and joint assemblies, whether welded, riveted, or friction-grip bolted. Other recommendations are concerned with the co-ordination of this research and the provision of design information, based on the results obtained, to practising structural engineers.

The Department is discussing the implementation of these recommendations with the Institution of Civil Engineers and other professional organizations, but meanwhile the Council has decided to make the report immediately available. The Council has also continued to examine various proposals and applications for support and development work in industry, and it hopes soon to complete a number of preliminary studies of such projects, particularly costly ones. These are all activities which clearly fall well within the scope of the Council, which, it should be remembered, unlike the Advisory Council on Scientific Policy, is an executive body, and as such is an important tool of that Council for carrying out its policy.

Nevertheless, while the steady attrition of the annual reports of the Department of Scientific and Industrial Research tends to rob them of interest, which shifts to those of the Advisory Council on Scientific Policy, there are passages in the present report which suggest the reflexion that the Council sometimes trespasses on the functions of the latter body, while failing to supply the information for a sound judgment as to the wisdom of the policy it advocates. Comment on the statement that the Council has found insufficient research effort in fields of science such as biophysics, geophysics, meteorology and oceanography, which lie between the main disciplines, could be premature in view of the promised report on the relations between the Department and the universities, and in any event the Council is obviously seeking to implement a recommendation of the Advisory Council on Scientific Policy. Indeed, it is the findings reported to the Advisory Council last year that are now guiding the Council's own policy.

The main interest of the present report, however, lies, as already stated, in the description of the new method of allocating the Department's resources. On its appointment, the Council believed that the inherited system of determining the man-power and finances available for the Department's work in agreement with the Treasury for five years at a time—subject to the annual approval of estimates by Parliament—would permit the Department's activities to be planned in advance with a reasonable assurance that, so long as could be foreseen, they would not suffer a financial cut. Rapid changes in the scientific and technological scene and the increasing cost of tools of research have, however, since made it almost impossible to forecast with any accuracy the nation's requirements as long as five years ahead; and two years ago, at the launching of the Department's second five-year plan, the Council expressed the view that it was possible to estimate the needs of research fairly accurately two years ahead but only provisionally five years in advance.

These doubts have been confirmed by events. In last year's report, the Council referred to the unexpectedly rapid growth of applications for research grants from universities and colleges of technology. This year it points out that the accelerated expansion of scientific departments in the universities must be matched by an increase in awards for postgraduate training, if it is to yield its full benefit. The Department is providing almost £300,000 for a space research programme, which only became possible after the five-year plan was prepared, and increasing use of the results of research in the conduct of Government business in itself leads to unforeseen demands on some of the Department's own research stations. Even more unpredictable are the requirements for very expensive pieces of scientific equipment, such as radio telescopes and particle accelerators.

Earlier this year, it thus became clear to the Council that adherence to the budget of the five-year plan would mean that much that should be done would be left undone, and that a more flexible financial instrument was needed. The new experimental scheme, which has been agreed with the Treasury, while providing reasonable assurance of future funds, is regarded as more adaptable to rapid and unpredictable change. Under it the Council will attempt to forecast its needs for five years in advance and will agree with the Treasury, subject to approval by Parliament: (1) the expenditure for the following year; (2) reasonably firm estimates for the two succeeding years (from which major departures will be made only if there are important changes in the scientific or financial situation); and (3) less-definite forecasts for the fourth and fifth years.

This system means that each year the Council will revise the estimates and bring them forward one year, so that they gradually become firmer. If the scheme works satisfactorily, the Council should be able to plan each year's expenditure alongside a fairly clear indication of likely expenditure in the years immediately following, and with some regard to longer-term plans. Whether this flexibility will be achieved at the cost of some decrease in security for research projects remains to be seen, but it would seem to be important, if confidence is not to be undermined, that the Department's annual budget should be large enough to provide a reasonable margin for adjustments.

The Council's understanding with the Treasury is that subject to the approval of Parliament the sums made available for the Department's work in what would have been the last three years of the second five-year plan will be more than 20 per cent above the original provision in the plan. Whether these provisions will give a sufficient margin of flexibility can scarcely be judged on the information disclosed in this report. What can be said is that, if the Council's annual report is to retain its importance and value, the legitimate segregation of detailed information to reports on particular phases of the Department's activities should not be carried to a point which hinders any general appraisal of the work of the Department as a whole. With Parliament taking a steadily increasing and healthy interest in the Department's work, it is the more essential that Parliament and public should be given ample information on which to judge the value of the work already being done, the needs and opportunities that are still not met and the way in which the Department's work impinges on that of other bodies contributing to the nation's research effort.

A. T. GREEN AND THE BRITISH CERAMIC RESEARCH ASSOCIATION

The A. T. Green Book

Dedicated to Arnold Trevor Green. By his colleagues of the British Ceramic Research Association. Pp. x+309. (Penkhull, Stoke-on-Trent: British Ceramic Research Association, 1959.) n.p.

Dr. A. T. GREEN, who had been director of research of the British Ceramic Research Association from its formation in 1948, retired from this post at the end of 1959. His colleagues in the Research Association have paid him a worthy tribute by publishing this collection of original papers. In doing so, they have not only recorded their feelings of affection and respect for Dr. Green but also have shown what a lively and successful example of a research association the British Ceramic Research Association is.

The first chapter describes the development of the British Pottery Research Association and the British Refractories Research Association, the two bodies which were amalgamated in 1947 to form the present British Ceramic Research Association. The second chapter deals with the growth of the research programme of the present Association and traces the continuity and development of the programmes from the two pre-amalgamation bodies. Green was appointed director of the British Refractories Research Association in 1937. He had, for seventeen years, been associated closely with the first director, Dr. J. T. Mellor, in the development from the small beginnings in 1917. It is interesting to note that in 1933 Green had sponsored work in the Refractories Department of the University of Sheffield on the use of magnesia as a refractory.

The British Potteries Research Association, although beginning its activities much later than the Refractories Research Association, started off in much more favourable financial circumstances, and in 1938 it purchased "Beechfield", a large house with extensive grounds at Penkhull in which stand the present laboratories of the Ceramic Research Association.

The success of Dr. Green's work is aptly summarized by A. E. Dodd at the end of his chapter on some features of the work of the Research Association, as follows: "At no stage in the forty years covered has it been possible to point to a specific experiment and say 'This revolutionized ceramics'. Yet this has been the period during which 'pot-banks' and 'brick-yards' have, for the most part, become modern factories. This same forty years is the period—the lifetime—of service given to the industry by Dr. A. T. Green".

But this quotation does not quite express the extraordinary facility with which Green had persuaded gradually an old-fashioned industry, divided into many small independent firms, to support and use the results of the laboratory investigations. Not only did Green do this, but also he always maintained the background and interest in fundamental scientific investigations, and in the period before his retirement he made special efforts to convince the industry that in the future they must look for far more long-term work from the Research Association than they had in the past. This present volume shows how this new programme has taken shape.

In all there are 27 separate papers in the volume, and it is impossible to comment in any detail on all of them; many of them are statements of the present position of work in hand. Of these the first technical paper on thermal shock in furnace structures is a good example. The experimental observations are clearly stated, and the lines along which understanding has to be sought are carefully set out; no attempt is made to present a final answer. The experimental results are quite fascinating; the pattern of the fracture in refractory bricks subjected to a temperature gradient is shown to be very largely associated with the size of the brick, the 3-in. brick cracking parallel to the hot face, the 6-in. brick cracking perpendicular to it and the 9-in. brick cracking in two places perpendicular to the heated face. It is shown that the most probable explanation lies in the distortion of the sides, and it is made quite clear that the semi-infinite slab approximation would not be appropriate in considering this problem.

The paper on the constitution of bone china contains a very interesting historical survey of the developments in the understanding of the nature of this material and the function of the raw material components; the problem does not appear to be solved completely.

The newer items that are coming into the programme are well exemplified by the article on special ceramics in which the work in progress in the Association using titanates and non-oxide ceramics such as boron nitride is described. This is a good example of A. T. Green looking ahead and taking steps to influence the direction of his research programme. Work on sintered oxides started in the laboratories in 1953, and in 1955 he accepted a Government contract for work on new dielectric materials. These programmes have been characterized by the fundamental way of viewing the problem.

The more homely aspects of the work in the Association are typified by the chapter on glass colours and the effect of the newer detergents on the durability of these colours. Other papers deal with strain in wall tile glazes, the firing of intermittent kilns, extrusion research, rheology and ceramic casting-slip control, chrome-magnesite refractories, to mention only a few. In all, there are twenty-six contributions in this well-deserved tribute to Dr. Green.

R. W. Douglas

THE WEST RIDING OF YORKSHIRE

The Place-Names of the West Riding of Yorkshire
By A. H. Smith. Part 1: Lower and Upper Strafforth and Staincross Wapentakes. Pp. xi+346. Part 2: Osgoldcross and Agbrigg Wapentakes. Pp. xi+321. Part 3: Morley Wapentake. Pp. xiii+278. (English Place-Name Society, Volumes 30, 31 and 32.) (Cambridge: At the University Press, 1961.) 35*s*. net each volume.

Since its foundation in 1924, the English Place-Name Society has produced 32 volumes dealing with 21 counties. The present three volumes form the first instalment of a total of eight concerned with the West Riding, and they cover almost all the county to the south of the Aire. The next three will cover the rest of the county; the seventh volume will be devoted to the interpretation—linguistic and historical—of the material thus assembled; and the eighth will consist of a general index to the place-names of Yorkshire as a whole. We must, therefore, wait for a discussion of the detailed evidence provided by this instalment; but, in the meantime, we can see what these volumes promise for the future and how they compare with the volumes that have already appeared for other counties.

Over the years, the Place-Name Survey has come to include an increasing number of minor names and field names, and this tendency is especially evident in these West Riding volumes. Many of the lesser names have no record earlier than the Tithe Awards of the mid-nineteenth century; but there is also a large number of names with forms from as early as the thirteenth century and even from the twelfth. All these names, together with the street-names of the larger settlements, are given a good deal of space in the present volumes. Complicated changes in the parish boundaries of the West Riding have involved many difficulties in the presentation of the material, but we are guided skilfully through the complexities of boundary changes. What is more, the area is highly industrialized, and, from beneath the modern built-up areas (with so many modern names), Prof. Smith has, in effect, disinterred an older landscape. The clearing of the wood, the draining of the marsh and the progress of rural settlement were activities that have left memorials in the place-names we use, often unthinkingly, to-day. Embedded in the detailed evidence there are many fascinating thumb-nail sketches of the history of individual names. The discussion of the name Halifax, for example, starts off with a review of early fanciful interpretations, and then proceeds with a piece of detective-like deduction. Another interesting sketch is that of the name Pontefract, which replaced the earlier Tateshale, now represented by the adjacent parish of Tanshelf.

This is the largest and most formidable county survey of place-names yet undertaken. The area is considerable; but, fortunately, the material is plentiful. These first three volumes whet our appetite for Volume 7 and for some general conclusions. The bulk of the names are Anglo-Saxon, but there is a strong Scandinavian element among them, and we look forward to a picture of the relative importance of English and Scandinavian names in different parts of the Riding. An idea of the bearing of this evidence on Yorkshire dialects will also be welcome; through the West Riding ran the boundary between Mercians and Northumbrians. We also look forward to knowing more about the West Riding Celtic names, not very numerous, but of especial interest because the Britons maintained the independent kingdom of Elmet here until well into the seventh century. Such parish-names as Monk Bretton, West Bretton and Walton suggest their own story. To the south, outside what may have been Elmet, there is Celtic evidence in the parish-names of Ecclesall and Ecclesfield near Sheffield, and in that of Wales not far to the east. In the north, just beyond the area dealt with in the present volumes, the name of Leeds is also Celtic in origin. Another category of names is that associated with Norman influence; the names are not many, but they give a feudal and French flavour to parts of the map of the West Riding, for example Hooton Pagnell and Laughton en le Morthen.

Two previous studies in the Survey (also by Prof. Smith) have dealt with the North Riding and the East Riding. The completion of the West Riding volumes will bring to an end a most ambitious undertaking: The completed survey of the place-names of the whole of Yorkshire will carry our knowledge of the evolution of the life and landscape of England a most important stage forward. At a different level of comprehension and enjoyment, it is pleasant to turn over the pages and see the rich and curious variety that characterizes English place-names. Parish names such as Cridling Stubbs, Purston Jaglin and Thorpe Salvin cannot fail to excite our curiosity. These three volumes are not only for scholarly reference but also for delightful browsing.

H. C. DARBY

SCIENTIFIC ATTITUDE TO MANAGEMENT

Management Sciences, Models and Techniques

Proceedings of the Sixth International Meeting of the Institute of Management Sciences, Paris, 7–11 September, 1959. Edited by Prof. C. West and Prof. Michel Verhulst. Vol. 1: Pp. xxix+602. Vol. 2: Pp. xiv+511. (London and New York: Pergamon Press, 1960.) 200*s*. net per set.

ESTABLISHED six years ago, the Institute of Management Sciences has made much more progress than even its enthusiastic founder members had cause to hope for. This is reflected in the *Proceedings of the Sixth International Meeting*, which was held in Paris during September 7–11, 1959, and which run to a weighty two volumes of concentrated report.

The meeting was supported by about a thousand participants from all parts of the world. Not unexpectedly, many of those attending came from the United States, and, again not unexpectedly in view of Britain's relative backwardness in management education, there were comparatively few British representatives. At present it would appear that other European countries and countries in the Commonwealth are showing greater interest in the scientific approach to problems of management than is the case in Britain.

Six years ago the complexity of management problems made it doubtful whether the scientist could make a noticeable contribution over a short period of time. During those six years the number of scientific personnel entering the management field has increased significantly, and there has been a corresponding if, as yet, limited advance along a number of sectors. Techniques like simulation, linear programming and the theory of decision-making have been applied to management tasks, sometimes with success verging on the phenomenal. Attention has also been paid to problem areas in production and inventory control, as well as to the place and performance of research and development departments.

These and related topics were all considered at the Paris meeting. Prof. Myron Gordon, of the Massachusetts Institute of Technology, showed that even the optimum amount of profit which a business should distribute can be analytically determined. Contributions from Norway and the Argentine gave examples of how little-developed industrial countries are using tools like operations research to stimulate their economies. From Britain, Prof. R. W. Revans presented a case-study to show how our hospital services need scientific help in their management, while F. de P. Hanika, now of Churchill College, Cambridge, presented a thoughtful paper describing the role of management sciences in training and education for management; his shrewd admixture of theory and practice should appeal to managing directors and teachers of management subjects in university or technical college.

Of the papers presented, however, a large proportion was directed to the area of business games. During the past few years there has been rapid progress in the development of analytical descriptions of entire business enterprises. These games are proving increasingly useful in management training as well as for research in investigating group interactions in industry and commerce; participants were able to sample games which had been developed and played in the United States, France, Denmark and Great Britain, and *Management Sciences, Models and Techniques* contains detailed descriptions of the game as well as comments from the players.

The two volumes make a noteworthy contribution to the application of science in management thinking and, by inference, indicate how ill-equipped many practising managers are to meet the challenge of the next decade. The need for a good basic education with more than a modicum of mathematics shines like a beacon on every page of this not inconsiderable book. So does the need for management scientists to eschew that extrapolative jargon which is already threatening to choke clear thinking and make them intelligible only to one another.

For such an important work the book is unevenly produced. Although intended for English-speaking readers, many outstanding American contributions are presented only as the briefest of abstracts; quite a number of contributions in French are set out in expansive detail, including the extraneous. It would appear that, for speed and economy, conference papers issued to participants have been printed by a number of different firms; when brought together to form a substantial book the effect is patchy and does not make for easy reading. Full contributions in one language with abstracts in the other might lead to the desired economies and produce more satisfying proceedings.

T. H. HAWKINS

TROPICAL FLORAS IN THE TERTIARY IN SOUTHERN ENGLAND

The Lower Tertiary Floras of Southern England. I.

Palaeocene Floras; London Clay Flora (Supplement). By Marjorie Elizabeth Jane Chandler. Pp. xi+354+Atlas of 34 plates. (London: British Museum (Natural History), 1961.) 200*s*. net.

A LARGE part of this book is a supplement to *The London Clay Flora* by Mrs. Clement Reid and Miss Chandler, published by the British Museum (Natural History) in 1933. In this joint work they gave a description fully illustrated with photographic reproductions of the fossil plants, mostly fruits and seeds, found in the London Clay, which is part of the Early Eocene of the south of England. They devised special methods of investigating the internal structure of these fossils, which are partially lignitized and partially pyritized and uncompressed. A great deal of information about their histology could be obtained and enabled close comparisons to be made with the corresponding organs of existing natural orders of flowering plants. It was possible to assign most of them to natural orders and, in a few instances, to existing genera. 234 species were described in the original work of 1933 : most were assigned to new genera and species. Miss Chandler now adds a dozen new genera and more than a hundred new species to the list. There are, in all, more than 50 natural orders represented in the London Clay. The geographical distribution of these orders to-day suggested that the London Clay flora was that of a tropical rain forest with a similar floristic composition to that of the present Indo-Malayan region. The Tethys Sea at that time included the present Mediterranean and extended from the Indian Ocean to western Europe, and this flora spread along the adjacent land areas. Miss Chandler's later work fully supports these views.

But, in addition to supplementing our knowledge of the London Clay flora, Miss Chandler makes important additions to our knowledge of the other Palæocene floras of England.

The Second World War interrupted much of her work, but in the previous eight years and in the period after 1944 much collecting has been done and there are many interesting and useful data about new localities. In the present work under review we find a systematic treatment of the Early Tertiary floras of southern England from Thanet, Woolwich and Reading Beds of the Pre-Ypresian to the Ypresian with the Oldhaven, Blackheath Beds and the London Clay. The geological relationship of these beds and the separate basins of the London and Hampshire areas is clearly explained. Their floras all suggest tropical conditions. There is no evidence for supposing that a cool climate existed during the Palæocene.

The systematic descriptions of the floras and their species occupy about three-quarters of the volume and are treated in a similar manner to *The London Clay Flora* by Reid and Chandler. The volume of illustrations contains 34 plates, which include more than 1,030 excellent reproductions of photographs of fruits and seeds.

Miss Chandler is to be congratulated on a great achievement. The work is marked throughout by accuracy, and the descriptions are always clear and unambiguous.

John Walton

K. TERZAGHI: FOUNDER OF THE SCIENCE OF SOIL MECHANICS

From Theory to Practice in Mechanics Soil

Selections from the writings of Karl Terzaghi. With bibliography and contributions on his life and achievement prepared by L. Bjerrum, A. Casagrande, R. B. Peck and A. W. Skempton. Pp. viii + 425. (New York and London: John Wiley and Sons, Inc., 1960.) 96*s*.

PROF. KARL TERZAGHI is one of the most interesting figures in the world of engineering science, and many believe him to be the greatest civil engineer of our day. Due to his pioneering research in Istanbul, leading to the publication of *Erdbaumechanik* in 1925, the study of the mechanical properties of soil was given a rational basis, and the science of soil mechanics, which is now studied by civil engineering students throughout the world, took its place among the other engineering disciplines.

Soil mechanics is itself divided into a number of specialities, for example the stability of earth dams, the design of pavements, the heaving of soil due to frost, etc., and it is astonishing how many of these various topics were first tackled systematically by Terzaghi, leaving it to others to develop along the lines he first indicated. This might have been deduced from his *Soil Mechanics in Engineering Practice* (1948) ; but it is brought out very clearly in this collection of his early papers, which trace in a fascinating way the development of soil mechanics, as we now know it, with the man. To the younger generation Terzaghi is already becoming a mythical figure. His James Forrest Lecture at the Institution of Civil Engineers in 1939 stirred their elders into a realization that soil mechanics was of vital concern to them ; now, in this work, all may come closer into contact with him and learn from his directness and integrity.

A few of the collected papers published here would have been available to readers in a big city with a good library. But the *Proceedings of the First Soil Mechanics Conference* (1936) are somewhat rare, and many will welcome the opportunity of having Terzaghi's contributions to it on their own shelves. Most of the other papers will be quite new, however, and of these the professional reports are the most valuable. Apart from their interest they provide a model of good prose.

The book has two particularly valuable sections. One, contributed by Prof. Skempton, assesses the importance of the concept of effective stress, discovered by Terzaghi, from which so much of the theory of soil mechanics is derived. The other, by Prof. Casagrande, is a biography of Terzaghi, which fills in some of the detail of the image which surrounds him. As he approaches his eightieth year, we are grateful to those who made this collection and edited the work. Few men of this stature emerge in each generation.

Kevin Nash

MELBOURNE SYMPOSIUM ON ANTARCTIC METEOROLOGY

Antarctic Meteorology

Proceedings of the Symposium held in Melbourne, February 1959. (Commonwealth of Australia: Bureau of Meteorology.) Pp. xviii + 483. (London and New York: Pergamon Press, 1960. Published for and on behalf of the Director of Meteorology, Melbourne, Australia.) 105*s*. net.

NO criticism of the contributors to scientific symposia such as the one here presented would be implied by the comment that, in some cases, it may be doubted whether the papers are of such lasting value as to justify a hard-covered book selling at a high price. There are, after all, the appropriate scientific journals as media for publication. This particular symposium was, however, a special case, held under the official auspices of the Special Committee for the International Geophysical Year and the Special Committee for Antarctic Research shortly after the conclusion of the International Geophysical Year.

The symposium (*Nature*, 183, 1503 ; 1959) was in fact a meeting of workers from the International Geophysical Year expeditions, then on their way home. In view of the fact that it was held so soon after the observations had been made, and with so little time for digestion, the content appears remarkably good. As might be expected from the numbers involved in this major scientific onslaught on Antarctica, the problems tackled are extraordinarily diversified—one dare not say comprehensive: there is, for example, little about oceanography or the sub-antarctic sea ice, though there is some interesting material for considering the role of the stratosphere; and, of course, local effects could only be sampled.

The forty papers printed were distributed between sessions on (1) local effects, (2) synoptic analysis and forecasting, (3) synoptic influences on lower latitudes, (4) general atmospheric circulation, (5) snow and ice characteristics, (6) heat and mass exchanges, and (7) climatology. In some of these fields, including both synoptic meteorology and the antarctic stratosphere, the book provides by far the richest reference material so far in print; the synoptic examples are, of course, largely drawn from a period far too short to be fully representative, and some of the contributors (if given longer experience in antarctic meteorology) might with advantage have drawn more on comparisons with earlier published synoptic situations. The work of Meinardus, Simpson, Palmer and Ramage—to quote a few—seems under-represented in the references used.

Until recent years only one or two general reference books existed from which the meteorologist working in antarctic regions, or his colleague concerned with antarctic effects on the weather and climate of the southern hemisphere homelands, could obtain guidance. The present work will be a very valuable addition to the libraries which such workers can consult—though for this purpose a book should have an index. It seems likely to take its place, for several years to come, among the four or five most used works on antarctic meteorology.

H. H. Lamb

MATHEMATICS AND LANGUAGE

Type-Token Mathematics

A Textbook of Mathematical Linguistics. By Dr. Gustav Herdan. (Janua Linguarum, Series Maior, Vol. 4.) Pp. ii+448. (The Hague: Mouton and Co., 1960.) 54 guilders.

THE application of statistical methods to linguistic problems goes back at least as far as Malone's suggestion (in 1778) that the order of writing of Shakespeare's plays might be ascertained by such tests. In the past fifteen years it has gained additional interest from the developments of 'information theory' and projects for mechanical translation. Various methods have been evolved, and some quite novel statistical techniques discovered: but a laborious search of both statistical and linguistic publications is needed to discover what has been done. A book which attempts a survey of the whole of this field therefore deserves some welcome.

Type-Token Mathematics has much the same scope as Dr. Herdan's earlier book *Language as Choice and Chance* (1956). It is considerably more than a rewritten and enlarged version, as it contains both new ideas and new material; several chapters are reprints of recent papers by the author. Its material falls into two parts (closely commingled): mathematical and philosophical. The mathematical parts are vitiated, unfortunately, by the extreme shakiness of Dr. Herdan's statistical technique: the innocent linguist must be urgently warned not to adopt any procedure that he recommends without advice from a statistician. Two examples will suffice to put the statistician on his guard: it is not true that if we use a significance test at the 1 per cent level "we shall be wrong once in 100 times with our assumption of a real difference" (page 105); and it is not true that "deviations [from the mean] exceeding three times the standard deviation" are very unlikely to occur by chance (page 287) when we are considering a variate which has (approximately) a Poisson distribution with an expectation of less than one (as on page 290). In the latter passage, it is regrettable that Dr. Herdan gives unreliable formulæ for α (the expected frequency in a second sample of a word occurring a small number of times in the first) and its variance, as the techniques for making reasonably accurate estimates form one of the most interesting developments of statistics applied to language. Dr. Herdan nowhere mentions these techniques, although his bibliography includes the paper containing them (Good, 1953).

The philosophical portions of the book are occasionally stimulating, but more often irritatingly vague and confused. A fairly extensive bibliography is given; unfortunately it fails to include several of the references in the text, leaving the reader to search for, for example, "Oettinger, 1957", with no further clue. In view of the sub-title it should be added that the book is essentially an exposition of Dr. Herdan's own, generally controversial, views—a perfectly proper purpose for a book in general, but most inappropriate for a 'text-book'.

A reader whose knowledge of both statistics and information theory is sufficiently sound to save him from being misled or confused may find some interesting material and some interesting ideas in the book, but is unlikely to find it good value for money. The beginner in these fields must be, reluctantly, warned to keep off it.

G. H. Toulmin

MATHEMATICS AND QUEUEING

Mathematical Methods in the Theory of Queueing

By A. Y. Khintchine. Translated by D. M. Andrews and M. H. Quenouille. (Griffin's Statistical Monographs and Courses, No. 7.) Pp. 120. (London: Charles Griffin and Co., Ltd., 1960.) 32*s*.

THIS beautiful little book breathes reason and modesty from cover to cover. It is written somewhat in the style of the classical French texts of fifty and more years ago—a style which allowed sufficient space for an honest exposition, and was not cramped by the present-day fashion for condensation and the pressure of costs. The publisher and the translators are to be congratulated for introducing the work to the English-speaking world, and the book will be of undoubted value to every mathematician interested in the theory of queues. A particularly pleasing feature is the way in which the results are developed; the mathematics is 'done', not merely indicated, and nowhere does the author state a result and refer the reader elsewhere for the details. Thus there is a satisfying aspect of completion about the exposition.

Part 1 is devoted to the theory which depends only on the arrivals and not on the nature of the service-time distribution. A 'simple' stream is defined to be one which displays stationariness, absence of after-effects, and orderliness; that is, the probability of k arrivals in any interval of time depends only on the length of the interval, and not on any arrivals before the start of the interval, and there cannot be two or more arrivals at the same instant. The theory of the simple stream is developed, and then extended by relaxing one or more of the conditions just stated. In particular, some properties of the stream without orderliness are obtained, and streams with limited after-effects are investigated.

In Parts 2 and 3 problems of service are considered. In a queueing system with several lines of service (in parallel) a customer on arrival may find that there is no service immediately available. He may wait until service is available, or may leave the line without waiting. In the former case one speaks of a "system allowing delay", in the latter case of a "system with loss". It is perhaps worth pointing out that Chapter 11 (the last) presents the general theory of a single-line system with an arbitrary service-time distribution. The distribution function for the waiting time is found in principle by a fairly simple method. The contents of the chapter are taken from a paper by the author, published in 1932, and present material which will be new to many readers.

The theory of queues has undergone considerable development in recent years. Some mathematicians think the development has gone too far. Whether this is so or not the book under review will serve to show that the phenomenon of queueing represents another human experience which has bowed to the forces of applied mathematics; the concepts that have been built around this experience have proved to be of the right kind, and sufficient in number, for the mathematical development to go 'with a bang'. Anyone who is in doubt as to whether applied mathematics is a living subject will find it rewarding and reassuring to read this book.

L. S. GODDARD

ARCHÆOLOGY OF OLD SMYRNA

The Annual of the British School at Athens
Nos. 53–54, 1958–1959. Pp. xii+312+74 plates. (London: The British School at Athens, 1960.) 105*s*. net.

WITH this number begins the serious publication of Old Smyrna, dug some ten years ago. Most of the articles on other subjects, to take these first, are also rather austere. Sinclair Hood and Piet de Jong describe a Minoan kitchen, Sinclair Hood, G. Huxley and N. Sandars a cemetery of the Late Minoan III Period, both near Knossos. Analysis of amber beads leaves their provenance uncertain. J. Leatham and Sinclair Hood describe some submarine work on sunken fish-ponds and harbours; work which, like the Mycenæan boat off Lycia, confirms that at present diving offers the bleakest prospects in archæology. R. Hope Simpson finds the rag of a Mycenæan settlement near Menidi, in Attica. J. M. C. Toynbee describes four interesting Roman portraits, dating from the Claudian to the Decian Period, in the Piræus Museum; and John Boardman describes a farmhouse inhabited about 450–350 B.C. at Pindakas in Chios, a site remarkable chiefly for a coin-hoard of about 350 B.C. Boardman has interesting things to say about the first-named 'magistrates' issues of Chios, and about her earliest bronze coins, which he would date about 375 B.C.

J. M. Cook introduces Old Smyrna in a topographical and historical résumé—a model of masterly brevity badly needed in this volume. The settlement at the north-east end of the Gulf goes back to Protogeometric times. "Aeolic" at first, it had become Ionic by about 800 B.C., which might agree with the story of its seizure by Colophon. The eighth century was the era of rounded houses. An earthquake about 700 B.C. destroyed the walls, which were not immediately replaced, while the new houses were rectangular. About 600 B.C., when a new wall had scarcely been finished, a new temple only begun, the Lydian Alyattes raised a siege-mound (still surviving, to the north-west) and took the city. After that, though quite densely inhabited by the fourth century, Old Smyrna never recovered its status, and was deserted for the present site before 300 B.C. J. K. Anderson, who describes the Corinthian pottery found, shows that Alyattes sacked the town well before the Early Corinthian style had run its course. Prof. Cook finds space for a mass of topographical information, subsidiary but never irrelevant.

John Boardman describes the Attic vases, some from good workshops. But the core of this instalment is an extremely painstaking hundred pages, by R. V. Nicholls, on the City Walls. The illustrations, through all their intricacies, maintain a high level of scientific exactitude and consistency; although even Mr. Nicholls grows weary in time of using all the symbols and *sigla* recommended by Sir Mortimer Wheeler, and finds (for example, in Figs. 7 and 10) that he can simplify with advantage. Somewhere in the text are many excellent points; for example, that the mound to the south-east of Gordium could also be a siege-mound, that the staked ditch of Iliad XII is more likely to be Iron Age than Bronze Age, that the step on the inner face of our Walls 2 and 3 might have supported a wall-plate to drain the walls' *chemi-de-ronde*, and that mud-bricks in the mid-seventh century changed to a size and shape more suitable for the new two-storey houses. It is, alas, typical that this last sensible conclusion should be mixed up with pages of metrological discussion (Nicholls believes, prematurely I feel, that only two constant lengths of foot existed and are known for the Classical Period.) These reach no firmer conclusions than one might expect, and lead by the way (p. 105) to such things as single bricks with dimensions on conflicting scales. Even Le Corbusier has not got to that. On important matters, like the arrival in the Greek World of the true arch, the citation of authorities seems most one-sided. For all its acuteness, the article is almost impossible to read. Page after page, one has to endure sentences like this: "The brick dimensions quoted on p. 101 are also of great interest in this regard, notwithstanding that it is not always possible to evaluate the different degrees of reliability with which they have been taken". Mr. Nicholls evidently knows his Herodotus and Homer. Yet, as stylists, they must mean nothing to him. Similarly, modern Athens has the most wonderful museums in the world, and the ugliest streets. Humanity, in its own home, has been sterilized.

HUGH PLOMMER

Genetica

By Prof. Enrique Sanchez-Monge y Parellada. Pp. xii+437. (Madrid: Enrique Sanchez-Monge y Parellada, Instituto Nacional de Investigaciones Agronomicas, 1961.) 380 pesetas.

THE present work by Prof. Sanchez-Monge is based on a previous book by him and Dr. Ramon Esteruelas published in 1952. The rapid development of genetics during the past few years has made rewriting necessary, though the objectives which animated the first work are unchanged. These were, states the preface, briefly to put into the Spanish language all the fundamental bases of modern genetics so that the reader may appreciate the importance of this discipline in pure and applied biology, medicine, psychology, agronomy, forestry, taxonomy, the improvement of plants and animals, microbiology and virology.

Such a book was highly necessary in Spain, where, it is alleged, outmoded ideas which should have been abandoned years ago still have a firm hold on the curriculum.

The present work differs in its general plan from the first one, principally in the change of title, and in the omission of two chapters on the improvement of plants and animals. It begins with an introductory chapter on reproduction, and then goes on to chapters on Mendelian heredity, qualitative and quantitative, the latter being preceded by a brief introduction to statistical analysis. It continues with an account of sex determination and sex-linked inheritance, types of evolutionary change, the role of cytoplasm in heredity, the genetics of populations and the physiology and nature of the gene. Then follows a chapter on genetical aspects of evolution, with special reference to mechanisms of speciation and to the species concept. Finally there are two chapters dealing with the genetics of micro-organisms and of man. Considering the wide field that the author has surveyed it is not surprising that treatment sometimes fails to achieve correct emphasis. For example, the chapter on human genetics contains no mention of the work of Penrose or Haldane, nor of the contributions of Harris in the domain of human biochemical genetics.

Five appendices contain: a short history of genetical discoveries, the international rules regarding symbols, a glossary of technical terms, a collection of problems and a list of recommended books. The book contains an admirably clear, concise and on the whole accurate account, in 437 pages, of present-day genetics, and is recommended to all students who read Spanish. It is well produced and illustrated and relatively cheap, though of somewhat inconvenient size. Each chapter contains a useful bibliography and there is a good index.

S. C. HARLAND

Proceedings of the Third World Orchid Conference

London, May 30–June 2, 1960. (Sponsored jointly by the American Orchid Society, The British Orchid Growers' Association and the Royal Horticultural Society.) Pp. 442+10 plates. (London: The Royal Horticultural Society, 1960.) 42*s*.; 6 dollars.

THE third World Orchid Conference, held in London at the beginning of June 1960, brought together more than five hundred persons, from thirty different countries, whose common interest was the study of the family Orchidaceae, albeit from many different points of view. Professional growers, amateurs and scientists were among those attending, and the numerous papers delivered, though with a distinct bias towards cultivation, covered a wide range of aspects of orchidology. This volume gives a general account of the programme and proceedings of the conference and includes all the papers delivered during its thirteen sessions.

The Conference was given an excellent send off by Dr. W. T. Stearn of the British Museum (Natural History), who entertained the members with a scintillating and highly humorous account of the development of orchidology from classical times up to the present day. Following this, each session dealt with some special aspect of the subject or with one of the more important groups of cultivated orchids. The former include such diverse subjects as culture methods for amateurs, the principles of orchid nutrition, the ecology of orchids, diseases and pests, aspects of the orchid trade, the classification of orchids, growing orchids in rooms, and orchids in botanical gardens. The papers on cultivated orchids deal with the genera *Cattleya, Vanda, Phalaenopsis, Cymbidium, Dendrobium, Cypripedium, Odontoglossum, Miltonia* and their close allies. These last-named papers include accounts of the latest methods of cultivation, progress in breeding and criteria used in judging. Altogether there are forty-eight papers and these taken together provide an adequate cross-section of the various activities which are being pursued to-day with respect to this remarkable and entrancing family.

The general format of the book is pleasing, the print being easy to read. There are ten excellent coloured plates of orchids which gained awards in the Royal Horticultural Society's Chelsea Show held just before the Conference and nearly thirty monochrome illustrations. In addition many of the papers contain illustrative text-figures. Altogether this is a book which everyone interested in orchids will require, containing as it does many of the latest ideas bearing on the cultivation, breeding and classification of these plants.

V. S. SUMMERHAYES

Advances in Agronomy, Vol. 12

Edited by A. G. Norman. (Prepared under the auspices of the American Society of Agronomy.) Pp. xi+464. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 12.50 dollars.

THE present volume fully maintains the standard set by recent issues of *Advances in Agronomy.* It contains eight reviews of United States work each of which is long, detailed, exhaustive and dull. Research papers are often summarized in single sentences and more-or-less contrary findings by different authors are recorded consecutively with serene impartiality and little or no critical comment.

Of the articles in this volume, that by Rich and Thomas on the clay fraction of soils is unusual in including many references to work done outside the United States and in dealing with recent work—real 'advances'. "Fertilizers in Forestry" (Stoeckeler and Arneman) also makes a good attempt at bringing together work done in many countries. Two papers on soya beans (81 pages in all) leave the reader with the impression that a great deal of research on this crop is proceeding, but that very little of it is likely to lead to increases in yield before 1980.

"Water Infiltration into Soils" (Parr and Bertrand) catalogues many fascinating pieces of apparatus and indicates the difficulties in measuring infiltration in the field. Fergus and Hollowell give a good historical account of red clover and research in progress on it; this paper includes a good account of husbandry in the field.

Of the rest, the paper on "Agronomy in the South-west United States" seems misnamed—it describes the agriculture of the area; the paper on grass and legume seed-production and testing omits all reference to the inoculation of legume seed, and in a long section on diseases dismisses bacteria, viruses and nematodes in one sentence. G. V. Dyke

Flora of Peru

By J. Francis Macbride. (Botanical Series, Vol. 13, Part 5, No. 1.) Pp. 536+ii. (Chicago: Field Museum of Natural History, 1959.) 8.50 dollars.

Mr. MACBRIDE continues his *Flora of Peru* with a volume devoted to the Araliaceae, Haloragaceae and a number of difficult gamopetalous families such as Ericaceae (including Vacciniaceae), Myrsinaceae, Ebenaceae, Gentianaceae, Apocynaceae and Convolvulaceae. The accounts of important genera such as *Buddleia* and *Strychnos* are also here. This work, as before, is a useful compilation as a basis for monographic work by specialists, and the author is an adept at pointing out the "interlocking characteristics" of the "species groups at present designated as genera" in some families. His numerous remarks in this vein force one to the conclusion that it is no good having 'specialists' in some of these families unless they have first-class brains and time for concentration on a wide survey over a long period. Mr. Macbride is old-fashioned enough to use the illegitimate name *Erythraea* for *Centaurium*, and he ignores the name *Gentianella* for a segregate genus covering the many Andine gentians, which he maintains in *Gentiana*. On p. 152, *Samolus Valerandi*, to which the South American *S. floribundus* Kunth is reduced, is unfortunately misprinted *S. Verlanderi*.

N. Y. Sandwith

The Atomic Problem

A Challenge to Physicists and Mathematicians. By Lancelot Law Whyte. Pp. 56. (London: George Allen and Unwin, Ltd., 1961.) 7*s*. 6*d*. net.

The author calls for a drastic revision of the principles of physics in order to find answers to unsolved problems in that branch of science. In particular he wishes to retain the "principle of atomism" and to discard "linear co-ordinate systems, dimensional parameters, two-entity interactions and time-invariants". Many of his propositions could be interpreted in different ways, but there is an unambiguous demand for a theoretical deduction of the fine structure constant; there is also a clear request for including irreversibility in the basic laws of the new physics. While not attempting to substantiate his programme the author expresses the hope that others might follow his directives.

The fundamental principles of a science are due for revision if unsolved problems prove *insoluble*. In contemporary physics this situation does not arise: existing theories have neither spent their force nor are they tied to any rigid preservation of all their details. Current attitude to irreversibility belongs to the best secured domains of physics. The problem of the fine structure constant will attract more attention than at present if it acquires any direct experimental implications. Perhaps the author believes that theoreticians are a complacent lot; they still continue to interpret new discoveries in terms of old theories unless incompatibility is clearly recognized. It would not be wise to prophesy now which of the author's propositions will materialize in a future revision. R. Eisenschitz

An Introduction to Infinitely Many Variates

By Prof. Enders A. Robinson. (Griffin's Statistical Monographs and Courses, No. 6.) Pp. 132. (London: Charles Griffin and Co., Ltd., 1959.) 28*s*.

The word 'Variates' in the title means 'random variables'. The first twenty pages give an account of probability theory as a normed measure over an abstract space. The next fifteen discuss limit theorems: followed by sixteen on Hilbert space and linear operators. These latter ideas are used in twenty pages on the spectral representation of stochastic processes and thirty pages on stationary processes. There is a large and useful collection of exercises. There is an extensive use of advanced mathematical ideas and notations, but practical problems are not entirely ignored (for example, section 7.9 on wavelet theory).

The monograph has been designed to give undergraduates an introduction to advanced techniques early in their statistical training. To my mind it is too condensed for this, and makes too many errors. Students at this level will not be familiar with the Radon-Nikodym theorem, and therefore will not know that it is stated wrongly on p. 13—nor the inversion theorem on p. 30—nor the definition of continuity on p. 19. But the book could be valuable for readers who can spot such mistakes and require a brief introduction to limit theorems and spectral analysis. The book would have been better had it been more carefully written and had it not been so curt, for the material is well organized. D. V. Lindley

Chemistry of Carbon Compounds

A Modern Comprehensive Treatment. Edited by Dr. E. H. Rodd. Vol. 4, Part C: Heterocyclic Compounds. Pp. xviii+1465–2201. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1960.) 130*s*.

With Volume 4 of this work the section on heterocyclic compounds is complete and the whole project draws near to its conclusion. This volume continues the systematic treatment of six-membered ring systems containing two hetero-atoms, proceeds to those with more than two and hence to seven-membered and larger heterocycles. Separate chapters present excellent accounts of azine dyes, of purines, of nucleosides and nucleotides and of pteridines. About half the volume is devoted to a survey of alkaloids, largely written by A. R. Pinder, with contributions by K. W. Bentley and G. F. Smith. They and the other contributors are to be congratulated on a high order of achievement. The more familiar terrain and the outer reaches of the subject are charted with competence and lucidity that belie the magnitude of the tasks involved.

This is no mere cataloguing, but a genuine appreciation and appraisal of the state of these chemical topics, close to the date of publication. Once again a team of authors and a redoubtable, indefatigable editor have put the world of organic chemists in their debt. James D. Loudon

THE CROSS-ANTENNA OF THE PROPOSED BENELUX RADIO TELESCOPE

By Prof. W. N. CHRISTIANSEN* and Dr. J. A. HÖGBOM

Benelux Cross-Antenna Project, Leyden

SEVERAL years ago, C. L. Seeger of the Netherlands Foundation for Radio-astronomy proposed[1] that a very large radiotelescope of the Mills cross type[2] should be built in Europe for high-resolution studies in radio-astronomy. The main feature of this radio telescope was to be its resolving-power of one minute of arc at a wave-length suitable for observations of discrete radio sources and of the structure of nearby galaxies.

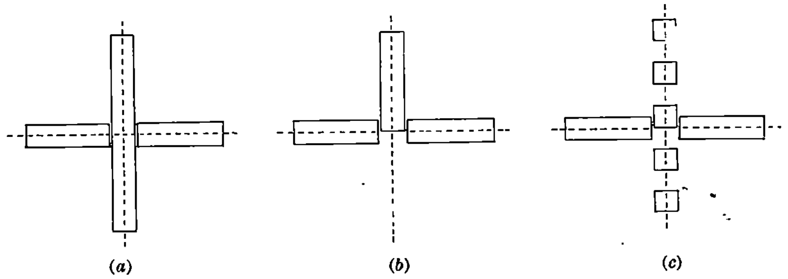


Fig. 1. Antennæ with similar beam-width. *a*, A Mills cross; *b*, a T-antenna; *c*, a displaced-centre grating cross

The dimensions of this antenna would be so large that it would be necessary to distribute radio-frequency amplifiers along the antenna in order to overcome a loss of sensitivity which would be introduced by severe attenuation in the transmission lines between antenna and receiver. It was suggested that these amplifiers could be made to serve an additional useful purpose, by using them in a way similar to that in the large grating-interferometer at Nançay in France[3]; the input of each amplifier would be connected to a small section of the antenna and the outputs of the amplifiers would be split into n separate channels. The output of each channel would then be correctly phased and combined with the outputs of one channel of each of the other amplifiers. The result of this is that each channel could be made to respond to radio emissions from a slightly different direction in the sky, so that with no loss in sensitivity the antenna would have n separately usable beams instead of one. By this means observing time can be reduced by a factor of n; the necessity for this is obvious when it is realized that with a

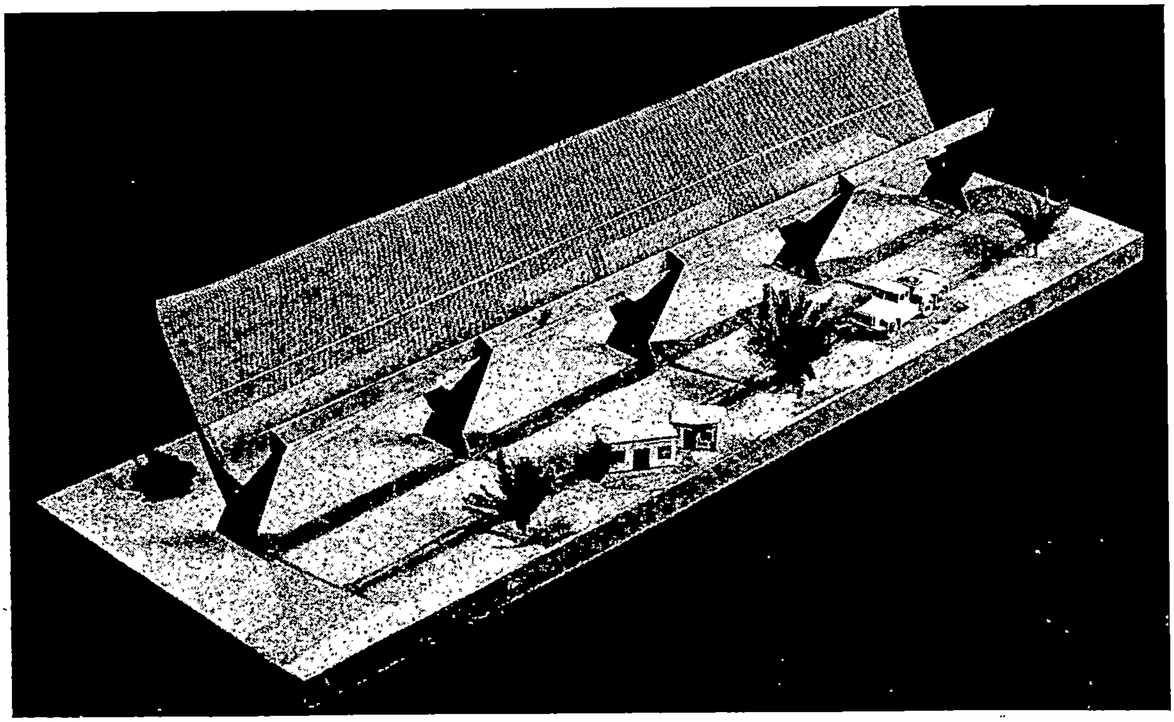


Fig. 2. A model of one section of the north–south array of the proposed Benelux cross. The dimensions are 15 m. × 85 m.

---

* On leave from the University of Sydney.

single 1′-arc beam, approximately 50 years would be needed to complete a survey of the sky.

As a result of this proposal, a committee with Netherlands and Belgian representation was set up under the chairmanship of Prof. J. H. Oort to investigate the possibility of building such an instrument. The committee is known as the Council of the Benelux Cross Antenna Project. A report was prepared[4] specifying the wave-length to be used (73 cm.), the size (600,000 m.^{2}), the resolving power (1′ arc) and emphasizing the necessity of designing the antenna to have very low response outside the region of the principal response.

Funds for the design of the large Cross Antenna were provided jointly by the O.E.E.C. Committee on Scientific and Technical Personnel, the Netherlands Organization for Pure Research (Z.W.O.) and the Belgian Ministère de l'Instruction Publique.

Recently, we have prepared a report[5] in which a suitable design for the cross-antenna of the Benelux radio telescope is submitted; we shall describe its main features here, since some of these are novel and may be of use to those interested in the planning of other large radio telescopes.

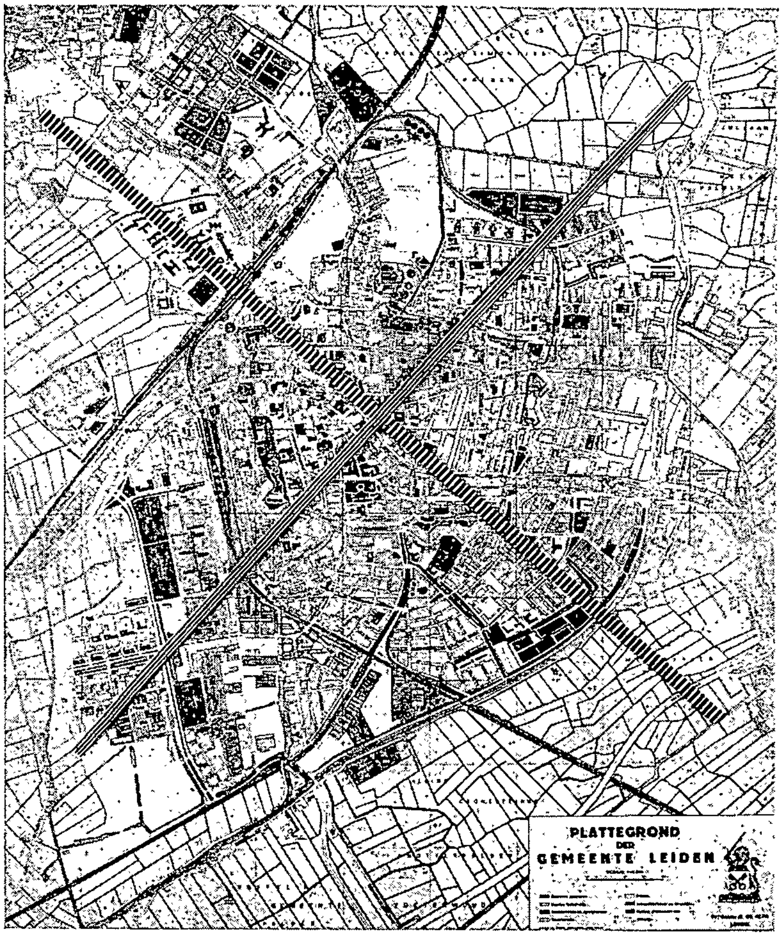


Fig. 3. Suggested arrangement for the 5,000 m. × 5,000 m. Benelux cross-antenna. The antenna is superimposed on a map of Leyden to provide a scale

The original Mills cross[2], which had a beam-width of 50′ arc, consisted of a north–south and an east–west array of parallel dipoles; the pencil-beam response of the antenna was produced at the intersection of the fan-shaped responses of the two arrays by a multiplication process first used by Ryle[6] in radio-interferometry. The angle of elevation of the principal response of the cross could be changed by readjusting the phases of currents transmitted to the receiver by the individual dipoles of the north–south array; this changed the angle of elevation of the fan beam of the north–south array and hence the direction in which this beam intersected the beam of the east–west array. The scanning of the sky in hour-angle was produced by the rotation of the Earth. One feature of this cross was that the 'steering' of the antenna response was effected without mechanical movement of any part of the antenna.

For an antenna which has an aperture (in square wave-lengths) three or four orders of magnitude greater than that of the first Mills cross, the use of simple fixed dipole arrays is impracticable, since the number of dipoles required is about 10^6–10^7 and the number of separately phased groups of dipoles is 10^4–10^5. The number of active elements in the antenna may be reduced by using parabolic reflecting surfaces; but these, except in special cases, must be capable of being tilted to allow the direction of the principal response of the cross-antenna to be varied.

In two of the large Mills crosses which are being built or planned, one in the U.S.S.R. and one in Australia[7], line arrays of dipoles are backed by cylindrical parabolic reflectors. For the east–west array these reflectors are capable of movement about the long axis of the array; in the north–south array the reflector is fixed, and the direction of the fan-beam response of the arm is varied by phase-changes in the dipole-array, situated in the line-focus of the parabolic reflector, in a similar fashion to that of the original Mills cross.

For the projected Benelux cross, which would have dimensions several times greater than these two crosses, such an arrangement would involve the separate phase-adjustment of more than 10^4 closely spaced elements for each change in declination of the antenna response. We have tried, therefore, to find a solution to the problem that will involve the phase-adjustment of only a limited number of elements. If the north–south array is divided into sectors each of which can be tilted in declination, the number of elements requiring separate phase adjustment can be reduced by a factor of about 100; moreover, the phasing difficulties are considerably reduced because the coupling between the sections can be made negligible. The difficulty which is introduced by this arrangement, however, is that each element tends to 'shadow' its neighbour as the array directed away from the zenith. If we separate the elements to avoid this shadowing, then multiple antenna responses are found; these are similar to those of a grating interferometer[8], and for the purposes for which the Benelux cross is designed, highly undesirable. We have found, however, that it is possible to overcome these difficulties.

It is known[9] that one of the four arms of a cross-antenna (Fig. 1*a*) can be removed without restricting the spatial harmonic response of the antenna, or in other words, without increasing the beam-width of the principal response of the antenna.

If, in such a T-antenna (Fig. 1*b*), we remove alternate sections of the asymmetrical arm, and place them on the opposite side of the antenna, as shown in Fig. 1*c*, then we have a cross-antenna in which one array is broken up into spaced sections. The unwanted grating-responses of this arm have been suppressed, however, by the displacement of its centre with respect to the centre of the continuous array. With this arrangement, we can now tilt the sections of the north–south array over a wide range of angles without having one section fall in the shadow of its neighbour.

It will be seen that the antenna design just described does not provide a complete solution to the problem, since the proportion of the north–south arm which is filled by the antenna elements changes with the angle of elevation. This, and non-uniformity of illumination of the elements of the north–south array, will produce the effect of a 'ripple' along the north–south array and unwanted secondary responses of the antenna will appear.

The unwanted responses can be reduced or eliminated if the response of the east–west array is made negligible in these directions, since the cross response in any direction is proportional to the product of the responses of the east–west and north–south arrays. One way to do this would be to make the elements of the east–west array larger (in the north–south direction) than those of the other array. Since there are considerable advantages in making the elements of both arrays similar, a better solution is to break up the east–west array into two parallel arrays, spaced so that zero responses fall in the direction of the principal unwanted 'ripple' responses. Alternatively one may break up the east–west array into four parallel rows instead of two, and this may have economic advantages; the suppression of unwanted responses, moreover, is slightly superior to that of the two-row arrangement.

With this design, in which all elements are similar and have the same orientation, it is obviously possible to use the very economical frame and stretched-wire type of construction for the parabolic cylindrical reflectors[9]. A photograph of a model of such an antenna, with an off-aperture line-feed designed for accessibility and to reduce unwanted responses, is shown in Fig. 2. Fig. 3 shows the general arrangement of the cross-antenna in which four lines of parabolic reflectors, each 5,000 m. × 15 m. in aperture, are used for the east–west array, and 240 elements, each 85 m. × 15 m., make up the north–south array. The performance of this cross-antenna has been calculated, and it has been found that the discontinuous structure of the north–south array does not produce any significant deterioration in performance when compared with the theoretical performance of a uniform structure. On the other hand, the number of places at which current phases have to be changed is relatively small, and these can be individually monitored, so that one might hope to keep a more accurate phase control than would be possible with a uniform structure in which very large numbers of elements are involved.

[1] Seeger, C. L., Benelux Cross Antenna Project (BCAP), *Tech. Rep.* No. 1 (1958).

[2] Mills, B. Y., and Little, A. G., *Austral. J. Phys.*, **6**, 272 (1953).

[3] Blum, E. J., *Ann. d'Astrophys.* (in the press).

[4] Van de Hulst, H. C., and Seeger, C. L., BCAP *Tech. Rep.* No. 2 (1960).

[5] Christiansen, W. N., and Högbom, J. A., BCAP *Tech. Rep.* No. 3 (1961).

[6] Ryle, M., *Proc. Roy. Soc.*, A, **211**, 351 (1952).

[7] Messel, H., *Nature*, **188**, 528 (1960).

[8] Christiansen, W. N., Mathewson, D. S., and Pawsey, J. L., *Nature*, **180**, 944 (1957).

[9] Ryle, M., and Hewish, A., *Mon. Not. Roy. Astro. Soc.*, **120**, 220 (1960).

ELECTRICAL PROPERTIES OF ORIENTATED LIPID ON A BIOLOGICAL MEMBRANE

AN ELECTROSTATIC DIFFUSION BARRIER AND ION PUMP

By Dr. J. W. L. BEAMENT

Agricultural Research Council Unit of Insect Physiology, Department of Zoology,
University of Cambridge

THE layer of lipid over the cuticle of insects is said to contain an orientated monolayer of lipid molecules on a substrate of the tanned lipoprotein of the cuticle membrane[1-3]. It is this monolayer which is believed to provide both the extreme impermeability of the cuticle to water[2-4] and also the unusual wetting properties which the surface may exhibit[4-7]. It has been suggested that the polar ends of the lipid are strongly attracted to the tanned lipoprotein, so that the monolayer remains stable even when, as in aquatic insects, the non-polar ends of the lipid are in contact with liquid water for long periods[5]. Now it is known that certain types of monolayer film at an air/water interface may be associated with surface potentials[8]; but as such films become unstable if a further aqueous phase is brought into contact with their outer surface, investigation of their electrical properties is largely confined to the use of an air-electrode in the form of a radioactive source. If, however, the insect cuticle does have such a monolayer, it should produce an electric potential; since that monolayer is stable in the presence of aqueous phases on either side, then those properties should be readily measurable. Investigation of the electrical properties of the membrane therefore presents a means of testing the hypothesis that a monolayer occurs, for that hypothesis has been based entirely on the study of the relationship between the

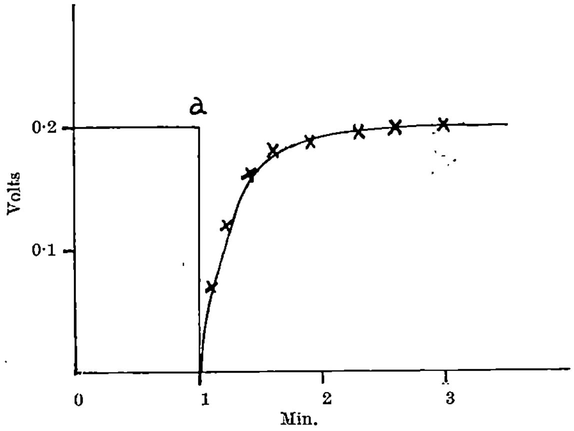


Fig. 1. Open-circuit voltage across a membrane preparation short-circuited for 5 sec. at *a*

membrane and water: either through measuring permeability or surface adhesion.

Electrostatic Potential across the Membrane

A membrane may be prepared from the integument of the cockroach *Periplaneta* by removing the cellular epidermis. When this membrane is arranged so that both sides are in contact with 1 per cent solutions of sodium chloride, there is a potential difference of the order of 200 mV. across the membrane, the morphological outside being positive with respect to the inside. Ten such membranes have given values between 170 and 235 mV., measured by a valve voltmeter of input impedance greater than 10^9 ohms. This potential depends on the grease (lipid) layer of the cuticle being intact, and can be destroyed by lipid solvents and by detergents. A potential difference of the same value is present across a cuticle membrane from which excess superficial grease has been removed by washing in the surface of clean water[2,5], that is, across a membrane believed to have only the orientated monolayer of grease on its surface. A similar electrical phenomenon has now been obtained with an artificial membrane coated on one side with grease from a cockroach (see below).

Using either a membrane with a natural thickness of grease (0·25μ) or with a monolayer, and with 1 per cent solutions of sodium chloride on either side, a small current will flow through a resistance connected between electrodes which dip into the solutions. If an ammeter of low resistance is used, a current of about 5 μamp. flows momentarily, and the potential across the electrodes falls very rapidly to an immeasurably low level. However, if connexion between the electrodes is then broken (except for the electrometer measuring the inter-electrode voltage) the original potential difference of 200 mV. reappears in the course of a few minutes. This process of recovery is exponential in form (Fig. 1), and by some 5–10 min. after 'shorting' the full potential difference has apparently been regained. The process of 'shorting' the electrodes may be repeated indefinitely—on each occasion the open-circuit potential of 200 mV. is re-established: one preparation has continued to behave in this fashion for 110 days with several hundred such treatments. But it is essential to maintain fresh solutions on both sides for recovery to be complete after short-circuiting in this fashion. If a high resistance is connected between the electrodes, then the potential difference across it falls rapidly to an intermediate value (for example, 50 mV. across a 22-M.ohm resistance in a typical experiment), and provided solutions of 1 per cent sodium chloride are maintained on both sides of the membrane, current continues to flow through the resistance. If the solutions on each side of the membrane are not maintained by perfusion, and the electrodes are connected through a resistance, the potential difference falls slowly. If the two electrodes are directly connected, and the preparation is left for several hours without perfusion, when the circuit is then opened, a lower potential difference than 200 mV. appears across the electrodes in the following few minutes.

Movement of Ions

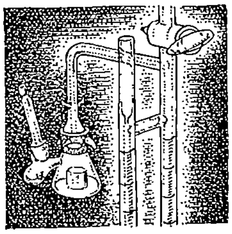
In a typical experiment, which started with two identical solutions of 1 per cent sodium chloride on either side of a membrane initially developing 205 mV. potential difference, the electrodes were short-circuited for 24 hr., and the circuit then opened; the potential difference was 90 mV. It was found that there was a difference of 2·1 *p*H units between the two sides—the solution against the morphological inside of the membrane having become more acid. A difference of 2·1 *p*H units between two solutions corresponds to 120 mV. This, added to the 90 mV. remaining across the electrodes, gives 210 mV., which is, within the experimental error of the methods used, in agreement with the initial open-circuit voltage of 205 mV. across the membrane at the start of the experiment. If the two sides of the membrane are then perfused with fresh 1 per cent sodium chloride solutions, the original potential difference of 205 mV. may be obtained. It is clear from this and other similar experiments that ions are transported across the membrane to produce two different solutions when electric current flows through the membrane, that is to say, the device will transport ions against a concentration gradient. Stable equilibrium, in the sense that no potential difference may be measured between the two sides of the membrane, is reached when the concentration potential between the solutions on either side counterbalances the electrostatic potential on the membrane; but this is reached only after several days.

Origin of the Electrostatic Potential in the Membrane

Using a membrane with a thick layer (0·25μ) of grease on it, and 1 per cent solutions of sodium chloride on both sides, a voltage may be applied across the preparation by connecting a suitable generator, such as a battery. If the membrane is momentarily subjected to an externally applied potential difference of 6 V. of the same polarity as the 200 mV. naturally found on the membrane, an open-circuit potential difference of about 4 V. can immediately afterwards be measured. These excess voltages are here called 'over-charge'. If the applied voltage be of opposite polarity to the natural potential difference of the membrane, then the open-circuit potential difference immediately after treatment is found to be reversed (reverse-charge). Intermediate values of over-charge and of reverse-charge potentials can be produced by using smaller polarizing

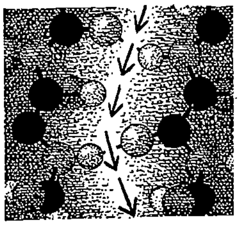
IT HAPPENED THIS MONTH...

a glance at yesterday in relation to today



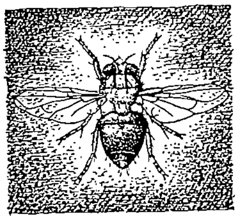
IN JULY—(1873)—Joseph Lister reviews a series of experiments supporting the germ theory of fermentation. This work utterly disproves the rival oxygen theory and shows all reported instances of spontaneous generation have been due to faulty experimentation. There is no evidence that any chemical possesses the one characteristic that distinguishes all true fermentation—self-propagation. However, it has been shown that emulsin, a peculiar albuminous principle existing in sweet and bitter almonds, can break up ten times its weight of amygdalin. "In this sense, then, as intervening between the growth of organisms and the resultant decompositions, the theory of chemical ferments might be welcomed as a valuable hypothesis."[1]

Within a short time, this theory became fully established, and the chemical ferments elaborated by yeast became known as enzymes. It has taken considerably longer for the self-propagating properties of certain chemicals to become a major subject of research. Currently the relation of the nucleic acids to genetic duplication and virus replication has become one of the focal points of research. Whether you are concerned with enzyme action or nucleic acid chemistry, the biochemical intermediates available from Schwarz BioResearch—plain or labeled with C^{14}, H^3, S^{35}, or N^{15}—may provide useful tools for your research.



IN JULY—(1933)—a report from the Lister Institute (London) considers the effect of proteolytic enzymes on the oxytocic hormone. Gulland and Macrae[2] find that two commercial trypsin preparations inactivate this hormone at widely different rates. A comparison experiment using quantities with equal tryptic activity clearly indicates that the inactivating enzyme is not trypsin. Nor is it a papainase, arginase, or prolinase. Aside from the fact that this inactivating enzyme accompanies proteolytic enzymes, there is no evidence adduced here that the oxytocic hormone contains peptide linkages.

Twenty years later, du Vigneaud received the Nobel prize for investigations which established the polypeptide structure of oxytocin through amino acid analysis, systematic degradation, and chemical synthesis. Chemists at the Yeda Research and Development Company at the Weizmann Institute (Israel) have synthesized a wide variety of peptides, polyamino acids, and intermediates for peptide synthesis which are distributed by Schwarz BioResearch. A special catalog listing these compounds is available upon request. Write for a quotation on your individual requirements.



IN JULY—(1953)—the Journal of Histochemistry and Cytochemistry discusses the function of the sarcosomes of insect flight muscle. It is suggested that these small spherical bodies play a role similar to that of mammalian mitochondria. ATP is synthesized in the sarcosomes by oxidative phosphorylation, and the energy-rich phosphate is made available for actomyosin contraction by diffusion into the adjacent muscle fibrils. Following contraction, ADP diffuses back into the sarcosomes (the sarcosomal membrane being impermeable to AMP), and the cycle is repeated.[3]

Adenosine phosphates have long been an important specialty in the Schwarz BioResearch line. We supply the various isomers of adenylic acid, as well as ADP, adenosine 3′:5′-cyclic phosphate, adenosine 5′-phosphoramidate, and several grades of ATP. The 5′-mono-, di- and tri-phosphates of guanosine, uridine and cytidine have recently been added to our list. Many of these compounds have been radiolabeled and all are listed in our regular catalog. Send for your copy.

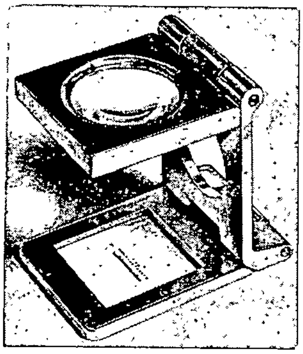
1. Lister, J.: On the germ theory of putrefaction and other fermentative changes. Nature 8:232 (July 17) 1873. 2. Gulland, J. M., and Macrae, T. F.: The oxytocic hormone of the posterior lobe of the pituitary gland. IV. The action of preparations of animal proteolytic enzymes, and some observations on the nature of the hormone. Biochem. J. 27:1383, 1933. 3. Levenbook, L.: Mitochondria of insect flight muscle. J. Histochem. 1:242 (July) 1953.

SCHWARZ BIORESEARCH, INC. • Dept. 7H • Mount Vernon, New York

BIOCHEMICALS • RADIOCHEMICALS • PHARMACEUTICALS *for research, for medicine, for industry*

MAXTA

FOLDING MICROMETERS



Here at last is the ideal pocket instrument to give you really accurate measurements. A clear cut and robust graticule is built into the base of a folding magnifier so that the image can be placed in contact with the object being measured. An accuracy of measurement down to 0.002″ is comfortably achieved.

A large variety of patterns is available to suit most requirements, but above all this micrometer is for on-the-spot measurement be it in drawing office, laboratory or on the factory floor.

Full particulars and prices gladly sent on request.

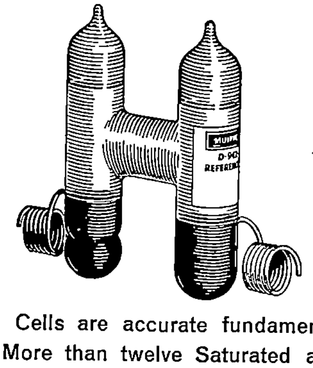
Graticules LIMITED

57-60 HOLBORN VIADUCT
LONDON, E.C.1
CENtral 2717-8

WESTON

CELLS

STANDARD AND INDUSTRIAL REFERENCE TYPES



MUIRHEAD Weston Cells are accurate fundamental standards of voltage. More than twelve Saturated and Unsaturated types available—choose one to suit your application. Ask for Publications 425 and 430.

MUIRHEAD

PRECISION ELECTRICAL INSTRUMENTS

MUIRHEAD & CO. LIMITED, BECKENHAM, KENT, ENGLAND
Telephone: Beckenham 4888
MUIRHEAD INSTRUMENTS LIMITED, STRATFORD, ONTARIO, CANADA
Telephone: 271-3880
MUIRHEAD INSTRUMENTS INC., 441 LEXINGTON AVE., NEW YORK 17, N.Y., U.S.A.
Telephone: Murray Hill 2-8131

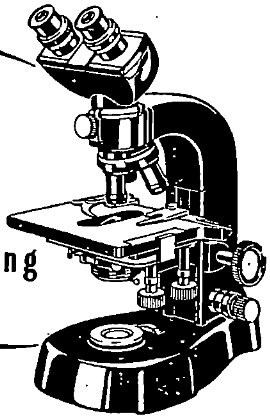
514

BECK

OPTOMAX

MICROSCOPE

Designed for the discriminating



Pubn. No. 251

R. & J. BECK LTD · 69/71 MORTIMER ST.
LONDON W.1
Pre-eminent for more than a century

The Scorah **OXY-COAL GAS BLOW PIPE**

specially for working BOROSILICATE GLASSES (Pyrex, Phoenix, Hysil, etc.)

- Five jets and central pilot light — flame size selected INSTANTANEOUSLY.
- Rotating turret head for easy jet selection.
- Auxiliary supply of oxy coal mixture for hand torch.

Minimum oxygen consumption
Maximum economy

A modern oxy-coal gas blow-pipe of sturdy construction evolved through years of practical experience in glass manipulation. A nearly silent flame from pin point to one capable of working 100 mm. tubing at a flick of the turret. To the inexperienced it gives confidence, to the experienced it is an assurance of efficiency and successfully completed work. A unique instrument for the research laboratory or workshop. Send for Leaflet BP/N2.

Obtainable only from

Price **£25.0.0**

L.V.D. SCORAH M.Sc. 44, NORTHFIELD RD.
BIRMINGHAM, 30
phone: KINgs Norton 1885

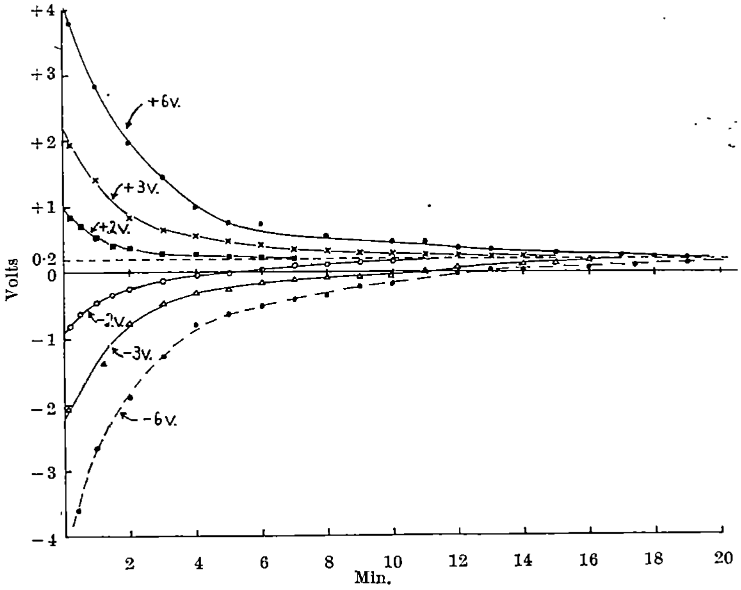


Fig. 2. Decay of over-charge voltages, and of reverse-charge voltages across a thickly greased membrane under open-circuit conditions. Labelling figures on each curve show the potential difference and polarity of the generator initially applied across the membrane to create the over-charge or reverse-charge. The untreated preparation has 200 mV. across it

voltages (Fig. 2), but potential differences in excess of 6 V. applied to the preparation will not raise the open-circuit voltage above 4 V. Both over-charge and reverse-charge potentials decay under open-circuit conditions over a period of about 20 min. to give the normal potential difference of 200 mV. (Fig. 2). ('Open-circuit' here means either with the valve voltmeter continuously attached to the electrodes, or momentarily attached to obtain potential difference values.) If, during the decay process, the electrodes are momentarily connected by a low resistance, the potential difference between electrodes falls to a very low level just as with a normal preparation (Fig. 1). And like a normal preparation, over the following minutes, the over-charged, or reverse-charged, preparation will produce a potential difference between electrodes when returned to open-circuit conditions (Fig. 3). However, the potential difference which reappears is that which the preparation would have reached by decay, if it had not been short-circuited. In other words, the process of recovery of the normal potential difference from conditions of over-charge or of reverse-charge is independent of the load placed across the membrane.

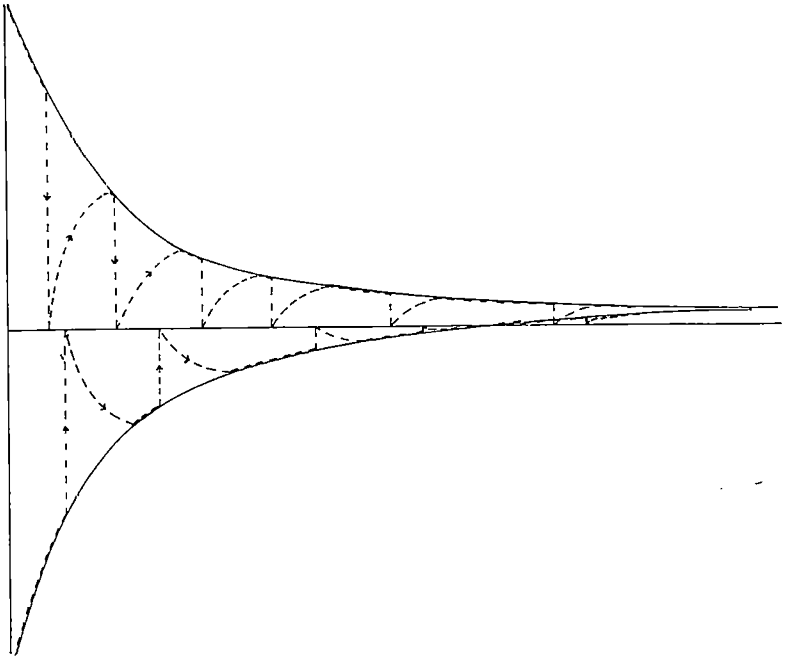


Fig. 3. Semi-schematic. The dashed line shows the form of recovery of the preparation of Fig. 2, over-charged or reverse-charged by 6 V., when repeatedly short-circuited momentarily during its decay. The natural decay is shown by the unbroken curves

However, when an external voltage source is applied across a water-washed membrane (which has only the basal monolayer on it), no such temporary over-charged or reverse-charged states can be produced. The excess potential difference created on the thickly greased membrane must therefore be associated with the grease above the monolayer. When an artificial membrane is used, covered on one side with a thick layer of grease, or with a monolayer only, little if any potential difference can at first be measured when the membrane is mounted and perfused with 1 per cent solutions of sodium chloride on both sides. A small polarizing voltage (0·5–1·0 V.) applied across the membrane will suffice to produce a standing potential difference of about 200 mV. from this preparation, and then it will behave almost identically to a membrane taken from a cockroach. If the grease is thick, temporary overcharge phenomena can similarly be obtained. However, if a polarizing voltage of reverse polarity is applied to an artificial membrane which has only one monolayer on its surface, then a relatively stable potential difference of about 200 mV. occurs across the preparation, with reverse polarity, that is, with the greased side of the membrane negative to the ungreased side. The best artificial membranes so far produced do, however, lose their open-circuit potentials in the course of a few days, despite maintaining fresh 1 per cent solutions on either side. They can, however, be repolarized by an externally applied voltage at any time.

It is useful, in explaining these phenomena, to make reference to the phenomenon of the 'electret'[9] as well as to the surface potentials of monolayers at air/water interfaces. An electret is a material having a permanent electrostatic state, which is analogous to the permanent magnetic moment of a magnet. It has been known for many years[10-12] that a permanent electrostatic moment can be produced in a block of a suitable wax by melting it and allowing it to solidify in a powerful electric field; it is perhaps significant in the present context that beeswax is among the materials most suitable for making electrets, and it has indeed been suggested that it is the long-chain primary alcohols (such as are believed to comprise insect waxes[3,13]) which are essential components in electret-forming waxes. Although controversy still surrounds the explanation of some electretic phenomena in physical and theoretical terms[14-16], it would seem most satisfactory to assume that electron-displacement towards the polar group along the long chain of the alcohol molecule gives it its electrostatic moment. Crystallographic evidence has been advanced to support the idea that, in a hard wax electret, crystallites of wax are all aligned, whereas in normal wax they are randomly arranged, and that such alignment has been brought about in the molten wax under the influence of the applied electrical field, with the result that all the electrostatic moments are fixed, orientated, when solidification occurs[16]. A hard wax electret is virtually permanent. (Swann[17] suggests that slow thermal derangement of the domains in an electret will occur, producing degeneration, but that the half-life of the system is several years).

So far as the monolayer of grease on cockroach cuticle is concerned, the aligning force acting on the polar molecules is the high adhesion of the polar groups to the tanned lipoprotein substrate of the cuticle surface. Since this will also align the electrostatic moments of the molecules, an electret voltage would be expected to appear. It is interesting that a potential difference of 200 mV. across the probable thickness of the grease monolayer is the equivalent of an electrostatic gradient of 20,000 V./cm., which is precisely the highest electrostatic gradient quoted by several investigators of hard wax electrets[14,15,17]. Now according to the theories proposed from experiments on water permeability and surface properties[2-5,7], the bulk of the grease overlying the monolayer on a natural insect cuticle is not molecularly organized—on the cockroach it is a viscous fluid, and thermal agitation will ensure that the molecules are randomly arranged. Hence both the natural cuticle of the cockroach, with its thick grease, and also the cuticle washed by a water surface until only a monolayer remains, would be expected to produce the same electretic potential difference corresponding to one monolayer—which is found experimentally. If, on the other hand, an electric field is imposed on the thick grease, temporary alignment of all the grease molecules will be produced, and immediately after the application of such a polarizing field, the phenomena of over-charge or of reverse-charge, reported above, appear. The decay of these higher-energy states of induced molecular alignment is a direct consequence of the thermal derangement of the molecules in the thick grease (which may be from a normal or from an inverted orientation, depending on the polarity of the polarizing voltage which was applied); but the basal monolayer cannot be so deranged, and the preparation returns to a potential difference of 200 mV., regardless of whether it is short-circuited or not. The decay cannot, in fact, be influenced unless some further energy source is introduced into the system.

In a monolayer preparation, over-charge and reverse-charge potentials cannot be induced, because there is no thick grease to align. On the artificial membranes so far used, it is clear that the adhesion of the polar groups in the grease to the substrate is by no means as great as it is to insect cuticle. A combination of thermal agitation and electrical strain will thus prevent the formation of a good monolayer *ab initio*; but in an appropriately polarized electric field applied across such a membrane, a monolayer can be aligned—or indeed an inverted monolayer can apparently be stabilized. When this monolayer is inverted there are some indications that the surface adhesion of the monolayer to water is greatly increased, so that we have a direct confirmation of the theory of monolayer inversion and the production of hydrofuge surfaces already proposed[5]. However, it is seen from the experimental results cited here that, on an artificial membrane, the polar attractions to the substrate are not sufficient to maintain the monolayer against thermal and electrical strain, and it very slowly decays, whereas the structure on the more efficient substrate of cuticle is seemingly permanent. This adds to the conclusion already reached on different grounds[3,6] that considerable energy is needed in the construction by the insect of its monolayer.

Energies of the Ion Pump

It must be clearly understood that a monolayer, with its distribution of charge, does not represent a 'battery' or source of energy, any more than a hard wax electret does. In the case of the hard wax electret, current will flow temporarily through a wire connecting two plates applied to the two sides of the block of wax. If the plates are repeatedly removed and replaced, current may repeatedly be drawn: the energy for this electric power is put into the system by the process of applying and removing the plates. In the case of the cell, made up of a permanently charged membrane, with identical solutions on either side, current can only be obtained in the external load for so long as the two solutions are maintained. Consider the well-known phenomenon of the diffusion or concentration cell (Fig. 4*A*). A potential difference is generated between two solutions of different composition separated by a membrane which is electrically inert, but through which components of the two solutions may diffuse. The potential difference between the two solutions is determined by the difference between the composi-

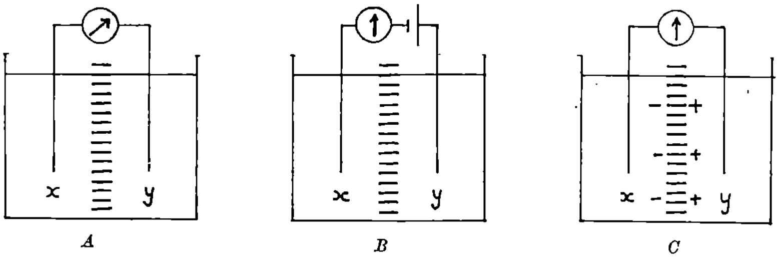


Fig. 4. See text

tions of the two solutions; it disappears when the solutions have become the same through diffusion, and the system has reached a state in which there is no thermodynamic difference across the membrane. However, if the changes in the two solutions, due to diffusion through the membrane, are countered by perfusing the two sides of the membrane with their respective original solutions, the potential difference across the membrane can be maintained indefinitely; energy is introduced into the system in removing the products of diffusion.

If a source of potential difference (not of energy) is inserted into the leads connecting the electrodes of such a cell (Fig. 4*B*) current does not flow in the external circuit when the two solutions are at compositions which are not identical, but which are such as to generate a potential difference equal and opposite to that of the introduced source of potential. This represents the equilibrium state in which energy cannot be derived from the difference between the solutions. However, this situation is electrically identical to one in which such a source of potential is introduced at another point in the circuit—across the membrane (Fig. 4*C*). It follows that if identical solutions are placed on both sides of a membrane with an electrostatic potential across it, the composition of these solutions will tend to change until they establish an electric potential exactly compensating that on the membrane, and that this condition will represent thermodynamic equilibrium in the system. It further follows that, in the device described in the early part of this article, the source of energy for the continuous flow of current through an external load is provided by the perfusing solutions of 1 per cent sodium chloride, in displacing the concentration difference which the membrane is endeavouring to establish on either side of itself.

The biological consequences of this system cannot as yet be fully foreseen. An electrostatically charged monolayer of lipid is capable of maintaining two solutions of different composition, concentration, *p*H, etc., on either side of itself, without the expenditure of energy, that is, a thermodynamic device of great biological advantage; it must be remembered that this it can only do because of the enormous amount of potential energy stored in the monolayer, both in its orientation order and also in its bonding to the protein substrate. Maintenance is not, however, necessarily a static matter; if the solutions on either side change from such compositions as to offset the membrane potential, ions may be transferred to counter that change. The membrane may, therefore, be the integral part of a biological 'ion pump'; the energy for this pump is not derived from any 'carrier' used in the transport process, but is a direct consequence of the organization of the membrane itself. Biologically speaking, the energy for using the pump might be provided by the manipulation of the concentrations of materials on either side of the monolayer. It is appreciated that, according to current ideas, cell membranes contain a bimolecular leaflet of lipid, rather than a monolayer, but it would be necessary only for there to be a net electrostatic field from the algebraic sum of the moments of the monolayers comprising the leaflet, for the system outlined in this article to operate; of course, across a complex differentiated bimolecular layer, local variations in potential and polarity might be very considerable.

I am grateful to Drs. R. H. J. Brown, K. E. Machin and J. W. S. Pringle for discussions during the course of this work.

[1] Beament, J. W. L., *J. Exp. Biol.*, **21**, 115 (1945).
[2] Beament, J. W. L., *J. Exp. Biol.*, **35**, 494 (1958).
[3] Beament, J. W. L., *Biol. Revs.* (in the press).
[4] Beament, J. W. L., *J. Exp. Biol.*, **36**, 391 (1959).
[5] Beament, J. W. L., *Nature*, **186**, 408 (1960).
[6] Beament, J. W. L., *Symp. Soc. Exp. Biol.*, **15** (in the press).
[7] Beament, J. W. L., *J. Exp. Biol.* (in the press).
[8] Adam, N. K., *The Physics and Chemistry of Surfaces* (Oxford Univ. Press, 1951).
[9] Eguchi, M., *Phil. Mag.*, **49**, 178 (1925).
[10] Jaeger, P., *Ann. Physik.*, **21**, 481 (1934).
[11] Theissen, P. A., Winkel, A., and Herrmann, K., *Physik. Z.*, **37**, 511 (1936).
[12] Mikola, S., *Z. Physik.*, **32**, 476 (1925).
[13] Beament, J. W. L., *J. Exp. Biol.*, **32**, 514 (1955).
[14] Gemant, A., *Phil. Mag.*, **20**, 929 (1935).
[15] Gross, B., *Phys. Rev.*, **60**, 360 (1944).
[16] Nakata, K., *Proc. Phys. Math. Soc. Japan*, **9**, 179 (1927).
[17] Swann, W. F. G., *J. Franklin Inst.*, **250**, 219 (1950).

VIRTUAL ABSENCE OF GLUTATHIONE INSTABILITY OF THE ERYTHROCYTES AMONG ARMENIANS IN IRAN

By Dr. J. E. BOWMAN
Department of Pathology, Shiraz Medical Centre, Nemazee Hospital, Shiraz
AND
Dr. D. G. WALKER*
Department of Biochemistry, University of Shiraz, Iran

THE glutathione (GSH) stability test of the erythrocytes developed by Beutler[1] and studies by the University of Chicago group[2] have resulted in the discovery of a genetic erythrocytic defect, namely, an absence or a deficiency of the enzyme glucose-6-phosphate dehydrogenase. This erythrocytic defect is found in some ethnic groups but not in others[3-6]. In general, American and North European Caucasians, Askhenazic Jews, American and Peruvian Highland Indians and Japanese do not have the defect. However, American Negroes, Sephardic Jews, Sardinians, East and West Africans, and some Asians, have the genetic defect to an extent such that tests for deficiency of this enzyme have become valuable anthropological tools. Recently we reported that approximately 8 per cent of Moslems in Iran are sensitive by the GSH stability test[7]. Several tribal groups studied thus far have also shown incidences of the

---

* Present address: Department of Biochemistry, University of Birmingham, Edgbaston, Birmingham 15.

defect similar to the Moslem group and even higher, for example, an incidence of 15 per cent in the Mamassani tribal group[8].

This report is concerned with an examination of 163 Armenians, 153 of whom reside in New Julfa near Isfahan. The subjects comprised 152 males and 11 females. Of this group, only one male was found to be sensitive by the GSH stability test of the erythrocytes, making this population the first thus far studied to be virtually negative for the defect in Iran. Unfortunately the one 'sensitive' person could not be traced for requestioning as to possible admixture with another ethnic group. Blood samples were taken from apparently healthy subjects only, but this does not exclude the possibility that this male could have had congenital non-spherocytic hæmolytic anæmia, which is another condition where erythrocytes from such persons also show the enzymic defect[5]. The virtual absence of the genetic defect in Armenians is of greater importance than the finding of the defect in this ethnic group.

According to Montague[9], Armenians are a composite of an Atlanto–Mediterran component and a greater Irano–Afghan element with some Nordic and Alpine elements. From studies of *ABO* blood groups published thus far[10], the Alpine component is very strong for there is an extremely high frequency of the *A* gene. This was confirmed in our *ABO* studies on this population (gene frequency : *p*, 0·3894 ; *q*, 0·1084; *r*, 0·5022, D/σ, + 0·3). The *cde* gene frequency was 0·3941, which is also European. These studies of blood groups of Armenians are distinctly different from those of Boué and Boué[11] on other populations in Iran and our own unpublished observations.

The Armenians once occupied a large part of the area which was the seat of the Hittite Empire (present-day Turkey) and they are believed to be descendants of these people. It is of interest to note that authorities are divided as to whether the Hittites, with their Indo-European language, came originally to Anatolia about 2,000 B.C. from the north-west from northern Europe or from the north-east across the Caucasus Mountains[12]. Be that as it may, there can be no doubt that there is a distinct northern European influence in Armenians from the present study and those of other workers. This northern European influence is strong, despite the fact that the Armenians throughout their long history and by virtue of being situated along the trade route between East and West have been in association with diverse ethnic groups which have and probably did have the gene for glucose-6-phosphate dehydrogenase deficiency, such as Phœnicians, Africans, Jews, Greeks and Iranians.

The Armenians in New Julfa were brought to Iran as Christian captives in 1604 and 1605 by Shah Abbas the Great from Julfa in what is now Soviet Armenia[13,14]. Rather than being treated as slaves, they were encouraged to create a prosperous trade centre between East and West in the area of Isfahan. The original number was about 25,000 ; the present population is about 6,000[14]. The decrease in size is due to movement of many to other parts of Iran and to other countries, mainly India. While religious intolerance has not been permitted in Iran for many centuries, the Armenians were not encouraged to intermarry with the surrounding population. According to Dr. Caro Minasian, an Armenian physician and scholar, Armenians who have intermarried live outside of the area of New Julfa. This fact is supported by our finding of a virtual absence of the erythrocytic defect and by the blood group data. This population is thus an excellent example of what has been termed 'religious isolates' by Montague[9]. Since the original group brought to Iran was fairly large, it is unlikely that the genetic defect was present in the population and then lost during the last 355 years as a result of 'genetic drift'.

Of further interest is the fact that both the area in which the Armenians now live and their original homeland have been, until recent years, regions of high endemicity so far as falciparum malaria is concerned[15]. Several reports have noted that the gene for glucose-6-phosphate dehydrogenase deficiency coincides with the incidence of falciparum malaria in many parts of the world and have suggested that this harmful gene might possibly be maintained as a result of balanced polymorphism with respect to this disease[6,16]. The Armenians in the Isfahan region represent one group which has resided in a malarious area for centuries without the appearance of this protective effect.

During the course of the survey, the subjects and others in the area were questioned about the ingestion of fava (broad) beans, which are agents having an adverse effect on carriers of the genetic defect[8]. Although practically all of them ate fava beans regularly in the spring of the year, neither the subjects nor the physicians in the New Julfa area were aware of any difficulty as a result of eating this food. This is unlike our experience with the Moslem and Jewish groups in Iran. Most of these people are familiar with the possible harmful effect of the *Vicia faba* bean.

It appears to be very important that other workers who have access to Armenians should make studies comparable with ours. The findings of either a high incidence of the genetic defect or a virtual absence such as we have found in Armenians in New Julfa would be of great interest to anthropologists and geneticists.

We wish to express our appreciation to Dr. Caro Minasian for making this survey possible and for his clarification of many points regarding the history of the Armenians. We thank Messrs. Galoost Galestian, Wayburn D. Davies and Parviz Haghighee for assistance on the survey, Misses Annerose Oemisch and Iran Haffarian for technical assistance in the laboratory and Dr. Garegin Saroukhanian for his translation of several Russian sources on malaria in Soviet Armenia.

[1] Beutler, E., *J. Lab. Clin. Med.*, **49**, 84 (1957).

[2] Carson, P. E., Flanagan, C. L., Ickes, C. E., and Alving, A. S., *Science*, **124**, 484 (1956).

[3] Alving, A. S., Kellermeyer, R. W., Tarlov, A., Schrier, S., and Carson, P. E., *Ann. Int. Med.*, **49**, 240 (1958).

[4] Beutler, E., *Blood*, **14**, 103 (1959).

[5] Childs, B., and Zinkham, W. H., in Ciba Foundation Symposium on Biochemistry of Human Genetics, 76 (Churchill, London, 1959).

[6] Motulsky, A. G., *Human Biology*, **32**, 28 (1960).

[7] Walker, D. G., and Bowman, J. E., *Nature*, **184**, 1325 (1959).

[8] Walker, D. G., and Bowman, J. E., *Proc. Soc. Exp. Biol. Med.*, **103**, 476 (1960).

[9] Montague, M. F. A., *An Introduction to Physical Anthropology*, 387, 454 (Charles C. Thomas, 1960).

[10] Mourant, A. E., *The Distribution of the Human Blood Groups*, 38 (Blackwell Scientific Publications, 1954).

[11] Boué, A., and Boué, J., *Sang*, **26**, 705 (1955).

[12] Ceram, C. W., *The Secret of the Hittites: The Discovery of an Ancient Empire*, 92 (Alfred A. Knopf, New York, 1956).

[13] *Encyclopaedia Britannica*, **2**, 378 (1955).

[14] Minasian, Dr. Caro A. (personal communication).

[15] Saroukhanian, Dr. Garegin (personal communication).

[16] Allison, A. C., *Nature*, **186**, 531 (1960).

OBITUARIES

Prof. H. W. Turnbull, F.R.S.

By the death of Prof. Herbert Westren Turnbull at Grasmere on May 4, Britain has lost a distinguished mathematician of the older school, while many mathematicians in England and Scotland have lost a personal friend.

Herbert Turnbull, a son of W. Peveril Turnbull, H.M. inspector of schools, was born in Wolverhampton in 1885. From Sheffield Grammar School he went as a scholar to Trinity College, Cambridge, being Second Wrangler in 1907, first in the first division of Mathematical Tripos II in 1908, and Smith's Prizeman in 1909. After a varied experience of lecturing at Cambridge, Liverpool and Hong Kong, he seemed for a few years to be likely to follow his father's career, teaching as he did at Repton for three years and becoming an H.M. inspector of schools. This pattern was quite altered in 1921 by his appointment to the chair of mathematics in the United College, University of St. Andrews.

The result was to associate his name permanently, and especially to Scottish mathematicians, with St. Andrews. He wrote there the great majority of his sixty or more published papers, and founded a school of algebra and geometry of the older type which, though younger mathematicians have moved to the more modern abstract disciplines, had value in its day. Turnbull was inspired immediately by J. H. Grace and Alfred Young, his associates and seniors by slightly more than a decade at Cambridge, and through them by the Continental masters Clebsch, Gordan and Capelli, with Cayley and Sylvester beyond again. The realm most congenial to him was that of the invariants and covariants of algebraic forms, as investigated by the Clebsch–Aronhold symbolic calculus, of which he became the last and in some ways perhaps the greatest master; but he was constantly aware of the applications to projective geometry. Also, when in 1927 Alfred Young began his new attack on invariants with the Frobenius–Schur group algebra of the symmetric group, Turnbull was fully alive to the new range thus opened, and some of his work consists of a reconciliation of the symbolic with the group-algebraic approach. His name will also be associated with memoirs and text-books on matrices and determinants.

An interest in the history of mathematics first found utterance in a small book, *The Great Mathematicians* (1929); but the discovery of hitherto unknown documents relating to the work of the Scottish mathematician, James Gregory (1638–75), produced his first historical work on a larger scale, the *James Gregory Tercentenary Memorial Volume*. Felicitously, this was ready in time for the James Gregory tercentenary celebrations which, somewhat resembling the Napier tercentenary celebrations of 1914, took the form of an international congress of the Edinburgh Mathematical Society—the University of St. Andrews and the Royal Society of Edinburgh being sponsors. This occasion, too soon overshadowed by rumours of war, will be remembered by many as the special one in which Turnbull, in the prime of life and ably seconded by his wife, showed himself as the host of hosts and moreover as an excellent musician, for he was a pianist of almost professional standard. To Scottish mathematicians these qualities were well known, in the annual June meeting of the Edinburgh Mathematical Society at St. Andrews and its four yearly colloquia held there.

Retiring in 1950, Turnbull as emeritus professor devoted himself to his second and major historical work, the editing of the correspondence of Isaac Newton. He lived to see the first volume, handsome and impressive, appear in 1959, and to know that the second was well under way.

His character was founded on the simplest faith, absolute sincerity and the desire to serve. His many friends, in their sympathy extended to his wife and their son Derwent, will remember the man first, and then the mathematician. A. C. Aitken

Prof. C. G. Jung

Carl Gustav Jung, who died on June 6, was born on July 26, 1875, at Kesswyl, in Switzerland. His father was a clergyman and he was brought up in his parsonage in the country. He attended school at Basle and was educated in medicine in that city, where he graduated.

In 1900 he became assistant in the State Mental Hospital and in the psychiatric clinic of the University of Zurich, later becoming senior staff physician there until 1909.

In 1902 he spent six months in Paris attending P. Janet's lectures and worked in Zurich with E. Bleuler. He first met Freud in 1906.

When Freud put forward his ideas and discoveries he had been greeted with scorn and derision until Bleuler made it known that his views were regarded sympathetically by the Zurich school. The interest of the Swiss certainly helped to direct the scientific world towards psychoanalysis, and Freud acknowledged his gratitude, stating, "The Zurich doctors made, as it were, the nucleus of the little band which was fighting for the recognition of psychoanalysis. Only in Zurich was there a possible opportunity to learn the new art and to apply it in practice".

Freud at this time wished to take a less prominent place in the Psychoanalytical Society and diplomatically transferred his presidency to Jung.

Jung must have been an impressive young man in those days, and Wittels described him in his well-known depiction as "Tall and upright of figure, like a young Siegfried". When I first saw him some thirty years ago he was middle-aged, but still well built, with a rather burly figure, a jovial red face, short clipped hair, and gold-rimmed glasses. He was a good lecturer and able to impress an audience with his profundity. With those who sought his views he had a kindly, serious manner and he was certainly 'a good mixer'.

The first important investigation of note which he made was his careful study of association. His research followed on the experimental work started by Galton in 1879 and Wundt a year later. This was 'controlled association' or the timing of words associated with those already provided. This led to the development of the conception of 'the complex' or group of ideas, emotionally charged, capable of influencing conduct and thought. Following on his work on association Jung did some valuable research on schizophrenia, published under the title of *The Psychology of Dementia Precox* (1909).

He remained president of the International Psychological Society until 1913, when he published his book on *The Psychology of the Unconscious*, which was a study of mythology. This showed such a deviation from psychoanalysis that he resigned. The Zurich and Vienna schools then drifted apart.

After this Jung advanced his well-known theory of psychological types. This was a development of philosophical views known from the earliest times and used by William James, who thought men were divisible into tough and tender, by Schiller who thought them idealists and realists and so on. However, the two main types Jung described were extroversion and introversion, but complicated by the addition of sub-classes of thinking and feeling, so that there were eight in all. Extroversion and introversion have become household words.

Jung moved further and further from clinical psychiatry and more and more towards an interest in mythology. His later works were, *The Secret of the Golden Flower* (1930), *Modern Man in Search of His Soul* (1933), *Psychology of Religion* (1939), and *The Integration of the Personality* (1940), etc.

Although Jung's investigation of myths is regarded with suspicion by many psychiatrists, since it is impossible to prove the correctness of an interpretation, and the application of myths to the understanding of clinical illness is of uncertain value, most accept that Jung's earlier work on association, complexes, schizophrenia and psychological types is of immense value.

He was a tireless writer and lecturer. He visited most countries at the invitation of learned societies and studied myths all over the world. The last of the triumvirate formed by him with Freud and Adler, his death represents the passing of a great epoch of psychiatry. CLIFFORD ALLEN

NEWS and VIEWS

Electrical Engineering at Glasgow : Prof. J. Lamb

DR. JOHN LAMB has been appointed to the James Watt chair of electrical engineering in the University of Glasgow, in succession to the late Prof. B. Hague. For the past five years Dr. Lamb has been a reader in electrical engineering in the Imperial College of Science and Technology, London. He is well known for his many contributions to knowledge in the fields of ultrasonics and the physics of materials. Dr. Lamb is thirty-nine years of age. He graduated in electrical engineering in the University of Manchester in 1943, and was awarded the degree of Ph.D. in 1946 and the degree of D.Sc. in 1957. In 1947 he joined the staff of the Imperial College. For the past three years, in addition to his readership, he has held the appointment of assistant director in his Department, involving administrative responsibilities that will no doubt have given him experience that will be of value in taking charge of a department. It may be expected that the appointment of Dr. Lamb will ensure that electrical engineering in Glasgow will be developed on modern lines and with a keen appreciation of the part that advances in basic physics must play in the future of electrical engineering.

Beilby Medal and Prize, 1961

THE administrators of the Sir George Beilby Memorial Fund, representing the Royal Institute of Chemistry, the Society of Chemical Industry and the Institute of Metals, have decided to make awards from the Fund in 1961—each consisting of the newly instituted gold medal with a prize of 100 guineas—to Dr. Constantin Edeleanu and Prof. Jack Nutting.

Dr. C. Edeleanu

DR. C. EDELEANU was born in Rumania, and wen to Britain in 1937. He was a pupil at the Morrison Academy, Crieff, and then went up to Cambridge in 1941. In 1946 he joined Dr. U. R. Evans, working on the stress corrosion of light alloys. In the course of that work he became interested in pitting reactions and developed the autocatalytic mechanism for pitting first proposed by Dr. Hoar. A somewhat surprising conclusion of the work was that the true rate of corrosion during the pitting of aluminium in a given solution was a constant. Direct experimental proof of this was, however, only obtained some ten years later. In 1950 he joined the Brown Firth Research Laboratories, where he was in charge of a section dealing with long-term alloy development. Stress corrosion, especially in high-pressure water systems and nuclear-reactor cooling systems, was of particular interest, and the work demonstrated that failures in such systems were, as a rule, associated with concentration effects arising either during heat transfer or in heating and cooling cycles. In 1956 he joined the Tube Investments Research Laboratory at Hinxton, to form a corrosion group. At first the work consisted mainly of studying the factors that control corrosion in some fused-salt systems of interest to the nuclear-engineering industry. One of the main results was to show that such systems could be treated in a somewhat analogous way to aqueous systems but that there were important differences. More recently, Dr. Edeleanu has returned to stress corrosion and, in conjunction with Dr. A. J. Forty, has attempted to interpret this type of failure in a more physical manner. This led naturally to the question of the part played by dislocations and other structural features in corrosion. With G. A. Bassett a start was made in this field, techniques such as transmission electron microscopy having played a useful part.

Prof. J. Nutting

PROF. J. NUTTING was educated at Mirfield Grammar School, Yorkshire, and the University of Leeds. He was then appointed research assistant to the late Prof. Preece at Leeds and was awarded the degree of Ph.D. in 1948 for a dissertation on the overheating and burning of steel. Afterwards he was employed by the British Iron and Steel Research Association and was seconded to the Cavendish Laboratory, Cambridge, where he worked with Dr. V. E. Cosslett and Prof. E. Orowan, developing techniques for the examination of metals with the electron microscope. In 1949 he was appointed demonstrator and in 1954 lecturer in the Department of Metallurgy, University of Cambridge—an appointment he held until 1960, when he returned to the University of Leeds as professor of metallurgy in the Houldsworth School of Applied Science. During his work at the Cavendish Laboratory he became convinced that the electron microscope could be more widely applied to problems in metallography. On transferring to the Metallography Department in Cambridge he had the opportunity of testing his convictions, and together with a group of research students a wide variety of metallographic problems was investigated.

Paul Instrument Fund Committee Grants

AWARDS of grants by the Paul Instrument Fund Committee have been made as follows: £1,550 to Dr. E. T. Hall, director of the Research Laboratory for Archæology and the History of Art, Oxford, for the design and manufacture of a recording instrument combining a drum recorder and servo transmitters with a spectrometer to give a direct reading of wavelength; £3,900 to Dr. P. B. Hirsch, lecturer in physics, University of Cambridge, for the construction of an electron optical instrument which will measure the energy distribution of the electron beam passing through small areas (diameter less than 1μ) in the crystal specimen which can be observed and selected on the electron microscope image; £3,000, in supplement of a previous grant, to Dr. H. Motz, Donald Pollock reader in engineering sciences, University of Oxford, for the construction of a linear accelerator working at 1·6 cm. (*J*-band); £2,284 to Mr. B. M. Turner, who is working in the Department of Zoology and Comparative Anatomy, Oxford, for the further development of micro-scale chromatography and electrophoresis techniques.

The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science".

U.S. Scientists in Universities and Colleges : 1958

A PRELIMINARY report on scientists and engineers engaged in research and development in colleges and universities in the United States for 1958, issued by the National Science Foundation (*Reviews of Data on Research and Development*, No. 27; April 1961), gives the number of those engaged as 69,919 in 377 colleges and universities, or 44 per cent of all scientists and engineers employed at colleges and universities in 1958, and the expenditure as 736 million dollars, compared with 410 million dollars in 1954. Of these, 47 per cent were employed in the biological sciences, 26 per cent in the physical sciences, 17 per cent in engineering sciences and 10 per cent in the social sciences. Operating expenditure for separating budget research and development at the universities and colleges accounted for 45 per cent of the total expenditure on research and development, agricultural experiment stations for 16 per cent, and Federal Contract Research Centres for 39 per cent. Sixteen institutions employing 1,000 or more employed 31,260 of this total of scientists and engineers, and twenty institutions with 500–999 another 14,026, while 206 institutions employed fewer than 25 scientists and engineers. While the total faculty engaged in research and development only rose by 3 per cent between 1954 and 1958, the number of faculty engaged full-time in research and development increased by 49 per cent.

Production of Gas from Coal in Britain

IN a written answer in the House of Commons on June 23, the Minister of Power, Mr. R. Wood, said that he had accepted the advice of the Wilson Committee that town gas offered the greatest scope for expansion in making gas, oil and chemicals from coal, but that new plants would be required to make gas more cheaply. He had therefore decided, in agreement with the Gas Council and the National Coal Board, to accept the recommendations that the development of high-pressure slagging gasifiers should be pursued; that the Gas Council should be responsible for further work on the standard Lurgi gasifier using British coals, on the Otto–Rummel gasifier and on any further gasifiers suitable for town gas, as well as for development on slagging gasifiers; and that the National Coal Board should be responsible for work on producer gas plants. He also accepted the Committee's view that the prospects of making oil from coal economically in Britain are so remote that no further technological work on the development of this process should be undertaken at present. The Department of Scientific and Industrial Research had accordingly terminated its development work on this process, but was continuing basic investigations on catalysis and keeping in touch with developments in making oil from coal. The Minister said that his Department, which had been carrying out research and development on the slagging gasification of coal as a raw material for oil synthesis, would continue this work until March 31, 1962, when the Gas Council would be responsible for all work in this field. The Gas Council and the National Coal Board were making a joint economic study of the possibilities of the Lurgi process which might later be extended to include slagging gasification. This study would define the scope for further developments of the process and provide a basis for further co-operation between the Gas Council and the Board in the total gasification of coal.

Research on West Indian Volcanoes

GEOLOGICAL work is in progress on volcanoes in the West Indies, by the Department of Geology and Mineralogy, University of Oxford, in co-operation with the Seismic Research Unit of the University College of the West Indies. In view of the large amount of geophysical data available on this region, it is desirable that a detailed petrological study of the volcanic rocks of the British Leeward and Windward Islands be undertaken. A succession of Oxford graduate students will work on specific problems during the next five years, under the supervision of Prof. L. R. Wager and Dr. G. M. Brown (University of Oxford) and with assistance in the field by Dr. G. R. Robson (Seismic Research Unit) and Dr. P. H. A. Martin-Kaye (Government Geologist, Windward Islands). Their work will include mapping of the structure of the volcanoes and the recognition and classification of the various phases of recent volcanic activity. Petrological and mineralogical work on the rocks and minerals will, in conjunction with the field work, lead to a better understanding of the origin of the rocks and of some of the causes underlying the development of volcanic episodes in the region.

Work has begun with studies by Prof. Wager on the petrology and mineralogy of plutonic blocks ejected by the Soufriere Volcano, St. Vincent (1959 visit), and by P. E. Baker on the strato-volcano, Mt. Misery, on St. Kitts (1961 visit). Other students will begin work shortly on St. Lucia and Dominica, which are characterized by having centres of activity concentrated in almost circular caldera-type depressions, in which tholoids and ash deposits predominate and in which fumarole activity and earth tremors have persisted in recent years.

Education by Television

As a tool for instruction, television is accepted and well established in the United States. Television made its first appearance in education as long ago as 1932 at Iowa State University, but it was not until 1953 that it was first used for normal instruction. By 1960, half a million school-children and 100,000 college students were receiving regular instruction by television. This situation is in marked contrast with that prevailing in Great Britain. In a pamphlet entitled *Educational Television: a Survey and a Plan*, which has been published privately, John Wellens shows that in Britain educational television is practically synonymous with the schools television service run by both the B.B.C. and the independent network (Pp. 44+8 plates. Guilsborough, near Northampton: John Wellens (ITT), Ltd., The Sun House, 1961. 5*s*.). The only exception consists of a handful of colleges and universities which have their own closed-circuit installations for giving instruction in special departments, such as medicine, dentistry and engineering. These installations are few and have only recently been introduced; in many cases they have not yet developed beyond the pilot stage. In some cases the installations have been put in, below economic cost, to promote the acceptance of television as a teaching tool. Two Middlesex schools have been linked by micro-wave equipment for the teaching of chemistry, but, none the less, Britain is now at the stage reached by the Americans approximately ten years ago. Within the past year British educationists have become aware of the activity in this field in the United States and, in his booklet, Wellens describes the present position in the United States and makes a practical evaluation for Britain.

Research Defence Society

THE Research Defence Society has published another of its pamphlets to make known the facts about experimental research involving the use of animals, and the conditions and regulations under which animal experiments are conducted in the United Kingdom. *Animal Experiments in the Control of Animal Diseases* is set out on the lines of previous pamphlets, that is, by posing a series of questions which the general public could rightly ask and by providing answers which are as objective and free from bias as humanly possible (Pp. 6. London: Research Defence Society, 1961. 6*d*.). In this new pamphlet the questions posed include: "Why are the treatment and prevention of animal disease important enough to warrant animal experiments ? Why is animal experiment necessary to help in combating animal disease ? Can you give examples of new agents for control of animal disease which would not have been developed without animal experiment ? Can you explain the paradox that animal experiments involving suffering are used to prevent suffering in other animals ? Why are animal tests necessary in the development of new drugs ? How do animal experiments help in the control of animal disease ? Why are animal experiments necessary in the safety testing of vaccines for use in animals ?"

The Museums Journal

EARLIER this year it was decided to discontinue the monthly *Museums Journal* and publish instead a monthly *Bulletin* and a quarterly *Journal*. The first issue of the latter publication (No. 1; June 1961) was awaited with interest. The decision of the Council is fully justified, and curators are certainly not disappointed, for the new format even exceeds expectations. Mr. R. L. S. Bruce-Mitford describes the Book of Kells exhibition, stressing the technical presentation; Mr. Ronald Alley, in a comprehensive analysis, proves the almost total failure of British art galleries to acquire modern pictures and sculptures of foreign schools; and Mr. Stanley Rubenstein deals with the complicated problems of copyright. On the purely technical side, Mr. R. M. Organ gives a new treatment for 'bronze disease', and Mr. A. M. Tynan describes the uses of 'Styrocell'. Mr. Hugh Wakefield details the practical nature of the purchase grants made by the Victoria and Albert Museum. The issue also includes the annual report of the Museums Association for 1960–61, and a well-balanced *Journal* concludes with notes on the contributors and research work in progress.

The Victorian American

IN 1960 the family of the late Harry T. Peters presented the 'American on Stone' collection of lithographs to the Smithsonian Institution (Publication 4466: *The Victorian American*. By Antony N. B. Garvan and Peter C. Welsh. Lithographs from the Harry T. Peters 'American on Stone' Collection. Pp. 30. Washington, D.C.: Smithsonian Institution). The collection touches on every phase of American life in the nineteenth century. Part of this interesting material is now on tour as a travelling exhibition, and the 100 lithographs listed in a well-prepared catalogue include those illustrating technical progress, suburban novelty, rural nostalgia, the family and the genteel female, sports, fashion, the sea, politics, warfare and humour.

Medical Research in East Africa

A NEW treatment for tuberculosis and investigations into the causes of eye diseases in Africans are among the many items referred to in the report of the East African Council for Medical Research for 1959–60 (Pp. ii+15. Nairobi: Government Printer, 1960. 2*s*.). In tuberculosis, the important finding has been that thiacetazone (thiosemicarbazone), TB1, is as efficacious in combination with isonicotinic acid hydrazide (INAH) in therapy and in preventing drug resistance as is *para*-aminosalicylic acid (PAS). TB1 is cheap and palatable; PAS is expensive and patients dislike it. They are apt, therefore, to take INAH alone and thus acquire resistance to infections. A high (about 10 per cent) and increasing incidence of initial drug-resistance has been revealed in new and presumably untreated cases. The investigations now in progress are to determine the optimum dosage. Surveys have been carried out to determine the most common eye conditions in different African communities. A higher incidence of non-leprous eye disease was found in lepers in the Central Province than in Lake Province, and this is probably due to trachoma and pterygium being common in the former area. In the foothills of the Ruwenzori Mountains of West Uganda, onchocerciasis is common; it was found that this filarial infection was the most important cause of blindness. Some investigations into the ability of the African to accommodate were made. Although during early life accommodation is poor, it does not deteriorate so rapidly in late middle-life as it does in Europeans. Colour vision was also tested, and it was confirmed that the incidence of red-green and other forms of colour-blindness is lower in Africans than in Europeans.

Diabetes Mellitus in Africans in Rhodesia

A SURVEY carried out recently on Salisbury Africans by Dr. M. Gelfand and W. R. Carr indicates that diabetes is less prevalent than in most other parts of the world, particularly in comparison with areas where more processed and highly refined foods are purchased (*Central African Journal of Medicine*, 7, No. 2; February 1961). The diets with traditionally high carbohydrate, low fat and low animal protein contents are probably the main factor involved in keeping the disease at a low level. There are increasingly large numbers of Africans whose diet is changing tremendously during short periods of time towards higher fat, refined sugar and animal protein consumption, but, as yet, they are insufficient in numbers to indicate whether this will have an effect on the incidence of diabetes and, *inter alia*, coronary thrombosis. The socio-economic background shows that most of the diabetic patients come from groups who work in the European economy, and whose diet has been altered to include more foods containing, in particular, refined sugar and fats. The clinical types encountered in Rhodesia correspond very closely with the European pattern. No variants such as *J* or *K* types are found. The African diabetics may require more insulin than the European for stabilization, but this may be due to lack of understanding of the need to follow a strict dietary routine. The level of blood-sugar was found to be higher than in a European group and this may be the reason for the difference. Although the insulin requirements of the African appeared to be high, hypoglycæmia coma developed if the requirements were exceeded. The symptoms of diabetes appear to be the same for the African and European, the main features being thirst, polyuria, weakness, loss in weight and pruritus vulvæ; but there were certain differences, such as the infrequency of pulmonary tuberculosis, gangrene and intercapillary glomerulosclerosis.

Ecological Aspects of Irrigation in Ancient Mesopotamia

A PAPER by Hans Helbaek, "Ecological Aspects of Irrigation in Ancient Mesopotamia" (*Iraq*, 22, 186; Spring and Autumn, 1960. Published by the British School of Archæology in Iraq), develops the argument that man first harvested his grains (barley and linseed, in particular) where the wild plants grew on hills in the Near East, and on soils fed by rain. Here he supposes the genetic constitution of the crop plants remained fairly constant. But some 7,000 years ago man sowed and grew his wild barley in the plain of Mesopotamia in rich land naturally irrigated by flooding (and later artificially irrigated by canals). Here presently the wild 2-row barley gave rise to a 6-row barley by a moderate genetic change causing the two lateral abortive spikelets to become fertile. Helbaek claims to have produced such a barley as a mutant from a 2-row wild barley by X-ray irradiation. Such 6-row barley was welcomed by early man, and spread to Egypt and elsewhere. Meanwhile, continued irrigation in Mesopotamia led to salt accumulation in the soil and the disappearance of crops, first of the sensitive Emmer wheat, then linseed, and finally of barley, and with that of man. Helbaek gives evidence also that while early man's *Linum* seeds (whether flax or linseed) remained constant in size for some thousand years by areas fed by rainfall, the size of seed steadily increased in areas with irrigation. Readers interested in the botanical history of crop plants and of the early growth of civilization made possible by crops should read and assess this short paper themselves.

Taxonomy of the Earthworm in India

EARTHWORMS, apparently sent simultaneously to the British Museum (Natural History) and to the American Museum of Natural History for identification, have directed attention once more to the deplorable state of the taxonomy in a genus that may be dominant in a considerable portion of the Indian Peninsula. Each of the species left in that genus by a revision by G. E. Gates in 1939, based on an over-all somatic rather than genital anatomy, has remained indefinable hitherto. Material now studied by Gates, at the University of Maine, has enabled recognition not only of two further characters for generic definition but also has permitted, for the first time, fairly satisfactory characterization of a species based on data such as variation in sexual individuals secured in lots of some size from several localities (*Ann. Mag. Nat. Hist.*, 3, No. 35; November 1960). Previously unpublished information included in his account was obtained from 90 specimens collected for him at three localities in the Jubbulpore region during the Second World War.

Geology of the Country around Monmouth and Chepstow

THE Geological Survey of Great Britain has now published a composite Memoir covering the geology of the country around Monmouth and Chepstow (Department of Scientific and Industrial Research Memoirs of the Geological Survey of Great Britain—England and Wales. By Dr. F. B. A. Welch and Dr. F. M. Trotter. With contributions by T. R. M. Lawrie, J. D. D. Smith and R. W. Elliot. Pp. viii+164+2 plates. London: H.M.S.O., 1961. 22*s*. 6*d*. net). Previously, this area was dealt with in separate Memoirs and Survey sheets, but the present Memoir is an explanation of New Series Geological Sheets 233 (Monmouth) and 250 (Chepstow)—an area occupying a total of 432 square miles, of which some 46 square miles are covered by the Severn Estuary; a little more than half lies within Monmouthshire, part in Herefordshire and the remainder in Gloucestershire. The Memoir details the geological sequence and history of the area, which extends from the Ludlow Series of the Silurian rocks through Old Red Sandstone, Carboniferous, Triassic, Jurassic to recent superficial formations. A general account of the geological structure of the region is included, also an analysis of those earth movements which have affected the area. Each geological epoch represented is treated individually, and bibliographies of the literature are provided. A short chapter deals with the igneous rocks of the area, and another concerns itself with the economic deposits. Two appendixes provide records of recent important bore-holes, and also a list of Geological Survey photographs of the area.

The Art of Interviewing

The British Association for Commercial and Industrial Education has produced a pamphlet which should find readers well outside the range of those for whom it was intended. Written by John S. Gough, of I.C.I., Ltd., the pamphlet *Interviewing in Twenty-six Steps* describes the approach to an interview designed to select a candidate for a job, the method of conducting it, the dangers and diversions to be avoided and the way the interview should be used in summing-up the candidate. The pamphlet is forthright, practical and has clearly been written by one who has tried out his twenty-six steps on many occasions before summarizing them in this form. Gough makes it clear that no skilled interviewer can guarantee success in selection; the more he practises, the less mistakes he should make. This pamphlet will help him to reduce his number of failures. Copies may be obtained from the British Association for Commercial and Industrial Education, 26*a* Buckingham Palace Road, London, S.W.1; price 3*s*. 6*d*.

Applications of Solar Energy

In *Solar Energy Technology: 1954–1959* (Library Association. Special Subject List No. 34. Pp. 32. London: Library Association, 1961. 3*s*. 6*d*.), T. Brimelow attempts to list all the significant literature on the subject published in Europe and the United States with the exception of items published in the popular and political periodicals of the U.S.S.R. The 329 items are arranged in sections which include: aero/space applications; air-conditioning; automotive engineering; biological science; electrical conversion; furnaces; heating; radio; refrigeration; water heating and water treatment. There are an author index and also a subject index, and a brief introduction by Dr. H. Heywood notes the salient features in present applications of solar energy.

Scientific Research in British Universities, 1960–61

Scientific Research in British Universities, 1960–61, issued by the Department of Scientific and Industrial Research, is an indispensable handbook for all those who endeavour to keep track of scientists in the United Kingdom and their fields of research (Pp. xiii + 513. London: H.M.S.O., 1961. 32*s*. 6*d*. net). The handbook describes the nature of research projects in progress, and indicates the scope of research carried out by various scientific departments and individual teams of investigators. Entries are made in alphabetical order of university, and subdivided into faculty and subject. Simple numerical headings of universities and subject allow ease of reference. Three indexes are provided, satisfying any search by name, subject heading or actual research.

Lady Tata Memorial Trust: Awards for 1961–62

The Trustees of the Lady Tata Memorial Trust, on the recommendation of the (European) Scientific Advisory Committee, have made the following awards for research on leukæmia and allied diseases in the academic year beginning October 1: *Grants for research expenses*, Dr. J. L. Amiel, Cancer and Radiopathology Research Centre, Paris; Dr. B. M. Braganca, Indian Cancer Research Centre, Bombay; Dr. G. W. Griffith, County Health Department, Anglesey; Dr. B. Lagerlof, Karolinska Institute, Stockholm; Dr. H. J. Woodliff, New Pathology Laboratory, Radcliffe Infirmary, Oxford. *Scholarship*, Dr. L. Chieco-Bianchi, Institute of Pathological Anatomy, Bari.

Awards in Radiological Research and Nuclear Medicine: James Picker Foundation

On behalf of the James Picker Foundation, the National Academy of Sciences—National Research Council is accepting applications for fellowships and grants in the field of radiology and nuclear medicine for the year 1962–63. Awards are made in four categories: (1) *Advanced Fellowships in Academic Radiology*. Completion of clinical training in radiology ordinarily will be a prerequisite, and the candidate must be prepared to devote a minimum of two years to course work in the basic sciences and to the application of the techniques and methods of these basic disciplines to radiological research. (2) *Post-doctoral Research Fellowships*, open to recent graduates who desire investigative experience and training in radiology or nuclear medicine. Applicants must hold the M.D., Ph.D. or Sc.D. degree or the equivalent. Awards are for a period of one year. (3) *Grants for Scholars*, to assist medical schools in supporting and developing junior staff members in radiology. Initial grants are made for two years. (4) *Grants-in-Aid of Research* are designed to encourage investigations in radiology and nuclear medicine offering promise of improvement in methods of diagnosis or treatment of disease. These grants may be made for two years. Applications for the fiscal year 1962–63 should be submitted by October 1, 1961, to the Committee on Radiology, National Academy of Sciences—National Research Council, 2101 Constitution Avenue, N.W., Washington 25, D.C.

The Institute of Physics and the Physical Society

At the first annual general meeting of the amalgamated Institute of Physics and the Physical Society, held in London on July 4, the following were elected to office: *President*, Sir John Cockcroft; *Vice-Presidents*, Dr. V. E. Cosslett, Prof. R. W. Ditchburn, Dr. W. H. Taylor and Dr. J. Topping; *Honorary Treasurer*, Dr. J. Taylor; *Honorary Secretary*, Dr. C. G. Wynne. Eight ordinary members of Council were also elected. This, the first elected Council of the amalgamated body, is very nearly the same as the 'caretaker' Council which has been in office since the amalgamation of the Institute and Society on July 5, 1960.

University News: Leeds

The Foundation for Management Education has arranged for grants totalling £16,600 a year for seven years to be made available to the University of Leeds. This will make possible the strengthening of the present teaching in management studies for students mainly in the Faculty of Technology; and the expansion of the work with residential and non-residential courses for upper and middle managers, and the establishment of a one-year postgraduate diploma course in industrial management. It will also enable the University to gain the assistance of a number of visiting lecturers, and to set up a number of scholarships and fellowships for research in the field of management and business studies.

The following appointments to lectureships have been announced: Dr. A. Chadwick (zoology); Dr. B. L. Shaw (inorganic and structural chemistry). Mr. F. P. G. Whitaker, at present lecturer in industrial management, has been appointed director of management studies in the Department of Economics and Commerce.

London

Dr. J. S. Webb, reader in applied geochemistry at the Imperial College of Science and Technology, has been appointed to the University chair of applied geochemistry tenable at that College. Dr. F. G. A. Stone, assistant professor at Harvard University, has been appointed to the University readership in inorganic chemistry tenable at Queen Mary College from July 1, 1962. The title of reader in biology in the University of London has been conferred on Dr. Agnes M. Hughes in respect of her post at the Royal Free Hospital School of Medicine.

Prof. J. N. P. Davies, professor of pathology at Makerere College, has been appointed to the University readership in morbid anatomy tenable at the Postgraduate Medical School of London. Mr. H. D. Ritchie, assistant director of the Surgical Unit at the London Hospital, has been appointed to the University readership in surgery tenable at the London Hospital Medical School.

Titles of reader in the University have been conferred on: Mr. R. W. Fearnhead (dental anatomy), in respect of his post at the London Hospital Medical College; Dr. T. G. Bonner (organic chemistry), in respect of his post at Royal Holloway College.

North Staffordshire

The University College of North Staffordshire, Keele, is to become Keele University in 1962. H.R.H. Princess Margaret, who is at present president of the College, has been proposed as the first chancellor of the new University.

The following appointments have been announced: H. Liebeck, to a lectureship in mathematics; Dr. M. Asgar Ali, to an Imperial Chemical Industries research fellowship; M. J. A. Smith, to be Mullard Research Fellow in physics; Dr. W. P. McCann, to be Leverhulme research officer.

Oxford

The first award of the J. H. Burn fellowship, at the Department of Pharmacology, for 1961–62 has been made to Mr. H. P. Rang.

Sheffield

The following have been appointed to readerships: Dr. L. Mirsky (pure mathematics); Dr. V. R. Pickles (physiology); Dr. F. A. Benson (electronics). G. Lederer has been appointed as a lecturer in pure mathematics. Dr. P. B. N. Neal is to be senior Research Fellow in mechanical engineering, and Mr. A. B. Hedley has been appointed to the Central Electricity Authority research fellowship in fuel technology.

Announcements

The David Anderson Berry Prize for 1961 of the Royal Society of Edinburgh has been awarded to: (*a*) Drs. H. B. Hewitt and C. W. Wilson (Westminster Hospital School of Medicine, London) for their quantitative estimation of the lethality of radiation on leukæmia cells *in vivo*; and (*b*) Dr. D. H. Sussdorf (research fellow in immunochemistry, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena) for his studies on the re-population of the spleen of X-irradiated rabbits by lymphoid cells from shielded regions.

Dr. J. F. Goodman has been appointed head of the Basic Research Department, Thomas Hedley and Co., Newcastle upon Tyne. Dr. Goodman joined Hedley as a research chemist in 1957, and in 1960 became head of the Physical and Analytical Group in the Basic Research Department.

Dr. Leo P. Vernon has been appointed as director of research of the Charles F. Kettering Laboratory in Yellow Springs, Ohio. Dr. Vernon, who is well known for his work on photosynthesis, is at present on sabbatical leave at the Nobel Institute, Stockholm, from his post as professor of chemistry at Brigham Young University. Dr. Vernon will assume his position at Kettering Laboratory on or about October 1.

The Plant Phenolics Group is holding a meeting on "Wood Extractives" at Royal Holloway College, Englefield Green, Surrey, during September 20–22. Further information can be obtained from the Honorary Secretary, B.L.M.R.A., Milton Park, Egham, Surrey.

"The Fishing Research Vessel" will be the major subject at a forum to be organized by the Food and Agriculture Organization in Tokyo during September 18–30. The meeting will also consider hydrographic survey ships, polar exploration vessels, hospital ships, lighthouse tenders, cable ships and non-magnetic ships. Further information can be obtained from the Food and Agriculture Organization, Rome.

The Systematics Association and the Challenger Society are arranging a symposium on "Speciation in the Sea", to be held at the Plymouth Laboratory of the Marine Biological Association of the United Kingdom, during September 27–29. Further information can be obtained from Norman Tebble, Zoological Secretary, Systematics Association, British Museum (Natural History), Cromwell Road, London, S.W.7.

An Advanced Study Institute on Underwater Acoustics is to be conducted during July 31–August 11 of this year in the Physics Department in the Imperial College of Science and Technology, South Kensington, London, S.W.7. Dr. Vernon M. Albers, chief scientist at the Ordnance Research Laboratory of the Pennsylvania State University, and Dr. R. W. B. Stephens, reader in acoustics at Imperial College, are co-chairmen of the Institute.

The Institute of Physics and the Physical Society is organizing a residential symposium on "Some Aspects of the Physics of Space Research" to be held at the Royal Military College of Science, Shrivenham, Swindon, Wiltshire, during September 20–22. Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

THE BRITISH SPACE DEVELOPMENT CO., LTD.

THE British Space Development Co., Ltd., has been formed in order to bring together a representative cross-section of that part of industry which is interested in the development and exploitation of space for commercial purposes and which believes that the use of space will, in the next two decades, become a major key to the continued prosperity of the United Kingdom and Commonwealth, politically and industrially. It is considered that a successful and economic entry into the new medium of space demands a unified effort on the part of British industry.

Briefly, the initial aims of the Company are as follows:

(*a*) To provide an organization to discuss with the Government and co-operate in any proposals that the Government may make in regard to a United Kingdom and Commonwealth space programme, which is either independent of European participation or which includes European participation.

(*b*) To provide the Government with an indication of the faith that British industry has in the exploitation of space and to show its readiness to play its part and to take its share of the risks as well as its share of the future benefits.

(*c*) To offer the Government an industrial body broadly based to act as an authority or agency to conduct research and development and to implement and operate a space programme and to consider and develop all the possible areas of exploitation of space.

(*d*) To provide those industries concerned with an organization to negotiate with other countries or foreign industrial organizations or consortia any matters concerning the commercial uses of space which are likely to have global implications, that is, communications, broadcasting, television, meteorological forecasting and navigational aids.

(*e*) To provide the greatest economy in the use of the limited number of technical personnel available in the country, particularly in the field of astrophysics and astronautical engineering, and to ensure that duplication of effort, which has been so prevalent in the United States and which has contributed to wasteful use of resources, is avoided in the United Kingdom.

(*f*) To encourage the provision of and to provide directly, in due course, facilities for the training of an adequate number of technical personnel in all those sciences associated with space research and the exploitation of space.

(*g*) To preserve for British industry a place in space and a proper share of the national and international benefits deriving from the development and use of space.

It is possible that the Company may develop into an international company, particularly as no enterprise in the world has as yet been envisaged where such a wide cross-section of industry has come together to pool its resources and effort in order to compete in this demanding, expensive but ultimately most lucrative new technical field with its limitless possibilities.

The British Space Development Co. now being formed will have an initial capital of £20,000. The following Companies will be the initial participants: Associated Electrical Industries, Associated Tele-Vision, British Insulated Callender's Cables, Decca Radar, Hawker Siddeley Aviation, Ltd., Pye, Rank Organization, Rolls-Royce, Plessey, Elliott Automation.

As one of its first major projects, the Technical Committee of the Company is now examining the technical and economic problems of the United Kingdom and Commonwealth satellite communications system which allows for integration with an American system.

Sir Bernard Lovell, professor of radio astronomy in the University of Manchester and director of the Nuffield Radio Astronomy Laboratories, Jodrell Bank, has been appointed scientific adviser to this newly formed Company, though he will retain his present position at Manchester and Jodrell Bank.

THE DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

THE report of the Council for Scientific and Industrial Research for the year 1960* records gross expenditure for the year ended March 31, 1960, of £12,008,536, compared with £10,059,376 in the previous year, and reduced to £10,342,691 by various receipts from industry and other sources for services rendered. Of the latter, £722,241 was from other Government Departments and £723,601 from industry, £613,592 in all being received by the National Physical Laboratory: the net increase on 1959 was £1,447,871. Grants to students amounted to £870,289, the number of research students in training being 2,377, of whom 942 (including 88 NATO awards) were new; advanced course studentships numbered 315 (of which 274 were new), first-year studentships 77, and research fellowships 75, of which 30 were held overseas and 45 in Great Britain, and 39 (including 21 NATO fellowships) were new.

Of the research students, 290 were in biology and biochemistry, 176 in chemical engineering, 747 in chemistry, 77 in electrical and 162 in other engineering, 143 in geology, 43 in the human sciences, 236 in mathematics and 503 in physics. Of the advanced-course students, 67 were in physics, 62 in mathematics, 20 in electrical and 42 in other engineering, 39 in geology, 28 in biology and biochemistry, 27 in chemical engineering and metallurgy, 25 in the human sciences and 5 in chemistry. All 77 first-year studentships were in chemistry, and chemistry (22), physics (21) and biology and biochemistry (18) claimed most of the research fellowships.

Grants for special researches totalled 525, of which 293 were new, amounting to £5,591,270; of these, 75 were in biology, 102 in chemistry, 37 in geology and geophysics, 3 in mathematics, 19 in astronomy, 65 in low-temperature work and investigations on the solid state, 60 (totalling £2,221,896) in nuclear physics, 90 in technology, 23 in the human sciences, and 24 (totalling £297,543) in space research. The report does not include the list of individual grants exceeding £1,000 or more, nor is the analysis of the research effort of the Department's research stations given in last year's report repeated.

---

* Department of Scientific and Industrial Research. Report of the Research Council for the year 1960. Pp. 65. (Cmnd. 1365.) (London: H.M. Stationery Office, 1961.) 4*s*. net.

Expenditure on the Warren Spring Laboratory was £402,854 gross, compared with £404,963 in 1959, and on the Torry Research Station, Tropical Products Institute and Government Chemist, which appear in these accounts for the first time, £177,860, £164,051 and £290,237, respectively. Otherwise, there are increases under all the separate heads of expenditure, the largest increases being in headquarters administration (£623,206 from £441,440); the National Physical Laboratory (£1,438,776 from £1,285,018); building research (£656,975 from £599,931); Geological Survey and Museum (£490,014 from £433,550); National Engineering Laboratory (£686,156 from £637,299); and road research (£718,056 from £618,763). Expenditure on fire research amounted to £158,441; on hydraulics research to £175,041; on radio research to £239,174; and on water pollution research to £137,100.

Annual grants to research associations amounted to £1,761,368 (£1,683,195 in 1959) and special grants to £17,823; £1,259,000 was contributed to the European Organization for Nuclear Research and £75,333 to NATO scientific schemes. Expenditure on headquarters overseas liaison increased from £113,929 to £132,684. Further progress is reported with the planned expansion of the staff, which rose by 3 per cent from 5,506 to 5,680 during the year, and it is estimated that, excluding stations transferred to or from the Department, the total staff has increased by just under 20 per cent during the past four years.

THE RADIO RESEARCH STATION, SLOUGH

THE Radio Research Station, Slough, gave visitors the opportunity of seeing some of its work during two open days on May 31 and June 1. Most of the Station's work is concerned with radio-wave propagation and with the fundamental properties of the ionosphere and the troposphere through which the waves travel. About half of the work is devoted to some aspect of experiments made on rockets and artificial satellites.

Among the exhibits concerned with the propagation of very-high-frequency waves through the troposphere was one which showed how the dielectric constant of the air had been measured continuously in an aeroplane. The measurement was made by determining the natural frequency of an open-ended electromagnetically resonant cavity. The measured distributions of dielectric constant were then related to certain aspects of radio wave propagation. Of other exhibits concerned with tropospheric propagation one showed a method for determining the azimuthal angle of arrival of waves propagated by tropospheric scatter from distances beyond the horizon, and another showed how the time-delays in multipath transmission over a distance of about 35 miles were being measured.

Equipment used for recording information about the ionosphere over Slough and over outstations at Falkland Islands, Singapore and Inverness was demonstrated. The results, together with those from other ionosphere observatories all over the world, are available for the use of all bona fide workers in the Station's World Data Centre. This is one of four set up in different countries by international agreement. One of the exhibits showed how the Research Station was making use of these recordings to investigate the nature of the ionosphere over the polar regions. Conditions of strong absorption (blackout) and of sporadic ionization at levels near 100 km. (*Es*) were shown to occur along spiral lines fixed in space relative to the Sun. When, as a result of the Earth's rotation, any given point on the Earth passes beneath one of these spirals, the appropriate phenomenon is observed. The two spirals, one for the blackout and the other for *Es*, curve in opposite senses, due, it is thought, to the fact that they are caused by the arrival in the atmosphere of particles of opposite sign. Another exhibit showed how the ionosphere over Halley Bay in Antarctica had varied throughout the International Geophysical Year.

The ionization produced in the ionosphere by the Sun's radiation varies throughout the day and the season as the angle of the Sun's rays, and possibly also the constitution of the atmosphere, changes. If changes caused by these two varying quantities could be eliminated the density of the ionization might be expected to be a measure of the intensity of the solar radiation. Methods for removing these variations were explained and the way in which the resulting 'ionospheric indices' varied with time were shown. One puzzling result was that the indices both for the *E* and *F* regions appear to have decreased steadily throughout the past 20 years even when the variations of the sunspot cycle have been removed.

The procedure was shown in which a digital computer was used to estimate the main parameters (height and peak electron content) of the *F* region from a knowledge of these quantities at a few key observing stations, and it was explained how this might help in the future for forecasting the nature of the ionosphere all over the world.

Once the world-wide *F* region is known, it is, in principle, possible to calculate the different rays by which waves will travel, on any frequency, from any one point to any other point on the Earth. The results of such calculations were compared with measurements of pulses received over the range of frequencies from senders in Malta and on Ascension Island. It is hoped that this kind of calculation, extended with the help of a digital computer, might lead to a simplified method for planning the frequencies used in point-to-point communications.

Another series of exhibits showed the work of the research station in the field of radio measurements. A resistive film inserted in a waveguide was used in an apparatus for the measurement of microwave power. This device had been compared satisfactorily with other standards from other laboratories and other countries.

Realization of the danger of intense radio-wave fields has led, recently, to a need for their measurement. A method of calibrating field-strength meters for these measurements was shown in which the intense field was produced in the space between the two plates of a 'strip waveguide'. In this way a field of the necessary intensity was produced from a comparatively feeble source of power and was confined to the guide, where it was harmless.

Other exhibits showed what was being done in research concerned with rockets and artificial satellites. Observations of the Faraday and Doppler effects on the waves emitted from satellites had led to deductions about the total electron content of the ionosphere overhead. Observations of the orbits of satellites were being used to make deductions about the density of the upper atmosphere and its variation.

An experiment to investigate the electron density in the ionospheric *D* region is to be performed in a rocket fired from Watheroo in Australia. This experiment will be done in collaboration with the University of Sheffield. The apparatus in the rocket will measure the parameters of a low-frequency wave-field set up above a transmitter radiating waves from the ground on a frequency near 200 kc./s. Part of the equipment was shown.

The Research Station is to collaborate with Canada and the United States in an experiment designed to investigate the upper side of the ionosphere. In this, satellites designed and launched by those working in the other countries will carry equipment to sound the ionosphere from above. The part of the Radio Research Station will be to receive telemetry from these satellites at Slough, Falkland Islands and Singapore, and to compare the results of the 'topside' sounding with the results of sounding made at these three places from the ground.

The Research Station is responsible for manning and running a station of the 'Minitrack' system set up by the Americans on a world-wide basis for the tracking of artificial satellites and the reception of their telemetry. The equipment is provided on loan by the Americans and is installed at a field site in Winkfield, about eleven miles from the Research Station. The visitors were taken there to see it. Measurements of position and records of telemetry made at this Station are sent back to America and are also available for the use of workers on the Station.

A World Data Centre for Rockets and Satellites, one of three, is run by the Station which is also responsible for tracking the position of the more important satellites for the use of optical and radio observers in Britain.

THE ROYAL AIRCRAFT ESTABLISHMENT, FARNBOROUGH

THE open days held at the Royal Aircraft Establishment, Farnborough, during June 9–10 were the first for six years. (The annual Air Show is, of course, that of the Society of British Aircraft Constructors, who hire the airfield for the occasion.) They gave a vivid impression of an Establishment that can afford to remain silent about its 56 famous years of service to scientific aviation (except through the single exhibit of a flying *S.E.5a*, the fighter designed at Farnborough of which Britain built 5,000 in the First World War), presumably because the present is so packed with excitement and achievement in the Establishment's main fields of transport aircraft, military aircraft, space, weapons, aircraft equipment and general research.

A central display hall, transformed from an aircraft hangar into a pavilion with flowers and fountains, summed up the whole display. It set out in its first illustrated stand the organization of the Royal Aircraft Establishment, with its headquarters at Farnborough, its second ultra-modern research centre and airfield at Bedford, and its well-equipped ranges for weapon trials. Subsequent stands, each devoted to one of the six main fields just noted, showed by models and highly informative backcloths how the different kinds of scientist and engineer in the Establishment make their different contributions to each. For example, study of transport aircraft problems was stated to include work on 'cabin conditioning', 'engine intake design', 'metal fatigue' and 'communications engineering' as four out of some thirty separate contributions—and similarly with the other fields. Two further stands showed the impressive range of major equipment for experimentation which the Establishment possesses to help it do its work, and the large-scale workshops, drawing-office and aircraft-servicing effort that lies behind the scientific achievements.

Both this central hall, and the 108 specialized scientific or technological exhibits, showed an Establishment pushing vigorously forward, with all its extraordinarily diverse activities concentrated on a few clearly defined objectives—to get air transport safer, cheaper, faster, more comfortable, more reliable, to get for the Army, Royal Navy and Royal Air Force a complete range of aircraft and weapons which will use the latest scientific advances to meet economically the needs of war as they are now envisaged, to develop continually improved aircraft equipment for navigation, reconnaissance, communications, power generation, automatic control and even landing, and to ensure that in spite of a late start Britain reaps the benefits of operations in space. Inquiries showed that the extent of 'space' activity, not only on the development of launching vehicles based on *Blue Streak* but also on the technology of satellites themselves, with special emphasis on a satellite system for world-wide communications, would almost justify a re-christening as the 'Royal Aircraft and Spacecraft Establishment'.

In advanced scientific research the variety of activity was astounding. For example, supersonic and hypersonic aerodynamics were to be expected, but the strength of the Farnborough school of low-speed aerodynamic research was equally evident, and remains equally necessary. The complexity of its subject-matter was exemplified by a wind-tunnel investigation of the effect of ground proximity on the lift of an aircraft model with swivelling jets, after the manner of the *Hawker P.*1127 vertical-take-off strike aircraft. Equally the 'slender-delta' designs of supersonic airliner have demanded for the solution of their landing and take-off problems a new aerodynamics, the extension of which into the unsteady 'gust case' was exemplified by an exciting experiment in which balsa-wood models were catapulted across a 24-ft. wide jet of air and their response in roll and yaw recorded by ciné-camera.

Another flourishing research school is in physics and chemistry of materials. The physical mechanisms of metal fatigue, including the details of crack development under cyclic loading, are now, in the main, scientifically determined, and are being rapidly related to the fatigue-life estimation of aircraft structures, showing, as one surprising result, that increase of temperature can sometimes extend fatigue life, because crack propagation in each cycle may halt sooner if plastic working is facilitated. Research on new materials has uncovered some impressive carbons with good strength and zero permeability, some new methods of construction in plastics reinforced with fibre-glass to give a strength-to-weight ratio 2·4 times better than 100 tons/in.^{2} steel, and a range of promising organic syntheses aimed at lubricants and polymers with improved high-temperature stability.

In the electronic field, an important new method of determination of the effective mass of charge carriers in semi-conductors was demonstrated, using observations of Faraday rotation, which was also shown modulating a beam of infra-red radiation at audio-frequencies. Promising circuits were exhibited, aimed at using semi-conductors to convert variable-frequency power from an aircraft's alternators to constant frequency. Research on aerials included the study of logarithmic-spiral forms at microwave frequencies, and some elegant work on corona breakdown on aircraft aerials at altitude. In the electronic equipment field, the miniaturized telemetry equipment, for transmitting pressures and other data to ground during weapon trials, stood out particularly.

Servocontrol is a well-known Farnborough activity. In the manned-aircraft field, it was interesting to see an aircraft being flown with all-electric signalling between the pilot's stick and the control surfaces. This is being used to facilitate a range of manœuvre demands. In the rocket field, the control of *Black Knight* by the swivelling of its two motors was demonstrated, together with equipment for stabilizing the attitude in space of the upper-atmosphere-research rocket *Skylark*. Equipment for studying the possible gyro-stabilization of a communications satellite so that its aerials will always point towards the centre of the Earth was also exhibited.

Research aimed at navigation and guidance was shown in impressive volume and quality. The propagation of very-low-frequency waves (of length around 20 km.) is now known to have remarkable stability as far as the antipodes even during magnetic storms, and research in a *Comet* aircraft flying from Farnborough has given good reasons to expect development of an airborne system for world-wide position determination within an error of at most 5 km. using only six ground transmitters. Inertial navigation has also been making extraordinarily rapid progress, with the Royal Aircraft Establishment again active, its longer-term researches being aimed, it appears, mainly at reduction in costs. Other titbits in this field were a tantalizing star-tracking demonstration and a glimpse of the future for high-speed computers in air traffic control.

It would be impossible to do justice to this huge exhibition in a short article, although a brief mention of the fascinating stands relating to water-drop or ice-crystal impact on aircraft, to new wind-tunnel techniques for aeroelastic research, and to rocket techniques for aerodynamic research at very high speeds, can scarcely be resisted. The days ended with experimental flying displays, showing among other things how the British army of the future may receive its supplies without parachutes, by low-level drops of equipment on special shock-absorbing skids, and how flight research is adding to our knowledge of the physical characteristics, and the effects on aircraft, of atmospheric turbulence.

Mr. M. J. Lighthill and his staff at Farnborough can be warmly congratulated, both on the story that they have had to tell and on the manner of its telling.

PETER SANDERSON

BRITISH BAKING INDUSTRIES RESEARCH ASSOCIATION

NEW LABORATORY WING

A NEW laboratory wing at the Baking Industries Research Station, Chorleywood, was opened on April 27 by Mr. Trevor Bowen, honorary president of Barkers of Kensington, and a former master of the Worshipful Company of Bakers.

In declaring the new north wing open, Mr. Trevor Bowen recalled the early days of the Research Association in 1947, when it had few members and a staff of only three, and, to indicate his appreciation of the services rendered by the Research Association, Mr. Bowen made a most generous gift of one thousand guineas as a contribution towards research.

The facilities of the new wing have been made essential by the growth in the Association's membership and activities. The work of the Association has outgrown the extra accommodation made available in 1951, when the present west wing was opened, and the need for additional and improved facilities has been a pressing one for some time.

The new accommodation consists of a two-storey wing running northwards from, and linked to, the original building of Chorleywood Lodge, designed to blend with the existing buildings and erected at a cost of approximately £40,000, including equipment.

The main feature of the ground-floor is the pilot-scale biscuit bakery and its associated laboratory. New pieces of equipment, built specially by members of the Research Association, are a model forced convection travelling oven by the Spooner Food Machinery Engineering Co., Ltd., and a De Vuurslag rotary biscuit cutter supplied by John Pelkman Engineering Co., Ltd. The biscuit section will thus have, virtually, a model biscuit plant which will enable the whole range of hard- and soft-dough biscuits to be studied. It is expected that this will enable previous work to be expanded on such problems of biscuit production as checking, or spontaneous cracking, in biscuits, on changes in biscuit dimensions in baking, and on the effect of various factors such as fat composition and storage conditions on the development of fat bloom on biscuits. The new equipment should also greatly facilitate the testing of biscuit flours and the development of standard procedures for this purpose. Sectioning and staining procedures have enabled a technique to be devised for investigating the starch, protein and fat distribution in biscuits and these investigations will be continued.

A new and well-equipped laboratory has been provided for the Organic Chemistry Section. This will give much-improved facilities for the work which has been in progress for some years on flour lipids, which is substantially supported by a grant from the United States Department of Agriculture. The work on lipids represents one facet of the attack on the problem of why flours differ in baking quality. Its immediate aims are to establish whether wheats of different varieties grown under identical conditions differ in lipid content, the influence, if any, of seasonal or environmental factors on the lipid composition of a single wheat variety and, finally, whether the lipid content or composition of a flour may be correlated with its baking properties. Also being studied are the fatty acid distribution in flour lipids, and methods for the separation of flour lipids in undegraded form.

These investigations, together with others in progress at the Research Station, will be greatly facilitated by the Buhler mill, which forms part of the equipment of the new Cereals Research Laboratory. This mill will provide much-needed facilities for milling wheat varieties on the site in connexion with the lipid project and other investigations, such as that of protein fractions being carried out in the new Physical Chemistry Laboratory. These provide another aspect of the problem of baking quality. Proteins of the gluten complex are being studied in the hope of establishing why wheats of similar protein content differ so markedly in this respect, and the work ultimately may lead to an understanding of the vast superiority of wheat protein over other cereal protein for bread-making. The present emphasis is on separation of gluten into its constituent proteins using starch-gel electrophoresis.

Also in the Physical Chemistry Laboratory are located the investigations into bread staling at present being pursued. These are concerned with those properties of wheat starch which influence the hardening of bread during the staling process with particular emphasis on the role of anti-staling agents. These agents increase the initial softness of bread and reduce the rate at which it hardens on ageing. Investigations of the interaction of anti-staling agents and starch are in progress in order to elucidate the basic mechanism of these effects.

An instrument and balance room has been provided on the first floor to house the more elaborate equipment used by all sections on different occasions.

At the southern end of the first floor the new Microbiology Laboratory extends the width of the building. There are a separate room for sterilization and another with filtered air-intake suitable for sterile techniques. The laboratory is concerned with all aspects of the microbiological deterioration of baked goods, but at present the work is mainly concerned with factors affecting mould growth in baked products, such as the effect of humidity, sugar concentration, and the degree of acidity or alkalinity of the mix. The use of mould inhibitors, such as propionic acid and its salts, as ingredients in bread and cake is also being studied. It is expected that legislation will shortly be passed to allow the use of these compounds, and the most effective ways of employing them and of overcoming side-effects, such as a reduction in bread volume, are being worked out.

Further facilities in the north wing are a combined office and laboratory for the Deputy-Director of Research, and another for the head of the Member Services Division, who is also responsible for applied research projects.

There is also office accommodation for two members of the newly formed Scientific Liaison Service set up to complement the work of the Industrial Liaison Service, which is staffed by trained bakers. The purpose of this Scientific Liaison Service (at present staffed by a chemist and an engineer) is to promote the application of the results of scientific research in the baking industry and to assess those problems in industry which require some research for their solution. These objectives are being attained primarily by personal visits and discussion, but also by lectures, demonstrations and collaborative experiments. A mobile laboratory has been equipped to take scientific apparatus to members' bakeries and has already proved its worth.

G. A. H. ELTON

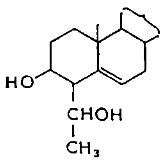
STEREOCHEMISTRY OF NATURAL PHENOLIC COMPOUNDS

THE aim of the Plant Phenolics Group symposium, held at the University of Bristol on December 16, was to discuss recent developments and current problems concerning the stereochemistry of natural phenols against the background of available methods for determining the stereochemistry of molecules.

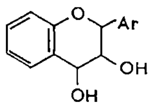
Dr. B. R. Brown (University of Oxford) dealt with the determination of relative stereochemistry.

The methods available for the determination of relative stereochemistry were illustrated by reference to the conformationally rigid, steroidal diol system (I) and to the conformationally non-rigid flavan-3,4-diol system (II). Infra-red measurements of C—O stretching frequencies enable reliable conclusions to be reached about the stereochemistry of the 3-hydroxyl group in (I) but not about the hydroxyl groups in (II), because of interference from aromatic vibration frequencies. Similarly, infra-red studies of hydrogen-bonding in (I) lead to reliable conclusions, but those from (II) do not, because of the conformational lability of the system. Ring formation to give carbonates or *iso*propylidene derivatives is diagnostic with (I) but not with (II), the *cis* and *trans* isomers of which both form cyclic compounds. In view of this, measurements of the rates of cleavage of flavan-3,4-diols with lead tetraacetate is in progress, a method which enables the *cis* and *trans* isomers of cyclohexane and other cyclic 1,2-diols to be differentiated. The method of molecular rotation differences has been applied with success to the steroids (Barton) and to the catechins (Freudenberg) and more recently by

Weinges[1] to correlate naturally occurring catechins, flavanonols, and flavan-3,4-diols (leucoanthocyanidins). The application of enzymic methods to sugars is well known and a recent investigation by Bokadia, Brown and Somerfield[2] indicates that it may have value when applied to polyphenols of the catechin type.



(I)



(II)

Prof. W. Klyne (Westfield College, University of London) described the determination of absolute stereochemistry.

The determination of the structure of an asymmetric natural product should not be considered as complete until its absolute configuration is known. The principal methods, with examples taken from different fields of organic chemistry, and chiefly from recent literature, are as follows:

(1) Chemical reactions, without displacement at the asymmetric centre, concerned: (*a*) degradations, for example, catechins (Hardegger *et al.*), cadinene (Herout and Šorm), and methymycin (Djerassi); (*b*) syntheses, for example, the conversion of representatives of the yohimbine–corynantheine–ajmalicine alkaloids and cinchona alkaloids into a common intermediate (Wenkert).

(2) Chemical reactions, with displacement at the asymmetric centre, concerned, for example, the classical studies of Ingold, Hughes and their colleagues on the steric consequences of nucleophilic substitution. Arguments by analogy include the work of Balenovic on β-aminobutyric acid, and of Witkop and of Sicher on ephedrine.

(3) Asymmetric synthesis (Prelog and Cram), for example, the catechins (Birch) and the diphenic acids (Mislow, also Berson).

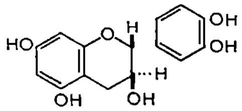
(4) Formation of quasi-racemates (Fredga), for example, the correlation of (+)-methylsuccinic acid and (−)-thiolsuccinic acid, which gave the first link between branched-chain compounds and the glyceraldehyde series.

(5) X-ray crystallographic methods do not, in general, distinguish between enantiomers, but an ingenious technique due to Bijvoet (Utrecht) which uses X-radiation of a wave-length which is partly absorbed by an atom in the crystal under examination permits a solution of the phase problem and determines the absolute configuration of the structure. This method, first applied to sodium rubidium tartrate, showed that by a fortunate chance the Fischer convention for glyceraldehyde is correct.

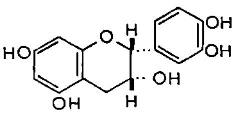
(6) Optical rotation evidence: (*a*) rotatory dispersion, (*b*) monochromatic rotations. All structural and stereochemical arguments which make use of optical rotations are based on analogy, and the usefulness of such arguments depends on the care with which analogies have been chosen. Recent instrumental developments have permitted the measurement of complete rotatory dispersion curves (that is, rotation : wave-length curves). Djerassi has found that "typical features of dispersion curves of alicyclic monoketones are on the whole only a reflexion of the immediate structural and stereochemical environment around that particular carbonyl group". A notable application of this principle was to the problem of the absolute configuration of the 'abnormal' diterpene, eperuic acid (Djerassi, F. E. King).

Dr. J. W. Clark-Lewis dealt with the stereochemistry of flavan derivatives.

The absolute configurations of the catechins were determined in 1957 and although the 2*R* : 3*S*-configuration of (+)-catechin (III) was unequivocally established, it was not until 1960 that the 2*R* : 3*R*-configuration of (−)-epicatechin (IV) received confirmation. The occasion of this symposium was therefore timely for reviewing the evidence for the configurations of the catechins. The diagnostic reaction for *cis*-flavan-3-ols is the elimination of toluene-*p*-sulphonic acid from tosyl esters, and for *trans*-flavan-3-ols is their rearrangement to 2-alkoxy-*iso*flavans; these two reactions have been used to characterize new catechins obtained by hydrogenolysis of leucoanthocyanidins and dihydroflavonols. Relative configurations of the catechins follow from the formation of enantiomeric 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)-propan-2-ols after reduction of (+)-catechin and (−)-epicatechin methyl ethers with sodium and liquid ammonia. Reduction with lithium aluminium hydride and aluminium chloride mixture is accompanied by a 1,2-rearrangement and leads, after methylation, to formation of (−)-2-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)-propan-1-ol from both diastereoisomers. It was shown that this rearrangement confirms the relative configurations of the catechins, and that it proceeds by benzyl-group migration. The absolute configurations of (+)-catechin and (−)-epicatechin are known from ozonolyses and from the application of Prelog's atrolactic acid method. The stereochemistry of several naturally occurring flavanoids can now be correlated with the stereochemistry of the corresponding catechins obtained by hydrogenolysis.



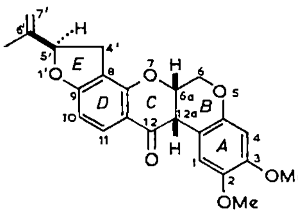
(+)-Catechin
(III)



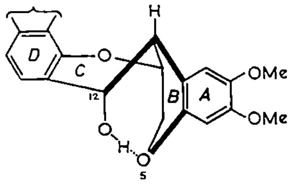
(−)-Epicatechin
(IV)

Dr. L. Crombie (King's College, University of London) described the absolute configuration of rotenone and its derivatives.

Work by Prof. G. Buchi (Massachussets Institute of Technology) and Dr. L. Crombie *et al.* has led to complete stereochemical proposals for the natural insecticide rotenone (V). By ozonolysis and oxidation the 5′ centre was extracted from the rotenone degradation product (−)-dihydrotubaic acid as (+)-3-hydroxy-4-methylpentanoic acid. The latter was related, by a series of transformations, to D-glyceraldehyde. Ozonolysis of the enol-acetate of 6′,7′-dihydrorotenone yielded the 6*a* centre as (−)-glyceric acid and the absolute configuration at 6*a* and 5′ is thus established. The B/C ring fusion of rotenone is the thermodynamically favoured form and this was shown to be *cis* by study of the stereoisomeric 12*a*-hydroxy*iso*rotenones, by hydrogenation evidence, and by infra-red methods. For example, the borohydride-reduction products of rotenone and its relatives show a hydrogen bond between the new 12-hydroxyl and the 5-oxygen which is possible only with a *cis*-fusion (cf. VI). The complete configuration of rotenone is thus (6*aS*, 12*aS*, 5′*R*) as in (V).



(V)



VI

Dr. W. D. Ollis, University of Bristol, spoke on the relative configuration of the rotenoids.

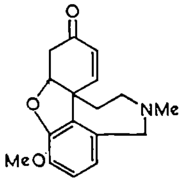
In collaboration with Prof. Carl Djerassi (Stanford University), it has been shown that the absolute stereochemistry of the eight natural rotenoids is closely related to that of rotenone (V). They all have the same relative stereochemistry at C_{6a} and C_{12a}; sumatrol and rotenone also have the same stereochemistry at $C_{5'}$. These stereochemical relationships have been established by optical rotatory dispersion studies on natural rotenoids and on derivatives of rotenone which were selected in order to assess the contributions of C_{6a}, C_{12a} and $C_{5'}$.

This work is complementary to that of Crombie and Büchi described in the preceding paper on the determination of the absolute stereochemistry of rotenone and indicate that the biosynthesis of the rotenoids is highly stereoselective. The stereochemical relationship between flavanoids, *iso*flavanoids and rotenoids, and possible biosynthetic implications were discussed.

Prof. D. H. R. Barton (Imperial College of Science and Technology, University of London) described alkaloid synthesis.

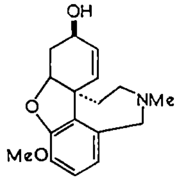
The biogenesis of many plant products may proceed through the oxidative coupling of phenolic precursors. In recent years several successful chemical syntheses have been based on this biogenetic idea; for example, oxidation of methylphloracetophenone and dehydration of the product gave the mould metabolite, usnic acid. Certain alkaloids may also be derived in principle by oxidation of suitable phenolic intermediates, the morphine group providing a particularly attractive example.

The structure of the Amaryllidaceae alkaloid galanthamine (VIII) was predicted on biogenetic grounds, and a chemical synthesis has now been completed. The hypothetical phenolic precursor was synthesized and its oxidation by a variety of reagents studied. Polymerization occurred readily but it was eventually possible to isolate the desired α,β-unsaturated ketone, narwedine (VII), in low yield. Reduction of narwedine gave a mixture of epimeric alcohols one of which was shown to be racemic galanthamine. A comparison of the physical properties of galanthamine and epigalanthamine showed that the hydroxyl group in galanthamine is hydrogen bonded to the ether ring oxygen atom, thus establishing the stereochemistry of these alkaloids. Narwedine racemized readily in hydroxylic solvents but crystallization of a mixture of (±)-narwedine (VII) and (−)-galanthamine (VIII) from ethanol gave (+)-narwedine. A study of this unusual phenomenon has led to the resolution of narwedine and galanthamine.



Narwedine

(VII)



(−)-Galanthamine

(VIII)

The biogenesis of Amaryllidaceae alkaloids is at present being investigated using precursors labelled with carbon-14. Preliminary experiments show that both tyrosine and the phenolic intermediate are incorporated into galanthamine in King Alfred daffodils.

BEN BROWN

---

[1] *Annalen*, 627, 229 (1959).

[2] *Proc. Chem. Soc.*, 280 (1960), and unpublished work.

INTERNATIONAL CONVENTION ON STARCH

THE annual International Starch Conference was once again held at Detmold, during April 26–28, and was attended by nearly 300 chemists from 25 nations. Twenty-five papers were presented to the meeting and they were classified as follows: (1) starch research; (2) industrial research; (3) derivatives.

A series of beautiful colour microphotographs were exhibited by Dr. A. H. Czaja (Aachen), showing the manner in which starch granules gelatinize in water. When suspended in a strong salt solution in the presence of I_2–KI the blue amylose fraction was seen to bleed out of the granule, leaving the violet amylopectin behind in the intact structure. No such bleeding of amylose was observed when the reaction was carried out in iodine-saturated water. Here the whole granule was stained violet throughout. When the granule was then damaged slightly by rubbing with a glass rod, the amylose blue colour was seen to develop. Potato, maize and rice starch behaved similarly.

When using a fluorescence dye, small starch granules were seen to fluoresce strongly (red) while large granules showed a weak green colour. No explanation of this behaviour, which is opposite to that expected, is yet known.

Acridine orange showed that granules of wheat starch were surrounded by a thin film of protein.

The renewed interest in the degradation of starch by physical means was discussed by S. Augustat (Potsdam-Rehbruecke), who had used a vibratory ball mill. In this machine the vessel does not rotate, but contains a large number of balls which are agitated with a frequency of 1,420 vibrations per min. The amplitude of the vibrations is about 2 mm., and the powder in the mill is kept in circulation by the airstream generated by the balls. After treating potato starch in such a mill for 5 hr. complete solubility in cold water was achieved together with a strong rise in reducing power. It was considered that this process may possibly have a future practical application.

The quantitative estimation of ether and ester groups in starch derivatives was described by Dr. R. J. van der Bij (Foxhol, Holland). A thin film of starch was cast on 'Pliofilm' and after removal the film was pressed between plates of rock salt. The estimation was carried out with the infra-red spectroscope, which gave a characteristic absorption band at 5·8μ. The intensity of the absorption was proportional to the degree of substitution, but a correction for the thickness of film had to be made.

The problems which arise through automation in the starch-producing industry were described by H. Runge (Hamburg). The starch industry is peculiar in that certain processes do not lend themselves to automation, so that an operative may be called on to perform his work in the conventional manner as well as by instrumentation. The main problem encountered in this industry was the education of a new generation of operatives who were able and willing to perform their tasks in this way.

A. Malyjev (Central Research Institute for the Starch and Starch-syrup Industry, Moscow) explained the use of the dextrose–sodium chloride double salt to facilitate the crystallization of dextrose in factories in the U.S.S.R. Dextrose syrup at 56° Brix was treated with 0·2 per cent sodium chloride (w/w) at 20° C. Crystallization was completed after 8 hr., and it was found that this was a much shorter period than experienced when crystallizing dextrose in the usual manner. The crystals obtained were larger and the yield (85 per cent) higher than normal. After crystallization the double salt was filtered off and decomposed in a syrup of 22° Brix, which had been cooled to 10° C.

The effect of certain cations on starch was discussed by G. Tegge and W. Kempf (Detmold) and Dr. S. Winkler (Berlin). Calcium and magnesium ions (such as occur in hard water) were found to lower the viscosity of starch pastes to a greater degree than sodium or potassium ions. It was maintained that the phosphoric acid ester of starch forms salts with these cations. It was claimed that the only true viscosity value could be obtained by having the ester in the free-acid form (called hydrogen starch). This form of starch was produced by washing the native, ungelatinized starch with $N/100$ hydrochloric acid for 10 min., followed by washing with distilled water for half an hour to remove the acid. Hydrogen starch was said to be unstable owing to slow autohydrolysis.

K. Pasirouk (Central Research Institute for Starch and Starch-syrup Industry, Moscow) described a new potato washing machine in use in Russian starch factories. Potatoes were freed from sand and grit by being shaken violently at 3,000 cycles/min. in a long stationary cylinder, through which a swift current of water passed. Potatoes vibrated with an amplitude of 5 mm. The machine dealt with 200 tons a day, each charge being 470 kgm.

A full account of the papers outlined above and of the remaining papers, together with the discussions which followed in each case, will be published in the journal *Die Stärke* in due course. E. Dux

THE NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

It is not always realized that the United Kingdom has one of the largest dairy industries in the world. Milk production in this country now exceeds 2,000 million gallons a year, and the milk has a value to the consumer of about £450 million, an amount of production which far exceeds that of many other well-known dairying countries such as New Zealand, Denmark and Holland. This vast and progressive British industry is continually increasing in efficiency, and much of the progress being made is directly attributable to the work done at the National Institute for Research in Dairying, the annual report of which for 1960 is now available*.

The Institute has grown greatly since the War. It now consists of ten main research departments, and has a staff of about 400 of whom some 30 per cent are members of the scientific and experimental officer grades.

The scope of the Institute's research programme is very great, ranging, as the report shows, from a study of many widely differing practical problems such as the use of slatted floors in the housing of dairy cattle and the types of chemical agents that can be approved for cleaning dairy equipment to basic scientific studies such as those of the Physiology Department on the pituitary gland and lactation, the biochemical properties of mammary gland mitochondria and the isolation, chemical structure and biological properties of the œstrogen mirœstrol.

No more than one or two of the many investigations now in progress can be referred to here. One particularly interesting example is concerned with the all-important question of the composition of milk on which, of course, the nutritive value of milk and milk products depends. It is now well known that much of the dietary energy absorbed by ruminants is absorbed in the form of the lower fatty acids which are produced in the rumen in different proportions from different types of feeding-stuffs. Collaborative work between two of the Institute's main research departments has shown that when isocaloric amounts of the lower fatty acids are given separately by ruminal infusion to lactating cows acetic acid increases not only the milk yield but also the fat percentage in the milk and the yields of fat, protein and lactose, whereas propionic acid has no effect on milk yield but increases the percentage of protein and decreases the percentage of fat. Butyric and lactic acids have no effect on milk yield but butyric acid increases fat percentage and lactic acid increases protein percentage. This type of information is already leading to a much better understanding of the factors that affect the composition of milk, and when further extended it should make it much easier than it is to-day to influence the composition of cows' milk within certain limits by suitable changes in the rations fed to the cows, a development which could be of far-reaching significance in the dairy industry.

Some of the other outstanding advances that are being made depend on the application of relatively new scientific methods to dairy problems. Work is being done, for example, on the differentiation and recognition of various strains and species of bacteria by paper chromatographic procedures, and the method is being applied in a detailed study of the bacterial flora of cheese. Then again gas chromatography is being applied to determine the volatile components of milk, and this may ultimately provide the basis of a rapid and reliable creamery platform test of milk quality. In another example a spectroturbimetric method has been devised which enables the mean fat globule diameter of homogenized milk to be rapidly determined. Indeed, it is abundantly clear from the report that every effort is being made to apply the most up-to-date scientific knowledge, methods and equipment to the art and practice of dairying in all its branches.

---

* National Institute for Research in Dairying. Report 1960. Pp. 164. (Shinfield: National Institute for Research in Dairying: University of Reading, 1961.)

The report includes a list which gives the titles and authors' names of the 165 papers which were published by members of the staff between October 1959 and September 1960. The Institute is to be congratulated on the variety, amount and high quality of the work reviewed in its report.

J. A. B. SMITH

FISHING IN MADAGASCAR

WITHIN the last decade, the fish *Tilapia melanopleura* has brought about a radical change in the diet and social habits of a large proportion of the population of Madagascar. Fish supplements the otherwise protein-deficient diet of the islanders, and fishing has become a useful and pleasant occupation practised every day by whole families.

The fish, along with other species of *Tilapia*, was first introduced by the Government ten years ago. The aim was to provide a source of much-needed protein to balance the Malgache menu of rice and manioc, fruit and a little honey, enlivened only occasionally by a small amount of meat or fish.

The *Tilapia* soon established itself in the rivers and lakes and multiplied rapidly. Fishing became popular. Encouraged by the success of this initial venture, the Government was stimulated to extend the output of fish still further by creating fishponds in the central part of the island where there is abundant water. Government agents went from village to village showing the inhabitants how to construct the ponds. By 1958 there were 40,000 village ponds; to-day there are 80,000, providing nourishing meals where formerly fish was seldom eaten.

A recent survey by Dr. Rudolph Kreuzer, Food and Agricultural Organization, Fisheries Division expert, shows that the average catch per adult is about 1·1 lb. of small fish each day, and even a small child can catch 4 lb. (*Sci. afrique*, 23; February 1961). Not surprisingly, the people who live near the rivers and lakes eat more fish daily than Europeans do in a week. But the quantities caught are related only to the fisherman's needs. There is still malnutrition in the dry areas of the island. Before this can be eliminated, it will be necessary to develop a fishing industry and overcome the distribution problems inevitable in the hot African climate.

A government pilot station on Lake Alaotra is already successfully producing smoked fish and fish meal. Dr. Kreuzer's investigations have shown that drying and salting would also be successful in the fishing villages along the western coast. There, shielded by a coral reef, a primitive technique, closer to hunting than fishing, is practised. First, a flotilla of outrigger canoes forms a semi-circle near the reef. By beating the paddles on the water, the men drive the fish inshore. Then the fishermen leap overboard to spear the catch in the water. Nets are used only to prevent the quarry escaping seawards.

Better methods of fishing and the development of economic methods of processing the catch would help towards correcting dietary deficiency where it still occurs. But formidable transport problems would still have to be solved, for dense bush isolates the west coast fishing villages from the rest of the island.

TERRESTRIAL EXOSPHERE AT HEIGHTS OF 1,000—1,700 km.

By MAX RÖMER

Sternwarte der Universität, Bonn

THE successful launching of the satellite iota 1, 1960 (*Echo* I), with its extremely small ratio of mass to area $m./A. = 10^{-2}$ gm./cm.^{2} first offered the opportunity of deriving atmospheric densities above a height of 1,000 km. For the time-interval August 12–December 19, 1960, it was possible to determine the densities for the heights 1,700 km. down to 1,000 km. Considering the large amplitude of the diurnal effect[1] and the relatively small eccentricity of the orbit it is impossible to use the density scale height H_ρ in the well-known air density formula by D. G. King-Hele, G. E. Cook and D. M. C. Walker[2]. The acceleration of a satellite is caused by the actual air density along the orbit. Therefore the 'individual orbital scale height' H characterizing a linear approximation to the logarithmic density function in the individual orbit was calculated[3]. For this calculation a model of atmospheric densities by H. K. Paetzold[4] was used augmented with the diurnal variation derived by W. Priester, H. A. Martin and K. Kramp[1]. The knowledge of the exact shape of the auxiliary model is not important because in the density formula only $\sqrt{H}$ is used. The computed densities hold for the orbital points of highest density, that is, for 14 hr. true local time.

The choice of the drag coefficient was discussed in detail[3] including the interaction of the charged satellite with the ionized component of the exosphere, and $C_D = 2{\cdot}5$ was adopted as a reasonable value[5]. According to R. W. Parkinson, H. M. Jones and I. I. Shapiro all orbital elements are disturbed by the solar radiation pressure during one revolution[6]. For orbits of small eccentricity the secular perturbation of the semi-major axis is very small. But for the calculation of air density by means of satellite accelerations all periodic variations of the orbital energy are important, the period of which is greater than the mean distance between consecutive orbital data. Considering the unequal time-intervals of acceleration and deceleration due to the non-uniform orbital

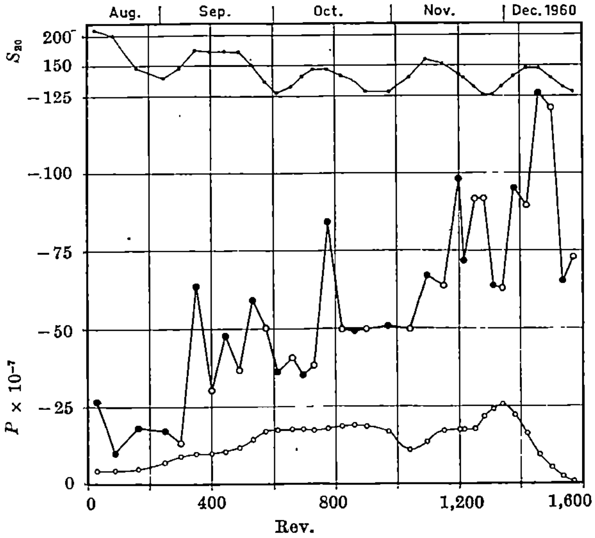


Fig. 1. Observed acceleration, acceleration due to solar radiation pressure and flux of solar 20-cm. radiation. The big circles of the curve in the middle of the diagram show the observed accelerations $\dot{P}_{\text{obs.}}$ of satellite *Echo* I. According to the different weight, mean values $\Delta P/\Delta t$ are represented by full circles and less weighted values dP/dt of the Bulletins of the National Space Surveillance Control Center, Bedford, Mass., by open circles. Accelerations due to solar radiation pressure $\dot{P}_{rp}$ are given by the small circles of the lower curve. The difference $\dot{P}_{\text{obs.}}—\dot{P}_{rp}$ yields the acceleration caused by aerodynamic drag. The flux of solar 20-cm. radiation S_{20} is shown in units 10^{-23} W./m.^{2} c./s. by the upper curve

velocity an approximate formula for the periodic perturbation of the period caused by solar radiation pressure (*rp*) was derived in co-operation with W. Priester:

$$\dot{P}_{rp} \simeq \frac{3 \times 8{\cdot}64 \times 10^4 \times P}{\pi} \cdot \frac{K \times \cos\theta_{\odot} \times e \times \sin(\omega - u_{\odot})}{m \times v} (\sin\Delta u_s - 2) \quad (1)$$

where P is period in days, $K = 330$ dyne, the product of area and radiation pressure if $\dot{S} = 2{\cdot}0$ cal./cm.^{2} min. $= 1{\cdot}4 \times 10^6$ erg/cm.^{2}/sec. is adopted for the total flux of solar radiation, e is orbital eccentricity, v orbital velocity in cm./s., m mass of the satellite in gm., $\theta_{\odot}$ the angle between the orbital plane and the line Sun–Earth, ω argument of perigee, $u_{\odot}$ angle of the Sun in the orbit measured from the ascending node and Δu_s is half of the angle of the Earth's shadow in the orbit. Fig. 1 shows that the acceleration due to solar radiation pressure cannot be neglected in the analysis of the observed accelerations of *Echo* I.

For the calculation of atmospheric densities the difference of the observed acceleration $\dot{P}_{\text{obs.}}$ and the acceleration due to solar radiation pressure $\dot{P}_{rp}$ was used. These densities were corrected to a flux of solar 20-cm. radiation $S = 170$ (unit 10^{-22} W./m.^{2}c./s.) using the standard reduction formula (ref. 7). The correlation of the observed density $\rho_{\text{obs.}}$ with the flux of solar decimetre radiation S was checked and the best representation is:

$$\rho_{\text{obs}} \propto S^{m(h)} \quad (2)$$

where $m(h) \simeq 2$ for altitudes 1,000–1,700 km. The calculated densities ρ_{170} for the standard flux $S = 170$ drawn in a ($\log\rho$, h)-diagram (Fig. 2) show a variation which can be explained by an 'annual' effect first found by H. K. Paetzold. The densities derived from *Echo* I by H. K. Paetzold[4,8], who uses a similar method which takes account of the large diurnal variation, are consistent within the limit of error if the influence of solar radiation pressure on the period is neglected. The values ρ_{170} were used by H. A. Martin and W. Priester[9] for expanding the density model (Bonn University Observatory model)[1] to 1,600 km. above the Earth ellipsoid.

Table 1. PHYSICAL PARAMETERS OF THE TERRESTRIAL EXOSPHERE

h 10^5 cm.	g cm./s.^{2}	$\log\rho$ gm./cm.^{3}	H_ρ 10^5 cm.	$\log p$ dyne/cm.^{2}	H_p 10^5 cm.	T/M °K.	M for $T = 1690$ °K.
600	819·5	0·140 – 15	111	0·142 – 5	123	121	14·0
700	796·5	0·770 – 16	120	0·806 – 6	136	131	12·7
800	774·5	0·427	132	0·504	154	144	11·3
900	753·4	0·115	145	0·252	182	165	9·7
1,000	733·1	0·818 – 17	160	0·042	228	201	8·1
1,100	713·6	0·555	179	0·896 – 7	307	264	6·5
1,200	694·9	0·330	205	0·772	398	333	5·2
1,300	676·9	0·135	237	0·677	515	419	4·1
1,400	659·6	0·970 – 18	281	0·600	647	513	3·3
1,500	642·3	0·831	333	0·539	795	614	2·7
1,600	626·9	0·712	399	0·490	957	721	2·3
1,700	611·5	0·609	484	0·448	1,129	830	2·0

If local thermodynamic equilibrium holds and the velocity distribution is Maxwellian[10] the connexion of the equation of state for ideal gases and hydrostatic equilibrium yields the differential equation:

$$\frac{1}{\rho} \times \frac{d\rho}{dh} = -\frac{m \times g}{k \times T} - \frac{1}{T} \times \frac{dT}{dh} + \frac{1}{m} \times \frac{dm}{dh} \quad (3)$$

where m is mean mass of particles, k Boltzmann's constant, T temperature and g acceleration due to gravity. If the exosphere is assumed to be isothermal above the critical height h_c, the gradient of temperature vanishes and equation (3) can be solved numerically with an assumed value of the mean molecular weight M and the temperature T at h_c as initial condition. The integration is stopped when the mean molecular weight begins to increase with increasing height and when a lower limit of $M = 1{\cdot}5$ is reached. The distributions of mean molecular weight passing between these two limits from the critical height up to 1,700 km. determine the temperature and the

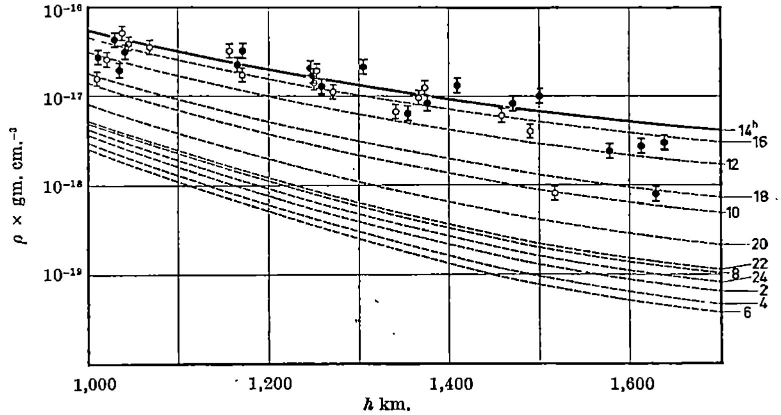


Fig. 2. Atmospheric densities at altitudes 1,000–1,700 km. above the Earth ellipsoid derived from satellite *Echo* I. The acceleration due to solar radiation pressure has been taken into account. The calculated densities (mean values represented by filled symbols, less weighted values by open symbols) hold for 14 hr. true local time and are corrected to mean solar activity ($S_{20} = 170 \cdot 10^{-22}$ W./m.^{2} c./s.). The 'fan shape' density model is the auxiliary model used for the calculation of the 'individual orbital scale height' H. The parameter of the curve gives the true local time. The time of year belonging to the densities is August 15, 1960, at $h = 1,640$ km. and December 18, 1960, at $h \simeq 1,000$ km.

lapse-rate of M in the exosphere. The temperature needed for the interpretation of the observed density distribution increases with increasing values of M at the critical height. For mean molecular weights of $M = 14, 16, 18$ at $h_c = 600$ km. the temperatures are 1,675° K., 1,900° K, 2,175° K. respectively.

One of the possible extrapolations of the density scale height $H_\rho = -\frac{dh}{d \ln \rho}$ leads to an isothermal exosphere with M = const. above 2,100 km. and to a pressure of $2 \cdot 02 \times 10^{-7}$ dynes/cm.^{2} at 2,100 km. The distribution of pressure can be calculated by:

$$p(h) = p(2{,}100 \text{ km.}) + \int_{h}^{2{,}100 \text{ km.}} \rho \times g \times dh \qquad (4)$$

The important physical parameters of the terrestrial exosphere are given in Table 1: acceleration due to gravity g, density ρ, density scale height H_ρ, pressure p, pressure scale height $H_p = \frac{p}{\rho \times g} = \frac{R \times T}{M \times g}$ and the lower limit of mean molecular weight M for T = 1,690° K. according to the H_ρ-distribution of the Bonn model. By means of the ratio T/M the mean molecular weight curve of an isothermal exosphere can be calculated for various temperatures. The unexpected small lapse-rate of the atmospheric density above 1,000 km. yields a rapid decrease of the mean molecular weight, that is, a rapid increase of hydrogen in the number density. For a detailed discussion of the problem see ref. 3.

I am grateful to Dr. W. Priester, who suggested this problem, for his co-operation and numerous discussions. Furthermore, I wish to thank the National Space Surveillance Control Center, Bedford, Mass., for supplying the orbital elements and the Heinrich-Hertz-Institut, Berlin-Adlershof, for the continuous dispatch of 20-cm. data prior to publication.

Note added in proof. According to recent investigations the orbital period of a satellite, which does not enter the Earth's shadow during one revolution, is not altered by solar radiation pressure. However, a comparison between the results plotted in Fig. 1 and the findings of R. W. Bryant (private communication) and of P. E. Zadunaisky *et al.* (*Smithsonian Astrophys. Obs. Spec. Rep. No. 61,* 1961) shows that formula (1) is a good approximation for the time-interval covered.

[1] Jacchia, L. G., *Smithsonian Astrophys. Obs. Spec. Rep.* No. 39 (1960). King-Hele, D. G., and Walker, D. M. C., "Atmospheric Densities at Heights of 180–700 km.", paper at Symp. Aeronomy, Copenhagen (July 1960). King-Hele, D. G., *Nature*, **186**, 928 (1960). Priester, W., Martin, H. A., and Kramp, K., *ibid.*, **188**, 202 (1960).

[2] Cook, G. E., King-Hele, D. G., and Walker, D. M. C., *Proc. Roy. Soc.*, A, **257**, 224 (1960).

[3] Römer, M., *Mitt. Univ.-Sternwarte Bonn*, No. 37 (Forschungsbericht d. Landes NRW No. 997, Westdeutscher Verlag Köln-Opladen, 1961).

[4] Paetzold, H. K., *Naturwiss.*, **48**, 39 (1961).

[5] Jastrow, R., and Bryant, R., *J. Geophys. Res.*, **65**, 3512 (1960).

[6] Parkinson, R. W., Jones, H. M., and Shapiro, I. I., *Science*, **131**, 920 (1960).

[7] Priester, W., and Martin, H. A., *Nature*, **188**, 200 (1960).

[8] Paetzold, H. K., "A Proposal for a Self-consistent Model of the Upper Atmosphere and its Variations", preprint (February 1961).

[9] Martin, H. A., and Priester, W., *Model of Atmospheric Densities* (Univ. Observatory, Bonn, February 1961).

[10] Öpik, E. J., and Singer, S. F., *Physics of Fluids*, **2**, 653 (1960), and *GRD–TN*–60–606 (June 20, 1960) with Supplement (October 14 1960).

USE OF THE ROTATING HANGING MERCURY DROP IN ANALYSIS

Anodic Stripping using a Rotating Mercury Drop

By Dr. J. J. ENGELSMAN and A. M. J. M. CLAASSENS

Philips Research Laboratories, N.V. Philips' Gloeilampenfabrieken, Eindhoven

A HANGING mercury drop has been used by several authors, for example, DeMars and Shain[1] and Kemula and Kublik[2], for the analysis of metals forming amalgams with mercury after electrolysis. Usually the metals in the amalgam are anodically dissolved by applying a potential which varies linearly with time. On the basis of preliminary calculations involving the diffusion conditions in and around the mercury drop, we were led to believe that the time constant of the dissolution process should be of the order of a few seconds. Consequently, it should be possible to obtain separate dissolution peaks for each of the components of a multicomponent drop of amalgam, using a rate of voltage scanning such as is normally used in polarography (5 mV./sec.).

A rotating mercury drop electrode as described by Barendrecht[3] might be used for this purpose. Delahay *et al.*[4] mentioned the use of a similar electrode for anodic stripping, but they did not give experimental results. The platinum electrodes of Barendrecht and Delahay were gold-plated; according to Kemula *et al.*[5], gold interferes during stripping of zinc and cadmium amalgams.

We carried out experiments with an electrode consisting of a platinum wire of 0·25 mm. diameter sealed into the end of a piece of soft glass tubing 5 mm. in diameter and filed flush with the glass as described by Ross *et al.*[6]. The glass tubing was fused to a cylinder of a chromium-iron alloy connected to the shaft of a synchronous motor (3,000 r.p.m.). Electrical contact was made via a graphite sliding contact. The platinum electrode was centred to better than 25μ.

The platinum was cleaned by the method of Ross *et al.*[6] and a mercury drop was made by constant-current electrolysis in a concentrated solution of mercurous nitrate, 1 N in nitric acid, using a mercury pool anode (2 m.amp. 3 min.). During rotation of the electrode, the mercury drop remains attached to the platinum.

For trace analysis an anodic stripping voltammogram is run after a period of cathodic electrolysis of a few minutes. In Fig. 1 are shown the anodic current peaks of zinc, cadmium, lead and copper in a 10^{-3} M potassium chloride supporting electrolyte, 2×10^{-6} M in zinc, cadmium, lead and copper ions. The solution was electrolysed for 5 min. at $-1 \cdot 5$ V., the rate of scan during stripping was 5 mV./sec. and silver wire coated with silver chloride served as a reference electrode.

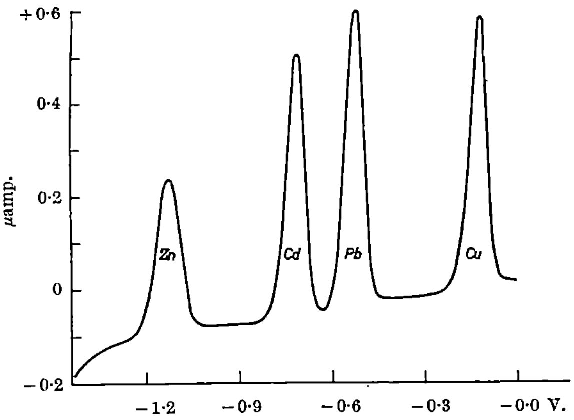


Fig. 1. Anodic current peaks of zinc, cadmium, lead and copper in 10^{-3} M potassium chloride, 2×10^{-6} M in the respective ions. Cathodic electrolysis: 5 min. at −1·5 V. vs. silver/silver chloride electrode. Rate of scan: 5 mV./sec.

The peaks are well separated and the areas of the cadmium, lead and copper peaks correspond to approximately 7×10^{-6} coulombs. The abnormal area and shape of the zinc peak may be attributed to the influence of platinum on dissolution of zinc from an amalgam, as mentioned by Kemula *et al.*[7].

The time-constant of about 4 sec. for the dissolution process allows the use of the rotating mercury drop for trace analysis of metal ions, described by van Swaay and Deelder (see following communication) as coulometric polarography. We intend to publish a mathematical treatment of this dissolution process in the near future.

[1] DeMars, R. D., and Shain, I., *Anal. Chem.*, **29**, 1825 (1957).

[2] Kemula, W., and Kublik, Z., *Anal. Chim. Acta*, **18**, 104 (1958).

[3] Barendrecht, E., *Nature*, **181**, 764 (1958).

[4] Mamantov, G., Papoff, P., and Delahay, P., *J. Amer. Chem. Soc.*, **79**, 4034 (1957).

[5] Kemula, W., Galus, Z., and Kublik, Z., *Bull. Acad. Polon. Sci., Sér. Sci. Chim., Géol. et Géogr.*, **6**, 661 (1958).

[6] Ross, J. W., DeMars, R. D., and Shain, I., *Anal. Chem.*, **28**, 1768 (1956).

[7] Kemula, W., Galus, Z., and Kublik, Z., *Bull. Acad. Polon. Sci., Sér. Sci. Chim., Géol. et Géogr.*, **7**, 723 (1959).

Trace Analysis with a Rotating Hanging Mercury Drop

By Dr. M. van SWAAY and R. S. DEELDER

Laboratory of Instrumental Analysis, Technical University, Eindhoven

Kemula[1] and Engelsman (preceding communication) have described a technique for the analysis of trace metallic impurities. A solution containing the trace impurities as metal ions is electrolysed under polarographic conditions with a rotating mercury drop cathode. With the resulting drop of dilute amalgam, an anodic polarogram is recorded whereby the metals, dissolved in the mercury, return to the solution. The resulting anodic current peaks correspond to the composition of the amalgam, which, in turn, is determined by the cathodic electrolysis time and the composition of the sample electrolyte.

We should like to describe the technique as 'coulometric polarography'. A refinement of this technique will be described, together with some results showing the applicability of the method to the analysis of non-amalgamating metals.

Both Engelsman and Kemula use the same electrolyte for the cathodic and anodic electrolyses. If different electrolytes are used for the two parts of the cycle, we are free to choose one or more 'stripping' electrolytes for optimum separation of the anodic current peaks. Moreover, the properties of the sample solution are less critical, so that time of preparation may often be reduced. The results depicted in Fig. 2 demonstrate this feature. Here traces of zinc, copper, nickel and chromium in a sodium phosphate sample solution were determined without noticeable interference from the phosphate medium.

Merck analytical grade reagents were used throughout. Water was purified in a mixed-bed ion-exchange column. Supporting electrolytes were purified by prolonged electrolysis with a mercury pool cathode and stored under nitrogen in glass vessels connected to the electrolysis cell via 'Teflon'-glass stopcocks.

The rotating electrode consisted of a 0·5 mm. platinum wire forced through a 0·45 mm. hole in a small 'Teflon' plug contained in the 1½-mm. bore of a short length of glass capillary tubing. The complete electrode assembly was ground flush with fine emery paper. The capillary was mounted with wax on the shaft of a 3,000 r.p.m. synchronous motor running on ball bearings. Centring to within 5μ was achieved by trial and error with the help of a binocular microscope and a stroboscopic flash unit. Electrical contact was made via a small insulated mercury well at the top of the motor shaft.

At the electrode, a mercury drop was generated by electrolysis in a saturated solution of mercurous nitrate. The size of the drop was deduced from the time-integral of the generating current. A single drop could be used for a large number of experiments. By the judicious use of stopcocks, a complete set of experiments could be made without opening the system to the atmosphere.

In Fig. 1 a schematic diagram is given for a typical electrolysis programme. In Fig. 2 a typical anodic

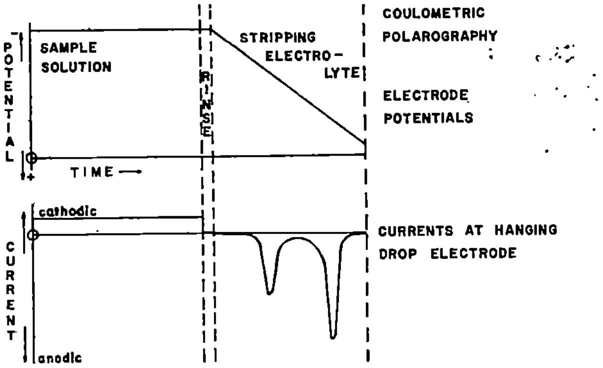


Fig. 1

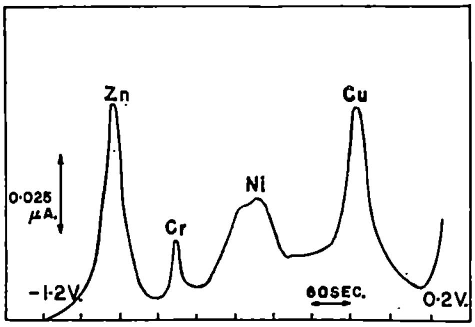


Fig. 2. Sample solution: 1 M Na_2HPO_4 with 5×10^{-7} M each of zinc, chromium, nickel, cupric ions. Electrolysis, 300 sec. at − 1·2 V. (saturated calomel electrodes). Stripping solution: 0·1 M KCNS. Voltage scan, −1·2 V. to −0·2 V. at 2 mV./sec.

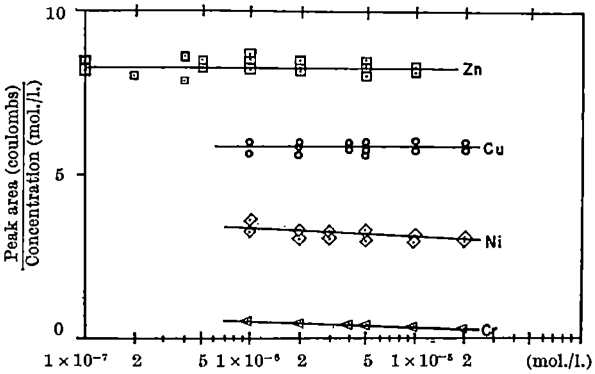


Fig. 3. Peak area/concentration versus concentration

Sample solution	Electrode potential	Stripping solution
Cu^{2+} in 0·1 *M* KCNS	– 1·0 V. (S.C.E.)	0·1 *M* KCNS
Zn^{2+} in 1 *M* KCL	– 1·2 V.	1 *M* KCL
Ni^{2+} in 1 *M* NH_3/NH_4Cl	– 1·35 V.	1 *M* NH_3/NH_4Cl
Cr^{3+} in 0·1 *M* KCNS	– 1·5 V.	0·1 *M* KCNS

Cathodic electrolysis time, 300 sec. } for all runs
Anodic voltage scan, 2 mV./sec. }

voltammogram is shown for an analysis of 5×10^{-7} *M* each of zinc, cupric, chromium and nickel ions. In Fig. 3 the relation between peak area and sample composition is shown for the same four metals. The electrolysis programme is described in the legend.

The graphs for nickel and chromium ions show a slight deviation from linearity, which necessitates the use of a calibration curve. The anodic current peaks for these metals also show a tendency to broaden out rather than increase in height with increasing concentration. However, these effects do not seriously impair the usefulness of the method for these 'non-amalgamating' metals.

Attempts to extend the voltage-range beyond –1·5 V. (saturated calomel electrode) have so far been unsuccessful, because a large irreversible cathodic current began to flow at this potential. The nature of the corresponding electrode process is not quite clear, but two possibilities suggest themselves :

(1) The protecting nitrogen gas may not have been sufficiently free of oxygen to prevent reduction of oxygen to hydroxyl or peroxide, which may be responsible for the reduction current. It is unlikely, however, that this process accounts for all the current.

(2) The mercury drop may not have covered the platinum tip completely, so that reduction of hydrogen ions could take place at the exposed part of the platinum. This effect may be expected to show a strong dependence on the *p*H of the solution.

We intend to investigate both possibilities further in this Laboratory. If it proves possible to eliminate these interfering currents, the method will become useful for the analysis of a large group of metals in concentrations down to 10^{-9} *M* and possibly less.

---

[1] Kemula, W., Rakowska, E., and Zublik, Z., *J. Electroanal. Chem.*, 1, 205 (1960). Kemula, W., Zublik, Z., and Glodowski, S., *ibid.*, 1, 91 (1960).

FLUID–FLUID INTERFACES IN STEADY MOTION

By PROF. WALTER ROSE

University of Illinois, Urbana

GIVEN a capillary channel of constant cross-section (shape, area and orientation) and let a fluid, for example a wetting liquid, displace another immiscible one therefrom at a constant rate. The fluid–fluid interface, therefore, will be advancing at a steady velocity. I now set out to prove that this interface will everywhere and continuously have constant curvature, neglecting, as a simplification, distortions due to gravity.

The question is an important one, touched on briefly in the subject-matter and cited literature of the papers by Scriven *et al.*[1,2], and it is an essential one to the discussion of capillary flow if valid boundary conditions are to be assigned in solving the appropriate equations of motion[3].

By simple definition, a constant curvature surface (in the present case, a fluid–fluid interface having a closed-perimeter contact with the pore wall) is one where the sum of the reciprocals of the principal radii of curvature has the same value at every point on the surface. Elementary considerations lead to the realization that the curvature of a fluid–fluid interface at a particular point is proportional to the pressure difference between fluid pressures in each phase at contiguous points across the interface (that is, the so-called capillary pressure)[4].

Without loss of generality, let us limit attention to displacement phenomena in circular capillary tubes ; therefore, the interface either will correspond to the surface of a section of a sphere, if it is a constant curvature surface (as I believe), or the interface will have some other irregular shape (albeit with symmetry to the axis of the tube). In any event, since the motion is given as steady-state, the geometry of the interface will be fixed in time, without reference to the question whether or not a film (that is, a high concentration of surfactants) contaminates the interfacial region.

Suppose we think of a horizontal tube, and of a wetting liquid spontaneously displacing another less-wetting liquid of the same viscosity (assuring a steady motion) and density (avoiding all gravity constraints). The tube will be taken as very long, so that for most of the time when interface motion is occurring the losses of energy where the tube-ends are connected to inflow and outflow reservoirs can be neglected.

An observation of importance is that the axial component of fluid particle velocity will form (approximately) a parabolic profile in the radial direction at all distant positions ahead of and behind the advancing interface, whereas the interface itself moves as though the radial profile of the axial velocity component were (approximately) planar. The earliest references to this consideration which have come to my attention[5,6] are those cited by Gardescu[7].

As a term having phenomenological merit, we introduce the designation, 'fountain effect', to describe how the wetting liquid particles from the central pore space decelerate as they approach the slower moving interface region, acquiring a finite radial component of velocity as they spill over towards the wall region being vacated by the advancing interface. Correspondingly, a 'reverse fountain effect' is seen in the non-wetting fluid ahead of the interface, as fluid is displaced from the wall region and for the most part moves towards the tube axis, and as the fluid particles accelerate centrally ahead of the advancing interface.

Apparently, an integration of the equations of Navier and Stokes would yield a complete description of the fountain effect, and even though the boundary conditions may be specified, the integrability is hampered by the inherent non-linearities of the problem. Relevant numerical approximations have not as yet been attempted, as can be seen in the literature we have examined. In any event, the speculation of Barrer[8], as investigated experimentally in this laboratory at present[9], that the curvature of a moving interface depends on rate, undoubtedly is an important consequence of the fountain effect. That is, the changing interfacial curvature as rate of interface motion changes means that the capillary driving force is diminished effectively as an external stress is imposed to make the imbibing interface travel faster (that is, the rule of Le Chatelier).

Proceeding from Barrer's point of view, one might argue that the fast-moving fluid particles approaching the slower-moving interface along the central stream gain pressure energy in the loss of kinetic energy; hence, the interface is flattened, and in an extreme case the interface may even reverse curvature. This argument is incomplete, however, in simply emphasizing the Bernoulli condition without reference to viscous dissipation of energy; moreover, by itself no conclusion can be formed from the argument whether or not the moving interface has constant curvature throughout its areal extent.

Of interest in this connexion is the early work of Prandtl *et al.*[10], as extended later by Schlichting[11], who treated the somewhat corresponding problem of the transition in velocity distribution in the region near the entrance of a pipe. The boundary-layer methods, which are applied, have only limited application to the analysis of the fountain effect problem; however, some useful ideas are suggested by this cited work.

For example, taking the Lagrangian point of view (where the moving interface is thought to have a fixed position in the sense that the observer is moving with the same velocity as the interface), the wetting fluid will seem to have an outward component of radial velocity behind the advancing interface, and the non-wetting fluid will seem to have an inward component of radial velocity ahead of the advancing interface. Therefore, at all points on the surface of the interface the tangential component of velocity must be zero. This is because[12], if there is a two-dimensional solid film at the interface, the fluid particles of the two phases on each side must have the same tangential velocity as the film; likewise, if there is no film, the viscous stress will be continuous. Therefore, in either case, no tangential motion can occur in the interface region.

The foregoing continuity proposition is so important that it bears restatement: if all fluid particles in the near-vicinity of one side of the interface have positive components of radial velocity, while those of the second phase in the near-vicinity of the other side have negative components, and if the fluid particles of the two phases making up the interfacial region have the same tangential component of velocity, then this tangential component necessarily is zero at all points defining the interfacial surface.

Now, it will be readily apparent that, if the tangential component of the velocity vector is zero at the boundary of each phase forming the interfacial region, no pressure gradients exist in either phase tangential to the surface. In consequence, the capillary pressure differences between wetting and non-wetting fluids at each point in the interface region will have some constant value; hence the moving interface will be constant curvature throughout. This proposition, once accepted, leads to an obvious simplification in stating the boundary conditions necessary to analyse certain categories of capillary flows (Fara, H., and Rose, W., unpublished).

By parallel argument, we may conclude that interfaces in steady motion will be characterized by constant curvature no matter what the pore shape is, for tangential motions in the interface (whether it be a two-dimensional solid or liquid film) cannot occur. The criteria of Boussinesq[1], namely, the surface shear viscosity and the dilational surface viscosity, have no particular bearing on the problem at hand, but only enter into the description of the dynamics of interface movement during unsteady-states and/or when the cross-section of the pore channel changes with distance[13].

[1] Scriven, L. E., and Sternling, C. V., *Nature*, **187**, 186 (1960).

[2] Scriven, L. E., *Chem. Eng. Sci.*, **12**, 98 (1960).

[3] Channapragada, R., and Rose, W., *J. Geophys. Res.*, **66**, 1199 (1961).

[4] Adam, N. K., *The Physics and Chemistry of Surfaces*, third edit., 8 (Oxford Univ. Press, 1941).

[5] Nageli, M., *Bull. Acad. Sci. (Munich)*, **1**, 597 (1866).

[6] Wolff, H. C., *Trans. Wisconsin Acad. Sci.*, **12**, 550 (1898).

[7] Gardescu, I. I., *Trans. Amer. Inst. Mech. Eng.*, **88**, 351 (1930).

[8] Barrer, R. M., *Disc. Farad. Soc.*, No. 3, 61 (1948).

[9] Rose, W., and Heins, R. W., *J. Colloid Sci.* (in the press).

[10] Prandtl, L., and Tietjens, O. G., *Applied Hydro- and Aero-mechanics*, 25 (Engineering Societies Monographs, 1934; cf. Dover Publications Edition, 1957).

[11] Schlichting, H., *Boundary Layer Theory*, fourth edit., 168 (McGraw-Hill Book Co., 1960).

[12] Milne-Thomson, L. M., *Theoretical Hydrodynamics*, third edit., 541 (Macmillan Co., 1955).

[13] Schlichting, H., *loc. cit.*, Figs 5.7 and 5.15 on pp. 74, 90.

INTRACELLULAR DISTRIBUTION PATTERN OF RAT LIVER GLUTAMIC-OXALACETIC TRANSAMINASE

By Dr. HERBERT J. EICHEL and JOSEF BUKOVSKY

Department of Biological Chemistry, Hahnemann Medical College, Philadelphia

In recent years, serum glutamic-oxalacetic transaminase (GOT)-levels have been studied in many diseases in human beings and a variety of experimental states in animals[1]. Since it is possible that the increased serum GOT activity observed under abnormal conditions is a reflexion of tissue injury (hepatic, cardiac, etc.) resulting in the release of intracellular GOT which then enters the circulation, information concerning the intracellular localization of the enzyme is of basic interest. However, at the time the work described here was initiated (1958), detailed data were lacking on this point. Hird and Rowsell[2] had reported that rat liver mitochondria and supernatant fluid catalysed transamination between glutamate and aspartate. Müller and Leuthardt[3] found 32 per cent of the total homogenate GOT activity in the mitochondria isolated from isotonic potassium chloride homogenates of rat liver, but the enzyme was concentrated 2·3-fold in this fraction with respect to the original homogenate; the remainder of the activity was apparently present in an unseparated microsomal-supernatant fluid fraction.

In view of the incompleteness of these results, a study was undertaken in our laboratory[4] of the distribution of this enzyme among sub-cellular fractions obtained from rat liver and spleen homogenates by differential centrifugation. GOT activity was assayed by two procedures; that used most frequently was a modification of the spectrophotometric method of Karmen[5] developed for serum, and the other was the method of Greenwood and Greenbaum[6]. All tests were carried out at *p*H 7·6 in 0·033 *M* potassium phosphate buffer.

Normal male Wistar rats (200–400 gm.), fed a stock diet of 'Purina' fox chow checkers *ad libitum* to the time of death, were used. The animals were anæsthetized lightly with ether, bled by heart puncture, and the livers quickly excised and washed free of blood. Usually, 1–3 gm. of liver were removed from the left lateral lobe and homogenized with 9 vol. of ice-cold 0·25 *M* sucrose in a TenBroeck glass grinder. Homogenates were fractionated according to the method of Schneider and Hogeboom[7], with some changes[8], and nitrogen analyses on homogenates and all fractions were made by a micro-Kjeldahl procedure. About 50–70 per cent (average, 62 per cent) of the total GOT activity was found in the mitochondria, 20–40 per cent (average, 29 per cent) in the supernatant (soluble) fraction, and the remainder, due to mitochondrial contamination, in the nuclei (2 per cent) and microsomes (7 per cent). Only the mitochondrial enzyme had a higher specific activity on a nitrogen basis than that of the original homogenate (2 : 1), the specific activity of the supernatant being about equal to the homogenate activity. These results raised the following question: Is part of the enzyme associated with the soluble phase of the intact liver cell or does its recovery in this fraction represent an artefact caused by the procedure employed ?

We carried out several series of experiments[4] aimed at shedding further light on this problem. First, it was found that the unusual bimodal distribution pattern of GOT was unchanged when the fractionation medium was varied to favour the preservation of mitochondrial structure or for other reasons. Second, attempts were made to release GOT from the mitochondria by various physical and chemical treatments. Although exposure of the mitochondria (or homogenate) to the action of a Waring blendor, sonic oscillation, distilled water, or the detergent, 'Triton *X*-100', unmasked previously unreactive enzyme, that is, increased the measured GOT activity to varying degrees but several-fold in general, only a portion, at best, of the total mitochondrial activity was solubilized in the process. It should be emphasized that since the total activity of GOT of freshly isolated, intact mitochondria suspended in sucrose is at least doubled by such treatments, the activity of GOT recovered in the supernatant fraction is actually much less than 30 per cent of the total GOT present in the whole cell.

On the basis of these experiments and the specific activity data mentioned previously, it was concluded[4] that GOT is concentrated in the mitochondria of the normal rat liver cell. The picture was more equivocal concerning the activity found in the supernatant fluid. However, the fact that a significant amount of the enzyme was always present in this fraction, regardless of the nature of the homogenization and fractionation medium, spoke for the localization of some GOT in this part of the intact cell, and posed the question of whether there were any differences in the properties of the enzyme recovered in the mitochondria and supernatant. One step in examining this question is to investigate the activities of the enzyme in response to certain physiological stresses. In preliminary experiments, we studied the effects of starvation (72 hr.) and whole-body X-irradiation (700 r.) on the specific activities of GOT in mitochondrial and supernatant fractions of rat liver. While there was some indication that the activities of the two fractions responded differently, particularly after irradiation, the small number of animals involved precluded the drawing of definite conclusions. Interesting observations bearing on this question have been reported recently by Fleisher *et al.*[9] and Rosenthal *et al.*[10]. By a paper electrophoretic technique, the former authors demonstrated two fractions of GOT activity in water extracts of human and dog heart and liver minced in a blendor. One fraction migrated towards the anode and showed the known characteristics of serum GOT, while the other migrated towards the cathode and exhibited different affinities for α-ketoglutarate and L-aspartate and a different *p*H-activity curve. In our work[4], an attempt had been made only to differentiate the readily measurable mitochondrial GOT from that part of the mitochondrial activity which was unmasked and solubilized on blending, by assaying GOT at various *p*H values between 5·4 and 8. No difference was observed. Although the effect of *p*H on the activities of the supernatant and mitochondrial enzymes was not investigated, we entertained the possibility that the bifractional distribution of GOT might be related to the observation of Fleisher *et al.*[9]. Experimental support for such a relationship appeared in the recent abstract of Rosenthal *et al.*[10]. In agreement with our findings, although a completely different enzyme assay was used, they reported that GOT was found in the mitochondrial (70–80 per cent) and soluble fractions prepared from 0·25 *M* sucrose homogenates of rat liver. Furthermore, they indicated that the mitochondrial and soluble GOT activities of rat liver closely resembled the cationic and anionic forms of the enzyme described by Fleisher *et al.*[9] with respect to *p*H-activity curves and relative affinities for aspartate and α-ketoglutarate.

Regarding the intracellular localization of the enzyme, May *et al.*[11] recently found 45 per cent of the total GOT activity of rat liver homogenate in the mitochondria, 17 per cent in the supernatant fluid and 33 per cent in the nuclear fraction. An appreciable amount of GOT was also recovered in the soluble fraction prepared from rat brain homogenate[11]. We have shown that while GOT is apparently concentrated in the mitochondria of rat spleen[12], considerable transaminase activity is present in the supernatant fraction obtained from 0·25 *M* sucrose homogenates of this organ. It would seem that a bimodal distribution pattern of GOT is characteristic of many tissues, and it would be of interest to determine if the enzyme also exhibits chemical differences in the two fractions from tissues other than liver. In this connexion, it should be noted that the cationic and anionic forms of GOT were found in both heart and liver[9].

Fleisher *et al.*[9] showed that serum from several patients with myocardial infarction and viral hepatitis contained only small amounts of cationic GOT. From this, it might be inferred that in these diseases increases in serum GOT result chiefly from the release of the soluble, anionic intracellular GOT rather than the particle-bound, cationic enzyme. This would be consistent with the observation already made that, at least in rat liver, mitochondrial GOT is released in soluble form only after considerable structural damage to the particles. In this respect it is to be noted that the cationic enzyme was demonstrated in soluble preparations made by treating tissues in a blendor[9]. We have found that treatment of rat liver mitochondria (0·25 *M* sucrose) for increasing periods, either in a Waring blendor (2–10 min.) or sonic oscillator (5–20 min.; Raytheon model *DF* 101, 10 kc.), followed by centrifugation of the mitochondria for 1 hr. at 150,000*g*, renders soluble increasing amounts of the total mitochondrial activity present after treatment. However, from our experiments it would appear that about half the enzyme remains firmly bound to the mitochondrial membranes, and possibly the cristæ, and to attempt to release more, at least without special care, might invite denaturation of the GOT. Hence caution should be exercised in assessing and assigning significance to the proportions of the two forms of GOT present in extracts of blended organs[9].

Finally, it may be necessary to re-evaluate past studies in which liver homogenate GOT activity was examined in animals subjected to a variety of experimental conditions, that is, administration of hormones, instillation of toxins, exposure to ionizing radiations, effect of dietary deficiencies, carcinogens, etc. For example, since the soluble enzyme represents about 30 per cent or less of the total homogenate GOT of fresh preparations in sucrose, it can be calculated that as much as a 75 per cent decrease in its specific (or total) activity would yield only a 23 per cent decrease in the specific (or total) activity of overall homogenate GOT, provided there are no changes in the nitrogen content.

This work was supported by a contract (*AT* 30-1-2118) from the U.S. Atomic Energy Commission.

[1] Wroblewski, F., in *Advances in Clinical Chemistry*, edit. Sobotka, H., and Stewart, C. P., 1, 314 (Academic Press, New York, 1958).

[2] Hird, F. J. R., and Rowsell, E. V., *Nature*, **166**, 517 (1950).

[3] Müller, A. F., and Leuthardt, F., *Helv. Chim. Acta*, **33**, 268 (1950).

[4] Bukovsky, J., M.S. thesis, Hahnemann Medical College (May 1960)

[5] Karmen, A., *J. Clin. Invest.*, **34**, 131 (1955).

[6] Greenwood, F. C., and Greenbaum, A. L., *Biochim. Biophys. Acta*, **10**, 623 (1953).

[7] Schneider, W., and Hogeboom, G. H., *J. Nat. Cancer Inst.*, **10**, 969 (1950).

[8] Eichel, H. J., *J. Biophys. Biochem. Cytol.*, **3**, 397 (1957).

[9] Fleisher, G. A., Potter, C. S., and Wakim, K. G., *Proc. Soc. Exp. Biol. Med.*, **103**, 229 (1960).

[10] Rosenthal, O., Thind, S. K., and Conger, N., *Abs. 138th Meeting Amer. Chem. Soc.*, New York, 10 C (September 1960).

[11] May, L., Miyazaki, M., and Grenell, R. G., *J. Neurochem.*, **4**, 269 (1959).

[12] Eichel, H. J., and Bukovsky, J. (to be published).

PHARMACOLOGICALLY ACTIVE SUBSTANCES PRESENT IN THE CENTRAL NERVOUS SYSTEM

By Dr. PAUL V. BUDAY

Department of Pharmacology, University of Rhode Island, Kingston, Rhode Island

PSYCHOPHARMACOLOGY is a new interdisciplinary science. It attempts to correlate mental aberration, emotional disturbance, and behavioural changes with modifications in the normal biochemistry of the central nervous system.

In an attempt to interrelate brain function with the psyche (mind) a number of basic propositions must be acquiesced. Thus, the thesis of psychopharmacology rests upon the concept that the brain and psyche, although distinct entities, are actually intimately associated and mutually dependent. It adheres to the principle that animal behaviour is a consequence of a dynamic equilibrium between a primitive, emotional and instinctual system (rhinencephalon), and a recollective, rational and cognitive mechanism (cerebrum). Psychopharmacology also asserts that emotion may very well be a result of cortical disorganization, and that biochemically induced changes in the central nervous system, via drug action, will modify psychic reactions.

This concept of mind–brain interdependence is not novel. In the sixth century B.C., the Greek physician Alcmaeon taught that the brain was the organ of the mind. To-day, the mind is, in certain respects, still considered to be the subjective expression of the living brain. Certain psychotogenic (hallucinogenic) drugs, for example lysergic acid diethylamide (LSD), mescaline, etc., provide an opportunity to readily induce a reversible model psychosis which, at least superficially, mimics schizophrenia, thus furnishing a closer introspection into possible psychopathological mechanisms.

A host of phrenotropic agents has been introduced into the armamentarium of the psychiatrist and physician in the past several years. These medicaments are believed to act, in the main, by way of certain substances found in the central nervous system. The substances thus far posited to be concerned with brain and mental activities include: adrenaline, noradrenaline, dopamine (3-hydroxytyramine), 5-hydroxytryptamine (5-HT), histamine, acetylcholine, γ-aminobutyric acid (GABA), adrenochrome, and substance '*P*'. Structural configurations for these chemical materials are depicted in Fig. 1.

Also intimately concerned are the sundry enzymes which synthesize and biotransform these biogenic substances. It appears that the neuro-hormones following synthesis are stored, physiologically inert, in certain cytoplasmic organelles and protected by certain intracellular barriers from enzymic catabolism. Following release, these substances become physiologically active, react with some cellular or

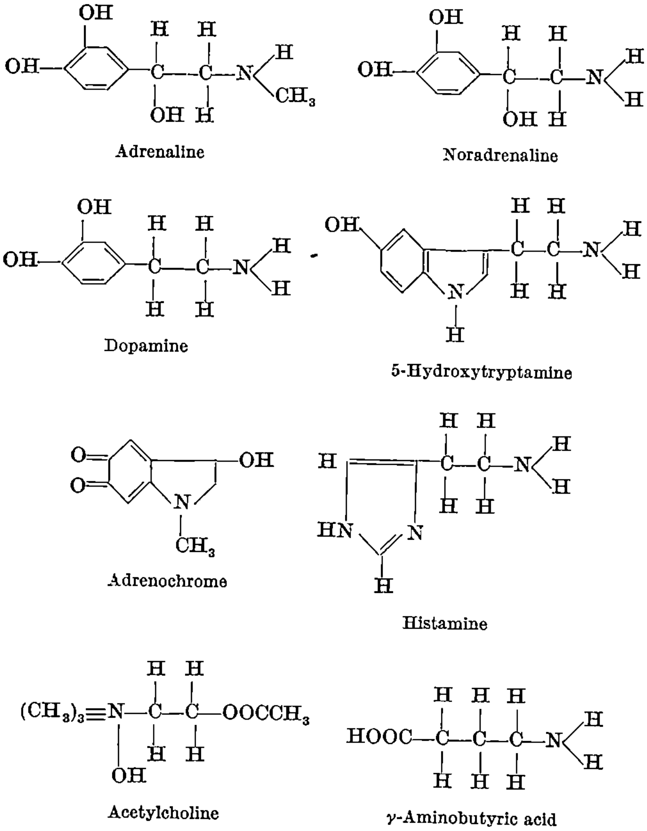


Fig. 1. Graphic configurations of some centrally active compounds

plasmatic membrane receptor, and thence regulate neuronal transmission or other functional processes. Biotransformation of the neuro-hormone then occurs via a specific enzyme system with subsequent termination of the substance's excitatory or inhibitory effects.

The central distribution of the neuro-hormones is well documented. The brain distribution of noradrenaline, the precursor of adrenaline, is disproportionate; highest concentrations are found in 'primitive' brain sites, namely, the hypothalamus, area postrema, medullary reticular formation and medial thalamus. Adrenaline, in lesser amounts, follows this distribution rather closely. Dopamine is located in highest concentrations in the corpus striatum, where peculiarly its metabolite noradrenaline is found only in minor quantities. Conversely, brain loci, for example the hypothalamus, replete in noradrenaline and its biocatalyst, dopamine-β-oxidase, contain paucid quantities of the parent molecule. 5-HT, synthesized from 5-hydroxytryptophan by the agency of aromatic L-amino-acid decarboxylase, follows the localization of noradrenaline rather closely. It is significant that only 1 per cent of the 5-HT content of vertebrates is found in the brain. The area postrema contains relatively high levels of 5-HT, but strangely enough no enzyme for its synthesis has been discovered here. Peripherally ubiquitous histamine is found in significant amounts solely in the hypothalamus and cerebellum, but histaminergic synapses have not been identified. Acetylcholine, reported by Pfeiffer *et al.*[1] to be derived from the naturally prevailing amino-alcohol dimethylaminoethanol, has been discovered particularly in the cerebral cortex, pons, thalamus, basal ganglia and other brain nuclei. An interrelationship between acetylcholine metabolism and that of adrenaline may exist for, theoretically at least, the monomethylaminoethanol chain cleaved from the latter compound may serve as a basis for choline synthesis[2]. GABA, a component of Florey's factor I, and manufactured via the agency of *l*-glutamic acid decarboxylase on glutamic acid, is present predominantly in the rhinencephalon ('visceral brain'), medulla, pons, and cortex. Adrenochrome, the highly labile indole oxidation product of adrenaline, has been discovered in erythrocytes and heart, and following convulsions, in brain tissue[3]. Substance '*P*', a biologically active polypeptide, has been detected in the intestines and brain, and in the latter especially in the basal ganglia, thalamus, hypothalamus, area postrema, and spinal cord.

A number of functions have been ascribed to these centrally active substances and the *modus operandi* of the various phrenotropic medicaments is largely predicated on their ability to modify the metabolism or receptor binding sites of these hormones or their metabolites. Hess[4] has postulated the existence of a subcortical (hypothalamic) integrating system composed of two reciprocally antagonistic divisions, namely, the 'ergotropic' and the 'trophotropic' nervous systems. The former mediates arousal, and an alerted mental state, skeletal muscle hypertonia, and an exaggeration of sympathetic activity. The latter centre, and anatomically the more anteriorly disposed, is concerned with sleep, apathy and psychic depression, a diminished skeletal muscle tone, and increased parasympathetic activity. Brodie and Shore[5] theorize that free (active) 5-HT and noradrenaline act as synaptic mediators for the trophotropic and ergotropic nervous systems repectively.

Marrazzi[6] has shown that adrenaline, noradrenaline, and 5-HT, as opposed to adrenochrome, are potent intercortical synaptic inhibitors, and he postulates that certain mental disturbances result from an imbalance between inhibition by adrenaline, noradrenaline or 5-HT and facilitation by acetylcholine of central synaptic, cholinergic transmission. Although acetylcholine activates Renshaw cells in the spinal cord, its, noradrenaline's, and 5-HT's inertness following electrophoretic application to medullary and reticular formation neurons implies a non-transmitting role for these substances in these particular areas[7].

Woolley and Shaw[8] have suggested that certain mental disturbances are caused by a metabolic deficiency of brain 5-HT. They have described a rather novel *in vitro* excitant action of this neurohormone on cortical oligodendroglia, and they hypothesize that 5-HT serves to augment cerebral metabolism through this neurotonic mechanism. Unfortunately, the careful clinical results of Feldstein *et al.*[9] do not support the concept of a relationship between 5-HT metabolism and acute or chronic schizophrenia.

The physiological significance of centrally partitioned histamine is still obscure. Indirect evidence for its participation in mental activity is exemplified by the antihistaminics, a number of which possess potent central actions. More direct evidence is gleaned from reports that histamine (and its methylated derivatives) are amine oxidase inhibitors.

Further, certain monoamine oxidase inhibitors affect histaminase (diamine oxidase) activity irreversibly. Histamine appears to be a natural synaptic inhibitor similar to 5-HT. Adrenaline blocks *l*-histidine decarboxylase[10], the enzyme responsible for the biosynthesis of histamine, and it may be that this hormonal effect provides a negative servo-mechanism for the regulation of central histamine-levels.

Feldberg[11] relegates a synaptic transmitting (facilitating) role to acetylcholine in the autonomic and central nervous systems. Because of this action on brain stem reticular formation synapses, Himwich and Rinaldi[12] propose that elevated brain concentrations of acetylcholine may exert antischizophrenic actions.

GABA, an amino-acid metabolite, is a synaptic and neuronal inhibitor. Purpura *et al.*[13] feel that this material is a transmitter substance in the sense that it acts as an inactivator of synaptic depolarization. It has been suggested that it may well be involved in neuro-excitability and in the etiology of certain brain abnormalities, for example epilepsy. GABA may also subserve the action of certain convulsive drugs, and the observation that monoamine oxidase inhibition elevates free brain GABA-levels tempts one to speculate that this explains the mechanism of the anticonvulsant action of certain 'psychic energizers'.

Hoffer[3] postulates that adrenochrome (chemically similar to LSD), or dihydroxyindole catabolites of abnormal adrenaline oxidation, are causes of schizophrenia. This concept, however, has been considerably criticized and this worker's findings have not been consistently substantiated. Adrenochrome may still have significance, nevertheless, for this hallucinogen is, at least *in vitro*, an inhibitor of both glutamic acid decarboxylase and cholinesterase.

Substance '*P*' is given a neuro-active role in the dorsal horns of the spinal axis, and a non-cholinergic transmitting function in the autonomic nervous system. It has been likened to a type of 'physiological tranquillizer' because of its meprobamate-like polysynaptic inhibitory properties in the central nervous system.

Vitally concerned with the action of the central neuro-hormones, especially the biogenic catecholamines and 5-HT, are the enzymes responsible for their biotransformation and inactivation. Monoamine oxidase is a prime example of such a biocatalyst the activity of which can be modified or destroyed by drug action with either a consequential deviation in normal metabolic pathways followed by its substrates or by a pile-up of these compounds. Monoamine oxidase is widely distributed in the animal body and is primarily located in mitochondria. Centrally, the enzyme is normally found where 'true' cholinesterase-levels are low, namely, the lenticular nucleus, hypothalamus, and pyramidal tracts. The primary functions assigned to monoamine oxidase are the metabolism of the relatively inert metanephrines[14,15] and other O-methylated catecholamine metabolites, and of degradating 5-HT to 5-hydroxyindoleacetic acid[16]. Dopamine, formed from dihydroxyphenylalanine by the action of aromatic L-amino-acid decarboxylase, is a better substrate for monoamine oxidase than noradrenaline, and hence enzyme inhibition by administration of a 'psychic energizer' shunts the metabolic pathway in favour of one catalysed by catechol-O-methyltransferase with the consequential accumulation of 3-methoxytyramine. This latter enzyme has been recently given the principal task of converting noradrenaline and adrenaline to the respective metanephrines[17,18], monoamine oxidase notwithstanding. Spector *et al.*[19] suggest that the latter enzyme may control cellular 5-HT and noradrenaline-levels, while catechol-O-methyltransferase inactivates blood-borne catecholamines. However, Belleau *et al.*[20] claim, rather convincingly, that noradrenaline is not acted on by monoamine oxidase at adrenergic effector cell sites. As may be noted, the implication of aromatic L-amino-acid decarboxylase in the formation of both the catecholamines and 5-HT leads one to believe that a closer correlation exists between the metabolic disposition of the catecholamines and 5-HT than heretofore suspected.

In summary, a number of pharmacologically active substances naturally occurring in the central nervous system have been briefly characterized and their potential physiological functions described. It seems, in retrospect, rather significant that of all the substances postulated to officiate as neuro-mediators or incriminated in the etiology of mental illness, only acetylcholine possesses neuronal and synaptic facilitating properties. It would appear that acetylcholine is the driving force in the nervous system whereas the remaining substances simulate physiological brakes. The phrenotropic agents widely employed in the pharmacotherapy of various forms of mental illness obviously modify brain biochemistry, some perhaps via mitochondrial receptor attachment[21], with apparently consequential effects on the psyche. However, it would be rather premature and naïve to hope that contemporary physiological, biochemical, and even psychopharmacological techniques of studying and modulating brain function will instigate dramatic and important insights into psychic activities. This problem is knotty and may perhaps have no solution as presently assailed. Nevertheless, break-throughs into this twilight zone bridging mind and brain may be possible if studies are performed with determination and if moderately permeated with imagination.

[1] Pfeiffer, C. C., Jenney, E. H., Gallagher, W., Smith, R. P., Bevan, jun., W., Killam, K. F., Killam, E. K., and Blackmore, W., *Science*, **126**, 610 (1957).

[2] Du Vigneaud, V., Chandler, J. P., Simmonds, S., Moyer, A. W., and Cohn, M., *J. Biol. Chem.*, **164**, 603 (1946).

[3] Hoffer, A., in *Psychotropic Drugs* (Elsevier, New York, 1957).

[4] Hess, W. R., in *Diencephalon, Autonomic and Extrapyramidal Functions* (Grune and Stratton, New York, 1954).

[5] Brodie, B. B., and Shore, P. A., in *Psychopharmacology Frontiers* (Little, Brown and Co., Boston, 1959).

[6] Marrazzi, A. S., in *Brain Mechanisms and Drug Action* (Charles C. Thomas, Springfield, 1957).

[7] Curtis, D. R., and Koizumi, K., *J. Neurophysiol.*, **24**, 80 (1961).

[8] Woolley, D. W., and Shaw, E., *Proc. U.S. Nat. Acad. Sci.*, **40**, 228 (1954).

[9] Feldstein, A., Freeman, H., Hope, J. M., Dibner, I. M., and Hoagland, H., *Amer. J. Psychiat.*, **116**, 219 (1959).

[10] Werle, E., *Biochem. Z.*, **311**, 270 (1942).

[11] Feldberg, W., *Brit. Med. Bull.*, **6**, 312 (1950).

[12] Himwich, H. E., and Rinaldi, R., in *Brain Mechanisms and Drug Action* (Charles C. Thomas, Springfield, 1957).

[13] Purpura, D. P., Girado, M., Smith, T. G., Callan, D. A., and Grundfest, H., *J. Neurochem.*, **3**, 238 (1959).

[14] Evarts, E. V., Gillespie, L., Fleming, T. E., and Sjoerdsma, A., *Proc. Soc. Exp. Biol., N.Y.*, **98**, 74 (1958).

[15] Champagne, J., D'Iorio, A., and Beaulnes, A., *Science*, **132**, 419 (1960).

[16] Sjoerdsma, A., Smith, T. E., Stevenson, T. D., and Udenfriend, S., *Proc. Soc. Exp. Biol., N.Y.*, **89**, 36 (1955).

[17] Axelrod, J., *Science*, **127**, 754 (1958).

[18] Bacq, Z. M., Gosselin, L., Dresse, A., and Renson, J., *Science*, **130**, 453 (1959).

[19] Spector, S., Kuntzman, R., Shore, P. A., and Brodie, B. B., *J. Pharmacol.*, **130**, 256 (1960).

[20] Belleau, B., Burba, J., Pindell, M., and Reiffenstein, J., *Science*, **133**, 102 (1961).

[21] Abood, L. G., *J. Neuropsychiat.*, **1**, 92 (1959).

ANALYSIS OF THE THYMUS INFLUENCE IN LEUKÆMOGENESIS

By Dr. J. F. A. P. MILLER

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W.3

THYMECTOMY markedly reduces the incidence of lymphocytic leukæmia in mice which would otherwise develop the disease spontaneously[1], or following exposure to ionizing radiations[2], chemical carcinogens[3] or virus inoculation[4]. Grafting thymus tissue to thymectomized mice restores the susceptibility to the development of lymphomas[1,3,5,6]. Thymectomy might prevent leukæmogenesis by effecting removal of one or more of the following: (*a*) the source of a leukæmogenic virus (either present from birth or activated by external leukæmogens); (*b*) the site of multiplication of such a virus (assuming that multiplication is a prerequisite for leukæmia induction); (*c*) the only type of cells capable of undergoing leukæmic transformation; (*d*) the source of a humoral factor involved in leukæmogenesis.

Presence of Leukæmogenic Virus in Non-Thymic Tissues

Evidence has been presented in previous papers[6,7] to show that the thymus does not act by simply providing the source of a leukæmogenic virus. Recovery of the agent from non-leukæmic tissues of virus-infected thymectomized mice[7] and restoration of incidence of lymphoma in such mice by grafting thymuses from normal mice as late as six months after thymectomy[6] strongly suggest that the virus is widely distributed in its host and not simply confined to the thymus. These results have recently been confirmed by other workers[8].

Virus Multiplication in Thymectomized Mice

It is possible that a leukæmogenic virus must reach a critical concentration before it can induce leukæmia in its host and that multiplication can only occur in thymus tissue. The question whether virus can multiply outside thymus tissue is being studied in this laboratory. Extracts of non-leukæmic tissues of thymectomized, virus-inoculated mice have been injected into successive generations of thymectomized mice after the operation. Since it has been shown that new-born or suckling mice are more susceptible to leukæmic filtrates than adult mice[9] it was decided to perform the operation of thymectomy in the first week of life. The technique used for baby mice was similar to that employed in this laboratory for adult mice[6], and the immediate operative mortality was no higher than 15 per cent. Subsequent mortality, however, was very high in mice that had been thymectomized at 1 day of age, more than 50 per cent of these dying between 2 and 4 months (whether they had been inoculated with virus or not), suggesting that the thymus at birth may be essential to life. Mortality at 3 months of age was much lower in mice thymectomized between 3 and 5 days of age. A group of mice thymectomized at that age were inoculated with leukæmic virus one day after the operation and killed 2–3 months later in order to prepare extracts from their spleens, livers and brains. Checks were made for the total absence of thymus tissue at death. The extracts were inoculated into a group of intact 3–5-day-old mice and into a second group of 3–5-day-old thymectomized mice. Extracts were prepared from the latter when they were about 3 months old and were inoculated in turn into further groups of intact and thymectomized baby mice. The incidence of lymphomas in the intact mice receiving the extracts at each generation is recorded in Table 1.

Table 1. LEUKÆMOGENESIS IN INTACT *C3Hf/Gs* MICE BY VIRUS SERIALLY PASSAGED THROUGH THYMECTOMIZED MICE

Inoculation given	No. of intact mice treated	Leukæmia incidence (per cent) and age in months
Passage *A* filtrate	26	73 (3–6)
Virus passaged once through thymectomized mice	10	80 (3–6)
Virus passaged twice through thymectomized mice	8	63 (4–6)
Virus passaged thrice through thymectomized mice	11	73 (3–7)

Only preliminary results from three generations are available, and these suggest that the leukæmogenic virus has not been lost by serial transfer through thymectomized hosts. The possibility, therefore, remains open that multiplication might occur in extra-thymic tissues. Such extra-thymic multiplication is not accompanied by neoplastic transformation in lymphoid cells, and, as a corollary, it may be that only replication within the thymus can be associated with leukæmogenesis.

Origin of Leukæmic Cells

The next problem to be considered is whether the thymus acts as a source of potentially malignant cells. It is possible that thymus lymphocytes differ from other lymphocytes in their susceptibility to neoplastic transformation. Experiments employing thymectomized hosts either genetically[8,10,11] or immunologically[12] tolerant of foreign thymus grafts have been set up by various workers in an attempt to settle this question. The results depended on the transplantation behaviour of the lymphoid tumour arising from the grafted thymus. In some cases the tumour behaved as if it had originated from lineal descendants of cells regenerating from the donor thymus tissue[11,12], which could then be assumed to have acted as a source of potentially malignant cells. In other cases, however, the neoplastic cells had the immunogenetic character of host-type cells[10,12] which must have populated the graft. Transplantation behaviour of tumour cells that have resided in an F_1 environment is not always clear cut, especially when the parental strains do not differ at the H-2 locus, and is always open to criticism in view of the phenomenon described by Barrett and Deringer[13].

The problem of the origin of leukæmic cells has recently been studied here in $(AkXT_6)F_1$ hybrid mice the cells of which can be identified at metaphase by the presence of an unusually small chromosome. The hybrids were inoculated at birth with leukæmic filtrate, thymectomized at 1 month of age and grafted subcutaneously with an intact thymus from a healthy 1-day-old *Ak* or T_6 mouse. The tumours developing in the graft were transplanted to *Ak*, T_6 and F_1 mice and also analysed cytologically for the presence or absence of the T_6 marker chromosome. Preliminary results are shown in Tables 2 and 3, from which it is apparent that: (*a*) the *Ak* thymus is much more effective than the T_6 thymus in restoring susceptibility to lymphoma development; (*b*) leukæmic cells arise in most cases from lymphoid cells of the host, suggesting that the thymic influence responsible for the leukæmic change is a non-cellular indirect one.

Table 2. Incidence of Lymphoid Tumours in *Ak* or T_6 Thymuses Grafted to Virus-infected $(AkXT_6)F_1$ Mice

Treatment given	No. of mice in group	No. of mice with lymphoid tumours and age at diagnosis
Passage *A* at birth	12	11 (2–4 months)
Passage *A* at birth, thymectomy and *Ak* thymus graft at 1 month	7	5 (3–5 months)
Passage *A* at birth, thymectomy and T_6 thymus graft at 1 month	8	1 (4 months)

Table 3. Immunogenetic and Cytological Analysis of Lymphoid Tumours arising in Thymus Grafts in Virus-inoculated Thymectomized $(AkXT_6)F_1$ Hosts

No. of mouse	Thymus graft	Transplantation results*			Cytological analysis		
		Growth in *Ak*	Growth in F_1	Growth in T_6	No. of *Ak* cells found	No. of F_1 cells found	No. of T_6 cells found
1	*Ak*	0/5	4/6	0/4	3	25	0
2	*Ak*	0/4	6/6	0/3	0	31	0
3	*Ak*	0/5	3/5	0/3	1	20	0
4	*Ak*	3/4	3/5	0/4	34	5	0
5	T_6	0/5	6/8	0/8	0	21	3
6	*Ak*	0/6	6/6	0/3	5	36	0

* Numerator, number of takes; denominator, number of animals.

The Question of a Thymus Hormone

Law[10] was the first to suggest that a non-cellular factor from the thymus might be exerting an influence in leukæmia development. In a series of experiments, as yet unconfirmed, Metcalf has given evidence for the existence of a lymphocytosis-stimulating factor from the thymus and suggested that this factor might be the non-cellular influence by which the thymus appears to determine leukæmogenesis[14]. In our laboratory, repeated injections of normal *Ak* thymus extracts have failed to restore the usual leukæmia incidence in thymectomized *Ak* mice. Non-cellular thymic extracts are thus probably not capable of replacing the thymus environment so far as leukæmogenesis is concerned.

The possibility of a humoral circulating factor from thymic transplants is suggested by the results obtained with intrasplenic thymus grafts. In contrast to subcutaneous grafts no lymphoid tumours arose from intrasplenic grafts in thymectomized irradiated mice[15], suggesting that a circulating leukæmic factor had been removed by the liver. In this laboratory, however, thymuses from intact day-old mice transplanted into the spleen of thymectomized virus-inoculated mice of the isogenic line have developed lymphocytic neoplasms, but the incidence of such tumours has been lower than that recorded for subcutaneous grafts in parallel experiments (Table 4).

Table 4. Development of Lymphocytic Neoplasms in Thymectomized *C3Hf/Gs* Mice bearing Intrasplenic Thymus Grafts

Inoculation at birth	Site of thymus graft*	No. of mice in group	No. of mice developing lymphoid tumours and age at diagnosis	Incidence (per cent)
Passage *A*	subcutaneous	26	20 (3–8 months)	77
Passage *A*	intrasplenic	38	17 (3–5 months)	45
Nil	intrasplenic	14	0	0

* Grafts were performed in all cases 6 days after thymectomy at 1 month of age.

Conclusions

It is apparent from the above series of experiments that the thymus does not influence leukæmogenesis by providing the source of a leukæmogenic virus nor the cells most susceptible to leukæmic transformation. It is doubtful whether it is required merely as a growth medium for the virus. It is known, however, that the thymus provides an environment wherein lymphocytes (whether produced in the thymus itself or by the rest of the lymphoid complex) can proliferate much more actively than in any other lymphoid tissue[16]. What determines this high local proliferative activity is unknown: it may be the non-cellular factor mentioned above or Metcalf's lymphocytosis-stimulating factor. It is possible, therefore, that neoplastic change occurs in virus-infected lymphocytes only if they proliferate at a rate characteristic of thymus lymphocytes. This suggestion could be put to experimental test, for example, by preventing virus-infected host lymphocytes from entering the thymus by isolating the latter in the anterior chamber of the eye or in a millipore diffusion chamber. Experiments of this type have been in progress for some time and their results will be published and discussed at a later date.

I am grateful to Prof. Alexander Haddow for his interest throughout this work. The investigations have been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

[1] McEndy, D. P., Boon, M. C., and Furth, J., *Cancer Res.*, **4**, 337 (1944). Law, L. W., and Miller, J. H., *J. Nat. Cancer Inst.*, **11**, 253 (1950).

[2] Kaplan, H. S., *J. Nat. Cancer Inst.*, **11**, 83 (1950).

[3] Law, L. W., and Miller, J. H., *J. Nat. Cancer Inst.*, **11**, 425 (1950).

[4] Gross, L., *Proc. Soc. Exp. Biol. Med.*, **100**, 325 (1959). Levinthal, J. D., Buffett, R. F., and Furth, J., *ibid.*, **100**, 610 (1959). Miller, J. F. A. P., *Nature*, **183**, 1069 (1959).

[5] Kaplan, H. S., Brown, M. B., and Paull, J., *Cancer Res.*, **13**, 677 (1953).

[6] Miller, J. F. A. P., *Nature*, **184**, 1809 (1959); *Brit. J. Cancer*, **14**, 93 (1960).

[7] Miller, J. F. A. P., *Nature*, **187**, 703 (1960).

[8] Levinthal, J. D., and Buffett, R. F., *Proc. Soc. Exp. Biol. Med.*, **106**, 426 (1961).

[9] Miller, J. F. A. P., *Brit. J. Cancer*, **14**, 83 (1960).

[10] Law, L. W., *J. Nat. Cancer Inst.*, **12**, 789 (1952). Law, L. W., and Potter, M., *Proc. Nat. Acad. Sci.*, **42**, 160 (1956).

[11] Kaplan, H. S., Brown, M. B., Hirsch, B. B., and Carnes, W. H., *Cancer Res.*, **16**, 422 (1956).

[12] Miller, J. F. A. P., *Brit. J. Cancer*, **14**, 244 (1960).

[13] Barrett, M. K., and Deringer, M. K., *J. Nat. Cancer Inst.*, **11**, 51 (1950); **12**, 1011 (1952). Hauschka, T. S., *ibid.*, **14**, 723 (1953).

[14] Metcalf, D., *Ann. N.Y. Acad. Sci.*, **73**, 113 (1958); *Third Canadian Cancer Conf.*, **3**, 351 (1959).

[15] O'Gara, R. W., *Proc. Amer. Assoc. Cancer Res.*, **3**, 48 (1959).

[16] Andreason, E., and Ottensen, J., *Acta Physiol. Scand.*, **10**, 258 (1945).

EFFECTS OF HEAVY WATER ON POLIOVIRUS MULTIPLICATION: RESULTS AND SPECULATIONS ON MECHANISM

By Dr. DAVID KRITCHEVSKY, Dr. RICHARD I. CARP and Dr. HILARY KOPROWSKI
Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

THE multiplication of poliovirus (*MEF*-1 or *CHAT* strain) in cells previously grown in media containing deuterium oxide results in a five- to tenfold increase in burst size[1,2]. In these experiments, two stable tissue culture lines (HeLa and *MS*) and freshly explanted cells of rhesus monkey kidney were grown in concentrations of deuterium oxide, ranging between 20 and 40 per cent, prior to exposure to poliovirus. Incubation of these cultures at 37° C. resulted in increased virus multiplication in all the strains tested. When they were incubated at 40° C., virus strains which would not normally replicate at this temperature grew in the cultures. One example was the attenuated type 1 virus, the *CHAT* strain, which did not grow on monkey kidney cells at 40° C. in water medium, but replicated when the growth medium contained deuterium oxide or 50 per cent concentration in the overlay.

More recently, Lwoff and Lwoff[3,4] have described the effects of high concentrations of deuterium oxide on the multiplication of poliovirus I, rt. 38·5° at different temperatures, and have discussed possible mechanisms responsible for the results. In their experiments they added deuterium oxide (50 or 75 per cent) to a stable tissue culture line of human cancer cells, the *KB* line, immediately after infection with virus and found marked differences in virus yield as compared with water media in relation to the incubation temperature of the cultures. Thus at 33° C. there was a marked reduction in virus yield; at 37° C. more virus was produced; and considerably greater multiplication of virus occurred at 39·5°. Lower concentrations of deuterium oxide were apparently not tested, or had little or no effect.

Study of progeny of poliovirus grown in deuterium oxide at 40° *C.* The mutagenicity of deuterium oxide in certain bacterial and phage systems has been recorded[5–7] and the possibility that growth of poliovirus in deuterium oxide at elevated temperatures may give rise to mutants was investigated. The test system consisted of monkey kidney cell monolayers exposed to the attenuated *CHAT* or the virulent Mahoney strains of type I poliovirus and incubated at 37° and 40° C. respectively. One set of virus cultures was maintained at 37° C. throughout the experiment. A duplicate set was kept at 40° C. for 18 hr., then transferred to an incubator held at 37° C. Four days later the number of plaques appearing in all the plates were counted and ratios of those observed in cultures kept for a limited period at 40° C. to those observed in cultures kept consistently at 37° C. calculated. The results shown in the last column of Table 1 are expressed as differences between plaque ratios obtained for the test virus and standard *CHAT* virus.

The character of *CHAT* virus grown in deuterium oxide at 37° C. has not changed whereas different plaque ratios have been obtained for *CHAT* virus grown in deuterium oxide at 40° C. However, it should be emphasized that these ratios were obtained for virus grown in cultures kept at 40° C. for a limited time only (18 hr.). If cultures were kept at 40° C. during the entire incubation period no plaques of *CHAT* virus–deuterium progeny were detected. Since, however, growth of this progeny in cultures exposed to 40° C. for a limited time has also been observed following second tissue culture passage (without added deuterium oxide) it is felt that its character differs from that of the parental *CHAT* virus.

Table 1. Ability of Virus Strains to reproduce at 40° C.

Virus strain	No. of exps.	No. ratios determined	Ratio 40°/37° plaques*
Mahoney	9	9	+ 1·64 ± 0·36†
37°-D_2O-*CHAT*	7	8	− 0·02 ± 0·03
40°-D_2O-*CHAT*	7	12	+ 0·28 ± 0·03

* Ratio 40°/37° test virus—ratio 40°/37° *CHAT*.
† S.E.

Comparative effect of deuterium oxide and X-rays. Speculations concerning the mechanisms of action of deuterium oxide should take into account the fact[2,8] that tissue culture cells grown in deuterium oxide tend to become enlarged. Virus multiplication is increased in X-ray-produced giant cells[9], and it has been shown that the size of the irradiated cells is due to continued synthesis of large molecules in cells that have lost their ability to divide[10]. In preliminary experiments the *CHAT* strain of type I poliovirus[11] was grown on monkey kidney cell monolayers maintained in (*a*) normal growth medium, (*b*) in deuterium oxide and (*c*) irradiated by 300 r. Duplicate cultures were kept at 35° and 40° C. respectively. As judged by the average plaque size of the virus shown in Table 2, the effect of deuterium oxide seemed to be more pronounced than that of X-rays, but these conclusions may be revised after these cells have been subjected to different dosages of X-rays.

Table 2. Effects of Deuterium Oxide and of X-Irradiation (300 r.) on the Multiplication of *CHAT* Poliovirus on *MK* Cells at 35° C. and 40° C.

Medium	X-ray	D_2O (per cent)		Average plaque size 35° C.	No. of plaques per plate 40° C.
		Medium	Overlay		
H_2O	—	—	—	2·60	0
D_2O	—	40	—	2·63	0
H_2O	+	—	—	3·00	0·5
D_2O	—	—	50	3·14	4·0
D_2O	—	40	50	3·66	9·0

Speculations concerning mechanism of action of deuterium oxide. The mechanism by which deuterium oxide influences virus growth would appear to involve the extent of randomization of the virus structure and could manifest itself either by altering the stability of the hydrogen bonding in the molecule or by affecting the transition temperature of the helix-random coil transformation. Lwoff[12,13] has discussed the effects of temperature on virus multiplication.

Blout and Lenormant[14] have shown that the strong infra-red absorption bands of deoxyribonucleic acid (DNA) observed at 1,680 and 1,645 cm.$^{-1}$ are shifted to 1,660 and 1,625 cm.$^{-1}$ in deuterium oxide solution. The 1,660 and 1,625 bands are also observed with dry DNA and they conclude that this shift indicates stronger hydrogen bonding. A similar shift in the infra-red spectrum of RNA has now also been observed[15,16]. Thus the absorption which RNA normally displays in the 1,700–1,720 cm.$^{-1}$ region is shifted to 1,670–1,690 cm.$^{-1}$ in deuterium oxide solution. This absorption is thought to reflect base interaction (as between adenine and uracil) and to involve proton transfer.

Tne general effect of deuterium substitution on the strength of hydrogen bonds and on helix random coil transformations has been discussed by Scheraga[17]; and more recently it has been shown that differences in helix-coil transition temperatures depend on the specific chemical system under study being reduced by deuteration in poly-γ-benzyl-L-glutamate[18] and increased by deuteration in ribonuclease[19].

The deuterium effect, however, is most likely dependent on changes in the stability of the hydrogen bonding of the protein and possibly of the RNA. Interaction between the peptide bonds and the aqueous medium could affect the apparent half-life of a given virus particle; and increased stability of the hydrogen bond, such as could be conferred by deuteration, might then lengthen the effective half-life of the virus, resulting in increased multiplication.

Experiments now in progress may shed further light on the mechanism of the action of deuterium oxide.

This work was supported, in part, by grants from the National Science Foundation (No. *G*–14241*R*), the U.S. Atomic Energy Commission (Contract No. *AT*(30–1)–2299), and the National Institutes of Health, U.S. Public Health Service (Grants 2*G*–142 and *E*–1799).

[1] Carp, R. I., Kritchevsky, D., and Koprowski, H., *Virology*, **12**, 125 (1960).

[2] Manson, L. A., Carp, R. I., Defendi, V., Rothstein, E. L., Hartzell, jun., R. W., and Kritchevsky, D., *Ann. N.Y. Acad. Sci.*, **84**, 685 (1960).

[3] Lwoff, A., and Lwoff, M., *C.R. Acad. Sci., Paris*, **251**, 3131 (1960).

[4] Lwoff, A., and Lwoff, M., *C.R. Acad. Sci., Paris*, **252**, 223 (1961).

[5] Zamenhof, S., and Demerc, M., *Amer. Naturalist*, **77**, 380 (1943).

[6] DeGiovanni, R., *Ann. N.Y. Acad. Sci.*, **84**, 644 (1960).

[7] Konrad, M., *Ann. N.Y. Acad. Sci.*, **84**, 678 (1960).

[8] Siegel, B. V., Lund, R. O., Wellings, S. R., and Bostic, W. L., *Exp. Cell Res.*, **19**, 187 (1960).

[9] Levine, S., *Virology*, **10**, 257 (1960).

[10] Levine, S., and Kritchevsky, D., *Exp. Cell Res.*, **15**, 422 (1958).

[11] Koprowski, H., *Spec. Pub. N.Y. Acad. Sci.*, **5**, 128 (1958).

[12] Lwoff, A., and Lwoff, M., *Ann. Inst. Pasteur*, **98**, 173 (1960).

[13] Groman, N., Lwoff, A., and Lwoff, M., *Ann. Inst. Pasteur*, **98**, 351 (1960).

[14] Blout, E. R., and Lenormant, H., *Biochim. Biophys. Acta*, **17**, 325 (1955).

[15] Shimanouchi, T., Tsuboi, M., Kyogoku, Y., and Watanabe, I., *Biochim. Biophys. Acta*, **45**, 195 (1960).

[16] Kyogoku, Y., Tsuboi, M., Shimanouchi, T., and Watanabe, I., *Nature*, **189**, 120 (1961).

[17] Scheraga, H. A., *Ann. N.Y. Acad. Sci.*, **84**, 608 (1960).

[18] Calvin, M., Hermans, jun., J., and Scheraga, H. A., *J. Amer. Chem. Soc.*, **81**, 5048 (1959).

[19] Hermans, jun., J., and Scheraga, H. A., *Biochim. Biophys. Acta*, **36**, 534 (1959).

MECHANISM OF MUTAGENESIS INDUCED BY FORMALDEHYDE

THE ESSENTIAL ROLE OF THE 6-AMINO GROUP OF ADENYLIC ACID (OR ADENOSINE) IN THE MEDIATION OF THE MUTAGENIC ACTIVITY OF FORMALDEHYDE

By Dr. THOMAS ALDERSON

Department of Genetics, University of Cambridge

In a previous communication[1], I have established by means of a chemically defined and axenic treatment medium that formaldehyde exhibits no mutagenic activity towards *Drosophila melanogaster* by the larval feeding method, unless adenylic acid is present in the treatment medium. A reaction-product formed between formaldehyde and adenylic acid in the treatment medium was postulated as the metabolite concerned in the mediation of the mutagenic activity of formaldehyde. The principal metabolite was assumed to be an adenylate dimer ($\overline{AA}$), where two adenylic acid molecules are linked together through their amino-groups by a methylene bridge. The stereochemical properties of this metabolite and its subsequent utilization in deoxyribonucleic acid synthesis, together with mechanisms for its mutagenic activity, were discussed[1].

An essential role was thus ascribed to the 6-amino group of adenylate for the formation of the adenylate dimer, since the binding of formaldehyde by ribonucleic acid mono- or poly-nucleotides appears to be a specific function of the amino-groups of their bases[2]. It is now found that adenylate is effective when present in the treatment medium as any of the forms adenosine-3′-phosphate, adenosine-5′-phosphate, or deoxyadenosine-5′-phosphate. The simple replacement of the 6-amino group of adenosine and adenosine-5′-phosphate by a hydroxyl group (to give inosine and inosine-5′-phosphate) completely removes the ability of these compounds to mediate the mutagenic activity of formaldehyde. Although adenosine is as effective as the adenine ribonucleotides, adenine shows no ability to mediate formaldehyde-induced mutagenesis and is actually anti-mutagenic on a formaldehyde-containing yeast–glucose treatment medium[3]. Adenine should be able to form a metabolite by reaction with formaldehyde similar to the one postulated for adenylate; but its free imino-group at the 9-position of the purine ring may be blocked by additional reaction with formaldehyde[4], thus preventing subsequent ribose sugar attachment; this condition would be expected to debar the utilization of adenine in nucleic acid synthesis.

The design of experiments[1,5], the method of treatment of larvæ[3], and brood pattern determinations of sex-linked recessive lethal mutation-rates[3] are as previously described. However, it has been found that a concentration of formaldehyde of

$1{\cdot}0 \times 10^{-2}\,M$, as against a previous concentration of $2{\cdot}0 \times 10^{-2}\,M$, results in an improvement in the mutational response and in the survival and development of the larvæ on the synthetic medium. This improved concentration was therefore used, as it more markedly illustrates the difference of effect of the various purine derivatives on the mutagenic activity of formaldehyde. Each nucleotide or nucleoside is used at a concentration of 0·1 per cent; adenine is used at an approximately equimolar concentration of 0·05 per cent. Ordinary medium is the oatmeal–treacle medium normally used for culturing *Drosophila* in this laboratory.

Table 1. EFFECT OF ADENOSINE, ADENOSINE-5′-PHOSPHATE, INOSINE AND INOSINE-5′-PHOSPHATE ON THE MUTAGENIC ACTIVITY OF FORMALDEHYDE WHEN NEWLY-HATCHED LARVAE ARE TREATED FOR 72 HR.

Treatment medium	*r*A	*r*H	*r*A5′-P	*r*H5′-P
Molarity of formaldehyde	$1{\cdot}0 \times 10^{-2}$			
Total larval developmental time in days	5·0	5·3	5·0	5·3
Per cent survivors	64	47	55	57
First broods				
No. chrom.	457	1,083	774	988
No. leth.	19	3	44	3
Per cent leth.	4·16	0·27	5·68	0·34
Second broods				
No. chrom.	370	679	630	523
No. leth.	7	1	27	0
Per cent leth.	1·89	0·15	4·28	0·00

No. chrom., total number of chromosomes examined.
No. leth., number of lethal chromosomes.
Per cent leth., percentage of sex-linked recessive lethal mutations.
*r*A, adenosine; *r*H, inosine; *r*A5′-P, adenosine-5′-phosphate; *r*H5′-P, inosine-5′-phosphate.

Table 1 illustrates the results obtained when newly hatched larvæ are treated on the formaldehyde-containing synthetic treatment medium for 72 hr., and then transferred on to ordinary medium to complete their development. The presence of adenosine (*r*A) and adenosine-5′-phosphate (*r*A5′-P) in the treatment medium produces a high mutagenic activity of formaldehyde. However, if either inosine (hypoxanthine ribonucleoside, *r*H) or inosine-5-phosphate (hypoxanthine ribonucleotide, *r*H5′-P) is substituted for the corresponding adenine derivative, no induced mutagenic activity is detected. In comparing these two series of experiments, three differences should be noted. Thus, chemically, the only difference between the series is that the amino-group at the 6-position of the purine ring in adenosine and adenosine-5′-phosphate is replaced by a hydroxyl group in inosine and inosine-5′-phosphate. Biochemically, the adenine derivatives enter the organism with the correct purine groupings for utilization in nucleic acid synthesis, whereas inosine and inosine-5′-phosphate have first to be aminated before utilization in nucleic acid synthesis; since this amination takes place intracellularly a reaction-product of formaldehyde with these hypoxanthine derivatives in the treatment medium is therefore not formed. Biologically, the developmental response of *Drosophila* larvæ to the adenine and hypoxanthine derivatives is approximately equal; this suggests that they are utilized by the organism to approximately the same extent, and that the hypoxanthine derivatives are incorporated into the nucleic acids as adenylic acid, since this nucleotide component is the only one utilized to any appreciable extent. It has been shown[6] that when pyrimidines are excluded from the diet of developing *Drosophila melanogaster* larvæ, the development response to inosine is virtually the same as that of adenosine.

Table 2. EFFECT OF GUANOSINE-3′-PHOSPHATE, URIDINE-3′-PHOSPHATE, AND CYTIDINE-3′-PHOSPHATE ON THE MUTAGENIC ACTIVITY OF FORMALDEHYDE, WHEN NEWLY-HATCHED LARVÆ ARE TREATED FOR 72 HR.

Treatment medium	*r*G3′-P + *r*U3′-P + *r*C3′-P	*r*G3′-P	*r*U3′-P	*r*C3′-P
Molarity of formaldehyde	$1{\cdot}0 \times 10^{-2}$			
Total larval developmental time in days	6·0	6·0	5·5	5·8
Per cent survivors	72	81	78	72
First broods				
No. chrom.	580	412	422	468
No. leth.	1	1	0	2
Per cent leth.	0·17	0·24	0·00	0·43
Second broods				
No. chrom.	470	493	417	462
No. leth.	1	0	1	0
Per cent leth.	0·21	0·00	0·24	0·00

*r*G3′-P, guanosine-3′-phosphate; *r*U3′-P, uridine-3′-phosphate; *r*C3′-P, cytidine-3′-phosphate.

It has been previously claimed[1] that the ribonucleoside-3′-phosphates of guanine, uracil and cytosine do not mediate the mutagenic activity of formaldehyde. Since the improved concentration for formaldehyde has been introduced, these compounds have been re-tested under the improved conditions; as seen in Table 2, the original findings were confirmed. In examination of the patterns of utilization of the ribonucleotide-3′-phosphates as growth requirements in *Drosophila melanogaster* larvæ, it has been shown[6] that the ribonucleoside-3′-phosphate of guanine, uracil and cytosine are not utilized when supplied in the absence of adenosine-3′-phosphate; consequently, metabolites formed by these compounds after reaction with formaldehyde would not be utilized in any event in this system. These compounds would therefore not be expected to mediate the mutagenic activity of formaldehyde.

Table 3 illustrates the results obtained when adenine (A), adenosine (*r*A), adenosine-3′-phosphate (*r*A3′-P), adenosine-5′-phosphate (*r*A5′-P), deoxyadenosine-5′-phosphate (*d*A5′-P), and inosine-5′-phosphate (*r*H5′-P) are tested for their ability to mediate the mutagenic activity of formaldehyde. For each treatment, the ribonucleoside-3′-phosphates

Table 3. EFFECT OF ADENINE, ADENOSINE, ADENOSINE-3′-PHOSPHATE, ADENOSINE-5′-PHOSPHATE, INOSINE-5′-PHOSPHATE, AND DEOXYADENOSINE-5′-PHOSPHATE ON THE MUTAGENIC ACTIVITY OF FORMALDEHYDE, WHEN NEWLY-HATCHED LARVÆ ARE TREATED FOR THE ENTIRE LARVAL LIFE

Treatment medium	A	*r*A	*r*A3′-P	*r*A5′-P	*r*H5′-P	*d*A5′-P
Molarity of formaldehyde	$1{\cdot}0 \times 10^{-2}$					
Total larval developmental time in days	5·0	4·5	4·7	4·9	4·3	5·3
Per cent survivors	54	53	46	51	63	45
First broods						
No. chrom.	685	732	1,378	1,331	1,413	874
No. leth.	1	49	56	58	4	10
Per cent leth.	0·15	6·69	4·06	4·36	0·28	1·14
Second broods						
No. chrom.	537	490	897	571	607	860
No. leth.	1	10	27	37	2	12
Per cent leth.	0·18	2·04	3·01	6·48	0·33	1·40

A, adenine; *r*A, adenosine; *r*A3′-P, adenosine-3′-phosphate; *r*A5′-P, adenosine-5′-phosphate; *r*H5′-P, inosine-5′-phosphate; *d*A5′-P, deoxyadenosine-5′-phosphate.

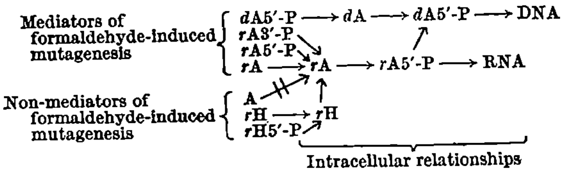
of guanine, uracil and cytosine are also added to the treatment medium, since an improvement in developmental time is found when these compounds are supplied as dietary supplements, though they are not otherwise concerned in the mediation of the mutagenic activity of formaldehyde (see Table 2). Adenine and inosine-5′-phosphate are seen not to be capable of mediating the mutagenic activity of formaldehyde, as expected from previous results with adenine[1] and inosine-5′-phosphate (Table 1). Adenosine, adenosine-3′-phosphate, adenosine-5′-phosphate, and deoxyadenosine-5′-phosphate are all found to mediate formaldehyde-induced mutagenesis ; but differences are observed in the mutagenic response produced by these adenine derivatives. Adenosine produces a significantly greater response in the first brood than adenosine-3′-phosphate and adenosine-5′-phosphate, whereas the response in the second brood is significantly greater with adenosine-5′-phosphate than with adenosine and adenosine-3′-phosphate. Second broods are usually sampled to detect any increase in the speed of germ-cell development, where such an effect may increase the mutagenic response in the second brood. Since the formaldehyde-containing medium loses its mutagenic activity after 24–48 hr., an increase in germ cell development may result in an increased number of cells passing into the sensitive stage of the second brood during the period of effective treatment. However, this does not appear to be the sole factor responsible here as the adenosine-5′-phosphate culture has a slower overall larval development-time than the adenosine, adenosine-3′-phosphate and inosine-5′-phosphate cultures. Deoxyadenosine-5′-phosphate shows a significantly lower ability to mediate formaldehyde-induced mutagenesis than the adenine ribonucleotides ; this may be because the concentration of deoxyadenosine-5′-phosphate used is not optimal for utilization by the larvæ. Data for the growth response of *Drosophila* larvæ to deoxyadenosine-5′-phosphate on the synthetic medium are not available.

As previously discussed[1], the mechanism of action of the postulated $\overline{AA}$ metabolite is mediated by way of deoxyribonucleic acid synthesis. However, the ribonucleotide derivatives of adenine (which are constituents of ribonucleic acid) are found to be more effective than the deoxyribonucleotide derivative (deoxyadenosine-5′-phosphate) tested, which is a constituent of deoxyribonucleic acid. Although relatively little is known of deoxyribonucleotide synthesis compared with that of ribonucleotide synthesis, it has been shown that ribonucleotides are effective deoxyribonucleic acid precursors[7].

It is thought that the synthesis of nucleotides follows a common pathway up to a point where a direct conversion of the ribo- to the deoxyribo-form takes place by simple enzymic reduction of the ribose sugar.

The nucleoside-5′-phosphates are the natural nucleotide precursors for ribonucleic acid, deoxyribonucleic acid, and nucleotide coenzyme synthesis. The ribonucleoside-3′-phosphates (which are products of alkaline hydrolysis of ribonucleic acid) are incorrectly phophorylated for direct utilization in these syntheses. In *Drosophila* larvæ the adenine ribonucleoside-3′- and 5′-phosphates are utilized, however ; but there is indirect evidence that dephosphorylation of these adenylic acids to adenosine precedes assimilation by the larvæ[6]. Thus, adenosine-5′-phosphate, adenosine-3′-phosphate, and probably deoxyadenosine-5′-phosphate would be converted to their nucleosides before assimilation. For reasons stated earlier, adenine, inosine, and inosine-5′-phosphate do not mediate the mutagenic activity of formaldehyde, although they are normally utilized by *Drosophila* larvæ. A schematic representation of these relationships is shown in Fig. 1.

Fig. 1. Schematic representation of the probable utilization by *Drosophila melanogaster* larvæ of the purines under consideration.


Mediators of formaldehyde-induced mutagenesis
*d*A5′-P
*r*A3′-P
*r*A5′-P
*r*A
*d*A
*d*A5′-P
DNA
*r*A
*r*A5′-P
RNA
Non-mediators of formaldehyde-induced mutagenesis
A
*r*H
*r*H5′-P
*r*H
Intracellular relationships

A, adenine; *r*A, adenosine; *r*A3′-P, adenosine-3′-phosphate; *r*A5′-P, adenosine-5′-phosphate; *r*H, inosine; *r*H5′-P, inosine-5′-phosphate; *d*A, deoxyadenosine; *d*A5′-P, deoxyadenosine-5′-phosphate; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

My acknowledgments are due to the British Empire Cancer Campaign for financial support of this work, and to Prof. J. M. Thoday for supplying the laboratory facilities.

[1] Alderson, T., *Nature*, **187**, 485 (1960).
[2] Fraenkel-Conrat, H., *Biochim. Biophys. Acta*, **15**, 307 (1954). Staehlin, M., *ibid.*, **29**, 410 (1958).
[3] Alderson, T., *Nature*, **179**, 974 (1957); **182**, 508 (1958).
[4] Feldman, M. J., *Acad. Nauk S.S.S.R.*, **25**, 12 (1960).
[5] Alderson, T., *Nature*, **185**, 904 (1960).
[6] Sang, J. H., *Proc. Roy. Soc. Edin.*, B, **66**, 339 (1957).
[7] Hammarsten, E., Reichard, P., and Saluste, E., *J. Biol. Chem.*, **183**, 105 (1950).

IMPROBABILITY OF THE CHELATION HYPOTHESIS OF AUXIN ACTION

By Dr. D. D. PERRIN

Department of Medical Chemistry, Institute of Advanced Studies, Australian National University, Canberra

The suggestion has been advanced on biological grounds that plant-growth regulators exert their physiological effects through complex formation with certain cations, such as one or more of the heavy metals[1]. For example, with indole-3-acetic acid (IAA), chelation has been postulated to involve either the carboxyl group and the aromatic ring[2] or the imine and the carboxyl groups[1]. Because chemical evidence for any such mode of action has been scanty and conflicting, and no quantitative stability constant data for aqueous solutions have been published, the present definitive study was undertaken.

Cupric ion is the obvious choice for experiments aimed at providing such evidence because, of all the cations normally found in plant tissue, it forms by far the most stable series of complexes with all known ligands. Cohen *et al.*[2] found the absorption spectrum of IAA in 50 per cent aqueous ethanol was changed when copper nitrate was added: this they attributed to the formation of 1 : 1 and 1 : 2 copper complexes which, from *p*H titration data, had stability constants of $10^{3 \cdot 2}$ and $10^{5 \cdot 4}$. Recaldin and Heath[3], repeating this work, were not confident of the interpretation of the observed changes; in addition, they found the stability constant of the 1 : 2 complex to be $10^{3 \cdot 0}$, which is about 1/250 of the previous value. Afterwards, Fawcett[4] showed that in the experimental conditions a major contribution to the results came from hydrolysis of free cupric ion, a factor that had not been considered. Armarego *et al.*[5] failed to find any correlation between the growth activity of phenoxyacetic acids and the stability constants of their copper complexes.

All the foregoing results were obtained using 50 per cent ethanol solutions and hence should not be applied to interpret observations made on purely aqueous media, especially as complex formation between IAA and metal ions *in vitro* in water could not be demonstrated by either Cohen *et al.*[2] or Recaldin and Heath[3]. This failure was presumed to be due to the attainable concentrations of free IAA being too low. The poor solubility of IAA in water precludes the use of the Bjerrum *p*H-titration method of stability constant determination, which is based on the displacement of a proton from the free acid by the cation which forms the complex. A further complicating factor is the hydrolysis of cupric ion, which involves a polynuclear process[6] so that its extent varies with the copper concentration as well as with the *p*H, making it necessary to work either at low *p*H values or at low copper concentrations (or both). This limits the usefulness of spectrophotometric methods. However, the copper-amalgam electrode[7] can be made the basis of a sensitive method which is free of these defects and which has been used in the present instance. The potential of this electrode depends on the activity of cupric ion in the solution. If a high concentration of an inert supporting electrolyte, such as potassium nitrate, is also present, the activity coefficient of cupric ion remains constant so that when a complexing species is added to the solution the potential measures directly the free ion concentration. Knowing this quantity, as well as the total copper and ligand concentrations, enables the stability constant of the complex to be calculated.

Experimental conditions (solution about *p*H 5 and initial copper concentration of 5×10^{-4} *M*) were chosen so that hydrolysis of copper ion was negligible[6], while, at the same time, advantage could be taken of the much greater solubility of the anions of the acids tested. The self-buffered solutions of IAA and α-naphthylacetic acids that were added were 1 *M* in potassium nitrate and 0·025 *M* in the acid plus its potassium salt; for the other acids a concentration of 0·05 *M* was used. Even at these concentrations, which in some cases represented supersaturated solutions, only very small changes in potential were observed and great care was needed for their measurement against a saturated calomel electrode. For this reason a potentiometer (Tinsley vernier) and galvanometer (Cambridge spot) combination was selected which could be read easily to 0·02 mV., and a Vibron electrometer *p*H meter with attached recorder was used to provide a continuous check on the *p*H of the solutions. The copper–amalgam surface was freshly exposed and solutions were kept at 20·0° C. in an atmosphere of nitrogen.

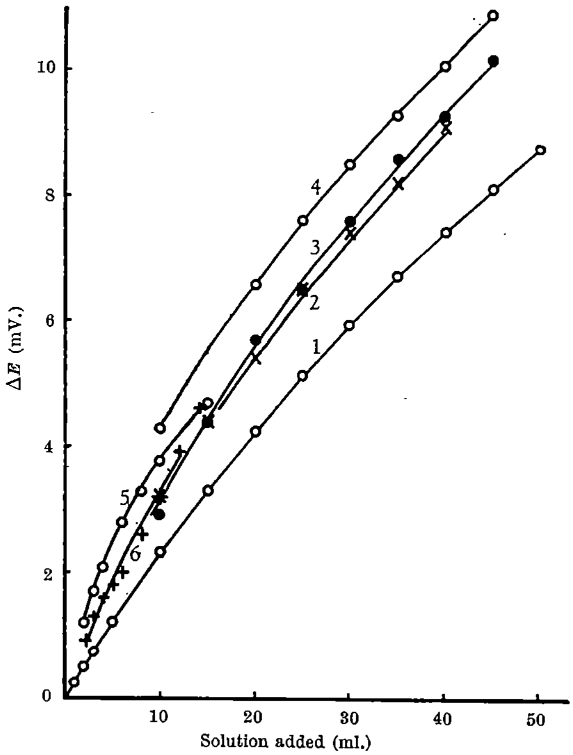


Fig. 1. Change in copper–amalgam electrode potential at *p*H 5·0 when 50 ml. 5×10^{-4} *M* copper nitrate in 1 *M* potassium nitrate was diluted with: (1) 1 *M* potassium nitrate; (2) 0·05 *M* 2,4-dichlorophenoxyacetic acid buffer; (3) 0·05 *M* 2,4-dimethylphenoxyacetic acid buffer; (4) 0·05 *M* 4-chlorophenoxyacetic acid buffer; (5) 0·025 *M* indole-3-acetic acid buffer; (6) 0·025 *M* α-naphthylacetic acid buffer. Solutions 2–6 were also 1 *M* in potassium nitrate

From the small but reproducible differences shown in Fig. 1 the stability constant, $K_1 = (\text{Cu}.L^+)/(\text{Cu}^{++})(L^-)$, where (L^-) is the anion concentration of the complexing species, has been calculated for the 1 : 1 complexes of each of the acids tested. The results set out in Table 1 show log K_1 to range from 0·8 to 1·7. This indicates that no greater degree of complex formation occurs than is known to exist in the cupric salts of simple carboxylic acids such as acetic and chloroacetic[8]. The slight increase of log K_1 values with the pK_a of the acids reflects only the commonly observed general parallelism between the strengths of binding of metal ions and protons in any related series of ligands. No evidence was obtained for the formation of 1 : 2, or higher, complexes, in agreement with the usual observation for many acids that log K_2 of their complexes is much less than log K_1.

The stability constants of the copper complexes of IAA and acetic acid are almost identical and, as the pK_a values of the two acids are comparable, the conclusion is inevitable that in IAA, also, only the carboxyl group plays any part in complex formation. For this reason, it appears justifiable to take the stability constants of complexes of other metals with IAA to be the same as for the corresponding acetates[9]. For zinc-, calcium- and magnesium-IAA complexes the constants would be about 1/10, 1/40 and 1/40, relative to copper.

Although the present results show the plant-growth acids of the types investigated to be only weak complexing agents, it is of some interest to assess the maximum possible extent to which they might form

Table 1. Ionization Constants of Some Plant-Growth Regulators and the Stability Constants of their Copper Complexes

Acid	pK ($\mu=0{\cdot}0005$)	$pK_{\text{conc.}}$ ($\mu=1$, KNO_3)	$\log K_1$ (copper complex)
2,4-Dichlorophenoxyacetic	2·90	2·78	0·8
4-Chlorophenoxyacetic	3·00	2·91	1·1
2,4-Dimethylphenoxyacetic	3·22	3·08	0·9
α-Naphthylacetic	4·22	4·04	1·4
Indole-3-acetic	4·54	4·36	1·7
Chloroacetic (ref. 8) (for comparison)		2·66	0·91
Acetic (ref. 8) (for comparison)		4·61	1·65

metal complexes under biological conditions, taking for this purpose the ions Cu^{++} and Zn^{++}. Based on figures for metal–acetic acid complexes[9], the reasonable assumption is made that changing the ionic strength of solutions of 1·0–0·15 only slightly increases $\log K_1$.

The total copper concentration in fresh plant tissue does not often exceed 1 p.p.m., that is, 17 μM. Young and growing tissue contains a higher free amino-acid concentration than mature tissues[10,11], but, even in the latter, total free amino-acid concentrations in excess of 5×10^{-3} M have been found in normal bracken, spinach beet and pea leaves[11]. It can readily be calculated from the latter figure (using 9·5 as the average pK of amino-acids, and 8·5 and 15·5 as the average log stability constants of their 1 : 1 and 1 : 2 amino-acid complexes at 20° (ref. 9)) that at pH 7 the free cupric ion concentration in plant tissues must be less than 10^{-11} M. Even then, this figure takes no account of the presence of competing ligands such as proteins, which must reduce the free copper concentration even further. Growth-stimulation has been demonstrated at concentrations of 10^{-9} M IAA[1], which, from the above results, must represent a copper–IAA complex concentration of less than 10^{-18} M. For similar calculations with zinc, an average zinc content of 3 mgm./kgm. green tissue has been taken. (This is an arbitrary figure because the zinc content of plants varies considerably with differences in soil zinc-level, but, from a summary of the literature[12], 1–10 p.p.m. seems to be the normal range). Taking an average log stability constant of 5·3 for 1 : 1 zinc–amino-acid complexes[9] leads to the conclusion that the concentration of zinc–IAA complex in plants must be less than 10^{-14} M. Both this and the copper value are so very much less than for any known biologically active substances as to make it extremely probable that metal-complex formation plays no part in the physiological activities of IAA and the other plant-growth regulators discussed above.

I thank Prof. R. L. Wain, of Wye College, University of London, for outlining the biological problem, and, together with Dr. S. F. Cox, of Imperial Chemical Industries, Ltd. (Australia and New Zealand), Melbourne, for kindly supplying the acids used in this work. I am grateful to Prof. A. Albert for helpful discussion.

[1] Heath, O. V. S., and Clark, J. E., *Nature*, **177**, 1118 (1956); *J. Exp. Bot.*, **11**, 167 (1960).

[2] Cohen, D., Ginzburg, B.-Z., and Heitner-Wirguin, C., *Nature*, **181**, 686 (1958).

[3] Recaldin, D. A., and Heath, O. V. S., *Nature*, **182**, 539 (1958).

[4] Fawcett, C. H., *Nature*, **184**, 796 (1959).

[5] Armarego, W. L. F., Canny, M. J., and Cox, S. F., *Nature*, **183**, 1176 (1959).

[6] Perrin, D. D., *J. Chem. Soc.*, 3189 (1960).

[7] Dawson, J. E., and Nair, C. K. N., *Soil. Sci.*, **69**, 239 (1950).

[8] Fronaeus, S., Doctoral Diss., Lund (1948).

[9] Bjerrum, J., Schwarzenbach, G., and Sillen, L. G., *Stability Constants. Part* 1: *Organic Ligands* (Chem. Soc., Lond., 1957).

[10] Allsopp, A., *Nature*, **161**, 833 (1948).

[11] DeKock, P. C., and Morrison, R. I., *Biochem. J.*, **70**, 266 (1958).

[12] Scharrer, K., *Biochemie der Spurenelemente* (Paul Parey, Berlin, 1941).

ENVIRONMENTAL FACTORS AND BRYOPHYTES

By KATHRYN BENSON-EVANS

Department of Botany, University College of South Wales and Monmouthshire, Cardiff

MOST plants appear to show a definite rhythm or periodicity in their vegetative growth, and to a more marked extent in their reproduction, connected with a periodicity in some factor or factors of their environment.

As early as 1925, Wann[1] suggested that all plants (with the possible exclusion of fungi and lichens) can be grouped into 'long-day' and 'short-day' plants depending on the photo-period or length of daily illumination which results in the production of 'flowers' and 'fruit'. Since then, the responses of higher plants to photo-periods, temperature, and auxins or growth substances and the interactions of these factors have been increasingly investigated. Relatively little, however, has been accomplished in the field of mosses and liverworts, probably because of the slight economic importance of these plants.

This article aims at reviewing some of the preliminary experimental work which has been performed with these groups, with particular reference to the environmental factors that influence vegetative growth and induce the sexual or sporangial condition.

Wann initiated work in this field by carrying out photo-periodic experiments using *Marchantia polymorpha* as his material, and his results indicated that *Marchantia* appeared to respond to photo-periods as do long-day plants in the higher plants. Voth and Hamner[2] repeated these experiments under improved conditions and again demonstrated certain definite photo-periodic responses in both vegetative thalli and sex organs, in both long- and short-day conditions, irrespective of the nutrient supply: (*a*) plants under long-day conditions were always larger and had greater dry weight than plants on comparable solutions under short-day conditions; (*b*) plants under long-day conditions produced a greater number of gametangiophores than similar plants on comparable solutions under short-day; (*c*) plants under short-day conditions produced a greater total number of gemmæ cups than plants on comparable solutions under long-days; (*d*) it was also noted that low humidity retarded the responses especially of the female branches and organs, whereas high humidity hastened the response.

Similar experiments were carried out at Cardiff using three other genera of the Marchantiales. In view of the results of Voth and Hamner, no attempt was made to culture plants on specific media, but turfs growing on their natural substrate were collected from the field. The experimental chambers were illuminated by six 4-ft., 40-W. fluorescent tubes supplemented by two red fluorescent tubes to make up the deficiency in waves from the red end of the spectrum. Heating was performed with a diffused heating spiral, and temperature was controlled by a cartridge thermostat to within ± 1° F. Intensity of light was about 800–1,000 ft.-candles at a distance of 1 ft. from the light source and measured at the surface of the plants. Day-length was controlled by Venner time switches. A second series of cabinets showed a greater fluctuation of temperature as they were not heated independently.

In all the species investigated, increased vegetative growth occurred in the long-day chambers, and sexual branches were stimulated on these plants; the plants under short-day conditions remained vegetative (Table 1).

Table 1

Species	Long-day				Short-day			
	Plants used		Plants responding		Plants used		Plants responding	
	♂	♀	♂	♀	♂	♀	♂	♀
Marchantia polymorpha	62	91	35	58	51	56	0	3
Preissia quadrata	62	177	41	141	40	72	0	0
Conocephalum conicum	94	119	88	89	52	61	0	0
Lunularia cruciata	85*	103	47*	86	38	67	0	2

* Denotes field observations (August).

Average maximum temperature, 65° F.; average minimum temperature, 46° F.

The response of *Marchantia polymorpha* to increased light intensity was checked. It was found that plants in experimental chambers having supplementary lighting always responded more rapidly than those in a lower light intensity, but that day-length influenced the actual number of gametangiophores that were produced (Table 2). This would appear to be comparable with the reactions of higher plants, since Mann[3] demonstrated a similar effect on floral initiation in *Xanthium*, where increase in light intensity increased the effect of the photo-period.

Table 2. PHOTO-PERIODIC AND LIGHT INTENSITY EFFECTS IN THE MARCHANTIALES

No. of gametangiophores produced in response to differing light intensities

Period of response (weeks)	14 hr. (500 ft.-candles)	14 hr. (1,000 ft.-candles)	18 hr. (1,000 ft.-candles)
1	0	0	0
2	0	3	30
3	0	7	35
4	2	7	35

Average maximum temperature, 76° F.; average minimum temperature, 58° F.

The response of *Lunularia cruciata* was of particular interest, since sexual reproduction in this form only occurs sporadically in the British Isles and in many other countries.

Experimentally, archegoniophores were only induced to develop during the months of December to March inclusive, when plants from the field, transferred to a temperature of 70° F. and long-day conditions, would produce archegoniophores within 7–8 days[4]. A temperature as low as 60° F. has been found to be just as effective early in the year and, owing to the rapidity with which *Lunularia* can produce sexual organs, the temperature stimuli need only be maintained for a few days. Later collections, that is, after March, remained vigorously vegetative, so that apparently there may be a low-temperature requirement which must be fulfilled before the stimulus of a rise in temperature. Other evidence of this is shown by plants grown from gemmæ, for these do not produce archegoniophores until the following spring, having first passed through the cold period of winter. Plants suffering from frost damage do not produce sexual branches; their apices give vegetative growth throughout the following season. Only plants kept at low temperatures, but protected from frosts, give rise to branches with reproductive organs when submitted to a rise in temperature and long days. Male branches have not been stimulated experimentally, but field records suggest that a summer temperature of 70–80° F. is necessary for their initiation.

Lunularia contrasts strongly with *Conocephalum conicum*, a native of Britain, which dies back in winter and regenerates from the apex each spring. It has been found that when dormant clones of *Conocephalum conicum* are transferred to growth chambers at 70° F. and long days, vigorous vegetative growth occurs, but after 2 weeks the apices begin to produce sexual branches. Clearly in *Conocephalum*, the death of the older parts of the thallus does not affect the ability of the regenerated branches to produce sexual organs.

Temperature apparently did not affect the response in this species or in *Preissia quadrata*, where the stimulus of long days is wholly responsible for the re-orientation of the growth of the thallus from the horizontal to the vertical plane, which is the first step to the production of the sexual receptacles. It would appear that this re-orientation is probably correlated with the synthesis of growth hormones, or the removal of a growth inhibitor, in the long days, since Rousseau[5] apparently demonstrated that the external application of auxins (2,4-dichlorophenoxyacetic acid, α-naphthalene-acetic acid, and β-naphthoxyacetic acid) to the vegetative plants of *Marchantia polymorpha* induced morphology comparable to the receptacles of normal plants. I have also induced a similar re-orientation of the thallus with receptacle formation in *Conocephalum conicum* using 2,4-dichlorophenoxyacetic acid, at a concentration of 10^{-4}, but no sex organs were formed. Other experiments indicated that application of high auxin concentrations to the normal young receptacles of these species in early summer inhibited the formation of sex organs. These observations are of interest in view of the work of Salisbury[6], who, among others, has demonstrated that the flowering of many higher plants is influenced by the application of auxin. High concentrations were inhibitory to the flowering, not only of short-day plants, but also of many species including long-day and indeterminate forms.

Normally the female receptacles remain very shortly stalked until fertilization has occurred. In *Conocephalum conicum* this occurs in the field in June, and the sporogonia develop soon after, but the stalk of the archegoniophore does not elongate until the following spring, usually from March onwards into the summer. Elongation of the stalk occurs by extension of the individual cells comprising the stalk and not by cell division.

Table 3. EFFECT OF VARYING CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE ARCHEGONIOPHORE STALKS OF *Conocephalum conicum*

Days after treatment	Concentration of 2,4-D					
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Control
	Average length of archegoniophore stalks in mm.					
7	2·0	1·5	2·0	2·0	1·5	2·0
14	5·0	11·0	11·0	4·0	2·0	2·0
21	5·0	11·0	11·0	12·0	2·0	2·0
28	5·0	11·0	11·0	18·5	2·0	2·0

Average maximum temperature, 62° F.; average minimum temperature, 58° F. Normal day-length for October–November.

We have[7] stimulated elongation in the archegoniophore stalks of *Conocephalum conicum* in October, by the external application of 2,4-dichlorophenoxyacetic acid, greatest elongation occurring at a concentration of 10^{-6} (Table 3). This suggests that normal elongation is brought about by a natural auxin produced within the gametophyte. As normal elongation occurs in the spring, the production of auxin may be stimulated by the greater temperature and photo-period of the spring days. To ascertain whether increased photo-period alone would stimulate endogenous auxin production, and thus bring about elongation, portions of the female thallus bearing archegoniophores were subjected to long-day conditions (16 hr.) at normal winter temperatures (January). No elongation occurred and so it was assumed that photo-period alone was not sufficient to stimulate auxin production, or at least in sufficient quantity to bring about elongation of the cells. A second group of plants was subjected to long-day treatment in a heated greenhouse at an average temperature of 60° F., when elongation of the stalks occurred (Table 4). Higher temperatures had a retarding effect, as did short days at both temperatures, indicating that the long photo-period combined with a temperature of 60° F. was optimal for cell elongation. This appears to be an effect similar to that observed by Knott[8], when studying the effect of temperature on the photo-periodic response of spinach. With the photo-period fixed at 15 hr./day, seed stalk elongation occurred sooner if temperatures during treatment were held at 60–70° F. than it did if held at a higher or lower range.

Table 4. EFFECT OF PHOTO-PERIOD AND TEMPERATURE ON ELONGATION OF THE ARCHEGONIOPHORE STALK OF *Conocephalum conicum*
Responses given for 5 archegoniophores after 4 weeks

Temperature		16 hr.		8 hr.	
Maximum	Minimum	Average length	No. responding	Average length	No. responding
40° F.	34° F.	2·0 mm.	0	1·5 mm.	0
62° F.	58° F.	19·0 mm.	5	1·5 mm.	0

That a natural auxin is present in the thalli has been demonstrated by Dickson[9], and Larue and Narayanaswami[10], who obtained positive results with *Avena* tests of the extracts of thallus tips of *Lunularia* and *Marchantia*. Preliminary experiments with analysis of such extracts by paper chromatography suggest that the auxin involved may be indole-3-acetic acid.

The sporophytes of the Marchantiales are small and more or less parasitic on the gametophyte, although some chlorophyll is formed. Hughes[11] has shown that in *Conocephalum conicum* sporophytes are complete in all their organs, 8 weeks after fertilization, but the slight elongation of the seta, typical of liverworts, had not occurred. With the temperature maintained at 70° F., half the plants were placed in long-day, and half in short-day, conditions. The development of the sporophytes was not found to be influenced by day-length.

Seta elongation has been induced by external application of auxin to young sporophytes of *Conocephalum conicum* in early autumn. This elongation was not influenced in any way by differences in day-length, and although capsules dehisced normally the spores were not viable.

Of the seven species so far investigated in the Jungermanniales, six appear to react as long-day plants, the sexual organs being stimulated to form in December and January, on plants placed in experimental chambers in long days, with a maximum temperature of 65° F. and a minimum of 46° F. Plants in short days remained vegetative at similar temperatures (Table 5).

Table 5. PHOTO-PERIODIC RESPONSES IN THE GAMETOPHYTE OF SOME JUNGERMANNIALS

Material	16 hr.		8 hr.	
	Plants used	Plants responding	Plants used	Plants responding
Diœcious forms	♂ ♀	♂ ♀	♂ ♀	♂ ♀
Riccardia pinguis	28 70	11 34	14 29	0 0
Cryptothallus mirabilis	5 10	3 9	5 10	2 7
Diplophyllum albicans	157	66 38	184	0 0
Monœcious forms				
Riccardia multifida	79	52	67	0
Pellia epiphylla	76	44	82	0
Lophocolea cuspidata	124	83	137	0
Lophocolea heterophylla	115	78	121	0

Average maximum temperature, 65° F.; average minimum temperature, 46° F., December–January.

Of interest in this group is the behaviour of *Cryptothallus mirabilis*. In Nature, the plants are found up to approximately 3 in. below the surface of leaf litter or a moss carpet overlying peat or humus. The thallus is often heavily infected with fungal hyphæ, which destroy the chlorophyll in many of the cells. The apices of young branches from which the sex organs arise are usually free from fungus. In this form the sexual organs are not produced in response to a particular day-length, but to a rise in temperature.

Experimentally, the organs formed in both long- and short-day conditions within 5 weeks of the plants being transferred from the field to the higher temperatures of the experimental chambers. Plants kept continuously in a warm greenhouse at 65° F. remained vegetative throughout the winter and the following season, suggesting that here, as in *Lunularia*, there may be a low-temperature requirement that must be fulfilled before the stimulation of a rise in temperature.

As in Marchantiales, maturation of the sporophytes is independent of day-length, but Crombie and Paton[12] have demonstrated that temperature affects seta elongation. They have shown that older sporophytes of *Pellia epiphylla*, left in the field until January or February, respond more rapidly to a temperature stimulus in the laboratory than do sporophytes collected in early autumn. They suggest that during the winter there is a slow synthesis of growth hormones (or removal of a growth inhibitor), which is stimulated to some extent by temperature and extra light. That temperature may well be the more important factor is indicated by the results of the experiments I have carried out in November on plants kept at an average temperature of 60° F. Application of various concentrations of 2,4-dichlorophenoxyacetic acid to plants bearing young sporophytes caused seta elongation to occur in the three liverworts investigated, maximum extension occurring at a concentration of 10^{-6} (Table 6). A study of seta elongation by time-lapse photography has also indicated that the extension of individual setæ of these genera often occurs over a period of two or three days, and that there is a period of growth immediately following sunset each day (Fig. 1). It is realized that growth could have been influenced by the light flashes used for the photographs, but if this light source were significant, one would have expected a more marked effect on the growth curves obtained.

Table 6. EFFECT OF VARYING CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON SETA ELONGATION IN SOME JUNGERMANNIALES

Species	Concentration of 2,4-D					
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Control
	Average length of setæ in mm.					
Pellia epiphylla	5·0	13·8	14·5	28·5	24·0	1·0
Cryptothallus mirabilis	1·0	4·0	16·0	18·6	17·2	1·0
Lophocolea cuspidata	0·5	0·5	12·5	22·5	21·0	0·5

Results after 14 days.
Average maximum temperature, 60° F.; average minimum temperature, 58° F., November–December.

Experiments by Asprey *et al.*[13] also have shown that external application of indole-3-acetic acid can produce seta elongation in young sporophytes of *Pellia*, and that addition of gibberellin gives greater extension than with the auxin alone—an average of 63 mm. in length as compared with 37 mm. in the former case.

Brian and Hemming[14] have presented evidence that gibberellin often depends on the presence of indole-3-acetic acid for its activity. Brian[15] suggests that gibberellin is produced naturally in plants in response to light, vegetative growth being proportional to the amounts of gibberellin and indole auxins which are formed. He suggests that this may well be true of the extension of *Pellia* setæ, and that gibberellins formed during daylight are utilized in the dark period.

Hughes carried out experiments on *Anthoceros laevis* in April and May when plants from the field had already discharged their antheridia and young sporophytes were formed on the dorsal surface of the thalli of the female plants.

Plants were placed in experimental chambers under long- and short-day conditions at temperatures of 70° F. and 50° F. in each case. After a month, the male plants were sexually fertile in short days at both temperatures, the antheridia developing on new lobes of the thalli. Plants under long-day conditions remained sterile. Subsequent experiments have shown similar results for the female plants (Table 7).

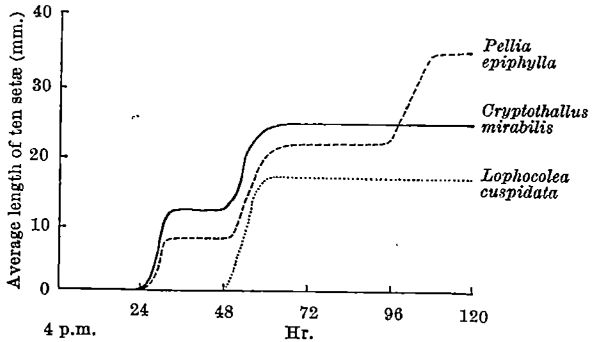


Fig. 1. Rate of seta elongation in three liverworts, based on time-lapse photographs 55° F.; hr. of darkness, 5.30 p.m.–8.0 a.m.

Table 7

Anthoceros laevis	18 hr.		6 hr.	
	70° F.	50° F.	70° F.	50° F.
Male plants	vegetative	vegetative	fertile	fertile
Female plants	vegetative	vegetative	fertile	fertile
Sporophytes	rapid vegetative growth	slow vegetative growth	slow vegetative growth	slow vegetative growth

When investigating the responses of the sporophytes, Hughes was able to show that after 8 weeks those sporophytes which had been maintained under long-day conditions exhibited almost unlimited power of vegetative growth due to the basal meristem, as compared with the low vegetative growth in short days. There was no difference, however, in the rate of spore production in long and short days. It was concluded that development of the sporophyte in this species is not induced by the photo-period except for a difference in the rate of the two sporophytes vegetatively. The length of the photo-period did not affect the embryonic tissue.

In the Sphagnales, as yet, I have made only a few preliminary observations on *Sphagnum plumulosum*. Material collected in August and kept in a glasshouse in short-day conditions produced sex organs in December. The male branches changed colour as they matured and appeared as red catkins in early February when the antheridia dehisced. Dissection of the apices of branches of the female plants showed that archegonia were present. Plants in experimental chambers under long-day conditions did not produce sex organs (Table 8).

Table 8

Material	16 hr.				8 hr.			
	Plants used		Plants responding		Plants used		Plants responding	
	♂	♀	♂	♀	♂	♀	♂	♀
Sphagnum plumulosum	134	115	0	0	110	107	54	31

Average maximum temperature, 76° F.; Average minimum temperature, 58° F.

In the field, plants were producing sex organs in late February and early March, capsules had formed by May, but these did not mature and dehisce until July. As yet there is no experimental data concerning the effect of temperature or day-length on the maturation of the sporophyte. The only record of auxin action in this group is the work by Patterson[16], who demonstrated that nearly mature capsules with unelongated pseudopodia gave maximum extension within 3–4 days when moistened with indole-3-acetic acid at a concentration of 10^{-6}. Higher concentrations of auxins, however, inhibited or entirely prevented elongation, a reaction similar to that recorded for the receptacles and stalks of the Marchantiales.

There is therefore some evidence that *Sphagnum plumulosum* may well be a short-day plant, but more confirmation is required.

Of the Bryales or true mosses, the only experimental work so far has been carried out by Hughes using *Polytrichum aloides* and *P. piliferum*.

In *Polytrichum aloides* mature sex organs are produced in the field from March until May, but arche-

Handbook of Organometallic Compounds

HERBERT C. KAUFMAN, John B. Pierce Foundation.

This handbook brings together all the known data on organometallic compounds that have been reported in the literature. For the individual compound, it gives the formula, name, molecular weight, state of aggregation, solubility, refractive index, density, vapour pressure, and all the other physical, chemical and thermodynamic properties which are available. The compounds are grouped in chapters according to the metal or other element which constitutes the central atom; and each is arranged essentially in order of increasing carbon content, followed by the non-carbon compounds. In addition to its broad appeal to chemists, this book should prove indispensable to the plastics industry, and to all those working on fuel and propulsion problems in the aircraft and missile fields.

6¼ × 9½, 1,400 pp., July 20th, 169s.

Rare Earth Alloys

KARL GSCHNEIDNER, Jr., University of California, Los Alamos Scientific Laboratory; consultant, Atomic Energy Commission.

This review of the rare earth alloy systems presents such information as phase diagrams, crystallographic and thermodynamic data, and physical and mechanical properties concerning these alloys assembled into a single, critical compilation. It should benefit those primarily engaged in research in the field of metals, and also those specializing in solid state physics, i.e. investigating intermetallic compounds.

145 illus., 6 × 9, 500 pp., July 20th, 96s.

Introductory Chemistry 2nd Edition

OTTO W. NITZ, Professor of Science and Mathematics, Stout State College, Wisconsin.

The second edition presents an exceptionally clear discussion of chemical principles and of the common elements and their compounds. The theoretical base of chemistry has been strengthened in this new edition, and the order of presentation has been provided where experience indicated their need. Coverage of the section on biochemistry has been widened, and the chapter on organic chemistry is now reorganized and extended. Sections on historical background and industrial methods have been revised and, in some instances, enlarged. There are some 40 new illustrations.

266 illus., 6 × 9, 600 pp., July 20th, 60s.

Linear Differential Operators

C. LANCZOS, Senior Professor, School of Theoretical Physics, Dublin Institute for Advanced Studies.

This book deals thoroughly with certain new and advanced topics in mathematical analysis, and is likely to interest engineers, mathematical physicists and other applied mathematicians. By examining the properties of certain mathematical entities, notably the Green's function, Professor Lanczos conducts a broad investigation into linear differential operators, taking as his central theme the identity of form and treatment between these operators and matrix algebra. Though the primary emphasis is on the analytical theory, concrete cases are cited to forge the link between theory and practice.

6 × 9, 580 pp., 58 diagrams, 3 tables, August 24th, 80s.

International Dictionary of Physics and Electronics 2nd Edition

The new revised and enlarged second edition of this famous dictionary, complete with multilingual indices in Russian, German, French and Spanish, is now published.

For a limited period (until August 30th, 1961) it is offered to the public at the specially reduced price of £9 5s. od. Thereafter it will be £10 5s. od. A leaflet giving further details may be obtained on request from the publishers.

PUBLISHERS SINCE 1848

Van Nostrand

358 Kensington High Street, London W14

DISTRIBUTORS FOR ELSEVIER

Immunochemical Approaches to Problems in Microbiology

Edited by MICHAEL HEIDELBERGER and OTTO J. PLESCIA,
Institute of Microbiology, Rutgers, The State University

A volume based on a recent Symposium designed to bring together scientists representing different disciplines to consider more effective utilization of immunochemical methods in the further development of microbiology.

The scientific papers and discussion by panelists and invited audience cover the following topics: Quantitative immunochemical aspects of microbial specificity: precipitation and inhibition techniques, multiplicity and specificity of bacterial antigens, immunochemistry of viruses, so-called non-specific factors in immunity, and biosynthesis of antigens and antibodies.

The panelists were Ö. Ouchterlony, Pierre Grabar, H. G. Kunkel, J. H. Rockey, T. Tomasi, J. R. Marrack, Paul H. Maurer, E. A. Kabat, J. Baddiley, Maclyn McCarty, James T. Park, D. L. Shrivastava, C. A. Knight, Lawrence Levine, Igor Tamm, Purnell W. Choppin, Becca Patras, Fred Karush, Raymond Latarjet, G. Biozzi, O. G. Bier, Elmer L. Becker, Otto J. Plescia, Manfred M. Mayer, Irwin H. Lepow, Robert A. Nelson, Jr., Myron A. Leon, Evelyn E. B. Smith, George T. Mills, Robert Austrian, Harriet P. Bernheimer, Brigitte A. Askonas, Felix Haurowitz, Maxwell Richter, G. J. V. Nossal, and O. Mäkelä.

402 pages with illustrations and index. £2 3s

Distributed by
RUTGERS UNIVERSITY PRESS
NEW BRUNSWICK, N.J.

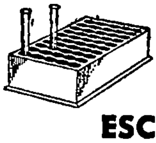
WANTED TO PURCHASE

Scientific books & periodicals

Entire libraries, and smaller collections;
sets and runs, foreign and domestic.

WALTER J. JOHNSON, INC.
111 Fifth Avenue, New York, 3, N.Y., U.S.A.
Cable Address: BOOKJOHNS, NEWYORK
British Office: ACADEMIC BOOKS, LTD.
17 Old Queen St., London, S.W.1
WHItehall 6631

BACK ISSUES OF JOURNALS IN
SCIENCE & THE HUMANITIES
ALWAYS REQUIRED BY:
WM. DAWSON & SONS, LTD.,
BACK ISSUES DEPT.,
16 WEST STREET,
FARNHAM, SURREY, ENGLAND.
TEL: FARNHAM 4664 CABLES: DAWBOOKS, FARNHAM



"E.R.L." RUBBER HOLDERS
FOR TEST TUBES, AMPOULES, etc.
Made in 8 sizes, either in black, synthetic or softer red organic rubber; price 20/- each

ESCO (RUBBER) LTD.
34/36 Somerford Grove, London, N.16

CYGNET meets the demands of science

The Associated Feed Manufacturers Research Laboratory, York Road, Belfast, is an excellent example of the top quality research accommodation now being provided for industry. Once again, Cygnet Laboratory Furniture provides the supreme craftsmanship and advanced design necessary for maximum efficiency.

Architects: Munce & Kennedy, A/ARIBA
Architects & Consultant Engineers,
University Street, Belfast, 7

CYGNET JOINERY LTD · HIGHER SWAN LANE · BOLTON · TEL: BOLTON 62121 (10 lines)

A New Reprint *In Preparation*

Zeitschrift für Kristallographie, Mineralogie und Petrographie

Volumes 1-50, 1877-1912

Approx. 40,000 pp. including General Index to Vols. 1–50

Cloth bound set	$1,475.00
Paper bound set	1,350.00
Single volumes, paper bound	25.00

Index to Vols. 1-50 also available separately

Address orders and inquiries to

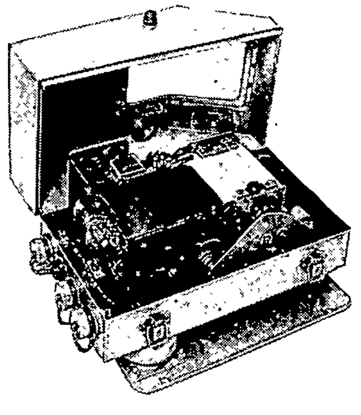
JOHNSON REPRINT CORPORATION

New York and London

111 Fifth Avenue
New York 3, N.Y.

17 Old Queen Street
London, S.W.1, England

Hendrey Spark Recorder Type 1487A



For full details and specification ask for Catalogue Sheet A4

Compact and weatherproof, this single channel recorder is light in weight and easily portable. The Perspex window gives a clear view of the heat-sensitive chart for approximately 6 in. of its length.

The instrument can also be used mounted on bench, wall or panel and is supplied with mounting plate incorporating three anti-vibration mountings which render the instrument shock-proof and vibration-proof to service standards.

An electro-magnetically operated stylus, operated by a separate switch not supplied with the recorder, provides transverse markings near the edge of the chart. Provision is made for an external alarm circuit which can be pre-set to operate at any deflection from zero to full scale by means of a control on the case.

Sensitivity—50 micro-amps. Full scale. Supply—12 Volt, D.C.

Hendrey Relays

HENDREY RELAYS LIMITED

392 BATH ROAD, SLOUGH, BUCKS. *Burnham* 609/611
MANUFACTURING ELECTRICAL ENGINEERS
CONTROL AND LABORATORY APPARATUS
A.I.D. AND A.R.B. APPROVED

TRANSIENT IMAGE BECOMES PERMANENT RECORD

with the new S & P direct-writing

OSCILLOGRAPH RECORDER

A highly-advanced and versatile unit with the important advantages of very high speed and sensitivity. Special pencil-type mirror galvanometers trace records on 6-in wide ultra-violet sensitive paper which requires no processing.

PERFORMANCE DETAILS

Permanent continuous or intermittent records can be made at frequencies from d.c. to frequencies in excess of 1,000 c.p.s. The system gives a well defined trace, at writing speeds above 12,000 in. per sec. Choice of 8 paper speeds, from 5 in. per min. to 150 in. per sec. 12 channels, but with 3 additional channels for time, event, datum reference lines, or for use as additional signal channels if desired. Matched amplifiers and power supply units can be supplied.

APPLICATIONS

Industrial, Scientific, Medical and Military . . . Because a transient tube image is replaced by permanent records for analysis, this unit will have special value for many purposes in universities and technical colleges. Other applications include: Stress analysis by electrical strain gauge techniques. Testing heavy electrical equipment. Records of electric power supply characteristics. Laboratory process analysis. Missile and gun performance testing, and general telemetery use. Cardio-vascular and respiratory investigation.

Please write now
for details and Publication No. 33/0

SAVAGE AND PARSONS LIMITED

WATFORD · HERTFORDSHIRE · ENGLAND · Tel: WATFORD 26071

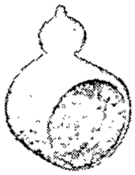
STATIC AND DYNAMIC STRAIN RECORDERS · RESISTANCE THERMOMETERS

SCINTILLATION COUNTING

Precision Nucleonic Instruments (Bulletins 14, 15, 16, 18, 19, 20, 21, 22, 24)
The Human Body Monitor shown above forms one of a series completed for Atomic Energy Establishments and hospitals in Britain, Canada, Germany and Australia by Nuclear Enterprises Low Level Division. Standard instruments and assemblies include non-overloading linear pulse amplifier NE 5202, pulse height selector NE 5103, EHT supplies, ratemeters, NE 8301 assembly for H3 and C14 counting and scanning scintillation spectrometer NE 8601.

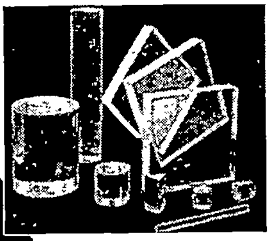
Spiral Flow Cell: For beta emitters in liquids and gases. Used for flow studies in biology and medicine, it gives 10% and 60% efficiencies for C14 (liquid and gas respectively).

Neutron Detectors: Boron polyester systems, greatly improved Hornyak systems, and highly directional fast neutron detectors.

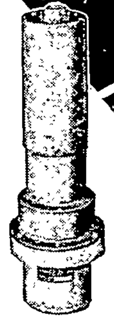


Liquid Scintillators & Scintillation Chemicals: Liquid and loaded liquid scintillators (lead, tin, cadmium, gadolinium, boron, etc.), terphenyl, POPOP, P.P.O., BBO, T.P.B., DPA, hyamine, dioxane, etc.

Crystal Scintillators: Wide range and high resolution NaI(Tl), CsI(Tl), and LiI(Eu) mounted scintillation crystals. Thicknesses down to 0·002″ and diameters up to 9″.



Plastic Scintillator NE 102: Available in complete range of rod, sheet, filament, well, sphere and tube form. Light output 65% anthracene, decay time 3·5 millimicroseconds. Linear dimensions from 1 micron to 1 metre.



Crystal-Photomultiplier Assemblies: Scintillation crystal head units offering improved resolution. NaI(Tl), CsI(Tl) and LiI(Eu) in integral and demountable assemblies with crystal sizes up to 9″ diameter.

SCINTILLATORS

The unique scintillation service afforded by Nuclear Enterprises (G.B.) Ltd. includes the highly efficient NE 102 plastic scintillator, liquid scintillators, neutron detectors, scintillation chemicals and an extensive range of crystals & crystal-photomultiplier assemblies.

New:

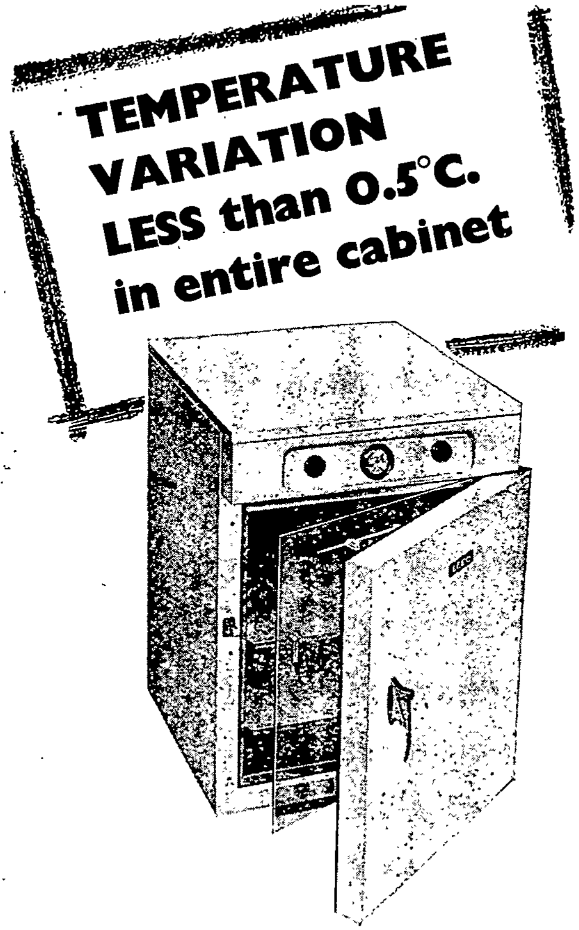
NE 150 plastic phosphor for pulse shape discrimination techniques and NE 160 a special high temperature plastic scintillator.

For full details write for Technical Bulletins (Nos. 9, 11, 12, 13, 23, and 25) and Catalogue to the Sales Division.

Nuclear Enterprises (G.B.) Ltd

Sighthill, Edinburgh 11, Scotland. Tel: Craiglockhart 5262
Associate Company: Nuclear Enterprises Ltd.,
550 Berry St., Winnipeg, 21, Canada.

ndh 8161



—that's the constant performance of the LEEC Precision Incubator. Immaculately designed and precision-built by specialist engineers to meet the exacting demands of modern laboratory practice. With interior lighting, inner plate-glass door and chrome handle with lock. Easy access to indicator bulbs and mains voltage setting. Four shelves—two 'perforated', two 'mesh'. Low running cost. Lightweight, only 200 lb.

LEEC
PRECISION
INCUBATOR
THE ONLY INCUBATOR WITH A 10 YEARS GUARANTEE.

Write for detailed brochure

LEEC

LABORATORY & ELECTRICAL ENGINEERING CO.

71–73 Goldsmith Street, Nottingham. Tel. Nottm. 48331

Measurement of Load by Elastic Devices

Accurate elastic devices were originally instigated as a convenient means of verifying machines for testing materials in tension and compression. This book defines the units of force employed, describes the more common types of elastic load measuring devices, and discusses the methods used for their calibration. **3s.** *(post 4d.)*

Overseas Geological Surveys

Outlines the major problems, achievements and work of the Overseas Geological Surveys during the year ended August, 1960. Illustrated. **4s.** *(post 5d.)*

Department of Scientific and Industrial Research

Report of the Research Council for 1960 showing a year of steady expansion and progress in all fields. (Cmnd. 1365) **4s.** *(post 4d.)*

HMSO

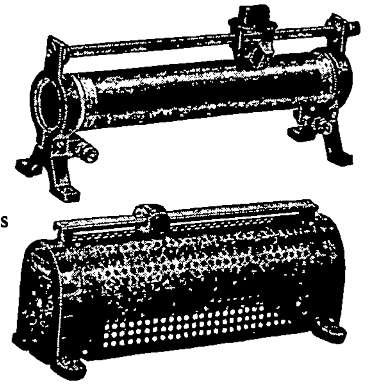
From the Government Bookshops
or through any bookseller

ZENITH
(REGD. TRADE-MARK)

TUBULAR SLIDING RESISTANCES

Wound on vitreous enamelled steel tubes with oxidised wire having a negligible temperature coefficient. Non-inductive windings on refractory formers are also available in many sizes.

Illustrated catalogue of all types free on request



The ZENITH ELECTRIC CO. Ltd.

ZENITH WORKS, VILLIERS RD., WILLESDEN GREEN
LONDON, N.W.2

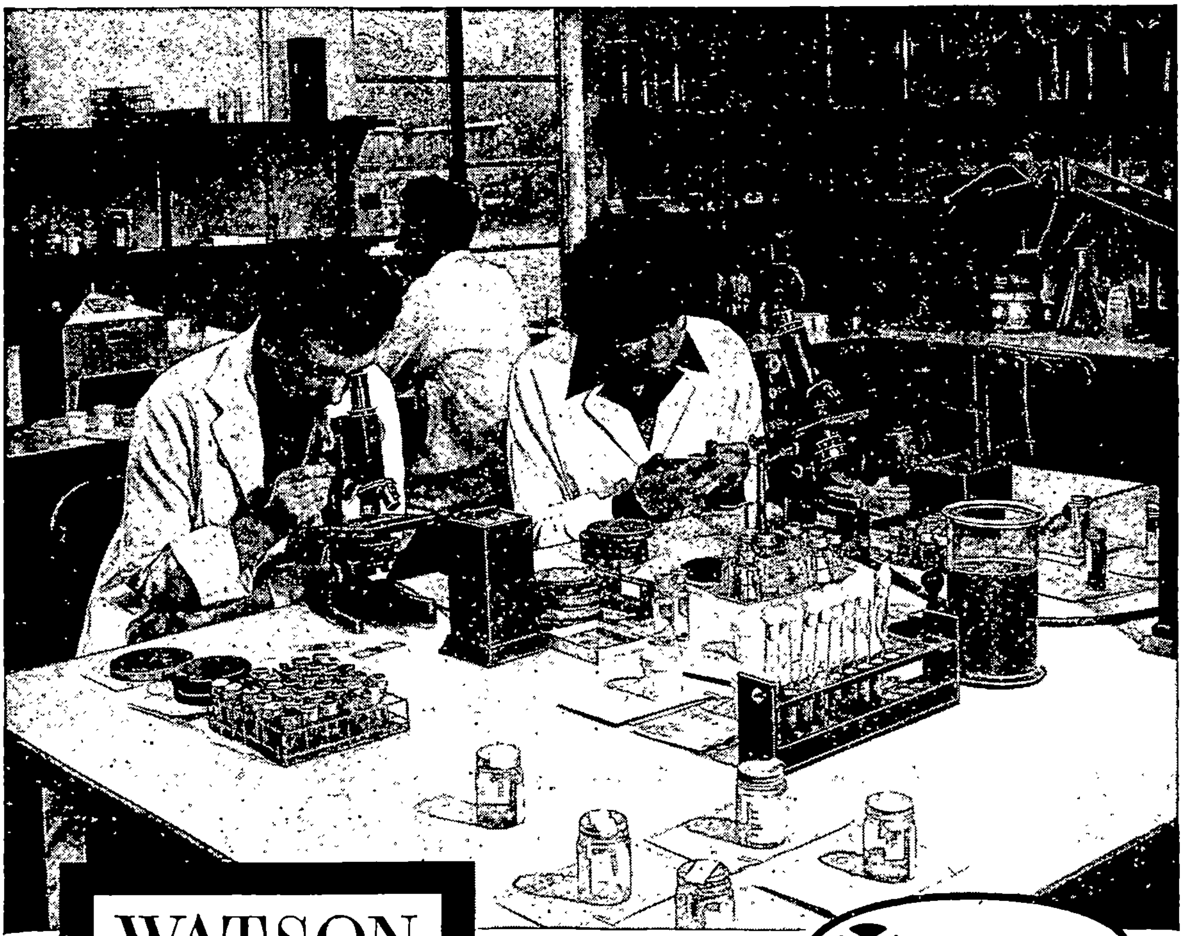
Phone: WILlesden 6581-5 *Grams:* Voltaohm, Norphone, London

MANUFACTURERS OF ELECTRICAL EQUIPMENT INCLUDING RADIO AND TELEVISION COMPONENTS

. . . . built for a lifetime of constant use

The 'SERVICE'

The "Service" is without doubt the finest student microscope today and has proved its high quality and serviceability in many school and university laboratories throughout the world. The simplicity of its design is allied with the highest standards of construction, resulting in a high quality instrument easy to work with and capable of giving a lifetime of satisfactory service in constant use. The comprehensive range of accessories, designed to make the "Service" suitable for more complex work is of equally high quality. See the Watson "Service" before you decide on your next microscope.

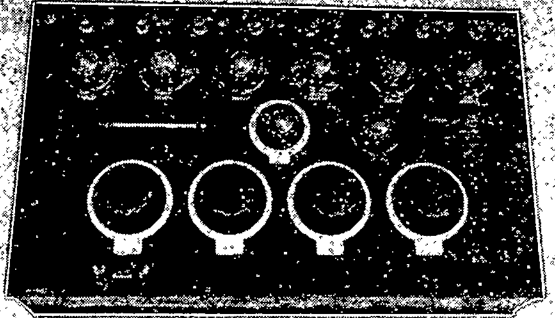


WATSON

A member of the PYE INSTRUMENT GROUP

W. WATSON & SONS LTD · BARNET · HERTFORDSHIRE

EXCEPTIONAL DISCRIMINATION



The Cambridge "Micro-Step" Precision Potentiometer is another "thoroughbred" from Cambridge Instrument Company. It has very great discrimination over a *single* range of 2 volts, and is designed to meet the requirements of high sensitivity *plus* precision, *plus* wide range.

Readings are in steps of 0.1µV up to 2 volts and the instrument is constructed in the form of a double potentiometer, but requires only one galvanometer.

The instrument has been designed to minimise the thermal EMFS generated, and these are extremely small indeed.

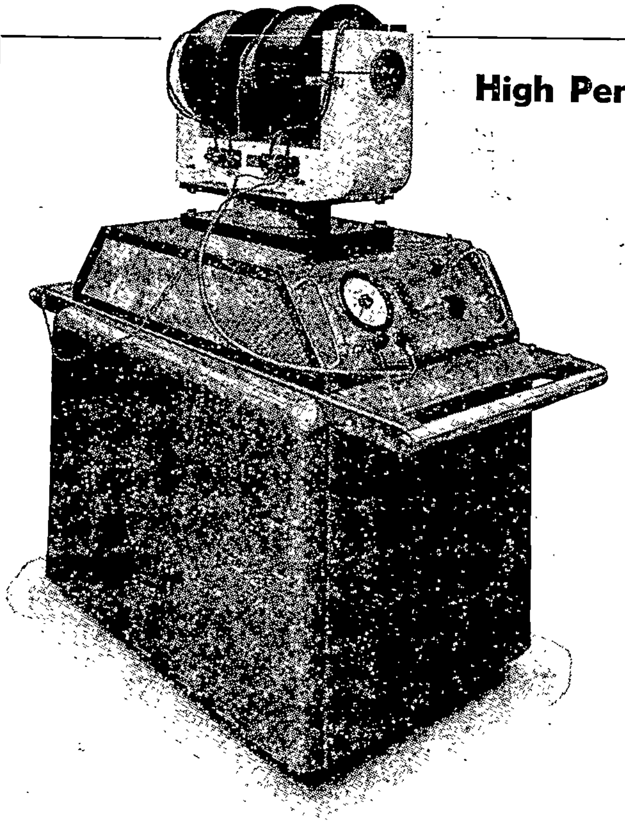
Write for List S.A.295N. For future publications, ask for Mailing Form N/7/61.

SPECIFICATION:

Range: 2 volts.
Discrimination: One part in 20,000,000.
Accuracy: Better than ± 0.001% on 2 volt dial.
Smallest Direct Reading: 0.000 0001 volt.
Temperature compensation for standard cell from 10°C to 35°C.

Cambridge "MICRO-STEP" PRECISION POTENTIOMETER

CAMBRIDGE INSTRUMENT COMPANY LTD • 13 GROSVENOR PLACE, LONDON SW1



High Performance ELECTROMAGNETS and Power Supplies

RUGGEDNESS

4″ Electromagnet Type A (4″ pole diameter)
Fields up to 25,000 gauss.
Homogeneity of at least 1 part in 10^5 in ½ cc.

ROTATION

Rotating Base Type AE
360° scale.
Positive friction lock.

STABILITY

Current-Stabilized Power Supply Unit Type B.

Also available:

1½″ Electromagnet Type C—for Teaching purposes.
7″ Electromagnet Type E—for Electron Resonance Work.
8″ Electromagnet Type D—for Electron Resonance and Nuclear Magnetic Resonance Work.
Direct Reading Magnetometer Type G.
Proton Resonance Magnetometer Type P.

NEWPORT INSTRUMENTS

(SCIENTIFIC & MOBILE) LTD
2 CRAWLEY ROAD, NEWPORT PAGNELL, BUCKS
Telephone : Newport Pagnell 401/2

gonial production frequently continues until July, particularly on plants where fertilization has failed. In the latter circumstances the number of archegonia produced is frequently increased to double the normal number. Where sporophytes have formed these are usually apparent by the end of July, covered by the white calyptra. The capsule takes shape in August and September, is fully formed by December, and matures to dehisce in March or April (Table 9).

Table 9. FIELD OBSERVATIONS OF THE MATURATION CYCLES OF *Polytrichum* SPECIES (J. G. HUGHES, 1955)

Developmental phase	*P. aloides*	*P. piliferum*
Sex organs mature	Early March	April
Vegetative phase of sporophyte	Short	Long
Onset of reproductive phase of sporophyte	May	November
Mature sporogonia	August	Following March
Capsules dehisced	Following spring	Early summer

Polytrichum piliferum

Date	Average length of apical meristem (cm.)	Developmental phase
Oct. 5	0·030	Vegetative, endothecium unsegmented
Oct. 23	0·035	Apical cell ceased to divide; endothecium segmented
Nov. 23	0·060	Endothecial block starts to swell at base; apical cell begins to vacuolate
Dec. 21	0·070	Little change
Jan. 18	0·085	Operculum and theca clearly demarcated
Feb. 15	0·110	Archesporial layer differentiated
Mar. 15	0·150	Archesporial tissue several cell layers thick; peristome apparatus developing
April 12	0·450	Sporangium fully developed

Female plants placed in experimental chambers in January under long- and short-day conditions, combined in each case with temperatures of 70° F. and 50° F., produced archegonia within two weeks in both long and short days, about 66 per cent responding in each case. Even after a month the plants in both cabinets at 50° F. were still vegetative (Table 10). From these results, it was concluded that day-length was not the governing factor in the production of sex organs in *Polytrichum aloides*, but that the rise in temperature in the spring months in the field may be the most important factor causing the sudden appearance of sex organs.

Table 10. PHOTO-PERIODIC RESPONSES IN *Polytrichum aloides* (J. G. HUGHES, 1955)

Period of response (days)	18 hr.		6 hr.	
	70° F.	50° F.	70° F.	50° F.
14	66 per cent response	—	66 per cent response	—
28	—	No response	—	No response

Twenty-five gametophytes (female) used in each case. Experiment conducted in January.

A second experiment was set up involving diurnal fluctuations of temperature in combination with the two photo-periods. By these experiments it was hoped to indicate whether the temperature stimulus was a light or dark reaction. The results indicated that there was a slightly greater response in the warm nights than in the cold (Table 11). A temperature stimulus of 20° F. proved sufficient to induce the development of archegonia in the laboratory two months before their earliest appearance in the field.

The sporophyte grows by means of an apical cell until a branch is formed. This does not enter into the reproductive stage until the apical cell loses the power to divide, and the whole original meristem is then incorporated into the developing sporangium.

Table 11

Response (per cent)	Light (hr.)	Temperature of light period (°F.)	Dark (hr.)	Temperature of dark period (°F.)
54	18	70	6	50
70	18	50	6	70
44	6	70	18	50
60	6	50	18	70

Plants of *Polytrichum aloides* bearing vegetative sporophytes were placed in experimental chambers in June, in 8 hr. and 18 hr. light respectively at normal temperatures for the time of year. At intervals of 3, 5 and 7 weeks, the cultures were exchanged and, once changed, remained in that cupboard up to a total period of 24 weeks. By sectioning ten sporophytes from each group, and measuring the total seta length and total meristem length, it was found that short days stimulated development of the capsule and spore production. Long days did not entirely inhibit spore production, but did have a distinct retarding effect on sporangium development. The length of the seta was also found to be influenced by the photoperiod, since in short days the vegetative stage is very limited, giving rise to a short seta, whereas in long days there is a late change to the reproductive phase and so a long seta is formed. To ascertain whether this was due to an increase in the number of cells, or to cell length, the setæ of both long- and short-day sporophytes were macerated and measured. Cells from the central strands were used as these were not as spiralled as the outer cells of the setæ. An average of 200 cells of each kind of seta (Table 12) indicated that it is a difference in cell length and not in cell number which causes the difference in seta length. It was concluded that *Polytrichum aloides* is only facultatively photo-periodic in spore production since, although spore production is markedly promoted in short days, it can also be finally achieved even in long days.

Table 12

Day-length	Response	Average length of 200 cells in seta (arbitrary units)
18 hr.	Slow*	3·71
6 hr.	Rapid*	2·61

* The true vegetative state is taken when the average meristem length is 0·04 cm.; the true reproductive state is taken when the average meristem length is 0·114 cm.

Polytrichum piliferum differs in that the sporophytes do not mature until March of the following year, so that sporangia pass through a cold winter period (in contrast to *Polytrichum aloides* where sporangia are mature in August of the same year) (Table 9).

Plants bearing vegetative sporophytes were removed from the field at monthly intervals, to growth chambers at 70° F., half being submitted to long days and half to short days, the field behaviour of the plants being studied as a control. Plants from collections made in October did not produce sporangia in the experimental chambers, although apical growth had ceased and some segmentation had occurred. This suggests that the character of the sporophytes as a branch of limited growth is inherent in the sporophyte itself and not contingent on environmental factors. Collections made on November 23 and afterwards, gave rise to small, normal capsules in both day-lengths. It was concluded that sporangium development was indifferent to day-length and that *Polytrichum piliferum* probably has a low-temperature requirement in its spore production which is normally satisfied in the field from late November onwards.

[1] Wann, F. B., *Amer. J. Bot.*, xii (6) (1925).
[2] Voth, P. D., and Hamner, K. C., *Bot. Gaz.*, **102**, 169 (1940–41).
[3] Mann, L. K., *Bot. Gaz.*, **102**, 339 (1940–41).
[4] Benson-Evans, Kathryn, and Hughes, J. G., *Trans. Brit. Bryol. Soc.*, **2** (4), 515 (1955).
[5] Rousseau, J., *Bull. de la Soc. Bot. Fr.*, **100**, 179 (1953).
[6] Salisbury, F. B., *Plant Physiol.*, **30** (4), 327 (1955).
[7] Benson-Evans, Kathryn, and Powell, Phyllis, *Trans. Brit. Bryol. Soc.*, **2** (2), 289 (1953).
[8] Knott, J. E., *Cornell Agric. Exp. Stat. Mem.*, **218** (1939).
[9] Dickson, H., *Ann. Bot.*, **46**, 483 (1932).
[10] Larue, C. D., and Narayanaswami, S., *New Phyt.*, **56**, 61 (1957).
[11] Hughes, J. G., Ph.D. thesis, University of Wales ,Cardiff (1955).
[12] Crombie, W. Mary, and Paton, Jean A., *Nature*, **182**, 541 (1958).
[13] Asprey, G. F., Benson-Evans, Kathryn, and Lyon, A. G., *Nature*, **181**, 1351 (1958).
[14] Brian, P. W., and Hemming, H. G., *Ann. Bot. Lond.*, 22 (1958).
[15] Brian, P. W., *J. Linn. Soc. (Bot.)*, **56** (366), 237 (1959).
[16] Patterson, P. M., *Bryologist*, **60**, 277 (1957).

CARBON DIOXIDE AND ETHANOL AS FACTORS CONTROLLING THE GROWTH OF ETIOLATED OAT SEEDLINGS

By Dr. C. L. MER
A.R.C. Unit of Plant Physiology, Imperial College of Science and Technology, London, S.W.7

When the airstream passing over etiolated oat seedlings is enriched with carbon dioxide to a concentration of 5 per cent for 3 days from the time of planting, then during the 5 days following cessation of treatment with carbon dioxide, the growth of the seedlings is modified in the following respects: (1) the mesocotyl, after an initial depression, shows a marked promotion[1-3]; (2) the coleoptile is depressed[1-3]; (3) the amount of reserves transferred from the endosperm to the embryo is reduced[2,6]; and (4) the mesocotyl retains a capacity to elongate after a brief exposure to light[4,5]. In the absence of carbon dioxide in the airstream, identical growth variations can be induced to occur during the interval mentioned, by treating the seedlings with 0·3 per cent ethanol through the roots for 3 days from the time of planting (references as above) and further, when the ethanol and carbon dioxide treatments are administered simultaneously, their effects on mesocotyl growth are independent and additive[4].

The coincidence between these effects on growth suggests that a single phenomenon may be under investigation, that is, the ethanol absorbed from the culture medium (London tap-water) might give rise *in vivo* to carbon dioxide to which both effects should be attributed; or, alternatively, treatment with carbon dioxide might induce ethanol formation, perhaps by occasioning a fermentative change in metabolism; in this event the growth responses observed should be regarded as ethanol effects.

The data to be presented favour the first alternative.

The effect of treatment with ± 5 per cent carbon dioxide combined factorially with ± 0·3 per cent ethanol on the ethanol content of 3-day-old seedlings has been determined on 10 replicate samples, each of 5 plumules, by distilling the volatile constituents under reduced pressure into an aliquot of acid dichromate and titrating the residual dichromate with sodium thiosulphate.

The mean ethanol contents, in µgm. per 5 plumules, are recorded in Table 1, exp. 1. It will be seen that treatment with ethanol alone increased the content in the plants, but the overall effect of ethanol on the internal content, as shown in the analysis of variance, did not reach significance. This was due to the highly significant negative interaction with carbon dioxide, which when administered alone reduced the amount of ethanol in the plumules but apparently did so more effectively when ethanol was additionally supplied.

Because of the variation in growth with treatment, determination of the ethanol in a given number of plumules means that different weights of material were assayed. As these samples were collected in darkness no correction could be made for the differences, but in a further experiment, the 5 plumules in each sample were briefly exposed to light for measurement. The ethanol contents were determined and submitted, as previously, to an analysis of variance (Table 1, exp. 2). Additionally, the total lengths of the mesocotyl + coleoptile + leaf of the 5 plumules in each treatment are quoted in italics, together with an analysis of covariance. From these length-measurements the content of water was estimated so that the concentration of ethanol in the plumules could be assessed. The content in µgm. and analysis of variance agreed with those of exp. 1, but the analysis of covariance shows that by allowing for variation in length, the depressive effect of carbon dioxide on the ethanol content of the seedlings was eliminated while the levels of significance due to applied ethanol and its negative interaction with carbon dioxide were both increased.

Table 1. Ethanol Content (in µgm./5 Plumules) of 3-day-old Oat Seedlings, after Treatment for 3 days in Darkness with ± Carbon Dioxide (5 per cent) combined with ± Ethanol (0·3 per cent)

Mean values of 10 replicates in µgm. Exp. 1, plants not exposed to light; exp. 2, plants exposed on the third day for measurement; the lengths of mesocotyl + coleoptile + leaf (in mm.) are quoted in italics

Treatment: Carbon dioxide	+	+	−	−
Ethanol	+	−	+	−
Exp. 1	6·74	9·05	15·56	13·73
Exp. 2	10·17	11·53	21·31	16·95
	122·4	*211·2*	*177·5*	*283·3*

Variant	Analyses of variance of ethanol content			Analysis of covariance (total length and ethanol content)	
	DF	Mean squares Exp. 1	Exp. 2	DF	Mean square Exp. 2
Carbon dioxide	1	455·6 ↓	685·6 ↓	1	0·9
Ethanol	1	0·6	22·5 ↑	1	138·8 ↑
Carbon dioxide × ethanol	1	42·5 ↓	81·8 ↓	1	137·8 ↓
Error	36	5·1	7·8	35	4·7

The arrows indicate the direction of the effect.
DF, degrees of freedom.

The mean percentage concentrations of ethanol (w/w) in these plumules were as follows: carbon dioxide and ethanol, 0·0096; carbon dioxide, 0·0063; ethanol, 0·013; controls, 0·0066. Evidently, the ethanol concentration within those plants treated with it did not approach that supplied externally. Further, treatment with carbon dioxide depressed the *in vivo* accumulation of ethanol when this was also administered in the culture medium, although under these conditions the content was higher than in the controls, indicating that some entry had occurred. Finally, as the same concentration of ethanol was present in the controls and under carbon dioxide, this treatment neither promoted the *in vivo* synthesis of ethanol nor did it bring about the decomposition of that occurring naturally.

The operation of this negative interaction between carbon dioxide and ethanol becomes clearly manifest when higher concentrations of ethanol are used in this type of experiment. In Fig. 1 are shown graphically the mean lengths of the mesocotyls and coleoptiles of etiolated oat seedlings, from eight treatments of a 2^3 factorially arranged experiment employing ± 0·5 per cent ethanol and ± 5 per cent carbon dioxide, both applied for 3 days from the time of planting, combined with exposure to dim red light (about 1 ft. candle) for 5 min. on termination of treatment with carbon dioxide and ethanol, when all seedlings were transferred in darkness to tap-water. The measurements of the third-day samples estimated the lengths of the plant organs at the time of exposure to light, and the differences in growth during the subsequent 5 days, displayed in the graphs, were after-effects.

In darkness (Fig. 1*A*) carbon dioxide (squares) initially reduced growth of the mesocotyl but afterwards occasioned a promotion, while 0·5 per cent ethanol alone (triangles) severely inhibited growth both during and after treatment. When, however, carbon dioxide was also administered, the initial depression of the mesocotyl due to ethanol gave way to rapid growth so that, ultimately, a length was attained equal to that reached in carbon dioxide alone (diamonds). The effect of illumination can be assessed by comparing the controls in Fig. 1, *A*, *B* (solid and open circles respectively); after exposure some elongation occurred, but more growth was shown by the mesocotyls of those plants treated with carbon dioxide. Ethanol alone severely inhibited growth, and again, simultaneous treatment with carbon dioxide relieved the inhibition so that a length was reached equal to that in carbon dioxide alone.

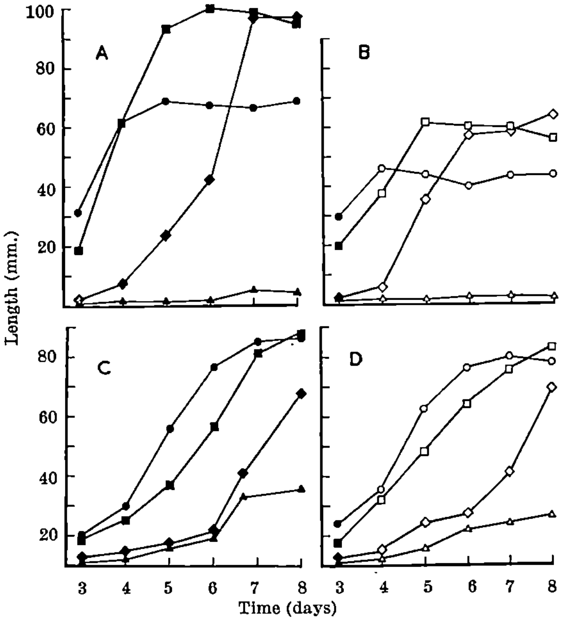


Fig. 1. Growth on successive days of mesocotyls and coleoptiles in darkness (*A* and *C*) and after a single exposure to dim red light for 5 min. on the third day (*B* and *D*) following treatment during the three days after planting, with: ◇ carbon dioxide + ethanol; □ carbon dioxide; Δ ethanol; O control
Solid points, in darkness; open points, after illumination

The behaviour of the coleoptiles is depicted in Fig. 1, *C*, *D* respectively, in darkness, and after exposure to light. The usual effects of light (transient promotion) and of carbon dioxide (generally depression) were recorded in this experiment. Ethanol alone inhibited growth, but again in the presence of carbon dioxide the inhibition was overcome; between the sixth and eighth days the rate of growth in darkness (1·02 mm./hr.) was approximately equal to that shown by the controls between the fourth and sixth days (0·98 mm./hr.). Similar effects were recorded for the plants exposed to light. Although the observations have not actually been made, the rates of elongation would suggest that the coleoptiles of the plants treated with carbon dioxide and ethanol together would ultimately have reached the lengths attained by their respective controls. The effect of this particular treatment may therefore be regarded as a delay in germination.

The inhibited seedlings (ethanol only) suffered no permanent injury; after transfer to water on the third day, root growth was resumed and in the last sample the second internodes were already quite long; the main apical meristem thus seems immune to externally applied ethanol.

The data as a whole suggest that ethanol has an inhibitory effect on growth which increases with the level of concentration. There is, as well, a secondary effect thought to arise from a conversion to carbon dioxide which then initiates a growth promotion. As these two effects are antagonistic the ultimate response observed will depend on the relation between these two factors. Thus, low concentrations of ethanol (0·2–0·3 per cent) are promotive whereas 0·5 per cent is inhibitory. In the first case the concentration in the tissues may not reach an inhibitory level and the promotive effect arising from its decomposition therefore appears. In the second case the inhibitory effect of ethanol masks the promotive effect of carbon dioxide.

This still leaves open the question as to why the inhibitory effect of 0·5 per cent ethanol can be eliminated by simultaneous treatment with carbon dioxide. Since both coleoptiles and mesocotyls behave in the same way some basic growth process seems to be affected by this dual treatment. The only data relevant are those in Table 1, which suggest that a high level of carbon dioxide prevents the *in vivo* accumulation of ethanol. Whether or not this is due to an activation of ethanol dehydrogenase by carbon dioxide is not at present known.

The variations in growth induced by these substances might indicate an effect on auxin metabolism, and this aspect of the problem is at present under investigation.

[1] Mer, C. L., and Richards, F. J., *Nature*, **170**, 179 (1950).
[2] Mer, C. L., *Ann. Bot.*, **21**, 13 (1957).
[3] Mer, C. L., *Ann. Bot.*, **23**, 177 (1959).
[4] Mer, C. L., *Nature*, **182**, 1812 (1958).
[5] Mer, C. L., *J. Exp. Bot.*, **10**, 220 (1959).
[6] Diamond, B. C., M.Sc. thesis, University of London (1960).

LETTERS TO THE EDITORS

COSMOLOGY

The Steady Universe with Charge Excess

RECENTLY, Lyttleton and Bondi[1] put forward the suggestion that the expansion of the Universe is caused by the absolute values of the charges of the proton and the electron not being equal, thereby giving a net charge to the hydrogen atom, and causing mutual repulsion in the universe, which to a first approximation may be considered as composed of a uniform distribution of hydrogen atoms. The analysis of Lyttleton and Bondi may be simplified by the use of the creation potential N suggested by Watson[2], and this simplification is the main purpose of this communication.

If there is creation, Maxwell's equations modify to

$$\nabla \times \mathbf{E} + \frac{\partial \mathbf{B}}{\partial t} = 0 \qquad \nabla \cdot \mathbf{B} = 0 \qquad (1.1),(1.2)$$

$$\nabla \times \mathbf{H} - \frac{\partial \mathbf{D}}{\partial t} = \mathbf{J} - \frac{1}{\mu_0}\nabla N \qquad (1.3)$$

$$\nabla \cdot \mathbf{D} = \rho + \varepsilon_0 \frac{\partial N}{\partial t} \qquad (1.4)$$

where the rate of creation of charge per unit volume is given by:

$$\nabla \cdot \mathbf{J} + \frac{\partial \rho}{\partial t} = \frac{1}{\mu_0}\left\{\nabla^2 N - \mu_0 \varepsilon_0 \frac{\partial^2 N}{\partial t^2}\right\}.$$

where μ_0, ε_0 are the permeability and dielectric constant respectively of free space. (The Giorgi system of units is used throughout.)

Suppose now that a steady state is assumed. All derivatives with respect to t vanish and equations (1.1) to (1.5) become

$$\nabla \times \mathbf{E} = 0 \qquad \nabla \cdot \mathbf{B} = 0 \qquad (1.6)\ (1.7)$$

$$\nabla \times \mathbf{H} = \mathbf{J} - \frac{1}{\mu_0}\nabla N \qquad (1.8)$$

$$\nabla \cdot \mathbf{D} = \rho \qquad (1.9)$$

the rate of creation per unit volume being

$$\nabla \cdot \mathbf{J} = \frac{1}{\mu_0}\nabla^2 N$$

A possible solution is clearly given by

$$\mathbf{E} = -\nabla V,\ \mathbf{H} = 0,\ \mu_0 \mathbf{J} = \nabla N,\ \rho = \varepsilon_0 \nabla^2 V \qquad (1.11)$$

and a solution of this type will be used in the next section. It will be noticed that, because of N, zero $\mathbf{H}$ is possible even when $\mathbf{J}$ is non-zero.

Suppose now that the charge on a proton is e and that the charge on an electron is $-(e - \Delta e)$. Let M be the mass of a hydrogen atom, its charge being Δe. Let n be the number of hydrogen atoms per unit volume (assumed uniform). The charge density is thus $n\Delta e = \rho_e$ and the mass density is $nM = \rho_m$.

Inside this, considered as a uniform spherical distribution the centre of which is the origin, the electric and gravitational intensities are respectively ($\mathbf{r}$ being the position vector from the origin)

$$\mathbf{E}_e = \frac{1}{3}\frac{\rho_e}{\varepsilon_0}\mathbf{r}\ , \quad E_m = -\frac{4\pi\gamma\rho_m}{3}\mathbf{r} \qquad (2.1),\ (2.2)$$

It will be observed that E_e satisfies equation (1.11) if

$$V = -\frac{1}{6}\frac{\rho_e}{\varepsilon_0}\mathbf{r}^2$$

The force on a hydrogen atom in this medium is, if $\mathbf{f}$ be the acceleration:

$$M\mathbf{f} = \Delta e \mathbf{E}_e + M\mathbf{E}_m = \frac{n}{3}\left[\frac{(\Delta e)^2}{\varepsilon_0} - 4\pi\gamma M^2\right]\mathbf{r} \qquad (2.3)$$

It follows from equation (2.3) that the sign of Δe is irrelevant. This point does not seem to have been mentioned by Lyttleton and Bondi, and so the theory applies equally well whether the charge of the electron or of the proton be the greatest in absolute value.

Using the relation between velocity and acceleration

$$\mathbf{f} = \frac{d\mathbf{v}}{dt} = \frac{\partial \mathbf{v}}{\partial t} + (\mathbf{v}\cdot\nabla)\mathbf{v} = (\mathbf{v}\cdot\nabla)\mathbf{v} \qquad (2.4)$$

it follows that

$$\mathbf{v} = \left[\frac{n}{3M}\left(\frac{(\Delta e)^2}{\varepsilon_0} - 4\pi\gamma M^2\right)\right]^{1/2}\mathbf{r} \qquad (2.5)$$

In order to obtain the proper behaviour at the origin, the arbitrary constant is put zero. This represents in the large a recession outwards with a velocity directly proportional to the distance. If the negative root were taken there would be an approach instead of a recession.

$$\mathbf{v} = \frac{\mathbf{r}}{\tau} \qquad (2.6)$$

where τ is the Hubble time.

The current flow vector associated with the field distribution discussed in section 2 is:

$$\mathbf{J} = \rho_e \mathbf{v} = n\Delta e \mathbf{r}/\tau \qquad (3.1)$$

Thus the rate of charge creation per unit volume is

$$\nabla \cdot \mathbf{J} = \frac{3n\Delta e}{\tau} \qquad (3.2)$$

This is equivalent to a rate of production of $3n/\tau$ hydrogen atoms per unit volume in agreement with Lyttleton and Bondi. Were the negative root taken in (2.5), there would be annihilation instead of creation of matter.

One further consequence of experimental data may be noted, as it does not seem to have been referred to by Lyttleton and Bondi. There is experimental reason to believe that[3]

$$\frac{8}{3}\pi\gamma\rho_m\tau^2 = U^{-1} \qquad (4.1)$$

where U is a number the value of which is about unity. It follows, therefore, that

$$U^{-1} = \frac{8}{3}\pi\gamma n M\left[\frac{n}{3M}\left(\frac{(\Delta e)^2}{\varepsilon_0} - 4\pi\gamma M^2)\right)\right]^{-1} \qquad (4.2)$$

whence

$$\frac{(\Delta e)^2}{4\pi\varepsilon_0} = \gamma M^2(2 + U) \qquad (4.3)$$

This implies that the electrical repulsion between two hydrogen atoms must be at least twice the gravitational attraction. Were U unity, it would be 3 times, and were the repulsion 5 times the attraction, as suggested by Lyttleton and Bondi, this would imply that $U = 3$.

I am indebted to Dr. R. A. Newing for discussions.

LL. G. CHAMBERS

Applied Mathematics Department,
University College of North Wales,
Bangor.

---

[1] Lyttleton, R. A., and Bondi, H., *Proc. Roy. Soc.*, A, **252**, 313 (1959).

[2] Watson, W. H., *Proc. Second Symp. App. Math. Amer. Math. Soc.*, 49 (1950).

[3] Klein, O., *Proc. 1958 Solvay Conf.*, 36 (1958).

PHYSICS

Topology of Linear Neutron Kinetics

IN the theory and interpretation of pulsed neutron measurements and in the analysis of random fluctuations in a nuclear fission reactor it is often helpful to study the kinetic behaviour of the time-dependent, space-independent model of the multiplying assembly.

For this purpose the neutron kinetics may be formulated as an integral equation as opposed to the more conventional set of first-order differential equations. This has some advantages in that: (i) The kernel functions of the Volterra-type integral equation have immediate physical significance. (ii) The integral equation is logically related to the topological representation of the fission process. It is also related in structure to the generating functions of probability theory. (iii) Numerical computation of resolvent kernels is usually more efficient than forward integration. (iv) Extension of the theory to non-stationary reactor kinetics and non-linear reactor topology is obvious.

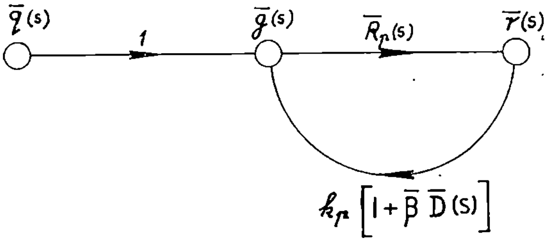


Fig. 1. k_p, Effective prompt multiplication constant; $\bar{q}(s)$, extraneous neutron source; $\bar{g}(s)$, mean generation rate of total importance; $\bar{r}(s)$, mean prompt regeneration rate of total importance; $\bar{R}_p(s)$, prompt importance regeneration kernel; $\bar{\beta}$, delayed neutron fraction; $\bar{D}(s)$, delay distribution function

The role of a nuclear reactor as a 'neutron amplifier' has been shown by Schmid[1]. His signal flow graph for linear topology in the frequency domain is given in Fig. 1. The integral equation of neutron kinetics then takes the form:

$$\frac{r(t)}{r_0} = 1 + \int_0^t G(t-\tau)\rho(\tau)\frac{r(\tau)}{r_0}d\tau \qquad (1)$$

where $G(t)$ is Green's function of neutron kinetics and, for the case of stationary kinetics, obeys the following relationship:

$$G(t) = L^{-1}\left[\frac{1}{s\{F(s)+\tau\}}\right] \qquad (2)$$

where

$$F(s) = \frac{1}{s}\left[1 - D(s)\right] \qquad (3)$$

and

$$\tau = \frac{l}{\bar{\beta}k_p} \qquad (4)$$

and

$$\rho = \frac{k_p - 1}{\bar{\beta}k_p} - 1 \qquad (5)$$

l being the mean neutron generation time.

On the basis of this theory, the computation of reactor frequency and transient responses is carried out completely automatically on a digital computer. Step, pulse, ramp, sine and arbitrary time-responses can be studied for fixed, circulating fuel and boiling-water reactors.

In Fig. 2 some solutions of the integral equation are given for linear variations in reactivity. The frequency responses of a boiling water reactor are given in Fig. 3 and Fig. 4.

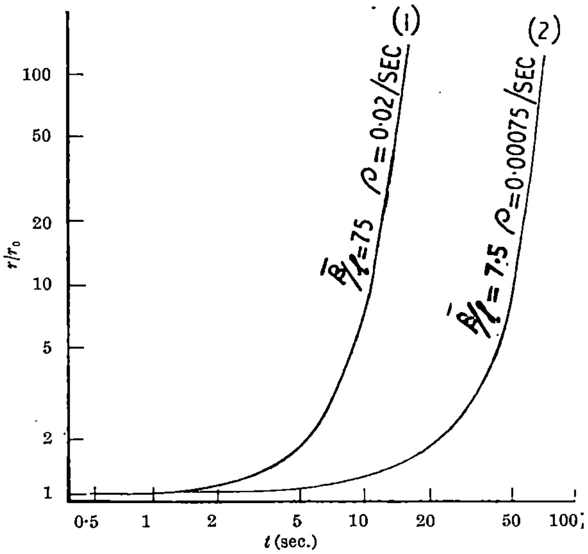


Fig. 2. Solutions of integral equation. (1) Water-moderated research reactor; (2) graphite-moderated power reactor

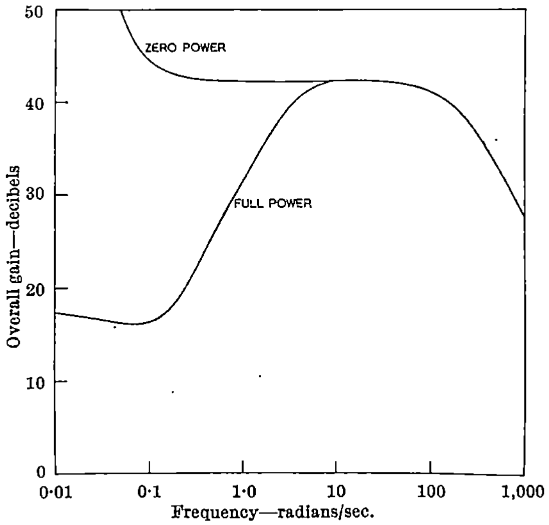


Fig. 3. Overall reactor transfer function

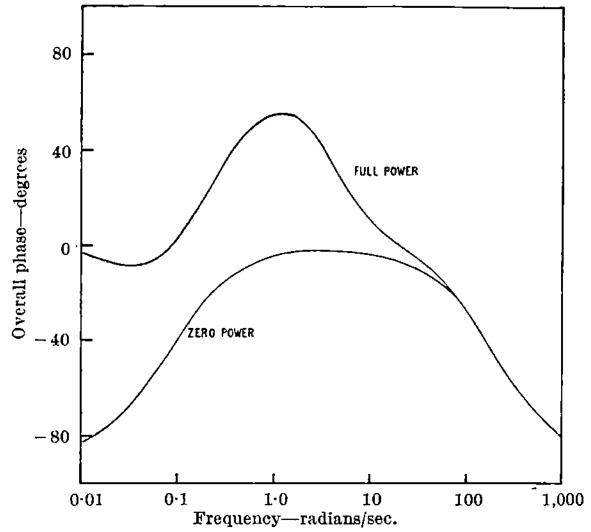


Fig. 4. Overall reactor transfer function

A full report will be published elsewhere.

The support of this work by the United Kingdom Atomic Energy Authority is gratefully acknowledged.

L. G. KEMENY

Nuclear Engineering Laboratory,
Queen Mary College.

[1] Schmid, P., Conference on Peaceful Uses of Atomic Energy, Paper *P*/232 (1958).

Molecular Emission Spectroscopy from 2μ to 12μ by a Michelson Interferometer

THE combination of a two-beam interferometer and Fourier transformation has a great advantage over conventional spectrometers in the effective use of available energy[1,2]. Further, when the interferometer is of a type which has circular symmetry like Michelson's, there is another gain[3], giving the Michelson–Fourier combination an effective luminosity that is the highest of all known spectroscopic systems[4]. It is most advantageously used in wave-length regions where the limit to the spectroscopic resolution obtainable in a given time of observation is set by detector noise. So in the infra-red region, considerable improvement in spectra over those given by conventional methods is to be expected. This has been realized in practice in the very near infra-red[5] and in the far infra-red[6,7]. This communication describes its application in the 2–12μ region, where many molecular vibrational bands are found.

To test the system, experiments have been made to obtain emission spectra of gases at relatively low temperature in cases where the spectra are well known from absorption measurements. The gas was contained in a heated cell with rock salt windows and, after chopping, the radiation passed through a Michelson interferometer to a detector, as shown diagrammatically in Fig. 1. The interferometer was designed and constructed for this work by the Light Division, National Physical Laboratory. It has, as special features, an optically worked glass slide to constrain the moving mirror, and disks of barium fluoride 10 cm. in diameter for the beam splitter and compensator plates, but details of this instrument will be the subject of a separate communication. Measurement of the displacement of the moving mirror to get path-difference was made by illuminating part of the interferometer field with mercury ($\lambda = 1{\cdot}014\mu$) radiation and recording fringes by a photomultiplier. The interferogram of the radiation of the emitting gas was sampled at equal intervals of path-difference by a signal derived from the record of the mercury fringes. The values of intensity at these points were digitized and punched on paper tape suitable for input to the digital computer *Ace*, where a programme for Fourier transformation gave the spectrum.

Fig. 2 shows part of the ν_2 band of ammonia obtained in this way. For this spectrum, the gas temperature was 70° C., the pressure was 11 mm. mercury and the detector was a Unicam Golay cell fitted with an indium antimonide filter. The maximum path-difference introduced was 2 cm., this limit being imposed by the fall-off in visibility of the 1·014μ mercury fringes. This is attributable to the rather

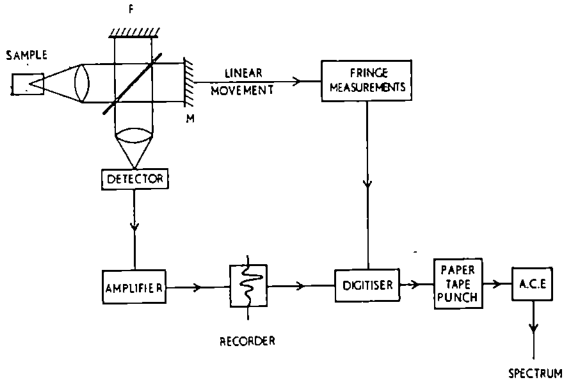
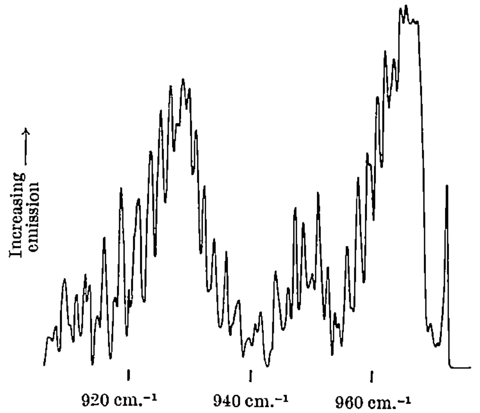


Fig. 1. Diagram of the arrangement of a Michelson interferometer used for emission experiments where the interferogram is digitized and recorded in a form for computer input



Conventional absorption spectrum

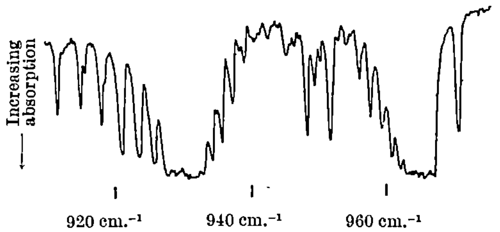


Fig. 2. Spectrum of part of the ν_2 band of ammonia in emission. The gas temperature was 70° C. and its pressure 11 mm. mercury in a cell of 10-cm. length. Below is shown a conventional absorption spectrum

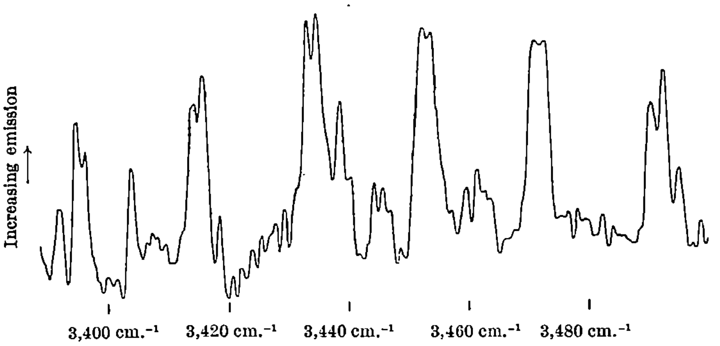


Fig. 3. Part of a spectrum near 3μ of ammonia in emission. The gas temperature was 75° C. and the pressure 600 mm. mercury in a 10-cm. cell

high pressure in the lamp used (Osram type *MBD* 125 W.). Following the convention which was adopted in far infra-red work[6], we define the resolution to be the reciprocal of the maximum path-difference, giving in this case a value of 0·5 cm.$^{-1}$. This is a restrictive definition, as may be seen by comparing the actual resolution of the emission spectrum with the conventional absorption spectrum shown below it. The latter was chosen from the literature[8] as most closely matching our resolution and pressure conditions. The resolution claimed for it is 0·4 cm.$^{-1}$, and it can be seen that the resolution in the emission spectrum is slightly better. When a comparison is made with the recent measurements of this band of ammonia at higher resolution by Mould, Price and Wilkinson, it is found that our resolution approaches their value of 0·1 cm.$^{-1}$, but the much lower gas pressure used in that work makes an exact comparison difficult.

The emission interferogram was recorded in about 1 hr. and the time taken by the *Ace* computer to calculate the part of the spectrum shown was about 15 min. The output of the computer is plotted automatically on a recorder, and the figures shown are direct reproductions of these plots.

Comparison with an emission spectrum made with a prism spectrometer[9] shows that much superior resolution and signal-to-noise ratio is possible with the interferometer in comparable times of observation.

Fig. 3 shows a small part of the spectrum of ammonia in emission near 3μ. Here the gas was at a temperature of 75° C., the pressure was 600 mm. mercury in a 10-cm. cell and the detector used was a lead sulphide cell with a germanium filter. The maximum path-difference was about 1·6 cm., giving a resolution of 0·6 cm.$^{-1}$. In this case it has not been possible to provide a suitable absorption spectrum for comparison, because in this region[10] three bands, ν_3, ν_1 and $2\nu_4$, all overlap and intensity distributions vary greatly. However, one expected feature, namely, the inversion splitting[11], can be seen in all the strong lines, despite the rather high gas pressure that was used.

From these results it can be seen that the Michelson–Fourier combination offers new prospects for the emission spectroscopy of sources of low brightness. The instrument described here is now being used for work on night-sky emission and also for observations on planetary atmospheres. Another possibility being investigated is the application to Raman spectroscopy with excitation lines of longer wave-length than those normally used. Finally, it is thought that advantages of this system, giving as it does the maximum product of solid angle and resolution, may be of importance in applications to artificial satellites, where the time-efficiency of the initial measurement of the radiation outweighs the disadvantage of having to compute the spectrum.

This work has been done as part of the research programme of the National Physical Laboratory and is published by permission of the Director. The Institut d'Astrophysique of the University of Liège has collaborated, and support for its members by the Geophysics Research Directorate (U.S.A.) through the European Office in Brussels is gratefully acknowledged.

H. A. Gebbie

Basic Physics Division,
National Physical Laboratory,
Teddington.

G. Roland
L. Delbouille

Institut d'Astrophysique,
Liège, Belgium.

[1] Fellgett, P. B., Symposium Ohio State (1952).
[2] Gebbie, H. A., and Vanasse, G. A., *Nature*, **178**, 432 (1956).
[3] Jacquinot, P., *J. Phys. Radium*, **19**, 223 (1958).
[4] Jacquinot, P., *Reports of Progress in Physics* (1960).
[5] Connes, J., and Gush, H. P., *J. Phys. Radium*, **21**, 615 (1960).
[6] Gebbie, H. A., *N.P.L. Symposium on Interferometry*, 1959, Paper (5–4).
[7] Gebbie, H. A., Stone, N. W. B., and Walshaw, C. D., *Nature*, **187**, 765 (1960).
[8] Tsuboi, M., *et al.*, *Spectrochimica Acta*, **13**, 80 (1958).
[9] Braunbeck, J., *Nature*, **185**, 754 (1960).
[10] Benedict, W. S., Plyler, E. K., and Tidwall, E. D., *J. Chem. Phys.*, **32**, 32 (1960).
[11] Dennison, D. M., and Hardy, J. D., *Phys. Rev.*, **39**, 938 (1932). See also *Infra-red and Raman Spectra*, by G. Herzberg, 422.

Pressure Differences in a Direct-Current Capillary Discharge

This communication deals with the difference of pressure which arises between the ends of the capillary of a d.c.-excited krypton discharge lamp as a result of the motion of ions and electrons in the electric fields[1] of the discharge. The effect is of importance in view of the adoption of the 0·606μ line emitted by krypton-86 as the new standard of length[2]. The wave-length of this line can be shifted, because of broadening due to pressure, by an amount which depends on the pressure and the current in the discharge; this shift is of the order of one part in 10^8 under the conditions for excitation by means of a d.c. discharge, which have been recommended by the International Committee of Weights and Measures[3].

The measurements were made by means of a special lamp, which is illustrated schematically in Fig. 1. The form of this lamp was basically the same as that recommended for excitation of the primary wave-length standard, having a capillary of 3 mm. internal diameter and 90 mm. length. However, instead of a single hot cathode, two were provided, one at each end of the capillary, so that the d.c. discharge could be run in either direction. A Pirani gauge head was connected to each end of the capillary, for the measurement of the pressure, and a side-tube connected to one of the ends enabled control of the vapour pressure of the krypton by the use of a

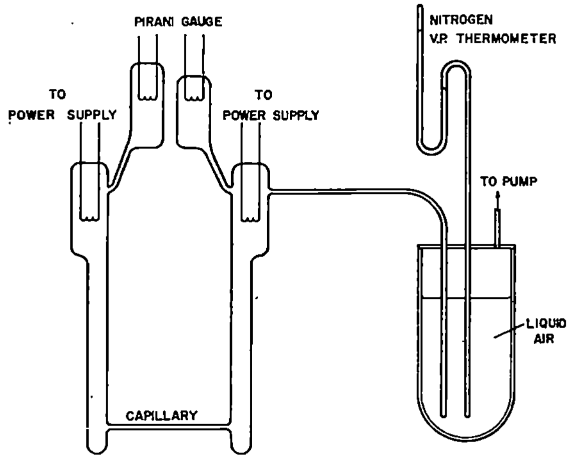


Fig. 1

cryostat. A nitrogen vapour-pressure thermometer was used to measure the temperature of the cryostat, and the resultant vapour pressure of krypton in the side-tube was deduced from the Meihuizen formula[4]. Although the pressure at one end of the capillary was controlled by the vapour pressure of krypton in the side-tube, which was held at temperatures down to 61° K., the temperature of the capillary itself was controlled by immersion in nitrogen at its normal boiling point.

The results of the measurements for a range of pressures and currents are shown in Fig. 2. The large relative differences of pressure between the two ends of the capillary are quite striking. Of particular interest in connexion with the primary wave-length standard is the pressure difference under the operating conditions as now recommended, namely, a current density of 3·0 m.amp./mm.^{2} with the krypton pressure controlled by its vapour pressure at the anode end, the temperature of which is at the nitrogen triple point. In this case the pressure is 32μ at the anode end and 21μ mercury at the cathode. According to typical values given for the shift due to pressure of the 0·606μ line of krypton-86 (ref. 5), the wave-length emitted by the gas in the discharge will vary along the length of the capillary by about 2·5 parts in 10^9. When the lamp is observed end on, as recommended at present, the possible error in the wave-length is not large compared with the accuracy specified by the International Committee (< 1 in 10^8), but it is clear that the effect must be taken into account if much greater accuracy is attempted with this form of source.

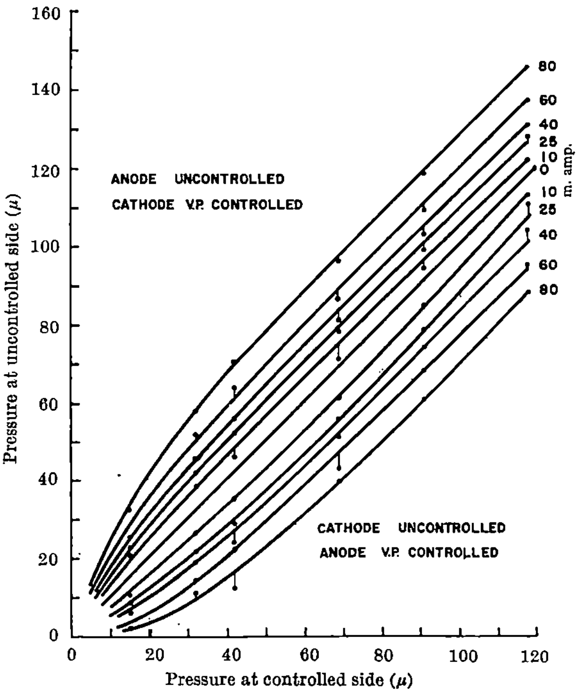


Fig. 2

D. S. Smith

Division of Applied Physics,
National Research Council,
Ottawa.

---

[1] Francis, G., *Handbuch der Physik.*, **22**, 195 (Springer, 1956).

[2] General Conference of Weights and Measures, Paris, October 1960.

[3] *Proc. Verb. du CIPM*, **27** (1960).

[4] Meihuizen, J. J., *Dampspanuingen en Vloeistof-en Dampdichthenden van Krypton* (Leyden, 1940). See also Fisher, B. B., and McMillen, W. G., *J. Phys. Chem.*, **62**, 494 (1958).

[5] Baird, K. M., and Smith, D. S., *Canad. J. Phys.*, **37**, 832 (1959).

METEOROLOGY

Radar Reflectivities of Hail Samples

In the past few years quantitative measurements have been made of the radar reflectivity of many storms, particularly severe ones in which hail and even tornadoes have occurred. In particular, Donaldson has published and discussed vertical profiles of reflectivity of storms in the eastern United States[1]. He finds that the absolute magnitude of the reflectivity increases with the severity of the thunderstorm in the sequence rain, hail, large hail and tornadoes; and that in hail and tornado situations, the maximum reflectivity is found aloft at a height which increases with storm severity.

The proper interpretation of such observations clearly depends on an understanding of the characteristics (as radar targets) of hail, both wet and dry. Recent computations by Herman and Battan[2] and measurements by Atlas *et al.*[3] have shed light on the problem of radar scatter by hail (particularly large hail); and Atlas and Ludlam[4] have calculated the reflectivities of hail of various size-spectra. The question arises as to exactly what size-spectra do in fact exist, and the reflectivities which can be expected from such spectra.

Power returned to the radar is proportional to the reflectivity of the target, that is, to the sum of the back-scattering cross-sections. For spherical target elements of diameter much less than the radar wavelength (Rayleigh scattering) the back-scattering cross-section is proportional to the sixth power of the particle diameter, of which the sum (ΣD^6) is called Z, the reflectivity factor[5]. It has become customary to examine Z in relation to precipitation-rate and liquid water (or ice) content in the cases of rain and snow in which Rayleigh scattering applies. Where hailstorms are concerned it is useful in making comparisons to describe the reflectivity of a storm in terms of the equivalent reflectivity factor Z_e, that is, that value of ΣD^6 which, assuming Rayleigh scattering from spherical water drops, would yield the same reflectivity as is in fact observed.

In the course of a hailstorm-observing programme * in western Canada (Alberta), 28 samples of hail were collected. Each sample represents all the hail which fell on a small area (of the order of 0·05 m.^{2}) during a hailstorm. The samples were collected from points over a wide area and on many different days; they represent a wide range of rates of hail-fall (0·02–40 mm. hr.$^{-1}$, melted) and of ice concentrations (0·01–6 gm. m.$^{-3}$). For each sample, Z_e was computed on the basis of reflectivities derived by Atlas and Ludlam[4] for both wet and dry hail at radar wave-lengths of 3·3 and 10 cm. Each of the 28 values thus computed was plotted against the corresponding value of R, the rate of precipitation of the hail as observed at the ground.

Fig. 1 shows the plot of Z_e versus R, for both wet and dry hail at wave-length 3·3 cm. The straight lines show the least-squares regressions, expressed by the equations:

$$Z_e = 4 \times 10^4 R^{0.88} \text{ (wet hail)}$$
$$Z_e = 1 \times 10^4 R^{1.08} \text{ (dry hail)}$$

Z_e being in mm.^{6} m.$^{-3}$ and R in mm. hr.$^{-1}$. Clearly, for the radar wave-length considered and for these particular hail distributions, wetting of the hail enhances Z_e by a factor of from 2 to 4. According to the recent reflectivity data mentioned above[2-4], such an enhancement is to be expected when the major contribution to the total reflectivity of the sample is made by hail of diameter less than about 1·5 cm. This is in fact the case in the Alberta samples where, in 21 of the 28 samples, 75 per cent of the total reflectivity at 3·3 cm. wave-length was contributed by hail of diameter less than 1·6 cm.

The regression lines are reproduced in Fig. 2 (d and e) with the corresponding ones for wave-length 10 cm. (b and c). Also shown (a) is the relation between Z ($= \Sigma D^6$) and R for the same 28 samples.

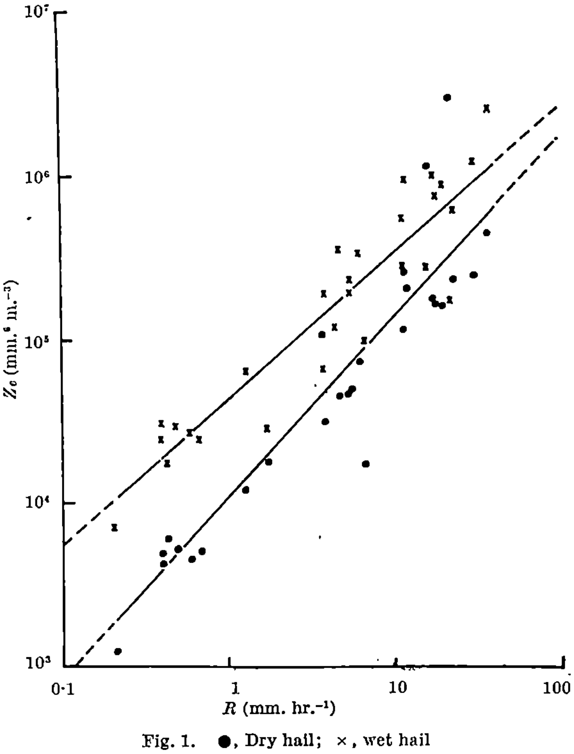


Fig. 1. ●, Dry hail; ×, wet hail

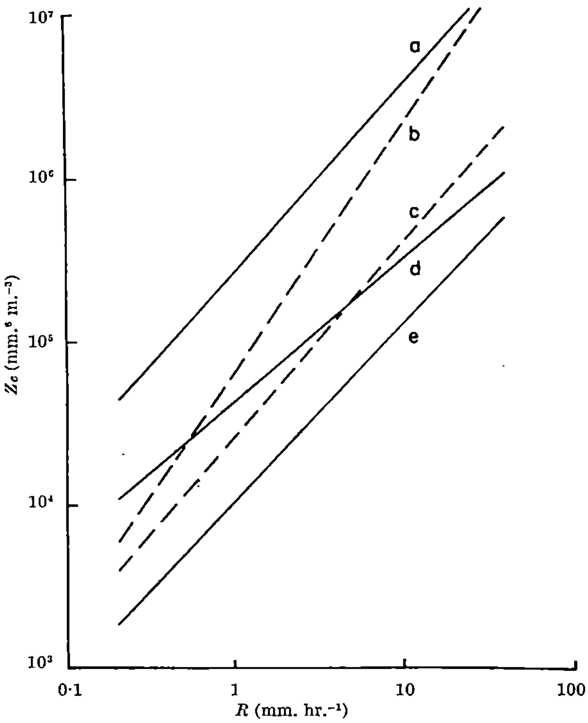


Fig. 2. a, $Z = \Sigma D^6$; b, Z_e wet 10 cm.; c, Z_e dry 10 cm.; d, Z_e wet 3·3 cm.; e, Z_e dry 3·3 cm.

This last relationship will clearly lead to an under-estimate of hailfall-rate on the basis of power returned to the radar. As in the case of the 3·3 cm. wave-length, so also does wetting of the hail enhance the reflectivity at the longer wave-length, particularly at high precipitation-rates.

Table 1. EQUIVALENT REFLECTIVITY FACTOR Z_e, MM.^{6} M.$^{-3}$

	LQ	M	UQ
Donaldson (ref. 1)	1.5×10^5	10^6	4×10^6
Alberta (wet)	3×10^4	2×10^5	5×10^5
Alberta (dry)	8×10^3	6×10^4	2×10^5

Donaldson[1] has published vertical profiles of the median (M), the lower (LQ) and the upper quartile (UQ) values of Z_e for hailstorms measured by means of 3·3 cm. radar, and it is of interest to compare values taken from his curves at a low level (5,000 ft.) with the Alberta values for the same wave-length (see Table 1). The Alberta values are lower than Donaldson's by factors between 5 and 20. The agreement may very well be better in view of doubts as to the calibration of Donaldson's radar; in fact, a reduction of his values by a factor of 6 is suggested by Atlas and Ludlam[4].

R. H. DOUGLAS

McGill University,
Montreal.

* Sponsored by the Alberta Research Council, the Canadian Meteorological Service and the National Research Council of Canada.

[1] Donaldson, R. J., *Proc. Seventh Weath. Radar Conf.*, B8 (1958); *Proc. Eighth Weath. Radar Conf.*, 115 (1960).

[2] Herman, B. M., and Battan, L. J., *Proc. Eighth Weath. Radar Conf.*, 187 (1960).

[3] Atlas, D., Harper, W. G., Ludlam, F. H., and Macklin, W. C., *Quart. J. Roy. Meteor. Soc.*, **86**, 468 (1960).

[4] Atlas, D., and Ludlam, F. H., *Imp. Coll. Sci. and Tech., London, Tech. (Sci.)*, Note 4 (1960).

[5] Battan, L. J., *Radar Meteorology*, 24 (Univ. of Chicago Press, 1959).

METALLURGY

Hydrogen Reduction Characteristics of Natural and Artificial Hæmatites

Published data on the rate and mechanism of the reduction process in iron oxides are frequently discordant. This lack of agreement is exemplified by two recent studes[1,2]. McKewan[1], for example, has reported the rate of reduction of hæmatite spheres in the size-range 0·6–2·5 cm. to increase continuously from 400 to 1,050° C. I found[2], however, that hæmatite particles in the size range 0·002–0·008 cm. showed a rate maximum at about 550° C. and a pronounced rate minimum at 700° C. approximately.

Since this difference in behaviour could be due to a particle-size effect, an apparatus-effect or to different physical properties of the materials, it was decided to repeat the work eliminating the first two of these variables, and using hæmatites similar to those employed in the two investigations in question[1,2].

For one series, approximately spherical particles of artificial hæmatite, 0·03 cm. diameter, were prepared by rolling pigment grade ferric oxide (as used by McKewan) in a jar for 1 hr. A screened fraction of this material was fired, in a platinum boat, for 2 hr. in an atmosphere of oxygen at 1,370° C. and then cooled slowly in oxygen to room temperature.

For the other parallel experiments, particles similar in size and shape to the artificial hæmatite were obtained by screening a washed natural hæmatite from Yampi Sound, Western Australia. Part of this material was subjected to the same treatment as the artificial hæmatite and another portion was dried in air at 110° C.

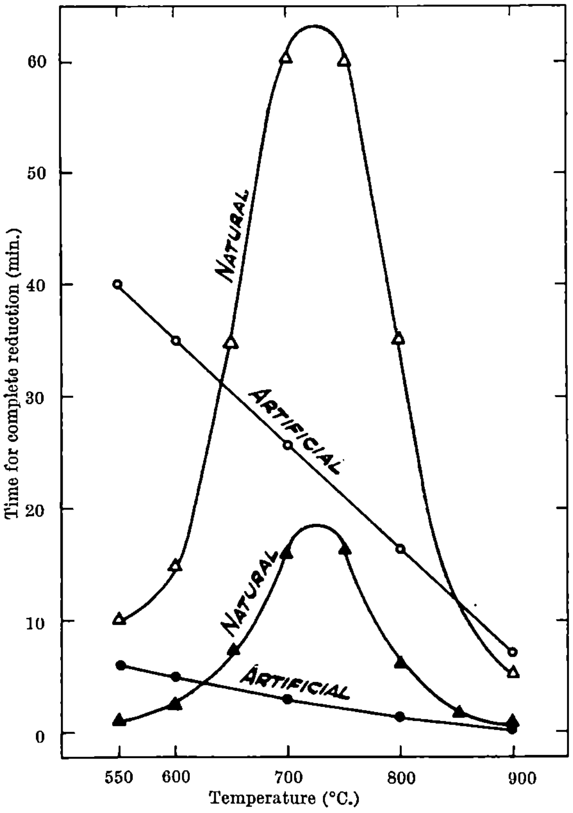


Fig. 1. Dependence on temperature of hydrogen reduction-rate for natural and artificial hæmatites. Δ, 0·03 cm. natural hæmatite, fired at 1,370° C. or dried at 110° C.; ▲, 0·003 cm. natural hæmatite dried at 110° C.; ○, 0·03 cm. artificial hæmatite fired at 1,370° C.; ●, 0·003 cm. artificial hæmatite dried at 110° C.

Particles of both artificial and natural hæmatite, approximately 0·003 cm. diameter, were also prepared from screened fractions of the raw materials and dried in air at 110° C. Using a procedure described in ref. 2, 100-mgm. samples of the various materials were reduced in a stream of hydrogen flowing at 2,000 ml./min. The distinctly different results given by the natural and artificial hæmatites are shown in Fig. 1.

In Table 1 some physical properties of the two hæmatites are shown. Both materials are weakly magnetic, and heating them to 500°, 700° and 900° C. respectively, in nitrogen, and quenching, did not alter significantly the lattice parameters shown in Table 1. The only significant differences found so far in the two hæmatites are that, under reflected polarized light, the artificial material shows a pronounced 'sub-structure', while the natural hæmatite shows none and the small amount of gangue material (mainly silica and alumina minerals) present in the natural hæmatite occurs as separate particles, whereas in the artificial material it is present with ferric oxide as composite particles.

Table 1. Physical Properties of Hæmatites

Property	Hæmatite	
	Natural	Artificial
Fe_2O_3 content (weight per cent)	99·1	99·5
Density (gm./cm.³)	5·20	5·10
Hexagonal parameters (Å.) *a*	5·035	5·033
c	13·75	13·75
Crystallinity*	Monocrystalline	Polycrystalline

* Reflected polarized light examination.

It has been clearly demonstrated that the origin of a hæmatite may influence markedly the dependence on temperature of its hydrogen reduction-rate. Until the reasons for this differing behaviour are discovered, it is apparent that care must be exercised in interpreting or comparing reduction data for hæmatites from different sources.

Thanks are due to Mr. I. D. Gray for experimental assistance and to Dr. H. K. Worner, director of research, for permission to communicate this work.

John Henderson

Broken Hill Pty. Co., Ltd.,
Central Research Laboratories,
Shortland, 2N,
New South Wales.

[1] McKewan, W. M., *Trans. Amer. Inst. Min. (Metall.) Eng.*, **218**, 2 (1960).

[2] Henderson, J., *Amer. Inst. Min. (Metall.) Eng., Metall. Soc. Conf.*, 8, Phys. Chem. of Process Metallurgy, 671 (New York: Interscience, 1961).

ENGINEERING

Power Requirements and Scale-up in Flotation

As part of a systematic programme for correlating mineral engineering process variables[1,2], flotation machines are now being investigated. There is no need to stress the engineering importance of the flotation process. First applied more than fifty years ago for the recovery of fine sulphide minerals from Australian gravity plant tailings, to-day it is the only method for treating the major base metal sulphide ores, and is becoming of increasing importance for non-metallic ores and coal. As a mineral engineering operation, it is often second only to fine grinding in power consumption. Despite this, empiricism largely prevails from choice of equipment to control of process variables.

Relationships between cell capacity, energy input, and flotation-rate have been suggested[3,4], and there are indications that cell power intensity and capacity are related[5]. However, the function of power in the flotation cell should not be misinterpreted. Apparatus of this type are essentially dissipative mechanisms in which the energy input ultimately appears as heat; any useful function (in this case, a separation of minerals) is incidental. Similar behaviour has been pointed out in connexion with ball milling[1], where the power is utilized in rotating an eccentric load, a negligible portion going into comminution. However, the power dissipation-level is responsible for the particular fluid-flow regime maintained within the cell, and this in turn is related to phenomena leading to flotation such as gas dispersion, particle suspension, and bubble-particle aggregation. Hence, the energy dissipation-rate which is related to the power requirements of the flotation impeller should be useful in correlating flotation cell performance with design factors.

For the present, flotation performance will not be considered beyond expressing the view that, once the power requirements can be related to the geometry and other mechanical features of cells, performance can then be related to the appropriate power number or index. The problem to be considered thus resembles the more familiar one of mixer evaluation in the chemical processing field, where correlations, using Power, Reynolds, Froude and Weber numbers, are well known[6-11]. In the following, the flotation cell will be treated as a baffled mixer; hence only the power number and the Reynolds number will be considered. To account for the effect of entrained air and suspended solids, air volume and pulp specific gravity are introduced, and the Power and Reynolds numbers are then defined respectively by:

$$N_p = \frac{Pg_c}{\rho N^3 d^5} \cdot \frac{1}{s(1-a)} \tag{1}$$

$$N_{Re} = \frac{\rho N d^2}{\eta} \cdot \frac{s(1-a)}{f_1 f_2} \tag{2}$$

Where P is net power (consumed power less transmission losses), g_c is the Newton's law conversion factor, d is impeller diameter, N is impeller rotational speed, ρ is density of fluid, η is viscosity of pure fluid, a is aeration volume, s is non-aerated pulp specific gravity, f_1 is a function of non-aerated pulp variables (ratio: non-aerated pulp viscosity/pure fluid viscosity), f_2 is a function of aerated pulp variables (ratio: aerated pulp viscosity/non-aerated pulp viscosity). f_1 and f_2 are still to be determined, though some aspects of the former have been investigated[12].

It is useful in equation (1) to replace d^3 by the cell volume, V, and to substitute power intensity Π for the term Pg_c/V. Also it will be noted that Nd is proportional to the linear peripheral speed πNd. So modified, equations (1) and (2) become:

$$N_p' = \frac{\Pi d}{\rho (Nd)^3} \cdot \frac{1}{s(1-a)} \tag{1a}$$

$$N_{Re} = \frac{\rho (Nd) d}{\eta} \cdot \frac{s(1-a)}{f_1 f_2} \tag{2a}$$

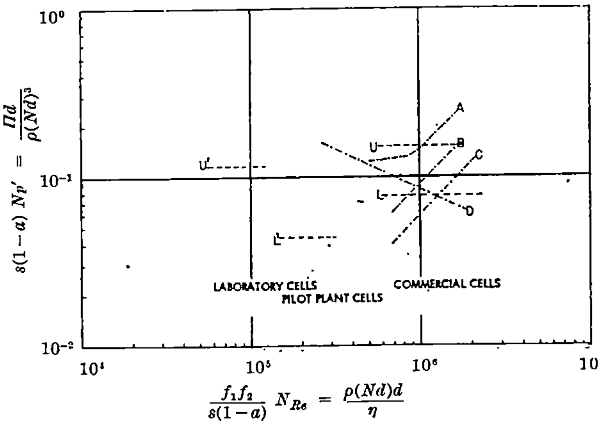


Fig. 1. Power factor – Reynolds number correlation for flotation cells

The literature is notably deficient in data for relating these dimensionless numbers. Without regard to the complex functions f_1 and f_2, there is little available on air entrainment volumes, and power figures where given at all usually are installed, rather than net values.

To make maximum use of the small amount of available data, attention was confined to Π, N and d. The constants for the fluid phase were ρ and η for water. With regard to the latter, Perry[6] suggests using the viscosity of the fluid phase when dealing with dispersions of gases in liquids; this is the procedure adopted here. Thus the groups become:

$$s(1-a)N_p' = \frac{\Pi d}{\rho (Nd)^3} \tag{3}$$

$$\frac{f_1 f_2}{s(1-a)} N_{Re} = \frac{\rho (Nd) d}{\eta} \tag{4}$$

Lines A, B, C and D (Fig. 1) are from approximate data supplied by four manufacturers. As in mixing, the radically different impeller design probably accounts for much of the difference in the relative positions of lines A, B and C.

The slope of unity for lines A, B and part of C follows from the scale-up criteria adopted, which (*a*) maintains constant peripheral speed for all cell sizes, and (*b*) estimates installed power from $\Pi \simeq$ constant. It will be noted from equations (3) and (4) that this is equivalent to plotting d against d. Line D is evidently based on different criteria. It is interesting to note that in baffled mixers N_p is almost independent of N_{Re} for $N_{Re} > 10^4$. Thus, in the absence of adequate mixer scale-up information (*b*) above is assumed, the value of Π being obtained from laboratory measurements, and for high Reynolds numbers N is calculated from $N_p \simeq$ constant[6,7,9].

Published and unpublished information for a range of commercial cells give values in the area enclosed by lines U and L. Estimated consumed power was used, and it is expected that net power values would be about 10 per cent lower. On the other hand, lines U' and L' enclose the area containing our own measurements on laboratory cells modelled after commercial cells, and here net power was used. Transmission losses for laboratory cells greatly exceed net power, and the use of consumed power would increase N_p about 10 times.

Thus, there is no reliable scale-up information between laboratory and commercial cells, or even for commercial cells alone.

In particular, the variation of peripheral speed and power intensity with cell size needs resolving. Once the relationships become established, they will provide a frame of reference for investigating the remaining operating variables. This should give an insight, for example, into the factors responsible for differences in the flotation-rate of the same material treated in different cells, both with regard to the size of cells and their design. In passing, it may be noted that this general correlation method should be applicable to the conditioning operation, which is likely to present fewer problems.

This possible correlation is brought to attention in the hope that adequate commercial data with which to establish this or some other correlation will now become available.

NATHANIEL ARBITER
COLIN C. HARRIS *

Henry Krumb School of Mines,
Columbia University,
New York, 27.

* On leave of absence from the University of Leeds.

[1] Arbiter, N., *Eng. and Mining J.*, **156**, 63 (1955).

[2] Harris, C. C., *Nature*, **183**, 530; **184**, 716 (1959).

[3] Arbiter, N., *Trans. Amer. Inst. Min. Eng.*, **190**, 791 (1951).

[4] Horst, W. E., *Trans. Amer. Inst. Min. Eng.*, **211** (*Min. Eng.*), **10**, 1182 (1958).

[5] Rose, E. N., *Trans. Amer. Inst. Min. Eng.*, **169**, 394 (1946).

[6] Perry, J. H., ed., *Chemical Engineers Handbook* (McGraw-Hill, New York, 1950).

[7] Brown, G. G., *et al.*, *Unit Operations* (John Wiley, New York, 1950).

[8] Coulson, J. M., and Richardson, J. F., *Chemical Engineering* (Pergamon Press, London, 1955).

[9] McCabe, W. L., and Smith, J. C., *Unit Operations of Chemical Engineering* (McGraw-Hill, New York, 1956).

[10] Johnstone, R. E., and Thring, M. W., *Pilot Plants, Models, and Scale-up Methods in Chemical Engineering* (McGraw-Hill, New York, 1957).

[11] Faust, A. S., *et al.*, *Principles of Unit Operations* (John Wiley, New York, 1960).

[12] Ward, S. G., *J. Oil Colour Chem. Assoc.*, **38**, 9 (1955).

CHEMISTRY

Nomenclature of Silica

FOR many years the term 'fused quartz' has been used for describing vitreous silica prepared by the fusion of crushed quartz crystals. In some quarters the term 'vitreous silica' has been reserved for material made from an acid-washed sand, usually containing larger amounts of impurities. Unfortunately, the term 'fused quartz' is often abbreviated to 'quartz' in the later pages of a publication, and sometimes even in the title. A quick glance at *Chemical Abstracts* subject index for 1956 revealed four abstracts dealing with "quartz", where it was obvious that vitreous silica was meant[1]. The electrophoresis of "quartz particles in a quartz cell" is mentioned in one abstract. It is highly unlikely that the electrophoresis cell was of crystalline quartz; it was presumably vitreous silica. The question remains, were the particles also of vitreous silica? A recent example of the misnaming of silica is a paper dealing with vitreous silica and probably cristobalite[2] and headed "Whisker growth from quartz". It contains the sentence "The quartz substrate . . . had a skin of devitrified quartz . . . ".

The term 'amorphous silica' is not used in any more consistent fashion. In general, it denotes an artificial silica filler or a naturally occurring material such as opal or diatomite. The artificial silica powder, of submicroscopic particle size and correspondingly high specific surface, may have been prepared by one of two types of process. A material made from an aqueous soil by drying will readily take up moisture; the particles will be porous. Silica powders prepared by a condensation of SiO_2 or SiO from the vapour phase will be less hygroscopic. A material such as 'Fransil', consisting of tiny spheres of silica glass, might be better classified as a fine vitreous silica. Diatomite is comparatively coarse, but extremely porous. If it has been calcined, it will have been partly converted to cristobalite or even tridymite, both toxic materials.

The chemical and biological properties of the various forms of silica depend markedly on the polymorphic form (vitreous silica, cristobalite, tridymite, quartz, coesite), particle size, porosity, and thermal history of the surface, even when the material is very pure. The examples cited show that there is a need for greater care and detail in the description of various forms of silica by means of such terms.

I. BERGMAN

Safety in Mines Research Establishment,
Ministry of Power,
Portobello St., Sheffield, 1.

[1] *Chem. Abstr.*, **50**, 6103*h*, 8285*e*, 15172*c*, 15173*i* (1956).

[2] Jaccodine, R. J., and Kline, R. K., *Nature*, **189**, 298 (1961).

Determination of Hydrogen in Gaseous Mixtures by Gas-Chromatography

IN the analysis of gaseous mixtures by gas-solid chromatography using hot-wire or thermistor detectors, it is usual to reduce the signal to the recorder, when necessary, by an attenuating system in the form of a potentiometric chain so that only a known proportion of the signal is applied to the recorder. This method results in the main components of a mixture being measured less accurately than impurities or components of small concentration. This is particularly true when hydrogen is a main component, as it has a thermal conductivity seven or eight times as great as most other gases or vapours, necessitating a large measure of attenuation to keep its peak on the chromatographic chart.

The gaseous mixtures commonly analysed in this Laboratory contain about 40 per cent hydrogen, and the volume of gas necessary to achieve sensible peak measurements for the nitrogen present results in a hydrogen signal from a katharometer bridge of about 115 mV. In the past this signal has been attenuated to about 2·3 mV. to allow the peak to be measured on the 3-mV. potentiometric recorder used. Thus 40 per cent of hydrogen is represented by 2·3 mV. or a deflexion of 214·5 mm., and an error of 1 mm. in measurement leads to an inaccuracy of about 0·5 per cent in the bridge signal.

It was therefore decided to reduce the signal to the recorder by applying an accurately determined e.m.f. in opposition to the output from the katharometer bridge. The conventional apparatus used had recorder sensitivities of 3, 5, 15, 25, 50 and 150 mV. for full-scale deflexion. Of these the 15 mV. range was selected as being the most practical one to use. On this scale, for a gas containing 40 per cent hydrogen an effective back e.m.f. of 105 mV. is required to keep the signal to a reasonable position on the chromatographic chart. This is achieved by applying an e.m.f. of 21 mV. (that is, 105 × 3/15 mV.) across the potentiometric chain in opposition to the signal to the recorder. The recorder then registers a peak from a signal of 10 mV., giving a deflexion of 186·4 mm. Thus an error of 1 mm. in measurement leads to an inaccuracy of only 0·05 per cent of the bridge signal.

This device was demonstrated by applying fixed signals from the katharometer bridge and noting that for each mV. applied from the test-box the expected decrease in recorder response was obtained. The device was then incorporated into new chromatographic units under construction and has since been used in the analysis of more than 200 gaseous mixtures containing hydrogen as a major component.

P. J. Kipping

Warren Spring Laboratory,
Stevenage,
Herts.

Heat Capacity and Resistivity Anomalies in Palladium Hydride

A recent paper on the electrical resistivity (ρ) of solutions of hydrogen in palladium[1] records maxima at 43° K. and 52° K. for the compositions (hydrogen/palladium) 0·60 and 0·54 respectively, whereas there is a linear fall of ρ when the hydrogen/palladium ratio is 0·48. A reproduction of the curves of ρ versus T by Schindler *et al.* is shown in Fig. 1. The maximum is evident in the graphs for the composition 0·60, but not for the composition 0·54; however, the authors state that for this composition there is a definite maximum in the points at the lower temperatures.

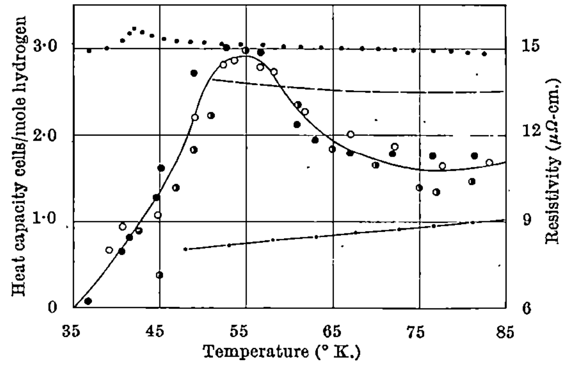


Fig. 1. Electrical resistivity and heat capacity per mole of hydrogen in certain solutions of hydrogen in palladium. ○, Cp H/Pd = 0·50 (Nace and Aston); ◑, Cp H/Pd = 0·25; ●, H/Pd = 0·125; ●●, ρH/Pd = 0·6; ——, ρH/Pd = 0·54; ●—●, ρH/Pd = 0·48

While the neutron diffraction work in ref. 2 leaves little doubt that there is hydrogen (hydrogen/palladium ~0·05) in the α-phase at room temperature, it is not unlikely that all the hydrogen is in the β-phase at lower temperatures.

Recent thermal studies in this laboratory have revealed slow exothermal processes on cooling solutions of hydrogen in polycrystalline palladium occurring near 150° K. and above 200° K. From the thermal drifts of the adiabatic calorimeter after various degrees of annealing, energies of activation of 6,400 and 2,900 calories were found for the upper and lower temperature processes respectively. In both cases graphs of the log of the rate of energy emission against $1/T$ gave good straight lines, except where annealing produced a state near equilibrium. This is in accord with anomalies in the heat capacity of hydrogen in palladium black (hydrogen/palladium = 0·50)[3] and one or both of these two processes may perhaps be thought of as connected with migration of hydrogens from the α- to the β-phase.

In view of the work on the resistivity[1] and of recent speculations concerning hysteresis in palladium hydride[4], heat capacity measurements for hydrogen in polycrystalline palladium with hydrogen/palladium ratios of 0·25 and 0·125 are of interest though perhaps not unexpected. The results, based on 1 mole of hydrogen in each solution, are given in Fig. 1 along with the earlier values for hydrogen/palladium equal to 0·50. The method used and the accuracy of the results are as given in ref. 3. Thus at hydrogen/palladium ratios of 0·125 and 0·25 the accuracy is respectively 2 per cent and 1 per cent of the heat capacity due to the hydrogen.

The heat-capacity maximum at 55° K. previously reported for a hydrogen/palladium ratio of 0·50 is not shifted at either composition and the results for both solutions lie on the same curve. The slow process occurring below 50° K. reported in ref. 3 was noted again. This is not connected with the two processes mentioned. By cooling the sample slowly through a temperature-range 30° above maximum, the slow process was 'annealed out' and all measurements were taken after this annealing.

The fact that the maximum is unchanged by composition at compositions of hydrogen/palladium below 0·50 might be taken to mean that all the hydrogen is in a phase of constant composition below room temperature. This would require migration of hydrogen from the α- to β-phase at low temperatures. However, if the hydrogen atoms are bound to palladium in such a way that there is little or no interaction between the protons, the heat capacities would not be affected very much by composition. This situation is unlikely. If the composition of the β-phase is constant, in view of the slow process occurring at the higher temperatures, it would seem that any process of ordering would most likely be one resulting in removal of some sort of rotational or librational process.

A comparison between the resistivity and heat-capacity data leaves little doubt that the maxima are connected. The results of Schindler *et al.*[1] indicate that the maximum in the resistivity curve may well be at 55° K. for a hydrogen/palladium ratio of 0·50. It is also not unlikely that the maximum in the heat-capacity curve would be shifted at higher concentrations of hydrogen. Heat capacities in this temperature-range at higher hydrogen concentrations will be studied.

Schindler *et al.*[1] suggest an anti-ferromagnetic transition as a possible cause of the maximum. The onset of rotational or re-orientation process used to explain the heat capacity maximum[3] would also produce an anomaly in g (ν) for the phonons and it is possible that the resistivity results might be explained on this basis. For compositions above Pd_2H it is not possible to connect the palladium atoms without bound hydrogen in endless chains[3].

Paul Mitacek, Jun.
J. G. Aston

Cryogenic Laboratory,
College of Chemistry and Physics,
Pennsylvania State University.

---

[1] Schindler, A. I., Smith, R. J., and Kramer, E. W., *Intern. Inst. Refrig.*, Copenhagen (1959).

[2] Worsham, J. E., Wilkinson, M. K., and Shull, C. G., *J. Phys. Chem. Solids*, 3, 309 (1957).

[3] Nace, D. M., and Aston, J. G., *J. Amer. Chem. Soc.*, 79, 3623 (1957).

[4] Everett, P. H., and Nordon, P., *Proc. Roy. Soc.*, 209, 341 (1960).

'Gel Filtration' of Organic Compounds

Following Porath and Flodin's description[1] in 1959 of gel filtration as a fractionation method, it has been regularly used in this laboratory for separation of proteins and polysaccharides from sugar cane juice and sugar factory syrups. Unpublished results of the Swedish workers suggested that the method might be extended to the fractionation of substances in a narrow range of quite low molecular weights. Because of the possibilities of the method materials other than cross-linked dextran have been investigated here as alternative media for gel filtration.

In our laboratory Mountfort has shown that 'Dowex 50' cation-exchange resin (Na^+ form) gives partial fractionation of a mixture of mono-, di- and tri-saccharides in aqueous solution. Other workers have noted similar phenomena[2,3] and even a number of monosaccharides have been separated on 'Dowex 50' (Ba^{2+} form)[4]. As fractionation without the complications of ion exchange is often desirable, styrene-divinyl benzene copolymers, samples of which were obtained in bead form from the Dow Chemical Co., were tested. However, even with the least cross-linked resin, substances as widely different as egg albumin and glucose could not be separated in aqueous solution. Likewise, when alcohol was used as the liquid medium, no separation of such large and small organic molecules as eosin and formic acid could be achieved.

Meanwhile Vaughan[5] reported separation of polystyrene fractions in benzene on a cross-linked polystyrene which swelled to a very high degree in that solvent. He also suggested that fractionation of low molecular weight materials might be possible on less swollen resins, paralleling the separation of glucose from a dextran of number average molecular weight 1,000 by Porath and Flodin. We have now achieved the separation of several organic compounds using not only benzene but also a number of other liquids in which the resin swells to some extent.

The grades of copolymer available contained from 2–12 per cent divinyl benzene (DVB) and were designated *X*2, *X*4, *X*8 and *X*12. The swelling of each in a variety of solvents is shown in Table 1, being expressed as the ratio of swollen volume to dry volume (lightly packed).

Using columns of 1·4 cm. × 45–50 cm. and benzene as medium, almost complete separation of cholesterol and furfural was obtained with *X*2, *X*4 and *X*8 resins, but rather poor separation with the *X*12 resin. Cholesterol was eluted ahead of the furfural in each case.

Table 1. Swelling of Styrene – DVB Copolymers

Solvent	Swelling ratios (× 100) *X*2	*X*4	*X*8	*X*12
n-Butyl alcohol	105	—	—	—
iso-Butyl alcohol	105	—	—	—
sec.-Butyl alcohol	105	—	—	—
tert.-Butyl alcohol	105	—	—	—
n-Amyl alcohol	105	—	—	—
iso-Amyl alcohol	110	—	—	—
Pentan-1-ol	105	—	—	—
tert.-Amyl alcohol	105	—	—	—
Diethyl ether	190	180	140	135
Acetone	195	175	135	135
Methyl ethyl ketone	220	180	155	175
Ethyl acetate	235	210	160	140
Butyl acetate	260	210	125	130
Methylene chloride	370	250	235	190
Chloroform	380	285	230	175
Carbon tetrachloride	315	230	130	140
Dioxane	310	235	160	135
Benzene	400	280	210	170
Toluene	395	260	180	155
Petroleum ether (60–80°)	145	105	100	100
'Shell *X*2' (100°)	165	130	105	100

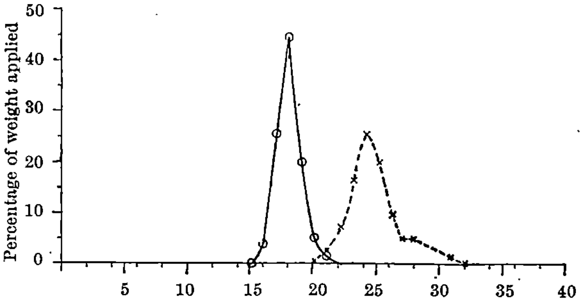


Fig. 1. Separation of resorcinol and 1-naphthylamine. ○, resorcinol; ×, 1-naphthylamine. Fraction volume, 5·1 ml.

With similar columns packed with *X*2 resin and using ethyl acetate or acetone, it was shown that it was possible to separate organic compounds with molecular weights in a much narrower range. Thus resorcinol and 1-naphthol (mol. wt. 110 and 144) when run separately in either solvent gave peak concentrations after different volumes of effluent and when run together were separated to a considerable extent. Resorcinol, although of smaller molecular weight, left the column ahead of the naphthol. Better separation of the two phenols was obtained in acetone than in ethyl acetate, although the resin swells less in acetone. By using a column 1·4 cm. × 140 cm. almost perfect separation of 0·4 gm. of each component was obtained.

To obtain quantitative data more readily, 1-naphthylamine was substituted for 1-naphthol. The phenol was determined by reaction with diazotized *p*-nitroaniline and the amine by its reaction with furfural in acetic acid. The separation of 0·1 gm. of each component in acetone solution is shown in Fig. 1.

Table 2. Effluent Volumes at Peak Concentrations

Substance	Molecular weight	Volume (ml.)
Acetaldehyde	44	165
Ethylamine	45	160
Ethanol	46	150
n-Pentanol	88	153
Furfural	96	167
Resorcinol	110	200
1-Naphthol	144	172
Alizarin	240	165
Quinalizarin	272	152
Cholesterol	387	126
Thymolphthalein	430	132

A number of compounds of varied structure were individually passed through a column 1·4 cm. × 135 cm. in benzene medium. The effluent volumes corresponding to peak concentrations of each were determined and are given in Table 2. The results suggest that gel filtration, as described by Porath and Flodin, is part of the mechanism involved in fractionation. However, the nature of each substance appears to be at least as important as its molecular weight. Thus, although cholesterol and thymolphthalein, both of high molecular weight, pass through the column ahead of the other compounds studied, a highly aromatic substance such as 1-naphthol will leave the column after aliphatic compounds of much lower molecular weight. In the aliphatic series the hydroxyl group causes relative exclusion (for example, compare ethanol and acetaldehyde). The extra hydroxyl group of resorcinol presumably explains its appearance ahead of 1-naphthol when these substances are run in acetone or ethyl acetate solution. In benzene solution resorcinol behaves differently and leaves the column a considerable time after such compounds as 1-naphthol and furfural.

In this case and with certain other substances, adsorption may play some part. When the benzene-soluble base from methylene blue was run through $X2$ resin, adsorption definitely appeared to occur, as there was a very large volume of effluent before the appearance of the dye, which was distributed in a much greater volume of solvent than usual.

The effect of the solvent is obviously great and the effect of the degree of cross-linkage of the resin is largely unknown. Much greater knowledge is required before a particular application or the optimum conditions can be predicted. However, the evidence suggests that here is a method which may assist in the isolation or purification of various natural or synthetic substances.

I am indebted to the Colonial Sugar Refining Co., Ltd., for permission to publish this communication.

B. CORTIS-JONES

Research Department,
Colonial Sugar Refining Co., Ltd.,
70 John Street,
Pyrmont, New South Wales.

[1] Porath, J., and Flodin, P., *Nature*, 183, 1657 (1959).
[2] Jones, J. K. N., Wall, R. A., and Pittet, A. O., *Chem. and Indust.*, 1197 (1959); *Canad. J. Chem.*, 38, 2285 (1960).
[3] Hough, L., Priddle, J. E., and Theobald, R. S., *Chem. and Indust.*, 900 (1960).
[4] Jones, J. K. N., and Wall, R. A., *Canad. J. Chem.*, 38, 2290 (1960).
[5] Vaughan, M. F., *Nature*, 188, 55 (1960).

Structures of Polymeric Titanium Ethoxide and Titanium Oxide Ethoxides

RECENTLY, Martin and Winter[1] suggested two new structures for trimeric titanium (IV) alkoxides. Their suggestion that $Ti_3(OR)_{12}$ may involve 6-co-ordinated titanium in a trigonal prismatic rather than an octahedral configuration is interesting but a decision on this must await the results of X-ray diffraction analysis. Besides providing a more symmetrical structure for the trimeric titanium chloride tributoxide they claimed that the trigonal prismatic structure also alleviates certain difficulties concerning the structures we proposed[2] for the polymeric titanium oxide ethoxides, which were based on the octahedral trimer structural unit. There are two points at issue. First, they consider on mechanistic grounds that the formation of the trilinear polymer species $Ti_{3(x+1)}O_{4x}(OEt)_{4(x+3)}$ "requires statistically unfavourable mutual orientation of two $Ti_3(OR)_8(OH)_4$ units before effective collision and expulsion of four water molecules could occur, etc.". Secondly, they mention that our structure for $Ti_{3(x+1)}O_{4x}(OEt)_{4(x+3)}$ involves an unknown 4-covalent bonding arrangement between oxygen and its four nearest neighbours. Concerning the first point it must be emphasized that our structure was developed to account for the variation of the number average degree of polymerization as a function of the degree of hydrolysis for a whole series of titanium oxide ethoxides. The mechanism of the hydrolysis was not considered, it is indeed unknown, and it is of no significance provided that a rapid rearrangement of the initial hydrolysis products occurs, thus resulting in the formation of the most stable structure. Moreover, we reported evidence which suggested that disproportionation takes place and this involves rapid structural rearrangements between members of the polymer series:

for example,

$$6\,Ti_9O_8(OEt)_{20} \rightarrow Ti_3(OEt)_{12} + 2\,Ti_6O_4(OEt)_{16} + Ti_9O_8(OEt)_{20} + Ti_{12}O_{12}(OEt)_{24} + Ti_{18}O_{20}(OEt)_{32}$$

An important feature of our structural series was the fact that the number average degree of polymerization is independent of the distribution of polymer sizes (as can be verified by inspection of the above equation) and is determined exclusively by the average degree of hydrolysis. The alternative structures for $Ti_3(OR)_{12}$ proposed by Martin and Winter will not account for the variation of the number average degree of polymerization with degree of hydrolysis even if the postulation that the titanium oxide ethoxides contain some terminal Ti=O groups were true. Thus although the formulation $(RO)^t_4(RO)^b_6Ti_3(O)^b_2Ti_3(OR)^b_6(O)^t_2$, where $(RO)^t$, $(RO)^b$, $(O)^t$ and $(O)^b$ are terminal alkoxide, bridging alkoxide, terminal Ti=O and bridging TiO groups respectively, will account for the compound $Ti_6O_4(OEt)_{16}$ the logical development of such a scheme would predict species which are not members of the $Ti_{3(x+1)}O_{4x}(OEt)_{4(x+3)}$ series, including a limiting infinite polymer $[Ti_3O_3(OEt)_6]_\infty$. We now report some infra-red spectra (in carbon disulphide solution) of $Ti_6O_4(OEt)_{16}$ and $Ti_{12}O_{12}(OEt)_{24}$ in comparison with that of $Ti_3(OEt)_{12}$. Briefly stated, it appears that as the degree of hydrolysis is increased there is a decrease in intensity of the bands at or near 2,960, 2,900, 2,840, 2,680, 1,370, 1,350, 1,135, 1,102, 1,063, 1,038, 915 and 890 cm.$^{-1}$, which involve in various ways the ethoxide groups. Simultaneously there is an increase in intensity of bands at around 790 and 770 cm.$^{-1}$ which was absent in $Ti_3(OEt)_{12}$ and which must therefore be assigned to Ti·O·Ti systems. Hydroxyl bonds were not detected in these spectra, and this eliminates the possible presence of Ti—OH groups in the oxide ethoxides. According to Barraclough, Lewis and Nyholm[3] a strong band at 1,087 cm.$^{-1}$ was assigned to the Ti=O group in titanyl *bis*-acetyl acetonate while bands in the 800–900 cm.$^{-1}$ region in other 'titanyl' complexes were considered to be due to Ti·O·Ti·O·Ti· systems. Accordingly we conclude that the bands near 800 cm.$^{-1}$ in $Ti_6O_4(OEt)_{16}$ and $Ti_{12}O_{12}(OEt)_{24}$ must be due to oxygen bridges between titanium atoms and that these compounds do not contain the Ti=O bonds proposed by Martin and Winter. It is noteworthy that Zeitler and Brown[4] considered that bands at 820 and 763 cm.$^{-1}$ in titanium oxide *n*-butoxide were due to Ti·O·Ti bonds. Thus, the trigonal prismatic alternative configuration for 6-co-ordinated titanium does not appear to have any supporting evidence.

The second objection to our structure, namely, the unfamiliar configuration of the quaternary oxygen, is more difficult to answer. The oxygen is situated at the apex of a tetragonal pyramid with titanium atoms in the basal positions. We tried several alternative structures in attempts to produce a tetrahedral co-ordination of the quaternary oxygens but without success. However, examples can be cited in which other first-row elements (for example, beryllium, boron) exhibit co-ordination numbers which are "not allowed" according to simple valency theory. Wheatley[5] has shown by X-ray diffraction on LiOMe that the oxygen is pyramidally bonded to four lithiums in this compound, and this provides a precedent for our Ti_4O configuration. There seem to be two alternative explanations of the 'pyramidal' oxygen. First, if the four Ti—O bonds are covalent and equivalent then it is essential to invoke the participation of 3-centred and 5-centred molecular orbitals involving oxygen and two or four titaniums, respectively. The Ti_4O system would then involve only six of the eight valence electrons of the O^{2-}. The second possibility would require the Ti_4O group to be unsymmetrical with two Ti—O bonds being essentially covalent and considerably shorter than the other two, which would involve electrostatic attraction. It is relevant to note that the intermolecular bonds in $Ti_3(OEt)_{12}$ are fairly weak[6] while the infra-red-spectrum[7] does not appear to distinguish bridging ethoxide groups from terminal ethoxide groups. This could be taken as evidence that the intermolecular Ti—O bonds involve weak electrostatic binding. Preliminary results[8] on the high-resolution proton magnetic resonance of $Ti_3(OEt)_{12}$ in carbon disulphide at room temperature revealed only one type of ethoxide group. This suggests that the bridging and terminal ethoxide groups are exchanging positions extremely rapidly, and this reinforces our view that the hypothetical kinetic arguments of Martin and Winter are not relevant to the determination of the structures of titanium oxide ethoxides.

D. C. Bradley
A. H. Westlake

Department of Chemistry,
University of Western Ontario,
London, Ontario.

---

[1] Martin, R. L., and Winter, G., *Nature*, **188**, 313 (1960).

[2] Bradley, D. C., Gaze, R., and Wardlaw, W., *J. Chem. Soc.*, 3977, (1955).

[3] Barraclough, C. G., Lewis, J., and Nyholm, R. S., *J. Chem. Soc.* 3552 (1959).

[4] Zeitler, V. A., and Brown, C. A., *J. Phys. Chem.*, **61**, 1174 (1957).

[5] *J. Chem. Soc.*, 4270 (1960).

[6] Bradley, D. C., Mehrotra, R. C., Swanwick, J. D., and Wardlaw, W., *J. Chem. Soc.*, 2025 (1953).

[7] Barraclough, C. G., Bradley, D. C., Lewis, J., and Thomas, I. M., *J. Chem. Soc.* (in the press).

[8] Bains, M. S., Bradley, D. C., and Stothers, J. B. (unpublished results).

The preceding communication by Bradley and Westlake is most interesting, for it provides new experimental data in the form of infra-red spectra and high-resolution proton magnetic resonance measurements on the polymeric titanium (IV) ethoxide and titanium (IV) oxide ethoxides.

Contrary to their above statement, however, one of the several logical developments of our scheme does, in fact, predict species which are members of the $Ti_{3(x+1)}O_{4x}(OEt)_{4(x+3)}$ series suggested by the workers at Birkbeck College, namely:

$$Ti_3(OR)_{12}+Ti_3(OR)_6(OH)_6 \rightarrow Ti_6O_4(OR)_{16}+2ROH+2H_2O$$

$$Ti_6O_4(OR)_{16}+Ti_3(OR)_6(OH)_6 \rightarrow Ti_9O_8(OR)_{20}+2ROH+2H_2O$$

$$Ti_9O_8(OR)_{20}+Ti_3(OR)_6(OH)_6 \rightarrow Ti_{12}O_{12}(OR)_{24}+ +2ROH+2H_2O$$

However, it differs significantly from their linear model in that the limiting polymer would be finite rather than infinite, with the formula $Ti_{12}O_{12}(OEt)_{24}$ rather than $[Ti_3O_4(OEt)_4]_\infty$ (ref. 1). It may be relevant that the painstaking investigations of the group working at Birkbeck College have led to the isolation and characterization of only two members of their $Ti_{3(x+1)}O_{4x}(OEt)_{4(x+3)}$ series, namely $Ti_6O_4(OEt)_{16}$ and $Ti_{12}O_{12}(OEt)_{24}$, which suggests that these may be structures of special stability. No obvious points of stability are to be expected on their model, and, in fact, highly hydrolysed oxide-alkoxides with conjectural formulæ as high as $Ti_{75}O_{96}(OR)_{108}$ have already been invoked[2] in discussions of ebullioscopic data.

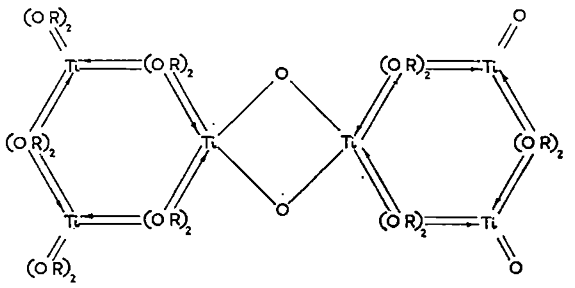


Fig. 1. $Ti_6O_4(OR)_{16}$

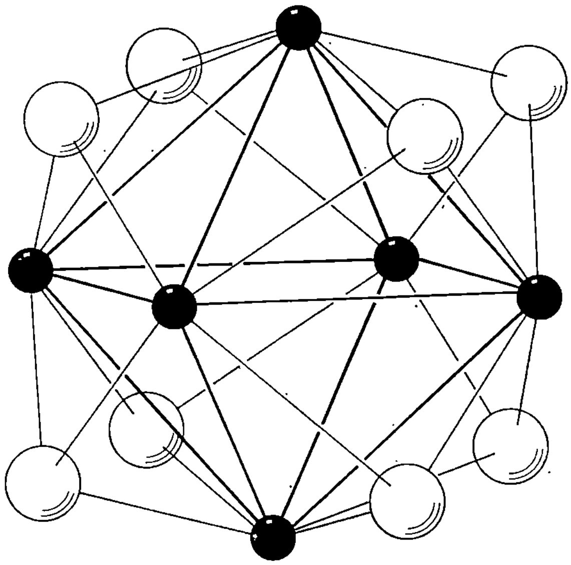


Fig. 2. Ti_6O_8 trisoctahedron

Structures for $Ti_6O_4(OEt)_{16}$ derived from a cyclic Ti_3-arrangement for the parent ethoxide, would also account very well for the observation[1,3] that on heating $Ti_6O_4(OEt)_{16}$ *in vacuo*, some $Ti(OEt)_4$ is recovered. This depolymerization could readily be initiated by the simple rupture of weak intermolecular bonds of the order of 5 kcal.[4,5] (see Figs. 1 and 2). That the ethoxy bridges are broken in this temperature-range is shown by the fact that *n*-alkyl-titanates are monomeric in the vapour state (for example, $Ti(OEt)_4$, b.p. 103° C. at 0·1 mm.)[4]. This depolymerization is rather more difficult to reconcile with the linear model for $Ti_6O_4(OR)_{16}$ (structure IV in our previous communication[6]), for each titanium atom is held in the structure by at least one strong covalent Ti—O—Ti bond, presumably of the order of 50–100 kcal. mole⁻¹, and a complete rearrangement would have to be postulated.

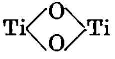
A further specific criticism made of our model is that it cannot accommodate the disproportionation scheme for oxide-alkoxide polymers. While we are not convinced of the occurrence of disproportionation in these compounds, Bradley and Westlake's criticism remains invalid, for numerical equations of the type illustrated here indicate that the number average degree of polymerization could also be independent of the distribution of polymer sizes for our model:

$$2[Ti_6O_4(OEt)_{16}] \rightarrow [Ti_3(OEt)_{12}]+[Ti_9O_8(OEt)_{20}]$$

$$3[Ti_6O_4(OEt)_{16}] \rightarrow 2(Ti_3(OEt)_{12}] + [Ti_{12}O_{12}(OEt)_{24}]$$

The case for disproportionation of $Ti_6O_4(OEt)_{16}$ was originally based on the time-independence of the number of osmotically active particles and the decrease in reactivity towards the Karl Fischer reagent which were observed on prolonged boiling in ethanol. Although Bradley, Gaze and Wardlaw[3] were unable to determine whether $Ti_6O_4(OEt)_{16}$ contained hydroxyl groups, they believed them to be absent, and therefore concluded that the reactive constituent must be bicovalent oxygen attached simply to titanium atoms, as in $\overset{\delta+}{\mathrm{Ti}}\text{—}\overset{\delta-}{\mathrm{O}}\text{—}\overset{\delta+}{\mathrm{Ti}}$. The decrease with time in Karl Fischer reactivity was then ascribed to progressive replacement of bicovalent by tricovalent oxygen as the molecular complexity of the oxide-alkoxide increased. This provisional interpretation has not been revised in terms of their subsequent $Ti_{3x+1)}O_{4x}(OEt)_{4(x+3)}$ model[1], and they have tacitly assumed that it continues to apply without modification.

It is not our purpose here to re-examine the case for disproportionation, but two comments will serve to illustrate that this thesis may be of doubtful validity. First, inspection of the linear model for $Ti_{3(x+1)}O_{4x}(OEt)_{4(x+3)}$ reveals that the number of bicovalent Ti—O—Ti groups (and of quaternary oxygen atoms) per titanium is, in fact, independent of molecular complexity, so that the decrease in reactivity to Karl Fischer reagent cannot be ascribed to this cause without either amending the original postulates or invoking long-range electronic effects. Secondly, the "apparent water content" of a crystalline sample of $Ti_6O_4(OEt)_{16}$ was found by them to decrease from 6·5 to 2·6 per cent simply by heating and recrystallizing the sample from ethanol (experiment *c*, ref. 3), so that the "active centres" cannot be the Ti—O—Ti groups as originally postulated.

With regard to the infra-red spectra reported by Bradley and Westlake, two points are at issue. First, we would agree with their conclusion that bands near 800 cm.$^{-1}$ in $Ti_6O_4(OEt)_{16}$ and $Ti_{12}O_{12}(OEt)_{24}$ must be assigned to oxygen bridges between titanium atoms. We direct attention again, however, to the unfamiliar configuration of the quaternary oxygen involved in the linear model, and stress that no simple Ti·O·Ti·O·Ti system is involved here. Examination of the alternative linear and cyclic structures for $Ti_6O_4(OEt)_{16}$ reveals that bonding of the type  occurs in both cases, and accordingly, their infra-red spectra in the 800 cm.$^{-1}$ region are unlikely to be dissimilar.

The second objection to our structure concerns the apparent absence of a Ti=O stretching frequency. Since the hydrolytic reaction scheme illustrated above represents only one of several possible schemes, the presence or absence of Ti=O groups in the hydrolysis products has little bearing on a decision between the linear and cyclic structures for the pure ester. For example, an attractive symmetrical close-packed $MoCl_2$-type structure can be envisaged for $Ti_6O_4(OEt)_{16}$ (Fig. 2), which does not involve terminal Ti=O groups. This structure, like our cyclic trimer, is again based on TiO_6-trigonal prisms with an octahedral skeleton of titanium atoms, with two terminal ethoxy groups attached to each. Each oxygen atom involved in the $Ti_6O_4(OEt)_4$-trisoctahedron is either 3- or 4-co-ordinated, in configurations, pyramidal or tetrahedral, both well established for oxygen.

In conclusion, we reiterate that the resolution of this polemic must ultimately await complete structure determinations of the ethoxide and two oxide-ethoxides, all of which can be obtained in crystalline form. Our original purpose remains, as stated previously, simply to direct attention to the fact that the linear model is by no means unique, and certainly, on the evidence available, does not yet enjoy the exclusive consideration claimed for it.

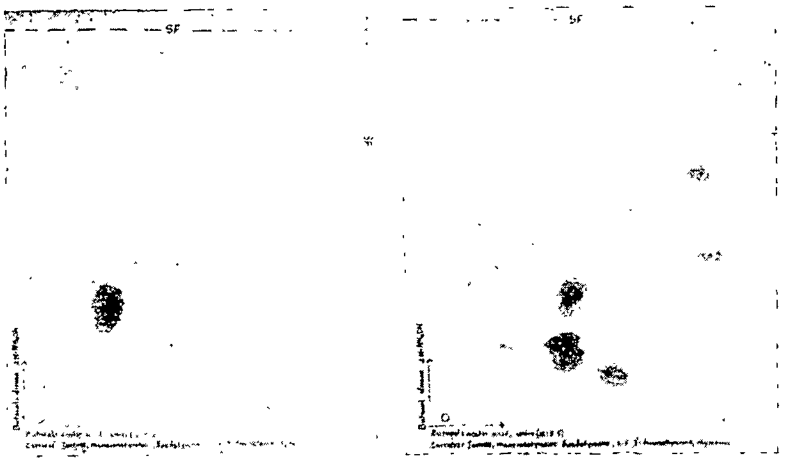
R. L. Martin
G. Winter
Central Research Laboratories,
Imperial Chemical Industries of Australia and New Zealand, Ltd.,
Newson Street, Ascot Vale, Melbourne.

[1] Bradley, D. C., Gaze, R., and Wardlaw, W., *J. Chem. Soc.*, 3977 (1955).
[2] Bradley, D. C., Gaze, R., and Wardlaw, W., *J. Chem. Soc.*, 469 (1957).
[3] Bradley, D. C., Gaze, R., and Wardlaw, W., *J. Chem. Soc.*, 721 (1955).
[4] Bradley, D. C., Mehrotra, R. C., Swanwick, J. D., and Wardlaw, W., *J. Chem. Soc.*, 2025 (1953).
[5] Martin, R. L., and Winter, G., *J. Chem. Soc.* (in the press).
[6] Martin, R. L., and Winter, G., *Nature*, 188, 313 (1960).

A Spray Method for the Simultaneous Detection of Iodide and Iodinated Amino-Acids on Filter Paper Chromatograms

For chromatographic identification of iodide and iodinated protein derivatives, it is often necessary to locate inorganic and organic iodide tracers on the same chromatogram. In paper chromatography a number of methods have been used for the detection of iodide. These include spraying with silver nitrate[1]; potassium chlorate, acid and starch[2]; 1 per cent aqueous palladium chloride[3]; a mixture of ferric nitrate and hydrogen peroxide[4]; the ceric-arsenite reagent[5]; and a mixture of $FeCl_3.6H_2O$, $K_3Fe(CN)_6$ and $NaAsO_2$ (*FFCA* reaction)[6]. Most of these tests for iodide interfere with those commonly used for the identification of iodinated amino-acids, namely, ninhydrin[7], diazotized sulphanilic acid[3], or its diethyl amide[8]. The ceric-arsenite and the *FFCA* reagents are the most sensitive (0·01 µgm. I$^-$) and can moreover be used for the detection of inorganic iodide, together with iodinated amino-acids on the same chromatogram[6,9]. The *FFCA* reagent, however, is not specific for iodine but also gives positive reactions with certain reducing substances[6]. The ceric-arsenite reaction has several disadvantages and is generally not used in the spraying technique[9].

The diazotized sulphanilic acid reagent can be used for the simultaneous identification of iodide as well as for iodinated amino-acids by spraying first with the diazotized sulphanilic acid, in which case a greyish or reddish-brown (depending on the concentration of iodide ions) spot locates iodide. This spot begins to disappear conveniently after 30 sec. and completely vanishes after about 2 min., thus leaving sufficient time for it to be marked. While the paper is still moist, 5 per cent (w/v) sodium carbonate is applied and the pinkish spots of the amino-acids appear. The iodine spots develop because of acid oxidation of iodide to iodine in the presence of sodium nitrite, since a mixture of 1·0 *N* hydrochloric acid and sodium nitrite, like sulphanilic acid and sodium nitrite (w/v), also give positive reactions with iodide. If the chromatogram is sprayed with sodium carbonate before it is sprayed with diazotized sulphanilic acid, the acid is neutralized and the iodine spots do not develop. In



a *b*

Fig. 1 (*a*) A two-dimensional chromatogram sprayed with diazotized sulphanilic acid to locate iodide. (*b*) The same chromatogram sprayed with 5 per cent sodium carbonate after the diazotized sulphanilic acid had been applied and the iodide spot had disappeared. Solvent systems: butanol/acetic acid/water (120 : 30 : 50) in one dimension; butanol/dioxan/2 *N* ammonium hydroxide (4 : 1 : 5) in the other dimension.
Carriers: 20 μgm. each of iodide, mono-iodotyrosine, diiodotyrosine, triiodothyronine, thyroxine

one- and two-dimensional chromatograms, quantities of 10 μgm. of iodide can still be easily detected by this method. Figs. 1*a* and *b* indicate the use of this test for the simultaneous identification of iodide and iodinated amino-acids.

A. VAN ZYL
M. BHAGA

Department of Physiology,
University of Cape Town.

[1] Laidlaw, J. C., *Nature*, **164**, 927 (1949).
[2] Roche, J., Jutisz, M., Lissitzky, S., and Michel, R., *Biochim. Biophys. Acta*, **7**, 257 (1951).
[3] Gross, J., and Leblond, C. P., *Endocrinol.*, **48**, 714 (1951).
[4] Block, R. J., Durrum, E. L., and Zweig, G., *A Manual of Chromatography and Paper Electrophoresis*, 319 (Acad. Press, Inc., New York, 1955).
[5] Bowden, C. H., MacClagan, N. F., and Wilkinson, J. H., *Biochem. J.*, **59**, 93 (1955).
[6] Gmelin, R., and Virtanen, A. I., *Acta Chem. Scand.*, **13**, 1469 (1959).
[7] Hird, F. J. R., and Trikojus, U. M., *Austral. J. Sci.*, **10**, 185 (1948).
[8] Winikoff, D., and Trikojus, U. M., *Biochem. J.*, **42**, 475 (1948).
[9] Smith, I., *Chromatographic and Electrophoretic Techniques*, **1**, 172 (William Heinemann Medical Books, Ltd., London, 1960).

BIOCHEMISTRY

Purine and Pyrimidine Metabolism in *Rhodospirillum rubrum*

WE have investigated the ability of the photosynthetic bacterium, *Rhodospirillum rubrum*, to deaminate aminopurines and aminopyrimidines.

Washed suspensions in 0·001 *M* phosphate buffer deaminate rapidly cytosine, methylcytosine and adenine to uracil, thymine and hypoxanthine, respectively. The products of the reaction were identified against authentic samples by paper chromatography with a variety of solvents, and the kinetics of the reaction followed either by eluting the spots and measuring the *E* values of the eluates at appropriate wave-lengths or, in the case of the adenine → hypoxanthine and cytosine → uracil conversions, by methods based on differential spectrophotometry. The method of Kalckar[1] was used for the first determination, and for the second determination we devised a similar method by which the amounts of cytosine and uracil could be determined by measuring ΔE at either 280 mμ or 250 mμ at *p*H 1·0. The production of 1 μmole of uracil per ml. of solution is indicated by a ΔE280 of $-$ 8·2 and a ΔE250 of + 3·74. The enzyme is soluble (not sedimented at 100,000*g* in cell-free extracts produced by sonic disintegration) with a sharp *p*H optimum for both pyrimidine and purine substrates at 7·0; it deaminates cytosine some ten times faster than adenine and about twice as fast as 5-methylcytosine. The chromatophores show no significant activity.

The specificity of the enzyme is indicated in Table 1. This shows that while guanine and *iso*guanine are probably deaminated to a very limited extent, neither 2,6-diaminopurine nor aza-adenine is attacked. Furthermore, aza-adenine does not inhibit deamination of adenine. With regard to pyrimidines, the specificity of the enzyme is more clear-cut; only those compounds carrying an amino group on position 6 are deaminated.

Table 1. SPECIFICITY OF THE PURINE/PYRIMIDINE DEAMINASE OF *Rhodospirillum rubrum*

Purines Compound	Activity	Pyrimidines Compound	Activity
Adenine	+	Cytosine	+
Guanine	?	5-Methylcytosine	+
*iso*Guanine	?	*iso*Cytosine	−
Aza-adenine	−	2-Aminopyrimidine	−
2,6-Diaminopurine	−	2,5-Diamino-4,6-dihydroxypyrimidine	−

Adenosine, when incubated with washed cells of *R. rubrum*, disappears rapidly ; no free inosine is ever detected, but after long incubation (over 4 hr.) traces of hypoxanthine are noted ; this suggests that adenosine is incorporated rapidly *in toto* into cellular material. Deoxyadenosine, on the other hand, is converted mainly into deoxyinosine ; small traces of hypoxanthine are observed after prolonged incubation. Cytidine was not attacked in any way ; deoxycytidine was not examined.

An attempt was made to demonstrate the presence of a deoxynucleoside transferase in *R. rubrum*. This enzyme will catalyse reactions similar to that recorded in (1). If such a reaction occurred, the adenine produced would be rapidly deaminated by the enzyme just discussed, while if it did not occur, the results of the reaction would be no different from the results observed with deoxyadenosine alone, that is, deoxyinosine would be the main product. Under our conditions, no deoxynucleoside transferase activity could be demonstrated in system (1), or when uracil was replaced by thymine and xanthine.

$$\text{deoxyadenosine} + \text{uracil} \rightleftharpoons \text{adenine} + \text{uracil deoxyriboside} \quad (1)$$

Hug and Werkman[3] found that whole cells and cell-free preparations of *R. rubrum* grown in darkness or light contain an active transaminase. In preliminary experiments in which adenine or cytosine were incubated with α-ketoglutarate we found that α-ketoglutarate had been aminated and adenine (or cytosine) had been deaminated. However, it soon emerged that this was not a new transaminase reaction, but that α-ketoglutarate was being aminated by the ammonium ions liberated during the deamination of adenine. Incubation of a washed suspension of *R. rubrum* with α-ketoglutarate and ammonium sulphate resulted in a marked formation of glutamate; under our conditions this was greater than when L-alanine was the amino donor.

It is hoped to publish this work in detail shortly. We thank the Royal Society for a grant to purchase a sonic disintegrator and Sigma Chemical Co. for a gift of purines and pyrimidines. This work was carried out in the Biochemistry Department, University of Liverpool. One of us (P. N. A. P.) carried out this work during the tenure of a Royal Thai Air Force scholarship.

T. W. GOODWIN
P. N. A. PASSORN

Department of Agricultural
Biochemistry,
University College of Wales,
Aberystwyth.

[1] Kalckar, H. M., *J. Biol. Chem.*, **167**, 429 (1947).
[2] Hug, D. H., and Werkman, C. H., *Arch. Biochem. Biophys.*, **72**, 369 (1957).

Intracellular Glycosidases of Dextran-producing Bacteria

EXTENSIVE work on the mechanism of dextran production from sucrose by bacterial extracellular dextransucrase has not, so far, clearly established the identity of the initial acceptors involved *in vivo* in dextran chain initiation[1,2]. As a step to obtaining such information we have examined the intracellular glycosidase and transferase activities of two dextran-producing bacteria. These were *Leuconostoc mesenteroides* (NRRL *B*512, NCIB 8590; from the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen) and *Streptococcus bovis* (strain I)[3].

Each organism was grown in an appropriate medium[3,4] containing sucrose (10 per cent w/v) plus sodium acetate (0·147 *M*). *Leuconostoc* cultures were incubated for 24 hr. at 25° and *Streptococcus* cultures for 5–6 hr. at 38°. During this time good growth occurred (*p*H fall from 7·0 to 5·8–6·0) but the dextran content remained sufficiently low to permit the cells to be collected easily. The centrifuged cells were washed twice in acetate buffer (0·05 *M*, *p*H 5·2) and disintegrated in 5–10 ml. of the same buffer at 2° in a Nossal cell-smasher[5]. After removal of cell debris by centrifugation the extracts were diluted with more buffer to give, from the cells from each litre of culture, 25 ml. of extract which was kept at 2° under toluene.

Digests were prepared containing cell-extract (0·25 ml.), buffer (0·25 ml., *Leuconostoc* extracts, acetate, 0·05 *M*, *p*H 5·2; *Streptococcus* extracts, phosphate–citrate, 0·15 *M*, *p*H 6·5) and sugar (10 mgm.). All sugars used were shown, by paper chromatography, to be pure or to contain only traces of sugar impurities. Controls omitting added sugar and cell-extract respectively were also prepared. The digests and controls were incubated for 24 hr. at 25° or 38° C. and analysed by paper chromatography, both before and after incubation, using ethyl acetate/water/pyridine (2 : 2 : 1) solvent and silver nitrate spray reagent[6].

The controls showed that neither the cell-extracts nor the added sugars alone produced any new sugars during incubation. Preliminary *iso*maltose, arbutin and sucrose digests, at various *p*H values, showed that the *Leuconostoc* carbohydrases were active over the *p*H-range 5·0–7·0, with maximum activity in the range 5·0–5·5, whereas the *Streptococcus* extracts were only active in the range 6·0–7·0, with maximum activity at about 6·5. The degree of hydrolysis of various sugars by the cell-extracts is shown in Table 1. In the disaccharide digests where strong hydrolysis occurred, there was also oligosaccharide formation by transferase action. *L. mesenteroides* was also grown in a 2 per cent maltose medium after passage through two maltose sub-cultures. The extract from these cells hydrolysed the same sugars as the sucrose cell-extracts at similar rates except for maltose, which was more rapidly attacked (spot-intensity of hydrolysis product + + +).

Table 1. HYDROLYSIS OF SUGARS BY EXTRACTS FROM DEXTRAN-PRODUCING CELLS OF *Leuconostoc mesenteroides* AND *Streptococcus bovis*

Sugar	Degree of hydrolysis*		
	L. mesenteroides extracts at *p*H 5·2		*S. bovis* extracts at *p*H 6·5
	25°	38°	38°
*iso*Maltose	+ + + + +	+ +	+ + + + +
*iso*Maltotriose	+ +	–	+ + + +
*iso*Maltulose	+		–
Melibiose	+ + +	–	+ + + +
Panose	+ +		+ + +
Maltose, leucrose	+	–	–
Nigerose, turanose, αα′-trehalose, methyl-α-glucoside	–	–	–
Sophorose	+ + + +		–
Gentiobiose, lactose, cellobiose	–	–	–
Sucrose	+ + + + + +	+ + + + + +	+ + + + + +
Raffinose	–	–	+ + + + + +
Melezitose	–	–	–
Arbutin, salicin, æsculin, linamarin, lotaustralin	+ + + + + +	+ + + + + +	–
Amygdalin, hesperidin, rutin	–	–	–

* Degree of hydrolysis assessed visually from the spot intensity, on the chromatograms, of hydrolysis products on the scale: + + + + + +, intense spot with substrate completely hydrolysed; down to +, barely detectable spot, trace hydrolysis only and –, no hydrolysis. Blank space, not tested.

Strong invertase activity was present in all cell-extracts, although it is evident that the *Leuconostoc* invertase is much more specific with respect to substrate than the *Streptococcus* one. Chromatographic analyses of hydrolysates of small amounts of the isolated sugars suggest that the oligosaccharides produced in the cell extract-sucrose digests are the well-known fructosylsucrose trisaccharides normally produced by invertase action.

The only α-glucosidase present to any extent in the cell extracts of both bacteria was an apparently constitutive α-1,6-glucosidase. The presence of this enzyme, which hydrolyses the repeating unit of dextran (*iso*maltose), in two unrelated dextran-producing bacteria is striking. The β-glucosidase activity in the *Leuconostoc* extracts also seems unusual in that it was only able to hydrolyse the β-link in glucosides with monosaccharide and aglycone residues and did not attack disaccharide derivatives of this type. Further, apart from sophorose, it was without action on β-linked reducing disaccharides. Attempts are being made to separate purer fractions of the β-glucosidase and α-1,6-glucosidase from the cell extracts.

This work was initiated as part of a programme supported by the Department of Scientific and Industrial Research (United Kingdom) and completed in the Plant Chemistry Laboratories (New Zealand). Acknowledgments for the gifts of sugars are due to Dr. D. H. Hutson, Royal Holloway College (nigerose), and Dr. M. J. Clancy, Lister Institute (sophorose).

R. W. BAILEY

Plant Chemistry Division,
Department of Scientific and
Industrial Research,
Palmerston North,
New Zealand.

E. J. BOURNE

Chemistry Department,
Royal Holloway College,
University of London,
Englefield Green,
Surrey.

---

[1] Koepsell, H. J., Tsuchiya, H. M., Hellman, N. N., Kazenko, A., Hoffmann, C. A., Sharpe, E. S., and Jackson, R. W., *J. Biol. Chem.*, **200**, 793 (1953).

[2] Bailey, R. W., Barker, S. A., Bourne, E. J., Stacey, M., and Theander, O., *Nature*, **179**, 310 (1957).

[3] Bailey, R. W., and Oxford, A. E., *Nature*, **182**, 185 (1958).

[4] Bailey, R. W., Barker, S. A., Bourne, E. J., and Stacey, M., *J. Chem. Soc.*, 3530 (1957).

[5] Nossal, P. M., *Austral. J. Exp. Biol.*, **31**, 583 (1953).

[6] Trevelyan, W. E., Procter, D. P., and Harrison, J. S., *Nature*, **166**, 444 (1950).

Isolation of Nigerose and Kojibiose from Dextrans

THE occurrence of 1,3-α linkage in some dextrans has already been suggested by many workers[1–5]. Barker *et al.*[3,4] have fractionated the partial acid hydrolysate of the dextran produced by *Betacoccus arabinosaseous* (Birmingham strain) on a charcoal column and recognized the presence of a trace of nigerose by paper chromatography. Jeanes *et al.*[5] have characterized dextrans from 96 strains of bacteria by periodate oxidation analysis and classified them into several groups on the basis of the proportions of 1,3 like linkages. According to their results, 29 of them appeared to contain more than 10 per cent 1,3-linkages and a few of them showed an extremely high content of 1,3-linkages (more than 30 per cent).

In 1956, Scott *et al.*[6] pointed out the presence of 1,2-linkage in some dextrans from the optical rotational shifts due to formation of a cuprammonium complex. According to their report, a few of them (for example, that from *Leuconostoc mesenteroides* NRRL *B*–1299 or *B*–1399) contain more than 30 per cent 1,2-linkages.

In spite of these observations, however, no oligo- or di-saccharides containing the heterogeneous glucosidic linkages have so far been isolated from the hydrolysates of dextrans. This may be due to the difference of the stability of 1,6- and other linkages against acid hydrolysis; the 1,6-linkage is much more stable than the other linkages. Therefore, by the usual method of hydrolysis in aqueous acid solution, heterogeneous linkages are more rapidly hydrolysed than the 1,6-linkage.

We have now found that acetolysis is more effective for the isolation of the heterogeneous linkages from dextrans. In contrast with hydrolysis, the 1,6-linkage is less stable to acetolysis, whereas the other linkages such as 1,2- and 1,3- are more resistant. For example, when the acetates of 1,6-linked isomaltose and 1,3-linked nigerose were subjected to acetolysis using the method to be described, the former was entirely cleaved into glucose in 6 hr. at 25° C., whereas the latter remained unchanged. By this method, we have now been able to isolate nigerose and kojibiose from the acetolysates of some dextrans. The dextrans used were prepared by the usual method from *Leuconostoc mesenteroides* NRRL *B*–421 and *B*–1299. The acetolysis reagent was a mixture of acetic anhydride, glacial acetic acid and concentrated sulphuric acid (24: 16: 3 by vol.). 20 gm. of dried and powdered dextran was suspended in 172 ml. of the above acetolysis reagent and kept at 30° C. for 7 days. The reaction mixture was then heated on a water-bath at 80° C. for 30 min. and poured into crushed ice. After neutralization with sodium carbonate, the whole solution was extracted with chloroform. On removal of the solvent, syrupy mixed acetates were obtained, which were deacetylated with sodium methoxide as usual. Yields of the acetolysates were 10–15 gm. from 20 gm. of the original dextrans. To ascertain if a reversible reaction had occurred, anhydrous glucose was treated exactly as described above; but no reversion product was detected by paper chromatography. The acetolysate of the dextran from *Leuconostoc mesenteroides* NRRL *B*–421 (29·7 gm.) was fractionated on a charcoal/'Celite' column by the gradient elution method. From the 5 per cent ethanol effluent, 7·8 gm. of nigerose and only 0·4 gm. of isomaltose were obtained. Nigerose was characterized as its crystalline octaacetate, melting point 149° C., undepressed on admixture with an authentic specimen.

In the same manner, 7·5 gm. of kojibiose and 1·0 gm. of nigerose were obtained from 22·0 gm. of the acetolysate of the dextran of *Leuconostoc mesenteroides* NRRL *B*–1299. Kojibiose was also characterized as its α- and β-octaacetates: α-kojibiose octaacetate, melting point 166° C.; β-kojibiose octaacetate, melting point 118° C. Neither acetates showed any depression on admixture with authentic specimens.

Thanks are due to Dr. F. R. Senti, of Northern Utilization Research Branch, Agricultural Research Service, U.S. Department of Agriculture (Peoria, Illinois, U.S.A.) for the gift of the strain of *Leuconostoc mesenteroides* NRRL *B*–1299.

KAZUO MATSUDA
HIROSHI WATANABE
KENTAROH FUJIMOTO
KIYOSHI ASO

Department of Agricultural
Chemistry,
Faculty of Agriculture,
Tohoku University,
Sendai, Japan.

---

[1] Abdel-Akher, M., Hamilton, J. K., Montgomery, R., and Smith, F., *J. Amer. Chem. Soc.*, **74**, 4970 (1952).

[2] Lohmar, R., *J. Amer. Chem. Soc.*, **74**, 4974 (1952).

[3] Barker, S. A., Bourne, E. J., Neely, W. B., and Stacey, M., *J. Chem. Soc.*, 2395 (1954).

[4] Barker, S. A., Bourne, E. J., James, A. E., Neely, W. B., and Stacey, M., *J. Chem. Soc.*, 2096 (1955).

[5] Jeanes, A., Haynes, W. C., Wilham, C. A., Rankin, J. C., Melvin, E. H., Austin, M. J., Cluskey, J. E., Fisher, B. E., Tsuchiya, H. M., and Rist, C. E., *J. Amer. Chem. Soc.*, **76**, 5041 (1954).

[6] Scott, T. A., Hellman, N. N., and Senti, F. R., *J. Amer. Chem. Soc.*, **79**, 1178 (1957).

Role of Lipid in the Hardening of the Cuticle in the Silkworm, *Bombyx mori*

Malek[1], and Dennell and Malek[2], have stated that the cuticle of *Periplaneta* becomes impregnated with a sterol and a protein rich in tyrosine, and Dennell[3] has given evidence that, in the development of the puparium of *Calliphora*, destruction of localized areas of the epidermis prevents the passage of sterol to the cuticle and also causes lack of tanning of the cuticle immediately above the operated areas.

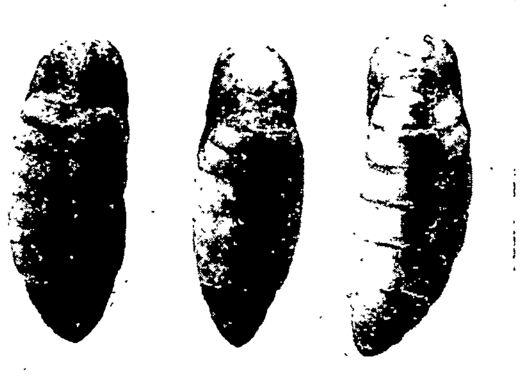
Table 1. HARDENING AND PIGMENTATION OF THE PUPAL CUTICLE TREATED WITH SOME ORGANIC SOLVENTS AT 25° C.

Solvent	Pigmentation	Hardening	Remarks
Methanol	(±)* ~ +	(±) ~ +	10–20 per cent of treated pupæ survived
Ethanol	(±) ~ +	(±) ~ +	,, ,,
Butanol	– ~ (±)	–	Died in a few hours
Acetone	(±) ~ +	(±) ~ +	10–20 per cent of treated pupæ survived
Ether	± ~ +	– ~ ±	Died in 2–3 days
Chloroform	– ~ (±)	–	Died in a few hours
Carbon tetrachloride	– ~ (±)	–	,, ,,
Benzol	– ~ (±)	–	,, ,,
Xylol	– ~ (±)	–	,, ,,
Ethyl acetate	– ~ (±)	–	,, ,,

* Brackets indicate that the effect was not usual. + = pigmentation (or hardening); ± = slight pigmentation (or hardening); – = no pigmentation (or hardening).

The lipid layer of the epicuticle of very young and soft pupæ of the silkworm *Bombyx mori* (race *N*124 × *C*124) has been removed within 30–150 min. of pupation either by immersion in ether for 10–15 sec. or by rubbing with cotton wetted with ether. After these treatments, pigmentation of the treated parts proceeds only slowly or imperfectly, and the cuticle remains unhardened. The pupæ thus treated shrank due to the evaporation of water, but some remained alive for a few days and showed an almost normal amber colour in the whole body or only in the region of the dorsal blood vessel (Fig. 1, *b* and *c*). This did not happen when methyl alcohol, ethyl alcohol, or acetone was substituted for ether. Pupæ treated with chloroform, xylol, benzene, ethyl acetate or carbon tetrachloride died within a few hours (Table 1).

Histochemical observation shows that the cuticle treated with ether is almost deprived of epicuticle lipid (see ref. 4). Replacement of the lipid by the ether extract from the cuticle or by 'Vaseline' prevented the pupæ from shrinking, but did not cause cuticle hardening to proceed.



(*a*) (*b*) (*c*)

Fig. 1. Pupæ 6 hr. after ether treatment. (*a*) Control; (*b*) and (*c*) treated pupæ

In agreement with Malek[1], and Jones and Sinclair[5], these results indicate that hardening and pigmentation of the cuticle are independent processes. However, it is not clear whether the lipid is directly involved in the hardening process, or whether it merely plays some part in facilitating the process. Microanalysis by paper chromatography has shown that the ether extracts from cuticles contain no phenolic substances.

I thank Prof. K. Hasegawa for his advice.

SHIGEMI KAWASE

Sericultural Laboratory,
Faculty of Agriculture,
Nagoya University,
Anjô, Aichi-ken, Japan.

[1] Malek, S. R. A., *Nature*, **170**, 850 (1952). **180**, 237 (1957).
[2] Dennell, R., and Malek, S. R. A., *Proc. Roy. Soc.*, B, **143**, 414 (1955).
[3] Dennell, R., *Ent. exp. et app.*, **1**, 268 (1958).
[4] Takahashi, *Bull. Nagano Seric. Exp. Stat*, **58**, 1 (1959).
[5] Jones, B. M., and Sinclair, W., *Nature*, **181**, 926 (1958).

Action of Neuraminidase on Haptoglobin

IN the course of work on the chemical structure of haptoglobin (Hp)[1], we have had occasion to investigate the action of neuraminidase on the Hp and on the complex of Hp with hæmoglobin (Hb-Hp). The neuraminidase employed was prepared as described by Mayron and Rafelson[2,3] from influenza virus, Asian strain (Jap. 305), and is free of proteolytic activity and glucosidase activities other than neuraminidase action. The Hp II preparations utilized were prepared as previously described[4], and are considered to approach the stage of theoretical purity. Such preparations contain 5·1 ± 0·1 per cent N-acetylneuraminic acid (NANA)[1]. The liberation of NANA from Hp II and Hb-Hp II was determined by the method of Warren[5] using three-times crystallized NANA as a standard. A typical enzyme incubation at 37° employed 2–5 mgm. of Hp II or Hb-Hp II and 0·1 to 0·3 unit of neuraminidase in 0·1 *M* phosphate buffer, *p*H 6·5, total volume 2–3 ml. One unit of enzyme activity is defined as that amount of enzyme which liberates 1 mgm. of NANA from an excess of neuramin-lactose in 1 hr. at 37°, *p*H 6·5.

Fig. 1 shows the percentage of NANA liberated as a function of time from Hp II and Hb-Hp II by the

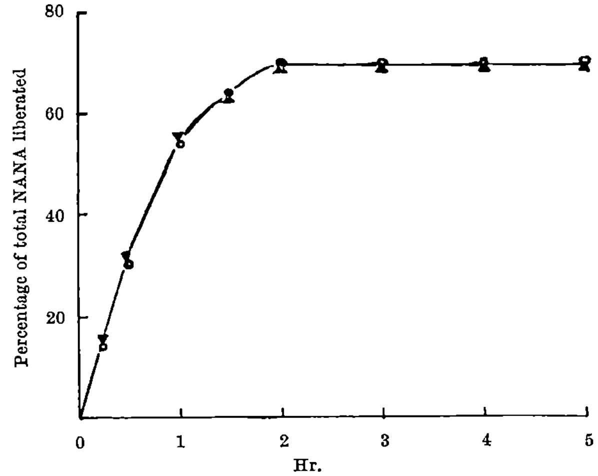


Fig. 1. Action of neuraminidase on Hp II and Hb-Hp. ○, Hp II; ▲, Hb–Hp II

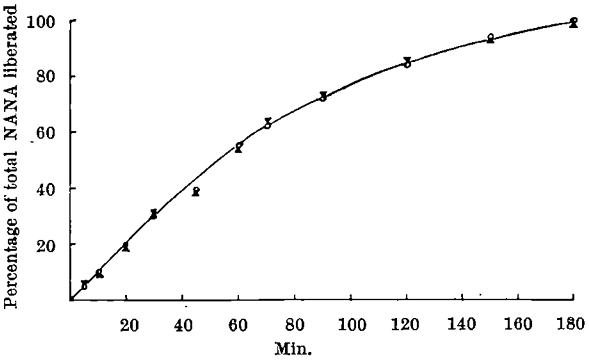


Fig. 2. Liberation of NANA from Hp 70 per cent NANA and from Hb–Hp 70 per cent NANA by 0·01 *N* hydrochloric acid at 80°. ○, Hp II 70 per cent NANA; ▲, Hb-Hp 70 per cent NANA

viral neuraminidase. It may be seen that the time-course of appearance of free NANA from the two substrates was identical, and that a maximum of 70 per cent of the total NANA was liberated in both cases. The *p*H optimum (*p*H 4·2) and the total peroxidase activity of the untreated complex (Hb-Hp), of the complex formed from untreated Hp II and of the complex formed from treated Hp (Hb-Hp 70 per cent NANA) were all identical. The conclusion which may be drawn from these results is that the 70 per cent of the NANA removed from Hp II and from Hb-Hp II participates neither in the formation of the Hb-Hp complex nor in the peroxidase activity of the complex. The possibility that the 30 per cent of NANA which is resistant to the action of neuraminidase may play a part in both the formation of the complex and in its peroxidase action cannot be excluded, although the following experiment suggests that this NANA is not involved in the formation of the Hb-Hp II complex.

Fig. 2 gives the results of an experiment in which the time-course of liberation of NANA by 0·01 *N* hydrochloric acid at 80° from Hp II previously treated with neuraminidase was compared with the liberation of NANA from the complex formed from this pretreated Hp II. The kinetic curves for the liberation of NANA from both substances were identical. These results are interpreted to suggest that the portion of NANA not liberated by the action of neuraminidase is not involved in the formation of the Hb-Hp II complex. If the 30 per cent of the NANA which is resistant to neuraminidase action were involved in the formation of the complex, it might be expected that kinetic differences in the release of NANA should have been observed.

This work was supported in part by a grant (*E* 2183) from the National Institutes of Health, U.S. Public Health Service.

Max E. Rafelson, jun.

Department of Biological Chemistry,
University of Illinois,
College of Medicine,
Chicago, Illinois.

Lise Cloarec
Jean Moretti
Max F. Jayle

Laboratoire de Chimie Biologique,
Faculté de Médecine,
45 Rue des Saints Pères,
Paris.

[1] Cheftel, R. I., Cloarec, L., Moretti, J., Rafelson, M., and Jayle, M. F., *Bull. Soc. Chim. Biol.*, **42**, 993 (1960).

[2] Mayron, L., and Rafelson, M., *Fed. Proc.*, **19**, 408 (1960).

[3] Mayron, L., Robert, B., Winzler, R., and Rafelson, M., *Arch. Biochem. Biophys.*, **92**, 475 (1961).

[4] Herman, G., Moretti, J., and Jayle, M. F., *Bull. Soc. Chim. Biol.* **42**, 817 (1960).

[5] Warren, L., *J. Biol. Chem.*, **234**, 1971 (1959).

Low-Angle Light Scattering and the Molecular Weight of Deoxyribonucleic Acid

It has been known for some time that the molecular weights obtained for deoxyribonucleic acid (DNA) by present-day techniques in light-scattering may be in error. This situation is not the result of theoretical barriers, but rather, the very considerable experimental problems to be solved in making reliable measurements of scattering below angles of 25°. Accordingly, considerable effort has been made to assess by indirect methods the validity of light-scattering molecular weights determined by extrapolation of data which extend to no lower than 25°. Doty, Geiduschek and Holtzer and more recently Butler *et al.* have given[1–3] detailed critical discussions of the nature of the indirect evidence and the remaining ambiguities.

In the case of molecules as large as DNA, measurements of light scattering must be made down to the angular region[1] of 6–10° to enable a reliable extrapolation to zero angle. Such measurements are necessary to determine the molecular weight of DNA and other very large macromolecules without appeal to any molecular models, but the experimental difficulties are so great that Doty has commented[1] that ". . . there is no likelihood of reaching this range [6–10°] because of practical difficulties".

The difficulties are, indeed, very great, but not insurmountable. I wish to report here measurements made on the scattering of a sample of calf-thymus DNA (prepared by a modification of the method of Signer and Schwander[4]) which sharply increase the likelihood of achieving reliable results at low angles. The measurements extend to 9°.

The scattering was measured in an instrument of my design the calibration and precision of which have been described[5] earlier. Its calibration constant determined by measurements on 'Ludox' is considered to be accurate to within a probable error of ± 0·3 per cent.

The results of the measurements are illustrated in Fig. 1. The plot has been made according to the equation :

$$\frac{Kc}{R_{\theta,u}} = \frac{1}{M_w}\left(1 + \frac{16\pi^2 n_0^2}{3\lambda_0^2}[\rho_G]^2 \sin^2(\theta/2) + \ldots\right) + 2A_2Q(\theta)c$$

where $R_{\theta,u}$ is the reduced intensity, c is the concentration (gm./ml.), K is $[2\pi^2 n^2(dn/dc)]/N_0\lambda_0^4$, M_w is the weight-average molecular weight, $[\rho_G]$ is the average radius of gyration, n is the refractive index of the solution and λ_0 is wave-length of the incident light *in vacuo* (here 4358 Å.).

The results can be fitted over the entire angular range of 9–45° by a straight line with a probable error of ± 4 per cent. Least-square calculations give $(Kc/R_{\theta,u})_{\theta=0} = 8\cdot2 \times 10^{-8}$ if all the data (9–45°) are included. The result is $7\cdot9 \times 10^{-8}$ when only the angular range 25–45° is considered and $8\cdot4 \times 10^{-8}$ for the range 9–17°.

The weight-average molecular weight obtained from the range 9–45° is $12\cdot2 \times 10^6$ and is independent of any assumptions concerning the shape. The

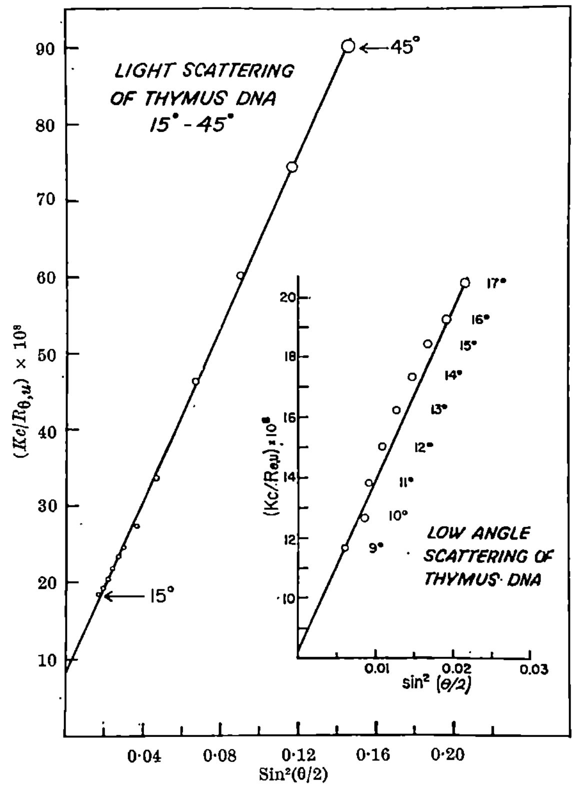


Fig. 1. The light-scattering of calf-thymus DNA in the angular region 9–45°. The straight line in both the main figure and the inset is that calculated by least squares for the entire angular region shown. The concentration of DNA was $3\cdot12 \times 10^{-5}$ gm./ml.

average radius of gyration, determined in the usual fashion, is 3700 Å. The reduced viscosity at zero shear for the sample reported on here was 45 as determined by measurements in a three-bulb viscometer.

The light-scattering data at higher angles, 50–135°, display a downward curvature which can be fitted reasonably well by a straight line in the range 60–135°. Extrapolated to zero angle, this line yields a value of 40×10^{-8} for the intercept. The interpretation[1–3] of the curvature is complicated: it may arise from polydispersity of molecular weights; it may be the result of variability in the shape of the molecules, nor is a combination of these two factors excluded. At present there is no unambiguous method of determining these properties independently for DNA in solution.

The experimental work reported here was completed at the Virus Laboratory of the University of California, Berkeley. It was supported partly by contract *NR* 121–175 between the Office of Naval Research and the University of California. A more detailed report of the experiments will be available shortly.

SIDNEY KATZ

Department of Biophysics,
School of Medicine,
University of Buffalo,
Buffalo, New York.

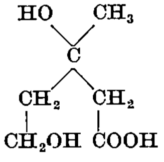
[1] Doty, P., *J. Cell Comp. Physiol.*, **49**, 27 (1957).

[2] Geiduschek E. P., and Holtzer, A., in *Advances in Biological and Medical Physics*, **6**, 431 (Academic Press, New York, 1958).

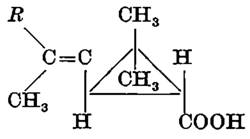
[3] Butler, J. A. V., Laurence, D. J. R., Robins, A. B., and Shooter, K. V., *Proc. Roy. Soc.*, A, **250**, 1 (1959).

Biosynthesis of the Pyrethrins

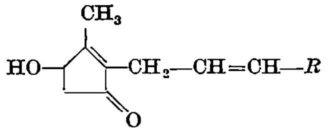
RECENT work[1] has indicated that mevalonic acid (I) is an intermediate in the biosynthesis of terpenoid compounds. We now wish to report some experiments which indicate that this acid is also involved in the biosynthesis of the 'pyrethrins', the insecticidal constituents of the pyrethrum plant, *Chrysanthemum cinerariaefolium*. The 'pyrethrins' are a mixture of four closely related esters[2] derived from two acids, chrysanthemum monocarboxylic acid (II, R = —Me) and pyrethric acid (II, R = —COOMe), and two keto-alcohols, pyrethrolone (III, R = —CH=CH$_2$) and cinerolone (III, R = —CH$_3$). It is customary to refer to the mixture of the two esters derived from chrysanthemum monocarboxylic acid and the keto-alcohols as 'pyrethrin I' and to that from pyrethric acid as 'pyrethrin II'. The two acids have carbon skeletons which can be built up from two isoprene units, and thus might be expected to be derived from mevalonic acid. The two keto-alcohols cannot be so regarded, but it is of interest to note that the methylpentenone ring could be formed in theory by the cyclization of mevalonic acid. We have now shown that this theoretically attractive route is not employed by the plant.



(I)



(II)



(III)

Because the pyrethrins are concentrated in the achenes[3] experiments on the incorporation of labelled compounds have been done on the flower head. Preliminary work with aqueous solutions of 2-^{14}C-acetic acid and DL-2-^{14}C-mevalonic acid showed that these two compounds moved slowly up the flower stem to the intact flower. A small amount of radioactivity was incorporated into the pyrethrins by this technique. A better incorporation was obtained by dropping an aqueous solution of the acid (I) containing a little detergent on the disk florets of the open flower. Finally, the highest incorporation was obtained by vacuum infiltration[4] of the substrate on ovules dissected from half-open flowers. This observation suggests that the pyrethrins are synthesized as well as stored in the achenes. It has also been found that the ovules from unopened and therefore unfertilized disk florets are slightly better at incorporating mevalonic acid into the pyrethrins than ovules from opened disk florets. This would seem to suggest, contrary to previous indications[5], that fertilization does not increase pyrethrin synthesis in the flower head.

The total pyrethrins were extracted from the plant tissue with acetone, and 'pyrethrins I and II' isolated by chromatography on silica gel/plaster of Paris[6]. The 2,4-dinitrophenylhydrazones of these compounds were prepared[7,8] and recrystallized to constant specific radioactivity. These derivatives were

[4] Signer, R., and Schwander, H., *Helv. Chim. Acta*, **33**, 1522 (1950).

[5] Bjorklund, R., and Katz, S., *J. Amer. Chem. Soc.*, **78**, 2122 (1956).

counted as thin films using an end-window counter. In a typical experiment DL-2-^{14}C-mevalonic acid (2 mgm., 2 μc.) was added to isolated ovules (2 gm.); after 24 hr. the incorporation of radioactivity into the pyrethrins had reached a maximum, corresponding to utilization of 1·4 per cent of the natural (+) isomer[9] of mevalonic acid. In a comparative experiment 2-^{14}C-acetic acid was incorporated to a much smaller extent, approximately one-tenth that of mevalonic acid.

Under strongly acid conditions in methanol 'pyrethrin II' yields with 2,4-dinitrophenylhydrazine the derivative of pyrethrolone methyl ether[7]. Using this method of splitting the esters in 'pyrethrin II', it has been found that while acetic acid is incorporated into the cyclopentenone ring, mevalonic acid is not. Thus, by difference, it is concluded that mevalonic acid is only incorporated into the terpene-like acids (II) of the pyrethrins. The exact location of the C-2 atom of mevalonic acid has not yet been determined; but it would seem very likely that it would be in the geminal methyl groups of the cyclopropane ring in both acids, and in a similar position in the isopropylidene group of 'pyrethrin I'. On the other hand, since the biosynthesis of the pentenones (III) does not involve mevalonic acid, it would seem to be either an example of the acetate route of biosynthesis that has been suggested by Birch and co-workers[10], or else to involve a precursor of mevalonic acid such as mevaldic acid[11] or 3-hydroxy-3-methyl-glutaric acid[12].

This work was supported in part by a grant from the Department of Agriculture, Kenya, and the Pyrethrum Board of Kenya.

MARY P. CROWLEY
HELENE S. INGLIS
M. SNAREY
E. M. THAIN

Tropical Products Institute, Department of Scientific and Industrial Research,
London, W.C.1.

[1] *Ciba Foundation 'Symp. Biosynth. Terpenes and Sterols* (Churchill, London, 1951).
[2] Harper, S. H., *Ann. Rep. Prog. Chem.*, **45**, 162 (1948).
[3] Martin, J. T., and Tattersfield, F., *Ann. App. Biol.*, **21**, 670 (1934).
[4] Purcell, H. E., Thompson, G. H., and Bonner, J., *J. Biol. Chem.*, **234**, 1081 (1959).
[5] Chandler, S. E., *Pyrethrum Post*, **4** (1), 10 (1956).
[6] Spickett, R. G. W., *Chem. and Indust.*, 561 (1957).
[7] Moore, B. P., *J. Sci. Food Agric.*, **7**, 740 (1956).
[8] Elliott, M., *Chem. and Indust.*, 685 (1958).
[9] Cornforth, R. H., Fletcher, K., Hellig, H., and Popják, G., *Nature*, **185**, 923 (1960).
[10] Birch, A. J., Blance, G. E., and Smith, H., *J. Chem. Soc.*, 4582 (1958).
[11] Eggerer, H., and Lynen, F., *Liebigs Ann.*, **608**, 71 (1957).
[12] Durr, J. F., and Rudney, H., *J. Biol. Chem.*, **235**, 2572 (1960).

Conversion of L-Galactono-γ-lactone into L-Ascorbic Acid by Plants

It has been inferred from feeding-experiments that cress seedlings[1], and also pea and mung bean mitochondria[2], are capable of converting L-galactono-γ-lactone into L-ascorbic acid. Similar results were obtained with mitochondria from cabbage leaves and cauliflower florets, and a relatively specific L-galactono-γ-lactone dehydrogenase was isolated from the latter[3]. The results (Table 1) confirm that this system may not be uncommon in the higher plants; *Sphagnum*, *Spirogyra* and *Psalliota*‡ gave inconclusive results. The figures in Table 1 are either the mean of duplicate estimations, or are taken from single, extended experiments which show how the concentration of ascorbic acid in the tissue varies during the period of feeding. Fig. 1 shows the results of one such extended experiment with parsley, in which the concentration of ascorbic acid in the laminæ fed with the lactone rose, during a period of 190 hr., from 1,582 to 12,010 mgm./100 gm. dry weight, that is, to approximately 12 per cent of the dry weight. During the same period, the value for the controls in de-ionized water fell gradually to 1,120 mgm./100 gm. dry weight, and at this time the experimental laminæ possessed more than ten times the concentration of ascorbic acid in the controls.

Table 1. EFFECT OF FEEDING WITH L-GALACTONO-γ-LACTONE ON THE CONCENTRATION OF ASCORBIC ACID IN CERTAIN PLANT PARTS

Plant material	Period of feeding (hr.)	Concentration of ascorbic acid (mgm./100 gm. dry wt.)		
		Initial value	Control samples in water	Experimental samples in lactone
*Slices of flesh from full-size, green hips of				
Rosa sherardi	66	3310	3540	5110
R. canina	66	1421	1500	2710
R. arvensis	66	342	344	1320
†Entire leaves of				
Parsley (*Petroselinum crispum*)	48	920	850	2450
Scurvy-grass (*Cochlearia officinalis*)	48	900	560	2650
Watercress (*Nasturtium officinale*)	24	620	650	2700
‡Procumbent yellow sorrel (*Oxalis corniculata*)	24	751	736	1587
‡Kentucky blue-grass (*Poa pratensis*)	33	331	304	1936
‡Maidenhair fern (*Adiantum capillus-veneris*)	56	531	397	1192

* The tissue slices were suspended and aerated at 25° C. either in de-ionized water or in a 0·5 per cent (w/v) solution of L-galactono-γ-lactone.

† The petioles of the leaves (except for watercress) were stood either in de-ionized water or in the solution of the lactone. The watercress leaves were floated on the appropriate liquid in Petri dishes.

‡ North American material, kept during feeding in a constant-temperature room at a temperature of approximately 22° C. under a daily regime of 14 hr. light and 10 hr. darkness; light intensity approximately 500 ft.-candles. The remaining material was British and was kept during feeding on a window-bench in the normal laboratory, with a diurnal cycle of approximately 11 hr. light and 13 hr. darkness.

Three pathways have been proposed for the synthesis of ascorbic acid in plants: (i) from D-glucose via D-glucurono-γ-lactone[1]; (ii) from

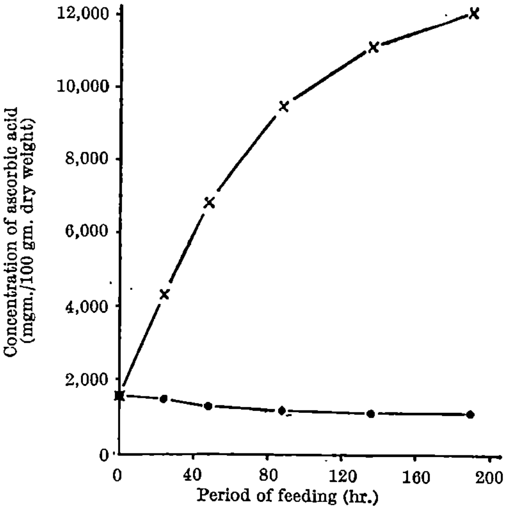


Fig. 1. Effect of feeding with L-galactono-γ-lactone on the concentration of ascorbic acid in the leaf laminæ of parsley†‡. ×, leaves with petioles standing in 0·5 per cent solution of lactone; ●, controls with petioles in de-ionized water

D-galactose via L-galactono-γ-lactone[1], and (iii) from D-glucose via glucose-6-phosphate and other phosphorylated intermediates[4]. Feeding with carbon-14-labelled compounds has led to the conclusion that, although D-glucurono-γ-lactone fed to strawberries may serve as a precursor in the synthesis of ascorbic acid[5], only pathway (iii) is involved in normal synthesis in this fruit and in cress seedlings[4,6]. On the basis of this view, any functional significance of the active system capable of converting L-galactono-γ-lactone into ascorbic acid remains to be elucidated; this problem is now being studied.

G. A. D. JACKSON
R. B. WOOD
M. V. PROSSER

Department of Botany,
University College of North Wales, Bangor, and
Department of Botany and Plant Pathology,
Ohio State University, Columbus.

[1] Isherwood, F. A., Chen, Y. T., and Mapson, L. W., *Biochem. J.*, **56**, 1 (1954).

[2] Mapson, L. W., Isherwood, F. A., and Chen, Y. T., *Biochem. J.*, **56**, 21 (1954).

[3] Mapson, L. W., and Breslow, E., *Biochem. J.*, **68**, 395 (1958).

[4] Loewus, F. A., Finkle, B. J., and Jang, R., *Biochim. Biophys. Acta*, **30**, 629 (1958).

[5] Finkle, B. J., Kelly, S., and Loewus, F. A., *Biochim. Biophys. Acta*, **38**, 332 (1960).

[6] Loewus, F. A., Jang, R., and Seegmiller, C. G., *J. Biol. Chem.*, **232**, 533 and 505 (1958); *Biochim. Biophys. Acta*, **23**, 205 (1957).

A Positive Feedback Mechanism of Adenosine Triphosphate Synthesis in Erythrocytes

IN previous papers, Nakao *et al.*[1,2] reported that adenosine triphosphate (ATP) is regenerated in erythrocytes stored for more than several weeks or treated with fluoride[3], when the cells are incubated with nucleoside and adenine at 37° C. In these aged or fluoride-treated cells, most of the nucleotides and other acid-soluble phosphate esters were decomposed almost completely and energy metabolism almost entirely stopped. To elucidate the mechanism of adenosine triphosphate synthesis in such erythrocytes, the following observations were carried out:

(1) As reported previously, inorganic phosphorus-32 (i^{32}P) and adenine-8-^{14}C were incorporated into ATP in long-stored erythrocytes after incubation with inosine, adenine-^{14}C and inorganic phosphorus-32. The incorporation of inorganic phosphorus-32 into ATP is inhibited by sodium-fluoride, mono-iodoacetate and arsenate, and adenine can be replaced by no other purine or pyrimidine bases, while inosine can be replaced by adenosine, deoxyadenosine, guanosine and deoxyguanosine though pyrimidine nucleosides were not effective[2]. These results show that ATP was synthesized from added adenine and added inorganic phosphate, and that glycolysis system and nucleoside phosphorylase seem to have some connexion with synthesis of ATP.

(2) The time-course of ATP generation during incubation with adenine and inosine in erythrocytes previously stored for 15 wk. or treated with sodium fluoride for several hours is shown in Fig. 1. The erythrocytes stored for 15 wk. or longer and those treated with sodium fluoride for 8 hr. showed a time-lag, in contrast to those stored for shorter periods or not treated with sodium fluoride.

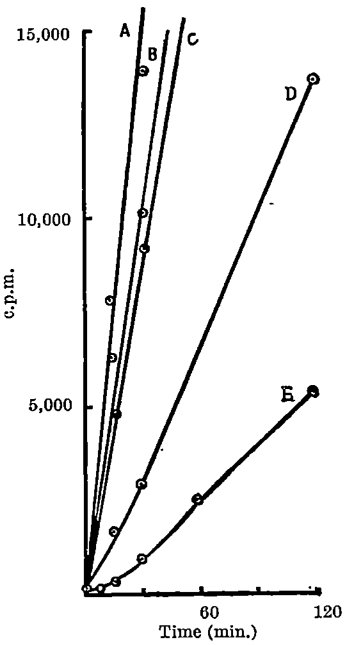


Fig. 1. Incorporation of adenine-8-^{14}C into nucleotide by intact erythrocytes. Reaction mixture contained 0·1 μmole (46·400 c.p.m.) of adenine-^{14}C, 5 μmoles of inosine, 0·7 ml. of intact cells, 2 μmoles of magnesium chloride, 10 μmoles of inorganic phosphate and 30 μmoles of *tris* buffer (*p*H 7·5); total volume, 1·32 ml.; temperature, 37° C. After incubation, the barium salt of nucleotides was isolated according to Kornberg (ref. 7) and its radioactivity was measured by means of a gas-flow counter. Pre-incubation with sodium fluoride of the blood stored for 4 wk. at 4° C. was carried out for 0(*A*), 2(*B*), 4(*C*), 8(*D*) hr. at 37° C. and (*E*) shows blood stored for 15 wk. at 4° C., in which no further pre-incubation was performed

(3) Hæmolysate obtained from fresh human erythrocytes was previously dialysed for 24 hr. at 4° C. and then passed through a 'Dowex 1' column in order to eliminate thoroughly all of the acid-soluble phosphorus compounds, using inorganic phosphorus-32 as an indicator. The incubation was carried out with a reaction mixture containing inorganic phosphate, magnesium, nicotinamide, adenine-8-^{14}C, either inosine or ribose-5-phosphate (R5P), and the pre-treated hæmolysate. When a trace amount of ATP was added to the medium, carbon-14 was found in adenosine monophosphate (AMP), but not in ATP, while no radioactivity was found either in AMP or in ATP, when ATP was not added to the reaction mixture. The time-course of the incorporation of adenine into nucleotide is illustrated in Fig. 2. It shows a remarkable increase in adenine-^{14}C incorporation with a time-lag in the case where diphosphopyridine nucleotide (DPN) was added and this time-lag disappeared when a greater amount of ATP was added to the medium. The results obtained from experiments (2) and (3) indicate that the existence of the lag phase was neither due to the inhibitory effect of a contaminating substance nor due to the induced

Table 1. SYNTHESIS OF ATP BY PRE-TREATED HÆMOLYSATE

Reaction mixture: Inosine, 50 μmoles, adenine, 5 μmoles, magnesium chloride, 10 μmoles, inorganic phosphate, 50 μmoles, DPN, 0·05 μmole, ATP, 0·05 μmole, nicotinamide, 66 μmoles, hæmolysate pre-treated as shown in the footnote of Fig. 2, 2·5 ml., total volume, 6·60 ml. Incubation was carried out for 60 min. at 37° C. After incubation, the acid-soluble fraction was analysed by Cohn and Carter's column chromatography on 'Dowex 1(*C*1)'

	Initial (μmole)	Final (μmole)	Difference (μmole)
Inosine	50·0	30·0	−20·0
Adenine	5·0	4·18	− 0·82
ATP	0·05	0·43	+ 0·38
ADP	–	0·51	+ 0·51
AMP	–	0·087	+ 0·087
Lactic acid	0	10·8	+10·8

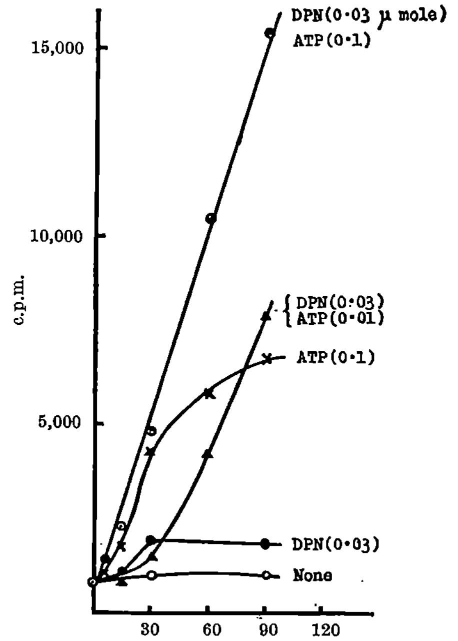


Fig. 2. Incorporation of adenine-8-^{14}C into adenine nucleotide by pre-treated hæmolysate. Reaction mixture contained 1 μmole (94·500 c.p.m.) adenine-8-^{14}C, 10 μmoles of inosine, 2 μmoles of magnesium chloride, 10 μmoles of inorganic phosphate, 10 μmoles of nicotinamide and 0·7 ml. of the pre-treated hæmolysate, namely, dialysed for 24 hr. at 4° C. and passed through 'Dowex 1' column (*Cl*) to eliminate thoroughly all the nucleotides, using inorganic phosphorus-32 as an indicator. The total volume was 1·32 ml. Incubation was carried out at 37° C.

formation of enzyme, but possibly due to the increase of coenzymes or of substrates.

(4) Not only incorporation of adenine-^{14}C into ATP, but also a net increase of ATP was determined by Cohn and Carter's ion-exchange chromatography[4] on 'Dowex 1', after the pre-treated hæmolysate was incubated with adenine, inosine, inorganic phosphate, magnesium, a small amount of DPN and of ATP, as shown in Table 1.

Preiss and Handler[5] reported in 1958 that R5P and ATP are converted to phosphoribosyl pyrophosphate (PRPP), and AMP and then PRPP and adenine yield AMP and inorganic pyrophosphate (PP) in acetone powder extract of human red cells. The presence of powerful adenylate kinase activity in human erythrocytes was reported by Tatibana *et al.*[6]. Moreover, there are a few observations indicating the existence of a considerable amount of glycolytic activity even in erythrocytes stored for more than several weeks. For example, when incubated with inosine, 2,3-diphosphoglyceric acid increased in erythrocytes stored for a long period[1,2] and lactate also increased in the erythrocytes when incubated with inosine and adenine or in hæmolysate when incubated with ATP and inosine. According to these results and the observations of others mentioned above, the mechanism of ATP generation might be explained tentatively as follows:

$$\mathrm{R5P} + \mathrm{ATP} \rightleftarrows \mathrm{AMP} + \mathrm{PRPP} \tag{1}$$

$$\mathrm{PRPP} + \text{adenine} \rightleftarrows \mathrm{AMP} + \mathrm{PP} \tag{2}$$

$$2\mathrm{AMP} + 2\mathrm{ATP} \rightleftarrows 4\mathrm{ADP} \tag{3}$$

$$4\mathrm{ADP} + n\mathrm{R5P} \rightarrow 4\mathrm{ATP} + X \tag{4}$$

(Warburg-Dickens system and Embden-Meyerhof system)

$$\text{Sum}: (n+1)\,\mathrm{R5P} + \text{adenine} + 3\mathrm{ATP} \rightarrow 4\mathrm{ATP} + \mathrm{PP} + X \tag{5}$$

The rapid formation of R5P from inosine and inorganic phosphate in erythrocytes or in hæmolysate is well established. R5P thus formed reacts with a trace amount of ATP still remaining in the cells, forming PRPP and AMP (1). ADP was synthesized from AMP, which was formed in both reactions (1) and (2), from remaining ATP, by the action of adenylate kinase (3). The ADP is converted to ATP in the course of R5P metabolism through Warburg–Dickens and glycolytic systems (4) (Fig. 3).

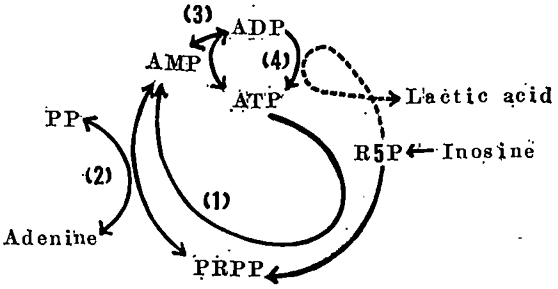


Fig. 3. A hypothesis of ATP regeneration in human erythrocytes

Thus, as a key substance, the trace amount of ATP remaining in the cells makes a cycle of reactions operative in synthesizing a greater amount of ATP; the further the reaction proceeds, the greater the increase in the amount of 'substrate ATP'.

MAKOTO NAKAO*
TAKASHI MOTEGI
TOSHIKO NAKAO*
SABURO YAMAZOE

Department of Biochemistry,
School of Medicine,
Gunma University, Maebashi.

HARUHISA YOSHIKAWA

Department of Physiological
Chemistry and Nutrition,
Faculty of Medicine,
University of Tokyo,
Tokyo.

---

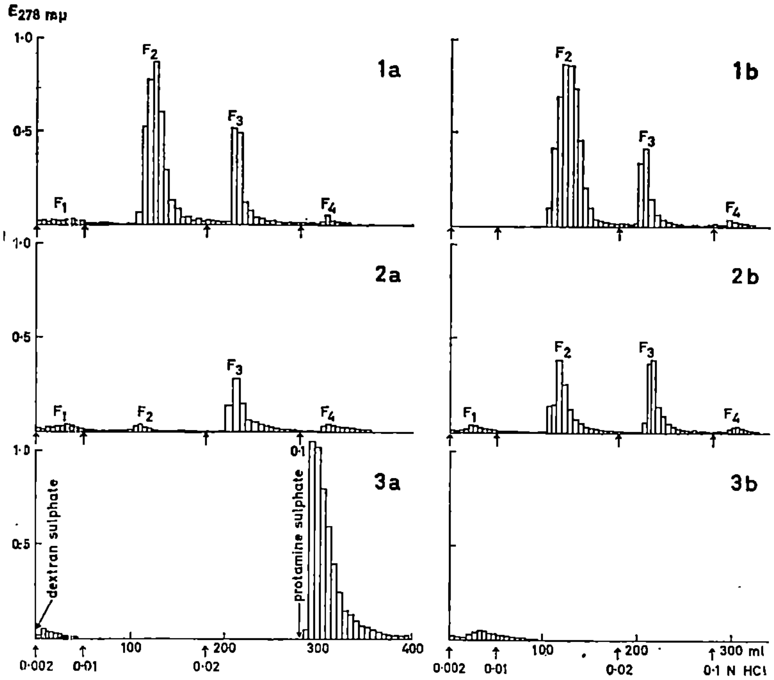
* Present address: Department of Biochemistry, University School of Medicine, Yokohama.

[1] Nakao, M., Nakao, T., Tatibana, M., Yoshikawa, H., and Abe, T., *Biochim. Biophys. Acta*, **32**, 564 (1959).
[2] Nakao, M., Nakao, T., Tatibana, M., and Yoshikawa, H., *J. Biochem.* (Japan), **47**, 661 (1960).
[3] Nakao, M., Nakao, T., and Tamazoe, S., *Nature*, **187**, 946 (1960); *J. Biochem.* (Japan), **49**, 487 (1961).
[4] Cohn, W. E., and Carter, C. E., *J. Amer. Chem. Soc.*, **72**, 4273 (1950).
[5] Preiss, J., and Handler, P., *J. Biol. Chem.*, **233**, 493 (1958).
[6] Tatibana, M., Nakao, M., and Yoshikawa, H., *J. Biochem.* (Japan), **45**, 1037 (1958).
[7] Kornberg, A., *J. Biol. Chem.*, **215**, 418 (1954).

Influence of Polyvalent Ions on Histones isolated from Different Tissues

WHEN dealing with fractionation of histones, we observed that the chromatographic pattern became markedly different depending on the cellular origin of the preparation. We also found that these differences reflected the polysaccharide content of the tissue.

The technique was as follows: deoxynucleoprotein was prepared from fresh frozen tissue through precipitation with 0·15 *N* sodium chloride[1] and an extraction was made with 0·2 *N* hydrochloric acid[1]. The extract, which will contain histones as well as polysaccharides, was gel-filtered at + 4° C. on a 'Sephadex' column, *G* 25, with acetate buffer of *p*H 4·0



Figs. 1–3. Effluent diagrams of histones chromatographed on carboxymethylcellulose columns (15 cm. × 1·4 cm.). All the extracts were applied to the columns in acetate buffer, I = 0·02 and pH 4·0. Elution with hydrochloric acid of the concentrations marked on the horizontal axis. 1*a*, Run of histones from chicken erythrocytes; 1*b*, the same run as 1*a*, but with protamine sulphate added to the extract before the application to the column; 2*a* and 2*b*, the corresponding runs of histones from calf liver; 3*a*, run of calf thymus histones to which has been added an equivalent amount of dextran sulphate before the extract has been added to the column. Thereafter elution with protamine sulphate. 3*b*, run of protamine sulphate (salmine) alone (from Nutritional Biochemicals Corporation)

and an ion-strength of 0·02 [2,3]. For separation of histone fractions, chromatography on carboxymethylcellulose (CMC) was utilized[4]. The stepwise elution was made with 0·002 N, 0·01 N, 0·02 N and 0·1 N hydrochloric acid. The elution was followed through measurements of the extraction at 278 mμ.

Fig. 1*a* shows the chromatographic pattern of extract from chicken erythrocytes during the stepwise elution of the CMC-column. Two distinct and homogeneous fractions of histone were obtained, the peaks F_2 and F_3. Almost the same pattern appeared with extracts from thymus of calf, pig and lamb and from lymph nodules of calf. The ratio F_2/F_3 shows only a slight variation within this material. All these tissues can be regarded as containing a high proportion of histones in relation to polysaccharides. The main difference between the two histone fractions, which are eluted with different strengths of the acid, should be that F_3 is a more basic protein than F_2. Besides, with reference to end-group analyses[4], F_2 and F_3 may represent two qualitatively different types of histones. Fig. 2*a* demonstrates the corresponding elution pattern from calf liver. In this case the histone fraction F_2 is almost non-existent. The same very low ratio F_2/F_3 was also obtained with extracts from other kinds of liver—pig, mouse and chicken—and from whole chicken embryos. All these tissues should have a relatively high amount of polysaccharides in relation to histones. The pattern from extract of calf white blood corpuscles certainly showed both F_2 and F_3, but the ratio F_2/F_3 was low and intermediate between the two groups.

To demonstrate that it was the polysaccharides, which interfered with the histone fractions, an equivalent amount of the acid polysaccharide dextran sulphate was added to thymus-histone attached to the CMC-column. Thereafter no histone could be obtained during the subsequent elution, because the formed histone-polysaccharide complex was not dissociated at the low hydrogen ion concentrations used, Fig. 3*a*. If thereafter an equivalent amount of protamine sulphate was added to the column, the less basic histone is replaced by the more strongly basic protamine in the polysaccharide-complex and the histones can now be eluted quantitatively (Fig. 3*a*). Fig. 3*b* demonstrates the simple chromatographic pattern with protamine alone, which shows that protamine will not interfere with the absorption peaks at 278 mμ of the histone fractions. Figs. 1*b* and 2*b* show the chromatographic pattern after addition of protamine to the histone extract from the two tissues exemplified here. The amount of the F_2-histone has increased, most highly from the polysaccharide-rich extract, 2*b*, while the change in amount of the F_3-histone is only slight. Entirely corresponding results were obtained with the other tissues examined. Thus the F_2-histone has obviously much greater affinity for acid polysaccharides than the F_3-histone.

Thus it seems clear that a quantitative analysis of the native DNA-histone relation cannot be made without a blockading of other acid polyvalent ions, especially of polysaccharide type, and it seems likely that also in the living cell there is the same competition for histones between nucleic acids and acid polysaccharides.

I. P. S. Agrell
E. G. Christensson

Zoophysiological Institute,
University of Lund.

[1] Davison, P. F., James, D. W. F., Shooter, K. V., and Butler, J. A. V., *Biochim. Biophys. Acta*, **15**, 415 (1954).

[2] Porath, J., and Flodin, P., *Nature*, **183**, 1657 (1959).

[3] Porath, J., *Biochim. Biophys. Acta*, **39**, 193 (1960).

[4] Phillips, D. M. P., and Johns, E. W., *Biochem. J.*, **72**, 538 (1959).

Side-Reactions during Hydrogenolysis of 2,4-Dinitrophenylhydrazones of Aromatic Keto-Acids

The identification of the keto-acid 2,4-dinitrophenylhydrazones is greatly facilitated by conversion to their respective amino-acids. The most commonly employed procedure is that first noted by Emil Fischer[1] and modified for application to organic systems by Towers *et al.*[2]. This involves low-pressure hydrogenolysis over a platinum catalyst, followed by paper chromatography of the amino-acids produced. The yields of the respective amino-acids vary between 30 and 98 per cent, and in general the conversion is free from side-reactions.

However, the hydrazones of the dicarboxylic acids, α-ketoglutaric and oxalacetic acids, may undergo some decarboxylation during this reaction. Thus, small quantities of alanine are produced from oxalacetic acid, and both α- and γ-aminobutyric acids, in addition to the expected glutamic acid, are found after hydrogenolysis of the 2,4-dinitrophenylhydrazone of α-ketoglutaric acid[2,3].

During recent work on the keto-acid excretion in phenylketonuria and tyrosinosis[4] another potentially important deviation from the theoretical keto→ amino-acid conversion could be demonstrated.

Hydrogenolysis of the 2,4-dinitrophenylhydrazone of phenylpyruvic acid employing the conditions recommended by Towers *et al.*[2], namely, a 12-hr. hydrogenation in distilled water at 25° C., using 30 lb./in.^{2} pressure, and 0·2 gm. of platinum oxide catalyst, yielded a mixture of two amino-acids. The main product was phenylalanine, but sizeable amounts of a non-aromatic substance with chromatographic properties of cyclohexylalanine could also be found (Table 1).

Table 1. CHROMATOGRAPHIC BEHAVIOUR OF HYDROGENATION PRODUCTS OF KETO-ACID 2,4-DINITROPHENYLHYDRAZONES

Keto-acid 2,4-dinitrophenylhydrazone	R_F of hydrogenation products			Relative intensity	Amino-acid formed
	1*	2†	3‡		
Phenylpyruvic acid	0·56	0·37	0·82	3+	Phenylalanine
	0·74	0·53	0·84	1+	Cyclohexylalanine
p-Hydroxyphenylpyruvic acid	0·39	0·28	0·64	Trace	Tyrosine
	0·37	0·32	0·84	1+	Hexahydrotyrosine
	0·73	0·55	0·84	2+	Cyclohexylalanine

Descending chromatography. No. 40 Whatman paper.

* Solvent 1: *n*-butanol (120 parts); glacial acetic acid (30 parts) water (50 parts).

† Solvent 2: 95 per cent ethanol (180 parts); water (10 parts); concentrated ammonium hydroxide (10 parts).

‡ Solvent 3: Liquid phenol (200 parts); concentrated ammonium hydroxide (1 part).

Spots identified by spraying with a 0·2 per cent solution of ninhydrin in acetone containing 2 per cent pyridine.

The chromatographic behaviour of the products was compared with cyclohexylalanine and hexahydrotyrosine prepared according to Waser and Brauchli (ref. 6).

When the 2,4-dinitrophenylhydrazone of *p*-hydroxyphenylpyruvic acid was hydrogenated under similar conditions, approximately equal amounts of hexahydrotyrosine (4-hydroxycyclohexylalanine) and cyclohexylalanine were obtained. Tyrosine, the expected product, was only present in small amounts (Table 1).

The production of the hydrogenated derivatives of phenylalanine and tyrosine represent an unwelcome side-reaction, which, however, is not unexpected.

Many workers have shown that under the conditions employed for hydrogenolysis of the keto-acid 2,4-dinitrophenylhydrazones, hydrogenation of the aromatic ring can also occur. The rate of hydrogenation of the ring has been found to be first order with respect to hydrogen pressure, zero order with respect to the concentration of the compound to be hydrogenated, and directly proportional to the amount of catalyst used[5]. Thus, under the very conditions used in my experiment, hydrogenation of phenylacetic acid proceeds rapidly, 50 per cent of the starting material being converted to cyclohexylacetic acid within 60 min.[5].

Cleavage of the phenolic group has also been described. Waser and Brauchli[6], in subjecting tyrosine to low-pressure hydrogenation over platinum, obtained a mixture of hexahydrotyrosine and cyclohexylalanine.

Factors favouring the replacement of the phenolic group by hydrogen are hydrogenation in an acidic medium[7], relatively low pressures, and a symmetrical structure for the substance to be hydrogenated[8]. Thus, *para*-substitution favours the replacement of OH-groups, while *ortho*-substitution retards it[9].

In concluding, it must be emphasized that side-reactions such as those reported in this communication do not detract from the usefulness of the hydrogenolysis of the α-keto-acid 2,4-dinitrophenylhydrazones as a step towards their identification.

This work was supported in part by grants *BT* 297(3) and research grant *B*–1620 (awarded to the Division of Neuropathology, College of Physicians and Surgeons, Columbia University) from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

JOHN H. MENKES

Department of Pediatrics,
The Johns Hopkins Hospital,
Baltimore 5,
Maryland.

[1] Fischer, E., and Groh, R., *Ann.*, **383**, 363 (1911).
[2] Towers, G. H. N., Thompson, J. F., and Steward, F. C., *J. Amer. Chem. Soc.*, **76**, 2392 (1954).
[3] Menkes, J. H., *A.M.A., J. Dis. Child.*, **99**, 500 (1960).
[4] Menkes, J. H., and Jervis, G. A., *Pediatrics* (in the press).
[5] Smith, H. A., Alderman, D. M., and Nadig, F. W., *J. Amer. Chem. Soc.*, **67**, 272 (1945).
[6] Waser, E., and Brauchli, E., *Helv. Chim. Acta*, **7**, 740 (1924).
[7] Levin, R. H., and Pendergrass, J. H., *J. Amer. Chem. Soc.*, **69**, 2436 (1947).
[8] Smith, H. A., *Catalysis*, edit. by Emmett, P. H., **5**, 175 (New York, 1957).
[9] Edson, N. L., *J. Soc. Chem. Indust.*, 53, 138 *T* (1934).

PHYSIOLOGY

Function of Taste Fibres in the Carp

THE carp (*Cyprinus carpio* L.) has a palatal organ supplied by nerve fibres running into the glossopharyngeal nerve. By dissecting and isolating small strands of the nerve branches running to the palate, it has been possible to obtain records of the electric response to the application of different sapid solutions to the mucous surface of the palatal organ.

It was found that such nerve preparations responded to a variety of test solutions such as 0·5 *M* sodium chloride, 0·005 *M* acetic acid, 0·01 *M* quinine and 0·5 *M* sucrose. These nerve preparations also responded very strongly to human saliva, as well as to the mucin covering the scales of the fish. The integrated response of such a nerve-strand to human saliva was generally quite as big as the response to 0·5 *M* sucrose and considerably greater than the response to 0·5 *M* sodium chloride.

A study of single fibre responses revealed that the saliva always stimulated fibres which responded positively to sugar. Fibres which responded to sodium chloride and acid or to quinine, but not to sugar, did not respond to human saliva, but for two exceptional cases. According to their response pattern the palatal chemoceptive fibres seem to be divided into six groups as will be seen from Table 1. Among these different fibres the types I, II and III are most common. So far we have only encountered two fibres of type VI.

Besides chemoceptive fibres and motor fibres, the palatal nerves contain fibres which respond very selectively to mechanical stimulation. The number of such fibres seems, however, to be relatively small

Table 1

Fibre type	Sodium chloride	Acetic acid	Quinine	Sucrose	Saliva
I	+	+	−	+	+
II	+	+	+	+	+
III	−	+	−	+	+
IV	+	(+)	−	−	−
V	−	+	+	−	−
VI	−	+	−	−	+

compared with the chemoceptive fibres. A comparison of the chemoceptive response of the palatal nerves with that from the nerves innervating the lips, the barbels and the gill rakers gives us the impression that the palatal nerves are the most important taste nerves of this species.

That human saliva is such a powerful stimulant of the palatal organ gives us, of course, a certain aspect on the angler's habit of spitting on the bait.

JIHEI KONISHI
YNGVE ZOTTERMAN

Department of Physiology,
Veterinärhögskolan, Stockholm 51.

Pharmacology of the Alkaloidal Fraction from the Bark of the Jamaican Shade Tree *Pithecolobium samath* Benth.

A PHARMACOLOGICAL screening programme of extracts of plants used as folk medicines in Jamaica for the preparation of the so-called 'bush teas' is being carried out in collaboration with a group under Prof. L. J. Haynes at the Department of Chemistry, and with Dr. P. C. Feng at the Department of Physiology, University College of the West Indies, Jamaica. The shade tree, *Pithecolobium samath* Benth., was selected for detailed study. The main alkaloid from its bark, pithecolobine, had already been isolated by Wiesner *et al.*[1], who have given its partial structure[2].

A crude alkaloidal fraction was isolated from the powdered bark of the shade tree by extraction with dilute hydrochloric acid. The extract was made alkaline and then washed with chloroform, and these washings were then extracted with dilute hydrochloric acid. The acid extract was made alkaline and re-extracted with chloroform. The chloroform was evaporated to give an oil. Paper chromatography of this oil in several solvent systems showed one major component, presumably identical to pithecolobine, and only traces of a second component were disclosed with Dragendorf's reagent[3].

This fraction (referred to as pithecolobine) when injected intraperitoneally into mice (40 γgm./gm. body-weight, *BALB/C* × *C*57 mice) produced characteristic convulsions within 2–4 min. The stages were: first hypersensitivity to auditory stimuli, followed by muscular twitching and vertical head tremor, clonus first appeared in the hind limbs and then became more generalized with jumping. Between convulsions the animal appeared flaccid with apparent paralysis of the fore and hind limbs. Clonic attacks were followed by rapid respiration, gasping, exophthalmos and death. With lower doses the severe clonic attacks did not occur and surviving animals were subdued for up to 90 min.

The LD_{50} at room temperature (20°) for pithecolobine injected intraperitoneally was 40 γgm./gm. body-weight, the LD_{50} was approximately halved at both 4° and at 36°. Doses of 12 γgm./gm. body-weight of pithecolobine reduced the quinalbarbitone sleeping time of mice by 20 per cent; higher doses (12–18 γgm./gm. body-weight) increased it by up to 100 per cent. The toxicity of pithecolobine for mice was approximately doubled by the doses of quinalbarbitone (450 γgm./gm. body-weight) used for sleeping-time determination. When mice were given pithecolobine intraperitoneally (14 γgm./gm. body-weight) the rectal temperature, normally 37° C., dropped to 33° C.; when given quinalbarbitone alone it dropped to 29° C. and when given both it dropped to 26° C. Intravenous pithecolobine reduced the arterial blood pressure in the anæsthetized cat (4 mgm./kgm.) and rat (1·5 mgm./kgm.) by about 20 per cent for 5 min., both before and after vagotomy. In the anæsthetized rat, hypotensive doses of pithecolobine caused a marked increase in the amplitude and decrease in the rate of respiration. Perfusion of the spinal cord of the frog[4] with pithecolobine (15 γgm./ml.) reduced or abolished the electrically stimulated reflex contractions of the femoral biceps.

The most striking peripheral effect of pithecolobine is its local anæsthetic activity. It is apparently twice as potent as cocaine in the frog lumbar plexus test for local anæsthetics[5]. The intradermal weal test on guinea pigs[5] showed that injection of 1–3 mgm. of pithecolobine caused local anæsthesia for up to 8 hr. Similar results have been obtained in man. Pithecolobine did not produce significant corneal anæsthesia[6] in guinea pigs or rabbits. Pithecolobine appeared to have a generalized depressant effect on isolated organ preparations; for example, on the electrically stimulated phrenic nerve diaphragm[7], on the contractions of the guinea pig ileum induced by acetylcholine, histamine, nicotine and barium, on the contractions of the rat fundus strip induced by 5-hydroxytryptamine[8], and on the Langendorff preparation of the isolated rabbit heart.

The nature of the convulsions caused by pithecolobine and its effects on respiration, sleeping-time, and possibly temperature, suggest that this substance has some action on the brain stem. These pharmacological actions of pithecolobine are of particular interest because of the very unusual structure[2] of this alkaloid. Chemical studies of the alkaloids of the shade tree are under way at the University College of the West Indies and we hope to investigate fully both the pharmacology and biochemistry of pure alkaloids from this source.

We wish to thank Prof. A. C. Frazer for his interest and encouragement, and Dr. R. Schneider, Dr. M. R. A. Chance and Dr. K. Sargeant for valuable advice and help. This investigation was supported by the Tropical Products Institute, Department of Scientific and Industrial Research.

Note added in proof. Further work suggests that the local anæsthesia produced by intradermal injections of pithecolobine is due to damage to nerve endings.

B. E. LEONARD
H. S. A. SHERRATT

Department of Medical Biochemistry and
Pharmacology, University of Birmingham.

[1] Wiesner, K., MacDonald, D. M., Valenta, Z., and Armstrong, R., *Canad. J. Chem.*, **30**, 761 (1952).

[2] Wiesner, K., and Orr, D. E., *Tetrahedron Letters*, **16**, 11 (1960).

[3] Munier, R., and Machboeuf, M., *Bull. Soc. Chim. Biol.*, **31**, 1144 (1949).

[4] Angelucci, L., *Brit. J. Pharmacol.*, **11**, 161 (1956).

[5] Bülbring, E., and Wajda, I., *J. Pharm. Exp. Therap.*, **85**, 78 (1945).

[6] Chance, M. R. A., and Lobstein, H., *J. Pharmacol. Exp. Therap.*, **12**, 344 (1957).

[7] Bülbring, E., *Brit. J. Pharmacol.*, **1**, 38 (1946).

[8] Vane, J. R., *Brit. J. Pharmacol.*, **12**, 344 (1957).

Urea Synthesis in the Living Rat Brain

In previous communications from this laboratory, the synthesis of urea-^{14}C from arginine-^{14}C was reported to occur in the living rat brain[1,2]. In these experiments only the rat brains were analysed for their radioactive urea content, and the objection was raised that perhaps these levels did not indicate endogenous brain urea synthesis, but were merely a reflexion of blood ^{14}C-urea levels, especially since it is known that urea readily diffuses across the blood brain barrier. Experiments were therefore designed to show that the brain ^{14}C-urea levels were independent of blood ^{14}C-urea levels and that the brain had the capacity to synthesize urea from arginine. This communication describes such experiments.

Guanido-^{14}C labelled L-arginine-hydrochloric acid was obtained through the courtesy of the California Corporation for Biochemical Research before it was commercially available and was further purified by descending paper chromatography in two consecutive solvent systems. Solvent 1: pyridine/acetic acid/water (50 : 35 : 15). Solvent 2: ethanol/water/diethylamine (76 : 23 : 1). Identification was determined using the ninhydrin spray[3], and a modification of the Sakaguchi test[4] in conjunction with coincidence of radioautography. The R_F of the arginine in the above two solvent systems was 0·40 and 0·46 respectively. The purity of the arginine-^{14}C was verified by chromatography of the arginine eluate from the second solvent system in separate solvent systems of 2,6-lutidine/collidine/water/diethylamine (1 : 1 : 1 : 2 per cent v/v), propanol/acetic acid/water (75 : 5 : 20) and 2,4-lutidine/collidine/water/diethylamine (1 : 1 : 1 : 2 per cent v/v), propanol/acetic acid/water (75 : 5 : 20) and 2,4-lutidine/collidine/acetic acid/water (1 : 1 : 5 per cent v/v : 2).

A 90-day-old male albino rat (Sprague–Dawley strain) was lightly anæsthetized with diethyl ether and the exposed saphenous vein was cannulated with a heparinized, polyethylene cannula. 5 min. later an intracisternal injection of 0·4 mgm. of guanido-^{14}C labelled L-arginine-hydrochloric acid was administered using a micro-calibrated syringe (total activity 10 μc. = 7·3 × 10^6 counts/min.). At intervals following the injection, 0·5 ml. samples of blood were withdrawn from the vein and analysed for the specific activity of radioactive urea. 60 min. after the injection, the animal was killed by decapitation and the brain analysed for radioactive urea. As a control for possible injection of urea-^{14}C as a contaminant of the arginine preparation as well as a control for urea formation from arginine as a result of the procedures employed, a litter-mate rat was killed and to the isolated brain was added 0·4 mgm. of guanido-^{14}C labelled L-arginine-hydrochloric acid. The brain was treated as described here.

Following the withdrawal of the blood samples from the experimental animal, 5 ml. of 15 per cent trichloracetic acid (TCA) were added and the samples were homogenized. 8 mgm. of unlabelled carrier urea were then added to the homogenates; the samples were centrifuged and the supernatant solutions were decanted and saved. The TCA from these fractions was then extracted with carbon tetrachloride. The samples were then taken almost to dryness with a rotary evaporator and the insoluble dixanthydrol derivative of urea was prepared and recrystallized to constant specific activity. The brain was homogenized and urea was isolated from the acid-soluble fraction by ion-exchange column chromatography. A derivative was prepared as described here.

Table 1. Specific Activity of Blood* and Brain* Urea Samples

Sample	Time (min. after injection)	Specific activity of urea (counts/min./mgm. urea)
Blood 1	18	3 × 10^3
2	20	3 × 10^3
3	30	3 × 10^3
4	40	5 × 10^3
5	50	6 × 10^3
6	57	7 × 10^3
Brain	60	250 × 10^3

* The whole rat brain contains 0·5 mgm. urea, and 0·5 ml. rat blood contains 0·29 mgm. urea.

Analyses for radioactivity and urea content were performed in a manner reported previously[1].

The results presented in Table 1 show that the specific activity of the brain urea is at least thirty times as great as the highest blood urea specific activity. The maximum urea-^{14}C contamination as determined by the specific activity of urea in the control brain is 6 × 10^3 counts/min./mgm. urea. If the brain urea were derived only from exogenous sources and was transported to the brain via the blood, then the brain urea specific activity could not be higher than that of the blood[5]. The fact that it is many times greater than the blood level, at all intervals in which blood was collected, is proof of endogenous brain urea synthesis from arginine.

Note: Ratner *et al.* have shown recently in partially purified rat-brain proteins that arginase is present; this is verified by our *in vivo* work[6].

This investigation was supported by a research grant *M*-4356 (*A*) from the National Institute of Mental Health.

R. K. Davies*
A. J. Defalco*
D. Shander*
A. Kopelman*
J. Kiyasu

Departments of Psychiatry and Biochemistry,
University of Rochester,
School of Medicine and Dentistry,
Rochester, New York.

---

* Scottish Rite Summer Medical Student Research Fellows.

[1] Sporn, M. B., Dingman, W., Defalco, A., and Davies, R. K., *J. Neurochem.*, **5**, 62 (1959).

[2] Sporn, M. B., Dingman, W., Defalco, A., and Davies, R. K., *Nature*, **183**, 1520 (1959).

[3] Toennies, G., and Kolb, J. J., *J. Anal. Chem.*, **23**, 823 (1951).

[4] Berry, H. K., Sutton, H. E., Cain, L., and Berry, J. S., *Univer. of Texas Publication 5109*, 22 (1951).

[5] Zilversmit, D. B., Entenman, C., and Fishler, M. C., *J. Gen. Physiol.*, **26**, 352 (1943).

[6] Ratner *et al.*, *Archiv. Biochem. and Biophys.*, **91**, 280 (1960).

Antibody Response in Rabbits following Injection of Sheep Erythrocytes into Lateral Ventricle of Brain

Immunological studies of the cerebrospinal fluid have been confined mainly to the passage of antibodies from plasma into the cerebrospinal fluid[1]. However, the investigation of exchange between cerebrospinal fluid and blood has revealed that various electrolytes[2], dyes[3] and proteins[4] may find their way from cerebrospinal fluid into general circulation. Furthermore, some data[5] indicate that red blood cells cross cerebrospinal fluid-blood barrier. It seemed, therefore, advisable to approach the immunological sequences following the injection of heterologous erythrocytes into lateral ventricle of the brain.

Table 1. PEAK AGGLUTININ AND HÆMOLYSIN TITRE ($\log_2$) IN THE SERUM AND CEREBROSPINAL FLUID (CSF) OF RABBITS RECEIVING SHEEP ERYTHROCYTES INTO THE LATERAL VENTRICLE OF THE BRAIN

Rabbit No.	Agglutinin									Hæmolysin								
	Before injection of sheep red cells		Day after the last injection of sheep red cells							Before injection of sheep red cells		Day after the last injection of sheep red cells						
			2	4	6	8	10	12*				2	4	6	8	10	12*	
	Serum	CSF	Serum						CSF	Serum	CSF	Serum						CSF
4	2	0	2	2	3	4	5	5	0	5	0	11	13	14	12	10	9	1
6	1	0	3	5	6	6	7	6	0	5	0	11	13	14	15	14	12	1
7	1	0	3	5	5	5	5	4	0	8	0	12	14	12	10	10	9	1
8	1	0	1	3	4	4	4	3	0	8	0	9	9	11	11	11	10	0

* Day when the rabbits were killed.

Young adult rabbits weighing 2·8–3 kgm. were used. A permanent cannula described by Feldberg and Sherwood[6] was implanted aseptically under pentobarbitone anæsthesia into the left lateral ventricle of the brain. The cannula was inserted at right angles to the bone, 7–8 mm. deep. The point of insertion lay 2 mm. lateral from the sagittal suture and 2 mm. behind the coronal suture. After the cannula was screwed into the skull and communication with the ventricular fluid was established, it was fixed to the bone by 'Texton' (S. S. White Co. of Great Britain, Ltd., London). Four days later the rabbits were treated for three successive days with a 5 per cent suspension (spectrophotometrically standardized) of washed sheep red cells. Two injections of 0·3 ml. each were performed daily, separated by a 1-hr. interval. The injections were all made into the lateral ventricle of the brain. After the last injection of antigen rabbits were bled every second day. The sera were heated at 56° C. for 20 min. and stored at −20° C. until titrated. Twelve days after the last injection the cerebrospinal fluid was withdrawn from the cisterna magna. The cerebrospinal fluid obtained was perfectly clear and not contaminated with red cells. Rabbit sera and cerebrospinal fluids were investigated for the presence of hæmolysin and agglutinin. The hæmolysin titration was essentially the same as described by Kabat and Mayer[7]. Hæmolytic activity was measured in 50 per cent units in the presence of five 50 per cent units of complement. Veronal buffered saline containing optimal concentration of magnesium and calcium ions was used for all washings and dilutions. The agglutinin content of sera and cerebrospinal fluids was determined by a semi-quantitative agglutination technique. Samples of rabbit sera and cerebrospinal fluids taken prior to the administration of sheep red cells were also investigated for the presence of hæmolysin and agglutinin.

Table 1 summarizes the results. It was found that cerebrospinal fluid, prior to the injection of antigen, did not exhibit hæmolysin and agglutinin activity, although natural antibodies were present in the serum. However, when the concentration of hæmolysin in the blood was sufficiently high it was possible to detect small amounts of these antibodies in the cerebrospinal fluid[8]. The injection of sheep erythrocytes into the cavity of rabbit brain was accompanied by an increase in antibody formation, the production of hæmolysin being much higher than that of agglutinin. Despite a high concentration of hæmolysin in the serum, only traces of that antibody were found in the cerebrospinal fluid. In a preliminary work it was noticed that rabbits which received only one intraventricular injection of sheep red cells manufactured specific antibodies at a very low level.

Bowsher[4] demonstrated that radioactive homologous serum protein, when injected into the subarachnoid space or ventricular cavities of the cat brain, was absorbed by different routes (leptomeningovascular, perineuro-lymphatic and/or ependymal). In rabbits we have also observed (unpublished experiments) that human γ-globulin inoculated into lateral ventricle may find its way to the general circulation, and provoke the appearance of specific precipitin in the serum. With rabbit serum taken 6 days after antigen injection a well-defined precipitin line was revealed by diffusion gel technique. The clearance of red cells from the cerebrospinal fluid, however, seems to have a separate mechanism. Essick[5] states that mesothelial cells may act as macrophages. Dupont *et al.*[9], using tagged red cells, have recently shown that the transfer of erythrocytes from cerebrospinal fluid to plasma is carried out by the mesothelial-lining cells of the subarachnoid space. These cells phagocytize erythrocytes and migrate towards the blood, thus causing slow appearance of radioactivity in the circulation. It may be supposed, therefore, that this slow removal of red cells from the cerebrospinal fluid to blood may act as a prolonged stimulus on antibody-producing apparatus, resulting in a significant increase of antibody in the serum. Finally, it seems that the intraventricular route of antigen administration offers a useful tool in the hands of an immunologist, and provides a new approach to the immuno-physiological examination of the central nervous system.

B. D. JANKOVIĆ*
M. DRAŠKOCI
K. ISAKOVIĆ

Microbiological Institute, and
Pharmacological Institute,
Faculty of Pharmacy,
Bulevar JNA 10, Belgrade.

---

* Present address: Neurology Research Laboratory, Massachusetts General Hospital, Boston, Mass.

[1] Stern, L., Kassil, G. N., Lockshina, E. S., Romel, E. L., and Zeitlin, S. M., *C.R. Soc. Biol., Paris*, **99**, 360 (1928).

[2] Krogh, A., *Proc. Roy. Soc.*, B, **133**, 140 (1945). Behring, E. A., *J. Neurosurg.*, **11**, 234 (1954).

[3] Weed, L. H., *J. Med. Res.*, **26**, 21, 51 (1914). Spirov, M., *Arch. Russ. Anat. Histol. Embryol.*, **6**, 257 (1927).

[4] Courtice, F. C., and Simmonds, W. J., *Austral. J. Exp. Biol. Med. Sci.*, **29**, 255 (1951). Fishman, R. A., *Amer. J. Physiol.*, **175**, 96 (1953). Bowsher, D., *Anat. Rec.*, **128**, 23 (1957). Van Wart, C. A., Dupont, J. R., and Kraintz, L., *Proc. Soc. Exp. Biol. Med.*, **103**, 708 (1960).

[5] Essick, C. R., *Contrib. Carnegie Inst. Washington*, **9**, 379 (1920). Simmonds, W. J., *Austral. J. Exp. Biol. Med. Sci.*, **30**, 261 (1952). Simmonds, W. J. *ibid.*, **31**, 77 (1953).

[6] Feldberg, W., and Sherwood, S. L., *J. Physiol.*, **120**, 3 P (1953).

[7] Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry*, 130 (Charles C. Thomas Pub., Springfield, 1948).

[8] Davson, H., *Physiology of the Ocular and Cerebrospinal Fluids*, 207 (J. and A. Churchill, Ltd., London, 1956).

[9] Dupont, J. R., Van Wart, C. A., and Kraintz, L., *J. Neuropath. Exp. Neurol.* (in the press).

Relationship between the Decrease in Urinary Excretion of Histamine and the Initiation of the Labour Process

As has been shown by Kahlson *et al.*, in rats fed on a histamine-free diet, the urinary excretion of histamine begins at about the fifteenth day of pregnancy and increases rapidly. The peak value occurs 1 or 2 days before birth, then falls steeply to the pre-pregnant level. The increase in histamine excretion depends on the number of young in the litter; the larger the number, the greater the increase in histamine excretion[1].

Investigations were made to demonstrate the relationship between the decrease in histamine excretion and the initiation of the labour process. Observations were made on white laboratory rats, fed on a normal diet. The method described in detail by McIntire, Roth and Shaw[2] was adapted to measure the amount of histamine in urine.

Table 1. Mean Daily Excretion of Histamine (μgm.)

		Control animals	Experimental	
Before pregnancy		50	50	
Days after mating	15	120	130	
	20	880	750	
	21	104	700	Administration of histamine
	22	60	750	
	23	—	200	
	24	—	60	
	25	—	100	

Two days before the time of the expected parturition, that is, just before the onset of the period of low histamine excretion, 1·5 mgm. of histamine hydrochloride daily, in three doses, was injected subcutaneously during light ether anæsthesia. In this way the pretermal decrement in urinary histamine was prevented, and the high daily excretion (500–850 μgm.) was maintained artificially for two days.

It has been observed that, after the last injection of histamine, urinary excretion fell gradually during 1·5–2 days to about the pre-pregnant level. The labour process began about 30 hr. after decrease of the histamine-level. In this way the pregnancy was prolonged for two days. Some of the young in the litter from such a prolonged pregnancy were born dead; others showed after-birth weak respiratory movements, and died soon afterwards.

Halina Wiskont-Buczkowska
Department of Physiology,
Medical Academy Gdańsk,
Poland.

[1] Kahlson, G., Rosengren, Elsa, and Westling, H., *J. Physiol.*, **148**, 91 (1958).

[2] McIntire, Floyd C., Roth, L. W., and Shaw, J. L., *J. Biol. Chem.*, **170**, 537 (1947).

RADIOBIOLOGY

Estimation of Time of Generation of Living Cells

The use of tritiated thymidine as a labelling agent has greatly facilitated examination of nuclear reproduction and cellular proliferation[1–4]. In such work, it has been customary to focus attention on the proliferation and fate of the cells that are labelled. Mendelsohn, however, has studied the fate of both labelled and unlabelled cells in tumours of mice and has used data obtained from both the labelled and unlabelled cell populations to derive the ratio of proliferative cells to total cells (the growth fraction)[5,6]. In the method presented here, applicable whenever a cohort of cells (that is, a fraction of the population) is labelled, the data for the labelled and unlabelled cells are analysed separately. In this way more information is obtained than when either only the labelled cells are studied or when data for the two cell populations are combined. The generation time of both labelled and unlabelled cells can be determined and compared, thus providing a method by which one aspect of toxicity of tritiated thymidine can be examined.

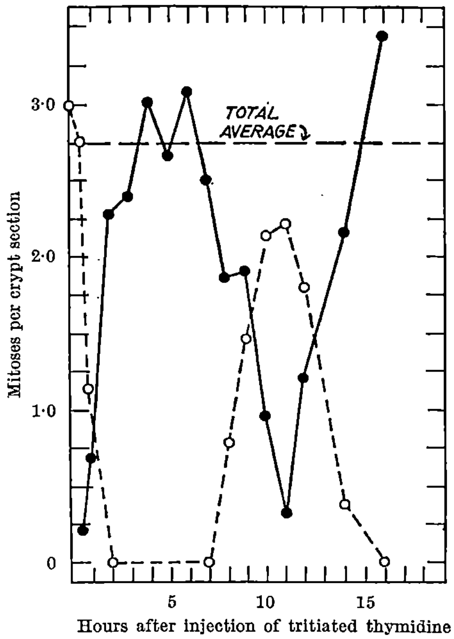


Fig. 1. Number of mitotic figures per crypt section as a function of time after injection of tritiated thymidine: dashed curve, unlabelled figures; solid line, labelled figures. 'Total average' represents the total number of mitotic figures per crypt section averaged over the 16-hr. observation period

The following experiment illustrates the use of this method in the determination of generation time and mitotic time of the labelled cells, and generation time of the unlabelled cells. CAF_1 mice, 93 days old, (weighing 25 gm.) received intraperitoneally 50 μc. of tritiated thymidine (360 mc./mM). Thereafter at intervals up to 16 hr., 1 or 2 animals were killed to obtain samples of the duodenum. Autoradiographs were made[7] of 3μ duodenal sections pre-stained by the Feulgen reaction. For each time of killing, the numbers of labelled and unlabelled mitotic figures (all stages) were counted in about 20 crypts of Lieberkühn (about 1,000 cells). The mean numbers of labelled (M_l) and unlabelled (M_u) mitotic figures per crypt section were calculated from these data and plotted as a function of time (Fig. 1).

Generation Time. In the case of the unlabelled cohort, the curve traced by M_u began at 3·0 mitoses per crypt section; at 2 hr. after injection the value was zero; the value began to increase again at 7 hr., and a peak value of 2·2 was reached at 11 hr. Thus a cycle was completed in 11 hr., which is an estimate of the generation time. If the duration had been measured between similar points (in the range $M_u = 1·5–0·5$) on the descending limbs of two successive cycles, it would have been about 12 hr. We therefore took the generation time to be 11·5 hr.

In the case of the labelled cohort, the curve traced by M_L began at zero and after passing through a peak of about 3 mitoses per crypt section (in agreement with the initial value for the unlabelled cohort) returned to a minimum value at 11 hr. The generation time was therefore estimated as 11 hr., in good agreement with that for the unlabelled cohort. The estimate based on the time elapsing between successive M_L values of 3 (approximately the maximum) was 11·3 hr. We therefore took the generation time to be 11·2 hr. This result is in good agreement with our previously reported figure of 11·5 hr.[8].

For 2–9 hr. inclusive, the values of M_L (1·85–3·07, Fig. 1) had standard errors of 0·25–0·54. The full statistical analysis of these and further data will be discussed in a later report.

Mitotic Time. The mitotic time T of the labelled cells can be estimated as follows: $T = G\bar{I} = G\bar{N}/P$ where for the labelled cohort, G is generation time, $\bar{I}$ is mean mitotic index, $\bar{N}$ is the average number of cells in mitosis (per crypt section) throughout the generation cycle, and P is the number of proliferative cells (per crypt section).

The generation time of the labelled cells (G_L) was 11·5 hr. The average number of labelled cells in mitosis ($\bar{N}_L$) was determined by taking the average of 11 consecutive hourly determinations of labelled cells in mitosis (M_L) (Fig. 1); $\bar{N}_L = 1{\cdot}91$. The number of labelled proliferative cells (P_L) was taken as the number of labelled cells (per crypt section) ½ hr. after the injection of tritiated thymidine, when the initial labelling was maximal and no labelled cells had gone through mitosis; $P_L = 15{\cdot}5$.

From these data it follows that T (for labelled cohort) $= 11{\cdot}2 \times 1{\cdot}91/15{\cdot}5 = 1{\cdot}38$ hr.

The same procedure can be used to obtain the mitotic time of the unlabelled cells when the number of unlabelled proliferative cells can be accurately assessed. As yet we have not established the number of unlabelled proliferative cells at the necessary time-intervals with sufficient accuracy to calculate the mitotic time of the unlabelled cells.

The agreement between the results for the labelled and unlabelled cells indicates that the dose of tritiated thymidine used (50 μc. per animal) did not affect the generation time. It should be noted that this result applies only to that part of the first generation-cycle that has been studied. It remains to be determined whether or not the duration of subsequent cycles is affected.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

R. J. M. Fry
S. Lesher
H. I. Kohn

Division of Biological and Medical Research,
Argonne National Laboratory,
Argonne, Illinois,
and
Radiological Laboratory,
University of California Medical School,
San Francisco.

---

[1] Hughes, N. L., Bond, V. P., Brecher, G., Cronkite, E. P., Painter, R. B., Quastler, H., and Sherman, F. G., *Proc. U.S. Nat. Acad. Sci.*, **44**, 476 (1958).

[2] Leblond, C. P., and Messier, B., *Anat. Rec.*, **132**, 247 (1958).

[3] Patt, H. M., and Maloney, M. A., in *Kinetics of Cellular Proliferation*, edit. by Stohlman, jun., F., 201 (Grune and Stratton, New York, 1959).

[4] Baserga, R., Kisieleski, W. E., and Halvorsen, K., *Cancer Res.*, **20**, 910 (1960).

[5] Mendelsohn, M. L., *J. Nat. Cancer Inst.*, **25**, 485 (1960).

[6] Mendelsohn, M. L., *Science*, **132**, 1496 (1960).

[7] Pelc, S. R., *Intern. J. App. Rad. and Isotopes*, **1**, 172 (1956).

[8] Lesher, S., Fry, R. J. M., and Kohn, H. I., *Exp. Cell Res.* (in the press).

Leukæmia in the *AkR* Mouse after X-Irradiation *in utero*

It is known that the incidence of lymphoid leukæmia in mice is influenced by X-irradiation of the adult[1]. In strains where the spontaneous incidence of the disease is low, it is increased; but it may be decreased when the incidence is already high[2]. In the high-leukæmia strain *AkR* mouse, it has been shown that, after doses of X-rays of between 50 and 500 r. to the new-born animal, there is a reduction of the usually high incidence of leukæmia, slightly among females but more markedly among males even at the lowest doses[3].

The work reported here is part of an investigation of leukæmia after small doses of X-rays to the mouse fœtus. Retrospective and prospective studies of leukæmia after diagnostic X-rays to the fœtus in man have given conflicting results[4]. The present experiment was designed to determine whether there is an alteration of incidence of leukæmia or latent period in the high-leukæmic strain *AkR* mouse after small doses of X-rays to the fœtus.

AkR female mice were mated to *AkR* males which were their respective litter mates and then put into one of three treatment groups after the appearance of copulation plugs: (1) those to be sham irradiated; (2) those to receive 5 rads at the level of the gravid uterus on day 7½ and again on day 14½ of pregnancy; (3) those to receive 25 rads at the level of the gravid uterus on day 7½ and again on day 14½ of pregnancy.

The X-ray source was a 'Resomax' operating at 300 kV. and 13 m.amp. (filtration 0·1 mm. tin + 0·5 mm. copper + 1·0 mm. aluminium). The dose-rate was 5 rads per min., at a treatment distance of 140 cm. The females were allowed to rear their litters, which after weaning at 6 weeks were kept 6 per cage (2 from each treatment group) under identical conditions, males and females separately.

All animals were observed and eventually killed when moribund. Diagnosis of leukæmia was verified by autopsy, blood count and blood and bone marrow smears. Any doubtful cases were investigated by bioassay. The histological appearance of the leukæmia occurring in all groups conformed to that of the spontaneous lymphoid leukæmia characteristic of this strain.

A total of 353 animals was observed, 178 males and 175 females, progeny alive at the time of the first case of leukæmia at 180 days and deemed to be at risk. Table 1 contains a summary of results obtained after all the animals had died. No real difference in incidence of leukæmia can be shown in the female groups. In the males, however, the group treated with 50 rads shows a lower incidence of leukæmia than that of the control or of the 10-rad groups.

The mean time to death from leukæmia in the female groups seems to be unaffected by irradiation. In the males, however, the mean time in the 50-rad group is longer than in the 10-rad or control groups. (An analysis of variance shows this lengthening to be significant at the 10 per cent level.) The times to death from leukæmia for individuals in each group show a normal distribution against the logarithm of the time. The tail of the distribution curve suggests that all animals would have succumbed to leukæmia if death from other causes had not intervened. In order to arrive at a more realistic mean for time to death from leukæmia in each group, allowance should be made for animals dying from other causes. The individual times of death for such animals have been

Table 1

Total dose (rads)	No. of animals at risk		Per cent leukæmia		Corrected mean time to death from leukæmia (days)	
	Males	Females	Males	Females	Males	Females
0	68	62	86·8	96·7	340 (333)±11	288 (288)±10
10	56	58	85·9	89·6	341 (333)±12	292 (290)±10
50	54	55	72·2	90·9	378 (362)±15	297 (294)±10

adjusted on the basis of a preliminary estimate of the distribution. The correction involves an addition of small magnitude which gives the probable time of death from leukæmia if the other cause had not operated. Corrected means are shown in the right-hand column of Table 1 with uncorrected means in parentheses.

It would appear that, after the doses of X-rays used, there is no evidence of either increase in leukæmia incidence or of accelerated disease in either males or females irradiated *in utero*. On the contrary, in males after 50 rads there may be a decrease in incidence of leukæmia with an increased mean time to death from leukæmia.

DONALD PORTEOUS

Radiobiology Department,
Christie Hospital and Holt Radium Institute,
Manchester, 20.

---

[1] Krebs, C., Rask-Nielsen, H. C., and Wagner, A., *Acta Radiol.*, Supp. 10, 1 (1930). Furth, J., and Furth, O. B., *Amer. J. Cancer*, **28**, 54 (1936).

[2] Kirschbaum, A., and Mixer, H. W., *J. Lab. and Clin. Med.*, **32**, 720 (1947).

[3] Reverdy, J., Rudali, G., Duplan, J. F., and Latarjet, R., *Le Sang*, **29**, 796 (1958).

[4] Stewart, A., Webb, J., Giles, D., and Hewitt, D., *Lancet*, ii, 447 (1956). Stewart, A., Webb, J., and Hewitt, D., *Brit. Med. J.*, i, 1496 (1958). Court Brown, W. M., Doll, R., and Bradford Hill, A., *ibid.*, ii, 1539 (1960).

HÆMATOLOGY

Hæmagglutinins from *Clerodendrum viscosum* Vent

THE capacity of certain plant proteins closely to simulate the action of various human blood group specific agglutinins is well known; the subject has been reviewed by me[1]. Almost all strong blood-group-specific plant agglutinins have been obtained from the genus Leguminosae; recently a good anti-A (anti-A_1) was found in the seeds of *Hyptis sauveolens* Poit of the genus Labiatiae[2]. Examination of other non-leguminous plants has now revealed specific agglutinins in the fruit pulp of *Clerodendrum viscosum* Vent (syn. : *C. petasites* Lour; *C. infortunatum* Cook *et al.*, non-Gaert and non-Linn), of the natural order Verbenaceae, a gregarious shrub commonly found throughout India, Burma, Ceylon and the Andaman Islands.

The fruit is about the size of a large garden pea. It has a relatively big seed which contains weak non-specific agglutinins. The fruit pulp was carefully separated from the seed, ground in three times its volume of physiological saline, and filtered. The filtrate, when tested for avidity by the tile method, and titrated by the conventional tube method, was found to agglutinate red cell suspensions of various ABO blood-groups and sub-groups in the following order: O (strongest), A_2, A_2B, B, A_1, A_1B; H-negative cells were not agglutinated. The reactions were not influenced by the Le^a or Le^b status of the erythrocytes. Thus the agglutinins of *C. viscosum* are anti-H or anti-O.

The agglutinin is inhibited, but not completely neutralized, by a 1/1,000 (w/v) aqueous solution of purified H-substance; it is not inhibited by identical concentrations of similarly purified A, B or Le^a substances. Similarly, it is inhibited, but not neutralized, by secretor salivas; it is not inhibited by non-secretor salivas. The agglutinin is inhibited (two tubes) by 2 per cent salicin; it is not inhibited by 2 per cent L-fucose. It thus belongs to the little-understood group of anti-H agglutinins, which are not inhibited by L-fucose, a sugar which is an important structural determinant of H-specificity[3].

The *Clerodendrum viscosum* agglutinin would be useful for making the A_1: A_2 distinction, particularly when used in parallel with the anti-A_1 agglutinin from *Dolichos biflorus* seeds. It is not recommended for general use in making the secretor : non-secretor distinction, for which the anti-H agglutinin from *Ulex europaeus* seeds would be more reliable. However, it could be used for this purpose by those familiar with its properties, particularly in countries such as India, in which other plant sources of anti-H are not readily available. It might also be useful, as an addition to the heterogeneous ranks of anti-H reagents, in research on the H-character or characters of erythrocytes and secretions.

I am indebted to Prof. W. T. J. Morgan, for the blood-group substances and sugars; to V. B. Kumar, for collecting the plants and for technical assistance; and to the Botanical Survey of India, Poona, for the identification.

G. W. G. BIRD

Blood Transfusion Department,
Armed Forces Medical College,
Poona, India.

---

[1] Bird, G. W. G., *Brit. Med. Bull.*, **15**, 165 (1959).

[2] Bird, G. W. G., *Brit. J. Hæmat.*, **6**, 151 (1960).

[3] Morgan, W. T. J., and Watkins, W. M., *Brit. J. Exp. Path.*, **34**, 94 (1953).

Hæmoglobin *P* in a Family of Southern Italian Extraction

THE larger part of abnormal hæmoglobins described in world literature has been observed, in sporadic form, among the people of Italy. As a matter of fact, Silvestroni and Bianco *et al.* have identified[1–3] not only many cases of hæmoglobin S, the presence of which in Italy was known for some time, but also cases of hæmoglobins L, D, G, K, H and A_2 abundant in non-microcythæmic (or thalassæmic) subjects.

Now another abnormal hæmoglobin, P, has been found in four members of an Italian family originating from Calabria. During work on the electrophoresis of the hæmoglobin of microcythæmics, a carrier of microcythæmia (or thalassæmia minima), F. Bettina, aged seventeen, was first found, and later three of her brothers (F. Francesco, aged

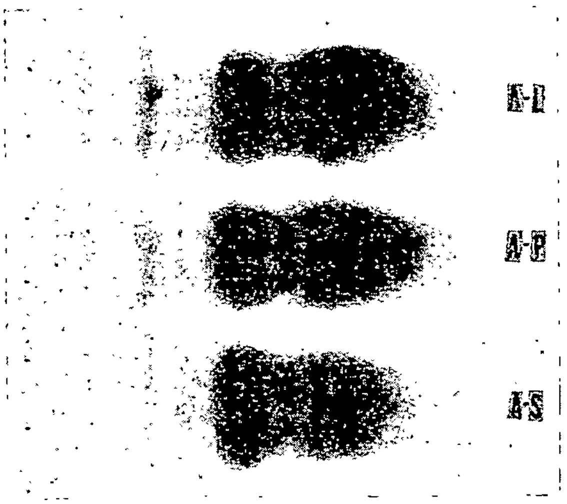


Fig. 1. Paper electrophoresis of hæmoglobins *A–L*, *A–P* and *A–S*, glycine buffer *p*H 8·8, ionic strength 0·04. Stained with bromophenol blue. Mobility of the hæmoglobin slow fraction identical in the three samples

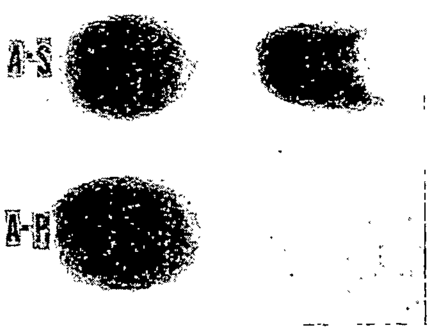


Fig. 2. Electrophoresis in agar gel of hæmoglobins *A–S* and *A–P*. Citrate buffer *p*H 6·2, ionic strength 0·05. Stained with bromophenol blue. Complete lack of separation of hæmoglobin *P* from hæmoglobin A_1

twenty-three ; F. Giuseppina, aged twenty-seven ; F. Maria, aged fifteen) and their grandfather on the mother's side (T. Francesco, aged seventy) were found carriers of a slow hæmoglobin ; all four were enjoying good health and their hæmatological pattern was perfectly normal, without presenting any character reflecting the simultaneous presence of microcythæmia, the fraction of hæmoglobin *a–r* being below 2 per cent and that of hæmoglobin A_2 between 2 and 2·60 per cent, whereas in the microcythæmic member it was 4·50 per cent. The slow hæmoglobin was present in all four subjects to the extent of about 50 per cent.

An investigation to determine the characters of this abnormal hæmoglobin has produced the following results :

(1) In paper electrophoresis at *p*H 8·6, the mobility of the slow hæmoglobinic fraction was equal to that of hæmoglobins *S*, *D* and *L* (Fig. 1).

(2) Even on starch-block and on starch-gel electrophoresis at *p*H 8·6 the slow hæmoglobin separated from hæmoglobin A_1 with the same mobility as that of hæmoglobin *S*.

(3) In paper electrophoresis and starch gel at *p*H 6·2–6·5 hæmoglobin A_1 and the slow hæmoglobin did not separate, and the hæmoglobin spot had the same aspect as that of hæmoglobin A_1 only.

(4) Even in agar gel at *p*H 6·2 the slow hæmoglobin did not separate from hæmoglobin A_1 (Fig. 2), contrary to hæmoglobins *S* and *L*, and like hæmoglobin *D*.

(5) In column chromatography using 'Amberlite *IRC*–50' at *p*H 6, the slow fraction did not separate from hæmoglobin A_1; the hæmoglobin band was, however, much wider than that of hæmoglobin A_1 alone, extending from this to the band of hæmoglobins A_2, *G* and *S* (Fig. 3).

(6) The ultra-violet spectrum of the slow hæmoglobin, isolated after starch block electrophoresis, was quite normal and identical with that of hæmoglobin A_1 of the same subject.

(7) In alkaline denaturation test, the isolated slow hæmoglobin behaved as hæmoglobin A_1 and denatured completely within 60 min.

These characters of the slow hæmoglobin examined correspond clearly with those described for hæmoglobin *P*[4–6]; in particular, the more specific characters of this hæmoglobin and which more clearly distinguish it from the other slow hæmoglobins (failure to separate from hæmoglobin A_1 in agar gel and during chromatography at acid *p*H) are certainly present in the hæmoglobin under examination.

The only discordant point appears to be that of electrophoretic mobility at alkaline *p*H, which in the initial observation of Schneider and Haggard[4,5] was defined as equal to that of hæmoglobin *L*, even though it has been observed in analyses between glass plates or electrophoresis of less than 18 hr. duration, that "its pattern was indistinguishable from that of hæmoglobin *A–S*". In previous analyses of other slow hæmoglobins, Silvestroni and Bianco *et al.*[3] have been able to demonstrate that the mobility of a slow hæmoglobin fraction is, within certain limits, dependent on its concentration in the hæmoglobin mixture, that is, the lower the aforesaid concentration, the more it decreases. This fact has been fully confirmed also for the present slow hæmoglobin, and comparisons of electrophoretic mobility have therefore always been made with mixtures of hæmoglobins containing the same percentage of slow hæmoglobin.

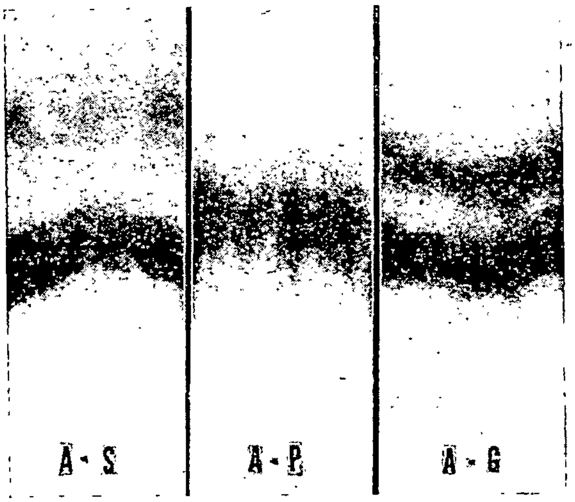


Fig. 3. Chromatography on columns of 'Amberlite *IRC*-50' of hæmoglobin *A–S*, *A–P* and *A–G*. Lack of separation of hæmoglobin *P* from A_1 and formation of a diffused hæmoglobin band

Therefore, as the difference of mobility is very slight and also so easily affected by the 'concentration factor', it can be stated, in conclusion, that it cannot have any value.

Hence the hæmoglobin described here is certainly a new case of hæmoglobin *P*: it seems that this is the fourth observation described in the literature after that of Schneider and Haggard[4,5], Dherte *et al.*[6] and Lambotte-Legrand *et al.*[7]; the second in which hæmoglobin *P* is present without any association of other slow hæmoglobins (*S*); and it is the first case of hæmoglobin *P* found in Italy.

E. Silvestroni
I. Bianco

Istituto d'Igiene, Università di Roma, Centro di Studi della Microcitemia e delle Anemie microcitemiche.

C. Brancati

Sezione di Cosenza del Centro Studi della Microcitemia e delle Anemie Microcitemiche,
Cosenza.

---

[1] Silvestroni, E., and Bianco, I., *La malattia microdrepanocitica*, second ed. (Pensiero Scientifico, Roma, 1955).

[2] Silvestroni, E., *Policlinico Sez. Prat.*, **66**, 1373 (1959).

[3] Silvestroni, E., Bianco, I., Lucci, R., and Soffritti, E., *Acta Gen. Med. Gemell.* **9**, 472 (1960).

[4] Schneider, R. G., and Haggard, M. E., *Nature*, **180**, 1486 (1957).

[5] Schneider, R. G., and Haggard, M. E., *Nature*, **182**, 322 (1958).

[6] Dherte, P., Lehmann, H., and Vandepitte, J., *Nature*, **184**, 1133 (1959).

[7] Lambotte-Legrand, J., Lambotte-Legrand, C., Ager, J. A. M., and Lehmann, H., *Rev. Hématol.*, **15**, 10 (1960).

PATHOLOGY

Sensitivity of Mice to Bacterial Lipopolysaccharide following Alteration of Activity of the Reticulo-endothelial System

It has generally been assumed that resistance of animals to the pyrogenic and lethal effects of bacterial endotoxin is largely dependent on the phagocytic capacity of the reticulo-endothelial system. Thus it has been shown that stimulation of the reticulo-endothelial system with non-lethal doses of bacterial lipopolysaccharide or endotoxin results in protection of the animals against a subsequent lethal dose of endotoxin[1]. Again, numerous workers have demonstrated that a 'blockade' of the reticulo-endothelial system by colloidal particles, for example saccharated iron oxide, thorotrast and trypan blue, increases the sensitivity of the animals to the lethal effects of these substances[2]. Recently, however, it has been shown that in certain circumstances it is possible for mice to possess an increased phagocytic activity, as measured by their ability to clear colloidal carbon, and yet be highly sensitive to the effects of endotoxin. This can be seen during the course of an intracellular infection[3], or following the administration of zymosan[4]. The apparent contradiction of these results has yet to be resolved, and it is not unlikely that such resolution may lead to an elucidation of the mode of action of the endotoxins.

Recently, Stuart *et al.*[5,6] have shown that certain fatty acid esters can produce marked stimulation or depression of the phagocytic activity of the reticulo-endothelial system, as measured by the granulopectic test[7]. Results of histological studies (to be reported elsewhere) have shown that glycerol trioleate (triolein) produces a marked increase in the activity of phagocytic cells of the liver and spleen, whereas ethyl stearate causes a striking reduction in their number; these effects are reflected in a respective increase or decrease of phagocytic indices (*K*) of mice treated with these substances. The use of these agents might help to clarify the role of the fixed phagocytic cells in protection against endotoxin.

Male mice from a single source, weighing 15–20 gm., were used throughout these experiments. Susceptibility of animals to a single endotoxin (Difco *Escherichia coli* 'O'111: *B*4 lipopolysaccharide) following treatment with the lipids was compared by two experimental systems. In one, the LD_{50} of the endotoxin in the variously treated animals was determined, whereas in the other the survival of groups of mice receiving an injection of slightly more than 1 LD_{50} of the endotoxin (for normal animals) was estimated. Groups of animals, previously treated with a non-lethal dose of *E. coli* lipopolysaccharide, were always included for comparison. Colloidal suspensions containing 20 per cent (v/v) triolein and 20 per cent (w/v) ethyl stearate were prepared as described previously[5]; the lipopolysaccharide was dissolved in isotonic saline in a concentration of 200 μgm./ml. The following dosage schedule was used throughout the experiments: triolein, 0·05 ml./20 gm. mouse (11·5 mgm.), ethyl stearate, 0·1 ml./20 gm. mouse (20 mgm.) and lipopolysaccharide 0·1 ml./20 gm. mouse (20 μgm.). All injections were given intravenously. 24 hr. later the mice, including appropriate groups of normal animals of the same age, were challenged by intraperitoneal injection of a saline solution of *E. coli* lipopolysaccharide. The dose used depended on the form of the experiment and is recorded in Tables 1–3. The mice were observed over a period of 48 hr.; LD_{50} values were calculated and compared by the method of Litchfield and Wilcoxon[6]. Tables 1 and 2 record the results of these experiments.

Table 1. LD_{50} of *E. coli* Lipopolysaccharide in Mice treated with Lipopolysaccharide and Simple Lipids

Treatment	Survival-rates following endotoxin doses of (in μgm.)					LD_{50} (μgm.) with 19/20 confidence limits
	500	400	300	250	100	
Normal	1/10	2/10	6/10	8/10	10/10	325 (276–357)
Lipopolysaccharide	5/8	8/10	7/9	9/10	9/9	630 (437–908)
Triolein	1/10	1/10	1/10	4/10	7/10	175 (132–234)
Ethyl stearate	3/10	6/9	8/10	8/10	9/10	445 (326–615)

Survival-rate = No. alive/No. tested.

Table 2. Survival of Animals treated with Lipid or Lipopolysaccharide following Challenge with 400 μgm. *E. coli* Lipopolysaccharide

Treatment	Survivors/tested	Survival (per cent)
Normal	19/40	47·5
Lipopolysaccharide	37/40	92·5
Triolein	6/40	15
Ethyl stearate	21/40	52·5

Table 3. Phagocytic Indices of Mice 24 hr. after Treatment with Lipids and *E. coli* Lipopolysaccharide

Treatment	Animals	Mean *K** values	Mean *α** values
Normal	10	0·037	5·4
Lipopolysaccharide	10	0·096	7·0
Triolein	10	0·105	7·7
Ethyl stearate	10	0·0125	3·4

* *K* and *α* values were determined at a dosage of 8 mgm./100 gm.

The granulopectic activity of mice 24 hr. after injection of the lipids or the *E. coli* lipopolysaccharide was determined by the technique of Halpern *et al.*[7]. The phagocytic index (*K*) and the corrected phagocytic index (α) were determined at a dosage-level of carbon of 8 mgm./100 gm. Results of these investigations are summarized in Table 3.

Although triolein stimulates as good, or better, phagocytic activity as does lipopolysaccharide treatment, these results demonstrate that this lipid renders the mice significantly more susceptible to endotoxic activity. Again, contrary to the findings with 'blockaded' animals, the removal of phagocytes following treatment with ethyl stearate does not appreciably enhance susceptibility to endotoxin. It is arguable that the endotoxin may be taken up by phagocytic cells other than those of the liver and spleen, although it should be noted that in normal animals these organs together remove more than 80 per cent of the endotoxin[9].

The increased susceptibility of animals following triolein is not so great as that recorded following *BCG* infection or zymosan treatment. This difference may be related to the finding that, after either *BCG* infection or administration of zymosan, a marked granulomatous response occurs, whereas the simple lipid apparently exerts its action only on those sessile phagocytic cells which are in contact with the blood. At this stage the results suggest that endotoxin activity is dependent on the rapidity of uptake by certain cells, particularly those of the reticulo-endothelial system. Certainly it seems that the more phagocytes present in the liver and spleen the more sensitive are animals to endotoxin; on the other hand, decrease in cell numbers appears to have, if anything, a slightly beneficial effect on the resistance of the animals.

Lipopolysaccharide treatment may involve not only an increase in phagocytic cells but also perhaps stimulation of intracellular mechanisms of detoxification. Nevertheless, the influence of endotoxin-neutralizing factors in sera cannot be overlooked in assessing the protective activity of these substances[10].

One of us (G. N. C.) is indebted to the Trustees of the Wellcome Foundation for provision of a research travel grant. The work of the other (A. E. S.) has been supported by a research fellowship awarded by the Scottish Hospitals Endowments Research Trust. We wish to thank Prof. R. Cruickshank and G. L. Montgomery for facilities and their encouragement.

G. N. Cooper
A. E. Stuart

Departments of Bacteriology and Pathology,
University of Edinburgh.

[1] Biozzi, G., Benacerraf, B., and Halpern, B. N., *Brit. J. Exp. Path.*, **36**, 226 (1955).

[2] Bennett, I. L., *Proc. Soc. Exp. Biol., N.Y.*, **81**, 248 (1952). Good, R. A., and Thomas, L., *J. Exp. Med.*, **97**, 871 (1953).

[3] Howard, J. G., Biozzi, G., Halpern, B. N., Stiffel, C., and Mouton, D., *Brit. J. Exp. Path.*, **40**, 281 (1959). Abernathy, R. S., Bradley, G. M., and Spink, W. W., *J. Immunol.*, **81**, 271 (1958).

[4] Benacerraf, B., Thorbecke, G. J., and Jacoby, D., *Proc. Soc. Exp. Biol., N.Y.*, **100**, 796 (1959).

[5] Stuart, A. E., Biozzi, G., Stiffel, C., Halpern, B. N., and Mouton, D., *C.R. Acad. Sci., Paris*, **250**, 2779 (1960).

[6] Stuart, A. E., *et al.*, *Brit. J. Exp. Path.*, **41**, 599 (1960).

[7] Halpern, B. N., Biozzi, G., Mene, G., and Benacerraf, B., *Ann. Inst. Past.*, **80**, 582 (1951).

[8] Litchfield, J. T., and Wilcoxon, F., *J. Pharm. Exp. Therap.*, **96**, 99 (1949).

[9] Noyes, H. E., McInturf, C. R., and Blahuta, G. J. B., *Proc. Soc. Exp. Biol., N.Y.*, **100**, 65 (1959).

[10] Freedman, H. H., *Ann. N.Y. Acad. Sci.*, **88**, 99 (1960).

Non-Specific Immunity in Pneumococcal Infection of Mice

The development of early, non-specific protection against experimental infections with Gram-negative micro-organisms has been observed following injection of bacterial endotoxin, lipopolysaccharide and lipid *A*[1]. Reports of similar activities of these substances against infections with Gram-positive organisms are less numerous and have been substantiated only in the case of infection with *Staphylococcus pyogenes* or *Mycobacterium fortuitum*[2]. In an attempt to assess the role of phagocytosis by cells of the liver and spleen in this non-specific protection, we have utilized certain fatty acid esters which are capable of profoundly increasing or reducing the activity of these cells[3]. One of the experimental models selected for this work has been infection with *Streptococcus pneumoniae* in mice. Infections with this organism have usually been carried out by the intraperitoneal route and are highly lethal, unless the infection is modified by the classical means of active or passive immunization. In an attempt to demonstrate other, perhaps less potent, protective mechanisms, we felt that a less-virulent type of infection might be more appropriate. This has been achieved by using a strain of *Strep. pneumoniae* type 8, preparing the challenge inoculum in 0·1 *M* phosphate buffer at *p*H 7·4 and injecting the chosen dose of organisms by the intravenous route. Details of this technique will be published fully elsewhere.

Male mice derived from a single source and weighing 15–20 gm. were used. The effects of glycerol trioleate (triolein) and ethyl stearate treatment on the infection were compared with those of non-lethal doses of *E. coli* 'O'111 : *B*4 lipopolysaccharide (Difco). The method of preparing the lipid suspensions has been described previously[3]. The following doses of the substances were given to individual groups of mice by the intravenous route: triolein, 0·05 ml. of 20 per cent (v/v) suspension (11·5 mgm.), ethyl stearate, 0·1 ml. of 20 per cent (w/v) suspension (20 mgm.) and *E. coli* lipopolysaccharide, 0·1 ml. of 200 μgm./ml. solution (20 μgm.). 24 hr. later all the mice, including appropriate groups of normal animals, were injected intravenously with *Strep. pneumoniae* derived from an over-night culture in horse digest broth, and diluted appropriately in phosphate buffer. The number of organisms in the challenge dose was determined by viable count using the Miles and Misra technique[4]. Injections of the lipids and lipopolysaccharides were repeated 24 hr. later. The mice were observed for seven days and time to death within the groups was recorded. In Tables 1 and 2 are summarized the results of two experiments, one in which a constant challenge dose (about 4×10^4 organisms) was used, the other in which three challenge doses differing by ten-fold dilutions were given to various groups of animals.

It has been shown (preceding communication) that mice treated with lipopolysaccharide and triolein have a significantly higher phagocytic activity than normal when measured by the granulopectic technique[5]. Conversely, a marked suppression of phagocytic activity occurs following administration of ethyl stearate. These findings may be attributed to a marked increase or diminution of the hepatic and splenic phagocytes (Stuart, A. E., unpublished work). The results described here suggest that phagocytic reduction does not significantly affect the survival or time to death of the mice. However, a striking degree of

Table 1. Survival of Mice treated with Lipids and Lipopolysaccharide following Challenge with *Strep. pneumoniae* (4×10^4 Organisms)

Treatment	No. survivors / No. tested	Survivors (per cent)	Mean time to death (hr.)
Normal	2/20	10	56
Triolein	6/20	30	73
Ethyl stearate	1/18	6	66
Lipopolysaccharide	18/20	90	108

Table 2. Survival of Mice treated with Lipids and Lipopolysaccharide receiving Graded Doses of *Strep. pneumoniae*

Treatment	Survivors/tested receiving challenge doses of:		
	$2\cdot5 \times 10^5$ organisms	$2\cdot5 \times 10^4$ organisms	$2\cdot5 \times 10^3$ organisms
Normal	0/10	1/10	9/10
Triolein	3/10	4/10	9/10
Ethyl stearate	0/10	1/10	6/10
Lipopolysaccharide	7/10	10/10	10/10

protection against infection can be achieved with lipopolysaccharide, and to a much less extent with triolein. There seems no doubt of the non-specific nature of this enhanced protection, which develops within 24 hr. This protection cannot be related to the properdin system, which has been shown to be effective only against Gram-negative organisms (ref. 1, *b*).

The difference in protection achieved with lipopolysaccharide and triolein is of particular interest. There is no observable difference in the ability of animals to clear colloidal carbon from the blood following treatment with either of these substances. It is generally assumed that the granulopectic test is a measure of an animal's ability to clear microorganisms from the blood as well[6]. This is substantiated by an experiment, the results of which are recorded in Table 3, in which the removal of viable pneumococci from the blood of animals was examined. Groups of mice treated with lipopolysaccharide or triolein were injected intravenously with approximately 1×10^6 organisms (derived from an overnight culture, as above) suspended in phosphate buffer. Immediately after injection and at 6-min. intervals thereafter up to 18 min., blood samples were removed from the retro-orbital venous plexus; ten-fold dilutions of the sample were prepared in phosphate buffer and viable counts were determined by the Miles and Misra method[4]. Logarithms of the counts plotted against time yielded approximately linear curves, and it was thus possible to express the rate of removal of organisms (K) in terms of the slope of the line.

The results suggest that the differences in effects observed with the lipopolysaccharide and glycerol trioleate cannot be explained in terms of rate of removal of the organisms from the blood. Rather it seems that the lipopolysaccharide treatment stimulates some factor which causes more rapid death of the organisms following their removal. This may be either a serum factor (non-specific opsonin) or an enhanced intracellular activity of the phagocytes stimulated by the lipopolysaccharide. Preliminary experiments suggest that opsonization *per se* may have no immediate influence in non-specific protection against the pneumococcal infection.

Table 3. Rate of Removal of Viable Pneumococci (K) in Mice treated with Lipid or Lipopolysaccharide

Treatment	Mean values of K	KT/KN
Normal	0·013	
Triolein	0·031	2·48
Lipopolysaccharide	0·035	2·68

KT/KN = Ratio of K values of treated to normal animals.

One of us (G. N. C.) gratefully acknowledges the receipt of a Wellcome Research travel grant. The work of the other (A. E. S.) has been supported by a research fellowship awarded by the Scottish Hospitals Endowments Research Trust. Our thanks are also due to Prof. R. Cruickshank and G. L. Montgomery for their encouragement and advice.

G. N. Cooper
A. E. Stuart

Departments of Bacteriology and Pathology,
University of Edinburgh.

---

[1] (*a*) Rowley, D., *Lancet*, i, 232 (1955). (*b*) Landy, M., and Pillemer, L., *J. Exp. Med.*, **104**, 383 (1956). (*c*) Dubos, R. J., and Schaedler, R. W., *ibid.*, **104**, 53 (1956). (*d*) Howard, J. G., Rowley, D., and Wardlaw, A. C., *Nature*, **179**, 314 (1957).

[2] Böhme, D., and Dubos, R. J., *J. Exp. Med.*, **107**, 532 (1958).

[3] Stuart, A. E., Biozzi, G., Stiffel, C., Halpern, B. N., and Mouton, D., *Brit. J. Exp. Path.* (in the press).

[4] Miles, A. A., and Misra, S. S., *J. Hyg.* (*Camb.*), **38**, 732 (1938).

[5] Halpern, B. N., Biozzi, G., Mene, G., and Benacerraf, B., *Ann. Inst. Bact.*, **80**, 582 (1951).

[6] Benacerraf, B., Sebestyen, M. S., and Schlossmann, S., *J. Exp. Med.* **110**, 27 (1959).

Increase in Specific Bactericidal Antibodies after Administration of Endotoxin

Many non-specific immune phenomena have been described in which animals became resistant to experimental infection following administration of antigenically unrelated materials. None has attracted more interest than the temporary state of enhanced resistance to infection with Gram-negative bacteria which develops in mice within hours after administration of antigenically unrelated bacterial endotoxins[1]. Among the many effects of endotoxins on the host is increased activity of the reticulo-endothelial system as shown by carbon clearance[2] and by removal of phosphorus-32 labelled endotoxin[3] or bacteria[4]. In addition, changes in the serum of endotoxin-treated animals have been reported, notably increased levels of properdin[5], bactericidins[6] and opsonins[7,8]. Passive transfer of opsonic capacity has also been demonstrated[4]. It is evident that these alterations in the host, individually or collectively, may contribute significantly to survival. Special emphasis has been placed on the non-specific nature of the humoral factors involved, because there has been abundant confirmation of activity against bacterial strains antigenically unrelated to the endotoxin used for stimulation. Cross-reacting antibodies[9] or a common antigen associated with the lipid complex of endotoxins[10] have therefore been suggested to account for these observations.

The serum bactericidal reaction against Gram-negative bacteria has been shown to depend on specific antibody, complement and divalent cations. We have developed a method for the determination of antibody based on this reaction[11]. Serum accessory factors, such as complement, are supplied in excess. The level of specific antibody in the serum under test is measured by the extent to which it restores bactericidal activity to absorbed human serum. In normal serum this method detects antibody against Gram-negative bacteria in amounts considerably less than

Table 1. BACTERICIDAL ACTIVITY OF MOUSE SERUM FOR *Salm. typhi* 0901

Serum (ml.)	Bacterial survival in serum from Normal mice (per cent)	Bacterial survival in serum from Endotoxin-treated mice* (per cent)
0·01	7	3
0·0066	59	3
0·005	72	23
0·004	87	46
0·0033	95	68

The bacterial inoculum was 10^6 cells.

* 24 hr. following 50 μgm. *Sh. shigae* endotoxin i.v. The quantity of serum giving 50 per cent killing was 0·007 ml. in the case of normal mice and 0·004 ml. for the endotoxin-treated mice.

Table 2. SPECIFICITY OF THE BACTERICIDAL ANTIBODY OF ENDOTOXIN-TREATED MICE

Treatment of mice	Relative bactericidal activity of serum*			
	Unabsorbed		After absorption with heat-killed *Salm. typhi* 0901	
	Salm. typhi 0901	*Esch. coli* 0127	*Salm. typhi* 0901	*Esch. coli* 0127
None	1·0	1·0	0	1·0
50 μgm. *Sh. shigae* endotoxin i.v. 24 hr. previously	1·7	2·5	0	2·3

* Supplemented with absorbed human serum as a source of complement.

those required for agglutination of bacteria or antigen-coated red cells, namely, 0·001 μgm. antibody nitrogen in the case of antiserum to *Salmonella typhi*. The quantity of serum which gives 50 per cent bacterial killing is determined, with satisfactory reproducibility, by plotting log serum dose against probits of bacterial survivors. Our work has dealt predominantly with the mouse, because non-specific resistance has been investigated almost exclusively in this species. Mouse serum is not bactericidal, because its complement is not functional in this system; supplementation with heterologous complement is therefore essential for the assay. In our tests pooled normal human serum, from which antibody had been absorbed by heat-killed bacteria, served as the source of serum accessory factors.

With this method we have found that after administration of endotoxin to mice, in amounts which elicit so-called non-specific immunity, there is an increase in serum bactericidal activity against several antigenically unrelated strains of Gram-negative bacteria. For example, within 3 hr. after administration of 50 μgm./mouse of a *Shigella shigae* endotoxin, increased bactericidal activity for other bacterial strains was discernible which persisted for more than 48 hr.; by 96 hr. the level returned to normal. Table 1 shows the level of this increased bactericidal activity at 24 hr. Absorption of the serum with one Gram-negative serotype removed bactericidal activity for that strain, whereas the increased serum bactericidal activity against strains of other genera was retained. An illustrative experiment is summarized in Table 2. Thus the factors in serum responsible for the elevation of bactericidal effect not only have the functional characteristics of antibodies, but also are individually absorbable as would be expected of specific antibodies.

Additional evidence for the antibody-like character of the bactericidin was also obtained. Serum from endotoxin-treated animals, after absorption with heat-killed *Salm. typhi* 0901, still possessed increased bactericidal activity for *Escherichia coli* 0127. This absorbed serum, added as a supplement to normal mouse serum in a bactericidal test against *Salm. typhi* 0901, did not increase the bactericidal activity of normal mouse serum. Thus the increased bactericidal activity is due to specific antibody rather than to some non-specific factors.

The rise in bactericidal titre after administration of endotoxin is assumed to be due to a transitory effect of endotoxin on antibody-forming cells. This may involve either release of preformed antibodies or a temporary increase in the capacity of these cells to produce antibody. These results point to a temporary elevation of a whole array of specific antibodies rather than the appearance of non-specific or cross-reacting bacterial antibodies. Experiments are in progress to determine whether the reported increase in opsonic factors in mice, following the administration of endotoxin, involves the same specificities.

J. GABRIEL MICHAEL
JAMES L. WHITBY
MAURICE LANDY

Laboratory of Chemical Pharmacology,
National Cancer Institute,
Bethesda 14, Maryland.

[1] Shilo, M., *Ann. Rev. Microbiol.*, **13**, 255 (1959).

[2] Biozzi, G., Benacerraf, B., and Halpern, B. N., *Brit. J. Exp. Path.*, **36**, 226 (1955).

[3] Howard, J. G., Rowley, D., and Wardlaw, A. C., *Immunol.*, **1**, 181 (1958).

[4] Benacerraf, B., Sebestyen, M. M., and Schlossman, S., *J. Exp. Med.*, **110**, 27 (1959).

[5] Landy, M., and Pillemer, L., *J. Exp. Med.*, **103**, 823 (1956).

[6] Rowley, D., *Brit. J. Exp. Path.*, **37**, 223 (1956).

[7] Rowley, D., *J. Exp. Med.*, **111**, 137 (1960).

[8] Jenkin, C. R., and Palmer, D. L., *J. Exp. Med.*, **112**, 419 (1960).

[9] Lee, L., and Stetson, C. A., *J. Exp. Med.*, **111**, 761 (1960).

[10] Benacerraf, B., and Miescher, P., *Ann. N.Y. Acad. Sci*, **88**, 184 (1960).

[11] Landy, M., Michael, J. G., and Whitby, J. L. (in preparation).

BIOLOGY

A Natural Population of Anomalous Branched Tapeworms, *Hymenolepis nana* (Cestoda: Hymenolepididae), in a Colony of *DBA/1* Mice

DURING work on metabolism of the dwarf tapeworm of mice (*Hymenolepis nana*), a natural infection with this parasite was found in a local colony of *DBA/1* mice (Curd's Caviary, Downey, California). Of 500 mice from this colony examined over a two-year period, 320 were infected with an average of 35 worms per mouse, varying from a single 12-in. worm to one or two thousand extremely small ones (demonstrating a 'crowding effect'). Among more than 12,000 worms recovered, approximately 5 per cent showed one of the following malformations or growth disturbances: (1) Partial twinning (Fig. 1*A*): forked posterior strobila; (2) lateral branching (Fig. 1*B*): new tissue outgrowth with varying degrees of differentiation; (3) dedifferentiation (Fig. 1*D*): groups of segments appearing as undifferentiated, vacuolated granular regions; (4) segment reversal: reversal of normal antero-posterior sequence of proglottides (Fig. 1*B*); (5) combination: dedifferentiation plus branch outgrowth (Fig. 1*C*); branch formation with segment reversal (Fig. 1*B*); multiple branching (Fig. 1*E*).

Cestode abnormalities reviewed by Foster, Faust, Clapham, Kuntz and Dollfus and others[1-7] include fenestration of groups of segments, fusion of strobilar loops, doubling and inversion of organs and other developmental irregularities, bud formation and dichotomy, spiral strobilization and polyradiate forms with additional suckers and marginal ridges. No one, however, has recorded recovery of numerous abnormalities from a single host population over a sustained period. The following circumstances are of added interest: (1) all aberrant worms were from a single commercial stock of mice, derived from the *DBA*/1 strain; (2) these hosts were maintained with a colony of Swiss Webster mice, with both groups showing a similar rate of infection, approximately 70 per cent, by *H. nana*. No branching was found among thousands of *H. nana* examined during a five-year period taken from 700 Swiss white mice from this colony. Nor were abnormal worms recovered from naturally infected mice from several commercial sources or from original Jackson Memorial Laboratory *DBA*/1 mice. Among infected Curd strain *DBA*/1 mice, approximately 60 per cent harboured abnormal worms, representing 800 or 5 per cent of the total recovered.

The commonest abnormality was a small nub or outgrowth of tissue showing considerable variation in length, orientation and degree of maturation. Less common were granulation and vacuolization, an apparent dedifferentiation or tissue destruction, bifurcation of posterior segments, and segment reversal. Multiple branching with varying degrees of maturation emphasize a capacity to bud or branch existing throughout the worm. Gravid branches were chiefly confined to twinned posterior ends. Abnormal worms were generally small, 3 cm. or less in length.

These malformations appear to be an interference with the normal pattern of differentiation and growth. New tissue development, usually confined to the neck area in tapeworms, appeared anywhere along the strobila, often accompanied by breakdown of organized regions. Subsequent differentiation and growth were delayed and abnormal. Branches usually showed more fully developed segments at branch-strobila junctions, the growing tip (equivalent to the neck of a normal worm) being carried out with the branch. No normal scolex was found, though neck-like, rounded branch ends often occurred (Fig. 1*B*, *C*). Twinned posterior ends formed by division of the terminal proglottides. This was followed by growth and maturation of the separate chains, their posterior portions therefore being most developed except for a terminal nub on the branch. Lateral branches probably formed similarly, but with sharply differing growth-rates for the two portions of the strobila.

These findings are of interest in terms of cestode growth patterns, antero-posterior differentiation and general growth control factors. Laboratory establishment of such an abnormal strain was therefore attempted. In three experiments, eggs cut from abnormal, gravid branches were fed in 0·1 ml. tap-water by pipette to six young non-infected *DBA*/1 mice. Gravid worms recovered were fed to normal white and *DBA*/1 mice and similarly passed through six generations of each host strain. No branched worms were recovered from any of these infections. In repeated feedings with intact abnormal worms to mice of Swiss, *DBA*/1 (Jackson Laboratory), and Heston/*A* (University of California Cancer Laboratory) strains, again only normal worms developed. Laboratory infection of Curd *DBA*/1 stock could not be attempted owing to absence of parasite-free animals from this source.

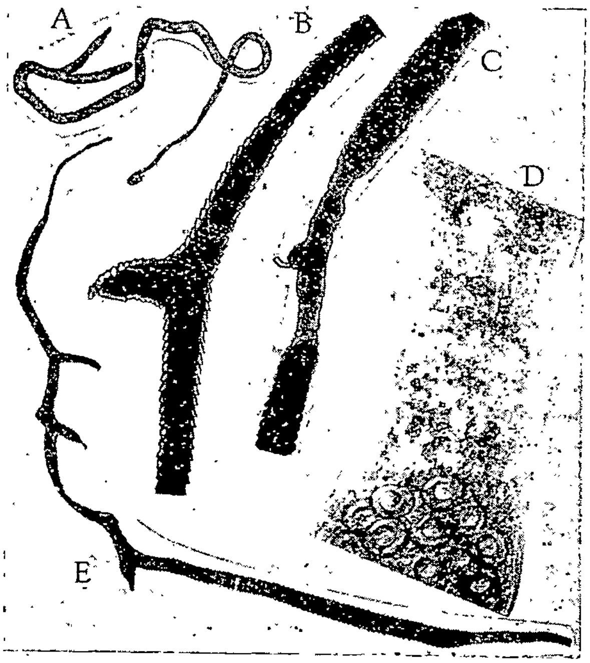


Fig. 1. Various expressions of tapeworm abnormality recovered from local strain of *DBA*/1 mice. *A*, Terminal twinning of strobila (× 5). *B*, Lateral branching showing terminal nub and reversed orientation of segments (× 10). *C*, Lateral branching with area of dedifferentiation (× 10). *D*, Detail of zone of granulation or dedifferentiation (× 110). *E*, Multiple branching, with division of segments of the most posterior branch (× 5)

Three groups of five grain beetles (*Tribolium confusum*), previously starved for seven days, were fed on abnormal gravid branches. No cysticercoids developed, owing either to reduced viability or insufficient numbers of eggs. However, normal cysticercoids developed when whole branched worms were exposed on filter paper to previously starved beetles. These cysticercoids produced only normal worms when fed to *DBA*/1 (Jackson strain), Heston/*A*, or Swiss Webster mice. Infections using grain beetle intermediate hosts were continued over four generations with the same result.

This phenomenon may represent a mutation activated by a specific host strain or an extraordinarily specific direct host-influence on an otherwise normal parasite. In either case, the high degree of dependence on the host for the expression of this abnormality emphasizes a remarkable specificity and an extreme degree of response of a parasite to its host.

This work was supported in part by a contract between the Office of Naval Research and the University of California, Los Angeles, *Nonr* 233(56).

Donald Heyneman*

Department of Zoology,
University of California,
Los Angeles,
California.

---

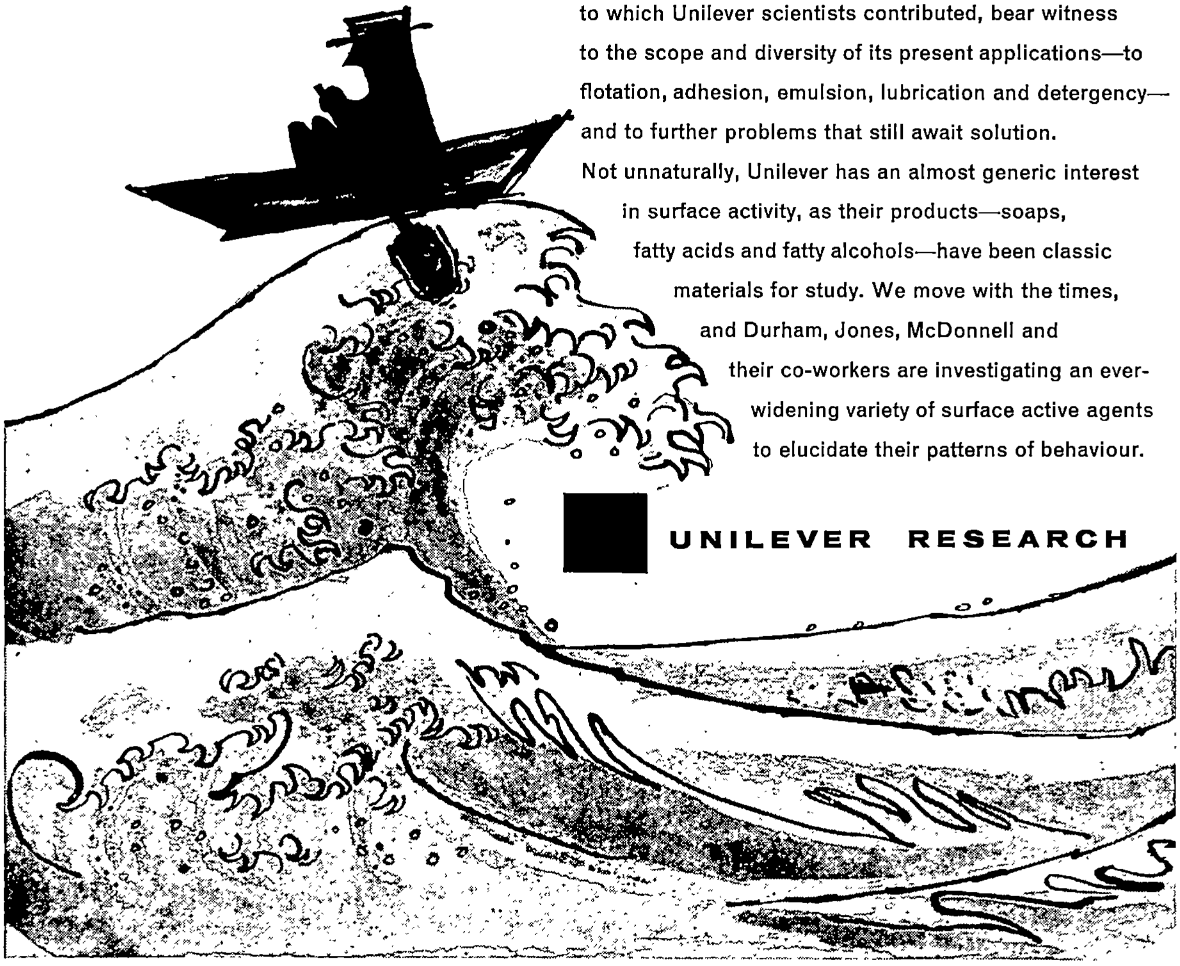
* On special leave of absence as Head, Department of Parasitology, United States Naval Medical Research Unit No. 3, c/o American Embassy, Cairo.

[1] Foster, W. D., *J. Parasitol.*, **2**, 7 (1915).
[2] Faust, E. C., *China Med. (Miss.) J.*, **39**, 800 (1925).
[3] Clapham, P. A., *J. Helminth.*, **17**, 163 (1939).
[4] Kuntz, R. E., *Proc. Helminth. Soc. Wash.*, **15**, 73 (1948).
[5] Dollfus, R. P., *Ann. Parasit. Hum. Comp.*, **26**, 274 (1951).
[6] Cram, E. B., *J. Parasitol.*, **14**, 54 (1927).
[7] Dobrovolny, C. G., and Dobrovolny, M. P., *Trans. Amer. Microscop. Soc.*, **54**, 22 (1935).

surface activity

For all practical purposes surface activity began with Adam—or so, for convenience, the detergent chemist thinks. He does not mean the biblical Adam, of course, although there is an implied analogy which, conceding important happenings prior to Adam's appearance on the scene, nevertheless considers him to have directed matters on to a systematic course, and to have made it clear that not only could knowledge of the subject lead to understanding, but understanding to greater knowledge. Surface activity, in a sense, is not a property of surfaces but an effect induced therein by unbalanced molecular forces, and our knowledge and understanding of surface phenomena are derived from the pioneer work of Willard Gibbs, Langmuir, Rideal, McBain, Hartley Harkins and of Adam himself. Not that the subject is yet exhausted. The four volumes of the Proceedings of the Second International Congress on Surface Activity, to which Unilever scientists contributed, bear witness to the scope and diversity of its present applications—to flotation, adhesion, emulsion, lubrication and detergency—and to further problems that still await solution. Not unnaturally, Unilever has an almost generic interest in surface activity, as their products—soaps, fatty acids and fatty alcohols—have been classic materials for study. We move with the times, and Durham, Jones, McDonnell and their co-workers are investigating an ever-widening variety of surface active agents to elucidate their patterns of behaviour.

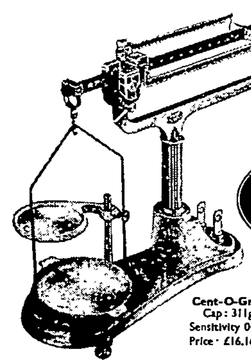
UNILEVER RESEARCH



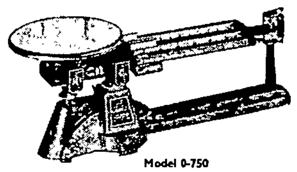
UR 4-7049-100

WEIGHT on your Mind?

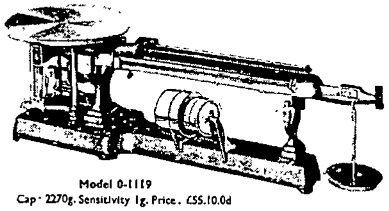
OHAUS



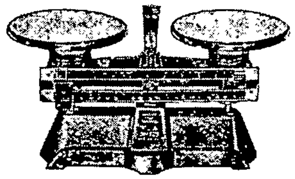
Cent-O-Gram
Cap: 311g.
Sensitivity 0·01g.
Price · £16.16.0d.



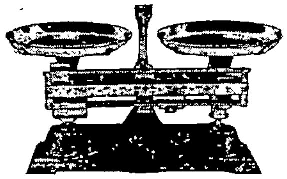
Model 0-750
Cap · 2610g. Sensitivity 0·1g. Price · £10 5.0d



Model 0-1119
Cap · 2270g. Sensitivity 1g. Price. £55.10.0d

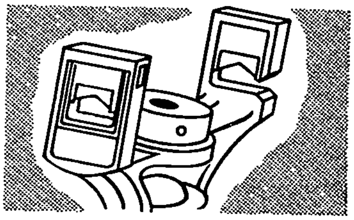


Model 0-1550 Cap: 2kg. Sensitivity 0·1g. Price £10.17.6d.



Model 0-1910 Cap: 5kg. Sensitivity 0·5g. Price: £49.15.0d.

These world-famous balances, made by the OHAUS Scale Corpn. of New Jersey, U.S.A., are justly renowned for their outstanding value. They incorporate special, built-in high-quality features, listed below, making them more durable, more accurate and easier to operate. 1. Self-aligning, semi-rigid agate bearings. 2. Dust-proof, self-locking bearing covers. 3. Patented scale-plates with threaded hubs. 4. Elliptical check rods and spindles. 5. Relief-etched stainless steel beams. 6. Hollow-ground steel knife-edges.



Self-aligning, semi-rigid agate bearings give greater sensitivity, better reproducibility, and longer life.

No. 1 of a series of advertisements highlighting special features of Ohaus balances.

For full details write your name and address in the margin of this advertisement and send it to—

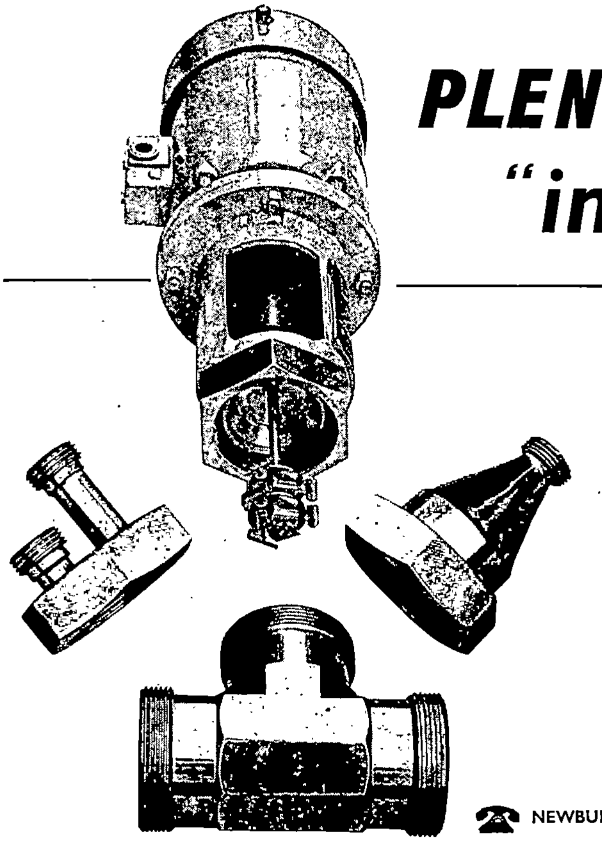
SHANDON

SHANDON SCIENTIFIC COMPANY LTD.
6, Cromwell Place, London, S.W.7. Tel. KNIghtsbridge 1131

THE

PLENTY "EASICLEAN"

"in-line-mixer"



Rapid homogeneous blending.

Suitable for light and viscous liquids.

Patent Impelator head.

Stainless steel construction.

Dairy couplings.

Full range of sizes (*laboratory model illustrated*).

PLENTY & SON Ltd.

Newbury, Berks., England.

☎ NEWBURY 2363 (5 lines) PLENTY, NEWBURY, TELEX TELEX: 84110

Calories/gm. in Species of Animals

ANIMALS of seventeen species, representing six phyla, were burned in a miniature bomb calorimeter[1]. The data are presented as calories/ash-free gm. in Table 1 and as a histogram, Fig. 1.

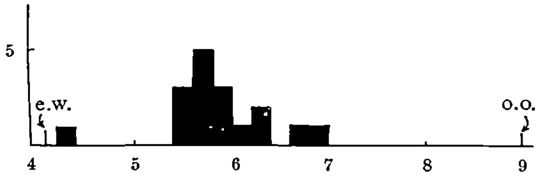


Fig. 1. Frequency distribution of the data in Table 1. The caloric values of egg white (e.w.) and olive oil (o.o.) have also been indicated.

Given a haphazard collection of species, any one of three mutually exclusive frequency distribution patterns might have been expected. These are listed below with the rationale of each :

(1) A normal, symmetrical distribution. This might be anticipated from the known biochemical similarities between animals. The variance would be related to slight biochemical differences and to errors of measurement.

(2) A skewed distribution with the modal frequency at or near the lower range limit. This might be anticipated, since there has always been selection for maximum number of reproducing progeny, but only sporadic selection for high-energy content/gm.

(3) Fairly great differences between major taxonomic groups with essentially normal distributions within each group. Many other properties of animals are common to a taxonomic category and differ between categories. Cal./gm. might be one such property.

The biological implications of the three possible distributions differ. The first distribution would imply that there is an optimal value of cal./gm., any deviation in either direction being selected against.

The second distribution would imply that there is some unique combination of components, characterized by a particular range of cal./gm., which just permits animals to maintain themselves. These components are essentially the same for all animals, and any combination with a lower cal./gm. value is non-viable. Higher values would be expected in animals that have temporarily accumulated fat, either because of unusually high food availability or as preparation for some period of fasting or stress.

Table 1. CAL./ASH-FREE GM. OF SEVENTEEN SPECIES OF ANIMAL

Common name	Species	Mean cal./ ash-free gm.	± 95 per cent confidence limit of the mean (corrected for small sample size)
Ciliate	*Tetrahymena pyriformis*	5,938	± 207
Hydra	*Hydra littoralis*	6,034	± 146
Green hydra	*Chlorohydra viridissima*	5,729	± 247
Flatworm	*Dugesia tigrina*	6,286	± 338
Terrestrial flatworm	*Bipalium kewense*	5,684	± 124
Acquatic snail	*Succinea ovalis* (without shell)	5,415	± 6
Brachiopod	*Glottidia pyramidata*	4,397	± 2,140
Brine shrimp	*Artemia* sp. (nauplii)	6,737	± 863
Cladocera	*Leptodora kindtii*	5,605	± 584
Copepod	*Trigriopus californicus*	5,515	± 277
Copepod	*Calanus helgolandicus*	5,400	± 197
Caddis fly	*Pycnopsyche lepido*	5,687	± 530
Caddis fly	*Pycnopsyche guttifer*	5,706	one determination
Spit bug	*Philenus leucophthalmus*	6,962	± 510
Mite	*Tyroglyphus lintneri*	5,808	± 446
Beetle	*Tenebrio molitor*	6,314	± 516
Guppie	*Lebistes reticulatus*	5,823	one determination

The third distribution would imply that cal./gm. is a secondary property in the evolution of animals and that the cal./gm. in any animal is a by-product of other selective requirements. This would be expected if, and only if, energy has generally not been limiting during the course of evolution.

So far as we have gone, the results suggest the second alternative distribution listed. Twelve of the seventeen determinations give results of 5,400–6,100 cal./gm. The one low value is from a moribund brachiopod (*Glottidia*). Two of the four high values were from animals that were about to go through a fast, the *Artemia* nauplii, since they were unable as yet to feed, and the *Tenebrio* larva, since it was about to pupate. The other two were from very well-fed situations, the *Dugesia* being from a laboratory stock culture fed on *Artemia* nauplii and the *Philenus* being taken from the field in late summer. The *Philenus* specimens included egg-laden females, but, unfortunately, they were not sexed on collecting. It seems likely that many herbivorous insects normally have superabundant food available[2].

Cal./gm. may provide an index of nutritional condition of animals in the field which would be considerably cheaper and as reliable as the elaborate sampling programmes now required.

This work has been supported by the National Science Foundation (*G*–9058). The calorimetric equipment was made available for testing on biological material by the U.S. Navy. We acknowledge the kindness of Dr. W. S. McEwan.

L. B. SLOBODKIN

Department of Zoology,
University of Michigan, Ann Arbor.

S. RICHMAN

Department of Biology,
Lawrence College,
Appleton, Wisconsin.

---

[1] McEwan, W. S., and Anderson, C. M., *Rev. Sci. Instrum.*, **26**, 280 (1955).

[2] Hairston, N., Smith, F. E., and Slobodkin, L. B., *Amer. Naturalist*, **94**, 421 (1960).

Manganese and Magnesium in the Grey Speck Syndrome of Oats

IT is generally recognized that the grey speck syndrome in oats is caused basically by manganese deficiency[1]. Opinions differ, however, on whether grey speck symptoms are due directly to manganese deficiency[1–3], or whether manganese deficiency functions indirectly by rendering the roots of the plant more susceptible to infection by rhizosphere organisms the toxic products of which give rise to the visual symptoms[4].

In consequence, the possibility of selecting breeding material resistant to grey speck is complicated, despite the variation in resistance reported by several investigators[5–7]. The present work is concerned with the investigation of possible differences in the critical levels of manganese in tissues of varieties of oats susceptible and resistant respectively to grey speck. For this purpose, the hexaploid oat variety Star (susceptible) and the diploid variety *S.*171 (resistant)[8] were used and grown in sand culture with a modified Shive solution and six levels of manganese, 0·0, 0·1, 0·2, 0·5, 2·0 and 5·0 p.p.m. Washed sand, de-ionized water and 'Analar' chemicals were used, but no elaborate precautions were taken to eliminate all manganese from the zero treatments.

Table 1. MANGANESE AND MAGNESIUM CONTENT OF OATS, SUSCEPTIBLE AND RESISTANT TO GREY SPECK DISEASE

Manganese treatment	Variety	Percentage Manganese Roots	Percentage Manganese Leaves	Percentage Magnesium Roots	Percentage Magnesium Leaves
0·0 p.p.m.	Star	0·00050	0·00050	0·45	0·55
	S.171	0·00256	trace	0·29	0·35
0·1 ,,	Star	0·00098	0·00175	0·44	0·50
	S.171	0·00300	0·00044	0·30	0·57
0·2 ,,	Star	0·00240	0·00200	0·46	0·45
	S.171	0·00350	0·00048	0·06	0·26
0·5 ,,	Star	0·00250	0·00800	*	0·27
	S.171	0·00500	0·00440	0·054	0·22
2·0 ,,	Star	0·00253	0·02000	0·25	0·35
	S.171	0·01250	0·01920	0·048	0·22
5·0 ,,	Star	0·00150	0·03900	0·23	0·48
	S.171	0·01350	0·02800	0·068	0·42

* No data.

The results (Table 1) show that for mature plants the manganese content of the leaves of Star is consistently higher than those of *S*.171 at the lower manganese-levels, but with manganese treatments of 2·0 and 5·0 p.p.m. there is no significant difference. The reverse holds for the manganese content of the roots, as in every case *S*.171 has the higher manganese content, and the difference between Star and *S*.171 is greatest at the highest manganese concentrations. Despite the low levels of manganese obtained in the leaves of both varieties at the 0·0 and 0·1 p.p.m.-levels, no typical grey speck symptoms were observed even in the susceptible variety Star. In contrast, other workers have reported grey speck symptoms associated with leaf concentrations of manganese in excess of those reported[3,9]. Our results are more comparable to those of Lundegårdh, who reported concentrations as low as 1 p.p.m. without grey speck symptoms[10].

In regard to magnesium, the content of the roots shows major varietal differences and an interaction between magnesium and manganese. The variety *S*.171 contains less magnesium in the roots at all levels of manganese than Star, but both varieties show a decrease in magnesium content when the manganese supply changes from limiting to adequate. The difference in the magnesium content of the leaves is not so marked, however, the decrease being most significant in *S*.171.

The relationship to grey speck resistance of high-leaf, low-root manganese content in the susceptible variety Star, and the low-leaf, high-root manganese concentration of the resistant variety *S*.171, is not clear. The analyses are for fully mature plants and may not represent the situation in actively growing material. It is possible that the results represent the effect rather than the cause of varying susceptibility to the grey speck syndrome. It has been established that redistribution of mineral constituents from the shoot occurs during the maturation of oats[11]. Thus, if at maturity manganese passes from shoot to root, then the higher manganese content of the roots and the lower content of the leaves in *S*.171 may reflect the greater mobility of manganese in this variety. The comparative lack of mobility of manganese in Star is then indicated by the high-leaf, low-root manganese concentrations, due to retention of manganese in the leaves without redistribution to the roots. Taking this view, susceptibility to the grey speck syndrome is primarily due to lack of mobility of manganese within the plant.

An alternative is that the mature plant analyses represent the situation in the growing plant, and in this case the grey speck resistance of *S*.171 may be due to the comparatively high concentration of manganese in the roots inhibiting the invasion and multiplication of ammonium-forming rhizosphere organisms, either directly or indirectly through the medium of a root exudate. Resistance to grey speck has been attributed by Gerretsen[6] to the root system having greater resistance to saprophytic organisms. However, more effective mobility of manganese seems more likely to be the basis of superior grey speck resistance, in that the syndrome appears to be primarily a disorder of photosynthesis, with root deterioration as a secondary factor. This is borne out by the rapidity with which deficiency is overcome when manganese-deficient cereals are sprayed with manganese sulphate.

Further work is in progress to determine the relationship of manganese mobility to resistance to grey speck, and the possible interaction of manganese and magnesium.

P. B. VOSE
D. J. GRIFFITHS

Welsh Plant Breeding Station,
Plas Gogerddan,
Nr. Aberystwyth.

[1] Samuel, G., and Piper, C. S., *J. Dept. Agric. S. Austral.*, **31**, 696 and 789 (1928).

[2] Twyman, E. S., *Nature*, **152**, 216 (1943).

[3] Leach, W., Bulman, R., and Kroeker, J., *Canad. J. Bot.*, **32**, 358 (1954).

[4] Gerretsen, F. C., *Versl. RijkslandbProefst., 's-Grav.*, **42**, 1 (1936), and *Ann. Bot., Lond.*, (N.S.), **1**, 207 (1937).

[5] Davies, D. W., and Jones, E. T., *Welsh J. Agric.*, **7**, 349 (1931).

[6] Gerretsen, F. C., *Landbouwk. Tijdschr., 's-Grav.*, **68**, 756 (1956).

[7] Toms, W. J., *J. Dept. Agric. W. Austral.*, **8**, 523 (1959).

[8] Kerr, D. H., *Scot. Agric.*, **34**, 157 (1954).

[9] Samuel, G., and Piper, C. S., *Ann. App. Biol.*, **16**, 493 (1929).

[10] Lundegårdh, H., *Med. Cent. Anst. Försöksv. Jordbr. Stockh.*, **403**, 1 (1931).

[11] Fagan, T. W., and Watkin, J. E., *Welsh J. Agric.*, **7**, 229 (1931).

Dependence on Light of Geotropism in Plant Roots

PLANTS grown in non-porous containers frequently show a lower concentration of roots on the periphery of the block of soil than similar plants grown in porous containers[1], and there is sometimes a tendency for the direction of growth of these peripheral roots to be more nearly vertical in non-porous than in porous containers[2,3]. Gradients of water, nutrient or gas concentration, or of soil temperature, have been suggested as causes of these phenomena, but there is also the possibility that light has an effect, since many non-porous containers made of glass or plastics are transparent, whereas porous 'clay' pots are opaque.

Seeds of China asters (*Callistephus chinensis* var. Ostrich Plume), stocks (*Matthiola incana* var. *annua*), *Calendula officinalis* var. Radio, tomatoes (*Lycopersicon esculentum* var. Amateur) and cucumbers (*Cucumis sativus* var. Market Cross) were sown on July 6, 1960, and transferred to John Innes No. 1 potting compost in the experimental pots on July 15. The pots were of colourless polyethylene, which is highly transparent to all wave-lengths of visible and infra-red radiation, and half of them were made opaque by coating them with black paint, on the outer surface, and on the inner surface down to the level of the soil. Opaque and transparent pots were arranged alternately on a bench in a glasshouse with natural illumination. Three plants of each species and from each kind of pot were examined on July 29 and at intervals of 3–6 days thereafter. An examination consisted in removing the plant from its pot and applying a 'cross of Lorraine' (Fig. 1) marked

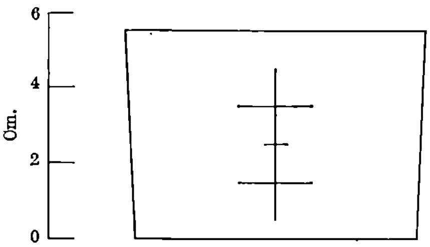


Fig. 1. Side view of a block of soil taken from a pot, with 'cross of Lorraine' superimposed for assessing roots

on a piece of transparent polyvinyl chloride, to four positions taken at random around the nearly vertical wall of the block of soil. In each position, the number of roots intersecting each of the two 2-cm. axes of the upper half of the cross and each of the two 2-cm. axes of the lower half were counted.

For all except tomatoes the increase, from one examination to the next, in the number of intersections counted was greater in the opaque than in the transparent pots. Thus, in order to compare the distributions of roots on the surface of the blocks of soil from the two kinds of pots when the total quantity of root present was much the same in both cases (Fig. 2), it was necessary to relate to the results from the transparent pots, obtained on August 15, corresponding results from opaque pots obtained respectively 16, 14, 6, 3 and 0 days earlier for stocks, calendulas, asters, cucumbers and tomatoes. One of the reasons for a low concentration of roots on the periphery of a block of soil from a transparent nonporous pot therefore appears to be that, for some species at least, light either inhibits the growth of the roots or causes them to turn back into the soil by negative phototropism.

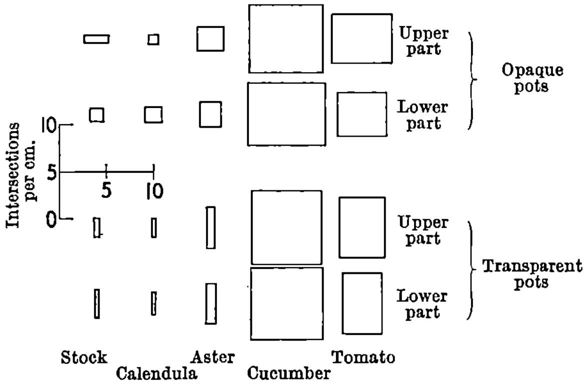


Fig. 2. Distribution of roots on the walls of blocks of soil taken from opaque pots and from transparent pots. The width of a rectangle represents the number of roots intersecting a vertical line and the height represents the number intersecting a horizontal line

Exposure to light influenced not only the concentration of roots but also their direction of growth, which was more nearly vertical in the transparent pots than in the opaque (Fig. 2). The difference was significant ($P<0{\cdot}001$) for all species, except cucumbers. The question arises as to whether this result is accurate or whether disturbing the roots, in order to examine them, had any effect upon the subsequent direction of growth. On August 22, plants of asters, stocks and calendulas which had been examined regularly (five times altogether) were compared with an equal number of plants which had been grown under similar conditions but which had not been disturbed. In opaque pots there was a greater tendency towards vertical growth in the disturbed roots than in the undisturbed of all three species, but the difference was only significant ($P<0{\cdot}01$) for asters (Table 1). For this species there was a similar and significant ($P<0{\cdot}01$) effect of disturbance in transparent pots, so that the result cannot be attributed only to the brief exposure to light at the time of examination, but also to the mechanical effect of taking the block of soil in and out of the pot. The roots in the upper part of the pot were those mainly affected.

Table 1. EFFECT ON THE ROOT SYSTEM OF *Callistephus chinensis* OF REMOVING THE BLOCK OF SOIL FROM THE POT IN ORDER TO EXAMINE IT

Type of pot	Position of roots	$\frac{\text{Intersections of horizontal axis}}{\text{Intersections of vertical axis}}$ Disturbed	Undisturbed
Opaque	Upper part	0·514	0·423
Opaque	Lower part	0·524	0·524
Transparent	Upper part	0·758	0·650
Transparent	Lower part	0·787	0·772

The fact that transparency of the pot wall significantly ($P<0{\cdot}001$) affected the direction of growth of undisturbed roots (Table 1) establishes that the results shown in Fig. 2 cannot be explained by a difference between transparent and opaque pots in the number of times the roots were examined. For tomatoes there was in any event no such difference. The question therefore arises as to whether the downward growth of the roots in transparent pots was a negative phototropism or a light-induced geotropism. The rims of the pots were separated by 8·5 cm. on the glasshouse bench and the polyethylene of the pot walls was somewhat diffusing. The light falling on the roots was therefore coming from many directions, but always with a component into the soil, so that the main effect of phototropism would be a reduction in concentration of roots on the surface and it would only account for vertical growth where re-entry of the root into the soil was prevented. Such a reduction was observed for four species, but not in the case of tomatoes. Since there is no reason to suppose that the roots could not re-enter the soil, one is forced to the conclusion that the vertical growth was a response to gravity.

To confirm this, three plants each of stocks, asters and tomatoes were grown in transparent pots, on a piece of wire netting supported 20 cm. above a reflecting surface of aluminium foil. The pots were enclosed on all sides by a wooden box, and their tops were covered by a piece of foil, blackened on its lower surface and perforated with small holes through which the stems of the plants passed. The arrangement stood in a glasshouse in natural light, which could only reach the roots from below. Root-tips could be seen through the transparent walls of the pots, and their positions were marked without disturbing them. They grew downwards, although this was now definitely towards the light.

Main roots are commonly supposed to exhibit positive geotropism, and lateral roots are sometimes plagiogeotropic although the evidence for this is conflicting[4]. In the present experiment, in the opaque pots there was in no case any evidence for a preponderance of vertical roots ; indeed there was even some evidence for a preponderance of horizontal growth (Table 1 and Fig. 1), especially in the upper part of the pot. In the transparent pots, on the other hand, all the species except cucumber showed a significant tendency towards vertical root growth. Cucumbers commonly produce roots on the surface of the beds in which they are grown commercially.

It now appears that exposure to light is a prerequisite for geotropism in some plant roots. This could be because light is necessary for the formation in the roots of a growth inhibitor, which afterwards becomes asymmetrically disposed under the influence of gravity. The formation of such an inhibitor could also account for the reduction in concentration of roots on the surface of blocks of soil from transparent pots, whereas negative phototropism only does so with difficulty, since roots of stocks, which showed the greatest reduction in concentration, grew towards the light when illuminated from below. The failure of previous investigators to observe a light-dependence of geotropism may be accounted for by their use of very young seedlings, as there may then be sufficient of the necessary growth substance present in the radicle for a geotropic response to take place in the dark.

Thanks are due to L. G. Morris and R. W. Soffe for valuable advice and assistance.

J. V. Lake
G. Slack

Glasshouse Research Department,
National Institute of
Agricultural Engineering,
Wrest Park,
Silsoe, Beds.

[1] Bunt, A. C., *Ann. Rep. Glasshouse Crops Res. Inst.*, 1959, 116 (1960).
[2] Hunter, J. A., *N.Z. J. Sci. Tech. Sec.* A, 34, 365 (1952).
[3] Breschke, K., *Gartenwelt*, 60 (3), 56 (1960).
[4] Rawitscher, F., *Bot. Rev.*, 3 (4), 175 (1937).

Mutation for Larger Fruit in the Raspberry

Raspberry breeders have long been concerned with the problem of breeding for larger fruit. Small fruits are a feature of wild raspberries from which present-day varieties are presumed to have been evolved by many generations of selection for improved size of fruit. It is therefore not surprising that size in raspberries is complex in its inheritance, and that breeders find difficulty in combining large fruit with the many other desirable characters required in modern varieties. The discovery of a mutant raspberry with larger fruits might be of importance as a means both of further improvement in this character and, if its inheritance were simple, of simplifying the breeder's task.

Such a mutant has been found in a commercial plantation of the variety Malling Jewel. It is characterized by an overall increase in the length and thickness of the fruiting laterals and the pedicels, enlarged floral parts, and an enlarged receptacle which carries fruit with more drupelets than the parent variety. The drupelets are also larger individually, and contain larger seeds. The laterals appear to develop more slowly, causing the fruit to ripen about 2 wk. later than the normal, but no differences have been found to distinguish the vegetative (first year) growth of the mutant from that of the parent stock.

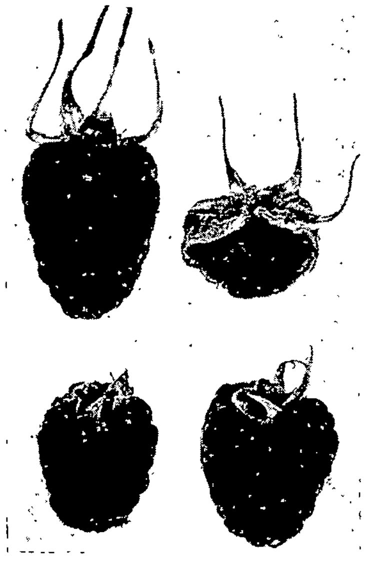


Fig. 1. Fruits of mutant Malling Jewel top left, mutant Malling Promise (top right), normal Malling Jewel (bottom left) and normal Malling Promise (bottom right)

Examination of pollen mother-cells from the mutant showed the normal diploid complement of fourteen chromosomes. Open-pollinated fruits from a plant propagated from the mutant and grown in isolation were used to raise a progeny of 114 seedlings: these all made normal vegetative growth but segregation for mutant types of fruiting lateral was evident in the second year. In addition to forms similar to the parental mutant, more extreme forms occurred which ripened later and had even larger laterals with malformed and frequently with sterile flowers. Classification of the seedlings gave normal, mutant and extreme mutant forms in the ratio of 40 : 56 : 18. This does not depart significantly from the expectation if the mutation were caused by a change in a major gene from the homozygous to the heterozygous state, and if the latter were intermediate in expression.

It seems that the change causes an error in the development of the axillary meristems, resulting in enlargement of all the tissues derived from them. The manifold effects of the gene are probably the result of what Grüneberg[1] has termed 'spurious' pleiotropism. Certain of these effects, such as the increase in seed size, are undesirable, but the growth of fruiting laterals in raspberries is governed by many minor gene complexes which give considerable independent variability to each component part. It is probable that the faults could be corrected by selection for these minor genes, for example by selecting for small seeds after crossing the mutant with a small-seeded variety such as Baumforth's Seedling *B*.

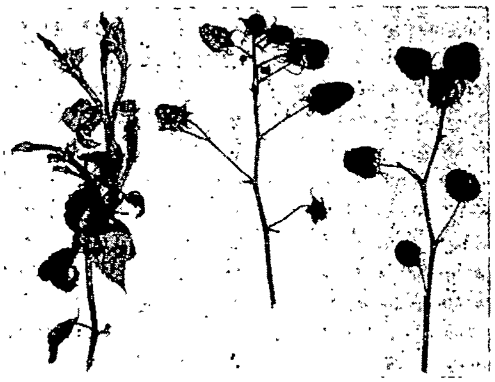


Fig. 2. Three forms of fruiting lateral which occurred in the progeny of mutant Malling Jewel: extreme, parental and normal

A second mutant raspberry has been found in a plantation of Malling Promise, but this mutant more closely resembles the extreme and presumably homozygous forms which segregated from the Malling Jewel mutant. It is conceivable that the same mutant gene may be more dominant in some genic backgrounds and may produce these sub-fertile forms when it is heterozygous ; alternatively the Malling Promise form may be a higher allele. An investigation is being made of the interactions of the mutant gene with different minor gene complexes to clarify these questions.

I am indebted to Mr. J. Morrison, of Forfar, Angus, for directing my attention to these mutants in 1958 and 1959, and to Miss N. N. Tun for counting the chromosomes.

D. L. JENNINGS

Scottish Horticultural Research Institute,
Invergowrie, By Dundee.

---

[1] Grüneberg, H., *Proc. Roy. Soc.*, B, 125, 123 (1938).

Growth of Ryegrass-dominant Pasture grazed by Cows, and Properties of New Zealand Butterfat

MARKED and regularly recurring seasonal fluctuations in the characteristics of New Zealand butterfat have been reported by Cox and McDowall[1]. The iodine value, for example, rises to a maximum of about 41 in mid-winter, falls gradually until the beginning of October and then rapidly during October and November to a mid-summer minimum of about 34, and rises again during the autumn and early winter to the mid-winter maximum. The carotene and vitamin *A* contents of the butterfat also have been shown by McDowell and McDowall[2] to change according to a regular seasonal pattern, with maximum values in the early spring and minimum values in mid-summer. These latter changes have been found by McGillivray[3] to be a reflexion of changes in the carotene and vitamin *A* contents of the blood plasma.

Seasonal changes in the properties of butterfat resulting from alternations between stall and pasture feeding of the cows, or from changes in the type of feeds given to stall-fed cows, have been reported by numerous investigators overseas, but it was surprising to find changes of this magnitude in the butterfat and blood plasma of cows grazing throughout the year on open pasture according to the general New Zealand practice. The causes of these changes have not hitherto been established.

In New Zealand almost all cows supplying the dairy manufacturing industry are calved down in the early spring. There are therefore consistent changes in the stage of lactation of the cows with progress of the dairying season, both for successive dairying seasons and for the different dairying districts. It is then a facile inference that the changes in butterfat properties are associated with change in the stage of lactation of the cows. In an investigation at this Institute with monozygous twin cows separated by several months in calving date, however, the butterfat from the later calving cow of each pair of twins was similar in properties to that from the earlier calving cow after the normal immediate post-parturition changes had been completed.

The indications were, therefore, that the changes in butterfat properties resulted from changes in the nature of the feed consumed by the cows, that is, changes in the character of the pastures. In this connexion it seemed significant that the sudden change in properties of butterfat in October–November is coincident with the rapid expansion in growth of clover in the clover-ryegrass associations, typical of most dairy pastures in New Zealand.

A change from ryegrass-dominant to clover-dominant pasture has been found by Worker and McGillivray[4] to cause some decrease in vitamin content of the butterfat, but the effect was relatively small and somewhat irregular. Further trials in which ryegrass and white clover were fed separately to twin cows have indicated that the effect of the sudden increase in the proportion of clover in the pasture consumed by cows is not sufficient to account for the observed changes in the characteristics of the butterfat.

At the same time as the proportion of clover in the mixed pasture shows a rapid increase, there is, during October–November, a transition in character of the ryegrass from the early spring lush soft foliage to the somewhat harder and more fibrous foliage which develops as the grass matures. Pure stands of *H*1 short rotation ryegrass were therefore prepared, for an investigation of the effect of maturity of ryegrass in the pasture on the butterfat properties. Portions of the area were kept closely grazed, so that the leaves were short (3–4 in.) and succulent, and the grass on the remainder of the area was allowed to grow to a height of approximately 12 in., at which stage the stalks were firming and becoming somewhat fibrous. Cows from a number of monozygous twin pairs were grazed on the two areas during September–December. Throughout this period it was found that the butterfat from the cows on the young leaves had the properties of normal early spring New Zealand butterfat, whereas that from the cows grazed on the longer-stemmed ryegrass had the properties and the carotene and vitamin *A* contents of normal October–November New Zealand butterfat. There were similar differences in the carotenoid contents of the bloods of the two groups of cows. The differences were maintained over the whole period of feeding of the two types of pasture. The average results given in Table 1 cover the period during which more detailed investigations of the fatty acid composition of both grass lipid and butterfat were made. Although the fats from both types of pasture were highly

Table 1. AVERAGE ANALYTICAL RESULTS FOR GRASS, GRASS LIPIDS, BLOOD AND BUTTERFAT FOR THE TWO TREATMENTS DURING SEPTEMBER 25–OCTOBER 6

Product analysed	Substance estimated	Average results		Difference (*I*)–(*M*)
		Immature grass (*I*)	Mature grass (*M*)	
Grass	Carotene (μgm./gm. dry matter)	545	400	145
	Lipid content (mgm./gm. dry matter)	86	46	40
Grass lipid	Linolenic acid (moles per cent)	62·0	53·0	9·0
Blood	Carotene (μgm./ml.)	21	15	6
Butterfat	Carotene (μgm./gm.)	11·5	10·3	1·2
	Vitamin *A* (μgm./gm.)	10·6	8·1	2·5
	Iodine value	43·2	35·6	7·6
	Oleic acid (moles per cent)	34·3	30·5	3·8
	Linoleic acid (moles per cent)	3·3	1·8	1·5
	Linolenic acid (moles per cent)	1·5	1·0	0·5

unsaturated, those from the immature pastures contained a higher proportion of unsaturated acids than those from the mature pastures. There was also a higher proportion of fat in the immature pasture. The differences in properties of the butterfats from cows grazing on the two types of pasture were thus associated with differences in both the nature and the amount of lipids present. It would seem, therefore, that the composition of butterfat from pasture-fed cows is markedly influenced by the type of pasture consumed, and that the seasonal changes in the carotene content of the blood and in the composition and properties of the butterfat from cows grazing typical New Zealand pastures result from seasonal changes in the state of maturity of the ryegrass in the pasture.

F. H. McDowall
W. A. McGillivray
J. C. Hawke

Dairy Research Institute,
and
Massey Agricultural College,
Palmerston North, New Zealand.

[1] Cox, G. A., and McDowall, F. H., *J. Dairy Res.*, **15**, 377 (1948).
[2] McDowell, A. K. R., and McDowall, F. H., *J. Dairy Res.*, **20**, 76 (1953).
[3] McGillivray, W. A., *J. Dairy Res.*, **24**, 95 (1957).
[4] Worker, N. G., and McGillivray, W. A., *J. Dairy Res.*, **24**, 85 (1957).

Intra-ovarian Pollination in *Eschscholzia californica* Cham., *Argemone mexicana* L. and *A. ochroleuca* Sweet

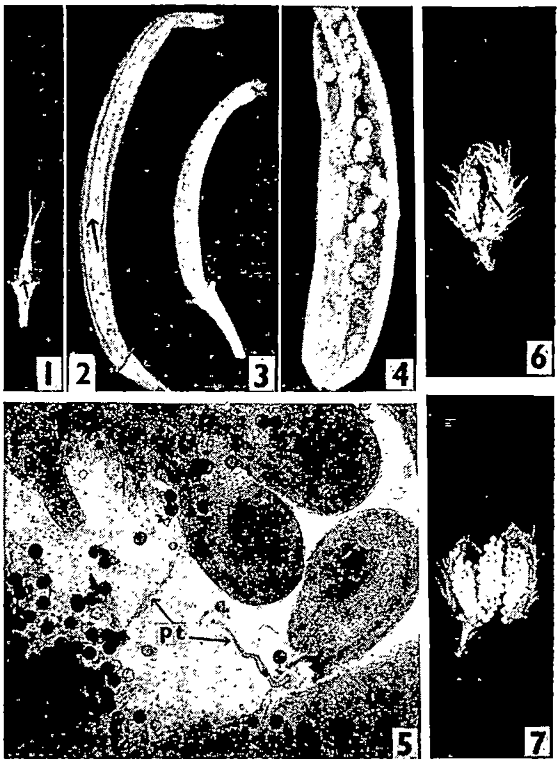
In an earlier communication one of us[1] described the results of intra-ovarian pollination in *Papaver rhoeas*. The present work deals with some observations of a similar nature on *Eschscholzia californica*, *Argemone mexicana* and *A. ochroleuca*.

The pollen suspensions were prepared in 0·5 c.c. of a boric acid solution—200 mgm./l. for *Eschscholzia* and 100 mgm./l. for *Argemone*. Each drop of the suspension contained 200–300 pollen grains.

Eschscholzia. The ovaries injected with pollen suspension grew normally (Figs. 1–3) although the size of the fruits was smaller than that of fruits from naturally pollinated ovaries.

The pollen grains germinated in the ovarian cavity (Fig. 5) and many pollen tubes entered the ovules. The growth of the fertilized ovules was slow in the beginning but the ultimate size (Fig. 4) attained by them was equal to that of ovules from naturally pollinated ovaries. The maturation of the ovaries and dehiscence of the capsules resulting from intra-ovarian pollination took almost the same time as in Nature (25–30 days). The seeds were viable and the embryos as large or larger than those which developed naturally. The seeds germinated and gave rise to normally flowering and fruiting plants.

Argemone. When a pollen suspension in 100 mgm./l. boric acid was injected the germination of the pollen grains inside the ovarian cavity was very low and scarcely more than 1 or 2 seeds were produced per fruit, or sometimes none at all. If a suspension of the pollen grains in double distilled water was used, seed setting was slightly improved (2–4 per fruit). However, when the pollen grains were introduced as such into the ovary through a vertical slit, they germinated profusely (50 per cent) and the seed set was very high. In *A. ochroleuca* about 60 seeds (Figs. 6, 7) and in *A. mexicana* as many as 140 seeds were produced per fruit ; in Nature the number of seeds varies between 150 and 200 in the two species.



Figs. 1–5. *Eschscholzia californica*, ovaries injected with a suspension of pollen in 200 mgm./l. boric acid. Fig. 1. Ovary at the time of anthesis and injection of pollen grains; the arrow in this and in Fig. 2 indicates the place of injection of the pollen suspension (× $\frac{3}{4}$). Figs. 2 and 3. 17 and 25 days after injection (× $\frac{3}{8}$). Fig. 4. Same as Fig. 3, cut vertically to show the developing ovules (× 2·25). Fig. 5. Transverse section of ovary 72 hr. after injection; note the pollen tubes (*pt*) from the germinating pollen grains in the ovarian cavity (× 47). Fig. 6. *Argemone ochroleuca*, ovary 12 days after insertion of pollen through a vertical slit shown by an arrow (× $\frac{3}{8}$). Fig. 7. Same as Fig. 6, split open to show developing ovules (× $\frac{3}{8}$)

In *A. mexicana* the growth of treated ovaries was slow and the maximum size attained by them was only two-thirds of the normal. In *A. ochroleuca* the treated ovaries grew to less than one-half of their natural size. Nevertheless, in both the species the growth of the ovules and the differentiation of the embryo were almost similar in the treated as well as in the naturally pollinated ovaries. The capsules ripened and dehisced in the same period (27–30 days after anthesis) in both sets and the seeds obtained by intra-ovarian pollinations were fully viable.

A. mexicana is a diploid ($n = 14$) and *A. ochroleuca* a tetraploid ($n = 28$)[2,3]. Natural hybrids between the two are rare and usually sterile[2]. This incompatibility could possibly be overcome by intra-ovarian pollination and work in this direction is in progress. The technique of injecting pollen grains into the ovary may be of help in overcoming some types of incompatibilities.

P. Maheshwari
Kusum Kanta

Department of Botany,
University of Delhi,
Delhi, 6.

[1] Kanta, Kusum, *Nature*, **188**, 683 (1960).
[2] Malhotra, S. K., *Curr. Sci.*, **29**, 282 (1960).
[3] Venkatesh, C. S., *Proc. Indian Sci. Congr., Bombay*, **47**, 401 (1960).

CYTOLOGY

Periodic Acid–Schiff Positive Giant Cells in the Mouse Thymus Cortex

HAMMAR[1] first described cells, laden with fat granules, in the thymus cortex of ageing humans. More recently, a detailed description of the histochemical reactions of these cells in the mouse thymus has been given by Loewenthal and Smith[2], who pointed out that these cells gave a strong red colour when stained with the periodic acid–Schiff reagent (PAS).

During a survey of the morphology of the thymus in high- and low-leukæmia strain mice, these cells were observed in large numbers in the cortices of ageing *AKR* and *C3H* mice. Their nuclei resembled thymic reticulum cell nuclei rather than those of the lymphocyte series. The cytoplasm of these cells was voluminous, and packed with granular material with the following staining reactions: positive to PAS (diastase resistant), pyronine and scharlach *R*; negative to toluidine blue (stained pale green), eosin and Feulgen. The cells appeared capable of phagocytosis, since they frequently contained pyknotic nuclear material, presumably lymphocytic in origin. These cells first appeared in the thymus in mice aged between two and four months, and their number increased with advancing age, although varying considerably in individual thymuses. These cells were found in increased numbers in thymuses of mice in the second half of pregnancy, if aged older than four months. The development of these PAS cells appeared to be related to changes in age of cellular elements within the thymus itself. One-week-old thymuses were grafted subcutaneously into six-month-old *C3H* mice and examined histologically one month after grafting. Although all host thymuses showed numerous PAS cells, none of the grafted thymuses showed such cells. Similar negative results were obtained with week-old thymuses grafted to pregnant six-month-old *AKR* hosts. These results appear to exclude the possibility that circulating humoral factors in ageing mice are primarily responsible for the development of these cells.

The location of the PAS cells was usually subcapsular, but frequently they were scattered uniformly throughout the cortex. The cells were not uniform in size and varied from 7μ to 26μ in diameter. The mean size of the cells increased progressively with advancing age. The PAS cells were surrounded by tightly packed lymphocytes. Frequently lymphocytes were seen which had invaginated the cytoplasm of the PAS cells and were lying in deep crypts, projecting into the cells.

In 7μ sections, one in three PAS cells was observed to have one or more mitotic lymphoid cells in contact with some part of the cytoplasm of the cell. A quantitative analysis was made of the frequency of mitotic lymphoid cells in the area surrounding PAS cells, relative to areas distant from these cells. In each thymus, twelve fields, $72\mu \times 72\mu$, were selected at random and drawn on graph paper and the total PAS area determined (area of PAS cells, plus area 5μ in width around each cell). Cortical tissue outside this area was designated 'non-PAS' area. The number of mitoses in both areas was determined and the stage of each mitosis noted. The results (Table 1) indicated that, in *AKR* thymuses, mitoses per unit area were three times more frequent in the PAS areas than in the adjacent 'non-PAS' areas. The results in thymuses of the low-leukæmia strain *C3H* indicated much less difference in the incidence of mitoses between the two areas. These results require extension to confirm the reality of this difference in strain.

Table 1

Strain	Age (months)	Sex	No. of thymuses	Mean percentage PAS area	Mean mitoses in PAS area, per unit area	Mean mitoses in 'non-PAS' area, per unit area	Ratio PAS mitoses 'non-PAS' mitoses*
AKR	6–10	M	13	27	95	32	3·1 ± 0·8
AKR	6–8	F	10	21	73	26	3·0 ± 0·8
C3H	8–10	M	12	24	59	42	1·4 ± 0·4

* ± *S.D.*

The analysis of the percentage of cells in various stages of mitosis in the two areas revealed, in *AKR* thymuses, a predominance of prophase mitoses in the PAS areas (11 ± 4 per cent versus 6 ± 3 per cent in the 'non-PAS' areas). The percentages of the other stages of mitosis were similar in both areas.

The significance of the association between lymphocyte mitotic activity and PAS cells in the thymus cortex is not known. The PAS cells may either stimulate mitosis in neighbouring lymphoid cells, or may attract cells about to enter mitosis. The predominance of prophase mitoses around PAS cells suggests that such stimulation or attraction may be related to the initial phases of mitosis, and that some of the mitotic cells may later move away from the PAS cells before mitosis is complete.

Since the PAS cells and associated mitoses occur only in post-involutional thymuses, they may represent some type of regulation of thymic lymphocyte mitotic activity, supervening on an earlier form of regulation present in the pre-involutional thymus.

D. METCALF
M. ISHIDATE

Cancer Research Laboratory,
Walter and Eliza Hall Institute,
Royal Melbourne Hospital P.O.,
Australia.

[1] Hammar, J. A., *Endocrinol.*, **5**, 543 (1921).
[2] Loewenthal, L. A., and Smith, C., *Anat. Rec.*, **112**, 1 (1952).

VIROLOGY

A Method of treating Asbestos Filter Pads for Virus Filtration

PRE-TREATMENT of Seitz-type filters with serum or with digest broth will reduce, to some extent, the often serious losses of virus caused by adsorption during filtration. Solutions of gelatin have been used recently instead of serum or broth with the same object in view. Although certain advantages are claimed for the use of gelatin, for example that most samples are antigenically inert, experience has shown that losses during filtration through asbestos filters treated with this protein substance are not uniformly negligible.

For some purposes it is desirable to avoid the addition of foreign protein to virus preparations, and so investigations were made to determine whether non-protein substances of complex molecular structure would diminish the virus-adsorbing properties of asbestos filter pads.

Sodium alginate, the sodium salt of alginic acid $(C_6H_8O_6)_n$, a polymer of beta-D-mannuronic acid, which is generally considered to be a non-protein substance, was used for this purpose and its efficacy was compared with that of gelatin. It is as well to mention, however, that protein estimations by the Folin-Ciocalta method revealed that the particular preparation used in these experiments contained a trace of protein (0·16 per cent calculated for dry weight). This protein no doubt occurred as an impurity and originated from the seaweed *Laminaria cloustoni*, from which the alginate was prepared. The concentration of protein in the 0·04–0·05 per cent solutions finally recommended for treatment of filter pads would be only 0·0006–0·0008 per cent. The effect of the alginate solution is attributable, therefore, principally to the complex polysaccharide molecule of alginic acid, in which the mannuronic acid units are connected in long chains by 1,4-β-glycosidic linkages.

Asbestos filter pads were treated before use with 25·0 ml. of solutions containing concentrations of either sodium alginate or gelatin in the range 0·0025–0·06 per cent; excess solution was then washed from the pads by passing 50·0 ml. of *M*/25 phosphate buffer through them. Untreated control pads were washed with 75·0 ml. of buffer only, to adjust the *p*H of the pad.

Preparation of viruses. The virus suspensions used in the experiments were first passed through clarifying filters to remove gross particulate matter. The results obtained therefore refer to relatively crude preparations and not necessarily to highly purified virus.

For all experiments on the virus of vesicular stomatitis, an egg-passaged strain of Indiana type (Ind. *C*.) was used, manipulations being performed with appropriate precautions to avoid inactivation by light[1]. The virus suspensions in physiological saline were made from embryo extracts.

Strains of foot-and-mouth disease virus of types *O*, *C* and Asia 1 were propagated in serum-free pig kidney or tongue epithelium tissue culture; culture extracts were diluted in *M*/25 phosphate buffer before filtration.

Filters. Asbestos filter pads of 3·0 cm. diameter were used throughout the series of experiments; all were of *E.K.* equivalent grade (Ford *G.S.* or Seitzwerke *E.K.*). Solutions of gelatin were made by dissolving gelatin powder (British Drug Houses, Ltd., Poole, Dorset) in warm distilled water, and then diluting the solution to give the required concentration of gelatin. The sodium alginate used in all experiments was of pharmaceutical grade and conformed with the *British Pharmaceutical Codex* specification. The following analytical data were kindly supplied by the makers (Messrs. Alginate Industries, Ltd., Bedford Street, London, W.C.2):

Moisture content		20·4 per cent
Calcium content		0·27 per cent
Lead content	(less than)	10 p.p.m.
*p*H		6·3
Viscosity of 1 per cent solution*		62 centi-stokes

* Measured in a B.S. U-tube viscometer at 25° C. and corrected for moisture content of the powder.

The calculated mean molecular weight was approximately 88,000; somewhat greater than that of serum albumins (65,400–72,300 when calculated from sedimentation and diffusion constants[2]).

Stock solutions of sodium alginate in glass-distilled water were prepared at a concentration of 0·05 or 0·1 per cent w/v, and these solutions were diluted for use as required. Residual solutions were always discarded seven days after preparation.

Mice. Samples of vesicular stomatitis virus were titrated in 8-day old mice of the Pirbright '*P*' strain; 4–6-day old mice of the same strain were used for titrations of the foot-and-mouth disease virus.

Titrations were made in groups of 8 or, occasionally, 6 mice per dilution with either 0·6 or 0·5 log intervals between dilutions of each series. The dose was 0·03 ml. for both virus systems, but inoculations were made intracerebrally with vesicular stomatitis virus and intraperitoneally with foot-and-mouth disease virus.

Calculation of results. The 50 per cent fatality end-point for each series was estimated by the Spearman–Kärber method to permit determination of the amount of virus lost during filtration.

The results obtained with suspensions of vesicular stomatitis virus before and after filtration are given in Fig. 1. They show that when filters are merely washed with *M*/25 phosphate buffer, to prevent inactivation of virus by *p*H changes while passing through the filter, the losses of virus during filtration in some cases exceeded 5 log units.

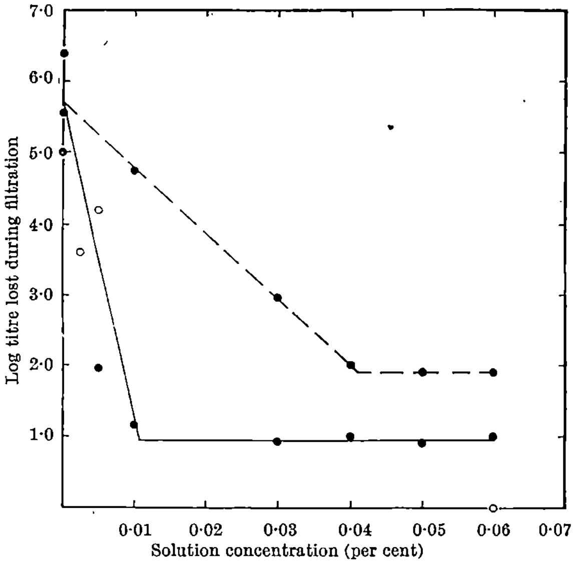


Fig. 1. Filtration of vesicular stomatitis virus through treated asbestos filters. Ford *G.S.* filter, ●; Seitz *E.K.* filter, ○; sodium alginate solution, ——; gelatin solution, – – –

Pre-treatment of filters with increasing concentrations of either gelatin or sodium alginate progressively decreased the losses of virus up to a finite value for each substance; but thereafter further increments of gelatin or sodium alginate in the solution failed to reduce filtration losses below the critical values of 1·95 and 0·9 log units, respectively. Losses in gelatin-treated filters were always greater than losses in filters treated with the same concentration of sodium alginate (Fig. 1).

When foot-and-mouth disease virus was filtered through either treated or untreated filters, the adsorption losses were considerably less than those encountered during filtration of vesicular stomatitis virus. This difference is probably a function of virus size, the

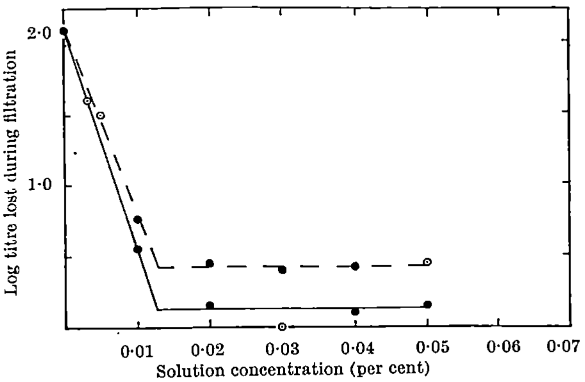


Fig. 2. Filtration of foot-and-mouth disease virus through treated asbestos filters. Mean values, ●; single values, ⊙; sodium alginate solution, ——; gelatin solution, - - -

infective component of the former virus being a roughly spherical particle 20–25 mμ in diameter, whereas that of the latter virus is a short rod-shaped body having an effective filtration diameter of 80–100 mμ. Nevertheless, more virus was lost in pads treated with gelatin solutions than in the corresponding pads of the sodium alginate series (Fig. 2). Irrespective of the concentrations used, it was impossible to reduce the filtration losses to less than about 0·15 log unit with sodium alginate or 0·43 log unit with gelatin.

These observations, together with the apparent inability of sodium alginate to act as an antigen, indicate that pre-treatment of asbestos filter pads with 0·04–0·05 per cent solutions of sodium alginate is a valuable means of minimizing adsorption losses during filtration of virus suspensions. The inferior results obtained with the protein, gelatin, may justify attribution of this effect to the sodium alginate *per se*, and not to the presence of infinitesimal amounts of protein impurity in the solutions used for treatment of the filters.

I am indebted to Dr. F. Brown for the protein estimations.

N. St. G. Hyslop

Research Institute
(Animal Virus Diseases),
Pirbright, Surrey.

---

[1] Skinner, H. H., and Bradish, C. J., *J. Gen. Microbiol.*, **10**, 377 (1954).

[2] Edsall, J. T., *The Proteins*, edit. by Neurath and Bailey (Academic Press, New York, 1953).

SOIL SCIENCE

Factors influencing the Composition of the Cellulose-decomposing Microflora on Soil Crumb Plates

Although many soil micro-organisms can decompose cellulose[1], each group of organisms does so under its own set of conditions. For example, *Cellvibrio* and *Cytophaga* develop around soil crumbs placed on filter paper which covers a mineral silica-gel medium, as shown by Winogradsky[2]. Other workers have reported that cellulose-decomposing fungi were the first organisms to attack 'Cellophane' buried in the soil[3].

It was sought to determine what causes one or the other species of the cellulose-decomposing flora to predominate and whether this is due to differences in technique or in the cellulose substrate used. For this purpose the composition of the cellulose-decomposing soil flora on filter paper and on 'Cellophane' were compared under the same conditions. Strips of filter paper (Whatman No. 1) and strips of 'Cellophane' (*PT* 300, British Cellophane Co.) were placed on mineral agar solidified in Petri dishes. Soil crumbs taken from the same soil sample were spread on strips of filter paper and 'Cellophane', respectively, and the Petri dishes were incubated for a few days at 30°.

Bacteria only (mostly *Cellvibrio*) developed on the plates covered with filter paper, while cellulose-decomposing fungi (mostly *Stachybotrys*) predominated on 'Cellophane' plates.

The failure of *Stachybotrys* to appear around soil crumbs on the filter paper could not be explained by its inability to attack the substrate, because a pure culture of *Stachybotrys* grew well on filter paper. To elucidate this question, the possibility of the bacterial growth interfering with the fungal growth was investigated. Soil crumbs were spread on filter paper placed on mineral agar plates containing 100 μgm./ml. chloramphenicol to inhibit bacterial growth, when only cellulose-decomposing fungi, especially *Stachybotrys*, developed.

In further tests a pure culture of *Stachybotrys* spores was mixed with a pure culture of *Cellvibrio* and the mixture inoculated on strips of filter paper on mineral agar. Under these conditions *Stachybotrys* failed to develop, or grew poorly, demonstrating the strong inhibitory effect of *Cellvibrio*.

Dobbs and Hinson[4] show that soil has a strong inhibitory effect on the germination of fungal spores; thus it was of interest to see whether *Cellvibrio* inhibited the germination of *Stachybotrys* spores. Direct microscopic observations of *Stachybotrys* spores in the presence of *Cellvibrio* showed that, although the spores germinated normally, growth was arrested after the development of the germination tubes. *Cellvibrio* inhibited the growth of the fungus only on media containing cellulose as the sole carbon source, and not when glucose or cellobiose was present.

No filtrable or diffusible substances responsible for the antagonism were detected. Direct contact between both organisms seems to be necessary to get the effect.

The predominance of fungi in the flora of soil crumbs spread on 'Cellophane' could easily be explained when it was shown that a pure culture of *Cellvibrio* either failed to grow, or grew only slightly, when 'Cellophane' was used as the substrate.

'Cellophane' has been much used recently in investigations of the cellulose-decomposing soil flora[3]. It is therefore important to point out that this substrate does not equally support the development of all the cellulose-decomposing micro-organisms, but seems to be more of an enrichment medium for the selection of cellulose-decomposing fungi.

A. Keynan

Israel Institute for Biological Research,
Ness-Ziona.

Y. Henis
P. Keller

Department of Soil Sciences,
The Faculty of Agriculture,
The Hebrew University,
Rehovoth, Israel.

---

[1] Siu, R. G. H., *Microbial Decomposition of Cellulose* (Reinhold Publ. Co., New York, 1951).

[2] Winogradsky, S., *Microbiologie du Sol* (Masson et Cie., Paris, 1949).

[3] Tribe, H. T., *Canad. J. Microbiol.*, **6**, 309 (1960).

[4] Dobbs, C. G., and Hinson, W. H., *Nature*, **172**, 197 (1953).

PSYCHOLOGY

Learning in Two Sense-modalities

In previous investigations[1,2] monkeys were taught to discriminate between shapes or rates of intermittence exclusively in one sensory modality (say visual) and were afterwards tested with the same shapes or rates exclusively in another modality (say tactile for shapes or auditory for rates). There was no evidence of cross-modal transfer of training. A variation of this procedure was also briefly described[1]. Four animals first learnt to discriminate between two test objects exclusively by touch (in the dark) and were afterwards tested for 30 trials with the same objects (in the light) under conditions where the objects could be discriminated either by touch or by vision. Only one animal performed significantly better than chance in the light. It was concluded that "even when monkeys are constrained to touch the very cues which served as the basis for successful somato-sensory discrimination of shape or size on the preceding day, there is still no clear indication of positive transfer on visual testing" (p. 62). In the present investigation the converse procedure was used: animals were first trained to discriminate between test objects by vision and/or touch (in the light) and were afterwards tested with the same objects for discrimination by touch alone (in the dark).

Three separate batches of respectively 8, 6 and 7 untrained rhesus monkeys (weight-range, 3·3–6·3 lb.) formed the three groups of subjects. All the subjects in a group were trained concurrently and all training was carried out under comparable conditions by the same investigator. However, group I animals were trained during March, group II during July and group III during November. (This consecutive training was necessary because all animals afterwards took part in different experiments.)

Standard apparatus (Wisconsin general testing apparatus) was used in conjunction with two food boxes placed 12 in. apart. The test objects were fastened to the lids of these boxes. Forty trials (non-correction) were given each day with the right/left positions of the positive test objects determined by the Gellerman schedule. During tactile training (in the dark) the animals' performance was observed by means of an infra-red telescope.

Animals of group I were first taught (see Table 1) to discriminate in the light between two relatively unsaturated hues, pink and cream. Animals of groups II and III were first trained on a quality discrimination in the light. In this test the positive (rewarded) object consisted of a piece of black foam rubber (2 × 0·75 × 0·3 in.) attached across the lid by a steel strip and two bolts. The negative object consisted of a black piece of wood of nearly the same dimensions as the rubber (but of different texture). The lids could only be opened by grasping and then pushing either the rubber or the wood. For their second test all 21 animals were trained to make this quality discrimination in the dark. However, animals of group III were concurrently given a further 100 trials on the visual form of this test according to a method described elsewhere[3]: visual trials in the light alternated with tactile trials in the dark (making 20 visual and 20 tactile trials each day), but were excluded from the learning scores relating to the tactile form of the test.

Table 1. Training Histories and Results on Quality Discrimination Test

Group I ($n = 8$)	Group II ($n = 6$)	Group III ($n = 7$)
Visual hue discrimination Mean = 275 trials	Quality discrimination (in the light) Mean = 192 trials	
Tactile quality discrimination test (in the dark)		
Animal: (1) 137 trials	93 trials	85 trials
(2) 162 „	116 „	55 „
(3) 133 „	98 „	243 „
(4) 157 „	97 „	43 „
(5) 60 „	84 „	318 „
(6) 108 „	120 „	46 „
(7) 124 „		62 „
(8) 161 „		

The figures refer to the number of trials required to reach the standard level of performance (10 or less errors in 100 consecutive trials).

Table 1 contains the results for the tactile test. There is considerable overlap between the scores of animals belonging to different groups. Animals of group I tended to have the highest scores and animals of group III, with two exceptions, tended to have the lowest scores. These two animals, although extremely hesitant and unresponsive on this their first tactile test, have not been excluded because on subsequent tactile testing their performance fell within the range relating to other animals. Statistical comparisons (Mann-Whitney '*U*' test) indicate a significant difference between the scores of group I and group II animals ($P = 0·02$); but not between the scores of group I and group III animals ($P = 0·14$) or between those of group II and group III animals ($P = 0·15$).

These results indicate that in the monkey combined visual and tactile experience of two objects may lead to significantly improved performance (group II animals) when the same objects are afterwards offered for exclusively tactile discrimination. A comparable investigation has not been found in the literature. There are at least three interpretations of the present results. First, since cross-modal transfer from vision to touch could not be demonstrated in a previous investigation[1] it is unlikely that memory of the actual visual quality discrimination improved the performance of the group II animals during later tactile testing in the dark. Secondly, non-specific familiarity with the test objects, acquired during training in the light, may have accelerated subsequent tactile learning. Thirdly, it is possible that obligatory tactile contact with the objects during testing in the light led to some measure of genuine tactile discrimination learning. Irrespective of its origin, this improvement in tactile learning resulting from visual-tactile experience of the test objects in the light was not as pronounced, at least under the present experimental conditions, as might have been expected.

This work was supported by the Research Fund of the Institute of Neurology and by the Medical Research Council. Dr. E. A. Carmichael gave it his active encouragement and provided facilities, as did Dr. E. T. O. Slater. I am also much indebted to Dr. S. L. Chorover for help with the preparation of this communication.

G. Ettlinger

Psychological Laboratory,
Institute of Neurology,
Queen Square,
London, W.C.1.

---

[1] Ettlinger, G., *Behaviour*, 16, 56 (1960).

[2] Burton, D., and Ettlinger, G., *Nature*, 186, 1071 (1960).

[3] Chorover, S. L., and Cole, M., *J. Physiol.*, 156, 35*P* (1961).

FORTHCOMING EVENTS

Tuesday, July 18

Council for the Preservation of Rural England (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 2.30 p.m.—Annual General Meeting. Mr. Harold Abrahams, C.B.E.: "The Amenity Societies, the National Parks Commission and their Common Cause".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant, Grade "B" (with a good honours degree, together with some research, industrial or teaching experience, and preferably a knowledge of some branch of modern applied mathematics such as operation research, numerical analysis or electronic computers) in Mathematics—The Principal, Brunel College of Technology, Woodlands Avenue, Acton, London, W.3 (July 19).

Lecturer (Grade II) or Assistant Lecturer (Grade III) in Clinical Pathology in the Department of Veterinary Medicine at the School of Veterinary Science, Langford—The Registrar and Secretary, The University, Bristol (July 19).

Research Sociologist in the Department of Nutrition, for an investigation of social factors affecting nutrition—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (July 19).

Lecturer or Assistant Lecturer (graduate with an interest in the philosophy of science) in Philosophy—The Registrar, The University, Leicester (July 20).

Assistant, Assistant Experimental Officer grade (preferably with a degree and some knowledge of languages), with the Agricultural Research Council's Weed Research Organization, near Oxford, for duties which include abstracting, indexing and responsibility for the Organization's Library—The Secretary, Agricultural Research Council, Weed Research Organization, Begbroke Hill, Sandy Lane, Kidlington, Oxford (July 22).

Computer Officer (graduate) in the Sub-Department of Numerical Analysis—The Registrar, The University, Liverpool (July 22).

Graduate (with a degree in biochemistry or related subjects), to take part in a chemical and biochemical investigation concerned with fruits and vegetables—The Director, Low Temperature Research Station, A.R.C., Downing Street, Cambridge (July 22).

Lecturer or Junior Lecturer (with a postgraduate degree in biochemistry and some teaching experience) in the Department of Biochemistry—The Secretary, The London Hospital Medical College, Turner Street, London, E.1 (July 22).

Lecturers (preferably with research and/or industrial experience) in Physical Chemistry—The Principal, Nottingham and District Technical College, Nottingham (July 22).

Research Assistant (graduate in science, preferably physics, and with a knowledge of electronics), for investigations into the measurement of radioactivity in livestock—The Registrar, The University, Leeds 2 (July 22).

Research Assistant in the Department of Geology, to collaborate in field and laboratory studies of Irish Dalradian rocks—The Registrar, The University, Liverpool (July 22).

Scientific Officer or Senior Scientific Officer (with a good honours degree, and preferably some postgraduate experience), to work on biochemical processes in seeds and seedlings—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (July 22).

Temporary Lecturer in Pure Mathematics—The Registrar, University College, Singleton Park, Swansea (July 22).

Research Student in X-Ray Crystallography, to work for a higher degree under the supervision of Dr. S. C. Nyburg on crystal structures and absorption spectra of transition metal complexes—The Registrar, University College of North Staffordshire, Keele, Staffs (July 28).

Scientific Officer, Senior Scientific Officer or Principal Scientific Officer (suitably qualified scientist, preferably with experience of blood grouping or serology), to extend the work of the Organization on antigens and biochemical variants in cattle blood—The Secretary, Animal Breeding Research Organization, Glenbourne, South Oswald Road, Edinburgh 9 (July 29).

Lecturer (medical or honours science graduate, with experience in general microbiology or microbial chemistry, and in research methods) in Bacteriology—The Secretary, The University, Edinburgh (July 31).

Lecturer or Senior Lecturer (preferably with a special interest and experience in dairy husbandry and postgraduate experience in animal physiology) in the Department of Agriculture—The Registrar, The University, Leeds 2 (July 31).

Plant Physiologist, Scientific Officer grade (with an honours degree and preferably postgraduate experience), to work on light intensity and leaf canopy development in orchards in relation to tree form and its modification for high yield—The Secretary, East Malling Research Station, Maidstone, Kent (August 1).

Postdoctoral Fellow in the Department of Chemistry, to work on mould metabolites—Prof. R. A. Raphael, Department of Chemistry, The University, Glasgow, W.2 (August 1).

Senior Technician in the Department of Agriculture, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 4).

Lecturer or Assistant Lecturer in Zoology (Entomology) at the University College of The West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 7).

Technician (qualified and experienced draughtsman, preferably with photographic experience) in the Department of Geography, University College, Ibadan, Nigeria, for work involving the preparation of maps for display and publication—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 12).

Senior Research Assistants and Research Assistants in Fluids Mechanics—Prof. K. Stewartson, Science Department, The University, South Road, Durham (August 15).

Chair of Pathology in Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 20).

Chair of Physics at Fourah Bay College, University College of Sierra Leone—The Secretary Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 31).

Lecturer (with an honours or higher degree in chemical engineering or equivalent qualifications, and preferably an interest in mass transfer or nuclear chemical engineering) in Chemical Engineering at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

Lecturers (3) (with special qualifications in inorganic, organic or physical chemistry) in Chemistry at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1, or The Registrar, University of Canterbury (New Zealand and London, August 31).

Scientific Officer (honours graduate with a special interest in plant breeding and genetics), for work on tree fruits under experienced leadership, as one of a team of fruit breeders—The Secretary, East Malling Research Station, Maidstone, Kent (August 31).

Scientific Officer (honours graduate), for research on problems arising out of the vegetative propagation of fruit plants—The Secretary, East Malling Research Station, Maidstone, Kent (September 30).

Assistant Lecturer (with a good honours degree, and preferably research and/or industrial experience) in Physical Chemistry—The Registrar, Hatfield College of Technology, Hatfield, Herts.

Assistant Professor (with Ph.D. or equivalent, and preferably with a background and experience in marine biology) of Biology, to give instruction primarily in animal physiology—Head, Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Chemist (with an honours degree in agricultural chemistry or chemistry, and preferably at least one years postgraduate training at an experimental station) in the Department of Agriculture, Uganda, for research work on soil fertility problems connected with tropical crop rotations, manures and fertilizers—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/9/09/C1.

Chemist (with some research experience in physics or biochemistry), to work on certain aspects of the chemistry of wheat protein; and an Assistant (male, with some experience in the management of laboratory animals), for a small animal unit concerned mainly with nutritional research on flour and bread—The Director of Research, Cereals Research Station, Old London Road, St. Albans, Herts.

Graduate (preferably with previous experience in tissue culture or virology), to be responsible for the maintenance and supply of tissue cultures needed for research on trachoma and related eye diseases—Dr. L. H. Collier, Trachoma Research Unit, Lister Institute, Chelsea Bridge Road, London, S.W.1.

Lecturer or Assistant Lecturer (with a degree in agriculture, or equivalent, and preferably with some tropical experience) in Agriculture at the Imperial College of Tropical Agriculture, University College of The West Indies, Trinidad—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

Lecturer (with an honours degree or equivalent qualification in metallurgy or physics) in the School of Metallurgy, University of New South Wales, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2, and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia.

Master (with a good honours degree) to teach Chemistry throughout the School—The Headmaster, King Edward VII School, Lytham.

Physicists, to carry out independent research and to take an interest in a broad range of applications of physics to the building industry—Dr. A. R. Collins, Director of Research and Technical Services, Cement and Concrete Association, Wexham Springs, Stoke Poges, Buckinghamshire.

Postdoctoral Research Fellow in Organic Chemistry—Prof. R. C. Cookson, The University, Southampton.

Postgraduate Research Assistant (with a knowledge of X-ray diffraction methods), for studies of liquid structure under the personal supervision of Prof. J. D. Bernal—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1.

Research Assistant (preferably with experience in cytology), to take part in a programme designed to study human chromosome abnormalities and associated disorders—The Secretary, Genetics Department, The University, Sheffield, 10.

Research Assistant, to help with current research on an immunological project connected with rheumatoid arthritis—The Pathologist, Stoke Mandeville Hospital, Aylesbury, Bucks.

Research Officer (Economic Botanist) (male, between 24–45, with an honours degree in natural science, two years postgraduate training, a good general knowledge of the subject, and preferably some field experience) in the Department of Agriculture, North Borneo, to deal with the selection and breeding of the major crops of North Borneo—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. BCD.63/21/09/C1.

Research Technician (with a knowledge of chemistry, physics or biochemistry) to assist in the study of biochemical changes to patients and experimental animals undergoing heart operations by means of heart-lung bypass procedures—The House Governor, Brompton Hospital, South Kensington, London, S.W.7, quoting PR2.

Senior Lecturer (qualified to teach microbiology to final degree and Diploma in Technology standard) in Microbiology—The Registrar (Department F), Bradford Institute of Technology, Bradford 7.

Senior Technician (Grade 1) or Scientific Officer for the Microbiological Assay Unit (Vitamin B_{12} and folic acid), City

General Hospital—The Group Secretary, Sheffield No. 1 Hospital Management Committee, "Lyndhurst", Nether Edge Hospital, Sheffield 11.

SENIOR TECHNICIAN I IN THE DEPARTMENT OF PATHOLOGY, for duties confined to experimental pathology—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Department of Scientific and Industrial Research. Report of the National Physical Laboratory for the year 1960. Pp. ix+176+8 plates. (London: H.M. Stationery Office, 1961.) 9*s*. 6*d*. net. [55

The British Coal Utilization Research Association. Annual Report 1960. Pp. 62. (Leatherhead: British Coal Utilization Research Association, 1961.) [55

Ciba (A.R.L.), Limited. Technical Notes, No. 220: Araldite-Jointed Drain. Pp. 8. (Duxford: Ciba (A.R.L.), Limited, 1961.) [55

Proceedings of the University of Durham Philosophical Society. Series A (Science). Vol. 13, No. 16: The Pupation of *Aphodius rufipes* L. (Coleoptera: Scarabaeidae). By D. C. Ungley. Pp. 161–167. Vol. 13, No. 17: The Development of Qualitative Analysis (1750–1850). Part 2: Mineral Water Analysis. By W. A. Campbell and G. E. Mallen. Pp. 168–174. (Durham: University of Durham Philosophical Society, 1960.) [95

Institution of Electrical Engineers. Annual Report for 1960–61. Pp. 16. (London: Institution of Electrical Engineers, 1961.) [95

British Medical Bulletin, Vol. 17, No. 2 (May, 1961): Foetal and Neonatal Physiology. Pp. 79–176+8 plates. (London: British Council, 1961.) 20*s*. [95

The Murphy Chemical Co., Ltd., The Control of Red Spider on Apples and Pears. Pp. 4. (Wheathampstead: The Murphy Chemical Co., Ltd., 1961.) [95

Research Defence Society. Conquest Pamphlet No. 13: Animal Experiments in the Control of Animal Disease: Outstanding questions posed and answered by the Research Defence Society. Pp. 6. (London: Research Defence Society, 1961.) 6*d*. [115

Building Research Station Digest (Second Series.) No. 10: Lifting Equipment for Cranes. Pp. 4. (London: H.M. Stationery Office, 1961.) 4*d*. [115

Air Ministry: Meteorological Office. Scientific Paper No. 7: Forecasting in the Falkland Islands and Dependencies. By S. D. Glassey. Pp. iii+30. (London: H.M. Stationery Office, 1961.) 3*s*. 6*d*. net. [115

B.B.C. Engineering Monograph No. 36: Some Aspects of Optical Lens Performance. Part 1: Secondary Flare in Lenses. By K. Hacking. Part 2: The Design of Lens Hoods. By W. N. Sproson. Pp. 19. (London: British Broadcasting Corporation, 1961.) 5*s*. [125

Embryo, No. 1, 1961. Pp. 48. (London: Scientific Society of the University Union, 1961.) 2*s*. [165

Department of Scientific and Industrial Research. Water Pollution Research 1960: The Report of the Water Pollution Research Board with the Report of the Director of the Water Pollution Research Laboratory. Pp. vi + 122 + 4 plates. (London: H.M. Stationery Office, 1961.) 7*s*. net. [165

The Society for Analytical Chemistry. Report of the Analytical Methods Committee, 1960. Pp. vi + 32. (London: The Society for Analytical Chemistry, 1961.) [165

Planning, Vol. 27, No. 452 (15 May, 1960): The Cost of Roads. Pp. 109–140. (London: Political and Economic Planning, 1961.) 3*s*. 6*d*. [165

The Impact of Chemical Physics on Technology. By Dr. J. S. Rowlinson. (Inaugural Lecture, 14 February 1961.) Pp. 12. (London: Imperial College of Science and Technology, 1961. Distributed by The Modern Book Co., Ltd., 19 Praed Street, London, W.2.) 4*s*. 6*d*. [165

The British Iron and Steel Research Association. Annual Report, 1960. Pp. 156. (London: The British Iron and Steel Research Association, 1961.) [175

Ministry of Agriculture, Fisheries and Food. Code of Practice for Ground Spraying. Pp. 5. Do's and Dont's for Users of Chemical Sprays. Pp. 2. (London: Ministry of Agriculture, Fisheries and Food, 1961.) [175

Report of Committee on Salmon and Freshwater Fisheries. Pp. viii + 151 + 13 plates. (Cmnd. 1350.) (London: H.M. Stationery Office, 1961.) 8*s*. net. [175

Infrared Physics, Vol. 1, No. 1 (March, 1961). (An International Research Journal.) Pp. 104. Annual subscription rates: (A) For Libraries, Government Establishments, Research Laboratories, etc. £7 (20 U.S. dollars); (B) for individuals who place their orders directly with the publisher and certify that the journal is for their personal use £3 10*s*. (10 dollars). (London and New York: Pergamon Press, 1961.) [175

Other Countries

United States Department of the Interior: Geological Survey. Professional Paper 347: Geomorphology and Forest Ecology of a Mountain Region in the Central Appalachians. By John T. Hack and John C. Goodlett. Pp. iv+66+6 plates. Professional Paper 358: Primary Textures and Mineral Associations in the Ultramafic Zone on the Stillwater Complex, Montana. By Everett D. Jackson. Pp. v+106. 1 dollar. (Washington, D.C.: Government Printing Office, 1960, and 1961.) [55

General and Comparative Endocrinology, Vol. 1, No. 1 (April, 1961). Pp. 90. Vol. 1 (6 issues) 1961: 18 dollars. (New York and London: Academic Press, Inc., 1961.) [95

ASLE Transactions, Vol. 4, No. 1. Pp. 196. (A publication of the American Society of Lubrication Engineers.) (New York and London: Academic Press, Inc., 1961.) 15 dollars per volume. [95

National Academy of Sciences—National Research Council. Nuclear Science Series. NAS–NS 3026: The Radiochemistry of Mercury. Pp. v+50. (Washington, D.C.: Office of Technical Services, Department of Commerce, 1960.) 50 cents. [95

Commonwealth of Australia. Department of Supply: Australian Defence Scientific Service. Annual Report of the Defence Standards Laboratories for the year ended 30th June, 1960. Pp. iv+66. (Maribyrnong, Vic.: Defence Standards Laboratories, 1961.) [95

Smithsonian Institution: United States National Museum. Bulletin No. 221: Type Specimens of Birds in the United States National Museum. By Herbert G. Deignan. Pp. x+718. (Washington, D.C.: Government Printing Office, 1961.) 2.75 dollars. [95

United States Department of the Interior: Geological Survey. Professional Paper No. 373: Aerial Photographs in Geologic Interpretation and Mapping. By Richard G. Ray. Pp. vi+230. (Washington, D.C.: Government Printing Office, 1960.) 2 dollars. [95

Companhia de Diamantes de Angola (DIAMANG). Servicos Culturais. Dundo-Lunda-Angola. Museu do Dundo. Publicacoes Culturais No. 50: Notas sobre a Siderurgia dos Indigenas de Angola e de Outras Regioes Africanas. Por J. Bacellar Bebiano. Sur Quelques Fossiles du Karroo de la Lunda, Angola. Par Prof. Carlos Teixeira. Pp. 116. (Lisboa: Companhia de Diamantes de Angola, 1960.) [95

New Zealand: Department of Scientific and Industrial Research. Bulletin 140: New Zealand IGY Antarctic Expeditions, Scott Base and Hallett Station. By T. Hatherton. Pp. 132. (Wellington: Government Printer, 1961.) 15*s*. [115

Laboratoire d'Astronomie de Lille. Astronomical Newsletter, No. 103. Pp. ii + 52. (Lille: Laboratoire d'Astronomie, 1961.) [125

United States Department of Commerce: Weather Bureau. Technical Paper No. 39: Verification of the Weather Bureau's 30-day Outlooks. Pp. iii + 58. (Washington, D.C.: Government Printing Office, 1961.) 40 cents. [165

Chicago Natural History Museum. Fieldiana: Botany. Vol. 30, No. 4: Orchids of Peru. By Charles Schweinfurth. Pp. v + 787–1005. (Chicago, Ill.: Chicago Natural History Museum, 1961.) 4.50 dollars. [165

Science Information Personnel: The New Profession of Information Combining Science, Librarianship and Foreign Language. By Leonard Cohan and Kenneth Craven. Pp. vi+74. (New York: Science Information, P.O. Box 624, Radio City Station, 1961.) 1.50 dollars. [165

Publications de l'Institut National pour l'Etude Agronomique du Congo. Série Technique No. 65: Le Comportement des Mélanges Herbagers *Trifolium repens* L. Graminées dans les Régions d'Altitude du Kivu. Par R. Compère. Pp. 32+9 photographies. (Bruxelles: Institut National pour l'Etude Agronomique du Congo, 1961.) 40 francs. [165

United States Department of Commerce: National Bureau of Standards. Miscellaneous Publication No. 233: Units of Weight and Measure (United States Customary and Metric)—Definitions and Tables of Equivalents. Pp. ii+62. (Washington, D.C.: Government Printing Office, 1960.) 40 cents. [165

Population Reference Bureau, Inc. *Population Bulletin*, Vol. 17, No. 3: Design for a Planet. Pp. 41–60. (Washington, D.C.: Population Reference Bureau, Inc., 1961.) 50 cents. [165

National Academy of Sciences—National Research Council. Science in Space: a Report by the Space Science Board. Chapter 1: Dimensions and Problems—a General Review. By L. V. Berkner and Hugh Odishaw. Pp. iv+41. 1 dollar. Chapter 6: The Sun. By Leo Goldberg. Pp. iv+24. 1 dollar. Chapter 8: Galactic and Extragalactic Astronomy. By Leo Goldberg and Edward R. Dyer, Jr. Pp. iv+49. 1 dollar. Chapter 9: The Biological Sciences and Space Research. By Joshua Lederberg and H. Keffer Hartline. Pp. iv+19. 1 dollar. (Washington, D.C.: National Academy of Sciences—National Research Council, 1960 and 1961.) [165

United States Department of the Interior: Geological Survey. Water-Supply Paper 1460–I: Effect of Irrigation Withdrawals on Stage of Lake Washington, Mississippi. By G. Earl Barbeck, Jr., Harold G. Golden and Edward J. Harvey. Pp. iii+359–388. (Washington, D.C.: Government Printing Office, 1961.) 20 cents. [165

Publications de l'Institut National pour l'Etude Agronomique du Congo. Serie Scientifique, No. 91: La Mesure de la Consommation du Bétail au Pâturage. Par A. Scaut. Pp. 86. (Bruxelles: Institut National pour l'Etude Agronomique du Congo, 1961.) 100 francs. [165

Organization for European Economic Co-operation: European Nuclear Energy Agency. Halden (Norway) Boiling Heavy Water Reactor Project—Second Annual Report. Pp. iii+151. (Paris: Organization for European Economic Co-operation—European Nuclear Energy Agency, 1961.) [165

Transactions of the American Philosophical Society. New Series, Vol. 51, Part 2: A New Fragment of Xenocrates and Its Implications. By Shlomo Pines. Pp. 34. (Philadelphia: American Philosophical Society, 1961.) 1 dollar. [165

Bulletin of the Museum of Comparative Zoology at Harvard College. Vol. 123, No. 8: Bird Speciation on the Australian Continent. By Allen Keast. Pp. 303–496. Vol. 123, No. 9: The Spitsbergen Arctoceratids. By Bernhard Kummel. Pp. 497–532+9 plates. Vol. 125, No. 1: Shell Morphology in the Larval and Postlarval Stages of the Sea Scallop, *Placopecten magellanicus*. By Arthur S. Merrill. Pp. 1–20+3 plates. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1961.) [175

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £8/8/-, payable in advance,
postage paid to any part of the world

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office

Si.

Save space
—use I.C.I. silicon.
Available in lump
or rod (single
crystal rod or
polycrystalline).

IMPERIAL CHEMICAL INDUSTRIES LIMITED, LONDON, S.W.1.

ICI

GENERAL
CHEMICALS
DIVISION

SN3

APPOINTMENTS VACANT

AUSTRALIA
UNIVERSITY OF NEW SOUTH WALES
LECTURER
SCHOOL OF METALLURGY

The University of New South Wales wishes to appoint a Lecturer to the staff of the School of Metallurgy. The successful applicant will work in the fields of physical metallurgy or metal physics, and should preferably have had formal training and postgraduate experience in the physics of the solid state. Applicants for this position should have an honours degree or equivalent qualifications in metallurgy or physics. Salary: £A.1,772 range £A.2,477 per annum. Commencing salary is determined by qualifications and experience. After passing a medical examination, the successful applicant will be eligible to join the State superannuation scheme. He will be eligible, after six years of service for twelve months study leave on full salary. The University will pay travel expenses for the appointee and his dependent family and will meet reasonable removal expenses for furniture and effects. With the approval of the University and its bankers, married men may be assisted by loans to purchase a home. Inquiries about the School of Metallurgy may be addressed to the Head of the School, Professor R. H. Myers.

Four copies of applications, including the names of two referees, should be lodged with the Agent-General for New South Wales, 56-57 Strand, London, W.C.2, and a copy forwarded by airmail to the Appointments Section, Box 1, Post Office, Kensington, N.S.W.

BRITISH DRUG HOUSES LTD.

have a vacancy for a

YOUNG HONOURS GRADUATE

to work in their RESEARCH DEPARTMENT in the STEROID FIELD.

The Company offers good conditions of employment.

Write, with full details of age, qualifications, and salary required, to:

Ref. HAC/VP, Personnel Manager,
The British Drug Houses Ltd.,
Graham Street, London, N.1.

TORRY RESEARCH STATION
D.S.I.R.

requires Scientific Officer for Humber Laboratory, Kingston-upon-Hull, Yorkshire, to work on problems of both fundamental and applied research concerned with fish-smoking; the transport of fresh and frozen fish; and the manufacture of fish meal. Qualifications: first- or second-class honours degrees in chemical engineering or in chemistry, with postgraduate training or experience in chemistry. Salary: £738 to £1,222.

Application forms from Director, Torry Research Station, Aberdeen, quoting TORRY 8Z.

ROAD RESEARCH LABORATORY (D.S.I.R.), Harmondsworth, Middlesex, requires Civil Engineers, Physicists, Mathematicians, Statisticians in the Senior Scientific and Scientific Officer grades for work in the Tropical Section. Duties concern problems of road building and road transport in tropical and sub-tropical areas, involving soil mechanics, traffic engineering, and economics studies. Section is based at Harmondsworth and work involves regular visits overseas with, in some instances, postings for periods of one or two years on specific projects: usual Foreign Service Allowances apply. Qualifications: First- or second-class honours degree. For Senior Scientific Officer some years of appropriate postgraduate research is required. Salary, within range: Scientific Officer, £773 to £1,267; Senior Scientific Officer, £1,392 to £1,714. Prospects of promotion to Principal Scientific Officer (range up to £2,478) with possibility of higher posts. Laboratory is expected to move to Crowthorne, near Bracknell, Berkshire in 1963-64.—Application form and further information from Director of Road Research (address above), quoting ROAD8YXW.

IMPERIAL CHEMICAL INDUSTRIES LIMITED

Fibres Division

The Fibres Division of Imperial Chemical Industries Limited invite applications from

SCIENCE GRADUATES

preferably women

with qualifications or experience in the fields of CHEMISTRY or TEXTILE TECHNOLOGY, to work as an

ABSTRACTOR

in their Technical Library

Conditions of employment include a five-day week, membership of staff pension fund, an employees profit-sharing scheme and canteen facilities.

Written applications, giving brief personal particulars, to:

Division Staff Officer,
Personnel Department,
IMPERIAL CHEMICAL INDUSTRIES LIMITED,
Fibres Division,
Hookstone Road, Harrogate, Yorkshire,

quoting Ref. T.40/C.

PHYSICAL RESEARCH CHEMIST

Applications are invited from physical chemists, preferably with several years' postgraduate experience, for a post concerned with fundamental studies on gelation at the British Gelatine and Glue Research Association, 2a Dalmeny Avenue, London, N.7.

The post carries F.S.S.U. benefits and other conditions usual in Research Associations.

Applications should be made to the Director of Research by August 14, 1961.

UNIVERSITY OF CANTERBURY
CHRISTCHURCH, NEW ZEALAND
LECTURER IN BOTANY

Applications are invited for the above-mentioned position. Applicants should be honours graduates in botany or microbiology of some British Commonwealth or foreign university and have had some previous experience of university teaching. They should also be qualified to lecture on fungi and bacteria to botany students, including postgraduate students, and to give a course on applied microbiology. Preference will be given to candidates with experience in microbiology. The salary will be £1,250 per annum, rising to £1,700 per annum. Approved fares to Christchurch will be allowed for the appointee, his wife and children; in addition, actual removal expenses will be allowed within specified limits.

Further particulars and information as to the method of application, may be obtained from the Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1. Applications close, in New Zealand and London, on August 15, 1961.

AGRICULTURAL RESEARCH COUNCIL
ANIMAL BREEDING RESEARCH ORGANIZATION

Animal Physiologist with research experience required to develop work in the field of adaption of animals to environment. Good laborator facilities including controlled climate chamber are available at Roslin, 7 miles from Edinburgh. These facilities have been designed primarily, bu not exclusively, for sheep. Salary according to qualifications and experience. Scientific Officer £800 to £1,222; Senior Scientific Officer, £1,342 to £1,654; Principal Scientific Officer, £1,716 to £2,418. Superannuation in F.S.S.U.

Apply not later than July 29, to the Secretary Animal Breeding Research Organization, Glenbourne, South Oswald Road, Edinburgh, 9, from whom further information may be obtained.

DEPARTMENT OF SCIENTIFIC AND INdustrial Research: Textile Technologist. Pensionable post, graded Principal Scientific Officer at Tropical Products Institute, London, W.C. for man or woman to supervise group concerned with research into, and laboratory examination of various aspects of animal and vegetable fibre pulp, paper and board-making materials, hides skins, etc., of tropical origin. Qualifications: normally first- or second-class honours degree in textile technology, chemistry, or physics, or Associateship or Fellowship of Textile Institute (or equivalent). Candidates without the prescribed qualifications may be considered if they have experience in the science or technology of one or more of the above commodities, and ability to supervise scientific and technical staff essential Interim salary scale (Inner London), £1,781 to £2,483. Starting salary may be above minimum Promotion prospects.—Write Civil Service Commission, 17 North Audley Street, London, W.1, for application form, quoting **S/5333/61**. Closing date August 15, 1961.

SCIENCE FOR SCIENTISTS AND TECHNOLOGISTS

PRE-OCCUPIED as they are with problems of providing sufficient quantities of concrete and glass to accommodate all the pressing applicants, university leaders have found it difficult of late to spare the time to consider the kind of teaching which will best serve the graduate scientist or technologist. In his statement to Convocation of the University of Leeds, the vice-chancellor, Sir Charles Morris, wisely resisted the temptation to deal with the expansion difficulties of the 'sixties and concentrated on the problems which are immediately in front of all university teachers and administrators and which all can help to solve.

Chief among these is the kind of science which should be included in undergraduate courses for scientists and technologists. Despite the accelerating pace of scientific discovery, the problem of adjusting syllabuses to meet the needs of students of pure science are, though difficult, not insuperable. Although much that was considered essential in the past necessarily has to be cut out, at regular intervals there is a rethinking of the principles on which courses and syllabuses have to be rebuilt, and this is done at not infrequent intervals.

With the applied sciences however, and this applies to medical as well as technological courses, the situation is quite different. Here knowledge of a pure science is not an end in itself. It is a piece of equipment which is necessary before the study of the applied science or the acquirement of professional skills can be initiated. The underlying danger is that the amount of knowledge of the pure sciences which teachers of technology demand may be much more than individual students can humanly be expected to absorb.

Nor are the difficulties confined to the students. Studies undertaken by pure scientists in teaching the applied scientists are becoming so exacting that the prospect of the pure science departments themselves becoming considerably changed in nature and direction is by no means remote. The prospective engineer or metallurgist or medical man regards his basic chemistry, physics and mathematics as so many obstacles to be got over before embarking on the studies that matter. Teaching physics or chemistry to such students involves much routine work and, to sustain student interest, demands not only much pedagogical skill but also a considerable quantity of evangelical zeal. To maintain continual interest, the teacher may have to resort to methods of teaching which may not always have been thought appropriate in university departments.

This is a problem which needs speedy resolution if departments of pure science are to continue to flourish both as centres of creative investigation and places where pure scientists themselves may be taught. Sir Charles Morris believes that if this problem is not solved, "the departments of pure science as centres for the advancement of knowledge and homes for education of the pure scientists of the future, may in many universities wither and die". The alternative is that our future doctors and technologists may be brought up with insufficient knowledge of the basic sciences on which the whole structure of medicine and technology rests.

To push this problem back to the schools would provide no lasting solution. Already the fear exists that, so far as science is concerned, too much is being demanded of the schools. Since the amount of pure science required by the technologists will grow more rather than less, it would seem inevitable that the universities will be required to teach more pure science to the applied scientists.

The content of courses for technologists was also examined by Sir John Cockcroft, Master of Churchill College, Cambridge, in his Trueman Wood Lecture delivered to the Royal Society of Arts on April 26. Since their training should enable them to deal with the technological developments of the future rather than the problems of the past, Sir John suggested that university courses in technology should have a strong component of basic science. All students of technology, for example, should become familiar with the fundamentals of electricity and magnetism so that they can design equipment from first principles rather than handbooks. They should also know the principles of heat and thermodynamics, fluid dynamics as well as solid-state physics, which form the basis for understanding those solid-state electronic devices which are changing the face of technology. All basic training should stem from a good working knowledge of mathematics, particularly calculus and differential equations. Sir John would find wide support for his contention that mathematics should take priority over all other subjects in the training of sixth-form scientists who wish to enter a university or go directly into industry. Many would also support his view that to-day there is too much of a divorce between mathematics and physics in some universities, but would hesitate in replacing mathematics entirely by theoretical physics in the training of technologists.

In the first one or two years of university education, potential technologists should study physics, chemistry and mathematics, and Sir John commends as a model the Cambridge Natural Sciences Tripos Part I in which three or more sciences, including mathematics, are studied.

The training of engineers should follow the same general principles that apply to the training of other technologists. In his first two years, the engineer should study those principles of engineering science which underlie his technology. Only then should he begin to specialize and spend a large part of his time on electronics, thermodynamics, power engineering or other specialist subjects. Support for Sir John's thesis came recently from the Institution of Chemical Engineers, which recommended that about 45 per cent of the teaching in a university should be devoted to pure science and mathematics. About an equal amount of time should be devoted to chemical engineering science and the remainder to other engineering science, report-writing and a foreign language.

Regard for the way basic science is being taught in Australian universities has been expressed by Prof. A. E. Alexander, professor of physical chemistry in the University of Sydney (*Vestes*, **3**, No. 4; December 1960). Prof. Alexander's reflexions were mainly concerned with chemistry and stem largely from the low staff–student ratio. At the University of Sydney enrolments for chemistry in 1960 were 2,250 and were accommodated in eight parallel lecture courses, these being taken by three lecturers, two temporary lecturers, and three teaching Fellows. In Australia little sixth-form chemistry is taught in the schools and Prof. Alexander sighs for the British system so that the university could be spared an intolerable burden of mass production and be able to spend more time on teaching chemistry more appropriate to a university (see also *Nature*, 189, 444, 1961). For some time, however, the problem of large student numbers has to be faced and raises anew the questions posed by Sir Charles Morris—what kinds of basic science should be taught to applied scientists, how much of it should be taught in the schools and the university, and what teaching techniques the universities should adopt to deal with the inevitable large numbers. American universities have paid much more attention to what should be taught and how it should be taught, and both Britain and Australia have much to learn from them.

The time has surely come when such fundamental decisions should no longer be left to heads of departments or *ad hoc* committees composed of members whose main interests are in research. Should not each faculty of pure and applied science initiate major research projects, carried out by senior research Fellows, to determine the content of their courses, the way it should be taught, where the teaching should be done, how much should be left to the schools, how often revision should be carried out, and who should be consulted before revision takes place?

GOALS FOR SCIENCE IN THE UNITED STATES

IN February 1960, General (formerly President) Eisenhower announced the appointment of a Commission on National Goals, and requested the members of this Commission to "develop a broad outline of co-ordinated national policies and programs" and to "set up a series of goals in various areas of national activity". The Commission's report, including sixteen essays written by selected individuals, was prepared and published in a volume entitled *Goals for Americans*.

For its essay on science, the Commission chose Dr. Warren Weaver, vice-president of the Alfred P. Sloan Foundation, who, aided by the advice and counsel of a number of distinguished American scientists, presented his contribution, which he called "A Great Age for Science".

As this article was written by one of America's leading scientists, and because of the number of requests for the material, it has been reprinted separately by the Foundation for special distribution*.

Of primary importance in Dr. Weaver's thesis is the need to recognize the importance of science teaching, and to reward teachers at all levels of the educational system. The fact that this need has been appreciated for some time, and is fairly self-evident, does not detract from its intrinsic importance. Concern is growing in the United States about the present-day neglect and disparagement of teachers, which, long-existent at the more elementary levels, has now obtained a foothold in the universities. The university tradition of great scholar-teachers, dedicated to research, but eager and willing to teach younger students, is being undermined. Nowadays, scientists are being offered research posts with the added 'advantage' of very little teaching duty, and many full-time research scientists, working at the universities and receiving salaries from research contracts and grants, are, in the words of Dr. Weaver, "intellectual eunuchs, not at all involved in the propagation of their kind". Furthermore, the premium placed on particular types of scientists for certain industries (notably the defence industry) often leads to emergent Ph.D.s being offered research posts with salaries exceeding those of the professors supervising the theses. These trends are tending to denigrate the academic dignity and fundamental importance of teaching, and the trend should be reversed. It is Dr. Weaver's contention that there are no secondary roles so far as teaching and research are concerned; they should be equal partners.

Much space is devoted to a discussion of the position of science within the framework of society. That an enormous gap has opened up between the thinking of scientists and the views and attitudes of mind of creative artists and moralists is serious enough, but even more disastrous is the chasm which separates the scientist from the layman. Dr. Weaver quotes from *The New Yorker* of March 5, 1960: "These are hard times for the layman. He is no longer thought competent to work out his own opinions on many matters, even many that touch him intimately. His very survival has become the property of committees and the subject of learned arguments among specialists. He has little to say . . . being largely ignorant of the information upon which plans for him are based".

---

* *A Great Age for Science.* By Warren Weaver. Pp. viii + 35. (Reprinted from *Goals for Americans.*) (New York: Alfred P. Sloan Foundation, 1961.)

Science is involved in many present-day social and political problems, and, in a democracy, these problems should be the concern of the citizens as well as the scientists. Further to this, science in the United States is now largely financed by Government agencies, which are under the ultimate control of the citizens. People ought not to have to subscribe to something they know nothing about.

These points make it imperative that individual citizens should have an improved understanding of what science is, and how it operates.

On more specific but nevertheless highly important goals, the question of financial support for science is discussed. Often there is not enough distinction made between research and development, and a cursory glance at budget statements of 'R and D' (Research and Development) may give a misleading impression of the amount of support given to 'R'. The figures indicate an enormous increase in the support of development, but the financial aid given to basic research has not kept pace. Although American defence commitments require enormous sums to be spent on development of missiles, rockets, etc., there is some criticism of the fundamental philosophy of State policy in this direction; military interservice rivalry and lack of understanding of military objectives lead to wasteful duplication of developmental projects, and 'crash programmes', undertaken more as a result of emotion than of reason ('space' is the perfect example), lead to a budget which is heavily overburdened on the developmental side. It is argued that, with the introduction of a little more common sense, the total annual national allotment for pure research, which at the moment is in the region of 8–900 million dollars, could easily and profitably be raised to 1,500 million dollars without increasing the overall research and development figures.

Attention is also directed to the part played by industry in promoting basic scientific research. Although most of the misguided, old-fashioned acrimony between academic and industrial scientists has now disappeared, only a very few of the best American industries have established a satisfactory liaison between the interests of the community and the interests of the individual. Other industries, although giving the appearance of following this pattern, do not, in fact, go far enough. Furthermore, although there has been great improvement, industries do not, in general, give enough support to research and education in universities and colleges, or to development of particular fields of science on which they themselves depend.

'Crash programmes', which work on the false assumption that 2 billion dollars equal one solved cancer problem, often lack adequately trained and able personnel, and, most important of all, there has not been established a basic theory to work on. What is needed is a broad attack at the roots of a problem by means of reasoned, fundamental research rather than a phrenetically active but superficial attack, which reflects adversely on national maturity and prompts Dr. Weaver to ask himself, "What's the rush ?"

ROTATION OF THE EARTH

The Rotation of the Earth

A Geophysical Discussion. By Walter H. Munk and Gordon J. F. Macdonald. (Cambridge Monographs on Mechanics and Applied Mathematics.) Pp. xix + 323. (Cambridge: At the University Press, 1960.) 70*s*. net.

THIS masterly book very well conveys the intellectual excitement of present-day geophysical research. From an examination of the places from which ancient eclipses were observed, Halley pointed out that the Moon's motion in longitude was gradually accelerating. Some of the best mathematical minds in the eighteenth century concerned themselves with the complex problem of providing a complete theory of the motions of bodies of the solar system, and Laplace in 1787 finally decided that this acceleration of the Moon was due to a decrease in the eccentricity of the Earth's orbit arising from gravitational perturbations by the planets. However, about the middle of last century, J. C. Adams showed that Laplace had neglected certain terms and that, in fact, only half the acceleration of the Moon could be explained by purely gravitational causes.

Gradually it became evident that the discrepancy was due to a secular increase in the length of the day, that is, a slowing up of the Earth's rotation. The much more detailed knowledge of the Moon's longitude, acquired through astronomical measurements in the past few hundred years, has shown that in addition to this secular acceleration of the Moon's longitude there are irregular changes with a time scale similar to that of the geomagnetic secular variation (about 50 years). The work of numerous astronomers, culminating in the famous paper of Spencer Jones in 1939, showed that these irregular fluctuations are also due to the changes in the length of the day. Since the War the Earth's rotation has been timed accurately over much shorter periods still by quartz clocks and an annual term in the Earth's rotation discovered.

About a hundred years ago astronomical determinations of the latitude of observatories became accurate enough to detect that the Earth's axis of rotation is wobbling, with respect to the Earth, a few tens of feet during periods of twelve months and fourteen months. The extraordinary accuracy of astronomical measurements by which these various phenomena have been discovered should be a cause of wonder: the irregular changes in the length of the day, for example, are about one part in 10^8. All these phenomena give very important information about the Earth, and Munk and Macdonald give a fascinating description of the analysis required to explain them. The secular deceleration of the Earth appears to result from the friction of the lunar oceanic tides; the irregular fluctuations in the length of the day from the turbulent motion in the Earth's core, which produces the magnetic field; and the seasonal changes from asymmetry in the atmospheric circulation in the southern and northern hemispheres. The exciting forces which cause the Earth to wobble on its axis are unknown, and this causes some difficulty at present in making reliable inferences concerning the properties of the interior of the Earth from the variation of latitude data.

However, on the whole, all the observations of phenomena so far referred to can be explained without having to assume any very startling new departures in geophysical thinking, and so, as Munk and Macdonald show, a mathematically satisfying and fairly tidy story results. However, for the completeness of their account of the idiosyncrasies of the Earth's rotation, Munk and Macdonald refer to the evidence, which has accumulated in recent years from palæomagnetic work, that large changes of the relative positions of the axis of rotation and the continents have occurred gradually over hundreds of millions of years. These theories of 'polar wandering' and 'continental drift' were vigorously debated thirty years ago by geologists and geophysicists and were then largely discredited. Munk and Macdonald discuss the possibility of producing large polar wandering, but they baulk at the idea of continental drift. Instead, they take refuge in a somewhat unsympathetic discussion of the palæomagnetic evidence, although these data are massive in quantity and are probably more statistically significant than some of the other observational evidence which they accept quite happily in the earlier part of their book. The beauty of the book is to show how evidence from such widely different fields as geology and positional astronomy must be woven together to understand this central problem of geophysics.

S. K. Runcorn

ASTRONOMY IN THE MAKING

The Controversy on the Comets of 1618

By Galileo Galilei, Horatio Grassi, Mario Guiducci and Johann Kepler. Translated by Stillman Drake and C. D. O'Malley. Pp. xxv + 380. (Philadelphia: University of Pennsylvania Press; London: Oxford University Press, 1960.) 48*s*. net.

As the links of the new astronomy were being forged in the seventeenth century, controversies, often heated and sometimes polemical, arose among the *cognoscenti*. On one hand the Aristotelian and Ptolemaic universe was upheld, having the respectability of authority and the blessings of the Church behind it; on the other the revolutionary ideas of Copernicus and Tycho Brahe were advocated by Galileo and Kepler, who also had their own novel contributions to make and who were supported by others of like mind. One of the difficulties of looking back on these formative years is so to stimulate the imagination that one can obtain something of the essence of the reality of the problems which had to be solved. It is only too easy to judge with a decision based on what we now know was later to be discovered and proved. Probably the best way out of this difficulty is, where possible, to read the actual arguments which took place and, moreover, to read them in the words of the actual protagonists concerned.

In the autumn of 1618 there appeared three bright comets, visible to the naked eye. The question arose whether these objects were meteorological in nature or whether they were truly celestial bodies and, if the latter, what really was the nature of their paths. The appearance of the comets aroused wide interest, many pamphlets about them appeared, and Galileo, then aged fifty-four, entered the arena of controversy and produced his brilliant polemic *Il Saggiatore*. This book is important not so much for its scientific content or its literary merit, high though this is, but primarily for an insight into Galileo's approach to scientific matters; modern scientific method is here seen as it was in its early stages.

Yet Mr. Drake and Dr. O'Malley have, in their book, done more than provide, for the first time, a translation of the whole of *Il Saggiatore*, for they have also placed this gem in its appropriate setting. This they have achieved by beginning with a translation of Gassi's disputation on the three comets and giving also his *Libra Astronomica*, which was published under the pseudonym of Lothario Sarsi. Interspersing Gassi's work is a translation of Guiducci's discourse on the comets (and really written by Galileo), and following Gassi's *Libra Astronomica* there is Guiducci's own letter about the matter written to his old professor, Tarquinio Galluzzi. *Il Saggiatore* comes next, and then there are appended Kepler's remarks on it taken from his *Tychonis Brahei Dani Hyperaspistes*. Thus, within the covers of one volume, we have the whole of the controversy over the three comets.

The works given are, of course, eminently readable, for their authors wrote well and the translations which have been made are excellent. Excellent also is Mr. Drake's nineteen-page introduction in which he sets out very clearly sufficient of the ramifications which lay behind the controversy and of the nature of the protagonists to make the translations illustrate their points clearly. Throughout the book there are references to notes which, given at the end of the volume, elucidate various points or obscurities in the main body of the text, and there is also an adequate index. Well printed in England, the price of the book may seem, perhaps, rather high, but nevertheless this excellent contribution to the history of science is much to be recommended.

Colin A. Ronan

THEORETICAL METEOROLOGY

Introduction to Theoretical Meteorology

By Prof. Seymour L. Hess. Pp. xiv + 362. (New York: Henry Holt and Company; London: Constable and Co., Ltd., 1959.) 60*s*. net.

This is an important new text-book written primarily for students, but equally suitable for those desiring an up-to-date review of the subject. The aim has been to provide a reasonably rigorous treatment which is not rendered unnecessarily difficult by advanced mathematical arguments. The first seven chapters are devoted to basic thermodynamics, the properties of water vapour and hydrostatic stability, and the next three chapters to radiation theory and its application to the Earth-atmosphere system. The remaining eleven chapters deal with dynamics, including viscosity, turbulence and modern numerical techniques.

The main emphasis is placed on large-scale properties of the atmosphere. The general circulation and the role of depressions and anticyclones in its maintenance receives a thorough treatment which brings the reader with a minimum of difficulty right to the frontiers of our knowledge of what is probably the most fascinating of all meteorological problems. On the other hand, smaller-scale phenomena such as sea-breezes are given a somewhat cursory treatment, and mountain waves are not mentioned at all, even though they have received much attention in recent years. Convection is discussed in more detail, but the impression given that a convection current behaves dynamically like a rising balloon subjected to viscous drag is misleading, since an isolated convection element is now known to be a rising vortex ring. The author's derivation of the equations of motion of the atmosphere is somewhat novel and will appeal to many, but may not satisfy those who are mathematically inclined. A more rigorous derivation, preferably using vectors, could well be appended.

These criticisms of detail must not be allowed to detract from the overall good quality of this book. The author's style is lucid and his presentation of some concepts, such as inertial stability and the vorticity equation, is particularly attractive. The examples at the end of each chapter are well designed and will help the student to acquire a knowledge of the factors which are of primary importance in the many and varied physical problems encountered in meteorology.

ROBERT P. PEARCE

INCOMPRESSIBLE AERODYNAMICS

Incompressible Aerodynamics

An Account of the Theory and Observation of the Steady Flow of Incompressible Fluid past Aerofoils, Wings, and other Bodies. Edited by Bryan Thwaites. (Fluid Motion Memoirs.) Pp. xx + 636. (Oxford: Clarendon Press; London: Oxford University Press, 1960.) 75*s*. net.

THIS book is the first of a new series of Memoirs on Fluid Motion which will also include volumes on laminar boundary layers and turbulence. It addresses itself to a class of problems which have been pushed off the centre of the aerodynamics stage by the current fashion for magnetogasdynamics, hypersonics, rarefied flows and all the other exotic fields associated with space flight or long-range rocketry—namely, the many non-linear problems of incompressible aerodynamics.

Nevertheless, these more old-fashioned problems of low-speed flight are neither irrelevant nor by any means solved or even fully understood, and the present volume is a welcome addition to the library of those in the aeronautical profession who are still concerned with aircraft which must take off, manœuvre and land with a reasonable degree of effectiveness and reliability; nor will it be found trivial by those many lovers of fluid mechanics who are fascinated by rotational and separated flow-patterns.

The basic thread which runs through this book is the subtle interplay of models of flow patterns suggested by ingenious experiments (both detailed measurements of pressures and visualization of flow fields by means of smoke, tufts and other devices) and approximate analytical techniques designed to fit the observed flow patterns into the fabric of classical aerodynamics. Never is the model over-simplified to make it amenable to analysis; never is mathematics pursued for its own sake to a sophistication beyond that of the physical picture. At all times, the relevance of the questions and of the answers to the design of more effective machines is clearly kept in mind.

Among the sections which I have found of particular interest, those which deal with stalling wings, separated flows over swept wings and thick wakes, appear outstanding; but this judgment is most likely a reflexion of my tastes. The treatment of almost all the difficult low-speed flight problems by ingenious methods is fascinating and a worthy monument to the men, largely at the National Physical Laboratory and the Royal Aircraft Establishment, who created a distinctive English style in aerodynamics, compounded of sound technique, balanced judgment and a profound intuitive feeling for the physics of fluid flow problems.

LEON TRILLING

INFRA-RED ARTS

Infrared Methods

Principles and Applications. By G. K. T. Conn and D. G. Avery. (Physical Chemistry: a Series of Monographs, Vol. 7.) Pp. viii + 203. (New York: Academic Press, Inc.; London: Academic Press Inc. (London), Ltd., 1960.) 6.80 dollars

A SPECTACULAR change in the attitude of chemists over the past twenty years has been their acceptance of physical methods for solving problems, particularly in organic structural work and in analysis. Among these methods is infra-red spectroscopy, which is now, and is likely to remain, a routine tool of the chemist. The arrival of 'simplified' commercial instruments is making the infra-red spectrometer as commonplace in laboratories as the refractometer and is creating a class of user who has no interest in spectroscopy for its own sake and little in the principles of operation and design of instruments; infra-red spectroscopy is no longer the preserve of the physicist and physical chemist. Any book with the broad title *Infrared Methods—Principles and Applications* is thus likely to have drawn to it the attention of a very large number of students and research workers. It must be said, however, that many of these will find the title misleading, for the principles are those involved in generating, dispersing, and detecting infra-red radiation, the applications are in the calibration of detectors, the production of monochromatic radiation, in the design of instruments for industrial gas analysis and plant control, and in pyrometry. This book is written from the point of view of the experimental physicist, and when the authors suggest that it is an introduction to infra-red studies for "the research student and the student in allied fields" it should be understood that they refer to the research student of spectroscopy or allied fields of physics. The average worker in other fields who wants to understand how an infra-red spectrometer works and what its limitations are will find it far beyond his needs. Authors write as they view the subject, but it does seem remarkable to find a work such as this in a series of monographs on physical chemistry. There is, without doubt, a demand for an up-to-date authoritative account of practical methods in infra-red spectroscopy written with chemical applications strongly in mind. This monograph does not satisfy it.

The chosen material is presented in a clear manner with a fair number of references to original papers. The book is beautifully produced, only two errors being noted, in figures I. 2 and V. 5.

E. S. WAIGHT

THE ELECTRON MICROSCOPE

The Electron Microscope

The Present State of the Art. By M. E. Haine, with a Chapter on Applications in Collaboration with Dr. V. E. Cosslett. Pp. xvi+282. (London: E. and F. N. Spon, Ltd., 1961). 55*s*. net.

THIS book, by the best British electron microscope engineer, with a chapter on applications written in collaboration with the doyen of British experts, is in many ways the last word on the electron microscope. It took the optical microscope 200 years to develop, from Leeuwenhoek to Abbe, from the 'dewdrop' to the apochromat. The electron microscope took less than 25 years from the primitive 'magnifying glass' of Knoll and Ruska, to the attainment of the present limiting resolution of about 6 Å., also by Ruska, in 1954, which, as readers of this book will soon become aware, is not likely to be much bettered in the future.

In the optical microscope two centuries of work went into correcting the aberrations, and the work is not yet quite finished. In electron optics it took only a few years before Scherzer demonstrated that the spherical aberration could not be corrected, and the question was only to find the optimum. In the electron microscope all the essential 'optical design' now boils down to a single curve (Fig. 1,7, p. 8), computed by the late G. Liebmann, one of the principal author's able collaborators, from which designers can pick their choice, according to the number of ampere turns which they are willing to embody in their objectives. The optimum theoretical resolution is about 2·7 Å. at 50 kV. and could be reduced to 1·5 Å. at 400 kV.—probably at the expense of contrast. The reasons why the practical resolution is only about one-half of the theoretical are fully and ably explained by Dr. Haine. It is particularly welcome that, apart from a detailed discussion of astigmatism, vibrations, creep, contaminations, etc., he describes, in full practical detail, the remarkable Haine-Mulvey Fresnel fringe test, so that every user of an electron microscope can test for himself the resolution limit of his own instrument.

The resolving power of the electron microscope has stopped at a particularly uninteresting stage. There are very few objects of 6 Å. size; organic molecules of this size have insufficient contrast, metal colloids tend to grow spontaneously to about twice this size, atomic lattice spacings are finer. (Only J. W. Menter was able to find an interesting example, by directly resolving the lattice of molybdenum trioxide, Fig. 10. 25, p. 270). If the resolution limit could be reduced below 3 Å., most atomic lattices could be resolved. The present book makes it clear why attempts to achieve this have failed, and are probably bound to fail for a long time—such as Scherzer's remarkable non-rotational lens, and the reviewer's suggestion of taking an imperfect electronic image, which is complete as it contains a record of phases as well as of amplitudes, and doing the corrections with light, in a second, optical process. The difficulties are so formidable that for a long time the electron microscope is bound to stay at the present limit—which, however, does not exclude interesting new special types, such as high-voltage instruments and interference microscopes, and the standard instrument will become a more and more convenient instrument for the user.

It is to the user—the biologist, the metallographer, the physical chemist—that this book is chiefly addressed. Correspondingly the mathematics is cut down to a minimum, but the critical attitude of Dr. Haine, who cannot suffer obscurities, has forced him to make this minimum so complete that it is somewhat doubtful whether most of the users of electron microscopes will not find it far above their heads. It takes a specialist to appreciate fully the amount of critical thinking which has gone into this book. I believe that it will have its greatest effect in the case of that small but important and growing class of users who are fully trained physicists (working mostly on imperfect crystals, such as metals) and who are willing to go to some pains to understand not only their object but also their instrument.

D. GABOR

ORGANO-METAL COMPOUNDS

Organo-Metallic Compounds

By Prof. G. E. Coates. Second edition. Pp. xiii+366. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1960.) 45*s*. net.

THE original stimulus for the investigation of organo-metallic compounds came from the possibility of their use in organic synthesis. Indeed, many chemists a decade or so ago were really familiar with only one class of compounds, namely, the Grignard reagents. To-day there exists an extensive subject covering compounds of almost all the known elements, which is rapidly developing and which is followed by many different kinds of chemist. The impressive advances which have been made have in many cases been the product of co-operation between theoretical, organic, inorganic and physical chemists. Synthetic organic chemistry is still, however, a major beneficiary from the investigation of these compounds, but there is an ever-increasing independence emerging in the studies in this field.

The first edition of this book (190 pages) appeared as a Methuen Monograph in 1956, having been written for the most part in 1953–54, with a section on the cyclopentadienyls having been added in proof. In the intervening years the amount of published work has been very great, so that even with certain condensations of material, notably that concerning organo-boron compounds, the second edition now comprises 360 pages and, in addition, has a larger page size. Such an expansion is inevitable and is completely justified in that this new and enlarged edition presents an almost complete picture of those compounds in which a direct metal–carbon bond is present. As in the previous edition the extensive organic chemistry of phosphorus, arsenic and silicon is omitted. The layout is little changed, but certain new theoretical and industrial aspects are now included. The main changes are to the sections on the alkali metals, boron, tin and the transition metals. This latter section has been expanded from 27 to 127 pages and now includes a thorough description of certain of the transition metal complexes in terms of molecular orbital theory. Throughout, complexes are discussed in terms of σ or π bonding.

The author's personal enthusiasm and experience in this field are clearly apparent, particularly in certain of the descriptions of preparations in which considerable detail is given; an aspect which should add considerably to the value of the book for those actually working in the field. Others will find the wide coverage of this book most helpful in obtaining an appreciation of the present position of organometallics. The book is marred unfortunately by a number of typographical errors. The first edition was cheap at 12*s*. 6*d*. The second edition is reasonably priced at 45*s*. in view of its increased coverage.

D. S. Payne

RADIATION CHEMISTRY OF ORGANIC COMPOUNDS

Radiation Chemistry of Organic Compounds

By Dr. A. J. Swallow. (International Series of Monographs on Radiation Effects in Materials, Vol. 2). Pp. xiii+380. (London and New York: Pergamon Press, 1960.) 84*s*. net.

In this book the author defines his objectives as the presentation of an overall picture of radiation chemistry and a review of the published work on the subject between 1895 and 1958. He begins with a brief introductory account of the interaction of radiation with matter, and a discussion of the transient species formed by radiation. The transient species are simple in the extreme compared with the highly complex systems to be discussed later in the book.

There follows a chapter on experimental methods which deals, only in outline, with radiation sources, purity of materials—a feature often overlooked, says the author, in some of the experimental work reported on—analysis of products, and dosimetry. In no sense is this a practical manual of techniques, but it surveys very adequately the background to the chapters which follow.

Very wisely, the author devotes the next chapter to the radiation chemistry of aqueous systems, partly because he can illustrate the fundamentals of the mechanism of radiation changes, and because of the importance of aqueous systems for the understanding of radiation effects on biological systems. The four chapters which follow deal with aliphatic and aromatic compounds, polymers and dyestuffs. In these chapters the author's task was difficult in the extreme for, as he says, mechanisms are not yet fully understood even for simple molecules. Thus, much of the material presented catalogues the results of radiation of a vast range of compounds, with comments on mechanism where this is even partially understood. I would have liked more comment on mechanism, no matter how speculative it had to be. The author deserves great credit for the thoroughness of his searches before presenting this—and the other material in the book.

The next chapter deals extensively with radiation effects on substances of biological interest, with limited reference to *in vivo* systems, and the final chapter deals with such diverse topics as the origin of organic compounds in the history of the Earth, radiotherapeutics, sterilization of pharmaceutical products, food, and detailed investigation of the economics of commercial radiation. This chapter contains the surprisingly few examples of commercially feasible radiation processes which have been discovered, in spite of the vast amount of irradiation experimentation in the laboratory. The importance of radiation damage to organic coolants and lubricants for reactors is also discussed.

Although it has a few stylistic defects, the book is written with great clarity, without unnecessary preamble or loquaciousness, in a refreshingly direct way. It covers an amazing range of material, which is referred to by a sensible reference system collected at the end of the book. The chapters are constructed according to well-established rules of learning—a brief statement of the aims of the chapter, the detailed discussion, then a brief statement of the results.

The main value of the book lies in the fact that it makes readily accessible the state of knowledge about particular systems. Its discussion of mechanism is necessarily brief; but since such a book as this in a subject which is rapidly growing will surely be revised, the author might consider greater elaboration of the discussion on fundamental aspects of the subject, whether the knowledge is speculative or tentative or otherwise, perhaps at the price of compressing some of the factual matter into tabular form. The present structure of the book reflects the way in which the subject has grown empirically, without a detailed and exact theoretical basis. Perhaps this situation will change in the cycle of experiment, theoretical interpretation, prediction, and further enlightened experimentation.

S. J. Thomson

PHYSICAL METHODS OF ORGANIC CHEMISTRY

Physical Methods of Organic Chemistry

Part 3. Edited by Arnold Weissberger. Third, completely revised and augmented edition. (Technique of Organic Chemistry, Vol. 1, Part 3.) Pp. xii+1799–2634+13. 184*s*. Part 4. Edited by Arnold Weissberger. Third, completely revised and augmented edition. (Technique of Organic Chemistry, Vol. 1, Part 4). Pp. xii+2635–3539+65. 195*s*. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.)

The general features of *Physical Methods of Organic Chemistry* have been indicated by the present reviewer when commenting on Parts 1 and 2, which were issued a year ago (*Nature*, 189, 954, 1961). Parts 3 and 4, which have now appeared, keep to the same general type in that they are mainly written by physicists for chemists and do not explain sufficiently the scientific significance of the types of measurements which they describe. However, organic chemists to whom the volumes have, by title, been commended will find Parts 3 and 4 of much more interest than Parts 1 and 2, since these survey the major optical and electrical techniques in use to-day for the investigation of molecular structure.

Part 3 deals mainly with optical measurements. Two sections on "Spectroscopy and Spectrophotometry" from the old edition have now been expanded to five chapters without inclusion of superfluous material. The chapter on "Ultraviolet and Visible Spectroscopy" (150 pages) can be commended as a well-balanced review in which technical improvements are brought to notice and the structural significance of experimental results is explained in simple terms.

In contrast, the chapter on "Infrared Spectroscopy" deals almost exclusively with intrumentation and techniques, but this is to be excused since Volume 9 of the general series has dealt with the subject.

A number of following chapters on colorimetry, fluorescence, light scattering and polarimetry have few novel features, but, following these W. Klyne and A. C. Parker have written a new chapter on optical rotatory dispersion to deal with the major recent development of polarimetry which has had, in the past five years. a profound influence on knowledge of structural organic chemistry. Half this chapter is devoted to a judiciously selected survey of recent experimental data together with their cognate theoretical interpretations. The chapter can be commended as a concise, well-explained summary of this important subject.

Again, a new chapter by C. G. and R. J. W. le Fèvre on the Kerr effect is a useful addition to the treatise, but the same can scarcely be claimed of a later new chapter on the Faraday effect which, as yet, has found little practical or even theoretical applicability. Old, but slightly revised, chapters on determinations of dielectric constants and of dipole moments complete this volume.

Part 4 has several quite new chapters. Microwave spectroscopy is accorded 35 pages and, following this, nuclear magnetic resonance is surveyed by H. S. Gutowsky in a well-balanced 130-page chapter. The use of this important technique receives sufficient description for the chemist who wishes to be conversant with its applicability without having to delve too deeply into quantum theory. Following this the treatment of paramagnetic resonance, in 70 pages, is too heavily mathematical and fails to give an adequate impression of the value of this type of measurement. There is no indication that this type of measurement may be of particular value to biochemists in elucidating the behaviour of such complex substances as active preparations of enzymes.

After these chapters, that on the determination of magnetic susceptibility, by P. W. Selwood, is old and conventional. The author has discussed applicability in terms of organic chemistry but has failed to indicate the growing significance of this technique for inorganic chemists, although these scientists are more and more concerning themselves with organo-metallic compounds of diverse types.

Old chapters on conductometry, electrophoresis and polarography have been revised slightly and two new ones on determination of transport numbers and controlled-potential electrolysis have been added. The theoretical significance and the synthetic applicability of this last technique fully merit the attention which it has been accorded; these aspects of the subject receive adequate treatment. Determination of radioactivity has been accorded a hundred pages and is not over long, for there have been major developments of experimental techniques since the last edition of this book appeared. Very properly the chapter deals mainly with the counting of carbon-14 and of tritium, but describes, in full, the chemical procedures required for the conversion of organic substances into samples suitable for radioassay. Finally, the chapter on mass spectrometry, thoroughly modernized, closes the book. It again can be commended for devoting more than half its length to applications in both pure and applied organic chemistry. Due note has been taken of the conjoint use of mass spectrometry and gas chromatography.

The high cost of these volumes will undoubtedly restrict their sale, except to reference libraries, but, considering the excellence of the printing and the clarity of the diagrams, they could scarcely be produced more cheaply. However, since these two parts contain the chapters to which reference will most regularly be made, potential users should seriously consider the advisability of purchasing individual parts of the work rather than the complete set of four volumes.

W. A. WATERS

CHEMISTRY AND THE NERVOUS SYSTEM

The Neurochemistry of Nucleotides and Amino Acids

A Symposium of the Section on Neurochemistry, American Academy of Neurology. Edited by Roscoe O. Brady and Donald B. Tower. Pp. xii+292. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1960.) 80*s*. net.

Phosphorus Metabolism of Brain

By Dr. P. J. Heald. Pp. vii+195. (London and New York: Pergamon Press, 1960). 42*s*. net.

THE Neurochemistry of Nucleotides and Amino Acids, being a symposium of multiple authorship, exhibits considerable variety of style and approach among the thirteen main contributors. The articles on adenine nucleotides (by E. G. Trams), on guanine nucleotides (by D. R. Sanadi) and on the phosphatide bases (by D. M. Greenberg) range far beyond the confines of neurochemistry. Dr. Greenberg's paper was originally delivered *in absentia*, and in its printed form is accompanied by a running commentary in footnotes from Dr. J. Stekol, who actually read the paper for Dr. Greenberg at the meeting on which this book is based. This novel variant of the 'invited discussion' may, as the sessional chairman observed, become a precedent for a new kind of symposium. Other notable contributions more directly oriented towards the nervous system are those by R. J. Rossiter and his co-workers on the cytidine nucleotides and by N. O. Kaplan on the pyridine nucleotides. The section on amino-acids contains a thoughtful review by H. Waelsch, with A. Lajtha, on protein metabolism in brain, and two papers on γ-aminobutyric acid which, however, deal mainly with metabolic minutiæ. Some of the recorded discussion might have benefited from rather more drastic editing, but Dr. Tower has written a useful summary which effectively rounds off this lively series of mainly neurochemical snippets.

Dr. Heald's book on the *Phosphorus Metabolism of Brain* is a slim volume with a high density of contained information, and, for a biochemical monograph, it has the distinction of not including a single chemical formula of the classical type. The opening pages summarize data on the composition of normal brain in respect of phosphorus compounds, the text being fully documented and embellished with several compendious tables. Such compression of material reflects much labour in compilation, but imposes some initial strain on the reader, although for reference purposes it enhances the value of the section. In later parts of the book emphasis shifts towards the functional correlates of the reactions described and the writing gains in fluency. Research work based both on investigation of brains of whole animals and of isolated tissue preparations is considered, particular attention being given to the turnover of nucleotide and other 'high-energy' phosphates (where there is common ground between Dr. Heald and certain participants in the symposium reviewed above). The interpretation of experiments in which electric pulses are applied to slices of brain suspended in saline is judicially discussed, and a phosphoprotein which Dr. Heald has investigated figures in an intriguing, if speculative, scheme of reactions linking phosphate bond energy with ionic migrations in nerve cells. Other topics covered include the synthesis of phospholipids and the metabolic effects of anæsthetics; the book as a whole provides a comprehensive and timely survey of a field of study where general biochemical concepts such as that of energy-rich phosphate bonds may be related to the special features of a highly differentiated organ.

R. V. Coxon

FLUORINE CHEMISTRY

Advances in Fluorine Chemistry

Vol. 1. Edited by Prof. M. Stacey, Prof. J. C. Tatlow and Dr. A. G. Sharp. Pp. vii+203. (London: Butterworths Scientific Publications, 1960.) 45*s*.

Chemie der Organischen Fluorverbindungen

Von Milos Hudlicky. Pp. xviii+416. (Berlin: Veb Deutscher Verlag der Wissenschaften, 1960.) 51.60 D.M.

ADVANCES in Fluorine Chemistry is an excellent beginning at fulfilling a critical need in the field of fluorine chemistry, namely, a systematic presentation and appraisal of the chemistry embodied in the various major areas of this rapidly expanding field. To cover all the areas with the attention that is their due is a monumental task. The editors of this book have selected a number of prominent British research chemists and teachers to write on five areas. These are: the halogen fluorides (W. K. R. Musgrave), transition metal fluorides and their complexes (A. G. Sharpe), fluoroboric acids and their derivatives (D. W. A. Sharp), the electrochemical process for the synthesis of fluoro-organic compounds (J. Burdon and J. C. Tatlow), and exhaustive fluorinations of organic compounds with high-valency metallic fluorides (M. Stacey and J. C. Tatlow). Each chapter is written with every attempt made not to be redundant. Whenever an earlier book or review article has made a contribution to the particular area under discussion it is cited as a prime source. As a result, in general, more emphasis is placed on the reactions and fundamental nature of the chemistry of both inorganic and organic fluorides than on the general preparative procedures, for the various authors are aware that a systematic fluorine chemistry is developing, is ready for more than a casual interpretation in terms of the science of chemistry and that the time has arrived to do so in earnest. Although there is a minor tendency, in some parts of the text, to extrapolate a bit beyond what may be construed as conservative, the facts are presented thoroughly enough to allow the reader to make his own interpretations, if he chooses to do so. At the same time, some of the writers of this book are courageous enough to be mildly critical of the authors they are citing when the occasion demands it. This is not a common practice, but appears with sufficient frequency to be stimulating.

This book is no less than its title advertises. It should prove helpful to the fluorine chemist in keeping abreast with the five areas discussed. If detailed data on a compended variety of fluorine compounds is what is being sought by the reader, he is referred to the more than adequate bibliography that follows each chapter. Typical of the better books by British authors, this book is up to date (1959), well written and discriminating. On the lighter side, the careful reader is in for a mild surprise when he reaches p. 11, and finds that gravity acts differently in England and in the United States.

In the introduction, Prof. Stacey states that this is the first volume in a series, and that further volumes written by chemists from several countries are to be anticipated.

The German edition of the book by Hudlicky is essentially a translation of the original edition in Czech reportedly brought more up to date by the addition of material that appeared in the chemical literature between the dates of publication. The author admits that the book is the result of his own attempts to enlighten himself in the area of fluorine chemistry.

The book meets rather well the purpose for which it was published, namely, to present in broad aspect the materials, conditions, yields and methods for preparing organic fluorine compounds. To this extent, the work is well done. No attempt is made to cover every appropriate compound produced or to compile the properties of those that are reported. Reaction equations, with conditions and yields, are used liberally to account for the results reported. Each such equation has a bibliographical reference and all information is cross-indexed by paper, author and compound, making it very simple to find any desired preparation. Consequently, even a student with a minimal comprehension of scientific German can use this book to good advantage.

There has been no attempt made to write this book for any purpose other than as a suitable reference text for information *apropos* laboratory practice in preparing organic fluorine compounds. There are a number of short chapters on nomenclature, materials for handling fluorine, reactions of fluorine with various substance, hydrogen fluoride and inorganic fluorides. To complete the book, sections on fluorine analysis, freons, and plastics containing fluorine are considered briefly.

The bulk of the text involves methods for putting fluorine into organic compounds, the various preparations of organic fluorine compounds, and their chemical reactions. A short chapter on the general physical properties of these compounds completes the book.

There is no serious attempt to present a critical analysis of fluorine chemistry nor is any serious distinction made between organic fluorine compounds and fluorocarbon compounds. Most conclusions as to the mechanisms and nature of the chemistry cited are left to the discretion of the reader.

To sum up, this book is in no way an erudite but rather a practical reference book, that furnishes a helping hand to both the neophyte and the practising fluorine chemist to find methods for preparing, handling and using fluorine-containing materials. It could serve as a useful adjunct to the chemist's bookshelf.

Richard Dresdner

A Laboratory Handbook of Pulp and Paper Manufacture

By Dr. Julius Grant. Second edition. (Incorporating the fourth edition of Stevens's "Paper Mill Chemist".) Pp. vii+523. (London: Edward Arnold (Publishers), Ltd., 1961.) 80*s.* net.

PAPER is so common that one forgets that, in comparison with metals, plastics and other sheet materials, it has some very peculiar properties. It is a fibrous assemblage and frequently contains more air than solids; it may vary widely in density, strength and other physical characteristics, and it may be treated with a large range of chemical additives.

This handbook, like Stevens's before it, is written for laboratory workers with some previous knowledge of paper. It should, nevertheless, appeal to a wider range of readers because it effectively summarizes the enormous amount of work put in by chemists into one industry. It outlines the essential pulp and paper processes, then describes, in more detail, the laboratory methods developed for the control of these processes, the analysis of raw materials and the physical testing and analysis of paper. Many methods are only briefly summarized, as numerous references are given to published standard methods, and to the original literature, which is now very large.

It must be difficult to decide what to leave out, and in one sense it is a pity that the book is confined so closely to practical laboratory methods. There is little reference to theory, to the large amount of published work on the visco-elastic properties of paper, or to the development of instruments for automatic control. There are some who believe that chemists have had their day in the paper industry and that the future lies with technologists trained in physics and engineering. Actually co-operation between all three will be essential, but a common ground of theory is vital to this. Even though present theory is incomplete it is a big help in the understanding of the properties of paper and of specifications for it.

There are three misprints in the brief section on statistical method; they would not upset a statistician but might confuse a laboratory worker unfamiliar with that subject. F. Lyth Hudson

Papers on Bacterial Genetics

Selected by Edward A. Adelberg. Pp. xlvi+400. (Boston, Mass.: Little, Brown and Company, Inc., 1960.) 4.50 dollars.

Papers on Bacterial Viruses

Selected by Gunther S. Stent. Pp. xxx+365. (Boston, Mass.: Little, Brown and Company, Inc., 1960.) 4.50 dollars.

THESE companion paper-back volumes are worthy successors to the collection, *Papers in Microbial Genetics; Bacteria and Bacterial Viruses*, selected by Joshua Lederberg in 1951. Yet these two supplementing volumes only reflect a portion of the enormous activity devoted to this field in the decade since the first collection. Of the 51 papers now presented, only five have been retained from the former collection.

To abate the disappointment of many readers who, no doubt, would have selected differently in some instances, each collection is preceded by the selector's introduction, which reviews the main papers on each theme and includes a wide bibliography: on viruses, 13-page review, 164 references; on bacterial genetics, 30-page review, 177 references.

The papers themselves are presented in their original type and style, from which a certain lack of uniformity results; there is dual pagination; but most clearly brought home by this method of presentation is the need for a standard method of reporting references.

The great benefit of this collection is that it provides for the student of microbial genetics a highly concentrated source of information on both the methods and the results of the main workers in this field. Also, the combination of the bibliography in the introduction and at the end of each paper, provides a fairly comprehensive guide to the other sources of related information.

To the specialist, therefore, these two volumes are almost 'essential'. L. B. Quesnel

A Laboratory Manual of Analytical Methods of Protein Chemistry

(Including Polypeptides). Edited by P. Alexander and R. J. Block. Vol. 1: The Separation and Isolation of Proteins. Pp. viii+254. (London and New York: Pergamon Press, 1960.) 50*s.* net.

THIS book is the first of three volumes devoted to practical techniques in the protein field, and contains seven chapters describing various methods for the separation and isolation of proteins. The first chapter (by Keller and Block) surveys the classical methods, such as precipitation with salts and organic solvents, for the separation of proteins. In chapter 2, Okunuki gives detailed descriptions of the methods, due mainly to Japanese workers, for the isolation of cytochromes, and bacterial α-amylase and proteinase. Chapter 3 comprises two sections. The first, by Keller and Block, reviews the techniques of adsorption and ion-exchange chromatography as applied to proteins, while the second, by Peterson and Sober, describes an ingenious variable-gradient device for column chromatography. In the next two chapters, Craig gives a full account of the use of dialysis and partition methods. The book ends with descriptions of the less commonly used techniques of multi-membrane decantation (Polson and Largier) and zonal density gradient electrophoresis (Svensson). It is to be hoped that the value of these methods will now be more widely recognized. Those readers who are not afraid of elementary mathematics will find that the theoretical bases of the various techniques are presented in a manner which does not engulf the practical aspects. A liberal number of plates and diagrams will enable workers in the less-affluent laboratories to construct much of the equipment described. In spite of a number of errors and a rather inadequate subject index, I feel that this volume will be extremely valuable to workers in the field of protein research. D. T. Elmore

An Introduction to the Chemistry of Heterocyclic Compounds

By R. M. Acheson. Pp. xiv+342. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 5 dollars; 35*s.*

THIS book has the laudable aim of supplying the student with a concise account of the properties and reactions of the more fundamental heterocyclic systems. In this it succeeds admirably. It covers the range from 3- to 6-membered heterocyclic compounds—with oxygen, sulphur, and nitrogen as the hetero-atoms taken singly and (for the larger rings) in pairs—together with their important benzo-derivatives and the fused systems of purines and pteridines. Naturally occurring compounds of relevant interest are discussed briefly or mentioned incidentally, but carbohydrates and alkaloids are excluded. Although the scope is therefore wide, the main theme is the inherent chemistry of the commoner heterocyclic compounds. This is treated in sufficient detail to allow comparisons to be made both within and between the various classes of compound. The presentation is clear and the discussion modern, being supported by pertinent physical data and based on mechanistic principles. Moreover, as befits an introduction, the reader is given adequate guidance to further sources of information.

Such books as this must surely put an end to those perfunctory postscripts with which exhausted writers of text-books usually dispose of the subject. If ultimately this is service enough, more immediately the service to students and teachers is immeasurable.

Theory of Markov Processes

By E. B. Dynkin. Translated from the Russian by D. E. Brown. Edited by T. Kováry. Pp. ix+210. (London and New York: Pergamon Press, 1960.) 60*s*. net.

THIS important and highly original work is devoted to a study of the foundations of the theory of Markov processes. It is written for the professional probabilist, and has little to offer to the non-expert, although any mathematician concerned with the teaching of measure theory will find it worth his while reading Chapter 1, in which the classical material is set out in a new and highly elegant form. This chapter also contains a careful account of conditional probabilities, some information about the new concept of "topological measurable spaces", and the best extension yet of the Daniell–Kolmogorov theorem on the construction of probability measures on function-space. The remainder of the book presents the first thorough formulation of the theory of Markov processes, starting with a whole collection of measures corresponding to all possible initial instants and all possible initial states, and allowing (as is now found convenient) the termination of the process at a random instant. The difficult theory around the concept of a 'strict' Markov process is set out in great detail. The present work is based on lectures given by the author in Moscow and Peking; it will be followed by a further volume devoted to the infinitesimal generators associated with Markov processes. It is very much to be hoped that the publishers will find it possible to translate this also.

D. G. Kendall

Combinatorial Analysis

Proceedings of Symposia in Applied Mathematics, Vol. 10. Edited by Richard Bellman and Marshall Hall, Jr. Pp. vi+311. (Providence, R. I.: American Mathematical Society, 1960.) 7.70 dollars.

THIS volume records the proceedings of the symposium held at Columbia University during April 24–26, 1958. It consists of twenty-four papers and covers a very wide range of topics, including finite geometry, algebra, and number theory in pure mathematics; and statistics, communication theory and transportation in the applied field. A fairly recent development has been the use of an electronic computer to investigate combinatorial problems, and several of the papers describe results obtained. Some of these depend mainly on algorithms and others on somewhat complicated search techniques.

The papers on finite geometries include one by R. H. Bruck on "Quadratic Extensions of Cyclic Planes", one by D. R. Hughes "On Homomorphisms of Projective Planes" and one by A. A. Albert on "Finite Division Algebras and Finite Planes". Those on number theory include a paper by A. L. Whiteman on "The Cyclotomic Numbers of Order Ten" and the paper by O. Taussky and J. Todd on "Some Discrete Variable Computations". Thus pure mathematics is well represented in current activity in combinatorial analysis. But perhaps the main motive for this activity has come from operations research. Thus there is a paper by R. E. Gomory on "Solving Linear Programming Problems in Integers", one by M. Gerstenhaber on "Solution of Large Scale Transportation Problems", and a paper by M. M. Flood on "An Alternative Proof of a Theorem of König as an Algorithm for the Hitchcock Distribution Problem". There is also a paper by R. Bellman on "Combinatorial Processes and Dynamic Programming", and a paper by A. J. Hoffman on "Some Recent Applications of the Theory of Linear Inequalities to Extremal Combinatorial Analysis". These titles are sufficient to demonstrate the dynamic character of present-day research in combinatorial analysis. Anyone who moves in this field will wish to consult the proceedings.

L. S. Goddard

Ciba Foundation Symposium on Quinones in Electron Transport

Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Pp. xii+453. (London: J. and A. Churchill, Ltd., 1961.) 60*s*. net.

THIS latest addition to the CIBA Foundation Symposia provides a most welcome review of recent work on the role of quinones in biological systems. As in previous volumes of this series, lectures by recognized authorities in the field are reproduced in full, the charts and diagrams often relating to hitherto unpublished material. Such treatment provides the atmosphere of the seminar rather than the formal lecture room, and the interest is further enhanced by the inclusion of the full discussion (diagrams again reproduced).

A detailed critique of a symposium volume of this type is quite out of place, but biochemists and organic chemists interested in the processes of biological redox systems will find stimulation and many useful references in this work. The symposium consists of the following lectures: isolation chemistry of ubiquinone (R. A. Morton, E. L. Crane, O. Isler and K. Folkers), coenzyme A and electron transport (D. E. Green); oxidation of quinones by mitochondrial preparations (E. C. Slater); oxidative phosphorylation (V. M. Clark, Sir Alexander Todd and P. J. Russell); biosynthesis of quinones (H. Rudney, A. J. Birch, O. Wiss and R. E. Olsen); biosynthesis of terpenoid side chains of quinones (F. Lynan); diet-induced changes in ubiquinone-levels (J. Green); function of vitamin K (C. Martius); spectroscopic observations of the redox reaction of ubiquinone in heart and kidney mitochondria (B. Chance); role of ubiquinone in the respiratory chain (E. R. Redfearn); histochemical studies (L. W. Wattenberg); role of plastoquinone in the electron transport system of photosynthesis (N. I. Bishop).

A. I. Scott

NATURAL RADIOACTIVITY IN TOBACCO AND TOBACCO SMOKE

By DR. V. C. RUNECKLES

Imperial Tobacco Co. of Canada, Ltd., Research Laboratory, Montreal

FROM time to time reports on the levels of radioactivity in tobacco and tobacco smoke have appeared in both the lay and scientific Press. Mulvaney[1], Spiers and Passey[2] and Turner and Radley[3] have reported experimental work on the levels of radioactivity in cigarette smoke, while Ash[4] has calculated figures for the levels of activity in cigarette main-stream smoke based on its potassium content and the natural abundance of the radioactive isotope of potassium (potassium-40).

Interest in the level of natural radioactivity in smoke has come from two directions. First, the suggestion has been made[1] that the natural activity of cigarette smoke, resulting mainly from its potassium-40 content, is a cause of lung cancer. Secondly, interest in smoke activity has been aroused by Ash's claims[4,5] that the level of activity in cigarette smoke is so high that the cigarette smoke can be defined as a radioactive material since, according to his calculations, its specific activity exceeds a level of 2,000 μμc./gm., the basis of the definition according to the British Factories Act[6] and the International Atomic Energy Agency[7].

It therefore seemed desirable to obtain experimental information on the level of natural radioactivity in cigarette tobacco, ash and main-stream smoke, and to compare the observed level in smoke with figures obtained by calculation from its potassium content. Accordingly, plain cigarettes of both Canadian and American manufacture (74 mm. and 70 mm., respectively) were smoked to a butt-length of 23 mm. on a constant-pressure smoking machine, taking a 35-ml. puff of 2-sec. duration at a frequency of one puff per min. Main-stream smoke was collected in a train of three water traps followed by a 'Cambridge' filter. Cigarettes were smoked in batches of fifteen. The 'Cambridge' filter was replaced after smoking two batches. The cigarettes were extinguished with carbon dioxide. A total of 300 cigarettes was smoked in each experiment.

The contents of the traps were combined with washings of water and methylene chloride (to dissolve organic materials), evaporated to dryness in 'Vycor' crucibles and ashed at 450° C. for 18 hr. The residues after ashing were boiled in aqua regia until almost entirely dissolved. The samples were then taken to dryness, dissolved in 5 *N* hydrochloric acid, and aliquots plated on stainless steel planchets for counting.

The 'Cambridge' filters were ashed for 18 hr. at 450° C. and extracted with hot concentrated hydrochloric acid. The extracts were evaporated to dryness, the residues dissolved in 5 *N* hydrochloric acid and plated as above.

In order to determine the amounts of radioactivity present in the cigarettes before smoking, fifteen cigarettes comparable to those smoked were cut to 51 mm. (Canadian) and 47 mm. (American) and exhaustively extracted with water for 5–6 days (Soxhlet). The extracts were dried, ashed and treated as above. Ash collected from the cigarettes smoked in these experiments was digested with aqua regia until almost entirely in solution, dried and treated as already described.

The amounts of activity in the butts were determined as described for the unsmoked cigarettes. The activity present in a 23-mm. length of cigarette before smoking was calculated by proportionality from the values found for the unsmoked cigarettes. Subtraction from the observed activities of the butts led to values for the amounts of smoke activity trapped within the butts.

Measurement of radioactivity was made with a Baird–Atomic model *A*2-2-27*PF* proportional flow automatic counting system using a model 822 detector tube with a 'Mylar' end window and 90 per cent argon–10 per cent methane counter gas.

Because of the low count-rates obtained from the aliquots, and in order to minimize counting errors due to fluctuations in background count-rate, sample and background measurements were alternated, each being counted for the duration 900 counts, and each determination being repeated in sequence 50 times, that is, until 45,000 counts had accumulated per planchet.

The efficiency of the counter for potassium-40 was determined by measuring the activities of weighed samples of potassium chloride (analytical grade). A sample of potassium chloride was dried *in vacuo* over phosphorus pentoxide. Approximately 20 gm. were weighed accurately and dissolved in 100 ml. water containing 0·5 per cent gum arabic. Replicate planchets were prepared by accurately pipetting 200 μl. quantities on to stainless steel planchets, which were dried beneath an infra-red lamp. Half the planchets were placed in a furnace at 450° C. for 18 hr. After heating, all planchets were counted until 14,400 counts had accumulated for each. The mean count-rates for the heated and unheated samples were 7·9 and 7·7 c.p.m. respectively, showing that no losses occurred during heating. The mean sample weight of potassium chloride was 42·58 mgm. and the mean count-rate 7·77 c.p.m. The calculated potassium-40 activity (see below) was 36·1 d.p.m. leading to a counter efficiency value of 21·5 per cent.

The experimental results obtained in this work are presented in Table 1, which shows that almost all the activity present in the unsmoked cigarette can be accounted for in the main-stream smoke, ash and butt. Furthermore, approximately 90 per cent of it

Table 1. NATURAL RADIOACTIVITY IN CIGARETTES, ASH AND MAIN-STREAM SMOKE PER CIGARETTE

Sample	Smoke B	Cigarette* A	Ash C	Trapped in butt D	Per cent recovery of activity $\left[\frac{B+C+D}{A}\right]\%$	Per cent transfer to smoke $\left[\frac{B}{A}\right]\%$
		μμc.				
1. Canadian 74 mm.	0·094	22·95	20·59	1·56	97·1	0·41
2. Canadian 74 mm.	0·084	—	—	—	—	—
3. American 70 mm.	0·159	21·20	17·90	0·52	88·8	0·75

* Activity of length of cigarette smoked to 23 mm. butt.

appears to remain in the ash, while less than 1 per cent is transmitted to the main-stream smoke. This finding is in agreement with the work of Spiers and Passey[2], who were unable to detect any activity in smoke, and concluded that the activity in the unsmoked cigarette remained essentially in the ash.

As pointed out above, each sample was counted until at least 45,000 counts had accumulated. For this number of counts the standard deviation is less than 0·5 per cent. However, considerably larger errors are associated with the various stages of sample preparation. The largest of these is undoubtedly associated with differences in sample size and thickness, giving rise to differences in sample geometry and self-absorption. While every effort was made to obtain uniform planchets, the pipetting of aliquots of the hydrochloric acid solutions and slurries led to uneven distribution of material.

The data for smoke activity are probably more accurate than those for cigarettes, butts and ash, since in this case sample preparation was more reproducible. However, the actual magnitude of the errors associated with these observations cannot be determined, but it would seem unlikely to exceed 10–15 per cent. In the light of this uncertainty, it is of interest to compare the observed activities for smoke with the value obtained by calculation.

Cogbill and Hobbs[8] found that potassium is the major metallic constituent of main-stream smoke, accounting for 135 μgm. of the 150 μgm. of metals in the smoke of one cigarette. The natural abundance of potassium-40 is 0·0118 per cent[9], and its half-life $1\cdot26 \times 10^9$ years[9], from which a simple calculation leads to a value of 0·113 μμc. as the potassium-40 activity in the main-stream smoke of one cigarette.

The figures for smoke in Table 1 are in general agreement with this calculated value. However, those for the Canadian cigarettes tested are somewhat lower, while that for American cigarettes is higher than 0·113 μμc. The American cigarettes smoked in this work and those used by Cogbill and Hobbs were blends of Burley and American flue-cured tobacco, while the Canadian cigarettes studied consisted exclusively of Canadian flue-cured tobacco. Since American flue-cured tobacco has a considerably higher potassium content than that grown in Canada, the difference in the level of smoke activity is perhaps not surprising.

If the 10–15 per cent figure suggested is a reasonable estimate of the error associated with these observations, it becomes clear that the measure of agreement between observation and theory suggests that the activity detected in main-stream smoke is due almost entirely to potassium-40, rather than to other isotopes, either natural or artificial. As Cogbill and Hobbs[8] point out, it is not surprising that potassium is the major metallic smoke constituent because it is one of the predominant mineral constituents of tobacco, and, as the chloride, possesses a distinct, though low, vapour pressure at the temperature of the combustion zone.

Table 2 lists the specific activities of the cigarettes studied and their main-stream smoke. In the case of the whole cigarettes the values are expressed as μμc./gm. of dry cigarette (tobacco plus paper), while in the case of smoke, the experimental values are expressed in terms of 'whole smoke'. These 'whole smoke' values were determined from the increase in weight of two 'Cambridge' filters in series when 15 cigarettes were smoked under the conditions described above, without the insertion of the water traps.

Table 2. SPECIFIC ACTIVITY OF CIGARETTES AND MAIN-STREAM SMOKE

Sample	Cigarette			Smoke		
	Activity μμc.	Wt. smoked gm.	Specific activity μμc./gm.	Activity per cig. μμc.	Wt./cig. gm.	Specific activity μμc./gm.
1. Canadian	22·95	0·675	34·0	0·094	0·026	3·62
2. Canadian	—	—	—	0·084	0·026	3·23
3. American	21·20	0·592	35·8	0·159	0·029	5·49
4. American	—	—	—	0·113*	0·072†	1·57

* Calculated from data of Cogbill and Hobbs (ref. 8).
† Reported by Cogbill and Hobbs.

The values for the specific activity of smoke are of primary interest in connexion with the suggestion that smoke can be defined as a radioactive substance[4]. Unfortunately, the figures on which Ash bases his claims are incorrect since they are based upon his calculated value of 0·01 mμc. of potassium-40 activity in the main-stream smoke of one cigarette. Thus when expressed in terms of 150 μgm. of smoke metals, the specific activity of smoke using the correct value of 0·113 μμc. per cigarette is 0·667 mμc./gm. Ash more recently[5] has used this value rather than the value of 6·67 mμc./gm. quoted in his original paper. However, all these calculations of specific activity are unrealistic since they are based upon the weight of metallic smoke constituents alone. A more reasonable basis for calculation is the weight of whole smoke, exclusive of gases, since the potassium-40 activity of smoke is distributed throughout its particulate matter as a whole. The results in Table 2 are calculated on this basis, and show clearly that the specific activities of the smoke from the cigarettes studied are several orders of magnitude less than the level of 2,000 μμc./gm.

Ash's view that smoke is definable as a radioactive substance[5] thus has its basis in the unrealistic use of smoke metals alone for calculating specific activity. However, his further statement that at 667 μμc./gm. the specific activity of smoke is only one-third of the level of the definition, but that this level should be reduced to one-tenth when applied to the population as a whole is without meaning. His point that it is generally agreed that the levels of radioactivity permitted for occupational exposure are ten times those permitted for the general public is irrelevant when dealing with specific activities; for permissible levels are based upon considerations of total accumulation of irradiation, irrespective of the specific activity of the source. As a consequence, the safety factor of one-tenth cannot meaningfully be applied to the 2,000 μμc./gm. level of the definition[6,7].

In connexion with the view that the radioactivity of tobacco smoke is the cause of lung cancer in the smoker, several questions can be raised. For example, how does the amount of activity inhaled by the smoker compare with his intake of natural activity in the diet ? How does the inhaled activity compare with the equilibrium-level of activity in the body ? How rapidly are mineral smoke constituents eliminated from the lung. How does the level of activity in the particulate phase of smoke compare with the natural level of radon and thoron activity in air ?

Little information is available in the literature on the absolute levels of the naturally occurring isotopes, in contrast to those of the isotopes strontium-89, strontium-90 and cæsium-137 which have come into prominence recently as constituents of 'fall-out'. Table 3 presents a collection of results obtained from various sources. Where so indicated, the levels of

Table 3. NATURAL RADIOACTIVITY IN MAN'S ENVIRONMENT

Source	Activity	Ref.
Air	10^{-4} μμc./ml.	10
Air	3×10^{-4} μμc./ml.	12
Human body	120 mμc. ^{40}K, total	10
Sea water	0·33 μμc. ^{40}K/ml.	10
Drinking water	1 to 10^{-4} μμc./ml.	10
Apples	1·00 μμc./gm.*	11
Almonds	7·24 μμc./gm.*	11
Boiled cabbage	1·21 μμc./gm.*	11
Boiled spinach	4·10 μμc./gm.*	11
Dried apricots	16·10 μμc./gm.*	11
Wheat bran	10·50 μμc./gm.*	11
Cow's milk	0·01 μμc./ml.	10

* ^{40}K activity of the potassium contents of these foods, calculated from the data of ref. 11.

activity are for potassium-40 alone, and are calculated from potassium content. A comparison of the results from Tables 2 and 3 shows that the specific activity of the unsmoked cigarette is higher than the specific activity of any of the foodstuffs listed, which were selected to cover a wide range of potassium contents. This difference is, however, to be expected, since tobacco has a relatively higher potassium content than most food crops because of the fertilization practices used in its cultivation. In addition, the tobacco in the cigarette is in a partly desiccated form, and it can be seen that the level of its activity is approached by those of the two dry food materials listed, namely, dried apricots and wheat bran.

Relating the level of smoke activity to total uptake of activity by the body, it should be emphasized that all food materials contain natural radioactivity to some extent. The average dietary intake of activity is difficult to estimate since it depends so much upon the nature of the diet, geographical location and, more recently, upon the contamination of foodstuffs by 'fall-out'. Mayneord[12] has shown that approximately 20 per cent of the dose-rate received by the body arises from its potassium-40 content, while external sources such as cosmic radiation and local radiation from the ground and building materials provide approximately 70 per cent. Internal radiation from isotopes other than potassium-40, for example, carbon-14 and the radium, thorium and uranium series, probably gives rise to less than 5 per cent of the total body irradiation.

Since experimental evidence suggests that potassium is by far the most important isotope in smoke, and since this same isotope is the major source of internal radiation in the body, it is of interest to look at the average diet from the point of view of its potassium-40 content.

The figures in Table 4 are calculated from values for daily potassium intake in the United States published by Anderson *et al.*[14]. Of the daily uptake of approximately 3 gm. potassium (2,560 μμc. potassium-40), 2·6 gm. (2,180 μμc. potassium-40) are excreted daily in the urine, while the remainder is eliminated in the fæces and sweat. The rate of total elimination is determined by the need to maintain an average bodily equilibrium level of approximately 133 gm. potassium (120 mμc. potassium 40) (ref. 12).

Table 4. DAILY POTASSIUM INTAKE, BASED ON *per capita* FOOD CONSUMPTION IN THE UNITED STATES

Source	Potassium (gm./day)	Potassium-40 (μμc./day)
Dairy products	1·15	960
Meats	0·46	390
Flour and cereals	0·20	170
Vegetables	0·56	470
Citrus fruits	0·10	80
Potatoes	0·59	490
Total	3·06	2,560

The calculated potassium-40 activity of cigarette smoke (0·113 μμc. per cigarette) is a very small fraction of the dietary intake, and, even in the case of the heavy smoker, smoking, say, 50 cigarettes a day, the intake of potassium-40 from smoke would be less than 0·25 per cent of the daily intake from food.

However, this extremely small amount of activity could be important if the potassium-40 remained in the lung for a long period, that is, if the lung accumulated mineral smoke constituents, including potassium, without elimination or absorption.

Table 5. FATE OF PARTICULATES IN RESPIRATORY TRACT IN STANDARD MAN

Distribution	Readily soluble compounds (per cent)	Other compounds (per cent)
Exhaled	25	25
Deposited in upper respiratory passages and ultimately swallowed	50	50
Deposited in lungs	25 (taken up in body fluids)	25 (12·5 per cent eliminated in first 24 hr.; remainder eliminated with a half-life of 120 days into body fluids)

Table 5 shows the fate of particulate matter in the respiratory tract as calculated for the standard man[15]. This indicates that, in general, soluble particulate matter is deposited in the lung to the extent of about 25 per cent and is then absorbed into the blood stream. Less-soluble materials are deposited to about the same extent, but about half of these are eliminated in one day while the remainder have a biological half-life of about 120 days. Since the potassium in smoke is likely to be in a readily soluble form, it would seem probable that it is eliminated quite rapidly. The work of Wolff and Purdom[13] suggests that this is so. They allowed dogs to inhale tobacco smoke containing various metal tracers and determined the level of activity of these tracers in the lung tissue at intervals after the smoking period. In the case of potassium-42 they found that nearly all activity disappeared from the lung during the smoking period or soon thereafter. Since potassium-40 and potassium-42 are chemically identical, these results suggest that potassium-40 taken into the lung during smoking is also rapidly eliminated from that organ.

These results combine, therefore, to suggest that cigarette smoking does not overburden the body from the point of view of irradiation by potassium-40, nor does it produce an appreciable increase in radiation to the tissue of the lung.

Relevant to this last point, it is of interest to compare the intake of potassium-40 activity from smoke with the natural activity of air, due to its content of radon and thoron. These gases decay with the production of heavy metal isotopes and in the process emit α-particles which are much more damaging to tissue than the β-particles of potassium-40. On the basis of 11 puffs of 35 ml. vol. in the case of the Canadian cigarettes smoked in this work, the specific activity of their main-stream smoke can be calculated as approximately $2 \cdot 4 \times 10^{-4}$ μμc./ml. In the case of the American cigarettes,

Appearing in July

A Course of Mathematics for Engineers and Scientists

C. PLUMPTON and B. H. CHIRGWIN, *Dept. of Mathematics, Queen Mary College, London*

Appearing this month is Volume I of this 7 volumes series covering the mathematics required by Science and Engineering Students during their undergraduate studies. The earlier volumes lead on from A level mathematics, while the later volumes are concerned with more advanced topics and methods used in science and engineering, and, with application, to classical mathematics. In the later volumes the honours student will find much that he will need in his studies. The emphasis throughout is placed on mathematical methods and their application to physical problems.

Volume I 350 pages illustrated Approx. 30s.

The Brain as a Computer

F. H. GEORGE, *Dept of Psychology, University of Bristol*

This important volume represents the first serious attempt to apply cybernetics to the problems of cognition. The emphasis throughout is on methodology, and attempts to provide a methodological framework within which cognitive problems may be tackled. The problems themselves are discussed also in terms of current neurophysiological evidence.

480 pages illustrated 63s. net ($9.50)

Progress in Materials Science (formerly *Progress in Metal Physics*)

Editor BRUCE CHALMERS

Volume 10, Paper 1 *Alloy Phases of the Noble Metals*

T. B. MASSALSKI and H. W. KING, *The Mellon Institute, Pittsburgh*

A valuable review of the experimental and theoretical developments in the noble metals and their alloys within the framework of the theory of alloys. Particular attention is devoted to phases with close-packed hexagonal structure, and reclassification of alloy phases.

77 pages illustrated 25s. net ($3.50)

Handbook of Numerical Methods for the Solution of Algebraic and Transcendental Problems

V. L. ZAGUSKIN

This book, translated from the Russian, will be extremely useful to engineers having no special mathematical qualifications, as the treatment of the material is not burdened too much with mathematical abstraction. The practical engineer will welcome the comparisons between the different methods given in the book and the Author's recommendations regarding the use of different calculating instruments.

216 pages 40s. net ($6.50)

Photometric Titrations

J. B. HEADRIDGE, *Dept. of Chemistry, University of Sheffield*

Photometric titration technique is discussed and reviewed from its introduction in 1928 up to the present day, covering all recent advances in the field. The book contains chapters on theory, apparatus and the application of photometric titrations in acid-base, oxidation-reduction, complexometric, precipitation and miscellaneous systems as well as a chapter on photometric end-point detection in coulometric titrations.

122 pages illustrated Approx. 40s. ($6.50)

Surface Phenomena in Metals and Alloys

V. K. SEMENCHENKO Edited by R. KENNEDY, *Royal College of Science and Technology*

This book, translated from the Russian, is concerned with the physics of certain surface phenomena, especially surface tension and adsorption, as manifested chiefly by pure metals and alloys, and explains the theory underlying these phenomena. A useful set of tables with data for a number of metals, many of them comprising the results by different authors, is appended.

460 pages £5 5s. net ($17.50)

All books gladly sent on 14 days' approval

PERGAMON PRESS

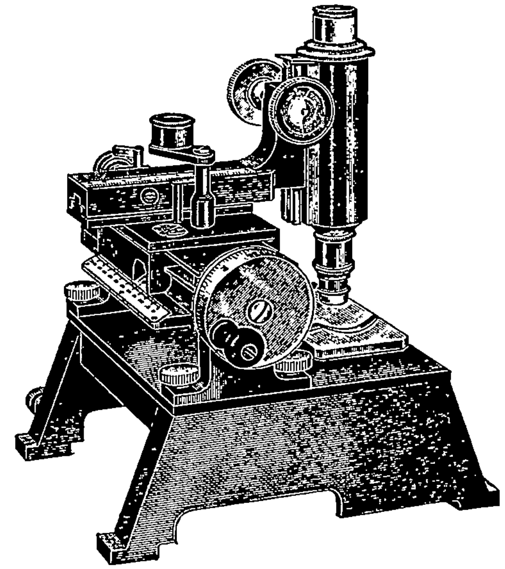
OXFORD LONDON NEW YORK PARIS

Headington Hill Hall, Oxford. 4 *&* 5 *Fitzroy Sq., London, W.*1. 122 *East* 55*th St., New York* 22, *N.Y.*

BECK

MICROMETER MICROSCOPE

Accuracy in measurement



Full particulars of:

R & J BECK LTD • 69/71 MORTIMER STREET
LONDON, W.1

GREEN'S PURE FILTER PAPERS

are
Competitive in price and made to published standards.

Ask for free descriptive book N.G.60, which gives you the specifications.

J. BARCHAM GREEN LTD.,
MAIDSTONE ENGLAND

'E.R.P.' PLASTIC TUBING

- WATERCLEAR AND FLEXIBLE
- STERILIZABLE AT 120° C.
- NON-PYROGENIC AND NON-TOXIC
- RESISTANT TO MANY CHEMICALS

ESCO (RUBBER) LTD. *34/36 SOMERFORD GROVE, LONDON, N.16*
Manufacturers of Rubber, Silicone Rubber and Plastic Products

CYGNET meets the demands of science

The Associated Feed Manufacturers Research Laboratory, York Road, Belfast, is an excellent example of the top quality research accommodation now being provided for industry. Once again, Cygnet Laboratory Furniture provides the supreme craftsmanship and advanced design necessary for maximum efficiency.

Architects: Munce & Kennedy, A/ARIBA
Architects & Consultant Engineers,
University Street, Belfast, 7

CYGNET JOINERY LTD • HIGHER SWAN LANE • BOLTON • TEL: BOLTON 62121 (10 lines)

approximately 9 puffs were taken per cigarette, leading to a figure of about 5×10^{-4} μμc./ml. The levels of radon–thoron activity in air are shown in Table 3. Using Mayneord's value of 3×10^{-4} μμc./ml., it is apparent that the levels of potassium-40 activity in smoke are of the same order of magnitude.

Further to this, Turner and Radley[3] have studied the α-particle activity of tobacco, due to its radium and thorium contents, and have concluded that the release of α-activity during smoking is extremely small. They state that even in the case of the heavy smoker, the additional α-activity taken into the lungs is less than 1 per cent of the atmospheric radon and thoron inhaled daily by any individual, approximately 2 mμc., a figure obtained from the approximate daily uptake of air, 2×10^{7} ml. (ref. 15) and its radon–thoron content, 3×10^{-4} μμc./ml. (ref. 12).

In summary, therefore, we can say that the observed activity of cigarette main-stream smoke appears to be due almost entirely to its content of potassium-40. The amounts of this activity taken into the lung, even by the heavy smoker, are minute compared with the bodily uptake from the diet, and are rapidly eliminated from the lung tissue. In addition, this potassium-40 activity only exceeds by a small amount the α-activity taken into the lungs of all individuals because of the radon and thoron in the Earth's atmosphere.

Finally, calculations of the specific activity of smoke, based upon a logical appreciation of its nature, lead to values far below the internationally agreed level at which any material can be defined as radioactive.

I wish to thank the Directors of the Imperial Tobacco Co. of Canada, Ltd., for permission to publish this article, and to acknowledge the assistance of Mr. B. J. Stirling and Mr. K. Woolrich in the experimental work.

[1] Mulvaney, D. K., *Lancet*, ii, 205 (1953).
[2] Spiers, F. W., and Passey, R. D., *Lancet*, ii, 1259 (1953).
[3] Turner, R. C., and Radley, M. M., *Lancet*, i, 1197 (1950).
[4] Ash, M., *Med. Services J. Can.*, **15**, 195 (1959).
[5] Ash, M., *Roy. Soc. Health J.*, **80**, 22 (1960).
[6] Factories Act (Ionizing Radiations). Special Regulations. Second Prelim. Draft, 8 (H.M.S.O., London, 1960).
[7] *Safe Handling of Isotopes*, 11 (Intern. Atomic Energy Agency, Vienna, 1958).
[8] Cogbill, E. C., and Hobbs, M. E., *Tobacco Sci.*, **1**, 68 (1957).
[9] Kohman, T. P., in *Radiation Hygiene Handbook*, edit. by Blatz, H., 6 (McGraw-Hill, 1959).
[10] *Handbook of Biological Data*, edit. by Spector, W. S., 470 (Saunders, 1956).
[11] McCance, R. A., and Widdowson, E. M., *The Chemical Composition of Foods* (H.M.S.O., London, 1940).
[12] Mayneord, W. V., *Roy. Soc. Health J.*, **80**, 22 (1960).
[13] Wolff, W. A., and Purdom, E. G., *Proc. Eighth Tobacco Chem. Conf.*, Richmond (1954).
[14] Anderson, E. C., Schuch, R. L., Risher, W. R., and Langham, W., *Science*, **125**, 1273 (1957).
[15] Blatz, H., and Kinsman, S., in *Radiation Hygiene Handbook*, edit. by Blatz, H., 1 (McGraw-Hill, 1959).

CLIMATE AND WEATHERED KARROO DOLERITES

By Dr. H. H. Weinert

National Institute for Road Research,
South African Council for Scientific and Industrial Research, Pretoria

Introduction

A very important road-building material in large parts of the Union of South Africa is Karroo dolerite, the weathered rock being used in the layers of road foundations and the fresh rock when crushed for surfacing chips. The performance of weathered dolerites is known to vary in different parts of the country. The material performs well in the western portion of the Union and badly in the eastern, the differences in performance being borne out by observation that the rate of alteration of the materials, after being placed in the road foundation, appears to increase. These facts led me to carry out research on the influences of environmental conditions on the alteration of basic igneous rocks with particular reference to Karroo dolerites.

The most conspicuous feature of the South African Karroo system is the huge mass of crystalline igneous rock which is called traditionally 'dolerite'; according to present nomenclature, however, it should be designated as 'diabase' of a very wide variety[1]. These dolerites are intrusive into the sediments of the Karroo era in numerous thin dykes and in sills up to a thickness of 200 m. or more. There is a marked southern limit to the extent of these rocks, crossing the southern portion of the Karroo areas in a roughly west–easterly direction (Fig. 1).

The rocks are dark-coloured generally, varying due to structure, texture and mineral composition from norite to quartz-diorite, with diabase forming the bulk of the whole occurrence. Differentiation may lead to light-coloured rocks exceptionally.

Weathering of Dolerites

Dolerite outcrops in the eastern parts of the Union of South Africa show a succession of weathering products consisting normally of four layers which can be distinguished readily by their colour and consistency. The lowest layer is the fresh rock or that which, in comparison with the rest, may be termed

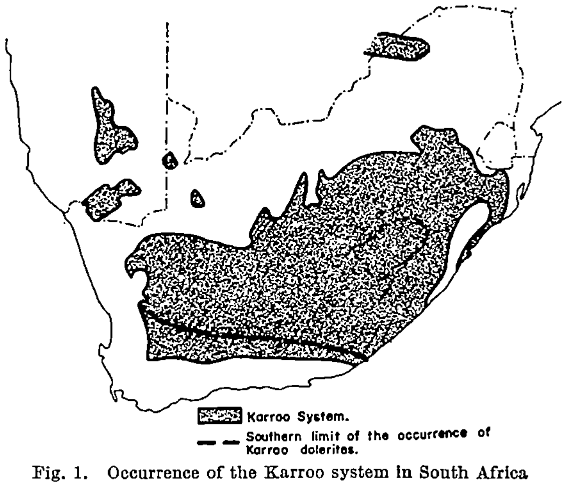


Fig. 1. Occurrence of the Karroo system in South Africa

'fresh'. This rock is dark in colour with the whitish feldspars standing out clearly in the brownish- to greenish-black matrix which consists of pyroxene, amphibole, different ores and occasionally olivine, biotite and quartz. The rock is hard and dense, giving a metallic ring when struck with the hammer. The next layer, the weathered rock, is still hard but brittle, breaking readily when struck with the hammer, and often showing exfoliation. The colour is brownish due to frequent staining with iron oxides. The feldspars are still clearly recognizable, while most of the mafic minerals have lost their dark colour. The third layer, the badly weathered rock, has lost its consistency completely and become a brittle to soft, yellowish-grey mass with an earthy feeling. The original structure of a crystalline igneous rock is still preserved, and the former planes of exfoliation are clearly visible: most of the original minerals, however, have lost their properties completely. The fourth layer is the residual soil on the top of such deposits. These soils vary, depending on climate and relief, being black clays in water-logged areas in most cases and deep red clays on slopes[2].

The weathering succession in dolerite outcrops in the western portions of the country differs somewhat from the profile given in the previous paragraph. The stages 'badly weathered' and 'residual soil' are not properly reached and the whole succession remains in the state 'weathered', as defined. However, the rock does not remain a hard material but merely disintegrates, finally becoming a dolerite gravel, the grains of which are the different minerals of the original rock, showing only partial chemical alteration. Physical weathering is clearly overshadowing chemical weathering in the western portions of the Union, this statement being in some contrast to others made previously[3].

An increasing amount of sand normally occurs towards the surface, being blown in, and consequently mixed with, the residual soil. The difference between clays, black or red, in the eastern parts of the country and sandy to gravelly soils with only little clay, is also expressed by the predominance of the soil clay minerals in the widest sense. Hydrous mica is predominant in the west, and other clay minerals, montmorillonite and kaolin in particular, in the east[4].

A statistical investigation on the percentage of secondary minerals in the different layers of the weathering successions has been carried out for a number of samples from different sites. Similar investigations had been made previously on certain basalts in Oregon, United States[5]; the South African approach is different from the American one in so far as the latter dealt with rocks from rather uniform environments in regard to topography and climate, while the South African investigation has covered materials from many different regions.

Quartz, feldspar, biotite, amphibole, pyroxene, olivine and magnetite were considered primary minerals and all others as secondary. The investigation was carried out with a polarizing microscope and counting was done with the aid of a micrometer. Special difficulties were experienced during the preparation of the badly weathered parts and the residual soils of the weathering succession. For grinding purposes, resins had to be used in order to harden these materials. All minerals which could be recognized individually were counted separately, and those too small for recognition under the microscope, such as clay minerals, were just counted as 'secondary'. The results of counting were arranged in two groups, primary and secondary minerals, and the relation was expressed as a percentage of the total number counted.

The results were compared with the field description of the weathering succession as 'fresh', 'weathered', 'badly weathered' and 'residual soil', and a remarkably good correlation of these designations with certain ranges of percentages of secondary minerals was observed:

Field description of rock	Percentage of secondary minerals
Fresh dolerite	< 25
Weathered dolerite	25–32
Badly weathered dolerite	> 32
Soil, residual from dolerite	> 70

The small range, 25–32 per cent, for the section 'weathered dolerite' appears to be the most remarkable result of this investigation, because it shows that the transition from the solid, fresh-looking rock to the thoroughly decomposed material seems to be a rather abrupt change. The number of samples studied so far has not allowed a range of percentage to be allocated to the group 'badly weathered'; the limit between 'badly weathered' and 'residual soil', however, may be expected somewhere between 60 and 70 per cent.

X-ray investigations have shown that montmorillonite and hydrous mica occurred in all samples, including those which were classified as 'fresh'. Montmorillonite is well crystallized in these samples and also in some of the 'weathered' and 'badly weathered' ones; it never appeared crystallized in the residual soil, but only as amorphous material. The transition from the crystallized to the amorphous states appeared to take place in the stages 'weathered' and 'badly weathered'. Kaolin may occur suddenly at a certain level in the 'badly weathered' stage, being then associated with an almost complete disappearance of all feldspars.

The results of the work on secondary minerals, as well as from X-ray and differential thermal analysis investigations, were the same for samples from the western and the eastern portions of the country as regards the classification from 'fresh' to 'residual soil'. In the western part of the country, however, the state 'weathered' was not passed.

Standard engineering tests carried out on those weathered dolerites which were intended for road construction indicated a regional distribution of the results. This was the case for the Atterberg limits in particular, where the liquid limit and the plasticity index were of special significance and importance. A line could be drawn across the Union with the values of 30 for the liquid limit and 10 for the plasticity index, larger values predominating to the east and lower values to the west of this line. This line intersected the coast line near Port Alfred and crossed the Orange Free State a little east of Bloemfontein; it then continued towards Rustenburg in the Transvaal, following thus a generally north–south course. This also agreed roughly with the types of weathering given above, disintegration prevailing on the western and decomposition on the eastern side of it.

Climatic Investigations

The Union of South Africa falls within three major climatic regions. They are the summer rainfall areas which cover the largest parts of the country, the winter rainfall areas of the Cape of Good Hope and the almost constantly dry desert regions of the north-west. There are remarkable variations within the large summer rainfall areas, concerning the duration of the rainy season and the quantity of water precipitated. The climatic investigations were concentrated on the summer rainfall areas because only those areas are bisected by the line which separated weathered doleritic materials on the basis of the Atterberg limits and which also agreed with the boundary between the types of weathering.

The Orange Free State was chosen for the first detailed investigation for three reasons: (1) the Orange Free State falls completely within the summer rainfall area; (2) the province is relatively small and in a central position within the country; (3) weathered dolerites are almost the only road-building materials within the province and a sufficient number of test results was available.

The climatic investigation included precipitation, potential evaporation calculated for a shallow free-water surface from the Meyer formula[6], relative humidity, wind speeds, temperature and a calculated ratio of evaporation to precipitation. All investigations were made for the months of January (rainy season) and July (dry season) separately, giving thus separate pictures for the climatically extreme parts of the year. The annual precipitation and a ratio of the relative humidities of January to those of July were also added.

The evaporation precipitation ratios were calculated so that the higher value was always divided by the lower one, giving results representing either a surplus of evaporation over precipitation (positive values) or the opposite (negative values); the numerical figures then are comparable. Contours were drawn on the basis of the values obtained, the contour 1·0 connecting places with equal evaporation and precipitation. The contour +3·0 for January appeared to be the most significant in comparison with the lithological boundaries.

Contours were drawn for each climatic indicator, the general contour pattern being compared with the boundary obtained from the test results. Those contours which agreed with this boundary were selected. The most remarkable feature of the results was that, while none of the contours referring to July agreed with the soils boundary, agreement was found for the January values and, furthermore, that all climatic indicators showing an agreement had something to do with water. Besides these monthly values, a close agreement occurred with the annual isohyet of 500 mm. and with a contour connecting all those points where July has the same relative humidity as January. This contour runs a little west of the zone occupied by the other contours. It appears to be of importance, as the Orange Free State falls completely within the summer rainfall area, where January would be expected to be more humid than July. However, the relative humidity of July is higher than that of January in the country west of this line. This fact indicated that the inflow of moist air in January is not high enough to compensate for the higher temperatures in this month.

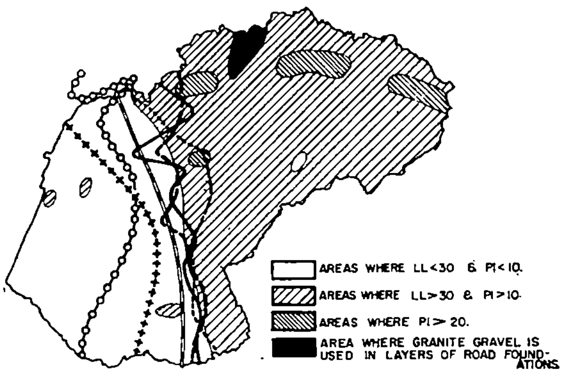

▭ AREAS WHERE LL<30 & PI<10.
▨ AREAS WHERE LL>30 & PI>10.
▧ AREAS WHERE PI>20.
■ AREA WHERE GRANITE GRAVEL IS USED IN LAYERS OF ROAD FOUNDATIONS

Fig. 2. Climatic and soils boundaries in the Orange Free State. – – –, Relative humidity of 55 per cent in January; + +, relative humidity in January = relative humidity in July; ——, 85 mm. isohyet for January; — —, evaporation/precipitation ratio + 3·0 in January; ○, boundary between hydrous mica (west of line) and other clay minerals (east) as predominant soil clay minerals; ■□■, 500 mm. isohyet of annual precipitation; *LL*, liquid limit; *PI*, plasticity index

The border between the predominance of hydrous mica and other clay minerals in South African soils as indicated by C. R. van der Merwe and H. Heystek[4] was also added. This border runs somewhat west of that defined by the Atterberg limits, which may be one indication that a certain percentage of clay minerals, montmorillonite in particular, must be present in the unsound weathered dolerites to make them differ from the sounder materials of another region.

The position of all these contours, relative to the subdivision of the weathered dolerites in the Orange Free State based on the Atterberg limits, is shown in Fig. 2.

These investigations were then extended over the whole Union of South Africa and contours were drawn again. The same climatic values as for the Orange Free State were sorted out and it was noted that their meaning was the same as before in the eastern Cape Province and the western Transvaal, the whole of Natal being east of the critical boundary. All contours then enter the Bechuanaland Protectorate. From 25° S., however, the 500 mm. isohyet of annual precipitation, together with the boundary of hydrous mica as the predominating soil clay mineral, turns east and re-enters the Transvaal again, but the others retain their approximate northerly direction (Fig. 3). This course of the contours leaves the deeply weathered Bushveld norites—the depth

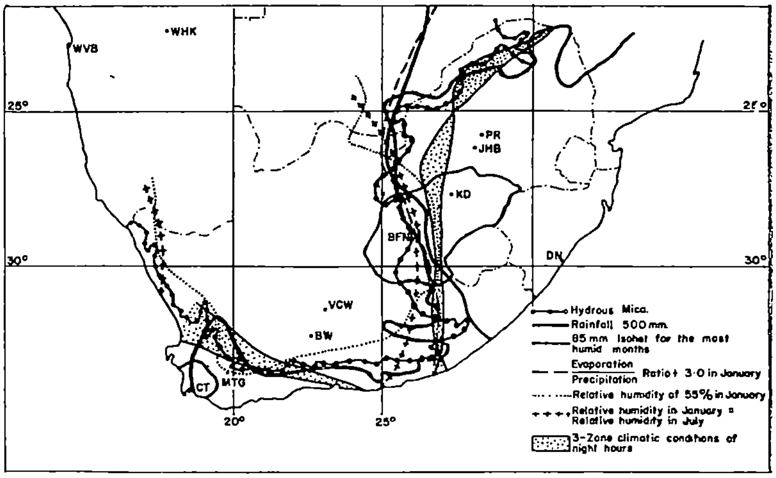

WHK
WVB
25°
25°
PR
JHB
KD
BFN
DN
30°
30°
VCW
BW
CT
MTG
20°
25°
Hydrous Mica.
Rainfall 500 mm.
85 mm Isohet for the most humid months
Evaporation / Precipitation Ratio + 3·0 in January
Relative humidity of 55% in January
Relative humidity in January = Relative humidity in July
3-Zone climatic conditions of night hours

Fig. 3. Climatic and mineralogical features concerning weathering of Karroo dolerites

of weathering is known to be more than 8 m.—on the eastern side of the zone created by the climatic contours. Dolerites do not occur in this part of the Union, but the norites can, however, be considered sufficiently similar to them for such comparison.

Assuming that the effect of water on weathering will increase if evaporation is reduced considerably, a separate investigation on precipitation, evaporation and the ratio of evaporation to precipitation was carried out for the night hours of January, that is, the period 1900–0500 hr., and July, that is, the period 1700–0700 hr.

Contours were again drawn for precipitation, evaporation from a shallow free-water surface and the ratios of evaporation to precipitation. The contour patterns of these three indicators, separately drawn for January and July, were compared with the soils boundary as obtained from the Atterberg limits and it was found that all patterns for January agreed with this boundary, but there was no agreement with the July contour patterns. Those January contours which agreed best with the soil boundaries were selected and used as critical values for the climatic effects on weathering. These critical values were: (1) evaporation during night hours 50 mm., its effect on weathering being greater with decreasing values; (2) precipitation during night hours 30 mm., its effect on weathering being greater with increasing values; (3) evaporation/precipitation ratio during night hours of 1·0, that is, evaporation equal to precipitation. A greater influence on weathering would be expected where surplus of precipitation occurs.

In order to gain an overall picture, numerical values were allotted to all areas of the country on the base of the above subdivision. These were 0 for weak and 1 for strong effects on weathering of the individual climatic conditions during night hours[7]. This evaluation led to the subdivision of the country into six different zones, each one designated by one of the numerical values 0, 1, 2, 3, 4 and 5 (Fig. 4), which means that the higher the numerical value of a given area the stronger the potential influences of all climatic indicators. Two relatively narrow zones, one of them bisecting the summer rainfall area and the other bordering the winter rainfall area of the Cape, have the value 3. The 3-zone of the summer rainfall area agrees well with the full-day contours, as already known from the Orange Free State, as well as with the soils boundary defined from the Atterberg limits and from the predominance of either disintegration or decomposition for that portion of its course which passes the western Transvaal, the Orange Free State and the eastern Cape Province. This zone becomes ill-defined in its northern continuation through the Transvaal, where the easterly type of weathering is known to cross this zone near Rustenburg and Brits, and here the 2-zone seems to take the place of the 3-zone. No conclusion can be drawn about the relevance of the 2-zone in the winter rainfall area of the Cape, where the bulk of rain falling during the colder part of the year might have a somewhat different effect on the weathering of basic igneous rocks. It may be mentioned, however, that more intense weathering of dolerites is known to occur in the vicinity of Calvinia, which is near the 3-zone.

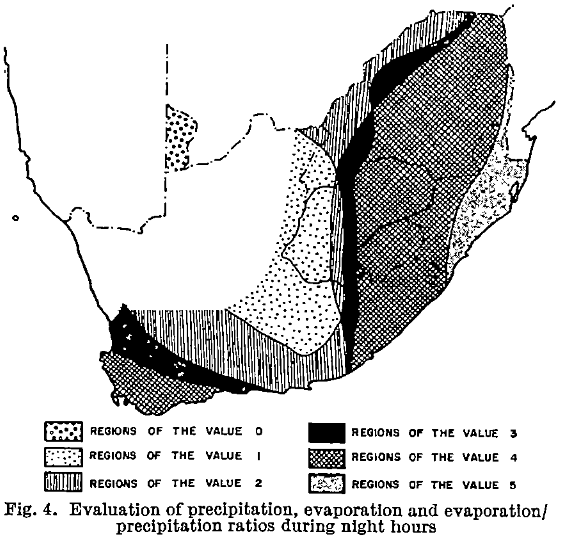


Fig. 4. Evaluation of precipitation, evaporation and evaporation/precipitation ratios during night hours

Conclusions

The comparison of the present climatic conditions of a country with very marked differences between a humid, warm and a dry, cool season, with the types of weathering and with some engineering properties of weathered basic igneous rocks, dolerites in particular, has emphasized clearly the importance of water. All climatic indicators which had an apparent bearing on weathering referred to the climatic conditions of the warmest and most humid month, and all of them were connected with the direct or indirect influences of precipitation or water. The close agreement of a number of climatic contours, demarcating regions with different types of weathering and, at the same time, with different engineering performances of the weathered materials, holds for the larger portion of the South African summer rainfall area. This climatic zone, however, becomes ill-defined towards the north, where increasing mean air temperatures as well as increasing soil temperatures might strengthen the influence of water, that is, a smaller amount of water at a warmer temperature may have the same effect on weathering and on engineering properties as a higher amount of water at lower temperatures. In the winter rainfall areas of the Cape Province no such close agreement of the various climatic contours occurred. A larger temperature effect may also appear here, because the rain falls during the colder part of the year.

Microscopic and X-ray investigations showed that the material alteration which the dolerites undergo during weathering is the same on either side of the climatic separation zone. The percentage of secondary minerals and the type and state of clay minerals were the same, independently of the local climate, if a certain state of weathering were reached.

The rate and progress of weathering, however, can be connected with the local climatic conditions. In the western parts of the country, that is, in the climatic zones 0 and 1 (Fig. 4), decomposition is reduced in favour of mere disintegration and does not normally pass the state 'weathered' of the climatic zones 4 and 5. In the eastern parts of the Union, that is, in the climatic zones 4 and 5, little disintegration occurs and the process of weathering is largely chemical in nature. The 2- and 3-zones are largely transition zones between the other two.

The differences in weathering also refer to the performance of the weathered materials if used for road-construction and, especially for foundation purposes, with decomposition leading to unsound materials and poor results, and disintegration leading to sound materials.

The climatic conditions of a given site or area, the amount of water available in particular, and probably also a combination of air and soil temperatures, may thus provide a means for defining the probable type of weathering and also for predicting the engineering behaviour of weathering or weathered basic igneous rocks, among which the Karroo dolerites are of great practical importance in South Africa.

This article is published by permission of the South African Council for Scientific and Industrial Research.

[1] Du Toit, A. L., *The Geology of South Africa*, third edit., 364 (Oliver and Boyd, Edinburgh and London, 1954).

[2] Maufe, M. B., *South African J. Sci.*, **25**, 156 (1928).

[3] Reiche, P., *A Survey of Weathering Processes and Products*, Univ. of New Mexico, Pub. in Geology, No. 3 (Albuquerque, 1950).

[4] Van der Merwe, C. R., and Heystek, H., "Clay Minerals of South African Soil Groups", *Soil Sci.*, **74**, No. 5; **79**, No. 2; **80**, No. 6, **81**, No. 5 (1952–1956).

[5] Scott, L. E., *Secondary Minerals in Rock as a Cause for Pavement and Base Failure* (Highway Res. Board, Proc. Thirty-fourth Ann. Meeting, 1955).

[6] Linsley, R. K., Kohler, M. A., and Paulhus, J. L. H., *Applied Hydrology*, 168 (McGraw-Hill Book Co., Inc., 1949).

[7] Weinert, H. H., *Climate and the Potential Performance of Weathered Dolerites in Road Foundations* (Second Southern African Regional Conf. on Soil Mechanics and Foundation Engineering, Lourenço Marques, October 1959).

MAGNETIC STORMS IN INTERPLANETARY SPACE AS OBSERVED BY *PIONEER V*

By EUGENE W. GREENSTADT

Space Technology Laboratories, Inc., Los Angeles, California

IN a preliminary report on the magnetometer experiment carried by the deep space probe *Pioneer V* (1960α), Coleman *et al.*[1] display a correspondence between fluctuations in average ambient magnetic field value at the probe and local a_k magnetic index values recorded concurrently at Fort Belvoir, Virginia, for the very active period March 30–April 3, 1960. Fig. 2 of their paper exhibits the satellite data and the a_k index plotted in parallel, showing agreement between the two traces for the intense magnetic perturbation of April 1 and for lesser events of March 31, April 1 and April 3. The present article extends the comparison between effects at the Earth and at *Pioneer V* over the entire period of regular acquisition of data in the interplanetary region, showing a correlation between 'storms' appearing at the two bodies, and between the interplanetary field and planetary magnetic index, a_p.

The trajectory of *Pioneer V* is shown in Fig. 1. Only field magnitude perpendicular to the axis of rotation of the spin-stabilized probe (arrows) was measured, and only relative changes in the measured component are considered here. Details of probe and magnetometer geometry have been published elsewhere[1,2].

Launched on March 11, 1960, the probe reached a distance of 500,000 km. by the middle of March 12, placing it well outside the terrestrial magnetic field[1,3,4]. The distance between the payload and the Earth increased continuously to approximately 12,000,000 km. on April 30, after which telemetry transmission became too infrequent for significant addition to the results.

Measurements were transmitted as several discrete messages each day, with each message averaging 30 min. duration and including many individual magnetic field readings. Individual readings were averaged over intervals of half a day for this work. The half-day interval has proved to be a convenient unit because it is long enough to diminish the influence of short-term transients, while not so long as to obscure the correlation between short magnetic storms. A smoothed graph of the data has been produced in turn by plotting, instead of each half-day field average $\bar{B}_i$, the mean of $\bar{B}_{i-1}$, $\bar{B}_i$, and $\bar{B}_{i+1}$. The upper curve of Fig. 2 presents this smoothed version of the average ambient field component at the payload, with concurrent geomagnetic storm periods and smoothed planetary indices below for comparison.

The magnetic index curve was prepared in the same manner as that used for payload data: the 3-hr. planetary index a_p, converted from published K_p values[5–7], was averaged over half-day intervals and a three-interval mean applied to the half-day averages.

Periods designated as separate geomagnetic storms by one or more reporting observatories[8,9] are represented in Fig. 2 by solid horizontal bars. Dashed bars appear in connexion with the March 14 event, where there is substantial disagreement on duration of the storm and later where some observatories reported single long events rather than separate occurrences.

Statistical correlation between payload field increase and separate geomagnetic storm incidence is particularly striking. The curve of smoothed payload

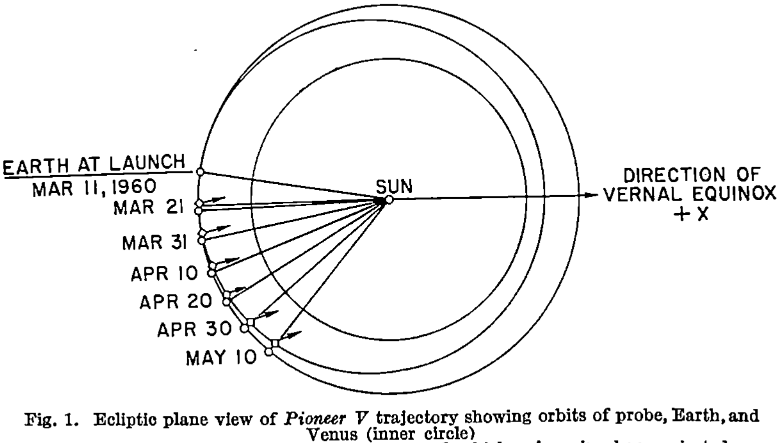


Fig. 1. Ecliptic plane view of *Pioneer V* trajectory showing orbits of probe, Earth, and Venus (inner circle)
□, Vehicle; ○, Earth. Arrows show direction of vehicle spin axis when projected on to ecliptic plane

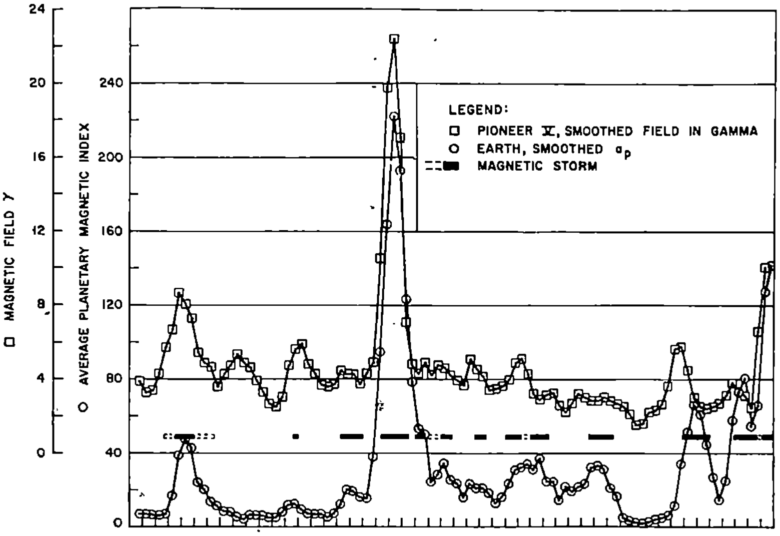


Fig. 2. Smoothed *Pioneer V* magnetic field, smoothed planetary index a_p, and geomagnetic storms

ambient field, characterized by a sequence of crests or excursions of diverse amplitude and more or less similar shape, has 14 maxima and a rapid rise before the graph terminates on April 30. Subsequent data, not shown, give a return to nominal field readings on May 1, so that a fifteenth peak may be added to those visible in the figure.

Of the 15 maxima in the satellite data, 14 are accompanied closely or followed within one day by the occurrence of magnetic storms at the Earth. Of the total of 13 individually reported geomagnetic storms occurring during the fifty days of observation (solid bars) every one is accompanied or preceded within a day by an excursion of the smoothed ambient field at the payload. The geomagnetic storm in the interval April 16–18 can be associated with a pair of local submaxima in the smoothed payload data, making as before a total of fourteen correlated peaks at the probe. The one exception to the agreement between probe data and storm reports is the excursion of March 19–21, but examination of magnetic records covering this interval shows that while no magnetic storm appeared in the reference list, disturbances in the Earth's field, noted particularly on College, Alaska, magnetograms, appear to have taken place (see discussion of magnetic index following).

The plot of smoothed planetary index in Fig. 2 consists of a series of irregular excursions comparable to those appearing in the *Pioneer V* curve. Qualitative agreement between the two measurements can be seen at once, with the extreme maxima of April 1 and 30 as outstanding parallel features. Some details of the March 30–April 3 period have been lost by smoothing, but evidence of correlation for separate peaks of April 1 and 3 can be found in the preliminary report mentioned earlier[1]. Once again, the payload excursion of March 19–21 appears uncorroborated at the Earth, despite the faint trace of corresponding activity in the planetary index. Indication of high-latitude terrestrial effects on March 19 and 21 is present, however, in magnetic indices from College. This can be seen in a comparison of the payload daily average field and College A_k index, reproduced in Fig. 3 for the interval March 12–28.

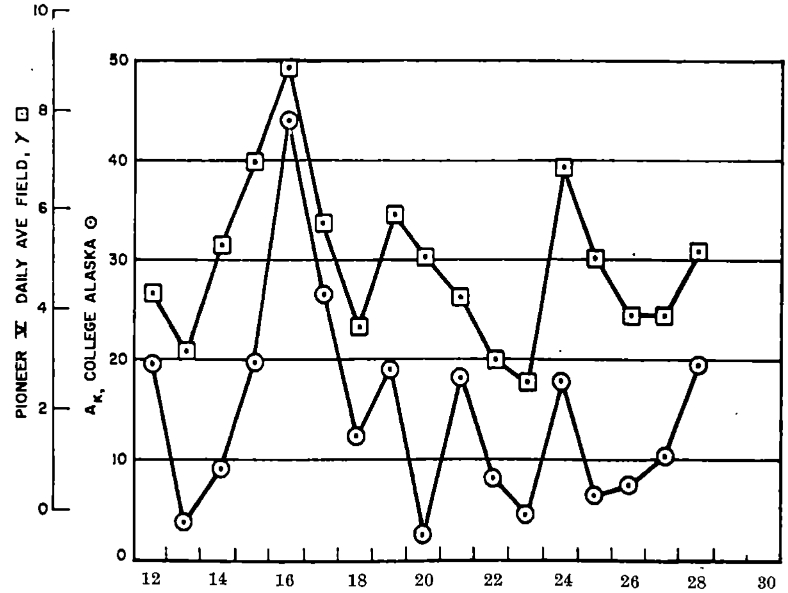


Fig. 3. *Pioneer V* daily average field and daily magnetic index A_k, College, Alaska

The data discussed here and presented in Figs. 2 and 3 show that relative increases in the average value of field amplitude measured by *Pioneer V* almost always occurred in approximate conjunction with magnetic storms at the Earth's surface and consistently with increases in irregularity of the Earth's field as measured by the magnetic indices a_p and A_k. It is tentatively concluded from this evidence that enhancement of at least one component of nearby interplanetary magnetic field regularly accompanies terrestrial magnetic storms. It is also concluded that this enhancement cannot be restricted exclusively to a component directed along radial lines from the Sun, since during an appreciable part of the observation period the probe magnetometer measured field magnitude in a plane nearly normal to the Sun-payload radius (normal to the arrows of Fig. 1).

A feature essential to the correlations between payload and planetary index curves and between payload maxima and terrestrial storms is the assumed correspondence of time-displaced events near the end of April. Preliminary comparison of unsmoothed data with terrestrial observatory magnetograms indicates that the extreme phase difference discernible in Fig. 2 for 'corresponding' events between April 21 and 29 cannot be accounted for entirely by the smoothing process, but is a real feature of the observed interplanetary and geomagnetic fields. Examination of unsmoothed data has also revealed that terrestrial effects were either delayed or persisted longer than corresponding interplanetary disturbances for some events earlier in the history of the flight, although never with phase differences as large as that seen late in April.

An increasing delay toward the end of the period of acquisition of data might be expected to result from the steadily advancing distance between Earth and probe, on the assumption the satellite was intercepting disturbances of solar origin travelling toward the Earth. However, based on the approximate 1,000 km./sec. average velocity usually inferred for transient solar-terrestrial magnetic effects, the expected delay between *Pioneer V* and the Earth, even for distances in effect at the end of April, would be less than 3·3 hr., and thus imperceptible in the scale of Fig. 2. Long delays due solely to increase in time of probe-Earth passage would require local velocities close to 100 km./sec. to account for the observed shifts, therefore ruling out solar effects moving by the payload at ten times this speed.

Suggested interpretations of the time displacements include a low payload-Earth velocity and a terrestrial delay mechanism, and the possibility that excursions observed in the interplanetary region and at the surface of the Earth are related respectively to different phases of the travelling storm phenomenon. Further investigation of the data is now in progress.

Acknowledgment is due to Dr. E. J. Smith and Dr. J. M. Kelso for useful discussions and to M. Saxe for help in preparation of the data used above. P. J. Coleman, jun., was responsible for reduction of the *Pioneer V* magnetic data.

The programme is sponsored by the United States National Aeronautics and Space Administration.

[1] Coleman, jun., P. J., Sonett, C. P., Judge, D. L., and Smith, E. J., *J. Geophys. Res.*, **65**, 1856 (1960).

[2] Coleman, jun., P. J., Davis, L., and Sonett, C. P., *Phys. Rev. Letters*, **5**, 43 (1960).

[3] Sonett, C. P., Judge, D. L., Kelso, J. M., and Sims, A. R., *J. Geophys. Res.*, **65**, 55 (1960).

[4] Sonett, C. P., *Phys. Rev. Letters*, **5**, 46 (1960).

[5] Lincoln, J. V., *J. Geophys. Res.*, **65**, 2198 (1960).

[6] Lincoln, J. V., *J. Geophys. Res.*, **65**, 2470 (1960).

[7] *Bull.* No. 12*h*, Intern. Assoc. Terres. Mag. Elec. (IATME).

[8] Lincoln, J. V., *J. Geophys. Res.*, **65**, 2467 (1960).

[9] Lincoln, J. V., *J. Geophys. Res.*, **65**, 4195 (1960).

OBITUARIES

Prof. W. T. Astbury, F.R.S.

With the death of Prof. W. T. Astbury on June 4, at the age of sixty-three, the world of crystallography, fibres and molecular biology lost an international figure.

A product of the 'Five Towns', eldest son of a large family, versatility at Longton High School earned a scholarship to Jesus College, Cambridge, where, with a break by service in the Royal Army Medical Corps, he graduated in physics in 1921.

The first phase, 1921–28, of his working life was one of tutelage and growth as assistant to Sir William Bragg, first at University College, London, then, from 1923, at the Davy Faraday Laboratory of the Royal Institution where "in that dawn 'twas bliss to be alive", the happy coterie including Müller, Shearer, Bernal, Yardley, Robertson, Robinson and Cox. There he learnt the lessons of simple and direct experiment, the fine tradition of 'Friday Evening' exposition, the value of collaboration. Notable early work on essential systematics and technique of X-ray crystal analysis were the famous space-group tables with Yardley and the α-ray integrating photometer. Bold attempts at key structures like tartaric acid and the acetyl acetones were necessarily sketchy; introduction to his life-work came in preparing fibre X-radiograms for Bragg's lectures "On Common Things".

In 1928, Astbury found a field ready for his talents as lecturer in textile physics, University of Leeds, Bragg sustaining him in early difficulties. Five years solid work produced papers in the *Philosophical Transactions of the Royal Society* on the structure of hair, wool and related fibres which laid for a generation the foundations of high-polymer structural studies. Briefly, inspired analysis of the vague X-ray diffraction pictures of biological fibres under varied conditions of humidity, temperature, tension and chemical treatments, related to optical, mechanical, chemical and technical features, led to a broad scheme of keratin structure: the $\alpha \rightleftharpoons \beta$ polymorphic transition as reversible intra-molecular chain folding, the folds plausibly related to side-chains and S—S links to explain 'set' and supercontraction. This brought academic and industrial recognition, welcome financial support, and award of the Royal Institution's quaintly worded Actonian Prize.

Extension to muscle and collagen established two broad classes of fibrous protein structure, while similar work on egg albumen and on protein layers emphasized chain folding as a key to fibrous and globular protein relations. These, with *ad hoc* studies of dyeing and tanning and his 'missionary' *Fundamentals of Fibre Structure*, impressed old trades with new knowledge. It was the creative period.

When not abroad, the rest of his life, from 1955 in doubtful health, was spent in Leeds developing such themes, as reader in 1937 (later honorary) and, from 1945, as professor of biomolecular structure. Polypeptides, cellulose, alginates; the vital structures of flagella, nucleic acids, muscle components; excursions on the abnormalities of cancer, influenza, rheumatism or '*pili torti*'; new tools; all was his oyster. About 150 publications in some thirty journals indicate his range and energy. His star shone brightly afar, attracting in all benefactions exceeding £100,000. Rather silent among his peers, he yet gave generous service to governmental and industrial planning committees, to the councils of scientific and professional bodies, to editorial boards, international conferences, industrial consultancy; and to furthering the work of others. Honours coming thick included an honorary D.Sc. Strasbourg, distinguished medals and monetary awards, foreign membership of the National Academies in Sweden and the United States, and (with A. V. Hill) honorary founder-membership of the British Biophysical Society. His quick wit and breezy style made him a popular choice for memorial lectures such as the Procter, Mather, and Sylvanus Thompson.

Astbury's importance lies in the questions he asked and when—stimulus rather than detailed answer. He was an individualist, poet, shy and unsure rather than leader, artistic amateur not scientific professional, Elia his fellow, his strength and weakness the courage and zest the egoisms of a boy, and 'ware the colleague who had to "be of no school and have no master". He brought his findings to market in the green ear, but would not clear the weeds nor suffer the system and technique necessary for the harvest. Inevitably the protein-structural breakthrough was lost, and it hurt. Yet at bottom he had the humility of giants, kindly to failure and to youth, and one warmed to the Croonian and Harvey lecturer who obediently yielded the pavement to a youthful fellow-dreamer chugging along his imaginary track. If he relished the quip 'physicists and stamp-collectors' his view of biological research was latterly more akin to Hinshelwood's than to Rutherford's.

> "I have ventured,
> Like little wanton boys that swim on bladders,
> This many summers in a sea of glory,
> But far beyond my depth."

He would rest content with the simple tribute 'salute to adventurers'. Married in 1922, he leaves a widow, son and daughter. I. MacArthur

When I joined Sir William Bragg's team in the Department of Physics at University College, London, in 1922, W. T. (Bill) Astbury was decidedly the most colourful personality there. He took me under his wing and helped me in every possible way. I think it was partly his natural kindness and partly the fact that his intense happiness in his recent marriage to a lovely young Irish girl made him feel fatherly towards all young people. He was so full of enthusiasm for his work that none of us could feel it to be drudgery, even though it entailed sitting for hours on end with one eye glued to a microscope taking readings of the movements of a gold leaf. He was so delightfully pleased with his own achievements, not only then but throughout his life, that it was impossible not to rejoice with him; and he did achieve miracles with what would have seemed to most people almost intractable problems. Any discussion in which he took part, whether it was about crystal symmetry or about politics, became at once a matter of the greatest interest; any game that he played (and especially our lunch-hour international table tennis tournaments at the Royal Institution) became the more lively because he took part: the normal game was often too slow for him, and he would initiate some twist (five marks extra for hitting the matchbox off the table) to make it more fun. I count myself fortunate to have been one of his friends and colleagues.

Kathleen Lonsdale

Mr. W. V. Lewis

William Vaughan Lewis, lecturer in geography at Cambridge and Fellow of Trinity College, was killed in a motor accident at De Witt, Iowa, on June 8. His work gave him a high standing among geographers in Britain and abroad. With his exceptional eloquence and vitality, he was a born teacher. He was a good friend and had an enduring influence on the lives of many people. His early death comes as a great shock.

Vaughan Lewis was born at Pontypridd in 1907, and was educated at Pontypridd County School. He went up to Gonville and Caius in 1926 and so began a life-long connexion with Cambridge. He read Part I of the Mathematical Tripos, then turned to geography, finding his enduring interest in physiography. After completing the Tripos in 1929 he remained, first as a student, and then as a University demonstrator. He became a lecturer in 1945, when 'freezing' of University appointments ended. In 1949, to his great pleasure, he was elected a Fellow of Trinity College.

Vaughan Lewis's research career began when Prof. (then Mr.) Steers took him to Scolt Head Island. He was intrigued immediately with problems of coastal deposition and morphology, turning his own attention to Dungeness. He was engaged mainly with coastal research, until in 1936 he visited Iceland and became a confirmed glaciologist. While this was the field of his greatest influence, he typically enlarged, rather than changed, his interests, later doing much research on Chesil Beach, the results of which remain to be published. He had also a secondary interest in hydrology.

His glaciological work, carried out mainly in Norway and Switzerland, was concerned particularly with aspects of the structure, flow and erosive influence of cirque and valley glaciers. Apart from his published contributions to glaciology, such as his lively exchanges on the cirque problem with D. W. Johnson, it should be recorded that he was personally responsible for making physicists interested in field-work on glaciology. He played a leading part in the affairs of the British Glaciological Society, which has much enhanced latterly the active development of glaciology in Britain and elsewhere. Vaughan Lewis initiated two intensive research projects in Norway. The results of the earlier investigation of cirque glaciers were published in a research memoir of the Royal Geographical Society, of which he was the editor. His personal interests in the Austerdalsbreen Valley glacier project are recorded in a recent joint paper in the *Glaciological Journal.* These investigations depended on the co-operation of numbers of workers in different fields, and on the active support of generations of Cambridge undergraduates and research students. Vaughan Lewis again and again caught the real interest of undergraduates and, naturally, gathered around him eager groups of volunteers for field-work. The range and vividness of his ideas were such that he had a catalytic effect on the work of others. He was always generous, and would continue to encourage and assist those whom he had inspired to undertake some investigation. He will be sadly missed in many places. Jean M. Grove

Mr. Geoffrey Parr

With the death of Geoffrey Parr on May 30 at the comparatively early age of sixty-one, a colourful and popular figure leaves the scientific world. Parr received his formal education as an electrical engineer at the Finsbury Technical College, and, after working for the Admiralty during the latter part of the First World War, became a lecturer and demonstrator at the City and Guilds Technical College. In 1926 he joined the Research Department of the Edison Swan Electric Co., Ltd., where the cathode-ray tube was undergoing some of its early developments. Then in 1932 he took charge of the same Company's Technical Service Department.

His real *métier* and flair, however, appeared to lie in the communication of knowledge—writing, publicizing, lecturing, editing and publishing.

In the middle 'thirties he published his first book, *The Cathode-Ray Tube and Its Applications*, which has since gone into many editions and become a standard work.

In 1941, Parr became the first editor of *Electronic Engineering* and did much to nurture that paper through its early phases and war-time difficulties. In 1948 he joined Messrs. Chapman and Hall, Ltd., as technical director, and remained in that appointment until his death, being responsible for the very considerable output of scientific books from this well-known house.

Recently, Parr wrote and published, in collaboration with Mr. J. Godfrey, *The Technical Writer*, a work which filled a much-felt void and should long remain an authentic guide to writing standards among scientific authors.

Apart from his professional work, Geoffrey Parr's 'side' activities were legion and these helped to absorb his boundless energy and enthusiasm. One of these was his association with Dr. Grey Walter in the development of the electro-encephalograph, for which Parr helped in the design and construction of the electronics and amplifiers, the whole forming the basis of a joint paper to the Institution of Electrical Engineers, entitled "Amplifying and Recording Techniques in Electro-biology" (*J. Inst. Elec. Eng.*, 90, 3; 1944).

He also lectured frequently to technical colleges and societies throughout the country, usually on some aspect of television. Parr interested himself in many scientific societies and helped in the practical management of several of them. Among these, and in particular, was the Television Society of which Parr had been a Fellow for nearly thirty years, and to which he devoted so much paternal care and devotion. Becoming honorary lecture secretary in 1936, honorary editor of the *Journal* in 1944 and honorary secretary in 1945, he gave unstintingly his free time and attention before, during and since the War years. In fact, he had only retired from the office of honorary secretary a fortnight before his death. He helped the Society survive the War and then grow into its present stature, and, to a large extent, he personally fostered all the important aspects of its development.

Geoffrey had a host of friends in the world of television, in technical publishing and journalism, and wherever men of science gathered; for his gaiety, informality and humanity hundreds will remember him gratefully and affectionately.

T. H. BRIDGEWATER

NEWS and VIEWS

Pure Mathematics at Aberystwyth :
Prof. V. C. Morton

PROF. V. C. MORTON retires in September from the chair of pure mathematics at Aberystwyth after thirty-eight years on the staff of the University College of Wales. Educated at King Edward VII School, Sheffield, and Merton College, Oxford, of which he was a scholar, he served in the Royal Corps of Signals during 1916–19. On his return he became Junior and Senior Mathematical Scholar of the University of Oxford. He lectured for a year at Brighton Technical College and in 1923 was appointed lecturer at Aberystwyth. He became independent lecturer and head of the Pure Mathematics Department in 1926 and professor in 1933. He investigated various geometrical configurations, his best-known work being contained in a series of papers on the cubic surface. Dean of the Faculty of Arts, twice dean of that of Science and vice-principal, he has served the College and the University of Wales in many capacities, most notably as acting-principal of the College in 1957–58. A gifted teacher and a stimulating lecturer, wise in counsel and disinterested in judgment, his integrity and magnanimity are an inspiration to his students and colleagues alike.

Prof. W. B. Pennington

DR. W. B. PENNINGTON, who has been appointed to succeed Prof. Morton, is thirty-eight years of age. He had a distinguished career in Cambridge culminating, after interruptions by war service in the R.N.V.R. (1943–46) and two years at Harvard University (1950–52), in a Research Fellowship at Jesus. Since 1953 he has been reader in mathematics at Westfield College, University of London. Dr. Pennington is well known to British mathematicians, not only for his contributions to the analytical theory of numbers, but also for his devoted activities in the service of the London Mathematical Society as member of Council, vice-president and *Journal* editor. With his forward-looking attitude to mathematics and the catholic range of his interests, extending to problems of mathematical teaching and administration no less than of research, Dr. Pennington has not stood aloof from the heart-searchings prevalent among mathematicians to-day, and his new appointment will enhance his opportunities for an effective part in the great debate. With his departure to Wales, Westfield College loses an able mathematician, a delightful colleague, an opening batsman and an accomplished baritone.

Physical Chemistry in Trinity College, Dublin:
Prof. D. C. Pepper

DR. DAVID CHARLES PEPPER has been appointed to the newly established chair of physical chemistry in Trinity College, Dublin. Prof. Pepper, born in 1917, was educated at Ushaw College, Durham, Bradford Technical College, from which he took the London degree of B.Sc., and Jesus College, Cambridge. Later, he was awarded the Cambridge degree of Ph.D. for work in the Department of Colloid Science in Cambridge. He was appointed assistant lecturer in Trinity College, Dublin, in 1945, and Fellow in 1951. He has been reader in physical chemistry since 1955, and has been a member of the Royal Irish Academy since 1953. Prof. Pepper's research career commenced with Sir Eric Rideal, with whom he worked on a variety of war-time topics, sponsored by the Scientific Advisory Council of the Ministry of Supply. These researches were concerned

—among others—with interactions between phenols and amine monolayers, catalytic oxidation of ethylene, the plastic and vinoelastic behaviour of solid–liquid dispersions and polymer gels, and the kinetics and mechanism of polymerization reactions. The last-named topic has formed the basis of Prof. Pepper's interests in Trinity College, Dublin, most of his work being in the field of polymer solutions or in studies of the kinetics and mechanism of cationic polymerization. His research school has revealed some of the special characteristics of these reactions, notably the effect of solvents both through their dielectric behaviour and as co-catalysts. His work has focused attention on the considerable kinetic differences between these ionic polymerizations and free-radical polymerizations. It is true to say that contemporary theory in this field is largely based on ideas developed in Prof. Pepper's research group.

U.S. National Bureau of Standards: New Appointments:

Mr. Martin T. Decker

Mr. Martin T. Decker has been appointed chief of the Tropospheric Measurements Section of the National Bureau of Standards Laboratories at Boulder (Colorado). Mr. Decker joined the Bureau staff in 1949, his early work being in the area of tropospheric forward scatter of radio waves. More recently his research has been primarily in air-to-ground communications, including air navigation and airborne television studies, especially the prediction of reliability. He has also made studies of the obstructing effects of various materials on radio waves at VHF, UHF and SHF. Born in Byron, Nebraska, in 1927, Mr. Decker received his B.S. degree in electrical engineering from the University of Nebraska in 1950. He has continued his engineering studies at the University of Colorado.

Mr. John L. Hague

Mr. John L. Hague, a national authority in the field of analytical chemistry, has been appointed chief of the Standard Reference Materials Section. Mr. Hague had been acting chief of the predecessor section, Analytical Chemistry, since March 1960. The Standard Reference Materials Section will play an important part in the Bureau's standard materials programme. In this programme, the Bureau makes available for distribution to science and industry more than 550 different standards of metals, ores, ceramics, chemicals, hydrocarbons, and more recently added radioactive materials, uranium isotopes, lithium ores, aluminium refractories, and phosphor materials. Standard materials are used to check, evaluate and standardize methods of analysis and analytical techniques and substances, and to develop new and improved methods and instruments. Mr. Hague joined the Bureau staff in 1930 as a laboratory apprentice. In 1932 he transferred to the Norfolk Shipyard and three years later was in charge of the Foundry Control Laboratory for ferrous and non-ferrous alloys. He returned to the National Bureau of Standards in 1935 and in 1951 became assistant chief of the Analytical Chemistry Section. Born in Kansas City, Missouri, in 1912, Mr. Hague received his B.S. degree in chemistry in 1938 from George Washington University and has done graduate work there. In 1958, Mr. Hague received the Department of Commerce Meritorious Service Award "for outstanding original contributions to methods of analysis necessary for controlling the production of complex metal alloys".

Mr. John K. Taylor

Dr. John K. Taylor, research chemist, whose entire 32-year professional career has been with the National Bureau of Standards, has been appointed chief of the Applied Analytical Research Section of the Bureau's Analytical and Inorganic Chemistry Division. The Applied Analytical Research Section is concerned with the development and application of methods for the analysis and characterization of solids, liquids and gases. Both classical and instrumental methods of analysis are employed, using both macro- and micro-techniques. The Section also provides analytical consultation services and assistance for other Bureau programmes. A major part of Dr. Taylor's work at the National Bureau of Standards has been concerned with the application of physical methods to chemical analysis, including the polarographic method and the limitations of voltammetric processes. Recently he has conducted research in high-precision coulometric methods of analysis. Dr. Taylor has written numerous papers on these subjects, and in 1960 was awarded the Department of Commerce Silver Medal in "recognition of his contributions to accurate electrochemical methods of analysis". Other areas of research interest for Dr. Taylor have included refractometry methods for separation of isotopes, and a programme for providing a series of uranium isotope standards. This last has consisted of high-precision analysis of uranium compounds and the blending of highly purified isotopic species for mass spectrographic analysis. Born in Mt. Ranier, Maryland, in 1912, Dr. Taylor received his B.S. in chemistry from George Washington University in 1934. He was awarded his M.S. and Ph.D. from the University of Maryland in 1938 and 1941, respectively. Throughout his professional life, Dr. Taylor has been an active supporter of science education. He is a member and past chairman of the Joint Board on Science Education of the Washington Academy of Sciences and is editor of *The Reporter*, a news-letter for science and mathematics teachers.

Public Investment and Export

Under the title "Public Investment and Export" an article by Sir Walter Benton Jones, Bart., which originally appeared in the *Times Review of Industry*, March 1961, has been, with the addition of some figures for public spending 1950–59, issued as a pamphlet (Pp. 7. Grantham: Sir Walter Benton Jones, Irnham Hall, 1961). Sir Walter asks the leading question: Has Britain reached a level of public spending which may jeopardize her power to compete in the expanding export markets of the world, which may soon become more competitive than at any time in history? Pointing out that public spending has risen from £3,692 million in 1950 to £5,099 million in 1955 and £6,237 million in 1959, whereas exports increased by 33 per cent in the first period and only 16 per cent in the second, compared with increases of 49 per cent and 20 per cent, respectively, in world exports, 52 per cent and 13 per cent, respectively, for the United States, and 21 per cent and 33 per cent for West Germany, he suggests that the high level of our public spending may be handicapping our exports. Sir Walter suggests that since Parliament ultimately fixes and controls public expenditure, Parliament should be concerned to regulate the level of public expenditure by public income after due regard to possible effects of taxation on production and export, and that what is primarily needed to stimulate export is a considerable easing of the burden of taxation. He does not suggest any particular reductions in expenditure and admits that much expenditure is desirable or essential, but the argument is one with implications which the scientist might be wise to consider.

Nutrition Society of Canada

At the fourth annual meeting of the Nutrition Society of Canada, held at the Ontario Agricultural College, Guelph, on May 30, the following officers were elected: *President*, Dr. E. H. Bensley, Montreal; *Vice-President*, Dr. R. H. Common, Macdonald College; *Treasurer*, Dr. J. A. Campbell, Ottawa; *Secretary*, Prof. E. V. Evans, Guelph; *Councillors*, Dr. J. M. Bell (1964), Saskatoon, Dr. L. P. Dugal (1963), Ottawa, and Dr. W. W. Hawkins (1962), Halifax. A feature of the annual dinner of the Society was the announcement of the winner of the second Borden Award of the Nutrition Society of Canada for research in nutrition. This year's winner, Dr. Donald Fraser, of the Research Institute, Hospital for Sick Children, Toronto, Ontario, was cited for his work on vitamin D-refractory rickets in children and on varied aspects of calcium and phosphorus metabolism. The special speaker at the dinner was Dr. Ross A. Chapman, assistant director, Scientific Services, Food and Drug Directorate, Ottawa, whose topic was "Research in the Food and Drug Directorate". The one-day Society programme included a session of short research papers and a symposium on "Current Problems in Nutrition".

New Thinking in School Mathematics

A revision of the school curriculum in mathematics is needed for two main reasons. First, the gap between school mathematics and new mathematics is increasing, though it is more than ever important that the average intelligent citizen should understand that mathematics is the true foundation of our technological society. Secondly, the shortage of mathematics teachers might be partially remedied by ensuring that no time is wasted by teaching valueless or outmoded material. The Organization for European Economic Co-operation arranged a survey and a seminar at Royaumont attended by about fifty representatives from Europe and North America. A report, edited by Howard Fehr, is now available (Pp. 246. Paris: Organization for European Economic Co-operation, 1961. 10 N.F.; 15*s*.; 2.50 dollars; 8.50 D.M.). Part 1 summarizes the proceedings of the seminar. There was a general demand for a reduction in old-fashioned geometry, the inclusion of modern ideas in algebra, possibly with an abstract basis, and the introduction of probability and statistics. The implementation of such a programme would require new text-books and equipment, revised courses for teacher-training, and the re-education of serving teachers. Part 2 gives the results of the survey of present practices and trends, with much valuable information concisely presented. The volume should be studied carefully by all teachers of mathematics; even if they quail at some of the ultra-radical suggestions, they will appreciate the insistence on pattern and structure as the essence of mathematics.

Coryndon Memorial Museum, Nairobi

The annual report of the Coryndon Memorial Museum for 1959 is, as usual, a record of much progressive work. This included the establishment of a Snake Park and Snake Study Centre surrounded by a Botanical Garden, to which there was immediate popular approval (Pp. 24+1 plate. Nairobi: Museums Trustees of Kenya and Coryndon Memorial Museum, 1961. 1*s*.). All Departments of the Museum are in urgent need of cabinets for storage, especially in view of the almost indescribable dust problem in dry, windy weather. A number of new exhibits was completed. These include a habitat group depicting the swordfish, two giant fossil human milk teeth from the Olduvai Gorge and a leopard habitat group.

Western Australian Museum

The annual report of the Western Australian Museum for the year ended June 30, 1960, records the separation as from May 27, 1960, of the Museum and the Art Gallery. The Trustees of the combined institution have been replaced by two Boards of five members each. Consequent on this separation the Art Gallery moved out of the present James Street wing. Appropriately, the report includes an illustrated short historical sketch of the Museum, together with a comprehensive statement regarding future policy in relation to the various Departments. An account is also given of the major expeditions undertaken during the year, and these include one to investigate the fauna and flora of the Bernier and Dorre Islands in Shark Bay, another to Northwest Cape, and assistance given to the Bernice P. Bishop Museum of Hawaii when it had a mollusc-hunting expedition in Western Australia. The practice of including a short summary of papers published by members of the staff is useful and worthy of commendation.

Treatment of Varicose Veins

Varicose veins of the lower extremities are a frequent and highly disabling condition. Their surgical management has undergone a great number of variations since varicosities were treated by puncturing and drainage in the Hippocratic era. The exact ætiology of varicose veins still remains obscure; the disease may tend to be progressive, and morbidity causes economic strain on the patient and the community. Yutaka Kato and Robert A. Nabatoff, of the Department of Surgery, the Mount Sinai Hospital, New York City, N.Y., have carried out complete stripping of varicose veins of the lower extremities under local anæsthesia (*Yokohama Med. Bull.*, 11, No. 6; December 1960). This permits, almost immediately, post-operative ambulation and minimizes thrombo-embolic phenomena. The number of incisions depends on the number of incompetent perforator veins. All incompetent perforators are accurately mapped out pre-operatively, and all are ligated flush with the deep veins prior to the stripping procedure. Since all the veins communicating with the diseased great saphenous system and all 'blow-outs' in other locations are interrupted, and since the remaining varicose vein segments are stripped out, very few, if any, injections of sclerosing solution are necessary following operation.

The American Philosophical Society

Year Book 1960 of the American Philosophical Society contains, besides the lists of present and former officers of the Society and of former and present members, the charter and laws of the Society, and a brief history of the Society by E. G. Conklin (Pp. 786. Philadelphia: American Philosophical Society, 1961). The largest of the reports of committees is that of the Committee on Research, which includes lists of the 170 grants totalling 165,609 dollars made in 1960 from the Penrose Fund and of the 53 grants, totalling 53,473 dollars, made from the Johnson Fund. Four fellowships, totalling 27,400 dollars, were awarded from the Darland Fund. Reports from recipients of grants occupy almost 400 pages of the 416 of the Committee's report. Among the biographical memoirs included in the volume may be mentioned those on R. C. Andrews, W. H. W. Baade, N. L. Bowen, O. E. Buckley, A. L. Day, Sir Walter Wilson Grey, M. von Laue, L. Loeb, S. A. Mitchell and E. C. Tolman.

Cigarette Smoke Condensate

THE Tobacco Manufacturers' Standing Committee has published a second edition of its Research Paper No. 4, *Cigarette Smoke Condensate: Preparation and Routine Laboratory Estimation* (by H. R. Bentley and J. G. Burgan. London, 1961). This largely follows the first edition published in 1959, which it supersedes; but in the new edition the section referring to routine laboratory estimation of the yield of cigarette smoke condensate has been revised and extended. The standard method given in the first edition has been modified in the light of experience in order to improve the reproducibility of results. In particular, collection of smoke particulate matter by means of a 'Cambridge' filter followed by drying to constant weight is recommended for general use on grounds of simplicity, in preference to electrostatic precipitation, which has been found to have some disadvantages for quantitative work. Consideration is also given to the definition of realistic smoking conditions to be used with automatic smoking machines, and parameters, based on observations of smokers, of one puff of 2-sec. duration and 25-ml. vol./min., are recommended. These values are useful to have, for it would clearly be an advantage if some such agreed parameters were to be adopted in all laboratories in the United Kingdom in which cigarette smoke is being prepared and investigated.

Colour in Factories

COLOUR in industry is not merely a form of decoration or even a welfare measure. It has certain functions to perform and the factors important for success have been established. The latest report in the Factory Building Studies series, based on several years work on the use of colour in various types of building, is a concise guide for factory managements and their architects or colour consultants (Factory Buildings Studies No. 8. *Colouring in Factories*. Published for D.S.I.R. by H.M.S.O. Price 7*s*. 6*d*., postage 4*d*.). It has two sections, both containing coloured illustrations. The first section describes the basic properties and effects of colour, linking them with certain facts about human eyesight, to form general principles of colour design. The second section explains the practical application of these principles. It indicates stage by stage the way in which colour for each element of the interior becomes disciplined so that a picture is formed of the characteristics essential to good factory colouring. The examples are mainly for daylight, but there are notes on the special problems arising where lighting is wholly or partly artificial. As lighting and colouring are interrelated, this work is complementary to the earlier work (No. 2 in the series) on *The Lighting of Factories*.

The Content of Jobs

To the steadily growing number of broadsheets which are being produced to assist all who are concerned with the personnel function of management, the Institute of Personnel Management has now issued one called *Job Analysis*. Prepared by H. E. Roff and T. E. Watson, of Management Selection, Ltd., the broadsheet defines job analysis as "the scientific study of statements of all the facts about a job which reveal its content and all the modifying factors which surround it". Accepting that much of what passes for job analysis to-day is scarcely scientific, the authors show how a systematic approach to the analysis of a job can help all those who have to deal with human problems arising from the establishment of wage or salary scales, and the intricacies of selection, training and safety. Great difficulties confront industrial management in classifying one job against another and the method described in *Job Analysis* provides a useful *modus operandi*. For detailed information the reader must seek elsewhere, and the broadsheet contains a valuable bibliography which will help him to do this. *Job Analysis* is obtainable from the Institute of Personnel Management, Management House, 80 Fetter Lane, London, E.C.4, price 7*s*. 6*d*.

European Organic Geochemistry Group

AT a recent meeting of members of various European geochemistry groups it was proposed that a European section of the Organic Geochemistry Group of the Geochemical Society should be formed. At present the Organic Geochemistry Group has 250 members, 45 of whom are resident in Europe. The Group held very successful meetings in Denver last autumn, and this year meetings are to be held in Cincinnati. As such, countries interested in such an organization include Italy, France, the United Kingdom, Germany and Holland. The possibility of a meeting in Milan during September 1962 is being considered. Persons interested in such an organization should write to Dr. G. D. Hobson, Department of Geology, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7.

Oliver Memorial Fund Award for Blood Transfusion Work

THE Committee of the Oliver Memorial Fund intends making its fourteenth annual award of the value of £50 to a British subject whose original work, or services in connexion with blood transfusion, is considered to be a notable contribution to the research organization or donor aspect of this subject. The Committee will welcome applications and, in addition, communications directing its attention to suitable candidates. Applications should be submitted before September 30, and should be sent to the honorary secretary, Mrs. F. E. Bedborough, 105 New Park Avenue, London, N.13.

Australian Post-Doctoral Fellowships in Protein Chemistry

THE Division of Protein Chemistry of the Commonwealth Scientific and Industrial Research Organization, Australia, is offering a number of post-doctoral fellowships. The Division has been engaged on a comprehensive research programme on protein structure and chemistry for the past twelve years covering chemical, physicochemical, biophysical and histological studies. The Division is well equipped for work in these fields and the applicant will be able to work with either fibrous or globular proteins. The fellowships have a three-year tenure and provide fares for the Fellows and their families to and from Australia. Salaries of up to £A2,720, depending on experience, are offered. Further information can be obtained from C. Garrow, Division of Protein Chemistry, C.S.I.R.O., Wool Research Laboratories, 343 Royal Parade, Parkville, N.2, Victoria.

Ramsay Memorial Fellowships

The Ramsay Memorial Fellowships Trustees have made the following awards of new fellowships in chemistry for the year 1961–62: a general fellowship to Mr. M. J. How at the University of Birmingham ; a Japanese fellowship to Dr. H. Suginome at the University of Cambridge; a New Zealand fellowship to Mr. B. A. Grigor at the University of Leicester; a Spanish fellowship to Mr. Fernan Alonso Matthias at the University of Cambridge; United States fellowships to Dr. K. D. Carlson at the University of Oxford and Dr. D. W. Setser at the University of Cambridge. The Trustees have renewed the following fellowships for the same year to: Mr. A. Bewick (general fellowship) at the University of Durham; Dr. D. G. Watson (Glasgow fellowship) at the University of Cambridge; Mr. H. Krabbendam (Netherlands fellowship) at the University of Edinburgh; Mr. G. A. Rodley (New Zealand fellowship) at University College, London; and Dr. Maria Covadonga Rodriguez (Spanish fellowship) at the University of Oxford.

Statistical Quality Control and Acceptance Sampling

The College of Advanced Technology, Birmingham, is organizing a full-time course of three weeks duration on "Statistical Quality Control and Acceptance Sampling", commencing September 11. The course is primarily intended as an introduction to the subject. Nevertheless, it provides the basic training necessary for quality control engineers. The first fortnight will be spent in covering the general principles and procedures of quality control. The final week will be devoted to lectures by specialists from industry, and to visiting the works of firms using quality control, where members of the course will be able to see the practical application of quality control methods, and discuss the techniques and associated problems. Further information can be obtained from the Bursar, College of Advanced Technology, Gosta Green, Birmingham 4.

Food Science Conference

The new Food Research Laboratories for the Commonwealth Scientific and Industrial Research Organization, Australia, are being opened at North Ryde, near Sydney, on September 18, and, to mark the occasion, a conference on "Food Science" will be held there during September 19–22. The conference will take the form of three symposia: "Chemical Aspects of Food Processing"; "Factors affecting Meat Quality"; "Food Preservation and the Organization of Plant and Animal Tissues". Further information can be obtained from the Chief, Division of Food Preservation, C.S.I.R.O., Private Bag, Homebush Post Office, N.S.W., Australia.

University News : Birmingham

The following appointments have been announced: Dr. L. Riddiford, senior staff Fellow in nuclear physics, to be senior lecturer in the Department of Physics; G. Martelli, staff Fellow in nuclear physics, to be lecturer in the Department of Physics; Dr. S. Swierszczewski, to be research Fellow in nuclear physics in the Department of Physics; Dr. J. H. Broadhurst, to be senior research associate, and D. E. Kidd, to be research associate, in nuclear physics in the Department of Physics; Dr. C. A. Barson and Dr. A. E. Pedler, to be senior research Fellows in the Department of Chemistry; R. J. Heitzmann, to be research Fellow in the Department of Chemistry; R. G. Bird, to be lecturer in the Department of Civil Engineering; B. K. Gazey, to be research Fellow in the Department of Electrical Engineering; Dr. B. Henderson, to be research Fellow in the Department of Physical Metallurgy; G. R. Ogram and B. Walker, to be research Fellows in the Department of Industrial Metallurgy; Dr. A. T. Kolnai, to be Lloyd-Muirhead Fellow in social philosophy in the Department of Philosophy, for one year; J. R. Turcan, staff tutor in the Institute of Engineering Production, to be research Fellow in the Department of Engineering Production; Dr. B. J. Key, to be lecturer in neuropharmacology in the Department of Experimental Psychiatry; Dr. R. R. Dils, to be lecturer in the Department of Medical Biochemistry and Pharmacology; D. Rimmer, to be lecturer in the Department of Economics.

Edinburgh

J. H. Turner has been appointed as lecturer in pathology. The following have been appointed Imperial Chemical Industries Research Fellows for a period of three years, with effect from October 1: Dr. I. Bihler (Hebrew University); Dr. J. Newham (Hull); C. McGreavy (Yale); Herman Wen-Geun Chew (Chicago); Dr. A. S. Milton (Oxford).

London

Dr. M. Gordon, senior lecturer in physical chemistry at the Imperial College of Science and Technology, has been appointed to the University readership in physical chemistry tenable at that College. The title of reader in demography in the University of London has been conferred on Mr. J. Hajnal, in respect of his post at the London School of Economics and Political Science.

Manchester

On an initiative jointly taken by Unilever Ltd. and the Department of Economics of the University, a Centre for Business Research is to be set up in Manchester. The Centre, which will be financed by money from industry, will, with the assistance of the co-operating firms, study some of the difficult problems of business decisions, and the ways in which economic, statistical and econometric knowledge can be used to help the decision-making of British business. Support has been guaranteed for an initial period of seven years, and it is hoped to appoint the first members of the full-time staff within a few months. The project will be directed by Prof. C. F. Carter, J. Johnston and B. R. Williams.

Sheffield

Dr. N. H. March, at present reader in theoretical physics, has been appointed to the newly created second chair of physics as from October 1.

The following new appointments have also been made: Dr. V. C. L. Hutson, to be lecturer in applied mathematics; J. W. Ross, to be junior research

Fellow in physics; Dr. I. Hawkes, to be lecturer in the Postgraduate School of Mining; Dr. C. J. Ball, to be lecturer in metallurgy; I. Livingstone, to be lecturer in economic statistics; P. E. Brown, to be senior lecturer in preventive medicine and public health; W. T. Baker, to be health physicist.

The Night Sky in August

NEW moon occurs on Aug. 11d. 10h. 36m. U.T. and full moon on Aug. 26d. 03h. 14m. The following conjunctions with the Moon take place: Aug. 5d. 17h., Aldebaran 0·3° S.; Aug. 7d. 18h., Venus 3° N.; Aug. 14d. 18h., Mars 2° S.; Aug. 23d. 15h., Saturn 3° S.; Aug. 23d. 23h., Jupiter 3° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Pollux on Aug. 24d. 11h., Venus being 7° S. There will be an annular eclipse of the Sun on Aug. 11, invisible at Greenwich. There will be a partial eclipse of the Moon on Aug. 26, visible at Greenwich. The Moon enters penumbra at 0h. 36·1m., enters umbra at 1h. 34·9m., leaves umbra at 4h. 41·5m., and penumbra at 5h. 40·4m., the magnitude of the eclipse being 0·992. Moonset at Greenwich on Aug. 26 is at 5h. 11m. Mercury is too close to the Sun for observation. Venus is a morning star, rising at 1h. 05m., 1h. 15m., and 1h. 50m. on Aug. 1, 15 and 31, respectively. Its stellar magnitude on Aug. 15 is − 3·5; the visible portion of the apparent disk increases from 0·683 to 0·792 during the month and its distance from the Earth increases from 96 to 115 million miles. Mars is too close to the Sun for observation. Jupiter sets at 3h. 50m., 2h. 40 m. and 1h. 30m. at the beginning, middle and end of the month, respectively. It is near the border of Sagittarius and Capricornus; its stellar magnitude on Aug. 15 is − 2·3 and its distance from the Earth 385 million miles. Saturn sets at 3h. 20m., 2h. 15m. and 1h. 10m. on Aug. 1, 15 and 31, respectively. It is in Sagittarius; its stellar magnitude on Aug. 15 is + 0·4 and its distance from the Earth is 845 million miles. There are no occultations of stars brighter than magnitude 6 visible at Greenwich. The Perseid meteors are active during the first half of the month. Maximum activity occurs on Aug. 12, the radiant is at R.A. + 3h. 04m., Dec. + 58°, and conditions for observation are very favourable.

Announcements

DR. ARNO SIGVARD EKLUND, of Sweden, has been appointed director-general of the International Atomic Energy Agency, in succession to Mr. Sterling Cole, whose four-year term of office ends on November 30, 1961. Dr. Eklund was secretary-general of the second United Nations Geneva Conference on the Peaceful Uses of Atomic Energy in 1958, and is chairman of the international board of management of the Organization for European Economic Co-operation High-Temperature Reactor Project *Dragon* at Winfrith Heath, Dorset.

DR. MORTEN SIMONSEN, lecturer in immunology, University of Copenhagen, has been appointed director of research at the McIndoe Memorial Research Unit, Queen Victoria Hospital, East Grinstead, Sussex, which is devoted to the study of tissue transplantation and associated problems of wound healing.

MR. J. P. CATCHPOLE, of the Department of Chemical Engineering, University of Birmingham, has been awarded the student's prize of the Institution of Chemical Engineers, for his paper entitled "Frequency Response Techniques for the Study of Packed Bed Characteristics". This is the first award of this prize, which is open to student members of the Institution of at least six months standing at the time of presentation or publication of the paper.

ARTHUR GUINNESS SON AND CO., LTD., have established a scheme of awards of cash prizes totalling up to £500, to be called the Guinness Awards for Science Teachers in Training. The scheme is administered by *The Science Teacher*, the editorial director of which, Mr. Maurice Goldsmith, is chairman of the Guinness Awards Advisory Committee. The object of the scheme is to encourage students in teacher-training to develop and report on their own original contributions to the pedagogy of science in all its branches. The scheme will begin to operate as from September 1961. Further information can be obtained from the Information Office, Arthur Guinness Son and Co. (Park Royal), Ltd., Berk House, 8, Baker Street, London, W.1.

AN international congress of aviation and cosmonautical medicine is being held in Paris during September 26–30. Further information can be obtained from the secretariat, C.E.R.M.A., *5bis* avenue de la Porte de Sèvres, Paris, France.

THE Faraday Society has arranged a general discussion on "The Structure and Properties of Ionic Melts" which will be held in the Department of Inorganic and Physical Chemistry, University of Liverpool, during September 5–7. Further information is available from the Secretary, Faraday Society, 6 Gray's Inn Square, London, W.C.1.

THE sixth annual conference of the British Society for the Philosophy of Science is being held at St. Hugh's College, Oxford, during September 22–24. The following are included in the programme: explanation in psychology; learning machines; genetic coding and information theory; statements about the universe. Further information can be obtained from Dr. M. B. Hesse, Whipple Museum, Free School Lane, Cambridge.

THE First International Conference on Timber Engineering will be held in the University of Southampton during September 18–23. General problems of timber construction, structural timber joints, structures of particular interest, testing of timber structures, timber shell structures, constructional problems and economics and comparisons are the main subjects which will be discussed. Further information can be obtained from Dr. H. G. Allen, Department of Civil Engineering, University, Southampton.

A SERIES of postgraduate full-time courses is to be held in the Nuclear Studies Section of the Department of Chemistry and Biology, Liverpool College of Technology, during the 1961–62 session. These include a seven-months course in radiation safety and health physics, a number of two-week courses in radiation safety, radiobiology and radiochemistry, and a one-week course in radiation chemistry. Further information can be obtained from the Principal Lecturer and Organizer in charge of Nuclear Studies, Chemistry and Biology Department, College of Technology, Byrom Street, Liverpool 3.

HAZARDS OF THE ANIMAL HOUSE

THE Laboratory Animals Centre continued its series of annual symposia with a discussion on "Hazards of the Animal House" at the Royal College of Surgeons of England, London, on May 30. As a first discussion of this problem, the symposium was concerned with the general assessment of the situation, indicating the risks involved in handling animals suffering from natural and experimental infections and outlining the methods by which these risks might be reduced. Attention was also directed to hazards from sources other than pathogenic organisms, including risks involved in experiments with radioactive material, allergic reactions to experimental animals and traumatic risks due to careless handling of animals.

Since, perhaps, the greatest hazard of the animal house is that the worker should be unaware of the risks to which he is exposed, it is natural that many of the papers dealt with various agents which could be transmitted from animals to man. Early in the symposium, Mr. Bywater referred to the fact that, although the first case of infection of man with *B* virus from a monkey took place in the 1930's, it seemed that the risk had not been appreciated until comparatively recently. Rules for the conduct of animal houses, aimed at the protection of not only the scientific worker but also the animal house staffs themselves, have been drawn up in the past few years. Such rules are of obvious value in dealing also with dysentery and other intestinal conditions, which are transmissible from monkey to man. Dr. Sommerville listed the natural infections of laboratory rats and mice which are transmissible to man. Lymphocytic choriomeningitis has not been encountered frequently in man in Great Britain, although it appears to be fairly common in the United States, sometimes producing an influenza-like syndrome, at other times a biphasic illness with an aseptic meningitis. The encephalo-myocarditis virus of mice is also of reasonably frequent occurrence, but other viruses are rare. On the bacterial side, *Pasteurella septica*, although found both in rodents and in man, has not often been clearly demonstrated as having passed from one to the other. However, since it has caused fatal illness in man, it should be taken seriously. Leptospirosis is also of importance.

A second group of infections is caused by viruses and bacteria transmitted from mice or rats inoculated in the laboratory with diagnostic material or with stock viral or bacterial cultures. In this second group, infection is much more frequent than by viruses and bacteria naturally carried by the animals, but the laboratory worker is usually on the lookout for the agent concerned. The worker may not always be ready, however, to realize the ease with which infection may be transmitted by inhalation or ingestion of dust from cages contaminated with excreta or nasopharyngeal material from the rodents.

Mr. McNeil dealt with the more specialized topic of the hazards associated with farm animals. He emphasized the importance of selection of healthy animals for experimental work both by clinical examination and by application of tests such as tuberculin tests and agglutination tests for *Brucellosis*. A quarantine period on arrival is valuable, since it also allows parasitic conditions such as ringworm, lice or mange to be dealt with. Most of the conditions which affect farm animals are likely to be familiar to the staff engaged in handling these species. Careless post-mortem technique, though perhaps not strictly part of the animal house routine, was referred to as a source of infection in such conditions as anthrax and swine erysipelas. Mr. Hodgman dealt with the hazards encountered in dealing with dogs and cats. He considered that the risks from disease are not of great importance in Great Britain, the main hazard being the traumatic one from bites and scratches. Conditions affecting dogs and cats and transmissible to man are more likely to be apparent in owners of pets than in laboratory workers.

Mr. Garratt spoke of the hazards associated with the use of radioactive substances in animal experiments. His main conclusion was that health hazards to personnel from external irradiations are usually negligible and those resulting from internal irradiation after ingestion or inhalation of radioisotopes could be prevented by careful handling. Careful disposal of waste was of great importance. In general, the attitude of the worker with radioactive substances in animal experiments might be summarized by the maxim: "Take care of your experiment and your health will take care of itself". Other specialized problems were dealt with by Dr. Cooper on the handling of large-scale experiments with polio virus, mainly from the laboratory point of view, and by Dr. Rees on precautions against tuberculous infection in the animal house and laboratory. Both these speakers were concerned mainly with the techniques which would reduce risks to the workers with the agents concerned and both referred to the advisability of vaccination of staff. Surgeon Commander Darlow dealt with the general topic of the provision of clean air as a factor in preventing the spread of infection in the animal house. He showed how considerable reduction in the number of airborne organisms could be effected by suitable air filtration. The use of ultra-violet light was recommended as the simplest method for killing airborne organisms. Mr. Porter dealt with the traumatic hazards not only from the animals themselves but also from damaged or incorrectly designed equipment. From the economic point of view, the minor accidents of the animal house (graphically illustrated by Mr. Porter) interfere considerably with efficient working. Cracked water bottles, damaged cages and bins all provide sharp edges on which fingers and hands can readily be cut or scraped, with consequent loss in working time.

Summarizing the discussion, the chairman, Dr. J. B. Brooksby, said that the first point that had been made was the necessity for awareness among research workers of the risks involved in handling any species. A remark had been made from the floor that any worker about to begin experiments with monkeys would do well to consult some of the laboratories concerned in handling the species in large numbers. Similarly, any worker dealing with any new or exotic species should try to obtain all possible information before beginning his experimental work. This symposium might, however, serve as an initial stage in the documentation of the various agents which should be considered as hazardous in the animal house. An important point seemed to be that the various risks were kept in their true perspective. Thus, *B* virus infection from the bite of an apparently healthy monkey was obviously far more serious than the unpleasant, but relatively trivial, infection by ringworm from a domestic animal. Just as the severity of infection varied from agent to agent, so the chances of infection from animal to man would vary, and the proper assessment of the dangers involved in any particular type of work would take into account these factors.

The action to be taken to avoid the hazards of the animal house would obviously relate to the degree of risk. Commander Darlow's paper indicated the way to the ultimate degree of protection possible, by designing a special building with air-filtration and all other apparatus necessary for restriction of airborne micro-organisms. Various practical steps could, however, be taken to reduce risks in accommodation already available. Several speakers emphasized the need for taking only healthy animals into the animal house. The value of tuberculin testing in monkeys was discussed, and there was some divergence of opinion as to its value. Restriction of risk in operative procedures could often be achieved by the use of hoods, anti-splash guards and other equipment of this sort. The point had also been made that spread from an animal often occurred after the object of the experiment had been achieved and there was no case, for example, for keeping tuberculous guinea pigs after the primary lesion had developed; they could be destroyed before caseation supervened. Another problem which was of great importance was staff discipline, particularly where large numbers of animals were being handled and much of the work was necessarily undertaken by staff recruited untrained. Training took place after appointment, when new staff members were undertaking potentially dangerous work. Attention to proper sterilization techniques and to the disposal of bedding and dirty equipment was most important. The use of proper equipment in a good state of repair was also a prime necessity for the avoidance of undue risks.

It will be realized that many of the steps that must be taken to control hazards are also those which would be taken in any animal house dealing with infective agents, in order to prevent cross-infection among the animals themselves, and the instruction of workers in the health hazard may help to achieve the discipline which will, in turn, prevent both the health hazard and the hazard to the experiment itself.

USE OF ANIMAL CELL, TISSUE AND ORGAN CULTURES IN RADIOBIOLOGY

FOR nearly fifty years the tissue culture method has proved a valuable experimental tool for radiation studies. By isolating a group of cells outside the body the effect on blood- and nerve-supply and the reaction of the organism as a whole are eliminated and the direct effects of radiation can be accurately assessed. The establishment of clone cultures and the introduction of chemically defined media in the past decade have brought a further simplification and standardization of experimental conditions which make it possible to obtain precise quantitative data. At a symposium convened by the New York Academy of Sciences in February 1961, the effects of ionizing radiations on cell structure and differentiation, on mitosis and growth of cells in tissues grown *in vitro* were reported and correlated with changes in synthesis of deoxyribonucleic acid (DNA); the influence of oxygen tension, sensitizing and protective agents on radiation action was outlined, and variations in radiosensitivity discussed.

L. J. Tolmach (St. Louis) and J. E. Fogh (New York), using HeLa *S*3 and *FL* amnion cells, reconfirmed the well-known fact that radiation affects cell division much more than cell growth. Cells unable to divide continue to increase in size, reaching a maximum 2–4 days following exposure.

Y. Onuki, A. Awa and C. M. Pomerat (Pasadena) investigated the effect of radiation on the chromosomes of leucocytes cultured from the human buffy coat. A dose of 400 r. increased the number of tetraploids by 7 per cent and the number of cells showing chromosomal damage by 50 per cent. F. E. Osgood (Portland) interpreted the effect of radiation on normal and malignant human hæmic cells *in vivo* and *in vitro* in terms of his α-cell–n-cell concept. The potentially dividing α-cells give rise, as a rule, to another α-cell and a differentiating n-cell. Doses of 10–400 r. immediately inhibit the division of α-cells while the number of fully differentiated and longer-lived n-cells remains, at first, unchanged. Their number begins to drop when the reduced α-cells fail to replace n-cells which have reached the end of their natural life-span. In this range of dosages, radiation did not seem to inhibit synthesis of DNA of α-cells as they continue to incorporate tritiated thymidine after their increase in number is stopped. Comparisons of hæmic cells as regards their radiosensitivity show that normal and malignant lymphocytes are the most sensitive cells though even in these cells there is a marked individual variation in sensitivity.

The great sensitivity of lymphocytes was also emphasized by Schrek and Trowell. R. Schrek (Hines, Ill.) examined the immediate and delayed effects of radiation on human and animal lymphocytes. A dose of 1,000 r. produced severe intranuclear vacuolization and death within 24 hr. following exposure, while 10 r. reduced the 10 per cent survival-time from 9·7 to 6·1 days. There was no difference in radiosensitivity of human lymphocytes from blood or lymph nodes, but lymphocytes from rats, guinea pigs and rabbits were slightly less sensitive than human lymphocytes. O. A. Trowell (Harwell) described the influence of various gas mixtures on the action of radiation on lymphocytes from rat mesenteric lymph nodes and stressed the importance of choosing the right effect and end-point for measurements. For example, by counting dead nuclei 5 hr. after exposure an oxygen sensitivity factor of 12 was found, while counts of surviving nuclei 24 hr. after irradiation gave a sensitivity factor of 2·7—a value which agrees well with most other biological materials. Trowell attributes his original result to a slowing down of pycnosis by oxygen.

Another aspect of the action of radiation which received much attention was the effect on synthesis of DNA as measured by the uptake of tritiated thymidine and its correlation with mitotic inhibition and cell death. H. Harrington (Cleveland), using *U*12 fibroblasts, reconfirmed the classic findings of Strangeways and Oakeley on the effects of radiation on cell division. A rapid drop in mitosis after 500 r. is followed 13 hr. later by re-appearance of cell division. Counts of thymidine-labelled mitotic figures suggested that there was no accumulation of cells in the *G*1 interphase period but a delay in the synthetic *S* and premitotic *G*2 period, using the terminology introduced by Howard and Pelc. Similar findings were reported by R. B. Painter (Columbus) and W. L. Hughes (Upton), who found no evidence that radiation influenced the rate at which HeLa *S*3 cells moved from the *G*1 to the *S* period. The small immediate inhibition of synthesis of DNA could not be correlated quantitatively with the lethal effect of radiation or was the position of the cells in the synthetic cycle related with the probability of their survival. The three authors agreed that in contrast to synthesis of DNA, synthesis of ribonucleic acid (RNA) was practically undisturbed, even after high doses of radiation.

J. E. Till (Toronto) observed a generalized dose-dependent prolongation of all three stages of the first synthetic cycle following exposure. However, the *G*1 and *S* periods were only slightly prolonged. The greatest effect was seen on cells which had completed synthesis of DNA and had moved to the *G*2 period, which was severely disrupted by radiation. Thus, the effects of radiation on synthesis of DNA seem to be the result, rather than the cause, of mitotic inhibition.

A. N. Stroud and A. M. Brues (Argonne, Ill.) gave an interesting example of differences in synthesis of DNA response in two strains of pig- and monkey-kidney cells. After exposure the monkey-kidney cells which normally have a low rate of thymidine incorporation showed an increase of 15–20 per cent more than that of the unirradiated controls, while the pig-kidney cells which normally have a high rate of incorporation show a striking decrease. P. F. Sanders, D. F. Petersen and W. L. Langham (Los Alamos) described methods for growing HeLa cell suspensions in the presence of tritiated thymidine and tritium water, for determining cell size and cell number and for measuring radioactivity. Beta radiation from tritium has a much greater inhibitory effect on proliferation when emitted from incorporated ^{3}H-thymidine in the nucleus than when merely present in water.

W. B. Looney (Baltimore) pointed out some limitations of labelled compounds in radiobiological studies. Erroneous interpretations may be made as a result of reduced synthesis of DNA unless differences in time-sequence of control and irradiated systems are taken into account. For example, the generation time of hepatocytes in regenerating rat liver is increased from 8 to 13 hr. after 3,000 r.; thus, the ratios of synthesis of DNA of irradiated and control specimens between 8 and 13 hr. would be the ratio of the activity in a second cycle of hepatocytes in the controls to that of the first cycle of irradiated hepatocytes.

T. R. Munro (Cambridge) described a technique which allows selected parts of single cells to be irradiated with α-particles and showed a film of irradiated chick fibroblasts. When the chromosomes were irradiated with a sufficient dose sticky bridges were seen at anaphase and the reconstruction of daughter nuclei inhibited. Neither of these effects was seen if the radiation was confined to the cytoplasm. He also described a method which allows single HeLa *S*3 cells and their progeny to be followed for a week. This technique should enable cell survival to be studied after localized radiation.

G. O. Gey (Baltimore) described a more elaborate analogous technique for studying population dynamics of lymphoblasts in culture. His apparatus photographed a number of accurately defined fields of cells at intervals of 15 min. and enabled the fates of large numbers of single cells to be studied.

H. B. Hewitt (London), using an *in vivo* bio-assay of mouse leukæmic cells, measured their response to radiation and its variation with different degrees of oxygenation. For whole-body exposure of mice breathing air or oxygen the dose producing the same survival curves of leukæmic cells was lower by a factor of 2·3 as compared with mice killed shortly before irradiation. On the other hand, survival curves for cells of a solid sarcoma were similar for living and recently killed mice and identical with the anoxic curve obtained for the leukæmic cells. He concluded that variations in radiosensitivity between different mouse tumours are due to differences in their oxygenation. J. R. Andrews and R. T. Berry (Bethesda), using the same technique, gave the survival curves of a different strain of mouse leukæmic cells which grow normally under anoxic conditions in the animal. The cells showed an oxygen factor similar to that found by Hewitt. They also reported preliminary results on the effect of dose fractionation and on sensitization by some pharmacological agents.

A. Goldfeder (New York), in an interesting paper, gave her results on electron-microscopic and biochemical studies of tumour mitochondria, and showed that variations in radiosensitivity of two types of mouse tumour cells could be correlated with differences in mitochondrial structure and metabolism.

R. Bases (New York) demonstrated the sensitizing action of actinomycin *D* and deuterium oxide in experiments in which Puck's single-cell technique was employed. A dose of 300 r. sterilized 5–10 times as many cells pretreated with actinomycin *D* than controls. Cells pretreated with deuterium oxide were also abnormally sensitive to radiation, an effect attributed to a presensitization by incorporation of the drug into the DNA of the cells.

D. E. Rounds (Pasadena) reported a protective effect of yeast RNA. RNA-treated cells of the Fernandes strain of amnion cells multiplied at a normal rate following radiation while untreated cultures showed immediate inhibition, but a minimum time of 8 hr. was necessary before protection could be seen. The magnitude of the protection varied considerably with different cell strains. A. Schleich (Heidelberg) reported her results on radioprotective properties of various compounds, among them histamine, thioctic acid, cysteine and cysteinamine, tested on whole-body irradiated mice, a strain of human malignant fibroblasts and solutions of thymine and DNA. The protection varied with the system tested but was consistent for all systems with cysteine and cysteinamine.

In contrast to the previous papers, which dealt mainly with the effects of radiation on established cell strains, J. G. Carlson, E. Borghese and I. Lasnitzki described the action of radiation on freshly explanted cultures of organized tissues, which retain their histological structure *in vitro*.

J. B. Carlson (Knoxville) pointed out the advantages of his grasshopper neuroblast technique for quantitative radiation studies. The large size of the neuroblasts, their position on the surface of the embryo, their constant number and fixed arrangement in relation to each other simplifies cell counts, facilitates their identification and allows radiation effects to be followed through several division cycles. A disadvantage of the method is the great sensitivity of cell division to changes in osmotic pressure.

As in vertebrate tissues, X-radiation produces a mitotic inhibition due to an arrest of cells in late prophase followed by mitotic recovery. Increasing the oxygen tension does not increase mitotic inhibition but reducing it diminishes the effect of radiation. Investigations of frequencies of chromosome aberrations induced by X-rays show a peak at middle prophase and anaphase.

E. Borghese (Rome) showed in a beautiful film the growth of mouse embryonic lung explanted by the organ culture technique and the effects of γ-rays on this tissue. In the controls, development and outgrowth of clear translucent bronchi could be observed. After exposure the explants became opaque, growth slowed down and finally stopped.

I. Lasnitzki (Cambridge) reported that irradiation can change the pattern of differentiation in organized tissues grown *in vitro*. Mouse vaginal explants grown by the organ culture technique form squamous epithelium with a superficial layer of keratin. Exposure to X-rays inhibits the squamous development and keratin formation both in normal- and œstrone-treated vaginas. This effect is unrelated to mitotic inhibition and closely resembles that seen after treatment with excess vitamin A. It also agrees well with results obtained in *in vivo* experiments.

The symposium reflected the present trend to measure the biological effects of radiation and showed the advantages tissue cultures offer for such work.

ILSE LASNITZKI

THE NATIONAL CHEMICAL LABORATORY

THE report of the National Chemical Laboratory, 1960*, comprises the report of the Steering Committee over the signature of Prof. C. E. H. Bawn, and the report of the Director, to which are appended details of membership of the sub-committees of the Steering Committee and of the staff of the Laboratory at December 31, 1960, and lists of publications and patents during the year and of Standard Samples of Organic Compounds.

On consideration of the Laboratory's research programme in greater detail, the Steering Committee agreed that the work of the Extraction of Metals Group should include fundamental work on the inorganic and physical chemistry of hydrometallurgy in addition to work for the Atomic Energy Authority on ores and minerals. While the Radioisotope Applications Unit, established at Harwell as an out-station of the Laboratory in 1957 and now at the Isotope Research Division of the Atomic Energy Research Establishment at Wantage, is regarded as contributing to the National Chemical Laboratory's mandate to cultivate instrumental techniques, the Steering Committee found no justification for the Department of Scientific and Industrial Research to undertake any work on the direct uses of high-activity sources of radiation.

Considering the building programme of the Laboratory in relation the future development of its research programme, the Steering Committee formed the opinion that, to cover the possible forward requirements, the present staff of 264 should be increased by about one-half in the next 5–7 years and doubled in the next 10–15 years. It anticipates that the need for Government research in the chemical field will inevitably grow with the continued expansion of the chemical industry, and to provide the buildings needed for a staff more than double the present complement it recommends that the Laboratory be moved to a larger site as near the present site as possible, in view of the close and important co-operation with the National Physical Laboratory. The site should be large enough not only to accommodate the buildings for the National Chemical Laboratory but also to provide in the long term for possible expansion there of other existing laboratories or for new laboratories. The Research Council has already agreed that the possibility of acquiring such a site should be explored.

Commenting on the problem of determining the best balance of effort in a national laboratory, the director, Dr. J. S. Anderson, defines the task of the National Chemical Laboratory as that of carrying out research in areas that are important but that cannot be studied so effectively by agencies less centrally placed. It should seek to place new developments on a firm basis of scientific understanding, to ensure that all its investigations enrich scientific knowledge, and to bring a deeply analytical approach to bear on those major problems of wide application, such as the prevention of corrosion, which fall within its scope. The present research programme is still being developed, and Dr. Anderson notes some material changes in the work of several research groups during the year. Work on the physicochemical properties of organic compounds has been converted into a much more comprehensive programme of chemical thermodynamics, though full effect cannot be given to the projected scheme until the new buildings are available. The Extraction of Metals Group, which hitherto has been working almost entirely in associations with, and at the request of, the Atomic Energy Authority, is now including in its programme some fundamental work on the more familiar metals of industry. Collaborative arrangements with the National Research Development Corporation for work on the development and application of ion-exchange membranes and of gold-selective ion-exchange resins was terminated, but some work in this field is being pursued at a more fundamental level. Work in the Inorganic Group on the extreme purification of semiconductor materials was also substantially reduced and effort transferred to applications for the substantial quantities of rare-earth elements that are becoming available.

---

* Department of Scientific and Industrial Research. *Report of the National Chemical Laboratory*, 1960 : *Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for* 1960. Pp. iv+70+4 plates. (London: H.M. Stationery Office, 1961.) 4*s*. 6*d*. net.

A small research unit was set up to continue the Director's fundamental research on the chemistry of solids, and in this unit the study of physical processes and chemical reactions on absolutely clean metallic surfaces, using the field-emission microscope, has commenced. A research fellowship has been established and work started on the related and very powerful technique of ion microscopy, which is capable of imaging individual atoms on the metal surface. The Chemical Thermodynamics Group made a critical examination of existing data, and, with the assistance of the Association of British Chemical Manufacturers, this has led to a recommendation that, on the organic side, the Laboratory should commence work on the aliphatic compounds containing oxygen and continue its work on phenols. The study of the purification and physical properties of phenol, cresols and xylinols has been completed for publication; work continued on the precise determination of the critical temperatures of certain pure hydrocarbons and the ebulliometric apparatus for measuring vapour pressure has been re-designed and re-built. In the Non-metals Group, particular emphasis was placed on boron–nitrogen compounds, and the systematic study of the chemistry of borazole and its derivatives promises to provide polymers with greater thermal stability than is usually associated with a molecular skeleton of carbon–carbon bonds. Compounds of promise as pre-ignition suppressors for motor 'petrol' have been obtained. Work on ion exchange materials, particularly ion-exchanging membranes, has been narrowed to a thorough study of selectivity and its exploitation, and increased attention given to polymers with potentially applicable electrochemical and electrical properties in the form of reversible redox polymers and high nolecular weight materials likely to exhibit semiconduction.

The Extraction of Metals Group has developed a modified rotary-film disk contactor for the solvent extraction of metals direct from solutions containing appreciable quantities of solids in which solvent and pulp flow parallel to the planes of the disks, and each stage contains several disks. This has given excellent recoveries of uranium, with acceptable solvent losses, from sulphuric acid-leached ore pulps and sulphuric or nitric acid-leached uranium concentrates. Promising results were also obtained in extraction of uranium by heap leaching with acid, and in co-operation with the Warren Spring Laboratory, the extraction and concentration of beryllium from concentrates containing 2–4 per cent beryllium oxide, derived by flotation from low-grade ores, has also been studied. Examination of the solution chemistry of beryllium, mainly concerned with developing methods for the selective recovery of beryllium from sulphate leach liquors, showed the pronounced tendency of beryllium to form polynuclear species in both weakly acidic and alkaline solutions.

The Inorganic Group has given considerable attention to the extraction of the reducible elements, samarium, europium and ytterbium, by sodium amalgam, and has developed a continuous process for recovering these elements from concentrates. The possibilities of a new and rapid method of extracting aqueous solutions of lanthanons with tributyl phosphate are being examined and the purification of gallium and arsenic is being studied with the view of the preparation of gallium arsenide as a semiconductor. The Corrosion of Metals Group is seeking a better understanding of the basic physico-chemical factors which control corrosion and oxidation processes, and of which account must be taken in devising solutions to specific problems. Special attention has been given to the relative importance of the surface preparation of mild steel and the anions present in the solution in which the steel is immersed. In the study of corrosion in moving water, attention has been given to the chemical nature of the corrosive products. The very high initial corrosion-rate of mild steel in oxygenated dilute chloride solutions at 200° C. has been determined, and relations have been established between the rate of corrosion of mild steel in batch cultures and the hydrogenase activity of sulphate-reducing bacteria. Work is also in progress on the distribution in soils of different species of sulphate-reducing bacteria, as well as a pilot investigation of the corrosion of mild steel exposed at two sites on the River Thames.

THE NATIONAL RESEARCH DEVELOPMENT CORPORATION

In its eleventh year of life, the National Research Development Corporation * negotiated more licence and option agreements with United Kingdom and overseas firms than in any previous year. Eighty-one such agreements were completed (as against sixty-one in the previous year) and, by June 30, 1960, some 456 such agreements were in force. Royalties and other payments received under licence and option agreements amounted to £259,000 as against £182,000 in the previous year. About half these receipts derived from overseas business, that is, from direct payments made by manufacturers abroad, and from royalties on goods exported abroad by United Kingdom manufacturers.

The outright sale of patent rights is another, but exceptional, form of exploitation activity, and is a fluctuating source of income.

By the end of 1960 the Corporation had a portfolio of 1,066 United Kingdom and 2,042 overseas patents and patent applications for inventions, derived chiefly from publicly supported research in the Government research establishments, research councils and the universities. The portfolio is under constant change. Unprofitable patents are allowed to expire, others lapse at the end of their periods of grant, and new accessions are continually being included.

At the end of 1960, the Corporation was providing financial assistance for thirty-five development projects. During the year, the Corporation's expenditure on development amounted to £427,000, and with its subsidiaries it has entered into commitments for £739,000 for development purposes. Recoveries of development expenditure amounted to £52,000.

---

* *National Research Development Corporation Bulletin*, April 1961.

One project which was commenced in the year under review was the automatic process plant control. This is concerned with the development of electronic automatic control equipment suitable for plant and process control automation and, for example, for the control of power stations and chemical plants. It contemplates the development of a range of standard equipment which will permit electronic analogue and mixed analogue/digital computing techniques to be used for this purpose, the individual elements being capable of assembly in a variety of ways to enable control systems to be designed as required for any particular plant.

The work is being undertaken by an established firm in the electronic computing and control engineering fields, which is sharing the cost with the Corporation. The project is still in its early stages, but work has started on the design of circuit elements and components, and on the study of potential plant applications.

FŒTAL AND NEONATAL PHYSIOLOGY

ALTHOUGH there have been great reductions in infantile mortality within the present century, it is only now being realized how much can be done to reduce mortality before birth and in the first days after it. The application of fundamental physiological principles to the management of premature infants has become a study in its own right. These principles were recently examined in the *British Medical Bulletin**, the contributions varying from investigations of blood pressures in new-born babies, the acquirement of immunity and immunological tolerance, protein and amino-acid movements in the fœtus and new-born animal, transport mechanisms in the placenta and red cells, enzyme development, the newer aspects of placental histology, fœtal endocrinology, and the chemoreceptors and their function in the aortic-pulmonary body.

Among the more outstanding experimental work is a review by Dr. R. Deanesley of the present position in fœtal endocrinology. Experimental operations by Jost, Wells and their collaborators, on pregnant rabbits and rats, and on fœtuses *in utero*, have shown that in these animals the pituitary and testes become active before birth and play a decisive part in differentiation. Thyroid and adrenal development is controlled by the fœtal pituitary. The developing fœtus at certain stages can react to exogenous hormones which reach it directly or through the placenta. The fact that the endocrine glands are active in the fœtus carries no implication that their secretions can affect the maternal physiology.

In their review of the fœtal kidney, Miss Pauline Alexander and Dr. D. A. Nixon show that development of function in the metanephros is spread over a period of time—some activities appearing earlier than others.

The onset of glomerular filtration precedes tubular activity so that large volumes of urine may be passed into the amniotic or allantoic sacs from an early age. Subsequent appearance of tubular activities results first in the production of a hypotonic and, later, a hypertonic urine. The maximal osmolarity of the urine is low by adult standards because of the low excretion of solute. The renal capacity to acidify the urine is limited in the early stages, in part by the very low excretion of phosphate. The glomerular filtration-rate appears to be somewhat labile at first, and the factors leading to its stability in later life are not known. Ratios for the clearance of *p*-amino-hippurate: inulin suggest that the supply of blood to the kidney is low in fœtal life in comparison with that in the adult, and this may limit its functional capacity considerably.

The stage of metanephric development at which a fœtus of any particular species attains an independent existence will determine its initial renal problems.

Dr. Heather J. Shelley has investigated reserves of glycogen and their changes at birth and in anoxia. However, there is no reliable information on the glycogen reserves of the new-born human infants, but, by analogy with other species, they probably have a high concentration of liver glycogen and a fair amount of muscle glycogen. New-born babies have been known to withstand anoxia for up to 30 min. (Bullough, 1958), so it is likely that their cardiac glycogen concentration is also high. Since lactation is not established in man until 24–48 hr. after parturition, the new-born baby must be particularly dependent on its reserves; if its liver glycogen falls as rapidly as in other species it will be in a precarious metabolic state for several days. If kept warm, the new-born baby can survive for several days without food, but if exposed to cold it later dies of hypoglycæmia (Mann and Elliot, 1957), probably the result of an attempt to maintain its body temperature (Bruck, 1959). It is therefore possible that factors which reduce the glycogen content of the fœtus *in utero* or at birth may jeopardize the infant's chances of survival after birth, and future work must determine to what extent the deleterious effects of maternal nutrition, placental insufficiency and birth asphyxia may be the consequence of inadequate glycogen reserves.

The ability of young mammals to withstand a total lack of oxygen was considered by Dr. Joan Mott. She believes that the practical conclusion to be drawn from the present state of knowledge of the conditions under which anoxic survival is possible is that the carbohydrate stores, particularly of the heart, should be protected from dissipation. Re-synthesis of carbohydrate does occur, but may be limited by the rate of entry of glucose into the cell. Thus the factor of vital importance is the store of carbohydrate already available within the cardiac cells. Repeated episodes of anoxia or hypoxia are cumulatively as well as individually dangerous, and it is probable that, if the *p*H is not restored to normal level, a stage is reached at which glycolysis may be completely inhibited. Repeated anoxic episodes may also exhaust the liver glycogen and lead to hypoglycæmia, so that maintenance of the circulation might be insufficient to protect the brain from injury.

---

* *British Medical Bulletin*, 17, No. 2 (May 1961): Fœtal and Neonatal Physiology. Pp. 79–176 + 8 plates. (London: British Council, 1961.) 20*s*.

From the available evidence showing the reaction of the new-born animal to environmental temperatures, Dr. June Hill suggests that the metabolism of the new-born of a warm-blooded species often differs from that of the adult animal in two important respects: first, the new-born animal has a lower basic metabolic rate than is appropriate for its surface area; and secondly, per unit surface area, the new-born animal has a smaller maximal metabolic response to cold. Thus the heat production of the new-born animal, both minimal and maximal, is lower per unit surface area than that of the adult. The degree of immaturity at birth probably determines to what extent the metabolism of the new-born deviates from the adult pattern.

In addition, the new-born animal often has a coat of lower effective insulating power than that of its parent; this is partly because the new-born is smaller than the adult, and is partly a specific attribute of immaturity. This means that, per unit surface area at a given environmental temperature, the new-born animal loses heat much more readily than the adult.

These facts underlie, and go far to account for, the difficulties which many new-born animals experience in maintaining a stable internal body-temperature.

LIFE AND SCIENTIFIC WORK OF SIR JAMES HALL

THE Bruce-Preller Lecture of the Royal Society of Edinburgh was delivered on June 5 by Dr. V. A. Eyles, formerly of the Geological Survey of Great Britain, who took as his subject, "The Life and Scientific Work of Sir James Hall, Bart., F.R.S. (1761–1832), Second President of the Royal Society of Edinburgh".

Sir James Hall, fourth Baronet of Dunglass, Haddingtonshire, was born on January 17, 1761. He was a great nephew of Sir John Pringle, Bart., the distinguished Army physician, a president of the Royal Society of London. Owing to the early death of Hall's father, Sir John took charge of his education. He attended the Universities of Cambridge, Geneva and Edinburgh. After extensive travel in Europe he settled down at Dunglass, though spending much time at his town house in Edinburgh. In 1786 he married Lady Helen Douglas, a daughter of the Earl of Selkirk.

Hall had been elected a member of the newly formed Royal Society of Edinburgh in 1784, and for many years took a prominent part in the affairs of the Society. He became president in 1812, being the first scientist to occupy the presidential chair. He was elected Fellow of the Royal Society of London in 1806.

Though chiefly remembered as a geologist of the Huttonian school, he was always much interested in chemistry, having been a student of Joseph Black, in Edinburgh. His first address to the Royal Society of Edinburgh was on Lavoisier's new theory of chemistry, to which he had been converted by Lavoisier himself, whom he had met a number of times in Paris.

Hall contributed a number of papers to the Edinburgh *Transactions*, chief among them being his "Account of the Effects of Compression in Modifying the Effects of Heat". This describes the long series of experiments by which he demonstrated that limestone, when heated under pressure, remains undecomposed. This paper has become a classic, and illustrates Hall's great skill as an experimentalist. Hall has been called the 'father of experimental geology', but might, perhaps more aptly, be called the 'father of geochemistry'. His skill as an experimentalist has tended to obscure the fact that he was also an extremely able field-geologist.

Hall was a man of many interests, including, in later life, politics, and he was member of Parliament for the St. Michael division in Cornwall during 1807–1812. He died in 1832, after suffering ill-health in his later years.

HOSPITAL IN-PATIENTS IN BRITAIN

A REPORT on the Hospital In-Patient Enquiry for 1959 gives provisional figures for that year and some comparisons with earlier years*. The report is based on data provided by hospitals (other than psychiatric hospitals) in the National Health Service, on forms giving a confidential summary of the case records of a 10 per cent sample of discharges (the term includes deaths). The purpose of the inquiry is to provide statistics to assist the administration of the hospital service and to supplement other statistics about mortality and illness in the community. It is organized jointly by the Ministry of Health and the Central Register Office. 1959 was the second year in which the inquiry had almost complete coverage of the relevant hospitals in England and Wales.

The tables in the report show the diagnostic distribution of case discharges in 1959 and in each year back to 1955, regional figures for selected important conditions back to 1956, regional figures for maternity cases according to the type of maternity care received, and the distribution of the cases in the 1959 sample by sex, age and diagnosis. It also includes the estimates of the population of hospital regions by sex and age at June 30, 1959.

There were, in all, 3,664,100 discharges from the relevant hospitals in 1959, or 807·3 per 10,000 population, compared with 3,554,700, or 788·0 per 10,000 in 1958, an increase of 2·4 per cent. The estimated numbers of discharges for most common causes of admission to hospital showed some increase, though much of this was probably due to more efficient use of hospital services rather than to increased illness.

Respiratory tuberculosis continued to decrease. There were 44,300 discharges in 1959, 9·8 per 10,000 population, compared with 48,800 and a rate of 10·8 in 1958. The rates for males fell in all regions except Liverpool, where an intensive mass radiography campaign was held early in the year.

---

* Ministry of Health and General Register Office. Report on Hospital In-Patient Enquiry for the year 1959. Part 1: Preliminary Tables. Pp. 16. (London: H.M. Stationery Office, 1961.) 1s. 6d. net.

There were 2,200 cases of acute poliomyelitis discharged, or 0·5 per 10,000 population, 1,000 fewer than in 1958; there has also been a steady rise in hospital discharges for cancer of the lung, from 22,400, 5·0 per 10,000 population, in 1955, to 30,600, 6·7 per 10,000, in 1959. Discharges for other tumours benign and malignant, have remained fairly steady.

Discharges for coronary disease continued to rise. The 1959 figure was 56,600 (12·5 per 10,000 population), 6,800 more than in 1958. For other heart diseases and hypertensive disease, combined, there were 5,500 fewer discharges in 1959 than 1958. 44,600 cases of varicose veins were discharged, 9·8 per 10,000 population, compared with 40,900 and 9·1 in 1958.

Figures for influenza, bronchitis and pneumonia all showed an increase over 1958, but whereas hospital cases of influenza fluctuate (2,600 in 1955 and 1956, 22,200 in 1957, 4,200 in 1958 and 10,000 in 1959), pneumonia and bronchitis have increased steadily from 58,000 and 35,400, respectively, in 1955 to 81,000 and 60,200 in 1959.

In the two years since 1957, discharges for head injuries increased by 20 per cent to 82,300, a rate of 18·1 per 10,000 population, and other injuries by 13 per cent to 219,200 and a rate of 48·3.

The number of maternity discharges in 1959 was 612,400, being 80·2 per 100 births, compared with 599,000 and 75·9 in 1958. Of all births, 60·5 per cent took place in hospital compared with 57·5 in 1958.

ORIGINS OF PETROLEUM

SIR ROBERT ROBINSON has put forward an intriguing new theory suggesting a duplex origin of mineral oils.* The prevailing view is that biologically derived organic matter aged and was transformed into petroleum; but the organic chemistry of this supposed petroleum has never been satisfactorily worked out.

In place of this conception, Sir Robert suggests a duplex mixture which, wherever it was situated, had plenty of time to find its way to the littoral regions of the oceans and there to become admixed with organic life. As in the full biological theory, life would be promoted by salts brought down by the rivers.

It was indicated by Oparin and Urey that the primeval atmosphere was probably largely methane, carbon dioxide and ammonia and the temperatures rather high. The atmosphere of Venus may be similar. It contains much carbon dioxide, and Hoyle has suggested that the clouds could consist of oil. Organic matter has been found in meteorites. Loss of hydrogen from methane would yield hydrocarbons and the thermodynamic equilibrium would be disturbed by escape of hydrogen from the atmosphere. Urey and Miller have shown that simple amino-acids, for example glycine and aspartic acid, can be produced in the laboratory naturally, by irradiation of such a mixture after cooling. Quite recently, Bahadur and Srivastava have made peptide from the same amino-acids by photosynthesis in aqueous solutions. The simple, early non-chlorophyllic organisms might well have used hydrocarbons as a source of energy, and possibly become covered with oil concentrated near the shores. It is hard to see that the distribution of oil which is now observed presents any insuperable obstacle. Almost all geochemical distributions are curiously uneven. The primeval oil may have been on the surface of the seas and brought to shore by solids such as small organisms and rock dust combined with the action of the tides. The release from miscelles, as suggested by Baker, is another possibility.

There are several mechanisms for straight-chain synthesis. Among these the most likely are the Fischer-Tropsch and oxo-synthesis. If olefines were present, or developed from products of oxo-synthesis, they could react with carbene to alkyl*cyclo*-propanes. The latter are known to give straight-chain α-olefines on pyrolysis as there is ample room for isomerization, branch-chain synthesis, aromatization and other cyclization, introduction of nitrogen and sulphur and so on in varied and inevitable side reactions.

---

* *Catalyst*, 6; Spring, 1961.

NEW AFTERGLOW EMISSION ASSOCIATED WITH METASTABLE NITROGEN ATOMS

By L. HERMAN and R. HERMAN

Observatoire de Paris-Meudon, France

ATOMIC two-body recombination is of importance if the atomic concentration is small enough. This is known to occur in interstellar space, for example. In laboratory experiments three-body recombination is usually the most important. When the gas pressure is small, the atoms diffuse towards the wall where they are de-activated before they recombine by two-body collisions. Addition of an inert gas reduces the diffusion effect. One may then observe strong emissions of atomic lines of forbidden transitions or atomic recombination spectra. These emissions are usually characterized by a strong afterglow since the recombination processes are slow or the life-times for the forbidden transitions very large, of the order of seconds. In the present article a new example is given of atomic recombination involving excited nitrogen atoms.

The discharge tube is a 1-l. glass bulb with two side-tubes sealed in. One contains a 2-mm. diameter tungsten rod which may be used as an internal electrode. The second tube contains phosphorus pentoxide and sodium trinitride powder. Phosphorus pentoxide eliminates the last traces of water vapour; sodium trinitride is used to produce small quantities of pure nitrogen.

In some experiments, in order to improve and accelerate the purification of nitrogen, a portion of the inner surface of the glass bulb has been coated with a thin layer of solid sodium. After careful evacuation, the glass bulb is filled with pure xenon

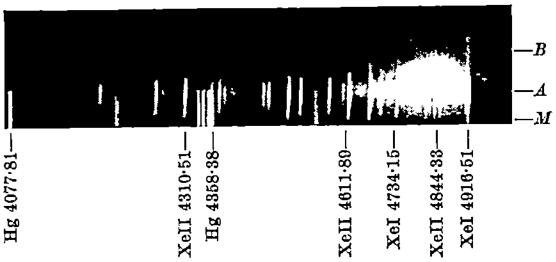


Fig. 1. Afterglow of a nitrogen-xenon mixture. *A*, high frequency discharge spectrum showing the XeI lines and the semi-continuous band system; *B*, short duration afterglow emission spectrum; *M*, mercury comparison spectrum

gas at a pressure of 20 mm. mercury and sealed off. The discharge and the afterglow spectra have been recorded using a conventional pulse technique and a rotating disk. The high-frequency generator had a pulse frequency of 50 cycles per sec. and a pulse duration of 10 μsec. The high frequency–high voltage output was applied directly to the tungsten electrode of the glass bulb.

The rotating disk was driven by a synchronous electric motor having the same frequency as the pulse generator. It is possible to obtain either the discharge or the afterglow spectrum, at the selected time delay, using the phase-shift circuit of the generator. Often it is interesting to obtain simultaneously the discharge and the early afterglow spectra. This can be achieved if the edge of the slit of the rotating disk is perpendicular to the slit of the spectrograph, and has been done for the spectrum shown in Fig. 1. Such a comparison is often useful: it is then quite certain that the experimental conditions are exactly the same for both the discharge and the afterglow spectra.

Fig. 1 shows a print of the spectrum obtained with the pulse generator, the rotating disk, and a glass spectrograph of *f*/8 aperture. The exposure time was 3 hr. This spectrum is obtained with a single exposure in which half the slit admitted light from the discharge (during the pulse of the generator), and the other half of the slit the early afterglow emission. The discharge spectrum *A* shows most of the XeI lines located in the blue and in the violet (the XeII lines in the red are not recorded in this spectrum). The remarkable feature is that the afterglow emission does not seem to be much excited in the discharge. The afterglow spectrum shows a vibrational structure which seems to merge into a continuum near 4790 Å. This continuous emission extends to about 4600 Å.

The experimental fact gleaned from Fig. 1 is that the semi-continuous band system has about the same intensity in the discharge as in the afterglow. It suggests strongly that the emission is associated with a slow reaction. It is emitted only if nitrogen is present. The emitting molecule is very likely diatomic. Any polyatomic molecule would be dissociated in the discharge in a matter of seconds. Since xenon and nitrogen are both necessary for the emission, the diatomic molecule is either an unstable XeN or one of the weakly bound excited nitrogen molecules.

Other semi-continuous spectra which also seem related to atomic forbidden transitions have been found in mixtures of xenon and oxygen. For example, there is a very strong green emission related to the forbidden 5577·35 Å. ($2p^4\,{}^1D - 2p^4\,{}^1S$) line. Contrary to the emission of Xe + N_2 investigated here, the 5577·35 Å. oxygen line lies at the limit of the semi-continuous emission. It corresponds to the asymptotic portions of the upper and lower molecular potential curves. In the present case (Xe + N_2) the wave-length limit should be at 5200 Å., corresponding to the forbidden transition of atomic nitrogen $2p^3\,{}^4S^0 - 2p^3\,{}^2D^0$. Actually, the short wave-length limit is about 4920 Å. This differs from the forbidden nitrogen line by 1,383 cm.$^{-1}$ = 0·171 eV.

There is, however, a large difference in the life-times for these two lines. The transition probability for the oxygen green line is 1 sec.$^{-1}$ while that of the nitrogen λ 5200 Å. line is 8×10^{-5} sec.$^{-1}$, about 10^4 times smaller. This difference may be the reason why the 5200 Å. line is not observed in the laboratory.

The emission may be due to recombination of two metastable or normal nitrogen atoms. The lowest states of neutral nitrogen are:

$$\mathrm{N}(2p^3\,{}^4S^0),\ \mathrm{N}(2p^3\,{}^2D^0) \text{ and } \mathrm{N}(2p^3\,{}^2P^0)$$

having the energies:

$$0{\cdot}00 \text{ eV., } 2{\cdot}37 \text{ eV. and } 3{\cdot}56 \text{ eV.}$$

The possible recombination processes are:

$$\begin{array}{l} M + \mathrm{N}({}^4S) \rightarrow M\mathrm{N}(S) \\ M + \mathrm{N}({}^2D) \rightarrow M\mathrm{N}(D) \\ M + \mathrm{N}({}^2P) \rightarrow M\mathrm{N}(P) \end{array}$$

where *M* is a nitrogen or xenon atom. The letters *S*, *P* and *D* in the right side next to *M*N indicate molecules built up with nitrogen *S*, *D* and *P* atoms. As is customary, we shall assume that the pairs of colliding atoms follow a very shallow potential energy curve.

If *M* is a nitrogen atom, the shallow potential energy curves will intersect other potential energy curves of stable molecular states, in the region of internuclear distances $r > r_e$.

For $\mathrm{N}({}^4S) + \mathrm{N}({}^4S)$, for example, intersections occur with $B^3\Pi_g$ at $v \geqslant 12$, $Y^3\Sigma_u^-$ at $v > 7$, $\alpha'\,{}^1\Sigma_u^-$ at $v > 6$, $a^1\Pi_g$ at $v > 6$, . . . For $\mathrm{N}({}^4S) + \mathrm{N}({}^2D)$, an intersection occurs with $C^3\Pi_u$ at $v > 3$.

However, there should be a chance for direct transitions between the unstable repulsive potential curves. The spectra emitted will be located in the following wave-length regions:

$$\begin{array}{lll} \mathrm{N}({}^4S) + \mathrm{N}({}^2D) \rightarrow & \mathrm{N}({}^4S) + \mathrm{N}({}^4S) + & h\nu \\ \quad 12{\cdot}139 \text{ eV.} & \quad 9{\cdot}756 \text{ eV.} & 2{\cdot}383 \text{ eV.} \\ \mathrm{N}({}^4S) + N({}^2P) \rightarrow & \mathrm{N}({}^4S) + \mathrm{N}({}^4S) + & h\nu \\ \quad 13{\cdot}331 \text{ eV.} & \quad 9{\cdot}756 \text{ eV.} & 3{\cdot}575 \text{ eV.} \\ \mathrm{N}({}^4S) + \mathrm{N}({}^2P) \rightarrow & \mathrm{N}({}^4S) + \mathrm{N}({}^2D) + & h\nu \\ \quad 13{\cdot}331 \text{ eV.} & \quad 12{\cdot}139 \text{ eV.} & 1{\cdot}192 \text{ eV.} \\ \mathrm{N}({}^2D) + \mathrm{N}({}^2D) \rightarrow & \mathrm{N}({}^4S) + \mathrm{N}({}^4S) + & h\nu \\ \quad 14{\cdot}522 \text{ eV.} & \quad 9{\cdot}756 \text{ eV.} & 4{\cdot}766 \text{ eV.} \\ \mathrm{N}({}^2D) + \mathrm{N}({}^2D) \rightarrow & \mathrm{N}({}^4S) + \mathrm{N}({}^2D) + & h\nu \\ \quad 14{\cdot}522 \text{ eV.} & \quad 12{\cdot}139 \text{ eV.} & 2{\cdot}383 \text{ eV.} \\ \mathrm{N}({}^2D) + \mathrm{N}({}^2D) \rightarrow & \mathrm{N}({}^4S) + \mathrm{N}({}^2P) + & h\nu \\ \quad 14{\cdot}522 \text{ eV.} & \quad 13{\cdot}331 \text{ eV.} & 1{\cdot}191 \text{ eV.} \end{array}$$

In these equations it is assumed that the emission takes place while the atoms are on the horizontal asymptotic part of the potential curve. If transitions from the shallow minima of the unstable potential curves are considered, slightly different wave-lengths may be obtained. This would explain the difference of 1,383 cm.$^{-1}$ discussed above.

Then our afterglow spectrum would correspond either to the first or the fifth process. The first reaction is more likely since the number of $\mathrm{N}({}^2D)$ atoms is much smaller than that of $\mathrm{N}({}^4S)$ atoms.

This work was supported in part by the Geophysics Research Directorate of the Air Force Cambridge Research Laboratories, Air Force Research Division, through its European office.

CHEMISORPTION AT SOLID-LIQUID INTERFACES

By A. R. BURKIN and G. HALSEY

Department of Metallurgy, Royal School of Mines, Imperial College of Science and Technology, London, S.W.7

ADSORPTION of solute molecules or ions from aqueous solutions at a solid surface may result in strong electron interaction between one or more atoms of the adsorbed material and the solid. In such cases water molecules must be displaced and if the adsorption sites correspond to close proximity of metal ions in the solid lattice the adsorption process is formally similar to the first stage in the formation of a co-ordination complex compound. For a simple hydrated metal ion M^{n+} and ligand L, assumed here to be uncharged, this is represented by the following reaction with equilibrium constant K_1:

$$[M(\mathrm{H_2O})_x]^{n+} + L \rightleftharpoons [M(\mathrm{H_2O})_{x-y}\,L]^{n+}$$

If this model of adsorption is applicable there should be a correlation between the extent of adsorption and the value of K_1 for an appropriate equilibrium in solution. Since the environment of a metal ion controls its electron energy-levels, direct comparison is impossible, but by use of a simple argument in the interpretation of some adsorption isotherms it is shown below that the model predicts the observed behaviour.

If certain transition metal ions are placed in order of increasing values of K_1 with a ligand which gives metal-oxygen or metal-nitrogen bonds, it is found that the order is the same for many different ligands (the Irving-Williams series). The values of log K_1 for complex formation with ammonia, extrapolated to infinite dilution, at 30° C. are [1] for Cu(II), 4·01; Ni(II), 2·67; and Zn(II), 2·18. It may be confidently predicted, therefore, that with primary aliphatic amines copper will form more stable complexes than will the other two metals, and the complexes of nickel will be slightly more stable than those of zinc. Thus such an amine should be adsorbed more strongly from an aqueous solution by copper oxide than by nickel oxide and more strongly by this than by zinc oxide, if the arguments presented above are correct.

Adsorption of *n*-dodecylamine on quartz occurs from solutions of *p*H around 9 by attachment of alkylammonium ions to the solid[2,3]. At higher amine concentrations multilayer formation occurs by abstraction of uncharged amine molecules. The results presented in Fig. 1 show that cupric, nickel and zinc oxides adsorb amine molecules, not the ions. The curves show the variation with *p*H of the minimum concentration of total amine (molecules and ions) in solution capable of giving 'pick up' of solid particles of the oxide of size such that they pass through a sieve with openings 0·211 mm. but not through one with 0·152 mm. openings. 'Pick up' is said to occur when the particles under the amine solution adhere so firmly to an air bubble that they do not readily break away when the bubble is raised through the liquid. For particles of similar size and mass it is generally agreed that pick up occurs when the surface coverage with a particular reagent reaches a certain value which depends on the system. The pick up curves for the three metal oxides correspond to lines of constant free-amine concentration in the solution. The analogous curve for quartz corresponds to constant alkylammonium ion concentration in solution. The lack of adsorption of the ions by the metal oxides from solutions of low amine concentration is probably associated with the much higher density of negative charge at the surface of quartz in solutions of *p*H 9–10.

In Fig. 2 adsorption isotherms measured at 30° C. are given for *n*-dodecylamine on cupric, nickel and zinc oxides. A full discussion of these will be presented elsewhere. In the case of cupric oxide a well-defined flattening of the curve corresponds to completion of a monolayer, and at higher amine concentrations multilayer formation occurs. Zinc and nickel oxides do not show completion of the monolayer but analysis of the curves indicates multilayer formation in the same region of amine concentration in solution as gives multilayers on cupric oxide. While the monolayer is being formed it is clear that the ability to adsorb amine from solution increases in the order ZnO < NiO < CuO.

The entropy changes on adsorption of amine to the metal oxides before completion of the monolayer will be of similar magnitude in each case. The enthalpy changes will, therefore, also be in the order CuO > NiO > ZnO as will the strengths of the bonds between the metal ions and the amine adsorbed relative to those between the metal ions and water. The strength of the bond between a metal ion and

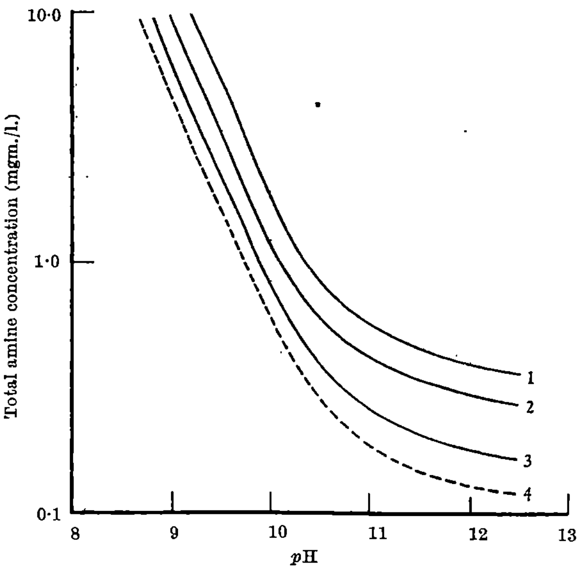


Fig. 1. Bubble pick-up curves for *n*-dodecylamine and (1) ZnO, (2) NiO, (3) CuO. Curve (4) shows the slope of lines of constant free amine concentration

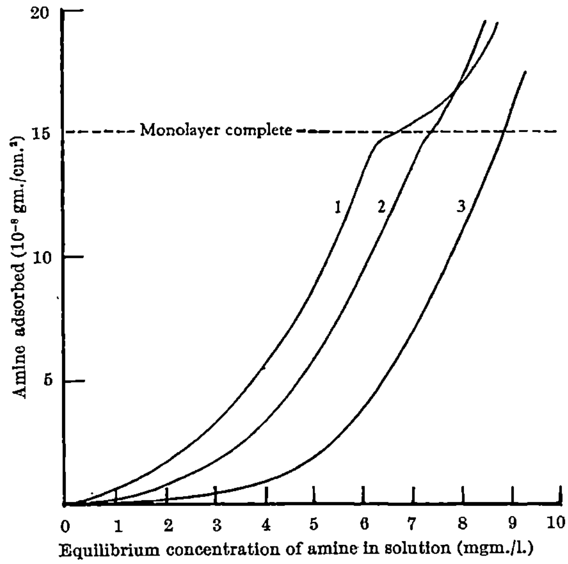


Fig. 2. Adsorption isotherms (30° C.) for *n*-dodecylamine in 10^{-3} *N* sodium perchlorate solution at *p*H 9·5 on (1) CuO, (2) NiO, (3) ZnO

ligand depends on the electronic structure of each. In particular, the ability to form π-bonds will significantly increase the bond-strength. Molecules containing an element such as sulphur in a low valency state can accommodate electrons from suitable levels in the metal ion in vacant levels and so give π-bonds. If the metal ions are in a solid lattice the electrons available for interacting with the ligand may either be isolated on the individual metal ions or they may be held in bands associated with the whole crystal if the solid is a semiconductor. In the latter case the relevant electron energy will be that at the surface and sensitive to lattice impurities in that region.

A large amount of data exists[4] showing that mercaptans, dialkyl sulphides, xanthates, dithiocarbamates and similar compounds containing divalent sulphur are adsorbed very strongly from solutions of suitable *p*H by solids containing metal ions which are in a suitable electronic state to form strong bonds with sulphur. With an oxidizing ion such as divalent copper, electron transfer often results, giving oxidized ligand and cuprous ions, whereas cuprous oxide, for example, simply adsorbs the reagent. If a solid has both anodic and cathodic areas on the surface, different adsorption behaviour would be expected at each kind of surface. In the case of galena and xanthate, adsorption occurs at the anodic regions[5] and greatly increased amounts are adsorbed when anodic bias is applied to the solid. The electron energy-levels are raised under anodic conditions so that the energy of electrons in the solid available for π-bond formation with vacant ligand orbitals is increased.

The views on the factors controlling chemisorption from solution set out above are strongly supported by experimental evidence. It is therefore possible to predict what reagents and treatment of the solid are likely to give strong adsorption under suitable conditions. This is of interest in infestigating the froth flotation process for separating minerals.

We thank the Department of Scientific and Industrial Research for a maintenance grant to one of us (G. H.) and the University of London Central Research Fund for a liquid scintillation counter.

[1] *Stability Constants*, Part 2 (Chem. Soc., Lond., 1959).

[2] Buckenham, M. H., and Rogers, J., *Trans. Inst. Min. Metall., Lond.*, **64**, 11 (1954).

[3] Burkin, A. R., and Soane, A. B., Third Intern. Cong. Surface Activity, Cologne (1960).

[4] Sutherland, K. L., and Wark, I. W., *Principles of Flotation* (Australasian Inst. Mining and Metall., Melbourne, 1955).

[5] Plaksin, I. N., *Proc. Intern. Mineral Processing Cong.*, 1960, 253. Plaksin, I. N., and Shafeyev, R. Sh., *ibid.*, 390 (Inst. of Mining and Metall., London, 1960).

MODIFIED CELLULOSE ION EXCHANGERS AND THEIR USE IN RIBONUCLEIC ACID FRACTIONATION

By Dr. L. BOSCH, Miss G. van der WENDE and Dr. H. BLOEMENDAL

Department of Biochemistry, The Netherlands Cancer Institute, Antoni van Leeuwenhoek-Huis, Amsterdam

SINCE their introduction by Peterson and Sober in 1956[1] modified cellulose ion exchangers have been widely applied for the fractionation of polyelectrolytes like nucleic acids and proteins. Two types of resins particularly proved to be of value. The first one is an ion exchanger containing basic groups derived from triethanolamine bound to cellulose through reaction with epichlorohydrin. This resin, called Ecteola-cellulose, has frequently been used for nucleic acid fractionation[2–4].

A second type of cellulose adsorbent displayed particular merits for the chromatographic separation of proteins. It contains carboxymethyl (CM-cellulose), diethylaminoethyl (DEAE-cellulose), triethylaminoethyl (TEAE-cellulose) and phosphate (P-cellulose) groups. Among these DEAE-cellulose may also be used for the resolution of nucleic acid mixtures[5].

The obvious need for improvement of the available fractionation techniques in connexion with metabolic work on nucleic acids, urged us to explore the possibilities of cellulose ion exchangers more systematically.

In two earlier papers[6,7] we described a fractionation and purification of microsomal (*m*-RNA) and soluble ribonucleic acid (*s*-RNA) from rat liver on the resin Ecteola. Although the results were valuable for the establishment of certain metabolic interrelationships between the two kinds of nucleic acids, definite disadvantages of this resin soon became apparent. Following adsorption of a polymer of relatively high molecular weight (for example, microsomal RNA) on Ecteola columns, complete recovery cannot be achieved without application of eluants of high *p*H, degradative for RNA, and destroying the secondary structure of DNA. Consequently, the value of

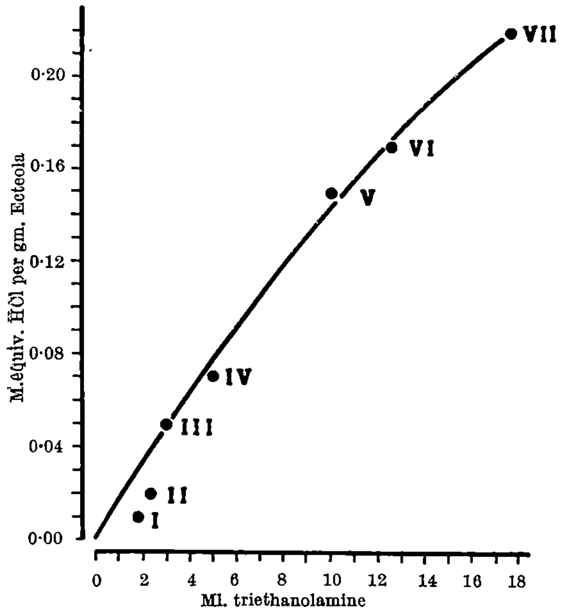


Fig. 1. Relationship between the amount of triethanolamine used for the preparation of Ecteola and the number of groups introduced as determined by potentiometric titration with hydrochloric acid. The resins were prepared as described in the text and designated Ecteola I to VII

Ecteola is rather limited although this resin is useful for the fractionation and isolation of smaller poly- and oligo-nucleotides and for analytical purposes. It is conceivable that a reduction in the density of binding sites on the exchanger would result in a weakening of the binding force between resin and the adsorbed polyanion. As a result the latter might then be eluted from the column by increasing the ionic strength of the eluant without necessarily raising the *p*H.

As an alternative such a weakening might be achieved by reducing the basicity of potential binding groups by introducing weaker bases than triethanolamine.

In this investigation both lines of approach have been followed. Various Ecteola resins with decreasing nitrogen content have been prepared and their capacities to fractionate soluble and microsomal RNA from rat liver have been compared. In addition, a new class of resins has been prepared by introducing nucleosides instead of triethanolamine into cellulose; the results of some preliminary fractionation experiments with the latter type of resins will be presented.

Experimental : Preparation of Ecteola-cellulose. Whatman cellulose powder (ashless, standard grade) was submitted for several hours to vigorous vibration in a sieve attached to the vibrator. Particles passing the sieve and having a diameter less than 0·05 mm. were used for the preparation of Ecteola according to Peterson and Sober[1].

Nucleoside cellulose was prepared by adding nucleoside instead of triethanolamine. Reactants were used in the following ratio: Whatman powder, 1 gm.; nucleoside, 1·9 m.mole; sodium hydroxide, 1 gm.; epichlorohydrin, 1 ml.; water, 2·5 ml.

Seven Ecteola resins were prepared according to Peterson and Sober[1], starting with constant amounts of cellulose (30 gm.) and epichlorohydrin (30 ml.) but increasing amounts of triethanolamine (1·75–17·5 ml.). Nitrogen content of the preparations as determined by potentiometric titration with hydrochloric acid, increased almost linearly with the amounts of triethanolamine, as shown in Fig. 1. Kjeldahl analysis yielded rather inaccurate results, probably due to the fact that very prolonged periods of heating with concentrated sulphuric acid were required for complete destruction. In most cases, however, about 90 per cent of all nitrogen groups determined by the Kjeldahl analysis could be accounted for by hydrochloric acid titration.

Fractionation of microsomal RNA from rat liver. Microsomal RNA was isolated from rat liver by means of phenol extraction[8,9], dialysed against 0·01 *M* phosphate, *p*H 5, and submitted to chromatography on the various Ecteola preparations. Some of the elution patterns, representative for our work on microsomal RNA, are illustrated in Fig. 2.

With increasing nitrogen content of the resins, a shift in these patterns towards the alkaline region is observed, and from Ecteola V onwards the amount of RNA requiring alkali for its elution rises. On Ecteola VII all the RNA emerges from the column in the alkaline region, indicating that fractionation experiments on resins of one particular nitrogen content only may not always yield a clear picture of the heterogeneity of the samples under investigation. Furthermore, it can be concluded from Fig. 2 that a fairly large proportion of *m*-RNA is recovered from various resins by sodium chloride elution. This fraction was designated as m_2-RNA, which means *m*-RNA recovered by washing with salt, although the varying proportions with which it is eluted in the salt region on various Ecteolas (Fig. 2) throws doubt on the homogeneity of this fraction. Ultracentrifugal analysis revealed that it is polydisperse[10].

Fractionation of soluble RNA. In earlier experiments[6] it was shown that a fraction of soluble RNA, which is involved in the transfer of amino-acids to the microsomes, also appeared in the salt region when fractionated on Ecteola. The question could therefore be raised whether microsomal RNA contains

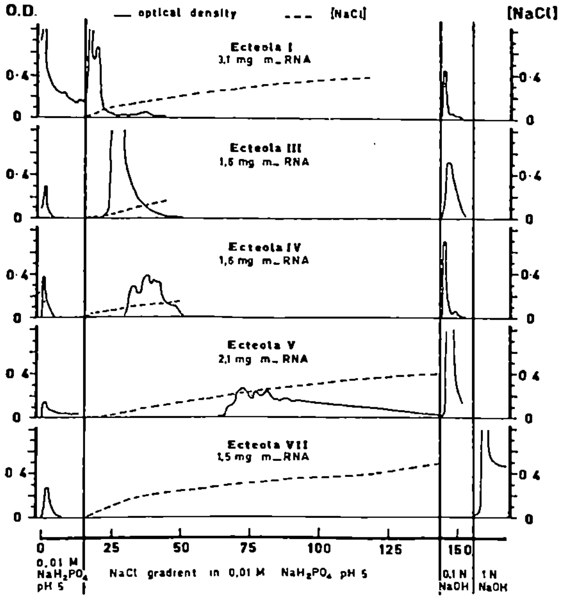


Fig. 2. Fractionation of microsomal RNA on Ecteola resins with increasing nitrogen content. Columns of 7 cm. length and 0·8 cm. diameter. A linear gradient elution system was established by applying a stock of 1 *M* NaCl–0·01 *M* phosphate (*p*H 5) solution and a mixing flask containing 1 l. of 0·01 *M* phosphate (*p*H 5). Optical density (O.D.) was read at 260 mμ

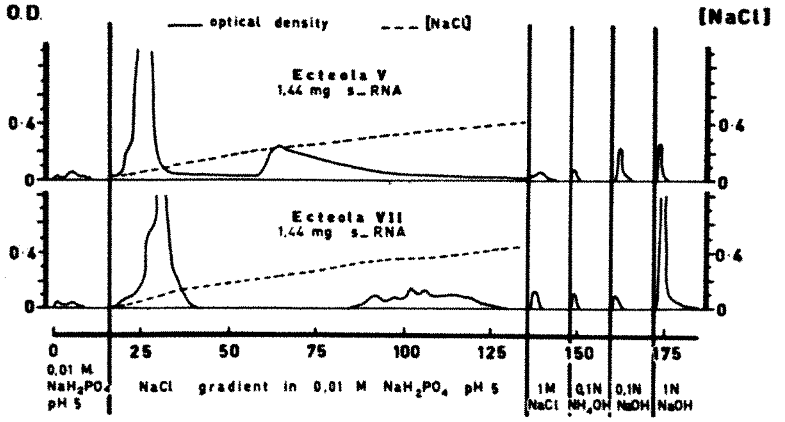


Fig. 3. Fractionation of soluble RNA on two Ecteola resins. For conditions, see legend, Fig. 2

soluble RNA, either as a native component or as a contamination due to inefficient washing of the microsomes. Support for the former assumption may be derived from communications by Smith *et al.*[11,12], who found that mouse-liver microsomes contain RNA (comprising 10–20 per cent of the total *m*-RNA) which will accept amino-acids, when incubated with enzymes at *p*H 5 under the usual conditions. Hence the behaviour of *s*-RNA on various Ecteola resins was examined, and some of the results are presented in Fig. 3. Chromatography of *s*-RNA on Ecteola V yields a fraction, earlier designated as s_3-RNA [6], which carries amino-acids and is indistinguishable from m_2-RNA, when fractionated similarly on Ecteola V.

A comparison of the elution patterns of *s*- and *m*-RNA on Ecteola VII, however, clearly demonstrates the non-identity of the fractions s_3 and m_2, and stresses once more the importance of using resins with varying densities of binding sites. A further characterization of the two fractions will be published elsewhere[10].

Two modifications in the elution procedure have been attempted: gradient elution with magnesium chloride in 0·01 *M* phosphate buffer of *p*H 5, and elution with increasing concentrations of phosphate, keeping the *p*H constant at 5. When *m*-RNA was fractionated on Ecteola IV only 10 per cent of the material could be recovered by applying the magnesium chloride gradient, the remaining 90 per cent appearing in the alkaline region. Similar results were obtained when the ionic strength was increased by applying a phosphate gradient.

Cellulose ion exchangers containing binding groups of lower basicity. The ease with which all kinds of groups may be introduced into cellulose suggested that nucleoside instead of triethanolamine might also react with cellulose through epichlorohydrin. According to this idea, four new ion exchangers have been prepared from adenosine, guanosine, cytidine and uridine, respectively. Preliminary results of *m*-RNA fractionation on the adenosine resin are illustrated in Fig. 4. More than 50 per cent of the polynucleotide adsorbed was recovered by applying a sodium chloride gradient elution system, the remainder by washing with ammonia and sodium hydroxide. *m*-RNA recovered by means of sodium chloride from the adenosine column and re-submitted to chromatography emerged from the column in exactly the same position, demonstrating a good reproducibility of this type of resin. Batches prepared at different times, however, did not always yield strictly identical fractionation patterns, necessitating a primary check before any new batch is going to be used.

Although the fractionation of *m*-RNA on guanosine cellulose has not been fully investigated so far, it appeared that *m*-RNA, when dissolved in 0·01 *M* phosphate, *p*H 5, is retained very weakly by the resin. Possibly elution has to be performed with buffers of very low ionic strength.

Uridine cellulose and particularly cytidine cellulose bind large proportions of *m*-RNA, recoverable by elution with alkali only.

Discussion. Ecteola and DEAE have a limited value for the fractionation of nucleic acids only due to the high *pK′* values of the two types of resins. (*pK′* of DEAE (in 0·5 *M* sodium chloride) is 9·5, that of Ecteola 7·5. Cellulose resins prepared from monoethanolamine (*pK′* 9) instead of triethanolamine (*pK′* 8·0) retained RNA more strongly than Ecteola did.) More favourable results might be expected by weakening the binding force between absorbent and polymer, for example, by replacing heteropolar linkages by van der Waals forces, hydrogen bonds, etc. In fact, a resin consisting of an insoluble polynucleotide seemed to meet these requirements ideally. Such a resin may be prepared by maximal loading of Ecteola with a synthetic polynucleotide of high molecular weight. Polyadenylic acid with a molecular weight of about 400,000, for example, is strongly bound by Ecteola[7], and can be recovered only by eluting with alkaline solutions. So far, exchangers of this type have not been investigated in this laboratory due to the fact that large amounts of polynucleotides are needed and were not available to us at the time. As an alternative, nucleosides were introduced into cellulose through reaction with epichlorohydrin, and preliminary results demonstrated that resins with satisfactory exchanging capacities were obtained. Of the four types of resins prepared, the purine cellulose exchangers showed the most promising fractionating properties. Uridine and cytidine cellulose retained

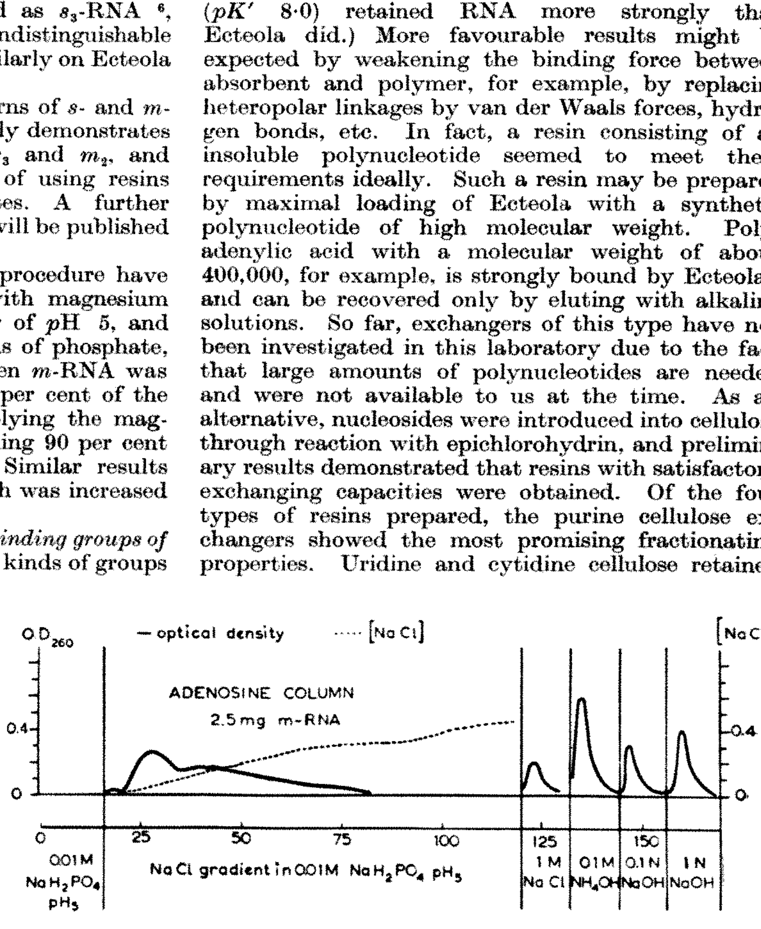


Fig. 4. Fractionation of microsomal RNA on adenosine cellulose. Column of 10 cm. length and 0·8 cm. diameter

m-RNA too strongly, and do not seem to have particular advantages over Ecteola.

The chromatographic behaviour of *m*-RNA on adenosine- and guanosine-cellulose is in accord with the lower dissociation constant of the NH_2-group of guanosine (pK'_a 1·6) as compared with that of adenosine (pK'_a 3·45) (ref. 13).

The extent to which ring opening of the purine and pyrimidine moieties occurs during preparation of the resin remains to be investigated. Strong alkali is required, and the temperature rises to more than 100° C. during the exothermic reaction between cellulose, epichlorohydrin and nucleoside. Potentiometric titration of adenosine cellulose did not reveal the presence of any additional basic groups and the pK'_a of the resin is the same as that of free adenosine. This may indicate that the purine structure remained unaltered during preparation of the ion exchanger. The strong retention of *m*-RNA by pyrimidine cellulose, however, throws doubt on this question so far as the pyrimidines are concerned.

[1] Peterson, E. A., and Sober, H. A., *J. Amer. Chem. Soc.*, **78**, 751 (1956).

[2] Bradley, D. F., and Rich, A., *J. Amer. Chem. Soc.*, **78**, 5898 (1956).

[3] Bendich, A., Pahl, H. B., Rosenkranz, H. S., and Rosoff, M., *Symposia Soc. Exp. Biol.*, **12**, 31 (1958). Bendich, A., Fresco, J. R., Rosenkranz, H. S., and Beiser, S. M., *J. Amer. Chem. Soc.*, **77**, 3671 (1955). Astrachan, L., and Volkin, E., *ibid.*, **79**, 130 (1957). Kondo, N., and Osawa, S., *Nature*, **183**, 1602 (1959); Kit, S., *Arch. Biochem. Biophys.*, **87**, 318, 330 (1960); *J. Biol. Chem.*, **235**, 1756 (1960). Tausig, A., and Creaser, E. H., *Arch. Biochem. Biophys.*, **83**, 436 (1959).

[4] Goldthwait, D. A., *J. Biol. Chem.*, **234**, 3245 (1959); **235**, 2025 (1960).

[5] Kit, S., *Nature*, **184**, 36 (1959).

[6] Bosch, L., Bloemendal, H., and Sluyser, M., *Biochim. Biophys. Acta*, **41**, 444 (1960).

[7] Bloemendal, H., Bosch, L., and Sluyser, M., *Biochim. Biophys. Acta*, **41**, 454 (1960).

[8] Gierer, A., and Schramm, G., *Nature*, **177**, 702 (1956); *Z. Naturforsch.*, **11** *b*, 138 (1956).

[9] Littauer, U. Z., and Eisenberg, H., *Biochim. Biophys. Acta*, **32**, 320 (1959).

[10] Bosch, L., van der Wende, G., Sluyser, M., and Bloemendal, H., submitted for publication in *Biochim. Biophys. Acta*.

[11] Smith, K. C., and Doell, R. G., *Fed. Proc.*, **19**, 318 (1960).

[12] Smith, K. C., Rebhun, S., and Kaplan, H. S., *Anal. Biochem.*, **1**, 202 (1960).

[13] Levene, P. A., and Simms, H. S., *J. Biol. Chem.*, **65**, 519 (1925). Levene, P. A., Bass, L. W., and Simms, H. S., *ibid.*, **70**, 229 (1926).

DIMER FORMATION AND THE TRANSFER OF GLYCEROL ACROSS THE ERYTHROCYTE MEMBRANE

By DR. W. D. STEIN*

Department of Colloid Science, University of Cambridge

A NUMBER of cell membranes have been shown to possess specialized systems which accelerate the movement down the concentration-gradient of specific metabolites. In the absence of such systems, these metabolites can cross the membrane slowly or not at all. Among such 'facilitated diffusion' systems[1] are those concerned in the movement of glucose into the human erythrocyte[2] and into muscle diaphragm[3] and the inward movement of sodium ions during the passage of the nerve impulse[4]. Most models that have been suggested for facilitated diffusion have involved hypothetical carriers in the cell membrane. The present investigation of the system which transfers glycerol across the human erythrocyte membrane[5] was initiated in the hope of isolating such a glycerol carrier. However, during the course of this work it has become clear that a model can be suggested which accounts for the facilitated diffusion of glycerol, without requiring the assumption of any membrane carrier at all.

It is perhaps useful to recall what constitutes the normal barrier to the movement of a molecule such as glycerol across the membrane, in the absence of any facilitated diffusion system[6]. The three hydroxyl groups of glycerol form strong hydrogen bonds with water. These hydrogen bonds anchor the glycerol in the aqueous phase, hindering its entrance into the hydrophobic lipid region of the membrane and hence reducing the rate at which it crosses the membrane. The membrane carriers that some previous workers have postulated have been 'alternative-hydrogen-bond formers' which can form hydrogen bonds with the glycerol molecules, competing with the water molecules. The carrier-glycerol complex has then its hydrogen-bonds internally saturated and can enter the cell membrane largely free of attached water molecules (Fig. 1*a*).

However, another method by which the glycerol molecule can have its hydrogen bond-forming capacity internally saturated is as follows (I am greatly indebted to Dr. D. A. Haydon of this Department for providing me with this idea, which is the core of the present theory): Two glycerol molecules may interact to form a dimer (Fig. 1*b*). Then each can act effectively as the 'carrier' for its fellow. On this basis I have derived the proposed model. I postulate the existence in the cell membrane of sites which bind pairs of glycerol molecules, thereby concentrating them and enabling them to interact with one another to form dimers. These dimers being free of attached water molecules (but still attached to the binding sites), are enabled to enter the hydrophobic region of the membrane. Inside the membrane the dimers dissociate from the binding site and can then traverse the membrane (either as dimers or, for the most part, having dissociated again into monomers). A summary of the evidence in favour of this model is presented here. Full details will be published elsewhere.

All the present measurements of red cell permeabilities have been made using the method of Orskov[7], in which the changes in optical density of a suspension of

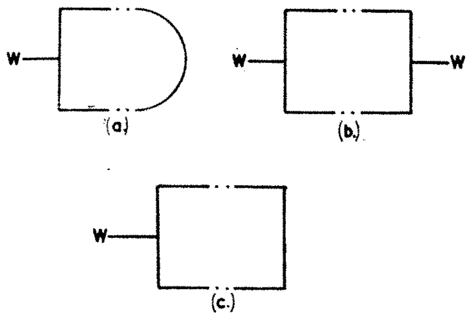


Fig. 1. Glycerol-'carrier' complexes: (*a*) glycerol + 'carrier'; (*b*) glycerol + glycerol; (*c*) glycerol + 1,3-dihydroxypropane. The left-hand molecule is, in each case, glycerol hydrogen-bonded to one water molecule

red blood cells on the addition of a solution of penetrating molecules is recorded. Analysis of the curves relating these changes in optical density with time shows that the curves are for the most part exponential functions. The half-time ($t\frac{1}{2}$) of such a curve (the time taken for the galvanometer deflexion recording the optical density to fall to half its initial value) can be related to the reciprocal of the rate at which the permeant crosses the membrane[5].

---

* Beit Memorial Research Fellow.

Postulate 1. *The glycerol acceptor site is bivalent.* (*a*) The activity of the glycerol facilitator is a function of *p*H. In Fig. 2, the reciprocal of the half-time for the entry of glycerol into the cell is plotted against *p*H for a number of buffer systems. Curve *A* in Fig. 2 is drawn on the assumption that one proton combines with a univalent glycerol acceptor to form the inactive complex, for example:

$$F + H^{+} \rightleftharpoons FH^{+} \qquad (1)$$

$$F + G \rightleftharpoons FG \qquad (2)$$

where F is the facilitator in the form capable of accepting a glycerol molecule G, FH^{+} is the inactive form, and FG is the glycerol-facilitator complex, which diffuses across the membrane. Curve B in Fig. 2 is drawn on the assumption that two protons are required to inactivate the glycerol-transferring system thus:

$$F + H^{+} \rightleftharpoons FH^{+} \qquad (1)$$

$$FH^{+} + H^{+} \rightleftharpoons FH_2^{++} \qquad (3)$$

$$FH_2^{++} \rightleftharpoons \text{inactive form} \qquad (4)$$

$$F + G \rightleftharpoons FG \qquad (2)$$

Clearly, it is this latter formulation that accords with the experimental results.

(*b*) Copper ions inhibit the glycerol facilitator. In Fig. 3, a plot of $1/t\frac{1}{2}$ against *p*M is presented. The free metal ion concentration was maintained with lysine as the metal buffer, using the copper di-lysine/lysine equilibrium. (The methodology of such diligand buffers will be presented elsewhere.) Again curve A is drawn for the metal equilibrium analogous to equations (1) and (2) involving a single copper ion, while curve B is drawn on the assumption that two copper ions are required to produce inhibition. Again the bivalent nature of the facilitator is manifest.

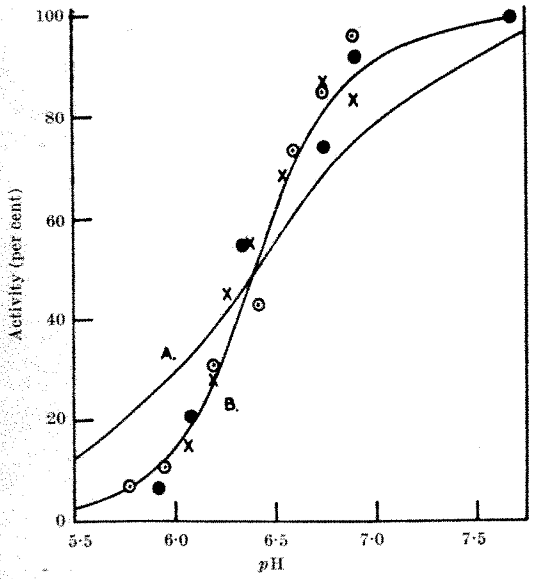


Fig. 2. Proton inhibition of the glycerol facilitator. Solid circles, histidine buffer; open circles, phosphate buffer; crosses, maleate buffer

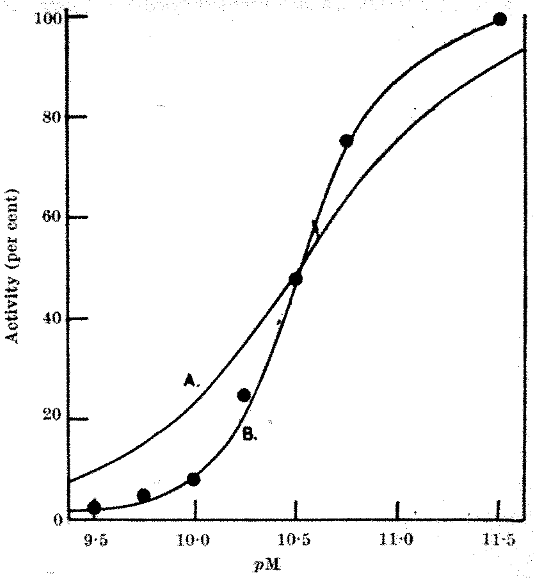


Fig. 3. Cu++ inhibition of the glycerol facilitator

(*c*) Both these experimental procedures are mute, however, as to the number of glycerol molecules present in the glycerol-facilitator complex. To investigate this, the activity of the system as a function of glycerol concentration was investigated. Red cells were equilibrated in successively increasing concentrations of glycerol and, after equilibration, a further quantity of glycerol, the same in each experiment, was injected into the suspension, and the optical density changes recorded. A theoretical analysis of the changes of cell volume with time in these conditions gave the following results:

The penetration of diethylene glycol, a molecule for which there is no reason to suspect that a facilitated diffusion system exists, is, at low concentrations, in very fair accord with the theory for penetration by simple diffusion. Somewhat surprisingly, the results for the penetration of glycerol also accord with simple diffusion theory. If, on the other hand, the theory for penetration by binding with a univalent carrier (equation (2)) is applied to the data for glycerol penetration, the absurd result is obtained that the dissociation constant of the carrier-glycerol complex is large and negative, that is, there is no affinity between glycerol and the membrane carrier. If, however, the bivalent theory is applied according to the equilibrium:

$$F + G \rightleftharpoons FG \qquad (2)$$

$$FG + G \rightleftharpoons FG_2 \qquad (5)$$

where FG_2 is the penetrating species, good accord is obtained below 1·3 M glycerol and an affinity constant of reasonable magnitude obtained.

The affinity constant for the first stage in the formation of the bivalent complex, that is, the interaction of single glycerol molecules with the acceptor sites according to equation (2) can be derived and is found to be 1·0 M. (The significance of this value will be discussed under postulate 3).

Thus it would appear that glycerol penetrates either by simple diffusion or by interaction with a bivalent acceptor, the results excluding the univalent acceptor model. But penetration by simple diffusion can also be excluded since glycerol penetration, but not that of diethylene glycol, is inhibited by protons (Fig. 2), by metal ions (Fig. 3), by substrate analogues (Fig. 4) and by a variety of other agents[8]. (See also an earlier paper[5] using a somewhat different experimental approach, where saturation of a membrane acceptor by glycerol could be demonstrated.) Thus the kinetic data demand that the facilitated diffusion system is bivalent towards the substrate glycerol as well as towards the inhibitors H^+ and Cu^{++}.

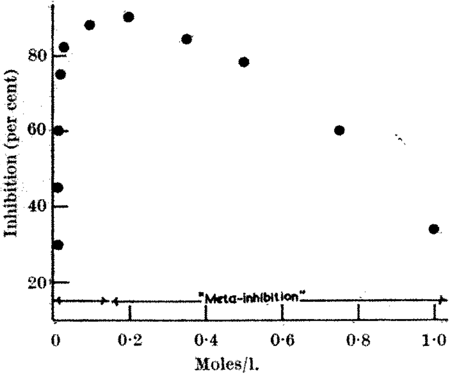


Fig. 4. Inhibition and meta-inhibition by 1,3-dihydroxypropane. Glycerol concentration fixed at 0·1 M, glycol concentration 0–1 M (abscissa)

Postulate 2. *Dimer formation leads to an increased rate of penetration.* The glycol 1,3-dihydroxypropane (or 103) is a competitive inhibitor of the glycerol facilitator. At a concentration of 0·01 M 103 (equal to one-tenth that of the glycerol added), the rate of entry of glycerol is inhibited by 50 per cent. But as the concentration of 103 is increased, the degree of inhibition passes through a maximum and then decreases again until at 1 M 103 the system is only 20 per cent inhibited (Fig. 4). This falling-off in the effectiveness of an inhibitor at high concentrations of inhibitor, or 'meta-inhibition', has here proved difficult to interpret, until now. However, let us suppose that glycerol and the glycol in the bulk of the solution interact as in Fig. 1*c* to form a dimer, albeit to a very slight extent. Then this complex will have a greatly increased rate of penetration across the membrane, as it has only one free hydrogen-bonding site available for binding to the water. On this view, then, meta-inhibition should bear no relation to the facilitated diffusion system. In fact, at concentrations of copper ions sufficient to block facilitated glycerol transfer completely, addition of 103 appears to restore the rapid transfer rate, and the same can be shown for hydrogen-ion inhibited cell preparations. Even more striking are the experiments using bovine blood. The bovine erythrocyte lacks the facilitated diffusion system for glycerol. However, if to a suspension of bovine red cells is added 0·5 M 103, there is a dramatic increase in the rate of transfer of glycerol and at a level of 1 M 103 beef red cells are, so far as their glycerol permeability is concerned, indistinguishable from normal human red cells (Fig. 5). 103 can, therefore, act as a membrane carrier for glycerol in both bovine cells and in inhibited human red cells.

Postulate 3. *The properties of the glycerol-binding site require no improbable assumptions as to energetics.*

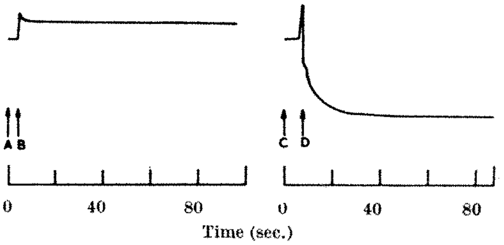


Fig. 5. Permeability of bovine erythrocytes to glycerol in the absence and presence of 1,3-dihydroxypropane. These are tracings of the experimental records of the optical density changes. At A, a suspension of bovine erythrocytes is added; and at B, glycerol injected to give a final concentration of 0·1 M. At C, the solution is changed and replaced by bovine erythrocytes equilibrated in 1 M 1,3-dihydroxypropane. At D, glycerol to 0·1 M is injected into this suspension

Direct experimental studies of glycerol dimer formation in dilute aqueous solutions are lacking. However, an estimate of the probable extent of dimer formation can be reached by considering the competition between water molecules and glycol molecules for each other according to the equilibrium:

$$AW + BW \rightleftharpoons AB + W_2 \qquad (6)$$

where A and B are hydrogen-bonding molecules interacting with water W to form AW and BW, and with each other to form AB. W_2 is the water dimer. Assume that all the hydrogen bonds AW, BW, AB and WW are equivalent and that only hydrogen bonds are concerned in the equilibrium. We can then apply the law of mass action to the equilibrium (6), and show that for a glycol concentration of 1 M and glycerol at 0·1 M, the extent of dimer formation (of the type of Fig. 1*c*) is some 0·1 per cent of the glycerol molecules. Now, a monohydroxy compound, such as in 1*c*, would be expected to penetrate the membrane about 10^4 times as fast as glycerol (ref. 6). Thus the increase in glycerol permeability in 1 M glycol should be then about $10^4 \times 10^{-3} = 10$-fold. In fact, at 1 M 103, glycerol penetration into bovine erythrocytes is speeded up some fifty-fold. This discrepancy between these two figures is not excessive considering the very drastic assumptions made in applying the law of mass action to equation (6). (In particular, the contribution to dimer formation by $-CH_2 \ldots CH_2-$ interaction has been ignored.) By similar arguments, it can be shown that to account for the experimentally observed value (1·0 M at 20° C.) of the affinity constant of single glycerol molecules for the binding site, 1,500 cal./mole of free energy must arise from binding other than at the hydroxyl group. This is a satisfactory figure for the interaction of 2 or 3 of the CH— residues of glycerol with hydrophobic sites on the glycerol acceptor.

We have seen then that interaction between glycerol and 1,2-dihydroxypropane, a molecule which can form dimers with glycerol, leads to a great increase in the rate of penetration of glycerol across the bovine erythrocyte membrane and that the extent of this increase is consistent with the extent of dimer formation to be expected in such solutions. Also, during the transfer of glycerol by the facilitated diffusion system across the human erythrocyte membrane, two glycerol molecules are bound at each acceptor site to form the penetrating complex. Finally, estimates of the energy of such interactions show that the degree of concentration of the glycerol molecules required for the system to operate is not beyond the capacity of a simple enzyme-like binding site.

The results strongly suggest, therefore, that the facilitated diffusion of glycerol occurs as a result of dimer formation between pairs of glycerol molecules bound at acceptor sites in the erythrocyte membrane.

It will be of interest to investigate whether this model can be applied more generally so as to include other facilitated diffusion systems.

I should like to thank Prof. J. F. Danielli (of King's College, London, where this work was commenced), and Prof. F. J. W. Roughton (of the Department of Colloid Science, Cambridge), for their hospitality in providing laboratory facilities for this work. Dr. D. A. Haydon and Dr. J. C. Kernohan of this Department were of invaluable help with their stimulating comments and discussion. I am indebted to the Royal Society and to Imperial Chemical Industries, Ltd., for financial assistance towards the cost of chemicals and apparatus.

[1] Danielli, J. F., *Recent Developments in Cell Physiology*, edit. by Kitching, J. A. (Academic Press, New York, 1954).
[2] Lefevre, P. G., *Symp. Soc. Exp. Biol.*, **8**, 118 (1954).
[3] Park, C. R., Bornstein, J., and Post, R. L., *Amer. J. Physiol.*, **182**, 12 (1955).
[4] Hodgkin, A. L., *Biol. Revs.*, **26**, 339 (1951).
[5] Stein, W. D., and Danielli, J. F., *Disc. Farad. Soc.*, **21** 238 (1956).
[6] Davson H., and Danielli, J. F., *The Permeability of Natural Membranes* (Cambridge Univ. Press, 1952).
[7] Orskov, S. L., *Biochem. Z.*, **279**, 241 (1935).
[8] Stein, W. D., Ph.D. thesis, University of London (1958).

SOLUBILITY OF DENATURED HÆMOGLOBIN VARIANTS IN ACID BUFFERS

By I. D. FRASER and Dr. A. B. RAPER
Pathology Department, Royal Infirmary, Bristol

WHEN methanol-fixed smears of human cord blood are exposed for 90 sec. at 37° C. to a 0·17 *M* phosphate–citrate buffer, washed, and afterwards stained with a Romanowsky dye, the degree of eosinophilic staining of individual erythrocytes varies from a negligible amount ('ghosts') to full coloration[1]. Erythrocytes in the blood of adults all appear as 'ghosts', and it is assumed that the fully coloured cells in cord blood are of fœtal type. It has not been proved that the retention of staining properties is in fact an expression of the insolubility of fœtal hæmoglobin (*F*) in the acid buffer, but we have obtained indirect evidence that this is so, and that the process allows a visualization of the hæmoglobin *F* present in individual cells.

The differential staining effect can only be obtained after denaturation of the hæmoglobin, either by methanol, acetone or heat. We therefore tested the solubility of hæmoglobins *A* and *F*, after acetone denaturation, in two ways: (*a*) after partial separation of a 1 : 1 mixture of hæmoglobins *A* and *F* by electrophoresis on 3 *MM* Whatman filter paper at *p*H 8·6, followed by immersion of the strip in acetone; (*b*) after precipitating a hæmolysate of cord blood with acetone. In the former case, immersion of the strip in the acid buffer for 2 hr. caused elution of most of the more anodically situated hæmoglobin (largely *A*), leaving the remainder (mostly hæmoglobin *F*) undissolved. In the second case, complete solution of 10 mgm. of denatured hæmoglobin *A* could be obtained in 10 ml. of the acid buffer, while with 10 mgm. of denatured hæmoglobin obtained from a cord blood hæmolysate containing 32 per cent of hæmoglobin *A*, only 28 per cent of the hæmoglobin was soluble in 10 ml. of acid buffer. Details of these experiments will be given later, but their results allowed us to proceed to investigate the distribution of hæmoglobin *F* in the erythrocytes of subjects with thalassæmia.

In smears from subjects with thalassæmia major, all the erythrocytes retained their hæmoglobin staining properties, reacting exactly as fœtal cells do (Fig. 1), when due allowance is made for the reduced thickness of many of the cells. In thalassæmia minor subjects in whom there was a measurable amount of hæmoglobin *F*, there was partial retention of hæmoglobin staining, and the degree of this did not vary from cell to cell (Fig. 2). This homogeneous picture was quite different from that given by cord blood or the blood of neonates. We interpret this as indicating that beyond the age of infancy hæmoglobins *F*

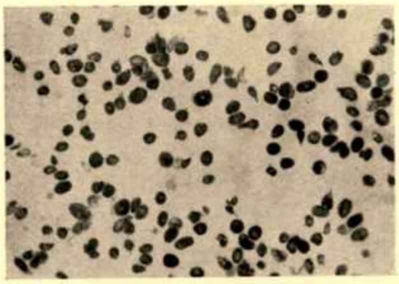


Fig. 1

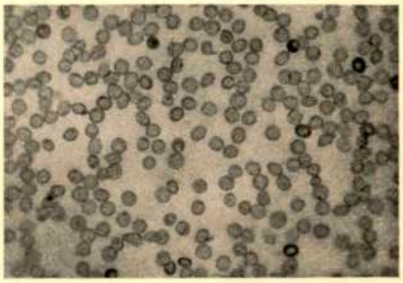


Fig. 2

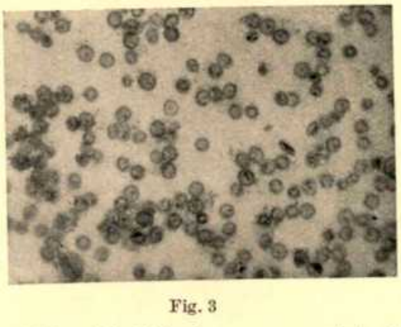


Fig. 3

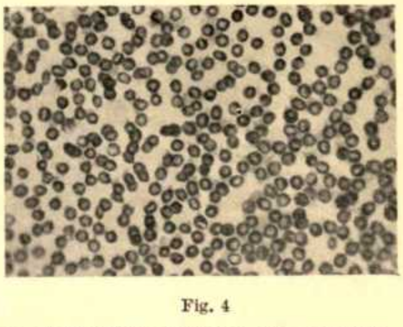


Fig. 4

Methanol-fixed blood smears, exposed to phosphate–citrate buffer at *p*H 3·4, and afterwards stained by May–Grünwald stain. (1) Thalassæmia major. (2) Thalassæmia minor. (3) Hæmoglobin *S*/*C* disease. (4) Sickle-cell trait and *A* must be synthesized together in each erythrocyte precursor. This double synthesis must occur in the marrow, the only erythropoietic organ in subjects with thalassæmia minor; and although there may be some extramedullary erythropoiesis in the case of thalassæmia major, exactly the same double synthesis must occur in other sites as in the marrow, since only one sort of cell appears in the blood.

It must be mentioned, however, that the proportion of 'insoluble' hæmoglobin retained in the erythrocytes from subjects of thalassæmia minor appeared excessive in relation to the low hæmoglobin *F* content of hæmolysates from these subjects. It is possible, therefore, that a part of their hæmoglobin *A*, though reacting normally to electrophoresis and alkali denaturation, may in fact be an aberrant molecular species, as postulated by Ingram and Stretton[2]. Some support for this is provided by the findings in congenital spherocytosis, where with proportions of hæmoglobin *F* in the same low range as in thalassæmia minor, there was no retention of hæmoglobin in the erythrocytes.

It was of interest to discover whether other hæmoglobin variants could be visualized in the same way as hæmoglobin *F*. Blood smears from subjects with various hæmoglobinopathies were submitted to the acid-lysis test, using buffers of several *p*H values. The results are shown in Table 1.

Where hæmoglobins *F* and *A* occur together, there was retention of hæmoglobin in the cells, and the degree of retention was roughly proportional to the amount of hæmoglobin *F*, with the exception, already noticed, of thalassæmia minor. The retention of hæmoglobin in the erythrocytes of a subject displaying hæmoglobin *H* is complicated by the facts that some hæmoglobin *F* was present, and that such persons are heterozygous for the thalassæmia gene. Hæmoglobins *C* and *D* are indistinguishable in their reaction from hæmoglobin *A*, while hæmoglobin *E* appears to be less soluble than hæmoglobin *A*. In the case of a subject with hæmoglobin *S*/*C* disease, every cell appeared to have retained about half its complement of hæmoglobin (Fig. 3). Since hæmoglobin *C* has been shown to be eluted from the cells of a hæmoglobin *C* trait carrier (*AC*), the insoluble fraction was presumed to be hæmoglobin *S*. Yet when the test was applied to sickle-cell trait (*AS*) subjects, carrying less hæmoglobin *S* than occurs in hæmoglobin *S*/*C* disease, almost the whole of the hæmoglobin appeared to be retained in every cell at *p*H 3·4 (Fig. 4) though all was eluted at *p*H 3·0. In sickle-cell anæmia the retention was even greater, being evident at *p*H 3·0. Some part of the retention in the latter case might be ascribed to the small amount of hæmoglobin *F* present, but this will not suffice to explain the difference between the *A* + *S* and the *S* + *C* subjects. We assume that either in its native state, or more probably during methanol denaturation, hæmoglobin *S* forms an insoluble complex with hæmoglobin *A* but not with hæmoglobin *C*.

Table 1. Retention of Hæmoglobin Variants in Fixed Erythrocytes Exposed to Acid Buffers

Cells containing hæmoglobins	Proportion of hæmoglobin *F* (per cent)	Retention of hæmoglobin in erythrocytes treated at *p*H: 3·4	3·0	2·0
A	< 1	0	0	0
F + *A* (16-week fœtus)	94	+ + +	+ +	+
F + *A* (thalassæmia major)	70	+ +	+	0
A + *F* (thalassæmia minor)	2·7–3·5	+	. . .	. . .
A + *F* (congenital spherocytosis)	3·3–3·5	0	0	0
S + *F* (sickle-cell anæmia)	8–17	+ +	+ +	0
A + *S* (30–45 per cent) (sickle-cell trait)	< 1	+ +	0	0
C + *S* (50 per cent)	. . .	+	0	0
A + *C* (40 per cent)	< 1	0	0	0
A + *D* (45 per cent)	< 1	0	0	0
A + *E* (30 per cent)	< 1	±	0	0
A + *H* (15 per cent)	3	+ +	0	0

+ + +, complete retention; + +, almost complete retention; +, about 50 per cent retention; ±, less then 33 per cent retention; 0, no retention.

We acknowledge receipt of specimens generously sent by Drs. E. R. Huehns, D. Stansfield, J. A. M. Ager, D. Speed and H. Lehmann.

[1] Kleihauer, E., Braun, H., and Betke, K., *Klin. Woch.*, **35**, 637 (1957).
[2] Ingram, V. M., and Stretton, A. O. W., *Nature*, **184**, 1903 (1959).

THYROGLOBULIN

By Dr. A. D. LITONJUA*
Thyroid Laboratory, Massachusetts General Hospital, Boston, 14

Effect of Acid

EDELHOCH has shown that alkali produces new sedimentable species of thyroglobulin which are smaller in molecular weight than the original material[1]. It was of interest, therefore, to find out if acid media produce the same dissociation effect.

Thyroglobulin was prepared in 1 per cent concentration in three buffer solutions according to a method in ref. 2: (1) 0·1 *M* phosphate buffer at *p*H 8·0; (2) 0·25 *M* acetate buffer at *p*H 3·5; and (3) 0·1 *M* hydrochloric acid–potassium chloride buffer at *p*H 1·5. Aliquots of each of these preparations were examined in the Spinco model *E* ultracentrifuge. Viscosity measurements were made in an Ostwald viscometer using standard procedures and calculations for intrinsic viscosities (ηi), expressed[3a] in centipoises per gm. thyroglobulin ($\eta i/c.$). Experimental temperature was 25° C.

The difference in the intrinsic viscosities of thyroglobulin at *p*H 8·0 and *p*H 3·5 was less than that at *p*H 8·0 and *p*H 1·5 (Fig. 1). Precipitation work to be described elsewhere showed the *p*H 3·5 preparation to be 50–60 per cent denatured, whereas *p*H 1·5 thyroglobulin was completely denatured. The latter result could account for the great difference in viscosity of the *p*H 1·5 material from either of the two preparations.

At *p*H 8·0, thyroglobulin showed three components, whereas two boundaries were evident at lower *p*H (Fig. 2). Molecular weight calculations using sedimentation values and viscosities of the different thyroglobulin preparations were made[3b]. The partial specific volume for thyroglobulin was taken as

---

* Fellow, China Medical Board of New York, Inc. Permanent address: Department of Physiology, College of Medicine, University of the Philippines, Manila, Philippines.

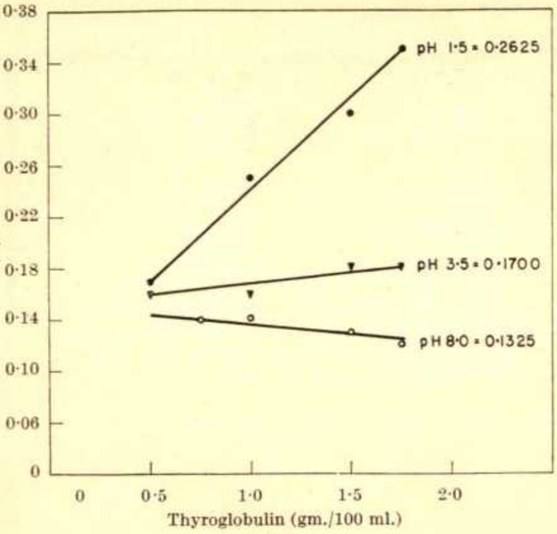
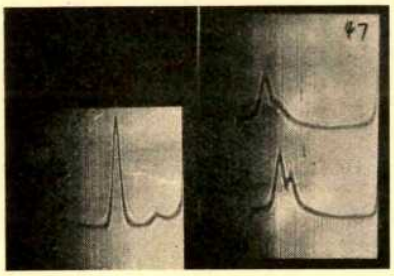


Fig. 1



(*A*) (*B*)

Fig. 2. Ultracentrifugal patterns of thyroglobulin. Direction of migration from left. All proteins, 1 per cent concentration. (*A*) Duration of spin, about 90 min. Thyroglobulin in 0·1 *M* phosphate buffer, *p*H 8·0. (*B*) Duration of spin, 47 min. Upper, thyroglobulin in 0·25 *M* acetate buffer, *p*H 3·5; lower, thyroglobulin in 0·1 *M* hydrochloric acid–potassium chloride buffer, *p*H 1·5

0·714 (ref. 1). Table 1 summarizes the different results obtained, together with the relative concentrations of each component derived from the areas under the peaks. At *p*H 3·5, a species, which was approximately one-half the molecular weight of the $S_{20,w}$ = 14·7 component of *p*H 8·0 thyroglobulin, was formed. The same finding was apparent at *p*H 1·5. The relative concentrations of the sedimentable components at *p*H 3·5 and *p*H 1·5 are approximately the same, so the $S_{20,w}$ = 8·0 or 8·3 components could not be denatured forms of thyroglobulin. It could be more plausible to explain these forms as arising from a dissociation phenomenon analogous to that observed by Edelhoch[1].

Table 1

	$S_{20,w}$	Relative concentration (per cent)	Molecular weight
Thyroglobulin, at: (1) *p*H 8·0	3·7 14·7 22·2	6 86 8	690,000
(2) *p*H 3·5	8·3 11·3	76 24	317,000
(3) *p*H 1·5	8·0 11·6	65 35	336,000

Evidence of Thyroid Protease Action

A MATERIAL rich in proteolytic activity against hæmoglobin can be obtained from thyroid glands by ammonium sulphate precipitation followed by methanol–sodium chloride fractionation[4]. However, this preparation is relatively ineffective against thyroglobulin[5]. Although an alternative method of thyroid protease preparation by acetone fractionation[7] provides substantial improvements in the yield and purity of the proteolytic activity against hæmoglobin, the activity against thyroglobulin is not affected, nor does treatment of the substrate by urea improve this activity. In previous work, the methods used for following proteolysis were those aimed at the detection of small peptide fragments or the known iodinated thyroid hormones. If, however, the main action of thyroid protease is to hydrolyse only a few crucial bonds in thyroglobulin, thus enabling other enzymes to act, then it is rather easy to see why these methods depict the enzyme as relatively ineffective against thyroglobulin. This report provides evidence for the premise.

Sheep thyroglobulin, prepared according to the method in ref. 2, was dissolved in 1 per cent concentration in either 0·1 *M* phosphate buffer at *p*H 7·0 or in 0·25 *M* acetate buffer at *p*H 3·5. The upper curve in Fig. 3*A* and the lower curve in Fig. 3*C* show the ultracentrifugal patterns of each thyroglobulin preparation. Thyroid protease showed one peak in either buffer (Fig. 3*A*, lower and Fig. 3*C*, upper).

After 2- and 6-hr. incubation at 37° C. in the Dubnoff shaker, aliquots of the *p*H 3·5 thyroglobulin and *p*H 7 thyroglobulin, each incubated with thyroid protease, were removed for examination in the Spinco model *E* ultracentrifuge and for viscosity investigation using standard procedures[3a]. Fig. 3*B* shows no change in the ultracentrifugal patterns of the *p*H 7·0 thyroglobulin hydrolysate. However, marked alterations were seen in *p*H 3·5 thyroglobulin (Fig. 3, *D* and *E*).

Molecular weights can be determined from the sedimentation constants and viscosities[3b]. The

Table 2

	$S_{20,w}$	Composition (relative percentage)	Intrinsic viscosity (centipoises)	Approximate molecular weight
I. At *p*H 8·0 1. Thyroglobulin, control	3·7 14·7 22·2	6 86 9	0·1299	690,000
2. Thyroid protease control	3·02	—	—	—
3. 2-hr. hydrolysate	2·9 (protease?) 13·83 20·70	36 >56 <18	0·1778	680,000
4. 6-hr. hydrolysate	3·02 (protease?) 13·83 20·80	32 >61 < 7	0·1841	720,000
II. At *p*H 3·5 1. Thyroglobulin, control	8·3 11·3	76 24	0·1626	317,000
2. Thyroid protease, control	2·51	—	—	
3. 2-hr. hydrolysate	1·96 17·44	>52 <48	0·1152	29,000
4. 6-hr. hydrolysate	1·42 15·48	>52 <48	0·0954	1,500

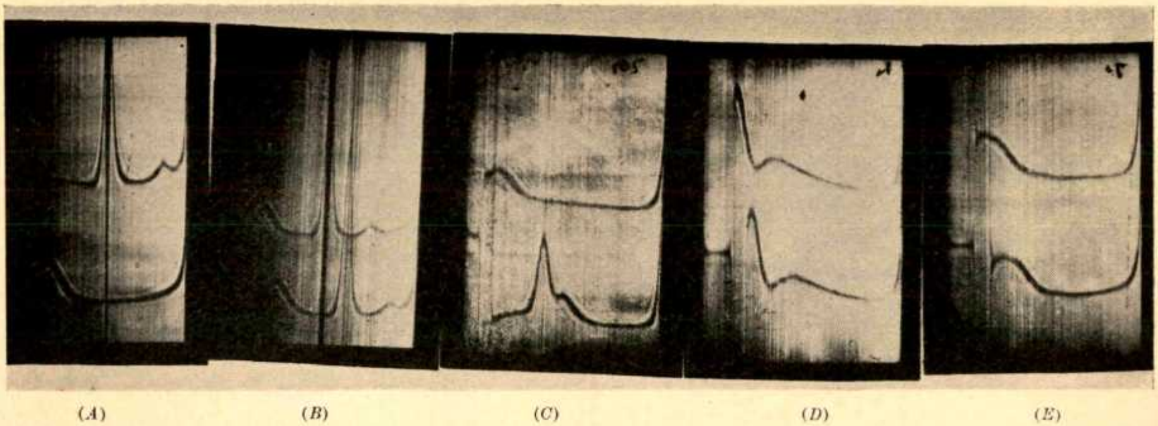


Fig. 3. Ultracentrifuge patterns. Centrifugation at 56,200 r.p.m. Direction of migration from left. (*A*) Duration of spin, 104 min. Upper, thyroglobulin in 0·1 *M* phosphate buffer, *p*H 8·0; lower, thyroid protease in same buffer. (*B*) Duration of spin, 90 min. Upper, 2 hr. thyroglobulin hydrolysate in 0·1 *M* phosphate buffer, *p*H 8·0; lower, 6-hr. hydrolysate, same buffer. (*C*) Duration of spin, 105 min. Upper, thyroid protease in 0·25 *M* acetate buffer, *p*H 3·5; lower, thyroglobulin in same buffer. (*D*) Duration of spin, 27 min. Upper, 2-hr. thyroglobulin hydrolysate in 0·25 *M* acetate buffer, *p*H 3·5.; lower, 6-hr. hydrolysate. (*E*) Duration of spin, 95 min. Upper, 2-hr. hydrolysate of *p*H 3·5 material; lower, 6-hr. hydrolysate. Faster moving component not visible in field at this point

viscosity measurements in this work were made on heterogeneous preparations, hence the calculated viscosity was the sum of viscosities of each component in the preparation. It was felt that this method, while not precisely accurate for molecular weight determinations, could give an approximation of what happened to the major component of the thyroglobulin preparation. As Table 2 shows, when no action of the enzyme was evident, the calculated molecular weight of the main thyroglobulin peak remained within the value of the control. The value of 0·714 was used as the partial specific volume of thyroglobulin[1]. Table 2 shows more than 50 per cent of *p*H 3·5 thyroglobulin reduced in a 2-hr. incubation to a component having a molecular weight less than one-tenth of the control. A further degradation occurred with progression of the incubation period.

With these observations and other work, it is postulated that thyroid protease exerts a 'priming' action on thyroglobulin by breaking it down into small polypeptide fragments. The formation afterwards of the thyroid hormones could take place as the result of such processes as the action of thyroid peptidases, action of serum peptidases, or reversal of biosynthetic pathways.

I wish to express my gratitude to Drs. M. J. Spiro and J. B. Stanbury for their advice and helpful suggestions.

This work was supported in part by Grant *A*-1880 of the U.S. Public Health Service.

[1] Edelhoch, H., *J. Biol. Chem.*, **235**, 1326 (1960).

[2] Derrien, Y., Michel, R., and Roche, J., *Biochim. Biophys. Acta*, **2**, 454 (1948).

[3] Colowick, S. P., and Kaplan, N. O., *Methods in Enzymology*, (*a*) **4**, 96 (1957); (*b*) **4**, 471 (1957).

[4] McQuillan, M. T., and Trikojus, V. M., *Australian J. Biol. Sci.*, **6**, 617 (1953).

[5] McQuillan, M. T., Stanley, P. G., and Trikojus, V. M., *Biochim. Biophys. Acta*, **7**, 319 (1954).

[6] Laver, W. G. and Trikojus, V. M., *Biochim. Biophys. Acta*, **16**, 592 (1955).

CORRELATION BETWEEN GROWTH-RATE OF THE SUCKLING OF VARIOUS SPECIES AND THE PERCENTAGE OF TOTAL CALORIES FROM PROTEIN IN THE MILK

By DR. F. W. BERNHART

Wyeth Institute for Medical Research, Radnor, Pennsylvania

BUNGE[1] proposed that there is a direct correlation between the growth-rate of the sucklings of several species (expressed as the number of days required for doubling the birth-weight) and the percentages of protein and ash in the fluid milk.

As a normal animal ingests only the amount of food necessary to satisfy the caloric requirement, maximal growth will occur only if this intake contains an adequate amount of protein (and all other nutritionally essential materials as well) for metabolic needs and for formation of new tissue. Powers[3] pointed out that as the requirement for calories determines intake of food, the percentage of total calories derived from protein is a better measure of the protein content of milk.

Using the percentage of total calories from protein as the criterion of protein content of milk, Powers[3] concluded that there is no correlation between the rate of growth of the suckling and the protein content. Brodie[4] concluded "that the situation is too complex for simple generalization although speed of growth, composition and physiological age are important factors in determining the evolutionary trend of milk composition".

Available data indicate that there is an inverse linear correlation between the percentage of total

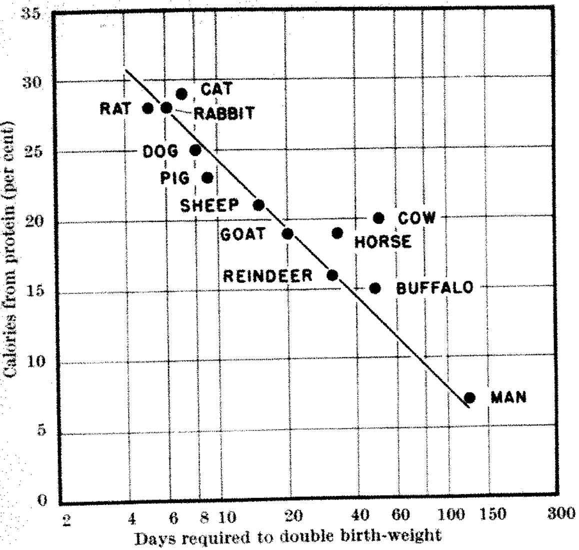


Fig. 1. The correlation between the percentage of total calories from protein and the number of days required to double the birth-weight. The formula of the line fitted by method of least squares is $y = 41 - 16{\cdot}5 \log x$ (data for cow not included)

calories supplied by protein in the milk and the logarithm of the number of days required to double the birth-weight of several species. The results for all species for which presumably reliable estimates of both variables have been found are listed in Table 1. A plot based on these results is shown in Fig. 1.

Early growth tends to follow the first-order reaction-rate[4]:

$$\frac{dW}{dt} = KW$$

where W is weight; t is time; K is first-order reaction-constant from which the following expression may be derived:

$$K = \frac{0{\cdot}693}{t_2}$$

where t_2 is days required to double the birth-weight.

The correlation between the log of K versus percentage calories from protein is positive and linear.

The caloric contents were calculated, using the factors 9, 4 and 4 cal./gm. for fat, protein and carbohydrate. Values for the time required for doubling the birth-weight were selected from work in which, so far as possible, optimal conditions were maintained. The values listed are considered to be the average for different varieties of a species.

The results indicate that the same relationship between protein supplied by the milk and rate of growth of various species is also true in man. The cow, however, appears to be an exception. In this species the percentage calories supplied by the protein of the milk is considerably greater than would be expected from the growth-rate. An excess of milk protein in relation to growth-rate is present, to a lesser extent, in the horse and buffalo. These three animals are herbivores with relatively slow growth-rates. A possible explanation for the excess of protein in their milks is that the food of the sucklings is also forage, and the higher protein content of the milk compensates for the lower protein content of the forage.

The elephant, not listed in Table 1, is of particular interest. Elephant milk has about the same protein content as human milk (7 per cent of calories)[3], but no exact information on rate of growth could be found. The Indian elephant is weaned at 4–5 years, puberty occurs at 14–15 years in the male and first calving at 15–16 years; full maturity is reached at the age of 40 and the life-span ranges up to 75 years. These similarities to man indicate that the elephant and human beings may have, during early life, similar rates of growth, predictable from the similar low protein content of the milk of these species.

Table 1. PERCENTAGE OF CALORIES FROM PROTEIN IN THE MILK AND GROWTH-RATE (EXPRESSED AS DAYS REQUIRED TO DOUBLE BIRTH-WEIGHT) OF VARIOUS SPECIES

Species	Total calories from protein (per cent)	Refs.	Days to double birth-weight	Refs.
Man	7	11	125	6, 7
Buffalo	15	12	48	17
Reindeer	16	12	31	3
Horse	19	12	33	18
Goat	19	11	20	12, 3
Cow	20	11	50	28
Sheep	21	12	15	19, 3
Pig	23	13	9	20
Dog	25	14	8	28, 2
Rabbit	28	15	6	21
Rat	28	16	5	8
Cat	29	15, 2	7	22, 4

According to Brodie[4] the speed of growth is an important factor in determining the evolutionary trend of milk composition rather than milk composition having a role in determining the growth-rate. That the protein content of milk is not the factor limiting the rate of growth was pointed out by Minot[5], as increasing the protein intake above that supplied by the milk has not been shown to cause an increase in the rate of growth. The only results have been obtained from human studies. The growth-curves of breast-fed infants during the period required to double the birth-weight[6] were practically identical to those obtained in an earlier investigation at the same location with a large number of infants preponderantly fed cow's milk formulæ supplying 2–3 times as much protein[7].

A rough approximation of the amount of protein supplied by milk in relation to needs of the suckling may be made by comparing the protein intake from milk with the protein requirement after weaning. The results in Table 2 indicate that the protein requirement of the weanlings of various species for optimal growth is about half the amount of protein supplied by the milk. A larger amount of protein supplied by milk, as compared with the dietary requirement after weaning, is necessary for the rapid rate of growth after birth in comparison with the markedly slower growth-rate after weaning[4]. In man, requirement for protein per kgm. body-weight after birth is 1·9 times the requirement at 6 months[9].

Table 2. COMPARISON OF THE PERCENTAGE OF CALORIES FROM PROTEIN IN MILKS WITH REQUIREMENT FOR OPTIMAL GROWTH AFTER WEANING

Species	Calories from protein (per cent)		Ratio	Source of protein	Refs.
	In milk (1)	Post-weaning require-ment (2)			
Man	7·2	4·4	0·61	Cow's milk	9
Pig	23	12	0·52	Cow's milk	23
Dog	25	17	0·68	Corn, yeast, casein	24
Rat	28	15	0·54	Casein plus 0·5 per cent methionine	25
Cat	29	19	0·66	Casein	26

These results indicate that generally the milks of the various species supply an amount of protein adequate for maximal growth but not much more.

The advantage of the evolutionary development of a milk composition which supplies enough protein for maximal growth of the offspring is evident. Nature's conservatism in limiting protein to the suckling is undoubtedly of benefit to the mother. The strain of lactation would be increased if excess protein were excreted in the milk, in view of the mother's limited stores of body protein and possible difficulties in obtaining an adequate protein intake to maintain positive nitrogen balance.

It is also possible that an adequate, though limited, protein intake may be of benefit to the offspring as well. Holt[10] has reviewed the recent literature on the effects of various protein-levels in the diet on body composition, enzyme-levels, effects of stresses, diseases and starvation and incidence of tumours. In several of these investigations diets supplying low or normal protein intake were found to be advantageous in comparison with those with higher amounts.

At the present time there is disagreement about the most advantageous level of protein intake of artificially fed infants. Gyorgy[27], a proponent of lower protein intakes for artificially fed infants, has used teleology as an argument; that is, there is a nutritional advantage associated with the low protein content of human milk because of purpose in Nature.

This teleological argument is strengthened by the present work, which supports the point of view that the same relationship between protein intake and growth-rate found in most other species is also true in man.

[1] Bunge, G., *Textbook of Physiological and Pathological Chemistry* fourth ed., 118 (1898).
[2] Abderhalden, E., *Z. physiol. Chem.*, **26**, 487 (1898); **27**, 408 (1898).
[3] Powers, G., *J. Pediat.*, **3**, 201 (1933).
[4] Brodie, S., *Bioenergetics and Growth* (Reinhold, New York, 1945).
[5] Minot, C. S., *The Problem of Age, Growth and Death*, 108 (Putnam, New York, 1908).
[6] Paiva, S. L., *Pediatrics*, **11**, 38 (1953).
[7] Jackson, R. L., and Kelly, H. G., *J. Pediatrics*, **27**, 215 (1945).
[8] Poiley, S. M., in *Rat Quality, A Consideration of Heredity, Diet and Disease*, 86 (Nat. Vitamin Found., New York, 1953).
[9] Committee on Amino Acids, *Evaluation of Protein Nutrition*, 16 (Nat. Acad. Sci. Pub. No. 711, Washington, D.C., 1959).
[10] Holt, jun., L. E., *Postgraduate Med.*, **27**, 783 (1960).
[11] Macy, I. G., Kelly, H. J., and Sloan, R. E., *The Composition of Milks*, (Nat. Acad. Sci. Pub. 254, Washington, D.C., 1953).
[12] Kon, S. K., *Milk and Milk Products in Human Nutrition*, 5 (F.A.O., Columbia Univ. Press, New York, 1959).
[13] Perrin, D. R., *J. Dairy Res.*, **21**, 55 (1954).
[14] Anderson, H. D., Johnson, B. C., and Arnold, A., *Amer. J. Physiol.* **129**, 631 (1940).
[15] Folin, O., Denis, W., and Minot, A. S., *J. Biol. Chem.*, **37**, 349 (1919).
[16] Luckey, T. D., Mende, T. J., and Pleasants, J., *J. Nutrition*, **54**, 345 (1954).
[17] Salerno, A., *Ann. Inst. Sper. Zootec. Roma*, **3**, 81 (1945).
[18] Nesini, R., Flade, E., Heidler, G., and Steger, H., *Arch. Tierzuckt.*, **1**, 91 (1958).
[19] Lewis, J. M., Mansfield, M. E., Garrigus, U. S., and Webb, R. J., *J. Animal Sci.*, **18**, 629 (1959).
[20] Forshaw, R. P., Maddock, H. M., Homeyer, P. G., and Catron, D. V., *J. Animal Sci.*, **12**, 263 (1953).
[21] Robb, R. C., *J. Exp. Biol.* (U.K.). **6**, 293 (1928–29).
[22] Dickenson, C. D., and Scott, P. C., *J. Nutrition* (U.K.), **10**, 304 (1956).
[23] Becker, D. E., Ullvey, D. E., and Terril, S. W., *J. Animal Sci.*, **13** 346 (1954).
[24] Gessert, G. F., and Phillips, P. H., *J. Nutrition*, **58**, 415 (1956).
[25] Harper, A. E., and Katayama, M., *J. Nutrition*, **49**, 261 (1953).
[26] Miller, S. A., and Allison, J. B., *J. Nutrition*, **64**, 495 (1958).
[27] Gyorgy, P., *West Virginia Medical J.*, **53**, 1 (1957).
[28] Spector, W. S., *Handbook of Biological Data*, 159 (Saunders, Philadelphia, 1956).

TREATMENT OF HOPS (*HUMULUS LUPULUS*, L.) WITH GIBBERELLIC ACID

Effect of Spraying with Gibberellic Acid on Yield and Composition

NASH AND MULLANEY[1] have described trials in which hops were treated with various concentrations of gibberellic acid. The maximum effect was observed when the plants were sprayed at the time of burr formation and accordingly a large-scale trial was carried out in which the Australian commercial variety Pride of Ringwood was sprayed with 12·5 p.p.m. of the potassium salt of gibberellic acid at this time. At harvest, the yield of the treated hops was 39 per cent higher than that of the controls, but on analysis the treated hops contained only 1·8 per cent of α-acid on which the brewing value largely depends, as compared with 10·2 per cent of α-acids in the controls. The overall yield of useful material for brewing was therefore depressed considerably by the treatment. It appeared possible, however, that the treated hops might have appeared ripe prematurely and that if they had been left in the field longer the resin content might have risen to the same level as that found in the controls.

The results reported here are of a similar field trial conducted under English conditions, using the variety Bullion (*Q*43). Samples were taken throughout the ripening period until resin synthesis was demonstrably complete. Twenty-five hills of two-year-old plants of the variety Bullion grown in the Withersdane Garden, Wye College, were sprayed with a solution containing 12·5 p.p.m. of the potassium salt of gibberellic acid on July 19, 1960, at which time the majority of the stigmas of the female plants were about $\frac{1}{8}$ in. long. Twenty-five similar hills served as the controls. Within a week of application the treated plants showed differences from the controls in their growth and development. The new leaves were noticeably paler green and the laterals were longer with more extended internodes. Furthermore, the cones in the treated plants developed more rapidly than those of the untreated plants, with the result that, 15 days after spraying, the dry weight per 100 cones of the treated hops was 4·32 gm. compared with 1·40 gm. with the controls. A summary of the analytical data obtained with these and later samples are given in Table 1. With the immature samples the normal methods of analysis for α-acid were insufficiently sensitive, but when the resin obtained from these hops was examined by ion-exchange chromatography[2] the presence of α-acids in the treated hops and their absence in the controls was clearly demonstrated. At the time of the second sampling (August 17, 1960), the visual difference between the treated and control samples had largely disappeared, but the treated hops still contained more resin and α-acids than the controls. Both samples contained a trace of steam-volatile oil, though when this was examined by gas chromatography none of the components of the essential oil of mature hops could be detected. At the third sampling (August 31, 1960), resin synthesis in both treated and control samples appeared to be complete. On the other hand, the essential oils continued to be synthesized until harvest (September 29, 1960), a point which has not emerged from earlier investigations.

Table 1. ANALYSIS OF CONTROL HOPS AND THOSE SPRAYED WITH GIBBERELLIC ACID (AFTER KILNING)

Sample	Date of sampling	Days after spraying	Nature of sample	Total soft resin (per cent) (ref. 3)	α-Acid/polarimetric (per cent) (ref. 3)	Essential oil (per cent)
1	4.8.60	16	Control	1·3	0·00*	—
			Treated	1·7	0·05*	—
2	17·8·60	29	Control	1·3	0·04*	0·03
			Treated	3·3	0·27*	0·025
3	31.8.60	43	Control	13·8	6·7	0·42
			Treated	13·8	6·7	0·38
4	14.9.60	57	Control	13·3	5·4	0·65
			Treated	13·5	5·7	0·78
5	29·9·60	72	Control	13·6	6·3	0·86
			Treated	13·7	6·5	1·00

* By ion-exchange chromatography (ref. 2).

At harvest the control hops yielded 3·25 lb. of green hops per bine while the treated hops only gave 2·59 lb. of green hops per bine. Both groups contained the same percentage of dry matter (23·3 per cent), soft resin, and α-acid. These results are therefore in complete contrast to those of Nash and Mullaney. The differences in the results of the Australian and British investigations cannot as yet be accounted for with certainty, but may be due to varietal or perhaps climatic conditions. For example, during the period of this trial 10·22 in. of rain were recorded at Wye, compared with a 25-year average value for this period of 7·27 in. The hours of sunshine per day and the mean temperature were also lower than the 25-year average. In view of these circumstances it is desirable to repeat the trial during at least one further year and the results of the more extended investigations will be reported in full detail later. Nevertheless, the present results are of interest in establishing that the earlier Australian results do not necessarily reflect a general effect.

We thank Dr. A. H. Cook, Dr. H. S. Darling and Dr. G. Harris for their interest and advice in these investigations.

R. STEVENS
Brewing Industry Research Foundation,
Nutfield, Surrey.

J. B. ROBERTS
I. H. WILLIAMS
Department of Hop Research,
Wye College (University of London),
Near Ashford, Kent.

[1] Nash, A. S., and Mullaney, P. D., *Nature*, **185**, 25 (1960).
[2] Howard, G. A., and Slater, C. A., *J. Inst. Brew.*, **66**, 305 (1960).
[3] Weston, E. W., *Rep. Dept. Hop Res. Wye Coll. for 1959*, 35 (1960).

Effect on Growth and Development

GIBBERELLIC acid has been shown to increase markedly the rate of elongation and the length of internodes in many plants[1]. Several workers have shown that juvenile characteristics, notably leaf form, can be prolonged or induced using this substance[2–4].

The rate of elongation of the hop during the vegetative or spring phase of growth is very considerable for a temperate plant. Shoots appear above ground in April and may reach a height of five metres by the end of the first week in June. Mature plants can sustain daily elongation rates of 8–10 cm. during this period. The leaf-form of the earliest leaves is entire; those developing later show a transition from the simple to the three, five or seven lobed 'adult' form. In the course of a series of experiments it was decided to examine in the summer of 1958 the effect of gibberellic acid on shoot-growth and leaf-form during the vegetative phase.

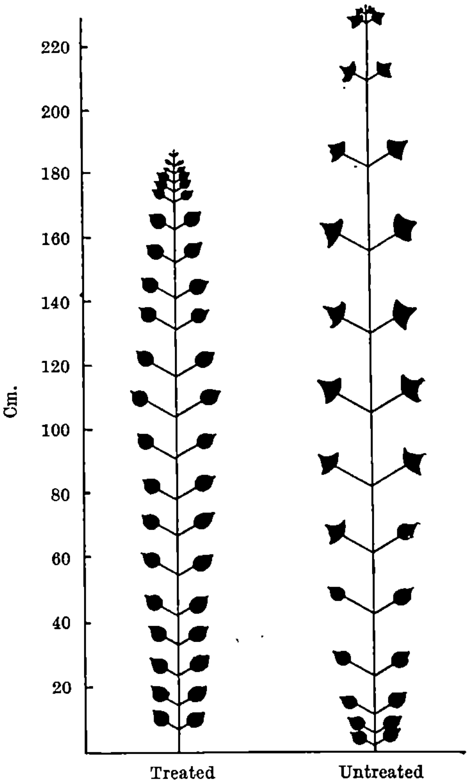


Fig. 1. Leaf-form and internode-length

Vernalized[5] seedlings were grown in a cool glasshouse. Treatment began immediately after the cotyledons had expanded. Gibberellic acid was applied, using a hand spray, at 5-day intervals at strengths of 1, 5 and 10 p.p.m. The treated seedlings elongated more rapidly initially, and the first two or three internodes were considerably longer than those of the controls. Thereafter the maximum internode-length of the treated plants did not exceed 14 cm. whereas the control plants developed successively longer internodes which reached a maximum length of 26 cm. when the experiment was concluded. The rate of elongation declined in the treated plants so that their mean height was significantly less than the untreated plants at the end of the experiment. The number of nodes developed by the treated plants was, however, a third greater than the controls. The treated leaves remained entire whereas all the untreated plants developed the typical lobed leaves before the ninth pair (Fig. 1).

It seems possible that the natural rate of elongation of the hop shoot, after the first few internodes have developed, represents the maximum capacity of cell elongation in this plant. In this case gibberellic acid may have had a secondary effect in speeding up the rate of meristematic development shown by the greater number of nodes which the treated plants developed.

The treated plants had a larger total leaf area than the controls, but their leaves were chlorotic, being very pale green in colour. This may have indicated a lower photosynthetic capacity than the controls where the leaves were normal in colour, and may have been responsible for the gradual decline in the rate of elongation of the treated plants.

From a fundamental point of view the present results are of some interest, but from a practical point of view it has yet to be shown that gibberellic acid will be of use in the growth of this crop.

I. H. WILLIAMS

Department of Hop Research,
Wye College (University of London),
Near Ashford, Kent.

[1] Brian, W., *Endeavour*, **16**, 161 (1957).
[2] Robbins, W. J., *Amer. J. Bot.*, **44**, 743 (1957).
[3] Njoku, E., *Nature*, **182**, 1097 (1958).
[4] Allsopp, A., *Nature*, **184**, 1557 (1959).
[5] Williams, I. H., *Rep. Dept. Hop Res. Wye Coll. for 1957*, 108 (1958).

CHROMATOGRAPHIC INVESTIGATION OF THE METHYL ALCOHOL/CHLOROFORM-SOLUBLE CONSTITUENTS OF HONEYBEE LARVAL FOODS

By N. G. PATEL, M. H. HAYDAK and R. LOVELL

University of Minnesota, St. Paul, 1

THE lipid content of the food given to the queen larvæ (royal jelly: RJ) remains fairly constant during the time of the larval growth, while that of the food given to 3–5-day-old larvæ (modified drone and worker jelly: MDJ and MWJ respectively) decreases considerably in comparison with the food of 1–2-day-old larvæ (drone and worker jelly: DJ and WJ)[1,2]. Since the nutrition plays a decisive part in the differentiation of caste in the honeybee[3], an investigation of any change[4] or heterogeneity[5] in the constituents of the larval food is important.

The larval foods were collected and stored as reported earlier[4]. In order to correlate the components contributed to the food by the glands, the hypopharyngeal, thoracic and mandibular glands were dissected from the freshly emerged bees and the 6-day post-emergence nurse-bees and placed in methyl alcohol/chloroform (1 : 2) and stored at – 40° C. with other larval food until further use.

For the analysis, each sample of the larval food was thawed and stirred, and a 1-gm. aliquot withdrawn for extraction. The extraction was carried out at room temperature in an Erlenmeyer flask with 20 ml. methyl alcohol/chloroform (1 : 2) mixture as the solvent and 20 min. stirring with the magnetic stirrer. The homogenate was filtered through a sintered-glass filter under suction. The process was repeated once with 10 ml. of the solvent mixture to assure complete extraction. Assays showed that close to 22 per cent of the material was extracted. The extraction of the glands was done by homogenizing them in the same solvent with the Potter–Elvehjem homogenizer and filtering through a 0·45μ pore-size 'Millipore' filter disk.

The separation of the constituents was done by ascending chromatography using thin films of silicic acid on glass plates[6]. 50 μl. of the filtrate samples were analysed by using different proportions of methyl alcohol/chloroform. 29 per cent methyl alcohol in chloroform gave the best separation. After the solvent front had migrated about 7 in., the plates were removed and dried at room temperature. Fluorescent spots were observed under 3660 Å. and 2537 Å. wave-length ultra-violet light. Following the ultra-violet observation, the replicated plates were treated respectively with different colour reagents, which included *p*-anisidine, ninhydrin, iron chloride, iodine vapour and concentrated sulphuric acid at 130° C. for half an hour. A composite representation of the various treatments is shown in Fig. 1.

It is seen from Fig. 1 that there is considerable complexity of constituents in normal and modified jellies of all castes. Apparently there is no difference in the long-wave (faint spots) ultra-violet fluorescent spots of young and old royal jellies (YRJ and ORJ). MDJ and MWJ are similar, but have one component less than DJ and WJ. YRJ and ORJ have one fluorescent component more than DJ and WJ with similar migration pattern, while the pattern of the MDJ and MWJ is different. These fluorescent spots could be of pteridine type as indicated by Remboldt and Hanser[7]. The one spot migrating to the top has originated in the mandibular glands of the nurse-bee and may be 10-OH-Δ-2-decenoic acid[8]. With the short ultra-violet light (dark spots), an additional difference is seen in MDJ and MWJ as compared with other jellies.

The colour reactions also illustrate the complexity involved and indicate from which gland a particular compound is contributed to different jellies. A positive reaction with iron chloride (a green spot turning yellow) is exhibited by YRJ, ORJ, MDJ, MWJ and extracts from the hypopharyngeal glands of nurse-bees; it is not present in DJ and WJ. Ninhy-

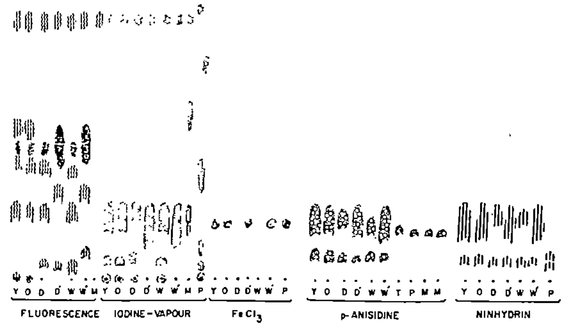


Fig. 1. Ascending chromatographic separation of honeybee larval foods and adult worker bee glands, revealed by various treatments on salicic acid plates

Y, young royal jelly (YRJ); O, old royal jelly (ORJ); D, drone jelly (DJ); D′, modified drone jelly (MDJ); W, worker jelly (WJ); W′, modified worker jelly (MWJ); M, mandibular glands of nurse bees; M′, mandibular glands of emerged bees; P, hypopharyngeal glands of nurse bees; T, thoracic glands of nurse bees

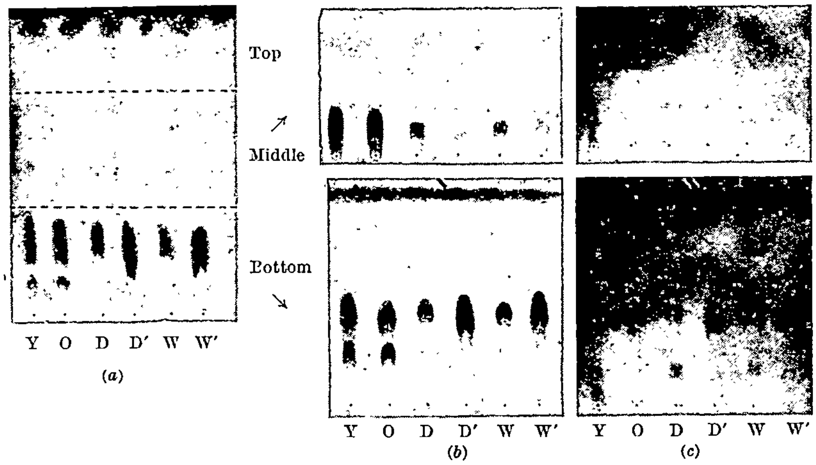


Fig. 2. Re-chromatographing of honeybee larval foods on silicic acid plates in different solvent systems. *a*, Solvent 29 per cent methyl alcohol in chloroform and sulphuric acid to char the constituents. The dotted line shows the divisions used for elution. *b*, Sulphuric acid charring. Upper photograph, middle division of (*a*) developed in 22 per cent methyl alcohol in chloroform; lower photograph, bottom division of (*a*) developed in 40 per cent methyl alcohol in chloroform. *c*, Iodine vapour treatment before charring. Upper and lower photographs correspond to upper and lower photographs of (*b*). Other abbreviations as in Fig. 1

drin- and *p*-anisidine-positive spots have similar migration patterns. The slower positive ninhydrin spots apparently originate in the hypopharyngeal glands of nurse-bees, while the migration of the *p*-anisidine-positive components from thoracic, mandibular and hypopharyngeal glands coincides with those fast-migrating spots of the jellies. The hypopharyngeal glands seem to contribute a larger share of *p*-anisidine-positive compounds. A preliminary paper-chromatographic separation shows that all the jellies have glucose and fructose, and sucrose is present in YRJ, ORJ, DJ and WJ while absent in MDJ and MWJ. On the other hand, MDJ and MWJ have maltose, which is absent in the rest of the samples.

The iodine vapour treatment indicating unsaturation exhibits four spots with a similar pattern in YRJ, ORJ, DJ and WJ, while there are only two components in MDJ and MWJ. These chromatograms were sprayed with concentrated sulphuric acid and heated to char the organic components (Fig. 2*a*). This treatment revealed other differences, but the resolution was not satisfactory. Better resolution was obtained by re-chromatography. Plates chromatographed with 29 per cent methyl alcohol in chloroform were divided into three as shown in Fig. 2*a*. The eluates from the top, middle and bottom sections were re-chromatographed in 5, 22 and 40 per cent methyl alcohol in chloroform, respectively. The top division did not reveal any significant qualitative differences as compared with the rest, which are shown in Fig. 2*b*. Quantitatively, the charring (Fig. 2*a*) in the top spots of YRJ, ORJ, DJ and WJ was at least twice that in MDJ and JWJ. This spot, as indicated earlier, may be 10-OH-Δ-2-decenoic acid. The concentration of re-spotted eluates in Fig. 2*b* at the point of origin was three times greater than that used earlier. The charring of the redeveloped plates was followed after the iodine treatment (Fig. 2*c*), so that the unsaturation could be compared with it. It is evident that the general pattern of the YRJ and ORJ remains the same and is not comparable with that of other jellies; however, there are some spots which are qualitatively similar though with quantitative differences. Unsaturation indicated by iodine vapour does not strictly correlate with the charring, particularly the slowly migrating components. This might be due to low concentrations of the components present and the amounts of reagents used to give coloured spots for visual observations.

A comparative study of the qualitative and quantitative changes of the components in relation to the age and different castes is of profound interest and importance. As is shown in this preliminary work, the biochemistry of the larval foods is quite complex. This complexity in turn is passed over to the nutritional phase and the phenomenon of dimorphism. The isolation, further characterization and incorporation of these biochemicals in a well-planned nutritional research investigation may help us to understand and control the formation of the queen and the workers.

[1] Shuel, R. W., and Dixon, S. E., *Can. J. Zool.*, **37**, 803 (1959).

[2] Haydak, M. H., *Ann. Ent. Amer.*, **50**. 73 (1957).

[3] Smith, M. V., *Cornell Univ. Agric. Exp. Stat. Memoirs*, **365** (1959).

[4] Patel, N. G., Haydak, M. H., and Gochnauer, T. A., *Nature*, **186**, 633 (1960).

[5] Weaver, N., and Law, J. H., *Nature*, **188**, 938 (1960).

[6] Stahl, E., *Pharmac. Weekblad*, **92**, 829 (1957).

[7] Remboldt, H., and Hanser, G. H., *Z. physiol. Chem.*, **319**, 203 (1960).

[8] Barker, S. A., Foster, A. B., Lamb, D., and Jackman, L. M., *Nature*, **184**, 634 (1959).

NERVOUS AND BIOCHEMICAL DISTURBANCES FOLLOWING HEXACHLOROBENZENE INTOXICATION

By Dr. F. De MATTEIS*, B. E. PRIOR and Prof. C. RIMINGTON, F.R.S.
Department of Chemical Pathology, University College Medical School, University Street, London, W.C.1

OUTBREAKS of cutaneous porphyria, involving several thousand cases, have occurred in districts of south-eastern Turkey since 1956[1]. Affected individuals, predominantly children, are said to exhibit photosensitivity, hepatomegaly and porphyrinuria but no abdominal or neurological symptoms. Poisoning by hexachlorobenzene, C_6Cl_6, introduced in bread made from dressed wheat intended for sowing, has been suspected as the cause of these disturbances. Ockner and Schmid[2] have confirmed such suspicions by administering hexachlorobenzene to adult male rats and observing neurological symptoms and increased urinary excretion of porphyrins and porphyrin precursors. Hepatomegaly with degeneration of liver cells was common in porphyric rats. There was no decrease in liver catalase or alteration in blood porphyrins or hæmoglobin, and no red fluorescence in the bone marrow *post mortem*, although the cortex of the long bones exhibited fluorescence. Of 33 rats started on the experimental diet, 13 died within the first month exhibiting terminal tremor, ataxia, weakness and paralysis but no major disturbance of porphyrin metabolism, while the remainder showed porphyrinuria, porphobilinogenuria and increased excretion of δ-aminolævulic acid after 2–8 weeks.

---

* British Council Scholar from the Institute of Clinical Medicine, University of Bari, Italy.

We have studied hexachlorobenzene intoxication in rabbits, guinea pigs, male and female rats and mice and have observed interesting differences in pattern of symptoms and chemical disturbances shown by each species. In addition, we have studied the susceptibility of the fœtal and new-born rat to poisoning from the hexachlorobenzene-fed mother. The biochemical picture seen in the rabbit most nearly resembles that described in man, and for this reason we have carried out most of our experiments with these animals. They are more rapidly affected by the poison and develop uroporphyrinuria, in contrast to the more usual coproporphyrinuria of the rat; disturbances of the central nervous system are relatively slight. Parke and Williams[3] found a single oral dose of hexachlorobenzene remained largely unchanged in the gut of rabbits after 5 days. There was no significant urinary or pulmonary excretion of metabolites. They found, however, increased urinary excretion of glucuronic acid, mainly in the free form. Cameron *et al.*[4] found subcutaneous injection of a dose as large as 0·5 gm. to rats produced no toxic effects.

Rabbits. Females (1·5–2·5 kgm.) were fed a diet prepared by powdering rabbit pellets ('Diet *S.G.* 1', Joseph Rank, Ltd.) incorporating 0·5 per cent of hexachlorobenzene, moistening with water, and then drying in shallow pans at about 60° C. Urine and fæces were collected separately. Five to six days were allowed for the establishment of basal excretion before institution of the drug. Pooled 2-day urines were analysed for uroporphyrin[5], coproporphyrin[6], porphobilinogen, δ-aminolævulic acid[7] and glucuronic acid[8]. Blood hæmoglobin and body-weight were recorded at the commencement and the end of the experiment. Tissue porphyrins[9] were determined after death. Organs were examined under ultra-violet light and those found to be fluorescent were prepared for ultra-violet microscopy. Paper chromatography was used for identification of the abnormal urinary constituents.

The general picture observed was development of increased urinary porphyrin, which was mainly uroporphyrin, after administration of the drug for about six weeks. This uroporphyrinuria became progressively more severe and was accompanied by a rise in coproporphyrin but a decline in glucuronic acid excretion, while there was no significant change in the levels of δ-aminolævulic acid or porphobilinogen. When determining the latter in the eluate from the 'Dowex 2' column[7], it was noticed that development of colour continued, reaching its maximum after about 6 hr. Chromatographic examination showed the substance responsible for this extra colour production to be not porphobilinogen but another Ehrlich reactor, possibly pyrrole-2-carboxylic acid. Rabbit urine is alkaline, and such a substance might be derived secondarily from a precursor (Gottschalk[10]). The identity of these materials and the relation they bear to administration either of the drug or of the heated diet (see later) are under investigation. The animals lost weight and suffered from oliguria and constipation, with diminished intake of food and water; their hæmoglobin remained unchanged. Unless killed earlier, they died after 8–12 weeks, having shown for the last week or so neurological symptoms in the form of tremor and paresis.

Out of 4 animals, 2 showed marked disturbance of porphyrin metabolism, another less marked, and another only slight disturbance. Nevertheless, all exhibited the same final neurological picture. Some of the biochemical changes in a typical example are shown in Fig. 1. Post-mortem examination revealed intense red fluorescence in ultra-violet light of the liver and adjacent tissue and of the bones, particularly of the metaphyseal region of the long bones but also along the diaphyphyseal insertion of the muscles. This picture of porphyrin distribution was confirmed by ultra-violet microscopy of suitably prepared transverse sections. There was no fluorescence in the bone marrow. The liver showed fatty change and patchy necrosis of varying degrees of severity. Under ultra-violet light, porphyrin was visible mainly in the centrilobular region. The degree of porphyrin fluorescence was related to the intensity of the porphyrin excretion during life. Necrosis, degeneration and focal calcification were seen in a few areas in muscles. No evident abnormality was found in the central or peripheral nervous systems in spite of the neurological symptoms shown by all animals.

Chemical analysis of organs showed massive quantities of a uro-type porphyrin in the liver, with only slight increase in the coproporphyrin and protoporphyrin fractions. Uro-type porphyrin was also detected in appreciable amounts in the serum and other organs examined. The qualitative reaction for porphobilinogen in the liver was negative. Paper chromatography of the uro fraction from the liver

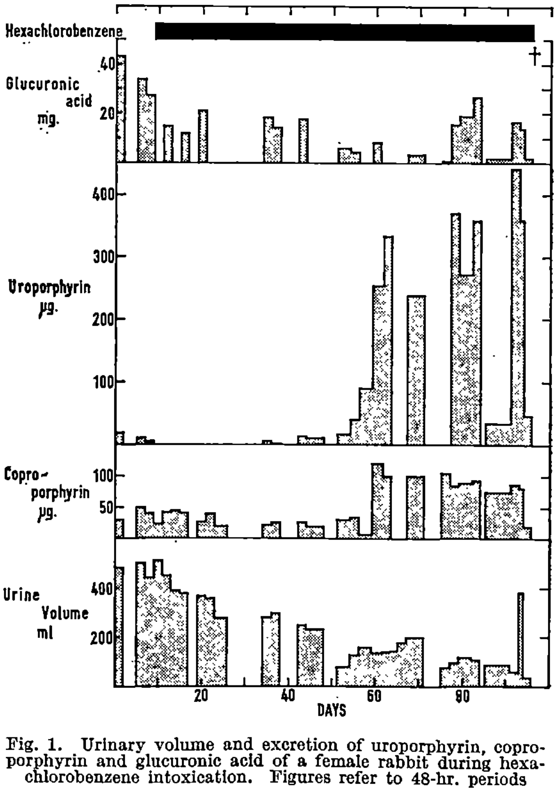


Fig. 1. Urinary volume and excretion of uroporphyrin, coproporphyrin and glucuronic acid of a female rabbit during hexachlorobenzene intoxication. Figures refer to 48-hr. periods

showed that it was mainly uroporphyrin III with some pseudo-uroporphyrin[11], whereas the uro fraction from the urine was predominantly pseudo-uroporphyrin. The urinary copro fraction was found to contain some coproporphyrin III together with pseudo-uroporphyrin and other porphyrins with chromatographic behaviour similar to hexa- and penta-carboxylic porphyrins.

Guinea pigs and mice. The level of dosage of hexachlorobenzene and means of administration in the diet were the same as for rabbits. These two species were found to be remarkably susceptible to hexachlorobenzene poisoning and to develop within 8–10 days very marked neurological symptoms. In guinea pigs, these took the form of continuous, rather coarse tremor of the extremities, interrupted by occasional clonic convulsions. There was also weakness, which was often more pronounced along one side of the body, so that the animals had difficulty in standing, could not right themselves if placed on the back and when moving in the cage they leaned against the wall for support. Mice exhibited a finer tremor involving the whole body, including head and tail. In place of convulsions, clonic contractions affecting mainly the hind limbs took place, so that the animals jumped like frogs. In both species there was a marked hyper-excitability, the symptoms being intensified by touch. If the drug was withdrawn within 24 hr. of development of nervous symptoms, recovery took place, but not otherwise. Death was preceded by progressive extreme weakness. All attempts to influence the symptoms described above by means of drugs such as hyoscine, chlorpromazine, phenylhydantoin, pyridoxine and asparagine were unsuccessful. Careful examination of the nervous systems of one guinea pig which died with typical tremor failed to reveal any structural changes.

Chemical analysis of the urine showed an early, marked increase in a δ-aminolævulic acid-like substance (see later), no significant changes being observed in porphobilinogen and porphyrin excretion except for a slight preterminal increase in coproporphyrin. Blood ammonia determinations were carried out in guinea pigs in view of the known ability of hexachlorobenzene to cause liver injury and the association of tremor and raised blood-ammonia in human cases of severe hepatic damage. All four guinea pigs so studied had ammonia values two to three times greater than their three normal controls. Liver histology, on the contrary, showed little evidence of damage apart from some fatty change.

Rats. The picture of hexachlorobenzene intoxication in rats resembles that in rabbits in its main features, with marked disturbance of porphyrin metabolism and only slight terminal neurological symptoms. The metabolic changes differed, however, in some important details. We observed a gradual increase in coproporphyrin and later of uroporphyrin with a terminal excretion of porphobilinogen which may be considerable. The identity of the latter was established by paper chromatography[12]; no chromogen reacting slowly with Ehrlich reagent was detected. The urinary coproporphyrin was identified chromatographically as mainly coproporphyrin III, only traces, if any, of the porphyrins intermediate between this and uroporphyrin being found. In addition to the picture described above, a prompt and sustained rise was seen in excretion of glucuronic acid and of a δ-aminolævulic acid-like material. The identity of the latter is still under investigation. Coincident with the terminal rise in porphobilinogen, there is an increased excretion of true δ-aminolævulic acid. Excretion of the δ-aminolævulic acid-like material and the rise in urinary glucuronic acid were both found to be associated with consumption of a diet prepared by heating and are therefore not relevant to intoxication by hexachlorobenzene.

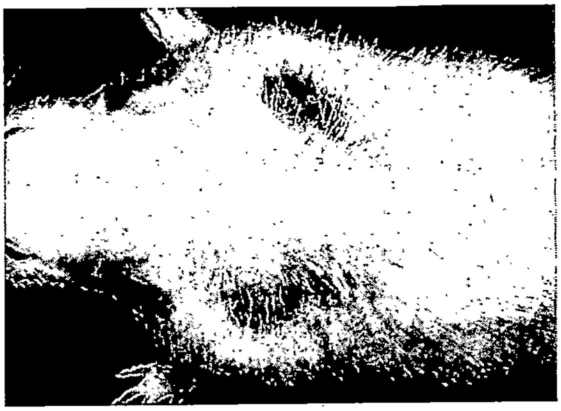


Fig. 2. Dermal lesions shown by a rat receiving hexachlorobenzene

In addition to the general picture of terminal neurological disturbance, some rats showed, at an earlier stage, cutaneous lesions in the form of depilated sores with hæmorrhagic crusts. These appeared before the animals developed porphyrinuria and were often symmetrically placed near the shoulder (Fig. 2). The animals showed signs of itching; hence scratching may have played some part in the development of these lesions. Quantitative tests for photosensitivity with a monochromator[13] were kindly carried out for us by Dr. H. A. Magnus but proved completely negative. Tests repeated during the phase of increased urinary porphyrin excretion failed to produce reaction at either 380, 400 or 420 mμ.

Two pregnant female rats were also given hexachlorobenzene in their food. One died, but the other was delivered normally and reared the young until they died with convulsions after 7–8 days. She was then given 3 normal week-old baby rats to foster. These died 3–4 days later with convulsions. The mother died 4 weeks later showing the usual picture. It would appear that the baby rat is particularly susceptible to the nervous effects of this poison.

The hexachlorobenzene used for the above experiments was the technical chemical, since examination had shown it to be free from less-highly chlorinated hydrocarbons. To eliminate the possibility that the observed toxic effects could be due to an impurity, some mice and rats were given pure hexachlorobenzene prepared as a standard for the estimation of halogen (British Drug Houses, Ltd.). These animals reacted in exactly the same way and after the same time as did those receiving the technical-grade product.

Discussion

Experiments with these four species of animal have confirmed the toxicity of hexachlorobenzene. Whereas cutaneous lesions dominate the picture in man and nervous symptoms are absent, our experimental animals have all shown striking neurological symptoms which were not associated with pathological change histologically evident in the central or peripheral nervous systems. It would therefore seem that these symptoms have a biochemical origin, and in this connexion it is interesting that affected guinea pigs had raised blood ammonia.

The nervous symptoms do not appear to be closely related to disturbed porphyrin metabolism, since in susceptible species they develop relatively rapidly and before pigment metabolism becomes markedly affected. Porphyrinuria must be looked upon as a chronic effect of continued hexachlorobenzene intoxication, not seen in those animals dying earlier from nervous complications.

The experiments on rats confirm Ockner and Schmid's[2] report of increased urinary porphyrin and porphobilinogen excretion after prolonged administration of hexachlorobenzene. A rise in true δ-aminolævulic acid usually occurs coincidently with the rise in porphobilinogen towards the end of the experiment. The cutaneous lesions we have seen in this species suggest chronic dermal irritation rather than photosensitivity.

The disturbance caused by the drug in the porphyrin metabolism of rabbits was more marked than in the rat and also resembled the human condition more nearly. As in clinical material from Turkey, uroporphyrin was the predominant urinary porphyrin and porphobilinogen was consistently absent. Post-mortem analyses revealed massive deposits of uroporphyrin III in the liver, appreciable uroporphyrin in the plasma and several other organs, including bones, but no significant increase of porphyrin in the bone marrow. The porphyrin in the urine was mainly of uro-type, including pseudo-uroporphyrin; chromatography of the copro fraction revealed that even here pseudo-uroporphyrin and hexa- and penta-carboxylic porphyrins were strongly represented. Such a distribution within the body strongly suggests a porphyria of hepatic type with possible interference to the uroporphyrinogen decarboxylating mechanism.

Increase in the excretion of glucuronic acid was not observed in the experiments on rabbits, although this has been reported by Parke and Williams[3] to follow administration of hexachlorobenzene. Cutaneous lesions were not seen; our rabbits were housed in diffuse light, and no tests for photosensitivity were carried out.

Many problems remain to be solved in connexion with hexachlorobenzene intoxication. Investigation of the pathogenesis of the porphyrin dyscrasia in different animals should help to throw light on the condition caused by this drug in man. The somewhat surprising findings of neurological disturbances arising in animals, which might well be due to the relatively high dosage-level employed, are of intrinsic interest and deserve further investigation.

Dr. W. R. Lee, of the Institute of Orthopædics, kindly prepared and examined by ultra-violet microscopy the sections of porphyrin-containing bone from our rabbits. We wish to thank Dr. J. Smith for advice on the histological material and Mr. R. Belcher and Mrs. A. Latter for technical assistance. Dr. M. D. Turner of the Royal Free Hospital kindly carried out the determinations of blood ammonia.

[1] Cam, C., *Dirim* (Istanbul), 34, 11 (1959). Schmid, R., *New Engl. J. Med.*, 263, 397 (1960).
[2] Ockner, R. K., and Schmid, R., *Nature*, 189, 499 (1961).
[3] Parke, D. V., and Williams, R. T., *Biochem. J.*, 74, 5 (1960).
[4] Cameron, G. R., Thomas, J. C., Ashmore, S. A., Buchan, J. L., Warren, E. H., and McKenny Hughes, A. W., *J. Path. Bact.*, 44, 281 (1937).
[5] Dresel, E. I. B., and Tooth, B. E., *Nature*, 174, 271 (1954).
[6] Rimington, C., Broadsheet No. 21, Association of Clinical Pathologists (November 1958).
[7] Mauzerall, D., and Granick, S., *J. Biol. Chem.*, 219, 435 (1956).
[8] Paul, J., Ph.D. thesis, University of Glasgow (1951).
[9] Schmid, R., and Schwartz, S., *Proc. Soc. Exp. Biol. Med.*, 81, 685 (1952).
[10] Gottschalk, A., *The Chemistry and Biology of Sialic Acids and Related Substances* (Camb. Univ. Press, 1960).
[11] Falk, J. E., and Dresel, E. I. B., *Biochim. Biophys. Acta*, 39, 485 (1960).
[12] Cookson, G. H., and Rimington, C., *Biochem. J.*, 57, 476 (1954).
[13] Magnus, I. A., Porter, A. D., McCree, K. J., Moreland, J. D., and Wright, W. D., *Brit. J. Derm.*, 71, 261 (1959).

MECHANISM OF SENSITIVITY TO HYDROSTATIC PRESSURE IN THE PRAWN, *PALAEMONETES VARIANS* LEACH

By PETER S. B. DIGBY
Department of Biology, St. Thomas's Hospital Medical School, London, S.E.1

MANY marine animals exhibit sensitivity to hydrostatic pressures of less than 100 lb./in.^{2}; some being sensitive to pressures equivalent to a depth of only a few centimetres of water. An increase of pressure causes active swimming upwards, a decrease usually causes passive sinking, but some forms swim actively downwards[1-3]. The mechanism has so far remained obscure.

The prawn *Palaemonetes varians* is sensitive to pressure, as are a number of Crustacea, and if confined in a bottle it will swim to the surface when a pressure of 40 lb./in.^{2} is applied and will sink again when it is released. The surface potential on a prawn, together with the electrode potential, investigated crudely by passing one copper electrode into the abdomen and placing the other in the water beside it, was found to be 50 mV. or more negative to the surrounding salt water. When the electrodes were connected through a 50-ohm galvanometer, the flow of current proved to be sensitive to pressure; although it declined rapidly, repeatedly increasing the pressure to 40 lb./in.^{2} for a few seconds, caused corresponding increases of current by up to 20 per cent. Investigation of the electric field in the water surrounding a prawn free in a shallow dish, showed that the ventral surface is the more negative. Vital staining with nile blue showed that the negative parts stain deeply, namely, the gills and the part of the abdomen bathed by the water coming from them. The potentials arise partly from differences of pH in the water bathing the two electrodes.

Since different parts of the prawn differ in potential, and small currents flow, it seemed possible that sensitivity to pressure might reside in the properties of a very thin film of gas produced electrolytically on some surface. To see if this might be feasible, a model was constructed in the form of a diffusion cell, consisting of a 6·0-cm. length of 7 mm. glass tubing with one end open and the other closed by a 'Cellophane' membrane. The tube was filled with

10 per cent gelatine and strong salt solution, and was supported vertically in the pressure bottle half-full of 3·5 per cent salt solution with the 'Cellophane' membrane below the surface and the open end above. One copper electrode was passed into the gelatine, the other into the surrounding salt solution. A diffusion potential was set up, the inside of the cell being negative to the salt solution as in the prawn. Single cells did not prove very sensitive to pressure at first, although they became so on standing for a day. When charged by a slightly larger outside potential, a freshly made cell immediately became sensitive to pressure.

As the prawn has different potentials across different parts of its integument, it may be regarded as two cells connected in parallel, one charging the other. In a further model therefore, two cells similar to that discussed here were mounted side by side in the same bottle (Fig. 1), producing potentials of −60 mV. and −20 mV., respectively, relative to the solution by virtue of their different internal salt concentrations. On connecting the two central electrodes through a 50-ohm galvanometer, the current recorded at once became sensitive to pressure, being 5–10 per cent greater at 40 lb./in.^{2}. This arrangement (Fig. 2) represents the free prawn, where the inner parts of the body surface of differing potentials are connected electrically by body tissues, the outer by sea-water. The current between these cells and the surrounding solution, an arrangement representing the prawn mounted on one electrode, is also sensitive to pressure. Experiments showed that the sensitive element is the weaker cell, charged by the stronger. The electric currents are very small and no gas can be seen until some hours have elapsed. Gas is, however, produced electrolytically in small amounts down to zero potential on a metallic electrode[4], and we may conclude that such a thin gaseous film forms immediately on connecting up. The layer may be only a few angstrom units thick, insufficient to produce obvious optical effects, but nevertheless having the compressibility of a gas and producing electrical effects which vary with pressure.

The outstanding question is whether such a gas film can be formed on the surface of an animal.

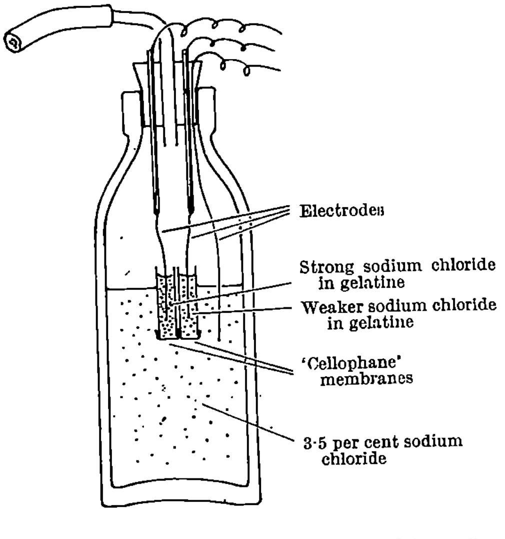

Electrodes
Strong sodium chloride in gelatine
Weaker sodium chloride in gelatine
'Cellophane' membranes
3·5 per cent sodium chloride

Fig. 1. Pressure bottle showing arrangement of two cells containing different concentrations of sodium chloride in 10 per cent gelatine

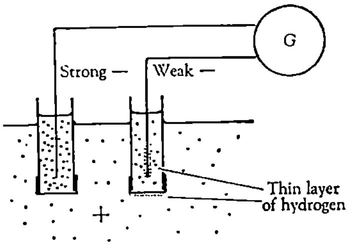


Fig. 2. Pressure-sensitive system, corresponding to arrangement in shrimp swimming free in water, when internal tissues take the place of the galvanometer

In order that gas shall be formed electrolytically, electron conductance must occur, as in the metallic electrode. That this is possible in the prawn may be demonstrated by applying a potential of 100 V. across the surface in distilled water, when hydrogen is freely evolved from much of the integument and, if a trace of copper sulphate is added, metallic copper is deposited. The hydrogen or copper ions are discharged by electrons passing through semi-conducting molecules or structures in sufficient numbers to produce visible amounts of discharge products at the high voltage. By the same procedure, hydrogen may be evolved from and copper deposited on a membrane of 'Cellophane' stained with methylene blue or coated with beeswax. At the lower voltages prevailing normally in the living animal, a correspondingly smaller number of ions will be discharged.

That such electrolytically produced gas layers occur in the prawn is suggested by the increase of sensitivity which follows the addition of salt and acid to the surrounding water. Further evidence is provided by experiments with surface-active substances that ionize to give charged complexes. Thus small amounts of 'Cetavlon' cetrimide B.P., which gives a negative bromide ion and a positive complex, completely destroyed the pressure-sensitivity of both the prawns and the diffusion cells. Small amounts of 'Teepol', which yields a negative complex, did not destroy this sensitivity in either, but sometimes enhanced it. As the positive complex which destroys the pressure sensitivity will migrate to the negative surface and there alter the charge, it is the most negative surface that is liable to blocking with 'Cetavlon' and is therefore first the gills. It is the outer surface which is coated because, after abolishing by 'Cetavlon' treatment, the sensitivity could be regained by immersing in sodium bicarbonate solution for some minutes to remove the substance, rinsing in acid water and transferring to fresh saline. The thin layers of hydrogen will, however, tend to be formed electrolytically on the outer surface wherever electrons can leak through the semiconducting structures in the integument under the influence of the normal potential drop across the surface.

Natural substances poison the surface, for prawns lose their sensitivity to pressure when crowded in a vessel with no seaweed and regain it when kept in a vessel containing seaweed. When lost, the sensitivity can also be restored by bicarbonate and acid rinses. Indeed the latter treatment, followed by the addition of a little salt, was found to induce or enhance sensitivity to pressure in the freshwater *Daphnia pulex*, which when newly caught showed only a trace of this reaction.

The widespread nature of charged surfaces in living biological material renders it likely that a capacity for pressure-sensitivity, based on the properties of a very thin film or perhaps partly adsorbed layer of electrolytically produced gas, may develop in a variety of situations where free hydrogen or hydroxyl ions are available against the surface of a charged membrane containing semi-conducting substances which allow a leakage of electrons.

Details of this work, which is continuing, will be published elsewhere. Certain initial experiments were carried out at the Kristineberg Zoological Station, Sweden; my thanks are due to the Director and his staff.

[1] Hardy, A. C., and Bainbridge, R., *Nature*, 167, 354 (1951).

[2] Knight-Jones, E. W., and Qasim, S. Z., *Nature*, 175, 941 (1955).

[3] Baylor, E. R., and Smith, F. E., *Recent Adv. Invert. Physiol.*, 21 (Oregon, 1957).

[4] Kortum, G., and Bockris, J. O'M., *Textbook of Electrochemistry* (Elsevier Pub. Co., London, 1951).

[5] Harris, E. J., *Transport and Accumulation in Biological Systems*, second edit. (Butterworth, London, 1960).

LOCATION OF POLYGENES

By Prof. J. M. THODAY

Department of Genetics, Milton Road, Cambridge

QUANTITATIVE genetics, hitherto, has been a subject distinct from Mendelian genetics in the sense that the specific genes segregating in any particular situation have not been handled as separate entities.

The first step of classical genetics is to identify the genes concerned as entities separable in heredity and to locate them on linkage maps so that thereafter they may be handled unequivocally in experiment. Our ability to do this depends on the classification of phenotypes into discrete groups, and the characteristic statistical techniques involved are those based on the χ^2 test of goodness of fit.

In the study of quantitative characters, on the other hand, the continuous nature of the relevant variation naturally leads to analyses of variance and, hence, to a biometrical approach. Using this approach has given us great insight into the problems, and we know that continuous and discontinuous heritable variation have in common the properties of segregation, dominance, interaction and linkage. It is established beyond doubt that the genes of biometrical genetics and the major genes of Mendelian genetics are subject to the same rules of inheritance for the same reasons; they are chromosomal[1].

Though the techniques of biometrical genetics are most elegant and informative, the resulting understanding of quantitative variation lacks the precision it would be given if we were to locate relevant genes with greater accuracy in linkage maps. Only when this has been done will we be able to look at a genic difference that has only been detected by its effect on a quantitative character and discover what it does and how it contributes to phenotypic variation.

The nature of the variation makes this difficult. In the best-studied examples, it is clear that the variation arises from genetic variation at a number of loci, and that the contribution of any locus to phenotypic variance is sufficiently small relative to that of the other loci and that arising from environmental causes and accidents of development to make the effects of that locus difficult to handle independently. To some extent this has affected our attitude to continuous variation, so that even those whose specific study is continuous variation, whether from a pure or applied point of view, seem rather often to act as if they thought that experimental control of the separate loci were in principle impossible. Statements occur in the literature such as "it is impossible to show the presence of these groups of linked polygenes by normal Mendelian methods"[2] or " . . . non-identifiability of genotypes and dependence on environment . . . have led to the development of a branch of genetics to which the name biometrical genetics is applied"[3]. Such statements may, however qualified by their authors, lead readily to a general feeling that any attempt to locate the genes of quantitative genetics would be defeated before it began. Those who are not specifically concerned with understanding quantitative genetics, but are interested in a character from some other point of view, may well be forgiven if they often seem to regard the demonstration that the character is inherited 'polygenically' as an end to investigation rather than a beginning.

Now it is clear that there is nothing in principle, though it may be difficult or laborious in practice, to prevent us from handling the genes concerned with quantitative variables by more or less classical genetic methods. Biometrical genetics is based on the fact that discontinuous variates, if there are enough of them, may be treated as if they were continuous variates, and it is implicit that the underlying genetic discontinuities are there to be uncovered. In fact, Johannson did just this by progeny testing and demonstrating that a number of different classes of inbred lines could be established from a strain varying in seed weight. Sax used a different approach when he used a marker to classify chromosomes and hence to detect linkage between major genes and quantitative genes[1], and the elegant experiments of Breese and Mather[4] go still further in this direction by splitting a chromosome into a number of component pieces and showing each of them to contain some of the relevant factors. But what has not been done is to exploit the possibilities fully by combining these two approaches.

My colleagues and I have had some success at this in the past few years, and it seems worth while to describe the basis of our techniques.

These depend upon the following simple principles: Suppose that we have found that a chromosome gives a higher value of a metric character than that given by a homologous chromosome marked with the recessive major genes a and b. We then test-cross individuals possessing this chromosome to the homozygous ab/ab stock, pick up equal numbers of the $++/ab$, ab/ab, $a+/ab$ and $+b/ab$ progeny and assay them for the metric character.

Let genes increasing the value of the metric character be H and their alleles L.

Suppose there is only one HL locus. Then the F_1 individuals test-crossed are either $H++/Lab$, $++H/abL$ or $+H+/aLb$ depending on the position of the HL locus in relation to the marker loci. Assuming the locus to be near enough to the markers for linkage to be detected, then the first two arrangements will give results such that one recombinant marker class is similar to one of the parental classes, and the other recombinant marker class is similar to the other parental class. The $+H+/aLb$ arrangement will give different results. Ignoring double crossing-over, the $++$ class will all be H, the ab class will all be L. A proportion of the $+b$ class will be H, the rest L, and reciprocal proportions of the $a+$ class will be H and L. The parental classes will therefore have distinct means but, since they are homogeneous, low variances. The recombination classes, on the other hand, will, as they are heterogeneous, have relatively high variances, their means being intermediate between the parental classes.

In investigating a particular chromosome, our first step is to search for a pair of markers which produce such results, that is to say, are outside the HL genes we wish to locate.

Now such results do not indicate there is only one locus. Suppose there are two, both between the marker loci, and suppose them to be in coupling. Then the F_1 individuals test-crossed are $+HH+/aLLb$. Again ignoring double crossing-over, the $++$ parental class of progeny are all HH, and the ab class all LL. The parental classes are homogeneous and different in mean. The recombination class $+b$ may be either HH, HL or LL, and the recombination class $a+$ either HH, LH or LL. Again the recombination classes are heterogeneous, and will have intermediate means and high variances.

The essential problem is to distinguish between the one locus and the two locus results. This is relatively easy. With one locus each marker recombinant class falls into two classes; they are either H or L. With two loci each marker recombinant class comprises three classes, HH, HL or LH, and LL. If, then, we progeny-test adequately a sufficient number of each marker recombinant class to determine how many classes it contains, we can determine whether one or two (or more) loci must be invoked to explain the difference in which we are interested. If we proceed far enough, we shall be able to assess not only the number but also the frequencies of the classes and hence obtain map-distances.

The investigation of a particular quantitative genetic difference thus proceeds in the following way: We first investigate which chromosomes are effective, by test-crossing with a stock in which, if possible, each chromosome of the haploid set is marked, and in the same breeding programme separate from one another the chromosomes of the genome in which we are interested. We then test the effective chromosome against a number of marked stocks in order to determine which are suitable markers. We then obtain adequate numbers of recombinant chromosomes and classify them by progeny-testing to see whether they fall into two or into three classes.

The feeling that the genes of quantitative genetics may not be readily located has been strengthened by the knowledge that much of the relevant genetic variation is cryptic. It is to this that Kempthorne referred in using the phrase "unidentifiability of genotypes" in the quotation referred to here. Such a situation occurs when two genomes, or more particularly two homologous chromosomes, differ genetically, but not in their phenotypic effects, because the relevant genes are in the repulsion phase. But this does not make it impossible to handle the genes concerned in breeding experiments. Consider again two loci: the heterozygote is HL/LH and will produce HH and LL gametes the presence of which can be detected in the progeny of such heterozygotes, for they will have a higher variance than the progeny of individuals homozygous for either chromosome. It was in fact just such a test that led Gibson and Thoday[5,6] to discover and locate the relevant loci in the population in which they were interested.

More sophisticated techniques can be derived easily to break down more complex situations so that they can be treated in this simple way, to deal with homozygous as well as heterozygous effects of the chromosomes being analysed, and to re-synthesize the analysed chromosomes to check conclusions. When it proves necessary to use markers themselves affecting the character concerned or marker stocks themselves possessing inconvenient combinations of relevant polygenes, the elegant breeding system of Breese and Mather[4] can readily be modified to include the only essential of our method, sufficient progeny-testing of sufficient recombinant chromosomes of each marker class to determine how many classes they fall into in terms of their effect on the quantitative character.

The main practical limitation of the technique seems to be the availability of suitable markers, and the time that can be given to the considerable work involved. No doubt there is also a lower limit of genetic differences that would make genes of slight effect difficult to isolate, because there is a limit to the reduction of other sources of variance that can be achieved, though inbreeding of the marker stocks can help. The technique may therefore lead to isolation of non-random samples of polygenes of larger than average effect, can only tell us what is the minimum number of loci we must invoke, and may not tell us how complex the loci identified are. Whether these will prove serious limitations we do not know.

We have, in fact, had considerable success using such techniques in the analysis of sternopleural chæta number loci in *Drosophila melanogaster*. We have explained a major response to artificial selection in a line 'dp1', of Thoday and Boam[7] in terms of two loci at about 28 and 32 centi-Morgans on the third linkage group. Wolstenholme has analysed the high-chæta number flies of the polymorphic population 'D+' of Thoday and Boam[8] and found them to be distinguished by two factors at about 49 and 51 centi-Morgans on the third linkage map. Using these and also other techniques made possible by lethality interactions of the genes concerned, Gibson has located two factors from the low-chæta number flies of the 'D+' population at about 27 and 47 centi-Morgans on the second linkage group. In the latter case we have for the first time shown how the relevant loci occur in the population from which the selection line is derived[6]. We are at present analysing second chromosomes derived from the populations of Millicent and Thoday[9,10] and have roughly located two factors in these at about 47 and 64 centi-Morgans.

None of these investigations has yet been pushed to the extent where we have tried to determine the relative frequency of the different classes, and in only one have we good evidence that two and not more than two loci are concerned. Nevertheless, we have gone far enough in each investigation to satisfy ourselves that the location of the genes is not too difficult, and are able to place them in the second and third linkage maps as shown in Fig. 1.

The precision of these locations is not great. We know 1 and 2 to be separate, and that both are between *h* and *eyg*; we know 3 and 4 to be about 20

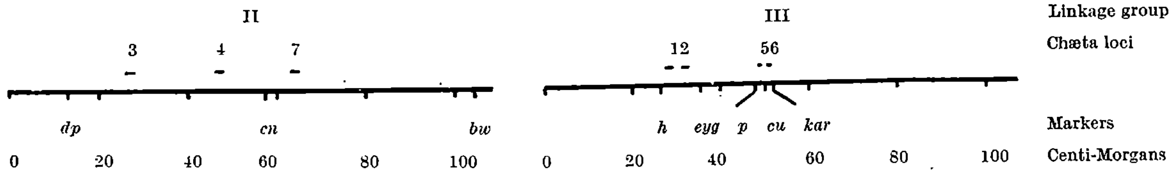


Fig. 1

centi-Morgans apart, that 3 is some 10 centi-Morgans to the right of *dp*, and 4 and some 8 centi-Morgans to the left of *cn*. We know 7 is to the right of *cn* and a long way from *bw*. We know that 5 is between *p* and *cu* and that 6 is to the right of *cu* and to the left of *kar*. The precision of the locations, and our knowledge of the chromosomes containing the relevant alleles are, however, sufficient for us to test future chromosomes against these and so identify new loci relatively easily.

Furthermore, we are now in a position to handle these loci in breeding experiments with sufficient precision to permit us to investigate their developmental effects. This should permit us to discover whether the different genes affect chæta number in different or similar ways. It may also permit us to discover other attributes of the various genotypes which are not so dependent on environmental and other sources of variance, and hence with some loci at least to classify genotypes directly from observations of phenotype that give discontinuity. It remains to be seen whether this will prove possible and if so whether it is only possible in special cases.

The present account of the principles of our methods is published with the view of encouraging others to approach their own problems in quantitative genetics with the possibility in mind of locating polygenes. An extensive attack on quantitative genetics made from this point of view as well as the biometric approach should be a great help in answering questions concerning the nature of polygenes, the randomness of their distribution in chromosomes, their relation to major genes and to heterochromatin, and whether or not they are a homogeneous or heterogeneous class of genes. At the same time, such an approach should bring new understanding of the consequences of artificial selection, and of the genetic architecture of natural and artificial populations.

[1] Mather, K., *Biometrical Genetics* (Methuen, 1949).
[2] Sheppard, P. M., *Natural Selection and Heredity* (Hutchinson, 1958).
[3] Kempthorne, O., *Biometrical Genetics* (Pergamon Press, 1960).
[4] Breese, E. L., and Mather, K., *Heredity*, **11**, 373 (1957).
[5] Gibson, J. B., and Thoday, J. M., *Nature*, **184**, 1593 (1959).
[6] Gibson, J. B., and Thoday, J. M., *Heredity* (in the press).
[7] Thoday, J. M., and Boam, T. B., *Genetical Research* **16**, 199 (1961).
[8] Thoday, J. M., and Boam, T. B., *Heredity*, **13**, 205 (1959).
[9] Millicent, E., and Thoday, J. M., *Science*, **131**, 1311 (1960).
[10] Millicent, E., and Thoday, J. M., *Heredity*, **16**, 199 (1961).

PREPARATION OF MITOCHONDRIA FROM THE PEEL OF APPLES

By Dr. J. D. JONES and Dr. A. C. HULME
Ditton Laboratory (Agricultural Research Council), Larkfield, Maidstone, Kent

TWO main difficulties in the isolation of active mitochondria from apple fruits are the low and well-buffered *p*H of the sap and the presence in the tissues of inhibitors, the chief of which has been thought to be of a polyphenolic nature, probably an oxidized *o*-dihydroxyphenol[1,2]. The successful isolation of mitochondria from the apple[3-5] has been achieved in recent years by the use of alkaline extraction media to neutralize the acid of the sap and by the use of peeled fruit, since the peel contains a relatively large amount of phenolic substances. The addition of ascorbic acid to the extraction medium has been advocated to keep these substances in the reduced form during the isolation procedures.

We are particularly interested in the metabolism of the peel of apples. Apart from the high concentration of phenolic substances in the peel[6] and their alleged effect on mitochondrial activity, peel tissue is difficult to disintegrate because of its leathery texture; satisfactory comminution cannot be achieved by grinding with sand in a mortar or by the use of a Waring blendor.

Grassman *et al.*[7] have shown that a polyamide material known as 'Perlon' or 'Ultramid' has a strong affinity for phenolic compounds, and Novikoff[8] has shown that the incorporation of polyvinylpyrrolidone (PVP) in the extraction medium preserves the morphological and biochemical integrity of mitochondria. Several workers[9-11] have advocated the use of PVP in the preparation of animal mitochondria, but its use has not been reported for mitochondria from plant sources.

A method has been developed for the preparation of mitochondria from the peel of Cox's Orange Pippin apples which incorporates the use of both 'Ultramid' and PVP and in which the tissue is disintegrated in a specially designed roller mill made in stainless steel. Briefly, the method is as follows: Peel (25 gm.) is added to a sucrose buffer solution (0·4 *M* sucrose) containing phosphate, citrate[12] ethylenediaminetetraacetic acid (EDTA) and 5 per cent PVP (molecular weight 25,000, pharmaceutical grade). The tissue is vacuum-infiltrated with the solution by placing in a vacuum desiccator for 10 min. and then releasing the vacuum. It is then passed through the diagonally serrated rollers of the mill and the ground material mixed thoroughly with the original solution to which has been added 5 gm. of 'Ultramid'. The tissue is then squeezed through two layers of muslin and the mitochondria separated from the filtrate according to the usual procedures for plant mitochondria. All preparative procedures are performed at 1° C. The particles are finally suspended in a solution of sucrose (0·3 *M*) and phosphate (0·2 *M*, *p*H 7·5) containing a small amount of adenosine triphosphate and adenosine monophosphate. These suspensions are used for assays of enzyme activity; uptake of oxygen and

Table 1. SUCCINIC AND MALIC OXIDASE ACTIVITY OF MITOCHONDRIA FROM VARIOUS SOURCES

Source of mitochondria	Avocado		Apple				Sweet potato	Etiolated pea leaf	Tobacco leaf
Ref.	(14)	(15)	(3)	(4)	(5)	Present work	(2)	(16)	(12)
Oxidation measured at									
Temp. °C.	25	30	30	30	25	25	25	30	30
*p*H	7·1	7·1	7·4	7·0	7·1	7·5	7·1	7·3	7·1–7·3
Qo_2 (N) on substrate:									
Succinate	338	336	276	210	300	640	432	1,196	116
Malate	94	—	133	195	—	518	—	200	106

outputs of carbon dioxide are measured by the standard Warburg manometric methods at 25° C. and at *p*H 7·5.

In presenting a summary of preliminary results, it is felt that the special features of the method may be of importance, not only to studies of the mitochondria of fruits, but also to studies of plant mitochondria and other enzyme systems in general. The use of PVP may also have a wider importance in relation to any large molecules precipitated and 'inactivated' by 'tannins'.

More than 100 active preparations have been studied which stain with janus green and satisfy the criteria for Krebs cycle activity of mitochondria[13], including oxidative phosphorylation. Pyruvate is readily oxidized in presence of 'sparking' amounts of malic acid. The best preparations have succinic dehydrogenase activity higher than that of most other preparations of plant mitochondria (Table 1).

The effect of the incorporation of PVP in the extracting medium varies with *p*H; and the uptake of oxygen and output of carbon dioxide to added succinate of preparations made with PVP over the range of *p*H 6·0–10·0 are shown in Fig. 1. It appears that these mitochondria are like those from other plant sources in that their activity is greatest when extraction is done at *p*H 7·0–7·5. Whereas in the absence of PVP the addition of ascorbic acid reduces darkening in the extracting medium, it does not enhance the activity of the particles prepared with or without PVP. The incorporation of polyamide ('Ultramid') in the extracting medium does not enhance the activity of the particles, but it has the useful physical effect of coagulating a gel, which is otherwise troublesome during the first centrifugation.

At this stage the role of PVP must be largely a matter for speculation. It does not appear to be acting merely to protect (by complex formation) the mitochondria from inactivation by polyphenols. A range of phenolic compounds (chlorogenic acid, caffeic acid, phloridzin and *o*-coumaric acid) added to the Warburg flasks at a concentration of 0·001 *M* causes at the most a 10 per cent diminution in the activity of succinic oxidase; gallic acid at the same concentration brings about a 25 per cent inhibition, and catechol and tannic acid are the only compounds tried which cause complete inhibition. Tannic acid inhibition is partially reversed by adding PVP to Warburg contents. Even a fairly concentrated extract of the 'phenolic fraction' from the leaves of Cox's Orange Pippin apples causes only 35 per cent inhibition. Apart from catechol and tannic acid all these compounds produce a slight initial diminution in the output of carbon dioxide when succinic acid (0·02 *M*) is added. At higher concentrations (0·02 *M*) chlorogenic acid causes an uptake of oxygen about equal to succinate, and when the two are added together the uptake of oxygen is 85–90 per cent of that expected from simple additions of the separate uptakes, while the output of carbon dioxide due to succinate is unaffected.

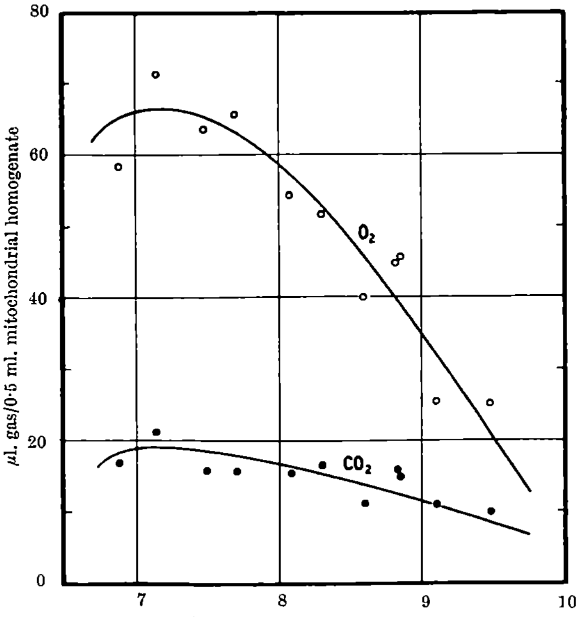


Fig. 1. Uptake of oxygen and output of carbon dioxide in the presence of 0·02 *M* succinate of mitochondria from apple peel prepared at various values of *p*H in the presence of PVP

Striking differences appear in the effect of the *p*H of the extracting medium, in the presence or absence of PVP, on the subsequent activity of the particles if the results are expressed on a nitrogen basis as compared with a given quantity of mitochondrial homogenate. Our methods are now sufficiently standardized and reproducible to justify this.

Results for preparations from comparable tissue are shown in Table 2. The rise in activity between *p*H 7·9 and 9·5 (extracting medium) without PVP, expressed on a nitrogen basis, agrees with the results of Lieberman[5] for mitochondria from apple pulp,

Table 2. μL. OXYGEN-UPTAKE (ACTIVITY) PRODUCED BY APPLE PEEL MITOCHONDRIA PREPARED IN VARIOUS WAYS
(Average rate per hr. over first 2 hr. in Warburg)

Without PVP			With PVP		
Final *p*H of extract used	Activity per unit N of preparation	Activity per 0·5 ml. of preparation	Final *p*H of extract used	Activity per unit N of preparation	Activity per 0·5 ml. of preparation
6·6	80	23	6·8	295	57
7·5	85	36	7·5	365	70
7·9	73	32	8·1	330	55
9·0	145	26	8·9	255	38
9·5	210	38	9·5	162	25

but this rise is almost entirely a reflexion of the fall of the nitrogen content of the preparation over this range of pH. The nitrogen content of the particles prepared with PVP varies between 0·112 and 0·230 mgm./0·5 ml. of homogenate whereas without PVP the range is 0·177–0·500 mgm. nitrogen with all the values below 0·300 mgm. being above pH 8·0.

It is suggested: (1) The relatively high nitrogen content of particles prepared without PVP at values of pH between 6 and 8 is a result of the formation of insoluble complexes between non-mitochondrial protein and phenolic substances in the peel, these complexes being spun down with the mitochondria. (2) At pH values above 8–9 these complexes are soluble and are not therefore spun down with the particles. (3) PVP forms soluble complexes with the phenolic substances which cannot therefore precipitate non-mitochondrial protein to contaminate the mitochondria. Suggestions (1) and (3) are supported by the results of Guttram and Higuchi[17], who showed that phenols form complexes with PVP, and by Swain (private communication), who showed recently that tannin extracts form inactive complexes with certain enzymes, the inactivation being reversed by PVP and some other polymers.

Until the uncertainty as to the true mitochondrial-nitrogen content of our preparations has been cleared up, we prefer to express our results in terms of the amount of mitochondrial homogenate (Fig. 1).

The general higher activity of preparations obtained with PVP, regardless of nitrogen content, may also be due to the removal by the PVP of inhibitors, whether they be of a phenolic nature or not.

Full details of this work will be published elsewhere.

[1] Hulme, A. C., Fifteenth Intern. Horticultura Cong., Nice 1959 (in the press).

[2] Lieberman, M., and Biale, J. B., *Plant Physiology*, **31**, 420 (1956).

[3] Tager, J. M., *Nature*, **182**, 1521 (1958).

[4] Hatch, M. D., Pearson, J. A., Millerd, A., and Robertson, R. N., *Aust. J. Biol. Sci.*, **12**, 167 (1959).

[5] Lieberman, M., *Plant Physiol.*, **35**, 796 (1960).

[6] Hulme, A. C., and Edney, K. L., *Phenolics in Plants in Health and Disease*, edit. by Pridham, J. B., 87 (Pergamon Press, London, 1960).

[7] Grassman, W., Homann, H., and Hartl, A., *Die Makromolekulare Chemie*, **21**, 37 (1956).

[8] Novikoff, A. B., *Symp. Soc. Exp. Biol.*, **10**, 92 (1957).

[9] Birbeck, M. S. C., and Reid, E., *Biochim. Biophys. Acta*, **20**, 419 (1956).

[10] Greenfield, R. E., and Price, V. E., *J. Biol. Chem.*, **220**, 607 (1956).

[11] Mahler, H. R., Wittenberger, M. H., and Brand, L., *J. Biol. Chem.*, **233**, 770 (1958).

[12] Pierpoint, W. S., *Biochem. J.*, **71**, 518 (1959).

[13] Millerd, A., *Encyclopaedia of Plant Physiology*, **12**, Part 1, 623 (Springer-Verlag, 1960).

[14] Millerd, A., Bonner, J., and Biale, J. B., *Plant Physiology*, **28**, 521 (1953).

[15] Romani, R. J., and Biale, J. B., *Plant Physiology*, **32**, 662 (1957).

[16] Smillie, R. M., *Aust. J. Biol. Sci.*, **8**, 186 (1955).

[17] Guttman, D., and Higuchi, T., *J. Amer. Pharm. A. Sci.*, **45**, 659 (1956).

AN AUSTRALOPITHECINE SCOOP MADE FROM A RIGHT AUSTRALOPITHECINE UPPER ARM BONE

By Prof. RAYMOND A. DART

University of the Witwatersrand, Johannesburg

TWO years ago, I compared[1a] several antelope cannon-bone scoops from the basal grey breccia at the Makapansgat Limeworks site with a late Victorian sheep cannon-bone scoop (or apple-corer) made by a Ledbury lad in Herefordshire in the last decade of last century. Another scoop from the same grey breccia has now been found, but it was made from the distal end of an australopithecine humerus and by the different technique (see Fig. 1, extreme right specimen) of spiral splitting. For comparison with this australopithecine humeral scoop, the first figure includes a cast of the Ledbury apple-corer, a rather ornate apple-corer that came from the sale of an old Natal settler's effects and an imitation of the Ledbury specimen made from an equally slender antelope cannon-bone by Mr. Ronald Klomfass, technical assistant in the Anatomy Department of the University of the Witwatersrand. When found in a Johannesburg curio shop, the purpose of the Natal apple-scoop was unknown to its purchaser, Mr. J. C. Cohn; he found its counterpart eventually in a cannon-bone scoop in the collection of the Pitt-Rivers Museum at Oxford. Later, when he learned about the repetitive occurrence of antelope cannon-bone scoops in the Makapansgat basal grey breccia, he kindly gave me this Natal specimen. Presumably, it had been brought from England some time last century, possibly from Oxfordshire, by the original settler (whose name unfortunately is unknown).

Since the original announcement in *Nature*, much corroborative evidence has been forthcoming (ref. 2 and through newspaper reports as well as correspondence) about cannon-bone scoops. Spatulæ of this sort were in such general demand by our edentulous forefathers for pulping crisp fruits and eating other pulped foods that they were freely reproduced in ivory (or silver) for folk more well-to-do. These imitations were often divided into screw-threaded halves so that the scoop-half fitted into the handle-half for easy transport[2b]. The geographical and temporal gap between Victorian England and australopithecine South Africa also has been bridged considerably by the series of bone spatulæ (see Fig. 2) to which Dr. A. J. Arkell has kindly directed my attention[3]. Incidentally, this series shows that cannon-bone scoops were even more prized by ancient Egyptians than by recent Europeans. The left-hand specimen is "certainly fossil" according to Dr. Kenneth Oakley and possibly Palæolithic; the second is typical of several excavated by Dr. Arkell himself from the Neolithic site at Shaheinab near Khartoum in the Sudan. The other two (Nos. 3 and 4) were found among the grave furniture of two Egyptian tombs of the third millennium B.C.

The Victorian cannon-bone scoops were made with the aid of a pen-knife; the ivory imitations were turned on a lathe, as the Natal specimen may also have been. Mr. Trevor Jones of Salisbury, Southern Rhodesia, aided me in discovering the bone-on-bone

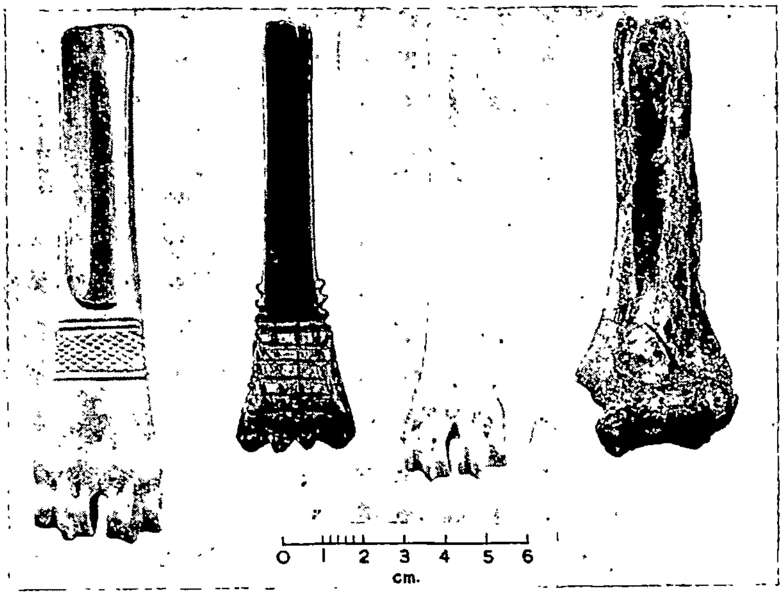


Fig. 1. An australopithecine and some recent bone scoops. The australopithecine humeral bone scoop from the Makapansgat grey breccia (extreme right) compared with —from left to right—a cast of the Ledbury bone scoop, the ornate Natal settler's specimen and an antelope cannon-bone scoop of similar calibre made recently in Johannesburg. Note the long spiral fracture of the australopithecine specimen and the abraded edges of the bone due to wear especially around its blade-like end

pounding or battering technique that produced the first australopithecine specimens described, and the precision labour it entailed[1].

This new australopithecine humeral scoop resembles more closely technically Dr. Arkell's cannon-bone spatulæ from the Nile Valley. It was prepared by the crack-and-twist technique, which was discovered by Mr. James W. Kitching and has been described elsewhere[4]. A single, well-controlled impact enough only to crack the bone enables one to twist the ends apart between the hands contrariwise.

Hazardous as it may appear to speculate from line-drawings how these Nile Valley bone spatulæ were prepared—especially when their surfaces have probably been smoothed by use, some possibly even on grindstones—the irregularity in the curvature of the broken bone surface of the Neolithic specimen (Fig. 2, No. 2) shows that it, at least, was first fractured by the same kind of crack-and-twist technique as *Australopithecus* employed at Makapansgat.

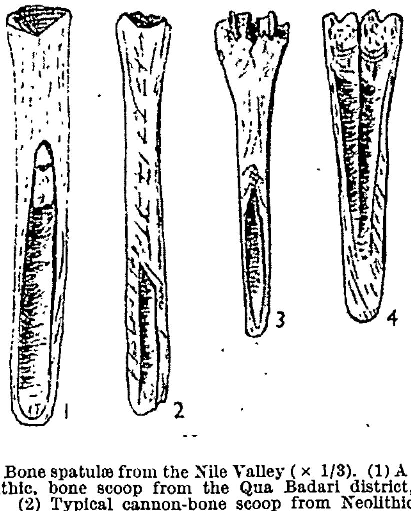


Fig. 2. Bone spatulæ from the Nile Valley (× 1/3). (1) A fossil (?) Palæolithic, bone scoop from the Qua Badari district, Middle Egypt. (2) Typical cannon-bone scoop from Neolithic site of Shaheinab, near Khartoum. (3) and (4) Cannon-bone scoops found among the grave furniture of two Egyptian tombs of the third millennium B.C.

This crack-and-twist technique must also have been used by *Sinanthropus* in China, to produce the spiral humeral fractures of antelope and other bovid bones at Choukoutien, by Kalkbank man in South Africa and by Mesolithic man in Portugal[4c,5]. So this early human bone-tool-making technique became dispersed over the entire Old World mass and has persisted from australopithecine times, when it was apparently invented, until very recently indeed.

This particular australopithecine scoop prepared from an australopithecine humerus also supports the inference made from the bowl-shaped condition in which the first female australopithecine occiput was found that *Australopithecus prometheus* was cannibalistic. The fact that it is the distal portion of a right humerus suggests that already some concept may have germinated in its fabricator's mentality that by its use he embodied some of his defunct opponent's vitality.

The value of the cannon-bone scoops or spatulæ lies not merely in the age-long persistence of the tradition they display but also in their overcoming the dental infirmities of mankind, for which they compensated. They explain the dental knives and scrapers and the food-pulping pestles and mortars of bone as well as the skull-bowls that accompany them. Upon these osteodontokeratic utensils domestic human family life was being built untold thousands of years before stone tools were adapted to its improvement. Von Koenigswald[6] has traced the human use of skull-bowls from *Sinanthropus* to Ngandong and recent times.

The interest of this australopithecine humeral scoop, like that of the australopithecine occipital skull-bowl, lies in the antiquity they reveal for human veneration of human bones above all other bones.

Acknowledgment is made to the South African Council for Scientific and Industrial Research, the Council of the University of the Witwatersrand, the Wenner-Gren Foundation and especially to the Wilkie Foundation of Des Plaines, Illinois, U.S.A., for the financial assistance which has maintained the field and laboratory work that has led to this and previous discoveries.

[1] Dart, R. A., (*a*) *Nature*, **183**, 844 (1959); (*b*) *S. Afr. J. Sci.*, **55**, 79 (1959).

[2] Higgs, J., *Countryman*, **55**, 347 (1958).

[3] Arkell, A. J., *Proc. Prehist. Soc.*, N.S., **23**, 234 (1957).

[4] Campbell, J. M., (*a*) *Nature*, **183**, 1542 (1959); (*b*) *Brit. Dent. J.*, **8**, 178 (1960).

[5] Dart, R. A., (*a*) *Illus. Lond. News*, **234**, 798 (May 9, 1959); (*b*) *J. Dent. Assoc. S. Afr.*, **14**, 164 (1959); (*c*) *Amer. J. Phys. Anthrop.*, N.S., **17**, 87 (1959); (*d*) *Amer. Anthrop.*, **62**, 134 (1960); (*e*) *S. Afr. J. Sci.*, **56**, 71 (1960).

[6] Koenigswald, R. von, *Anthrop. Papers, Amer. Mus. Nat. Hist.*, **43**, 211 (1951).

LETTERS TO THE EDITORS

PHYSICS

Absorption Spectrum of γ-Irradiated Lead Silicate Glass

In an earlier communication[1] it was stated that the absorption spectrum of γ-irradiated lead silicate glass showed three absorption bands at 1·7 eV., 2·3 eV. and 3·3 eV. respectively. The separation of the spectral curve into these bands was obtained by assuming that the peak at 3·3 eV. was uninfluenced by the presence of any other bands. The points in the vicinity of this peak were used to estimate the first Gaussian and the other bands were obtained by successive subtraction.

As the amount of available data increased, further work employing different methods has been done in order to confirm this separation, making only the assumption that the spectral absorption curve consists of a small number of Gaussian-shaped components.

This problem is well suited to solution by means of an electronic digital computer and a programme was written using the equation:

$$y = \Sigma A_i \exp B_i (x - c_i)^2$$

where A_i corresponds to the peak height of the absorption band at a wave-length of $1/c_i$, B_i is a function of the half-peak width of this band, and x is the reciprocal of the wave-length at the point considered. By a process of successive iteration it was found that the full spectral absorption curve in the region 350–1,000 mμ could be accurately represented as the sum of two Gaussian absorption bands with maxima at 2·54 eV. and 3·36 eV.

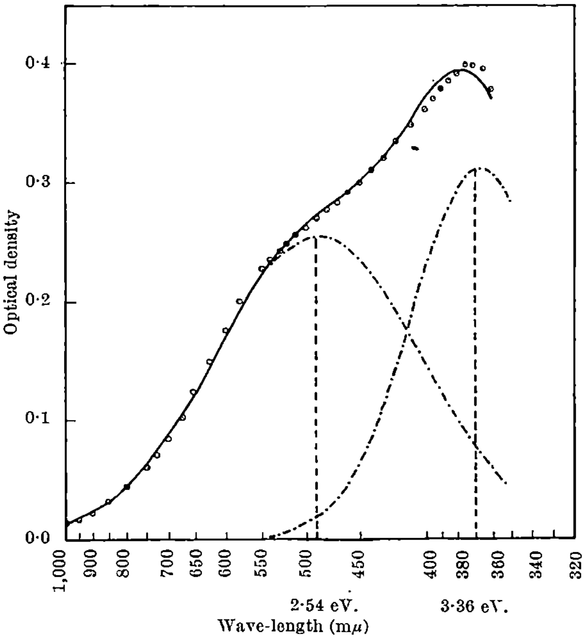


Fig. 1. Spectral absorption curve for γ-irradiated lead silicate glass. Dose, $3 \cdot 2 \times 10^4$ rads after 455 min.

The result shown in Fig. 1 is typical of many results so obtained, and it can be seen that there is now no evidence for an absorption band at 1·7 eV., but that there is considerable interaction between the two bands at their peak wave-lengths. The encircled points are the measured values and the full-line is the sum of the two Gaussian bands obtained by computation.

R. S. Barker
T. K. Harrison
D. A. Richardson
R. Rimmer

Research Laboratories,
Pilkington Brothers, Ltd.,
Lathom, Ormskirk.

---

[1] *Nature*, **187**, 135 (1960).

Rotational Analysis of Absorption Bands of Lead Monoxide

The low-lying states of N_2 and of CO arise from electron configurations[1] which may be written:

$$\pi^4\sigma^2,\ X^1\Sigma^+ \qquad (1)$$

$$\pi^4\sigma\pi,\ {}^3\Pi_r,\ {}^1\Pi \qquad (2)$$

$$\pi^3\sigma^2\pi,\ {}^1\Sigma^+,\ {}^1\Sigma^-,\ {}^3\Sigma^+,\ {}^3\Sigma^-,\ {}^1\Delta,\ {}^3\Delta \qquad (3)$$

In the group of molecules from CO to PbTe, the allowed absorption bands of longest wave-length may then be expected to be ${}^1\Sigma^+, {}^1\pi - X^1\Sigma^+$. This is found to be the case, for example, in SiS, where the systems $D^1\Pi - X^1\Sigma^+$ and $E^1\Sigma^+ - X^1\Sigma^+$ appear readily in absorption[2,3]. In addition, evidence about some other predicted states, notably $e^3\Sigma^-$, $I^1\Sigma^-$ and (?) ${}^1\Delta$, has been obtained from work[4,5] on the perturbations in $D^1\Pi$. In SiO, GeO and SnO there is no further strong absorption[6] until wave-lengths considerably shorter than those corresponding to the $E - X$ systems, indicating that more highly excited orbitals than (2) or (3) are here involved.

This comparatively simple situation, which obtains for the lighter molecules of the group, is in strong contrast with that for the heavier molecules, particularly for SnSe, SnTe and the lead compounds. In PbO, for example, the absorption region of longer wave-length ($\lambda > 2500$ Å.) shows at least six band systems[7]. The characters of the upper states of these systems have now been determined by rotational analysis of several bands of each system from plates taken on a 6·5-m. grating spectrograph. A summary of the results is given in Table 1. The results for the $A - X$ system agree with those from the less-extensive analysis of Christy and Bloomenthal[8]; however, their analysis of the $D - X$ system, which indicated that state D was ${}^1\Sigma^+(0^+)$ is incorrect.

In interpreting these results, two possibilities arise: either (i) new low-lying electron configurations other than (2) and (3) become of importance in the heavier molecules, or (ii) the coupling shifts to Hund's case-*c*. The latter is perhaps the more attractive suggestion. The case-*c* states of (2) and (3) are as follows :

$$\pi^4\sigma\pi^* \rightarrow 2,1,1,0^+,0^- \qquad (2)$$

$$\pi^3\sigma^2\pi^* \rightarrow 3,2,2,1,1,1,0^+,0^+,\ 0^-,0^- \qquad (3)$$

Table 1. Summary of Constants for ^{208}PbO

State		T_0	ω_e	$x_e\omega_e$	B_e	$10^3\ \alpha_e$	r_e Å.	Notes
E	0^+	34680	[440]	—	[0·2382]	[1·4]	2·18$_3$	1
D	1	30464	530·5	2·92	0·2711	3·1	2·046	2
C′	1	25720	[475]	—	0·248	1·8	2·14	2, 3
C	0^+	24108	[534]	[3·9]	0·254	2	2·11	4
B	1	22531	493·4$_7$	2·26	0·2646$_3$	2·6	2·070$_9$	2
a	?2							2
A	0^+	20088	451·7	3·33	0·2586$_9$	1·3$_8$	2·095$_3$	
X	0^+	0	721·8	3·70	0·3073$_7$	2·1$_2$	1·921$_6$	

(1) There are strong perturbations in state *E*, so that the constants for this state are uncertain. The values of v' for this system have been increased by 1, following a study of the isotope effect.

(2) Perturbations in *R*, *P* and *Q* branches at about the same value of J' are observed in some of the levels of *B*, *C′* and *D*. The form of these suggests that they are caused by one or more $\Omega = 2$ states.

(3) The system $C' - X$ was observed by Howell (ref. 9) as a single $v'' = 0$ progression.

(4) The values of v' for the $C - X$ system have been increased by 2, following a study of the isotope effect.

If these are all bound states, there are predicted three absorption systems $0^+ \leftarrow X$ and five systems $1 \leftarrow X$. The 0^- and 2 states would be identifiable in the absorption spectrum only through interactions with the $\Omega = 1$ states. The number and the character of the excited states so far observed suggest that their interpretation as case-*c* states may be the correct one. It is hoped that work, now in progress, of the numerous perturbations in these systems will help to elucidate this problem.

R. F. Barrow
J. L. Deutsch
D. N. Travis

Physical Chemistry Laboratory,
University of Oxford.

[1] Mulliken, R. S., *The Threshold of Space*, edit. by Zelikoff (London, 1957).

[2] Vago, E. E., and Barrow, R. F., *Proc. Phys. Soc.*, **68**, 538 (1946).

[3] Barrow, R. F., Deutsch, J. L., Lagerqvist, A., and Westerlund, B. (in the press).

[4] Lagerqvist, A., Nilheden, G., and Barrow, R. F., *Proc. Phys. Soc.*, A, **65**, 419 (1952).

[5] Nilheden, G., *Ark. Fys.*, **10**, 19 (1955).

[6] Barrow, R. F., and Rowlinson, H. C., *Proc. Roy. Soc.*, A, **224**, 374 (1954).

[7] Herzberg, G., *Molecular Spectra and Molecular Structure.* **1.** *Spectra of Diatomic Molecules* (New York, 1950).

[8] Christy, A., and Bloomenthal, S., *Phys. Rev.*, **35**, 46 (1930).

[9] Howell, H. G., *Proc. Roy. Soc.*, A, **153**, 683 (1936).

Kirkendall Effect in Gases

An experiment has been described[1] in which the motion of fine particles of ammonium chloride smoke in a diffusing gas mixture was considered evidence for a Kirkendall effect in gases. Only a rough semi-quantitative interpretation could be given at the time in terms of the usual phenomenological theory of the Kirkendall effect. Subsequent theoretical and experimental work[2,3] on gaseous diffusion has shown that the phenomenological theory cannot be correct for gases, but that the classical kinetic theory of gases is capable of accounting for the phenomenon quantitatively. We wish to point out that it is now possible to give a quantitative interpretation to the previous measurements.

The experimental results may be summarized in terms of the usual intrinsic diffusion coefficients. The observed motion of the finest particles of the smoke corresponded to a difference of intrinsic diffusion coefficients of $(D_2 - D_1) \simeq 0{\cdot}63$ cm.²/sec. for $1 = \mathrm{CF_2Cl_2}$ and $2 = \mathrm{H_2}$ at 20° C. and 1 atm., in the absence of gravity. This value is probably an upper limit, since the necessary correction for the rate of sedimentation of the smoke particles under gravity appeared to be somewhat too large as it was made.

If the smoke particles are much larger in diameter than the mean free path of the gas molecules, they should drift with the bulk velocity (that is, the mass average velocity) of the diffusing gas mixture and thus act as true Kirkendall markers. In this case the difference of the intrinsic diffusion coefficients is given by equation (23) of reference 2, and can be written as:

$$D_2 - D_1 = \frac{m_1 - m_2}{x_1 m_1 + x_2 m_2} D_{12} \qquad (1)$$

where the m's are the molecular masses, the x's are mole fractions, and D_{12} is the interdiffusion coefficient, which has been found[3] to have a value of 0·38 cm.²/sec. Taking $x_1 = x_2 = 1/2$ (at the diffusion boundary), we calculate from (1) that $(D_2 - D_1) = 0{\cdot}73$ cm.²/sec. This is larger than the experimentally observed upper limit of 0·63 cm.²/sec., so the smoke particles cannot be regarded as true Kirkendall markers.

If, however, the smoke particles are smaller in diameter than the mean free path, their behaviour is different, and the theory for such a case has been given by Waldmann[4]. According to his equation (7.20), the particles move with a velocity corresponding to:

$$D_2 - D_1 = \frac{m_1^{1/2} - m_2^{1/2}}{x_1 m_1^{1/2} + x_2 m_2^{1/2}} D_{12} \qquad (2)$$

from which we calculate $(D_2 - D_1) = 0{\cdot}59$ cm.²/sec. This is in good agreement with the observed upper limit. In equation (2) we have assumed that Waldmann's coefficients of diffuse scattering of the gas molecules from the smoke particles were equal to unity, which is in accord with experiment[5]. It is apparent that the finest smoke particles are probably smaller than the mean free path. This is physically reasonable, since the relevant mean free path in the experiments was of the order of 0·1μ.

Although the smoke particles were thus not true Kirkendall markers, they could have been used as such, since their velocity was proportional to the bulk velocity, although not equal to it. From equations (1) and (2) the relation is:

$$v = \left[\frac{m_1 - m_2}{m_1^{1/2} - m_2^{1/2}}\right]\left[\frac{x_1 m_1^{1/2} + x_2 m_2^{1/2}}{x_1 m_1 + x_2 m_2}\right] v(\text{smoke}) \qquad (3)$$

or:

$$v = [1 + 2(m_1 m_2)^{1/2}/(m_1 + m_2)]\, v(\text{smoke}), \quad \text{if } x_1 = x_2 \qquad (4)$$

where v is the mass average (bulk) velocity and v(smoke) is the velocity of the smoke particles.

E. A. Mason

Institute for Molecular Physics,
University of Maryland,
College Park, Md.

L. Miller
P. C. Carman

National Chemical Research
Laboratory,
South African Council for
Scientific and Industrial
Research,
Pretoria.

[1] Miller, L., and Carman, P. C., *Nature*, **186**, 549 (1960).

[2] McCarty, K. P., and Mason, E. A., *Phys. Fluids*, **3**, 908 (1960).

[3] Miller, L., and Carman, P. C., *Trans. Farad. Soc.* (to be published).

[4] Waldmann, L., *Z. Naturf.*, **14**, *a*, 589 (1959).

[5] Schmitt, K. H., *Z. Naturf.*, **14**, *a*, 870 (1959).

ENGINEERING

Effective Contact Stresses and Friction

The earliest statement concerning statical frictional forces between rigid bodies is usually attributed to Coulomb (about 1776). The principle of Coulombic friction is that the force (F) required to generate translation of one body relative to another is directly proportional to the external force (W) transmitted across the contact plane, so that:

$$F = \mu W \qquad (1)$$

where μ is the coefficient of (static) friction.

In 1950, Bowden and Tabor[1] published their, now well-established, concept of asperity contact giving rise to plastic junction. According to Bowden and Tabor the frictional force (F) is a product of the total area of the junctions (Aj) and their shear strength (Sj), hence:

$$F = AjSj \qquad (2)$$

and it was further postulated that the area (A) is given by the normal force (W) divided by the flow pressure (p) so that:

$$W = pAj \qquad (3)$$

and thus :

$$\mu = \frac{F}{W} = \frac{Sj}{p} \qquad (4)$$

and it has been found that for most metals the coefficient μ is independent of the normal load.

The purpose of this communication is to direct further attention to equation (3).

Terzaghi[2] was the first to state the law of effective stress in relation to inter-particle contact in a two-phase medium. Terzaghi's effective stress law states that the effective stress (σ^1) is given by:

$$\sigma^1 = (\sigma - u) \qquad (5)$$

where σ is the applied (external) normal stress and u is the positive hydrostatic pressure in the fluid surrounding the 'solid' particles.

This law entails two basic assumptions: (i) that a continuous surface exists which passes through the contacts between particles; (ii) that the area of contacts (plastic junctions) is insignificant compared with the total area of the continuous surface. Experimental work in the field of soil mechanics has established the validity of this law within practical limits[3].

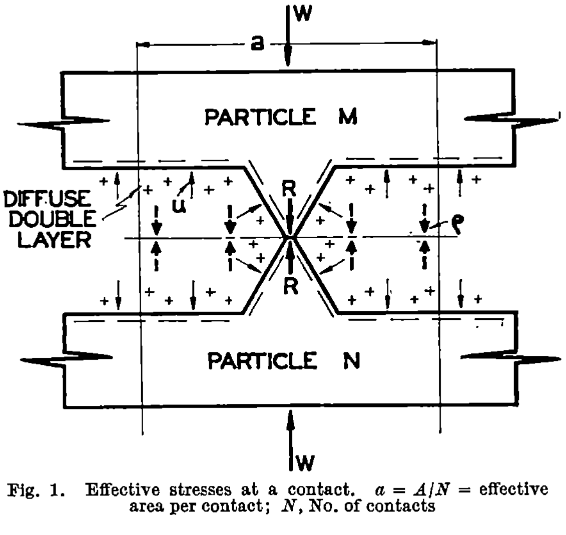


Fig. 1. Effective stresses at a contact. $a = A/N$ = effective area per contact; N, No. of contacts

Following a study of clay structure during recent years[4] I have extended equation (5) to take account of surface activity between proximate particles, and the effective stress equation now takes the form:

$$\sigma^1 = (\sigma - u + \rho) \qquad (6)$$

where ρ is the effective stress component derived from surface forces.

Consider the simplified system shown in Fig. 1.

The force R transmitted across the junction is made up of three parts.

$$R = W - \mu.a. + \rho.a. \qquad (7)$$

assuming that ρ, the resultant of all surface physico-chemical effects (for example, Van der Waals forces, double-layer repulsions, primary or secondary valence bonds at the junction), is attractive in character.

Then according to the Bowden–Tabor concept the frictional force (Fj) required to shear the junction is:

$$Fj = \mu R$$

thus:

$$Fj = \left(\frac{W}{N} - u.a. + \rho.a.\right) \qquad (8)$$

$$= \frac{W}{N} - \frac{uA}{N} + \rho\frac{A}{N}$$

The total frictional or shear force (F) is then given by:

$$F = \sum_{j=1}^{N} Fj = \sum_{j=1}^{N} \mu R = N\mu R$$

It follows that the shear stress (S) is:

$$S = \frac{F}{A} = \frac{N}{A}\mu R$$

$$= \frac{N}{A}\mu\left(\frac{W}{N} - \frac{uA}{N} + \rho\frac{A}{N}\right)$$

$$= \mu(\sigma - u = \rho) \qquad (9)$$

that is, the shear stress, on the basis of the assumptions given, is independent of the number of asperities.

Equation (9) is the effective stress expression governing friction in pure systems, that is, those where the junctions are all physically and chemically alike.

For composite materials, such as soils, the μ term is conventionally replaced by the term $\tan \varphi_e$ (Hvorslev, 1957), where φ_e is called the angle of internal friction or alternatively $\tan \varphi_e$ is the coefficient of macro-fraction and is a statistical mean of the component micro-friction effects[5].

Although equation (9) was derived with clay–water–electrolyte systems principally in mind it is thought to be of more general application. For example, if both u and ρ are effectively zero we have the condition of Coulombic friction applicable to dry granular materials; at the other end of the scale we may have conditions where both σ and u are insignificant compared with ρ, as with the internal structure of metals and certain adhesives. This latter concept may even be extrapolated to the inter-atomic level in the solid state.

Yet another possible application concerns lubrication. If, for the present, viscosity effects are neglected, then for invert surfaces ($\rho = 0$) the condition of 'dry' friction is obtained when $\mu = 0$, even though fluid may be present and the necessary condition for hydrodynamic friction is that $\sigma = u$. Between these two extremes there is a range of u values, the actual value of u and therefore that of the apparent coefficient of friction being dependent on the ambient conditions.

This latter approach is at present being considered in a study of high-speed skidding characteristics in relation to airfield pavements.

D. H. TROLLOPE

Civil Engineering Department,
University of Melbourne.

---

[1] Bowden and Tabor, *The Friction and Lubrication of Solids* (Clarendon Press, Oxford, 1950).

[2] Terzaghi, K., *Erdbaumechanik auf Boden – Physikalischer Grundlage* (Franz Deuticke, Vienna, 1925).

[3] Skempton, A. W., in Terzaghi, *From Theory to Practice in Soil Mechanics* (Wiley, 1960).

[4] Trollope, D. H., *Proc. Third Austral.-N.Z. Conf. S.M. and F.E., Sydney* (in the press).

[5] Boas, W., *Interparticle Forces in Clay-Water-Electrolyte Systems: A Symposium* (C.S.I.R.O., Melbourne, 1960).

CHEMISTRY

Quantitative Estimation of Peak Areas in Gas-Liquid Chromatography

QUANTITATIVE estimation of peak areas on gas-liquid chromatograms has been carried out by a variety of methods, which include triangulation, cutting out the peak areas and weighing the cut paper, planimeter tracing and use of an integrator built into the recorder. A new method recently proposed by Bartlet and Smith[1] is based on evidence that the peaks obtained with a well-designed chromatograph closely approximate a normal or Gaussian distribution curve.

The equation for this type of curve is given as :

$$Y = \frac{A}{\sigma\sqrt{2\pi}}\, e^{-\frac{1}{2}(X/\sigma)^2} \qquad (1)$$

where Y is the height of the curve measured at a distance x from the mean, A is the area and σ is the standard deviation. At the mean or central peak of the curve $x = 0$, $Y = h$ or peak height and equation (1) becomes :

$$h = \frac{A}{\sigma\sqrt{2\pi}} \qquad (2)$$

$$\text{or } A = 2{\cdot}507\, h\sigma \qquad (3)$$

The peak height h may be measured directly from the chromatogram and the standard deviation σ may be obtained from the peak width w at various heights since $w = \sigma$ at $0{\cdot}882h$, $w = 2\sigma$ at $0{\cdot}607h$, $w = 3\sigma$ at $0{\cdot}324h$ and $w = 4\sigma$ at $0{\cdot}134h$. The area under the curve may therefore be calculated from equation (3).

Bartlet and Smith also showed that σ is a linear function of the retention time t_R, and this has been confirmed in our studies with fatty acid esters. It is thus evident that the product of peak height and retention time may also be used as a measure of peak area. This is much better than using the product of peak height and standard deviation, since retention times are normally measured in any event and can be obtained with greater ease and precision than the standard deviations of the peaks.

The product of peak height and retention time was suggested by Pecsok as a measure of peak area[2], but no theoretical or experimental proof of its validity was provided and it does not seem to have been used very widely. The method was therefore compared in our Laboratory with the paper weighing and triangulation methods, using chromatograms of mixtures of fatty acid esters. Examples are shown in Table 1. Although the results obtained with the new method are not in perfect agreement with those of the other methods, they are nevertheless adequate for most biological studies, and the convenience of using the new method makes it very useful for routine analytical work.

In applying this method to peaks which are only partially resolved, it should be noted that the measured peak heights will be higher than the true peak heights by amounts which are determined by the degree of overlap and by the relative amounts of the components. A correction for this may be obtained by application of equation (1) as shown by Bartlet and Smith. Alternatively, if pure samples of the overlapping components are available, they may be chromatographed individually and correction factors obtained by direct measurement. In practice, the correction factors are very small, even for peaks with serious overlap, and although successive corrections are theoretically required, they are rarely, if ever, needed.

The present method of determining peak areas has an advantage over the paper-weighing method in that it allows the chromatogram to be saved for future reference. It is less laborious than triangulation and gives more reproducible results since it avoids the subjective variation involved in the drawing of triangles. It may also be noted that the units of measurement are unimportant as long as the same

Table 1. QUANTITATIVE ANALYSIS OF FATTY ACID ESTERS

Fatty acid methyl esters	Shorthand designation	Weighing method		Triangulation method		Present method		
		Wt. of cut-out area (mgm.)	per cent	½ height × base (mm.²)	per cent	Peak height (h) (mm.)	Retention time (t_R) (min.)	per cent
Palmitate	(16 : 0)	27·3	14·8	504	15·5	95	5·8	14·5
Oleate	(18 : 1)	49·5	26·8	900	27·7	88·5	12·2	28·4
Linolenate	(18 : 3)	69·7	37·7	1215	37·4	78	18·9	38·8
Behenate	(22 : 0)	38·3	20·7	630	19·4	17·4	39·8	18·2
Palmitate	(16 : 0)	33·8	15·0	635	15·7	114	5·8	15·1
Stearate	(18 : 0)	75·2	33·3	1341	33·1	128	11·0	32·1
Linoleate	(18 : 2)	76·1	33·7	1392	34·4	105·5	14·8	35·5
Erucate	(22 : 1)	40·5	18·0	680	16·8	17·5	43·5	17·3
From corn oil*	(16 : 0)	25·6	22·6	440	21·9	92·5	5·0	21·7
	(18 : 1)	45·6	40·3	805	40·1	80·5	10·5	39·7
	(18 : 2)	42·0	37·1	763	38·0	64·2	12·8	38·6
From rat plasma triglycerides*	(16 : 0)	48·3	44·6	885	45·0	162·5	5·0	44·7
	(18 : 1)	42·0	38·8	752	38·3	67	10·4	38·3
	(18 : 2)	18·0	16·6	328	16·7	24·5	12·6	17·0

Fatty acid esters were separated on a 6 ft. column packed with butanediol succinate (Craig ester) on Chromosorb *W* (1 : 4). A Barber–Colman chromatograph with a radium source for the ionization detector was employed. The column was operated at 200° and the flash heater and cell bath at 255°. Argon at 20 lb./in.² flowed at the rate of 150 ml./min.

* Minor peaks were not included in these calculations.

units are used for each component of the mixture. Thus, in the examples given, peak heights were measured in mm. and retention times in minutes. The convenience of this method and the probability that further improvements in instrumentation will give curves more closely approaching the Gaussian distribution curve suggest it as a method of choice for future quantitative work in gas-liquid chromatography.

K. K. CARROLL

Collip Medical Research Laboratory,
University of Western Ontario,
London, Ontario.

---

[1] Bartlet, J. C., and Smith, D. M., *Canad. J. Chem.*, **38**, 2057 (1960).

[2] Pecsok, R. L., *Principles and Practise of Gas Chromatography*, 145 (John Wiley and Sons, Inc., New York, 1959).

Compressibility and Specific Evaporation Resistance of the Monolayers of Long-Chain Alcohols and Glycol Monoalkyl Ethers

THE monolayers of long-chain compounds such as cetyl and stearyl alcohols are being used extensively to minimize the loss of water by evaporation in reservoirs[1]. Permeability of water vapour through a monolayer depends on, among others, the magnitude of the van der Waals type of cohesive forces of the film molecule with its neighbours, and on the adhesive force between the monolayer and the water sub-phase. Further extension of the *n*-alkyl chain and/or suitable modification of the terminal polar group would, therefore, make a monolayer more effective in reducing evaporation.

In view of various such considerations as structural, stereochemical and spreading property, and economic availability, we have been restricted to the C_{16}–C_{22} *n*-alkyl compounds and prompted to modify and thus increase the interaction of the polar end-group with the water sub-phase. Accordingly, we synthesized a series of glycol monoalkyl ethers, R–OCH_2CH_2OH (R being an *n*-alkyl chain containing 16–22 carbon atoms), and reported[2] their superior water evaporation resistance behaviour compared to the alcohols. This communication describes the preliminary results on the compressibilities and the specific evaporation resistances of the monolayers of these as well as those of the alcohols for comparison.

The preparation and purification procedures of the compounds used were indicated earlier[2]. The melting and softening point, micro-analysis, infra-red analysis and, in addition, equilibrium surface pressure[3] of the film spread from solid particles were used as criteria of purity. Monolayers were formed from solutions in A.R. distilled (60–80° C.) petroleum ether on a clean surface of double-distilled water in an all-glass Langmuir trough. The film was compressed by means of a paraffin barrier and the pressure was measured with a horizontal film balance.

From a study of the pressure/area isotherms, it has been observed that, initially, after a region of very low pressure (~ 1 dyne/cm.) the film is transformed into a so-called liquid condensed state. On further compression, the pressure rises steeply due to the formation of a highly non-compressible, compact film in the solid state. The transition pressures for liquid condensed to solid state vary from 10–25 dynes/cm. in the case of alcohols and 25–35 dynes/cm. in the case of glycol ethers when the chain length is increased from C_{16} to C_{22}. The calculated compressibility data $k = -\left(\frac{1}{A}\ \frac{dA}{d\pi}\right)$ are given in Table 1.

Table 1. COMPRESSIBILITY (25° C.)

n-Alcohol	Liquid condensed state $k \times 10^3$	Solid state $k \times 10^4$	Glycolmono-alkyl ether	Liquid condensed state $k \times 10^3$	Solid state $k \times 10^4$
C_{16}	5·43	13·22	C_{16}	7·86	12·82
C_{18}	5·03	6·80	C_{18}	7·29	7·82
C_{20}	4·59	7·09	C_{20}	—	—
C_{22}	1·93	6·97	C_{22}	5·02	6·98

The results for the C_{16} and C_{18} *n*-alcohols are in agreement with the reported data[4]. It is noticed that the liquid condensed state is more compressible than the solid state. However, the films formed by the glycol ethers are significantly more compressible than those of the alcohols in the liquid-condensed state, which may be attributed to differences in the dipole-repulsion property and the geometry of the polar end-groups in the two series.

Following the method developed by Langmuir *et al.*[5] the specific resistance (ν) of the film was evaluated from the expression:

$$\nu = \left[A(w-w_0)\ \frac{t}{M}\right]_{\text{film}} - \left[A(w-w_0)\ \frac{t}{M}\right]_{\text{no film}}$$

where A is the area of the desiccant (lithium chloride) exposed; w and w_0, the concentrations of water vapour in equilibrium with water and desiccant respectively; M, the mass of water absorbed by the desiccant in time t sec. The temperature of the desiccant as well as of the water surface were measured by using thermistor probes through a d.c. bridge circuit. All the precautions prescribed by La Mer *et al.*[6] were taken. The results obtained at 25° C. at different surface pressures are given in Fig. 1.

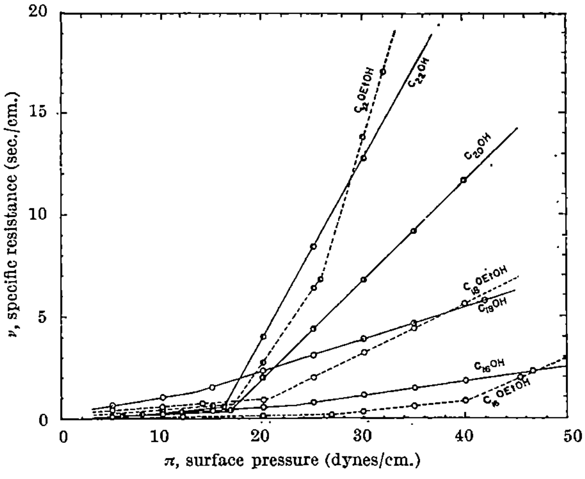


Fig. 1

The results for C_{16} and C_{18} *n*-alcohols are in agreement with reported data[6,7]. It is seen that, at lower pressures up to 16–18 dynes/cm., the resistance in all cases is very low (~1 sec./cm.). However, ν increases rapidly at higher pressures and also with the increase in the length of the chain. In the expanded condition of the film in the low-pressure region, the interaction between film molecules is very weak, requiring little energy to create a hole for the water molecule to escape. However, strong interaction between them is indicated by the sharp rise of pressure with further decrease in area when the formation of continuous, close-packed film starts through vertical orientation of, and strong intermolecular cohesive binding between, the long chains. The observed increase of ν with the length of the chain in both the series is due to an increase in the cohesive binding between the film molecules, and hence the increase in the activation energy required to create a hole in the film.

It is interesting to note that, between the two series, the ν-values in general are lower for the glycol ethers (shown as *R*–OEtOH in Fig. 1) than for the corresponding alcohols at the same pressure, and this is in accordance with the fact that compressible films are less effective. After about 30–40 dynes/cm. pressure, however, the C_{22}- and C_{18}-glycol ethers exhibit higher ν-values than the alcohols and this fact supports our observations reported earlier[2], that the glycol ethers are better retarders of evaporation than the alcohols at high (equilibrium) film pressure conditions.

That the superior performance of the glycol ethers compared with the corresponding alcohols may be ascribed to higher rate of spreading (surface solution of the ethers being greatly facilitated due to the greater interaction of the polar end with the water sub-phase), higher equilibrium film pressure in the significant temperature-range, and greater adhesive interaction with the water sub-phase, will be dealt with in a separate communication.

A. V. Deo
S. B. Kulkarni
M. K. Gharpurey
A. B. Biswas

National Chemical Laboratory,
Poona, 8.

[1] *a*, Mansfield, W. W., *Nature*, **175**, 247 (1955). *b*, Mansfield, W. W., **172**, 1101 (1953). *c*, Mansfield, W. W., *Austral. J. App. Sci.*, **9** (3), 245 (1958).

[2] *a*, Deo, A. V., *et al.*, *Nature*, **187**, 870 (1960). *b*, Kulkarni, S. B., *et al.*, (i) Ind. Pat. No. 70670 (1960); (ii) Australian Pat. No. 63866 (1960) and also U.S. Patent No. 62522 (1960).

[3] Cary, A., and Rideal, E. K., *Proc. Roy. Soc.*, A, **109**, 318 (1925).

[4] Nutting, G. C., and Harkins, W. D., *J. Amer. Chem. Soc.*, **61**, 1180 (1939).

[5] *a*, Langmuir, I., and Schaefer, V. J., *J. Franklin Inst.*, **235**, 119 (1943). *b*, Archer, R. J., and La Mer, V. K., *J. Phys. Chem.*, **59**, 200 (1955).

[6] La Mer, V. K., and Barnes, G. T., *Proc. U.S. Nat. Acad. Sci.*, **45** (8), 1274 (1959).

[7] Rosano, H. L., and La Mer, V. K., *J. Phys. Chem.*, **60**, 348 (1956).

Electronic Properties of Imidazole

The existence of semiconduction has been demonstrated in a variety of different classes of organic compounds such as condensed aromatic hydrocarbons, charge-transfer complexes, pyrolized polymers, and stable free radicals. With few exceptions, the electrical conductivities are small, and the energies of activation for the conduction process are high. But the fact that even small conductivities are displayed suggests that some kind of intermolecular interaction takes place which, in an electric field, gives rise to electronic conduction. It is suggested that this interaction is molecular orbital overlap. The extent of orbital overlap is a function of molecular structure and hence, if semiconduction arises from such overlap, it is not surprising that semiconduction activation energies correlate with properties which are also a function of molecular structure, such as the molecular ionization energy, electron affinity, and excitation energy of the ground-triplet state transition[1,2]. One of the entities which influences these properties is the number of (the more easily polarizable) π-electrons. As the number of these increases in a conjugated system, there is a trend toward smaller activation energies of conduction, indicating that the probability of achieving overlap is enhanced by the presence of a large number of π-electrons. However, if the overlapping of molecular orbitals gives rise to semiconduction, then any other factors which contribute to such overlapping should also encourage semiconduction, even if only few π-electrons are present in the individual molecule. Since intermolecular hydrogen bonds may provide such overlap, it might be expected that activation energies in hydrogen-bonded compounds should be lower than anticipated solely on the basis of the number of π-electrons.

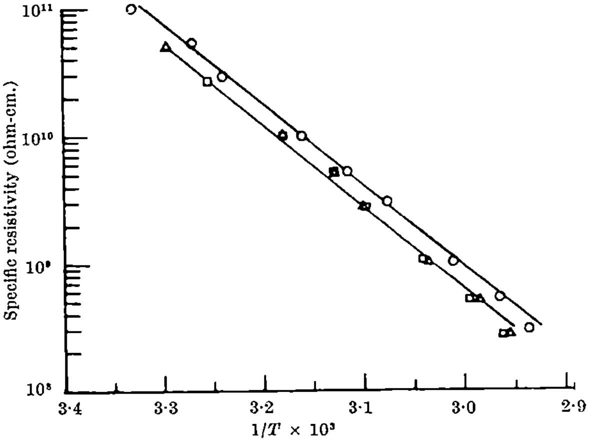


Fig. 1. Specific resistivity of imidazole. ○, run 2, 720 V./cm.; △, run 3, 72 V./cm.; □, run 4, 72 V./cm.

Supporting evidence for this view has now been found in a study of the resistivity of imidazole. Imidazole is extensively hydrogen-bonded as adduced from its relatively high melting and boiling points and from its spectra. In the present work, imidazole was purified by several recrystallizations, distillation at reduced pressure, and zone-refining. The resistivity was measured as a function of temperature on a compacted polycrystalline sample with the results shown in Fig. 1. The energy gap computed from the equation $\rho = \rho_0 \exp(E/2kT)$ was 2·6 eV. in the temperature range of 28–66°. Slightly higher resistivities were obtained at a higher potential stress; it is not known whether this is a significant effect. Energy gaps ranging between 2·8 and 3·0 eV. were obtained on several other samples which had not been as rigorously purified.

Energy gaps of compounds which contain only four π-electrons are not available for comparison with that of imidazole (which also contains four π-electrons). The energy gap of benzene (which contains six π-electrons) has not been experimentally determined but has been estimated[3] as approaching 5 eV. If the number of conjugated π-electrons were the sole factor which determines the energy gap, one might have expected that of imidazole to be much greater than the experimentally found value of 2·6 eV. It is concluded that the orbital overlap provided by hydrogen-bonding effectively facilitates semiconduction.

We thank Messrs. E. J. McGowan and J. Howard for valuable technical assistance. This research was supported by the Air Research and Development Command, United States Air Force.

S. Aftergut
G. P. Brown

General Engineering Laboratory,
General Electric Co.,
Schenectady, New York.

[1] Lyons, L. E., and Morris, G. C., *J. Chem. Soc.*, 3648 (1957).

[2] Aftergut, S., and Brown, G. P., *Nature*, **189**, 827 (1961).

[3] Eley, D. D., Inokuchi, H., and Willis, M. R., *Disc. Farad. Soc.*, No. 28, 54 (1959).

Formation of Boron Nitride in Diborane-Hydrazine Flames

Although reactions of metals with nitrogen or nitrogen compounds leading to nitrides are well known[1], the literature on combustion is devoid of descriptions of flames from the reaction between boron hydrides and nitrogen hydrides (ammonia, hydrazine). This communication reports a steady-state flame of diborane and hydrazine, in which the reaction products consist principally of boron nitride and hydrogen, according to the reaction $B_2H_6 + N_2H_4 \rightarrow 2BN + 5H_2$.

Even though the value of the heat of formation of boron nitride is not fully established (27–32 kcal./mol. in ref. 2, 60·2 kcal./mol. in ref. 3) the above reaction is sufficiently exothermic for it to proceed without difficulty. Examination of this system is complicated, however, by a rapid gas-phase reaction of boron hydrides with ammonia, hydrazine or amines, leading to a variety of solid 'adducts'[4], which prevents premixing of the components prior to combustion. To overcome this problem a novel burner was constructed, consisting of a closely spaced array of capillaries (Fig. 1) by means of which a 'multi-diffusion' flame is established in a laminar mixing zone. This type of burner is of general utility for combustion studies in which premixing of components is impractical or impossible, but where it is desirable to investigate the reaction products from flames approaching the premixed state.

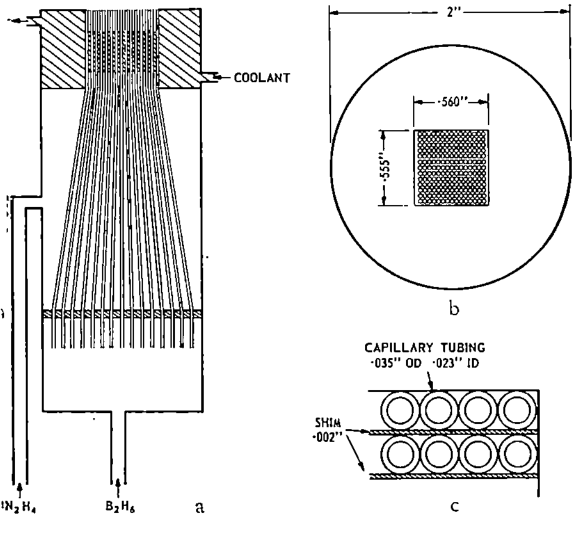


Fig. 1. Schematic diagram of capillary multidiffusion burner. *a*, Cross-section, showing arrangement of separate feeds for two incompatible gaseous components; *b*, top view; *c*, details of capillary assembly. Symmetrical assembly of tubes is essential to provide uniform gas flow at top of the burner. Metal shims are used as spacers between rows of capillaries

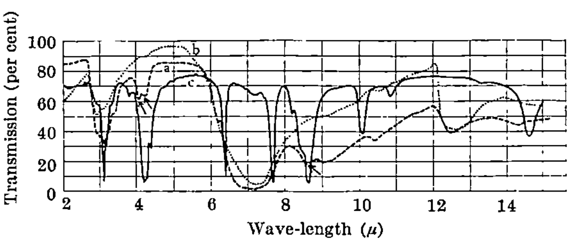


Fig. 2. Infra-red spectra of: *a*, solid reaction products from the B_2H_6—N_2H_4 flame; *b*, boron nitride; *c*, diborane-hydrazine adduct (Callery Chemical Co.). The arrowed absorption bands in *a* are due to the presence of small amounts of adduct

To achieve the boron nitride reaction a flat hydrazine decomposition flame is established first (requiring thermostatting the burner and fuel-feed above 100° C. to obtain an adequately high hydrazine vapour pressure). Subsequent addition of diborane through the separate feed system leads to a flame with copious evolution of solid reaction products consisting predominantly of boron nitride. As shown by infra-red (Fig. 2) and chemical analyses only small amounts of bound hydrogen appear as impurity in the solid.

By measurement of the flame temperature and the composition of the products of combustion an independent value for the heat of formation of boron nitride is being obtained.

This work was carried out under contract *NOrd* 7386 of the U.S. Navy.

W. G. Berl
W. E. Wilson

Applied Physics Laboratory,
The Johns Hopkins University,
Silver Spring, Maryland.

[1] Sidgwick, N. V., *The Chemical Elements and Their Compounds*, **1**, 663 (Oxford Univ. Press, 1950).

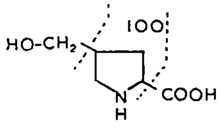
[2] *Gmelins Handbuch der Anorganischen Chemie*, eighth ed., Boron Supp., 159 (Verlag Chemie, Weinheim, 1954).

[3] Galchenko, G. L., *et al.*, *Proc. Acad. Sci. U.S.S.R.* (Chem. Section), **127**, 635 (1959) (English translation, Consultants Bureau, Inc.).

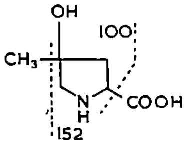
[4] Parry, D. W., *et al.*, *J. Amer. Chem. Soc.*, **80**, 4 (1958). Emeléus, H. J., and Stone, F. G. A., *J. Chem. Soc.*, 840 (1951).

Determination by Mass Spectrometry of the Structure of Proline Derivatives from Apples

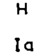
Hulme[1] and Urbach[2] independently isolated a new amino-acid from apples, and the isomeric structures I[2] and II[1,3] were proposed for these substances on the basis of elemental analysis, colour reactions and chromatographic properties[1–3]. The infra-red spectra of these compounds proved to be superimposable, and this indicated that the compounds were identical, but it did not permit a decision between the alternative structures[3].



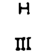
I



II



Ia



III

It seemed that this problem could now be resolved by mass spectrometry, a method recently introduced into amino-acid chemistry[4,5]. Therefore, small samples of the original isolates were procured from Dr. A. C. Hulme and Mrs. G. Urbach, respectively. For these samples we are grateful.

The ethyl esters of the two compounds were prepared by treatment of 1 mgm. of each substance with boiling ethanolic hydrochloric acid and by conversion of the product into the free ester[6]. The mass spectra of the ethyl ester of the two compounds (Fig. 1) were identical in every respect: this corroborated the presumed identity[3] of the two amino-acids which had been isolated independently.

The mass spectra exhibit a very intense peak at *m/e* 100 due to loss of the carbethoxy group (73 mass units) from the molecule, the molecular weight of which is 173. This evidence relates to the structure I, and this conclusion is also supported by a peak at mass 174, which exhibits the characteristics of an $M + 1$ peak[4]. An appreciable peak is also found at mass 31 (CH_2OH) and at mass 142 ($M - 31$), which can be reconciled only with the structure I. By contrast, one would expect from structure II a considerable peak at mass 158 ($M - 15$), but none at 31 and 142, because the loss of the methyl group would lead to a carbonium ion which would, in turn, be well stabilized by the high degree of substitution as well as the oxygen atom.

Furthermore, it is now possible to deduce the stereochemistry of this amino-acid, because the mass spectrum of the ester remains substantially unchanged after the sample is kept for 30 min. in the hot (140° C.) inlet system of the mass spectrometer. A substance with the *cis*-configuration of the carbethoxy- and hydroxy-methyl groups should, under these conditions, undergo lactone formation at least in part: because this does not occur, the *trans*-structure I*a* is assigned to this compound. By the same argument, the otherwise possible structure III is eliminated, because such a substance in the form of its ester should lactonize readily under the conditions specified, whether the configuration is *cis* or *trans*.

The assignment of the optical configuration is, of course, not possible from these experiments, and this must await the final correlation of this amino-acid with proline (presumably L-proline).

After the work described, one of us (F. C. S.) learned through correspondence (letter of January 5, 1961) that Dr. G. W. Kenner and Dr. L. F. Burroughs had examined a sample identified with that of Hulme[1] and concluded that "this turns out to be a hydroxymethyl compound rather than a methylhydroxy derivative of proline, and we hope to correlate it with methyl proline". Therefore, the identity of these compounds as the *trans* form of 4-(hydroxymethyl)-proline is now established.

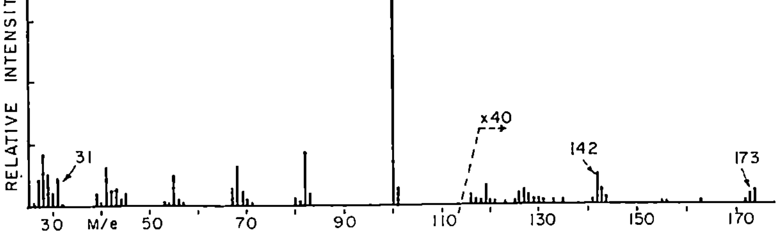


Fig. 1. Mass spectrum of the ethyl ester of the proline derivative isolated by Hulme from apples. Peaks above mass 115 are enlarged 40-fold. All peaks of ⩾ 1 per cent of the intensity at *m/e* 100 are shown below mass 116 and of ⩾ 0·05 per cent above mass 116. The spectrum of the compound isolated by Urbach was indistinguishable from that shown

K. BIEMANN
G. G. J. DEFFNER

Department of Chemistry,
Massachusetts Institute of Technology,
Cambridge, Massachusetts.

F. C. STEWARD

Department of Botany,
Cornell University,
Ithaca, New York.

[1] Hulme, A. C., *Nature*, **174**, 1055 (1954).

[2] Urbach, G., *Nature*, **175**, 170 (1955).

[3] Hulme, A. C., and Steward, F. C., *Nature*, **175**, 171 (1955).

[4] Biemann, K., Seibl, J., and Gapp, F., *Biochem. Biophys. Res. Comm.*, **1**, 307 (1959).

[5] Biemann, K., Lioret, C., Asselineau, J., Lederer, E., and Polonsky, J., *Biochim. Biophys. Acta*, **40**, 369 (1960).

[6] Biemann, K., and Vetter, W., *Biochem. Biophys. Res. Comm.*, **2**, 93 (1960).

[7] Burroughs, L. F., Dalby, S., Kenner, G. W., and Sheppard, R. C., *Nature*, **189**, 394 (1961).

Determination of Lead in Urine by Atomic Absorption Spectroscopy

MOST methods for the determination of traces (0·01–1 p.p.m.) of lead in urine require prior removal of organic matter and concentration of the lead by ashing. These methods have several drawbacks: the time required for ashing; possible interference from other metals such as bismuth; and the need for careful purification of a variety of reagents to keep the blank reading at a reasonably low level.

Several methods have been described in which the lead is co-precipitated with calcium oxalate and estimated colorimetrically[1] or polarographically[2]. However, it is doubtful whether much time is saved thereby, and there is evidence[3] that under these conditions lead is not completely precipitated from urine, especially where the patient has been treated with complexing agents such as ethylenediaminetetraacetic acid or 2,3-dimercaptopropanol.

Allan[4] has recently shown that trace quantities of copper in soils and plant ash can be determined by extraction with a chelating agent into an organic solvent and spraying this solution directly into the burner of an atomic absorption spectrophotometer.

I have found that lead can be quantitatively extracted from urine by ammonium pyrrolidine dithiocarbamate[5] and methyl *n*-amyl ketone at a *p*H sufficiently low (1·5–4·5) to avoid precipitation of the phosphates. The lead may thus be concentrated by a factor of 100 or more and determined by spraying the ketone solution into the 10-cm. air-coal gas flame of the atomic absorption spectrophotometer described by Box and Walsh[6]. The absorption of the lead line at 2833 Å. is measured and the instrument is calibrated by extracting and measuring a similar quantity of urine to which a known amount of lead has been added.

Since we are concerned only with the concentration of lead in the ketone phase the partial emulsification which invariably occurs does not affect the accuracy of the determination. After shaking with ammonium pyrrolidine dithiocarbamate and methyl *n*-amyl ketone the urine is allowed to stand for 5 min., and most of the lower layer is discarded, the last 5 ml. or so being transferred, together with the upper layer and the emulsion at the interface, to a centrifuge tube. After centrifuging for 5 min. a partial separation of the layers occurs; the upper layer is removed with a fine Pasteur pipette and the remainder of the liquid stirred thoroughly and centrifuged again. The upper layer is pipetted off once more, and the combined quantities are sufficient for a measurement.

Table 1 shows the recovery of added lead from normal urine, while Table 2 compares the results obtained for pathological specimens by the dithizone method and the atomic absorption technique. It will be seen that the results of the new method are in satisfactory agreement with those of the classical one.

Table 1. RECOVERY OF LEAD ADDED TO NORMAL URINE (P.P.M.)

Added	Recovered
0·10	0·10
0·20	0·21
0·30	0·27
0·40	0·41
0·50	0·56

50 ml. of urine (pH 4) containing the added lead was extracted with 0·5 ml. of 4 per cent ammonium pyrrolidine dithiocarbamate and 1·5 ml. methyl *n*-amyl ketone. Similar specimens to which 15 μgm. of lead had been added were extracted in the same way to serve as standards.

Table 2. COMPARISON OF COLORIMETRIC AND ATOMIC ABSORPTION METHODS FOR DETERMINATION OF LEAD IN URINE

Specimen No.	Lead content (p.p.m.) Colorimetric	Lead content (p.p.m.) Atomic absorption
1	0·03	0·04
2	0·04	0·07
3	0·06	0·07
4	0·07	0·07
5	0·18	0·14
6	0·26	0·28
7*	0·76	0·72

* From a patient undergoing treatment with ethylenediamine-tetraacetic acid.

The atomic absorption method is particularly well suited for the measurement of very low lead concentrations (<0·1 p.p.m.) as it is specific for lead and yields a negligible blank value. By using larger volumes of urine (100–200 ml.) it is possible to measure a lead concentration of 0·02 p.p.m. with a standard deviation of 0·002 p.p.m.

The method is also applicable to other heavy metals and indeed zinc and cadmium, for which the detection limit in aqueous solution is about 0·03 p.p.m.[7], can be determined by direct measurement on the urine itself without prior extraction.

I am indebted to Dr. Alan Bell and Mr. Graham Blanks for supplying the pathological specimens and for measuring their lead contents colorimetrically.

J. B. WILLIS

Division of Chemical Physics,
Chemical Research Laboratories,
Commonwealth Scientific and
Industrial Research Organization,
Melbourne,

---

[1] Taeger, H., and Schmidt, P., *Z. ges. exp. Med.*, **100**, 717 (1937).

[2] Baker, R. W. R., *Biochem. J.*, **46**, 606 (1950).

[3] Machata, G., and Neuninger, H., *Wien. med. Wochenschr.*, **110**, 39 (1960).

[4] Allan, J. E., *Spectrochim. Acta*, **17**, 459 (1961).

[5] Malissa, H., and Schöffmann, E., *Mikrochim. Acta*, 187 (1955).

[6] Box, G. F., and Walsh, A., *Spectrochim. Acta*, **16**, 255 (1960).

[7] Gatehouse, B. M., and Willis, J. B., *Spectrochim. Acta* (in the press).

BIOCHEMISTRY

Electrophoretic and Immuno-electrophoretic Patterns of Extracellular Diphtheria Proteins from Culture Filtrates of Different Iron Concentrations

IN cultures of *Corynebacterium diphtheriae* the toxin is produced only within a relatively narrow range of iron concentration. Excess of iron inhibits toxin formation, but the mechanism of this phenomenon is not explained. Toxigenicity is strictly associated with the presence of prophage in diphtheria bacillus cells, and it has been recently suggested that iron probably affects toxin production by causing induction of the prophage–host system[1]. At iron excess a certain amount of atoxic, extracellular protein is found in the culture filtrate, but their physico-chemical and immunological properties are little known. A closer characterization of these components, which will be hereafter referred to as 'Fe-proteins', appears useful for work on toxin formation.

Paper electrophoresis has shown extracellular diphtheria proteins to constitute a complex system, comprising at least six fractions, for which the following nomenclature has been proposed[2]: fraction *D*, amounting to about 65–80 per cent, more rapid fractions ρ_1 and ρ_2, and three fractions slower than *D*, namely: σ_1, σ_2, and σ_3 (Fig. 1). Diphtheria toxin, that is, the factor endowed with lethal and dermonecrotic properties, is localized in the cathodic part of fraction *D*.

The electrophoretic pattern of 'Fe-proteins' has shown striking difference as compared with the pattern of extracellular proteins obtained at iron concentration optimal for toxin production (Fig. 2). It is characterized by a strongly diminished content

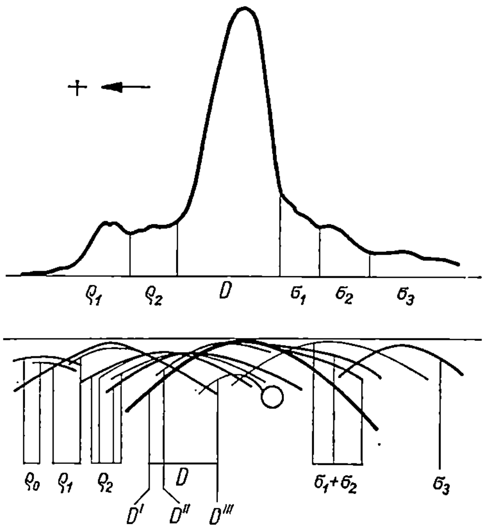


Fig. 1. Electrophoretic and immuno-electrophoretic patterns of extracellular diphtheria proteins obtained at iron concentration optimal for toxin production. Preparation of extracellular proteins obtained by precipitation with ammonium sulphate at 0·66 saturation from a 5-day culture of *C. diphtheriae PW*8. Iron concentration in the culture medium (acid hydrolysed casein), 0·12 μgm. iron/ml. Flocculation titre of the crude culture filtrate, 110 Lf./ml., flocculation activity of the preparation, 2080 Lf./mgm. protein nitrogen, dermonecrotic activity. 2×10^8–$2{\cdot}5 \times 10^8$ minimum reacting dose/mgm. protein nitrogen. Paper electrophoresis; Michaelis buffer pH 9·0; ionic strength, $\mu = 0{\cdot}1$. Densitometric curve after amido-black staining (ref. 2). Immunoelectrophoresis: Michaelis buffer, pH 9·0; ionic strength, $\mu = 0{\cdot}05$ (ref. 3)

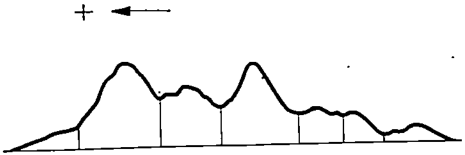


Fig. 2. Electrophoretic pattern of 'Fe-proteins'. Preparation of 'Fe-proteins' obtained by precipitation with ammonium sulphate at 0·66 saturation from a 5-day culture of *C. diphtheriae PW*8. Iron concentration in the culture medium, 1·0 μgm iron/ml. Flocculation titre of the crude culture filtrate less than 2·5 Lf./ml., dermonecrotic activity, 2·5 × 10^6 minimum reacting dose/mgm. protein nitrogen. Paper electrophoresis: Michaelis buffer, *p*H 9·0; ionic strength, $\mu = 0·1$. Densitometric curve after amido-black staining

of fraction *D*. In experiments involving determination of relative electrophoretic mobilities, it has been found that fraction *D* of the 'Fe-proteins' migrates with a mobility corresponding to the material of the anodic part of fraction *D* of extracellular proteins obtained at iron concentration optimal for toxin production. Running electrophoresis simultaneously on the same strip proved all the other fractions of the 'Fe-proteins' to be identical, with respect to their mobility, with corresponding fractions of extracellular diphtheria proteins obtained at iron concentration optimal for toxin production. In immunoelectrophoretic experiments the immunological identity of precipitating components of those fractions has been demonstrated.

The immuno-electrophoretic pattern of 'Fe-proteins' does not contain the *D'* line, which is the principal component of the pattern of extracellular diphtheria proteins obtained at optimal iron concentration[3]. When, however, immuno-electrophoretic patterns of extracellular proteins obtained at iron concentration optimal for toxin production were developed with anti-toxic sera absorbed with 'Fe-proteins' they were found to contain two additional precipitation lines besides line *D'* : line *D'''* and one localized within fraction ρ_2 (Fig. 3). These experiments have clearly demonstrated that excess iron inhibits the production of at least three precipitating components.

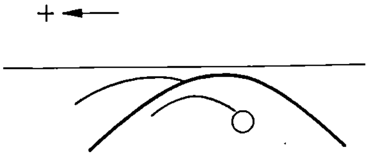


Fig. 3. Immuno-electrophoretic pattern of extracellular diphtheria proteins obtained at iron concentration optimal for toxin production. Flocculation activity of the preparation used, 1,900 Lf./mgm. protein nitrogen. The pattern was developed with anti-toxin absorbed with 'Fe-proteins'

It may be concluded that the culture filtrate of *C. diphtheriae PW*8 contains macromolecular components which differ in the mechanism of origin. The components of fractions ρ and σ are produced at iron concentration optimal for toxin formation and at a concentration which almost completely inhibits toxin production. Fraction *D* appears to be characteristic of culture filtrates of toxigenic strains of *C. diphtheriae* at iron concentration favourable for toxin production. The effect of iron, however, is a complex one, because the inhibition of production of at least three precipitating components, one of which may be identical with the toxin, is simultaneously observed.

The toxic activity of extracellular diphtheria proteins is localized within fraction *D*. However, the asymmetric distribution of the activity within this fraction, as well as the results obtained on electrophoresis in buffers of low ionic strength, clearly demonstrated its heterogeneity[2]. Fraction *D* contains also considerable amounts of glycoproteins, which may be only partially separated as the more mobile borate-separable fraction D_1 on electrophoresis in borate buffers[2]. It seems that an explanation of the part played by different macromolecular components present in the culture filtrate should throw some light on the mechanism of toxin formation.

BERTOLD J. RADOLA

Department of Bacteriology,
State Institute of Hygiene,
Warsaw.

[1] Barksdale, L., *Bact. Rev.*, **23**, 202 (1959).

[2] Radola, B. J., *Bull. Acad. Polon. Sci., Sér. Sci. Biol.*, **8**, 281, 287 (1960).

[3] Radola, B. J., *Bull. Acad. Polon. Sci., Sér. Sci. Biol.*, **8**, 493, 499 (1960).

Formation of 5-Hydroxytryptamine in an Aqueous Solution of 5-Hydroxytryptophan treated with X-rays

IT has been found that 5-HT (5-hydroxytryptamine) is formed by the action of ultra-violet irradiation on 5-HTP (5-hydroxytryptophan) aqueous solution, as a result of a decarboxylation process[1]. It is of interest to investigate whether the same process takes place under the influence of X-irradiation.

An aqueous 0·1 per cent solution of 5-HTP as a pure base, contained in a 'Pyrex' glass vessel, was irradiated with X-rays generated from a Philips machine, operating at 200 kV. and 20 m.amp. (The glass vessel was in close contact with the target.) The dose-rate was 1,818 r./min., measured by the ionization chamber. The solution was prepared in re-distilled water and exposed to X-irradiation for 10 hr. The thickness of the solution layer was 5 mm. The loss of water caused by evaporation during the exposure time was compensated every 2 hr. The irradiated solution was tested for 5-HT on atropinized œstrous rat uterus[2].

After the irradiation of the solution a brown coloration was noticed. This brown solution showed an oxytocic activity. In preliminary experiments oxytocic activity of the starting material (5-HTP) was examined and we found that 5-HTP *per se* possesses a slight activity on rat uterus, the sensitivity threshold being 0·2 mgm./ml. We were obliged, therefore, to work with the starting solution diluted below this threshold concentration of 5-HTP. After eliminating the interference with 5-HTP, we found that the solution still showed oxytocic activity. The inhibition experiments with lysergic acid diethylamide—a specific antagonist of 5-HT—showed that this activity is to be ascribed to 5-HT. The concentration of 5-HT in the irradiated solution was found to be rather low (0·004 per cent), the *G*-value being 0·2.

Preliminary analysis of descending paper chromatography of the irradiated solution (solvent system: butanol–acetic acid–water (4 : 1 : 5); Ehrlich's reagent) showed, besides 5-HTP ($R_F = 0·30$), four more spots with the following R_F values: 0·12; 0·36; 0·46 and 0·75. The spot with the R_F value of 0·46 corresponded to 5-HT in location, but not in colour.

It has been demonstrated that, in X-irradiated aqueous solutions of histidine[3] and probably tyrosine[4], decarboxylation occurs to a very low amount. This is possible, because in aromatic amino-acids there are a number of sites to be attacked by the free radicals produced from the water. Our results with rather small G-value indicate that 5-HTP (an aromatic amino-acid too) behaves similarly.

It has been noted[5] that 5-HT in aqueous solution undergoes many changes in its structure under the influence of different oxidizing agents. Taking into account the low conversion-rate of 5-HTP to 5-HT, one should consider the possibility of transformations of 5-HT molecules when under the influence of X-irradiation. It seems to us likely that the low amount of 5-HT formed could be partly explained by the action of the various oxidizing species produced by the X-rays.

We wish to thank Dr. A. J. Swallow, London, for his interest and helpful suggestions. 5-HTP was kindly supplied by the National Drug Company, Philadelphia, Pennsylvania.

M. Jović
Z. Supek

Biology Division,
Institute Rudjer Bošković,
Zagreb, Bijenička 54,
Yugoslavia.

---

[1] Doepfner, W., and Cerletti, A., *Experientia*, **14**, 376 (1958).

[2] Amin, A. H., Crawford, T. B. B., and Gaddum, J. H., *J. Physiol.*, **126**, 596 (1954).

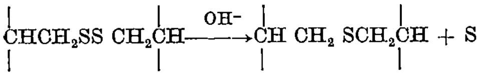
[3] Flemming, K., *Arch. Exp. Path. Pharmak.*, **239**, 425 (1960).

[4] Rowbottom, J., *J. Biol. Chem.*, **212**, 877 (1955).

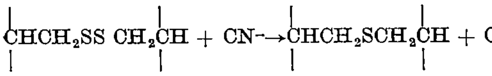
[5] Eriksen, N., *J. Biol. Chem.*, **235**, 1662 (1960).

Lanthionine Formation in Keratin

Reaction of wool keratin with solutions of alkalis or potassium cyanide leads to the elimination of one of the sulphur atoms of the dithio bond of the cystine residues with the formation of a thioether (lanthionine) linkage:



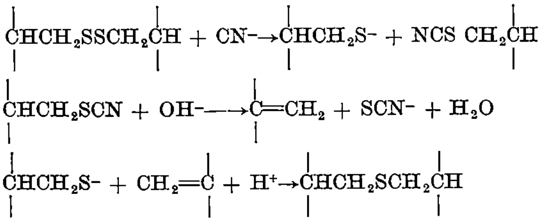
$$\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2SS\,CH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H} \xrightarrow{\mathrm{OH^-}} \underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H\,CH_2\,SCH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H} + \mathrm{S} \qquad (1)$$



$$\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2SS\,CH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H} + \mathrm{CN^-} \rightarrow \underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2SCH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H} + \mathrm{CNS^-} \qquad (2)$$

It is considered that the reaction with cyanide ions, which overall may be represented by equation (2), can follow two possible courses:

(a)

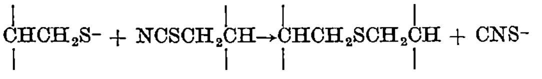


$$\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2SSCH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H} + \mathrm{CN^-} \rightarrow \underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2S^-} + \mathrm{NCS\,CH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H}$$

$$\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2SCN} + \mathrm{OH^-} \longrightarrow \underset{|}{\overset{|}{\mathrm{C}}}\mathrm{{=}CH_2} + \mathrm{SCN^-} + \mathrm{H_2O}$$

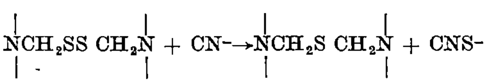
$$\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2S^-} + \mathrm{CH_2{=}}\underset{|}{\overset{|}{\mathrm{C}}} + \mathrm{H^+} \rightarrow \underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2SCH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H}$$

(b)



$$\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2S^-} + \mathrm{NCSCH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H} \rightarrow \underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2SCH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H} + \mathrm{CNS^-}$$

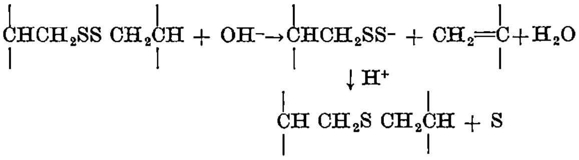
The β-elimination reaction (*a*) was first proposed by Swan[1], who later suggested the possibility of lanthionine formation by SCN- displacement[2] without removal of β-hydrogen atoms. This was confirmed by Earland and Raven[3], who studied the reaction of N-mercaptomethylpolyhexamethyleneadipamide disulphide with cyanide ions, and showed that a thioether bond is formed under conditions identical to those which produce lanthionine in wool:



$$\underset{|}{\overset{|}{\mathrm{N}}}\mathrm{CH_2SS\,CH_2}\underset{|}{\overset{|}{\mathrm{N}}} + \mathrm{CN^-} \rightarrow \underset{|}{\overset{|}{\mathrm{N}}}\mathrm{CH_2S\,CH_2}\underset{|}{\overset{|}{\mathrm{N}}} + \mathrm{CNS^-}$$

Since β-elimination is impossible, it must be concluded that a reaction such as (*b*) is very probably an important route for lanthionine formation in wool.

On the other hand, in the alkaline reaction, Swan[4] has shown the importance of a β-elimination mechanism:



$$\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2SS\,CH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H} + \mathrm{OH^-} \rightarrow \underset{|}{\overset{|}{\mathrm{C}}}\mathrm{HCH_2SS^-} + \mathrm{CH_2{=}}\underset{|}{\overset{|}{\mathrm{C}}} + \mathrm{H_2O}$$

$$\downarrow \mathrm{H^+}$$

$$\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H\,CH_2S\,CH_2}\underset{|}{\overset{|}{\mathrm{C}}}\mathrm{H} + \mathrm{S}$$

since $\alpha\alpha'$dimethylcystine is degraded only extremely slowly under these conditions, compared with free cystine.

The reaction of N-mercaptomethylpolyhexamethyleneadipamide with alkalis has now been examined, as it is probably a better model for wool[5] than Swan's compounds, in that the reaction, as with wool, is heterogeneous, giving the ruptured disulphide residues a greater chance to re-unite in a lanthionine linkage.

2·00 gm. of disulphide (sulphur, 4·20 per cent) were boiled under reflux for 5 hr. with 400 ml. of the solution of alkali. After reaction, the solid product was collected, washed with water and dried *in vacuo*. The results obtained are given in Table 1. The analytical methods used have been described previously[3].

Table 1

Solution	pH	Wt. loss (per cent)	Sulphur in product (per cent)	Disulphide sulphur (per cent)	NH_2 m.equiv. 100 gm.
—	—	—	4·20	4·07	1·5
0·10 M Na_2HPO_4	8·0	1·0	3·99	4·26	1·5
0·05 M $Na_2B_4O_7$	9·2	0	4·14	4·27	1·6
0·10 M Na_2CO_3	10·5	2·5	3·70	3·94	2·3
0·10 M Na_2CO_3*	10·5	11·0	3·39	3·28	2·7
0·10 M NaOH	12·0	35·0	2·23	1·90	4·7

* Boiled for 10 hr.

It is shown that a lanthionine type of reaction does not occur when the β $>$CH group is replaced by nitrogen, which must be regarded as strong supporting evidence for the β-elimination mechanism, and against mechanisms which involve formation by direct attack on the α-carbon atom[6].

We thank the Wool Textile Research Council for financial assistance with this work.

C. Earland
D. J. Raven

Department of Textile Industries,
Institute of Technology,
Bradford.

---

[1] Swan, J. M., *Proc. Intern. Wool Textile Res. Conf., Austral.*, *C*–25 (1955).

[2] Swan, J. M., *Current Trends in Heterocyclic Chemistry*, 65 (Butterworths, 1958).

[3] Earland, C., and Raven, D. J., *J. Amer. Chem. Soc.*, **80**, 3430 (1958).

[4] Swan, J. M., *Nature*, **179**, 965 (1957).

[5] Earland, C., and Raven, D. J., *Proc. Second Intern. Wool Textile Research Conf. England, J. Text. Inst.*, **51**, *T* 678 (1960).

[6] Zahn, H., Kunitz, F.-W., and Hildebrand, D., *Proc. Second Intern. Wool Textile Research Conf. England, J. Text. Inst.*, **51**, *T* 740 (1960).

Effect of Polyamino Carboxylic Acids on Catalase

CATALASES are known to be inactivated by the formation of compounds with cyanide[1], azide and hydroxylamine[2], so we thought it probable that the iron of the enzyme could be complexed with chelating agents, the effect being also to inactivate the enzyme. The chelating agents used were ethylenediamine tetraacetic acid (EDTA), cyclohexanediamine tetraacetic acid (CDTA), diethylenetriamine pentaacetic acid (DTPA) and NN′ethylene*bis*(2{*o*-hydroxyphenyl}) glycine (EHPG). The rate of decomposition of hydrogen peroxide by bovine-liver catalase in the presence of each of the above chelating agents was followed by a rapid titration method.

A decrease in the rate of destruction of hydrogen peroxide by the catalase was observed in the presence of all the chelating agents. With EDTA and DTPA, the inhibition reaches a maximum at a *p*H of about 8·5 and then decreases again with increasing *p*H. With CDTA, the inhibition increases slowly with increasing *p*H, whereas with EHPG the increase is rapid, although measurements could not be made at *p*H values greater than 10 due to a rapid decrease in the activity of the catalase, probably due to protein denaturation. In conclusion, it must be noted that the inhibition of catalase by these chelating agents is small compared to that of hydrogen cyanide, which has a value of K_i of $4{\cdot}6 \times 10^{-6}$ *M* at *p*H 7 (ref. 3), whereas at *p*H 8·5 (*p*H of maximum inhibition of catalase in the presence of EDTA and DTPA) the K_i values are $9{\cdot}2 \times 10^{-2}$ *M*, $9{\cdot}3 \times 10^{-2}$ *M*, $2{\cdot}6 \times 10^{-1}$ *M*, and $3{\cdot}3 \times 10^{-1}$ *M* for EDTA, DTPA, CDTA and EHPG respectively.

JOAN BOND
J. G. COOPER
Department of Pure and Applied Chemistry,
Royal College of Advanced Technology,
Salford, 5.

[1] Chance, B., *J. Biol. Chem.*, **179**, 1299 (1949).
[2] Foulkes, E. C., and Lemberg, R., *Enzymologia*, **13**, 302 (1949).
[3] Chance, B., *J. Biol. Chem.*, **179**, 1299 (1949).

Dextransucrase *V* and the Role of Metal Ions in Enzyme Catalysis

DEXTRANSUCRASE is a representative member of the general class of enzymes known as transglycosidases. In this particular example, the glucosyl group from sucrose is transferred to a suitable acceptor, which causes chain initiation, and dextran is formed. Polymer-formation is governed by many factors such as the nature of the acceptor molecule, *p*H and temperature[1].

This work was directed at the role of metal ions in enzyme catalysis[2]. This was initiated by some previous work which had suggested that magnesium plays a part in dextran synthesis[3,4]. These investigations had shown that a relatively linear molecule of dextran could be isolated from a magnesium-deficient diet inoculated with *Betacoccus arabinosaceous* (Birmingham). This is contrasted with the branched dextran isolated from a normal medium. With this in mind, it was felt important to study the effect of metal ions on the enzyme and to carry out a detailed kinetic investigation.

Using fructose-production as a measure of enzyme activity, the conversion of sucrose to dextran was examined under the influence of several metal cations. The details of the enzyme preparation and the analytical procedures have been reported[2]. In addition to testing the effect of the metal ions on the catalysis, the role of metal chelating agents, tetrasodium ethylenediamine tetraacetic acid (EDTA) and oxalic acid were investigated. All experiments were conducted in a 0·05 *M* acetate buffer of *p*H 5·2 and the reaction was carried out for 0·5 hr. in a constant temperature water bath at 25° C.

Of the metal ions tried as activators of this particular enzyme, notably calcium, zinc, magnesium, manganese and copper, only the first was effective. The reciprocal plot of initial velocity against calcium ion at various substrate concentrations is shown in Fig. 1. From the common intercept of these lines, a value for the dissociation constant of the enzyme metal complex may be evaluated. This turns out to be $9{\cdot}4 \times 10^{-5}$ *M*. The action of EDTA is shown in Fig. 2. The graph for oxalic acid was similar, except

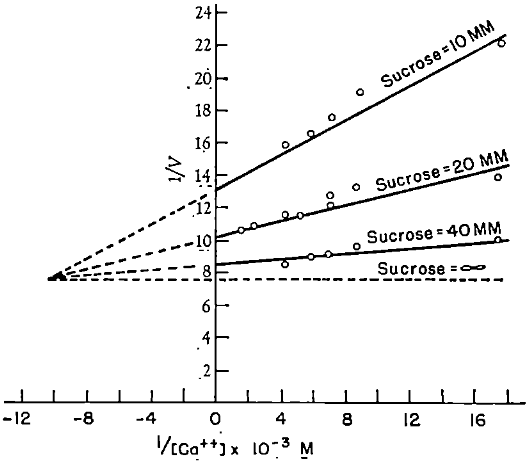


Fig. 1. Reciprocal plot of initial velocity (mgm. fructose/0·2 ml. enzyme/0·5 hr.) against calcium ion concentration. The dashed curve representing sucrose = ∞ was drawn through the intersection of the three other curves and the value for 1/*V* calculated from another experiment using enzyme and sucrose and an excess of calcium ion

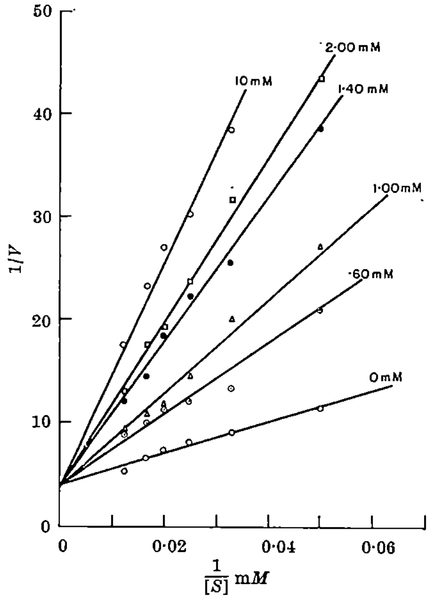


Fig. 2. Reciprocal plot of initial velocity (mgm. fructose/0·2 ml. enzyme/0·5 hr.) against sucrose concentration at various levels of EDTA

that it required twenty-five times as much oxalic acid to achieve the same results.

The plot in Fig. 1 showing the influence of calcium ions fits the first type of coupling activation as described in ref. 5. In this particular case of activation, the substrate will not couple efficiently with the enzyme unless the activator (in this case calcium ion) is already attached to the enzyme. Competitive inhibition is demonstrated in Fig. 2 for the action of the chelating agents. The action of oxalic acid and EDTA appears to be a removal of the calcium ion from the enzyme. This removal renders the catalytic site less active in its action on sucrose. In the presence of excess substrate the influence of the chelating agents is diminished by the law of mass action, as demonstrated by the convergence of the lines in Fig. 2 to a common intercept.

The ability of magnesium ion to influence the degree of branching has been mentioned[3,4]. In view of this fact, it is rather surprising at first sight that magnesium appeared to have no influence on the course of the reaction in this work. A possible explanation for this apparent anomaly might rest on the following argument: according to Bovey[6,7] there are at least two enzymes responsible for the synthesis of dextran. One enzyme operates by initiating chain-growth and liberating fructose. The other, the so-called 'branching enzyme', operates by transferring pre-formed units of oligosaccharides to the main chain. This latter enzyme would not liberate any reducing sugars; consequently it would not be detected by the methods of analysis used in this investigation.

W. BROCK NEELY
JULIE HALLMARK

Biochemical Research Laboratory,
Dow Chemical Co.,
Midland, Michigan.

[1] Neely, W. B., *Adv. in Carbohydrate Chem.*, **15**, 341 (1960).

[2] *J. Amer. Chem. Soc.*, Part IV, **81**, 4416 (1959).

[3] Barker, S. A., Bourne, E. J., Bruce, G. T., Neely, W. B., and Stacey, M., *J. Chem. Soc.*, 2395 (1954).

[4] Barker, S. A., Bourne, E. J., James, A. E., Neely, W. B., and Stacey, M., *J. Chem. Soc.*, 2096 (1955).

[5] Friedenwald, J. S., and Maengwyn-Davies, G. D., *The Mechanism of Enzyme Action*, 174, McElroy, W. B., and Glass, B., eds. (The Johns Hopkins Press, Baltimore, 1954.)

[6] Bovey, F. A., *J. Poly. Sci.*, **35**, 167 (1959).

[7] Bovey, F. A., *J. Poly. Sci.*, **35**, 183 (1959).

Degree of Polymerization of Hyaluronic Acid isolated from Human Aortic Tissue

SEVERAL reports have been presented on the degree of polymerization of hyaluronic acid isolated from umbilical cords, ovarian cysts and follicular fluid, synovial fluid and vitreous bodies[1-5]. The hyaluronic acid isolated from the tissues mentioned shows varying degree of polymerization.

In a previous publication Bertelsen and Marcker[6] discussed variations in the ratio chondroitin sulphuric acid/hyaluronic acid in aortic tissue with age. In this investigation the degree of polymerization of hyaluronic acid isolated from human aortæ has been determined by means of osmometry.

The aortæ were obtained 12–15 hr. after death. The adventitia was carefully removed by hand, and the media-intima parts of the vessels were stored in acetone for 4–5 months. Owing to the small amount of tissue in children, samples from individuals less than 2 yr. of age were pooled. Furthermore, three groups of aortæ from the new-born were included in the material, each of the groups consisting of 10–12 aortæ. Only macroscopically normal tissue was collected, and so, if the question were about elderly individuals with atherosclerotically changed aortæ, the changed parts were discarded.

The disintegrated, de-fatted tissue was incubated with a proteolytic enzyme, and the protein-split products were precipitated with trichloroacetic acid. The residual protein-split products were removed by repeated shakings with chloroform–*iso*amylalcohol[7] and subsequent dialyses against running tap-water. A more detailed description of the method of extraction was given in ref. 6.

In order to remove the chondroitin sulphuric acids, the aqueous solution of mucopolysaccharides was brought to *p*H 1·5 with 0·1 M hydrochloric acid in the presence of 0·1 M sodium chloride at 0° C. At this *p*H the ionization of the hyaluronate is suppressed, whereas the chondroitin sulphates are largely ionized. 1 gm. of 200–400-mesh amine ion-exchange resin 'Dowex 1–X8' in the chloride form per 25 ml. of the mucopolysaccharide solution was added, and the mixture was stirred for 2 hr. at 0° C. (ref. 8). After removal of the resin by centrifugation and filtration through a glass funnel, the *p*H was raised, and by slowly adding two volumes of cold absolute alcohol, the hyaluronate was precipitated. The precipitate was washed in absolute alcohol and ether and dried in a vacuum desiccator containing silica-gel.

To characterize the isolated substances, we used nitrogen and sulphur determinations, viscometric determinations and subsequent incubation with streptococcal hyaluronidase and paper chromatography. The nitrogen was determined by the micro-Kjeldahl procedure, and the sulphur contents of the hyaluronic acid preparations were determined by the method of Zinneke[9]. Viscometric investigations were performed in the micro-viscometer described in ref. 10. Paper chromatography of hydrolysates of the substances was performed in 2,6-lutidine/water (65 : 35) for 24 hr., and the chromatograms were developed by a 0·1 per cent ninhydrin solution in acetone.

The following tends to show that the isolated substances really were hyaluronate: the white powders were: (1) highly hygroscopic; (2) easily soluble in water, the solutions being clear and viscid; (3) the viscosities decreased considerably after incubation with bacterial hyaluronidase, giving the characteristic degradation curves of hyaluronic acid. Furthermore, (4) the paper-chromatograms of the hydrolysates only showed spots corresponding to authentic glucosamine, and (5) the amount of nitrogen in all samples was about 3·6 per cent (theoretically 3·4). The sulphur in the samples ranged from 0·6 to 0·8 per cent (with one exception), thus indicating only minor amounts of sulphated mucopolysaccharides.

The molecular weights were calculated from colloid osmotic pressure measurements performed by the micro-osmometer described in ref. 2. The concentration used in the osmotic pressure measurements was 5 mgm. of the isolated substance in 1 ml. 0·5 M potassium chloride. According to our experience the influence of the Donnan effect is then negligible.

The results are shown in Table 1. It will be seen that the degree of polymerization of hyaluronic acid in aortic tissue is independent of age and degree of atherosclerosis.

The isolation procedure used in this investigation is generally considered very sparing, and it is unlikely that any significant depolymerization occurred during the isolation.

Table 1. MOLECULAR WEIGHTS (*M*) OF HYALURONATE ISOLATED FROM HUMAN AORTÆ, CALCULATED FROM MEASUREMENTS OF THE OSMOTIC PRESSURES

Age (yr.)	Sex	$M \times 10^3$	Nitrogen (per cent)	Sulphur (per cent)	Gross appearance
0		44	3·62	0·69	No plaques
0		43			No plaques
0		35			No plaques
0·5–2*		51	3·59		No plaques
6	♂	30	3·18	0·63	No plaques
11	♂	48		0·61	No plaques
35	♀	48	4·29		Lipoid plaques
50	♂	43	3·52	0·76	Slight sclerosis
60	♂	47	3·62		Slight sclerosis
63	♀	40		0·73	Pronounced sclerosis
72	♀	42	3·44	1·30	Pronounced sclerosis
84	♂	60	3·80	0·81	Pronounced sclerosis

* This group consists of three aortæ.

Owing to the small amount of substance at our disposal, only some of the results with nitrogen and sulphur have been determined.

The molecular weights presented in Table 1 refer to apparent molecular weights, and not to true molecular weights; the latter, which are obtained by extrapolating to zero concentration, are, therefore, a little higher than those recorded.

It can be concluded that the hyaluronic acid isolated from aortic tissue seems to have a comparatively low molecular weight as compared with the molecular weights obtained for hyaluronic acid isolated from other tissues.

This work was aided by a grant from the Danish State Science Foundation.

SV. BERTELSEN

Department of Pharmacology,
20 Juliane Maries Vej.

K. MARCKER

Department of Physical Chemistry,
19 Blegdamsvej,
University of Copenhagen.

[1] Blix, G., and Snellmann, O., *Ark. Kemi Mineral. Geol.*, **19A**, 32 (1945).
[2] Christiansen, J. A., and Jensen, C. E., *Acta Chem. Scand.*, **7**, 1247 (1953).
[3] Jensen, C. E., *Acta Chem. Scand.*, **8**, 937 (1954).
[4] Jensen, C. E., and Zachariae, F., *Acta Endocrinol.*, **27**, 356 (1958).
[5] Blumberg, B. S., and Oster, G., *Science*, **120**, 432 (1954).
[6] Bertelsen, Sv., and Marcker, K., *Acta Pharmacol. et Toxicol.*, **18**, 1 (1961).
[7] Sevag, M. G., *Biochem. Z.*, **273**, 419 (1934).
[8] Simmons, N. S., Atomic Energy Project Univ. Calif. (Los Angeles) Chemistry Report 353 (November, 1955).
[9] Zinneke, F., *Z. Anal. Chem.*, **132**, 175 (1951).
[10] Dalgaard-Mikkelsen, S., and Kvorning, S. A., *Acta Pharmacol. et Toxicol.*, **4**, 160 (1948).

Incorporation of Deoxyribonucleic Acid by Mammalian Cells *in vitro*

TRANSFORMATION of bacterial cells in the presence of deoxyribonucleic acid (DNA) has been demonstrated[1-3], and has indicated that genetic information is carried by DNA. There is little unequivocal evidence for such transformations in higher forms of life, in contrast to the obvious transfer of genetic information that occurs by means of the gamete during fertilization. The two reported attempts to obtain these transformations through the mediation of added DNA have been conflicting[4,5]. On the other hand, evidence for the uptake of DNA by mammalian cells[6,7] has supported the idea that observed biological effects might be obtained. In this communication I present evidence for the incorporation of extracellular high molecular weight DNA into the nuclear DNA of cells of the Ehrlich–Lettré ascites tumour, when they are incubated *in vitro* (Table 1). Labelled homologous DNA for these experiments was first obtained by injecting formate labelled with carbon-14 intraperitoneally in mice bearing this tumour. After an appropriate time-interval, the tumour cells were removed and washed and the DNA isolated by the detergent procedure[8]. To determine the specific activity of the DNA used in the experiments, samples were hydrolysed with 70 per cent perchloric acid for 1 hr. at 100° C. and the bases, adenine and thymine, separated by paper chromatography using a solvent system of *iso*propanol, hydrochloric acid and water. The bases were eluted and the specific activity of each was determined for appropriately plated samples using a gas-flow counter for the radioactivity determinations.

Table 1. *In vitro* INCORPORATION OF DNA LABELLED WITH CARBON-14 BY EHRLICH-LETTRÉ ASCITES TUMOUR CELLS

	Specific Activity (counts/min./μmole base) Adenine	Thymine	Ratio adenine : thymine
Initial DNA, from *in vivo* labelling	3,852	1,395	1·00 : 0·36
Nuclear DNA after incubation	296	93	1·00 : 0·31

Incubation conditions: 2 ml. ascites fluid (0·5 ml. packed cells), 1 ml. 0·25 *M* sucrose—0·1 *M* phosphate buffer *p*H 7·4, 0·8 ml. 0·1 *M* glucose in 0·25 *M* sucrose, 0·2 ml. water. Incubated aerobically at 37° C. for 1 hr. in a metabolic shaking incubator.

In the incubation experiments tumour cells were obtained by draining the fluid from an abdominal incision in mice bearing a tumour growth of 7–10 days. The cells were incubated aerobically in a shaking incubator at 37° C. in the presence of ascites serum, 0·25 sucrose–phosphate buffer *p*H 7·4, glucose[9], and with samples of the intact labelled DNA dissolved in the buffer solution. After 1 hr. the cells were washed carefully with the sucrose–phosphate buffer and saline, and were then frozen in a dry-ice/acetone mixture. The frozen cells were homogenized in dilute citric acid (0·05 *M*) and fractionated by differential centrifugation to obtain preparations of clean nuclei. This procedure is of importance to ensure that the observed uptake is in nuclear material. The isolated nuclei were washed several times during the preparation with 0·01 *M* citric acid and were extracted finally with cold 4 per cent perchloric acid and lipid solvents[10]. The dry residual nuclear material was hydrolysed with 0·3 *N* potassium hydroxide for 18 hr. at 37° C. to separate the nuclear ribonucleic acid (RNA). DNA was obtained by acidifying the alkaline hydrolysate and extracting the residue with 4 per cent perchloric acid at 90° C. for 15 min. The DNA fraction was dried, then hydrolysed with 70 per cent perchloric acid as before and the bases separated by paper chromatography using the solvent system described.

The amount of DNA taken up by the nuclei under these conditions can be calculated from the total number of counts added before, and obtained after, the incubation. In these experiments it has been found that there is an observed uptake of 2–4 per cent of the added DNA by the nuclei of these cells. In looking further, in an examination of the specific activity of the nuclear DNA, it has been found that despite an expected dilution the ratio between the specific activity of adenine and thymine is similar to that of the original sample of DNA used in the experiments. While it is recognized that these observations might be explained by assuming a prior breakdown of the DNA followed by its re-synthesis in the cell nucleus, this seems unlikely, because of the difference in the degree of labelling of the bases adenine and thymine of DNA, under *in vivo* and *in vitro* conditions (to be described elsewhere). It is concluded that some intact extracellular DNA of high molecular weight can be transferred across the cell and be incorporated into the genetic fine material of these cells. Further attention is being directed to the conditions necessary for this transfer and incorporation and to the biological effects elicited by such incorporation.

This research has been supported by a grant (C–5172) of the National Cancer Institute of the U.S. Public Health Service.

E. R. M. Kay

Department of Biochemistry,
University of Rochester,
Rochester, New York.

[1] Avery, O. T., MacLeod, C. M., and McCarty, M., *J. Exp. Med.*, **79**, 137 (1944).

[2] Catlin, B. W., and Cunningham, L. S., *J. Gen. Microbiol.*, **19**, 522 (1958).

[3] Zinder, N. D., and Lederberg, J., *J. Bacteriol.*, **64**, 679 (1952).

[4] Benoit, J., Leroy, P., Vendrely, C., and Vendrely, M. R., *C.R. Acad. Sci., Paris*, **247**, 1049 (1958); *Trans. N.Y. Acad. Sci.*, II, **22**, 494 (1960).

[5] Medawar, P. B., *Nature*, **182**, 62 (1958).

[6] Gartler, S. M., *Nature*, **184**, 1505 (1959).

[7] Chorazy, M. R., Baldwin, H. H., and Boutwell, R. K., *Fed. Proc.*, **19**, 307 (1960).

[8] Kay, E. R. M., Simmons, N. S., and Dounce, A. L., *J. Amer. Chem. Soc.*, **72**, 1724 (1952).

[9] Kay, E. R. M., *Fed. Proc.*, **19**, 393 (1960).

[10] Smellie, R. M. S., Humphrey, G. F., Kay, E. R. M., and Davidson, J. N., *Biochem. J.*, **60**, 177 (1955).

Anticancer Properties of Thio-diglycollic Acid

In earlier publications[1–4] we have shown possibilities of preferential inhibition of tumour growth by antimetabolites of hexose monophosphate (HMP) pathway. A number of derivatives of thiophene-dicarboxylic acid have been prepared and tested for their anticancer properties. Results with thiophene 2,5 dicarboxylic acid (TDA) and 2,5 dicarbethoxy-3,4 dihydroxy thiophene (DICETOL) have already been published[3,4]. Results with other compounds will be published in detail elsewhere. One of the compounds synthesized in this series was thio-diglycollic acid (TDGA). In this compound carbon numbers 3 and 4 from thiophene have been completely dispensed with. This, in a way, amounted to an attempt to identify the active groupings which may be responsible for the anticancer properties.

Thio-diglycollic acid was synthesized by treating mono-chloro acetic acid with sodium sulphide, as described by Barkenbus and Landis[5]. This compound is extremely soluble in water. It was therefore isolated from aqueous solutions by continuous liquid-liquid extractions with ether.

Table 1. Analytical Data

Calculated	C = 32·00 per cent	H = 4·00 per cent
Found	C = 31·50 per cent	H = 3·60 per cent
Melting point:	Literature, 129°;	Found, 129–131°

The anticancer property of this compound was tested against two transplantable tumours: (1) Yoshida sarcoma (ascites) in rats and (2) solid fibrosarcoma in Swiss mice[6].

Thirty-six Wistar female rats, 2·5–3 months old, were transplanted intraperitoneally, under aseptic conditions, with 0·6 ml. each of freshly withdrawn Yoshida sarcoma ascitic fluid. After 8 hr., the animals were randomly divided into two groups—control and experimental—of 18 animals each. Control animals received 0·5 ml. of isotonic saline, while the experimental animals were given 2 mgm. of TDGA dissolved in 0·5 ml. of isotonic saline intraperitoneally. The treatment was given three times a day, with a total dose of 6 mgm. of TDGA/rat/day (40 mgm./kgm. of body weight). Multiple daily injections were necessary to maintain adequate drug concentration at the tumour site throughout the day. All the rats from the control group died between the fourth and ninth day after transplantation. TDGA administration to the experimental group was discontinued after the tenth day and survival observed. Out of the eighteen animals only two animals died 35 days after transplantation with solid growth in the peritoneal cavity. The remaining sixteen rats from the experimental group have survived for more than 5 months after transplantation, even though the drug administration was discontinued on the tenth day (Fig. 1). Since Yoshida sarcoma is a very rapidly growing tumour, it was anticipated that, even if a few viable cells had been left behind, the tumour growth would have flared up and killed all the transplanted animals within 10 days after discontinuation of the treatment. But the fact that sixteen out of eighteen animals have survived for more than 5 months after the discontinuation of the treatment suggests that Yoshida sarcoma tumour cells have been completely wiped out by TDGA treatment. All the animals from the experimental group are normal and do not show any toxic side-effects. The animals are still under observation.

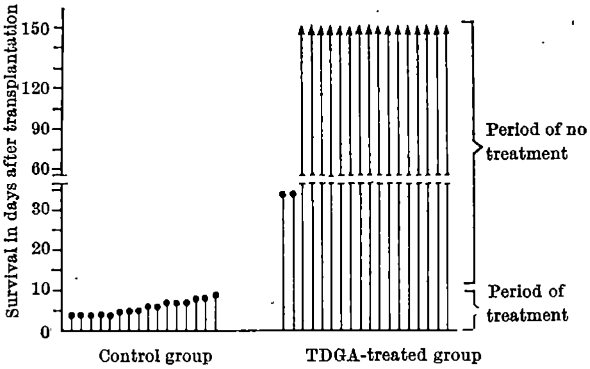


Fig. 1. Effect of TDGA on survival of Yoshida sarcoma (ascites) bearing rats. ●, Rats dead; ▲, rats living

TDGA is an acidic substance which was directly injected into the peritoneal cavity. In an auxiliary experiment, acetic acid in similar concentration was injected in the peritoneal cavity of Yoshida sarcoma-bearing rats and survival observed. It was found that the animals treated with acetic acid died more or less at the same time as the control group, suggesting that the therapeutic effects observed with TDGA were not due to acidity of the substance.

Influence of TDGA was also studied on transplantable solid fibrosarcoma in Swiss mice[6]. For this purpose, fibrosarcoma was implanted by subcutaneous injections of 0·5 ml. of tumour suspension, and the treatment started after the thirteenth day when the tumour attained measurable and uniform size in all the animals. TDGA was injected subcutaneously twice a day and the total daily dose was 6 mgm./day (240 mgm./kgm. of body weight). The animals were killed after 12 days treatment (twenty-fifth day after transplantation). The results are shown in Table 2. It will be seen that, although the results with solid fibrosarcoma were not as spectacular as with Yoshida ascites tumour, there was definite evidence of inhibition of tumour growth. The average weight of tumour from the control animals was 4·75 gm. as against 2·8 gm. in the TDGA-treated animals.

TDGA did not show any toxic side-effects with the therapeutic doses employed. Large amounts of

Table 2. EFFECT OF THIO-DIGLYCOLLIC ACID (TDGA) ON GROWTH OF FIBROSARCOMA IN SWISS MICE*

Treatment	Dose	Day of treatment after transplantation	Period of treatment (days)	Average tumour weight and range (gm.)
Treated	6 mgm./day	13th	12	2·829 (1·470–3·628)
Control	—	13th	12	4·755 (4·378–6·273)

* 6–8 weeks old Swiss female mice were used in these experiments.

TDGA, at a dose of 1,250 mgm./kgm. body-weight, were injected at one shot subcutaneously. Except for the skin necrosis seen at the site of injection, the treated animals did not show any toxic side-effects. Further work on the mechanism of action is in progress. TDGA has two acidic groups, and hence, in addition to studying its role as an antimetabolite of pentose phosphate pathway intermediates, its action as a potential bifunctional group is also under investigation.

M. B. SAHASRABUDHE
M. V. NARURKAR
L. B. KOTNIS
Atomic Energy Establishment,
Trombay, Bombay.

B. D. TILAK
L. C. SHAH
V. N. GOGTE
University Department of Chemical Technology,
Bombay.

[1] Sahasrabudhe, M. B., *Nature*, **182**, 163 (1958).

[2] Narurkar, M. V., Nerurkar, M. K., and Sahasrabudhe, M. B., *Proc. Second International Conference on Peaceful Uses of Atomic Energy, Geneva*, **24**, 255 (1958).

[3] Sahasrabudhe, M. B., Nerurkar, M. K., Narurkar, M. V., Tilak, B. D., and Bhavsar, M. D., *Brit. J. Cancer*, **14**, 547 (1960).

[4] Sahasrabudhe, M. B., Narurkar, M. V., Kotnis, L. B., Tilak, B. D., and Bhavsar, M. D., *Nature*, **184**, 202 (1959).

[5] Barkenbus, C., and Landis, P. S., *J. Amer. Chem. Soc.*, **70**, 684 (1948).

[6] Waravdekar, S. S., and Ranadive, K. J., *J. Nat. Cancer Inst.*, **18**, 555 (1957).

HISTOCHEMISTRY

Muscle Target Fibres, a Newly Recognized Sign of Denervation

THE muscle target fibre, a newly recognized sign of skeletal muscle fibre denervation, as observed in muscle biopsies from patients with neuromuscular disease, is described in this communication. From a series of forty-six biopsies showing the classic features of denervation[1] (associated with a variety of diseases), 27 specimens also contained target fibres. Eight specimens had 3–10 target fibres, four had 10–20 target fibres, and fifteen had more than twenty target fibres. The presence of target fibres could not be correlated with any particular associated disease. A concurrent series of 25 muscle biopsies showing the classic features of myopathy[1], associated with a variety of diseases, did not contain any target fibres. A concurrent series of 29 muscle biopsies having no pathological features did not contain any target fibres.

The name 'target fibre' is derived from the appearance on cross-section (Figs. 1*a*, 2*a*, *b*). Each muscle fibre so affected has three more or less distinct concentric zones, numbered 1, 2, and 3 from the centre outward, although these is some variability in this pattern. Occasionally zone 2 is absent. More than one target within a fibre is rare. On longitudinal section through the centre of the target fibre, five parallel-bordered zones appear (from one side to the other—3, 2, 1, 2, 3), running the length of the fibre present on the slide (Fig. 1*b*). In paraffin-embedded sections, target fibres are best demonstrated after fixation in Bouin's fluid and staining by the phosphotungstic acid hæmatoxylin or the Gomori trichrome method.

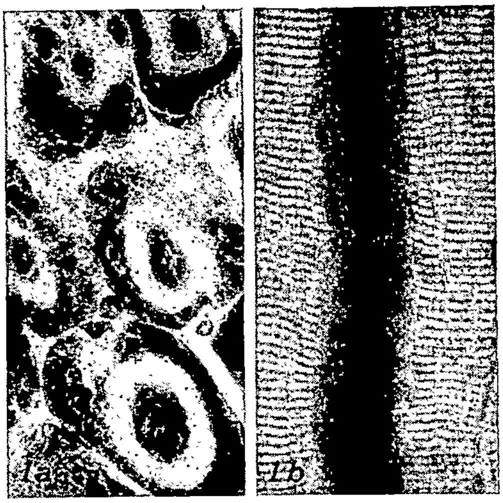


Fig. 1. *a*, Phosphotungstic acid hæmatoxylin stain (× 560); *b*, Gomori trichrome stain, polarized light. The anisotropism of zone 1 is masked by the stain (× 560)

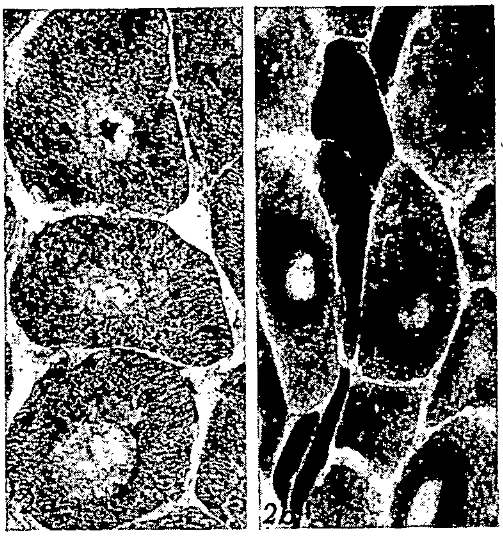


Fig. 2. *a*, Lactate dehydrogenase (× 225); *b*, alkaline adenosine triphosphatase (× 225)

Zone 1, in paraffin sections (Figs. 1*a*, *b*), contains compact wavy longitudinally running strands of myofibril-like material, which lack cross-striations and are uniformly anisotropic in polarized light. In fresh frozen sections, histochemical evidence of mitochondrial oxidative enzyme activity (succinate dehydrogenase[2], lactate dehydrogenase, cytochrome oxidase[3]) is completely absent (Fig. 2*a*). Evidence for the presence of glycogen (periodic acid–Schiff stain), for the means of glycogen formation (glucose-1-phosphate + uridine triphosphate via uridine-diphosphate-glucose to glycogen[4]), or for the means of glycogen breakdown (amylophosphorylase reaction[5]), which are sarcoplasmic reactions, is completely absent. Alkaline adenosine triphosphatase activity[6] is absent (Fig. 2*b*). In a few instances, within zone 1 there is a small region of high activity of the oxidative enzymes (Fig. 2*a*), the glycogen metabolism enzymes, and the periodic acid–Schiff reaction.

Zone 2 appears virtually unstained in paraffin sections. The concentration of myofibrillar material appears reduced, but there is a faint normal cross-striated pattern detectable in polarized light. Alkaline adenosine triphosphatase activity is increased. The mitochondrial oxidative enzyme reactions are usually decreased or absent, but infrequently they may be increased. The reactions associated with glycogen metabolism may be increased or decreased.

Zone 3 usually appears normal by all the methods used.

In the target fibres, the nuclei are not abnormal in appearance or location. There is not an increase of connective tissue or an inflammatory cell reaction around the target fibres. The target fibres are either of about normal diameter or are somewhat smaller than normal. They may appear in a group suggesting a motor unit, but such a pattern cannot always be determined, especially when their number is small or very large.

One must distinguish target fibres from the muscle fibres containing central cores in central core disease[7–9]. The central core fibres differ from target fibres in the following features: the central core, in paraffin sections or after histochemical staining, appears as a central uniform region (or regions) within the muscle fibre. With nearly all the ordinary histological techniques, the central core is more darkly stained than the surrounding non-core region. On longitudinal section, the central core has well-preserved cross-striations. There are no classic features of denervation in the muscle of central core disease, but there infrequently may be features of an active myopathy[9].

The work described here indicates that the target fibre represents a pathological alteration of the cyto-architecture of the human skeletal muscle fibre, which has been found only in association with denervation. It is concluded that the target fibre is a sign of skeletal muscle denervation. As the target appearance occurs in fibres of normal or somewhat smaller than normal diameter, it probably signifies recent denervation.

Most of the muscle biopsies reviewed were generously provided by Dr. G. Milton Shy. Prof. W. Blackwood kindly permitted study of the specimen shown in Fig. 1*a*.

W. K. ENGEL

Medical Neurology Branch,
National Institute of Neurological
Diseases and Blindness,
United States Department of Health,
Education and Welfare,
Bethesda, Maryland.

[1] Greenfield, J. G., Shy, G. M., Alvord, E. C., and Berg. L., *An Atlas of Muscle Pathology in Neuromuscular Diseases* (E. and S. Livingstone, Edinburgh, 1957).

[2] Nachlas, M. M., Walker, W. H., and Seligman, A. M., *J. Biophys. Biochem. Cytol.*, **4**, 29 (1958).

[3] Burstone, M. S., *J. Histochem. Cytochem.*, **8**, 63 (1960).

[4] Takeuchi, T., and Glenner, G. G., *J. Histochem. Cytochem.* (in the press).

[5] Takeuchi, T., *J. Histochem. Cytochem.*, **6**, 208 (1958).

[6] Padykula, H. A., and Herman, E., *J. Histochem. Cytochem.*, **3**, 170 (1955).

[7] Shy, G. M., and Magee, K. R., *Brain*, **79**, 510 (1956).

[8] Dubowitz, V., and Pearse, A. G. E., *Lancet*, ii, 23 (1960).

[9] Engel, W. K., Foster, J. B., Hughes, B. P., Huxley, H. E., and Mahler, R., *Brain* (in the press).

RADIOBIOLOGY

Assessment of Chronic Exposure to Radiostrontium by Urinary Assay

RADIOACTIVE contamination of the biosphere and particularly of the human population is a contemporary problem of high interest, and strontium-90 is perhaps the most important single radionuclide concerned. We require to know what levels are being incorporated into the human population at any given time. Since food is the major route by means of which environmental radiocontaminants reach human beings, attention has largely been turned to the monitoring and surveying of food and water. This communication proposes that urinary assays of strontium-90 and calcium may have considerable advantage for the estimation of the contemporary strontium-90 to calcium ratio of the intake, and for estimation of the strontium-90 to calcium ratio in newly forming bone. The same considerations would also apply to strontium-89 whenever this radionuclide is of inportance.

Several investigations have shown that, under typical conditions, the strontium to calcium ratios between diet, various tissues and secretions are roughly constant. Examination of results obtained by independent methods in many laboratories and countries has indicated that under usual dietary conditions the values of the ratio (strontium/calcium of sample (ref. 1)) to (strontium/calcium of precursor), called here the 'observed ratio', fall within a reasonably narrow range, with few indications of variations as high as a factor of 2. Some typical values are as follows: observed ratio (body/diet) for human beings, 0·25–0·50 (refs. 2–4); observed ratio (body/diet) for cattle, sheep, pigs, goats, 0·2–0·25 (refs. 2 and 5); observed ratio (milk/diet) for human beings, cow and goat, 0·09–0·16 (refs. 2 and 4).

An example of the usefulness of this concept is the estimation of the comparative levels of strontium-90 and calcium in the total dietary intake of dairy cattle by calculation from values in milk. There seems little question but that dividing the ratio strontium-90/calcium in milk by 0·1 (typical value for observed ratio (milk/diet)) would give an estimate of dietary intake more simply and more accurately than would efforts to measure total intakes directly or to sum the strontium-90 and calcium contributions of components of the feed and water. Also, an estimate of the strontium-90/calcium being laid down in the newly forming and exchangeable bone of the animals could be obtained by dividing this ratio in milk by 0·5 (typical value for observed ratio (milk/plasma)).

It is well known that urinary calcium excretion is highly variable between individuals and even from day to day in the same individual. It is assumed that urinary strontium would also be variable. Since many of the factors that affect the behaviour of calcium also affect that of strontium in the same way, there is the likelihood that variations would be smoothed by using the strontium–calcium ratio as the basis of measurement.

Table 1 presents results from experiments with rats designed to show the variation of the observed ratio (urine/diet) with time following the start of ingestion. In Experiment 1, 8 rats of about 80 gm. body-weight were placed in metabolism cages and fed commercial rat pellets; the drinking water throughout the work was labelled with calcium-45 and strontium-85 for double-tracer studies according

Table 1. STRONTIUM–CALCIUM RELATIONSHIPS BETWEEN DIET AND URINE OF RATS

Experiment	Time after start of dosage	Observed ratio (urine/diet)*
1	17 hr.	2·6 ± 0·97†
	41 ,,	2·8 ± 0·72
	64 ,,	3·2 ± 0·55
	88 ,,	3·0 ± 0·52
	12 days	3·3 ± 0·53
	24 ,,	2·8 ± 0·28
	35 ,,	4·1 ± 0·20
		Average 3·1
2	14 days	3·2 ± 0·37‡

* Observed ratio = Sr*/Ca* in urine ÷ Sr*/Ca* in diet.
† Mean ± standard error of mean of 8 animals.
‡ Mean ± standard error of mean of 22 animals.

to usual procedures[6]. It is noted that a statistically constant value of observed ratio (urine/diet) had been reached by 17 hr. after the start of ingestion of the radionuclides. In Experiment 2, 22 rats were used ranging in body-weight from 80 to 500 gm.; a mean value is reported since no significant differences were noted between age-groups. This work shows that urine values in rats do reflect intake of the previous few days, and that the variability was not excessive as shown by the values for the standard error of the mean.

It is next of interest to examine the literature for values of observed ratio (urine/diet) for human beings in normal health. Two investigations have been reported in which the stable strontium and calcium of normal diets and urine were estimated[7,8]. From other work as yet unpublished, Dr. Herta Spencer of the Montefiore Hospital has made available some results of fall-out strontium-90 and stable calcium in the diets and urine of several individuals; in this series, three 6-day balance trials were run on each individual and the averages are reported. It is seen from Table 2 that, in 16 individuals of 4–64 years, the range of observed ratio (urine/diet) was 0·43–1·1 with a mean of 0·67 ± 0·058; the variability was about the same as in the work on rats. The average value of 0·67 is in agreement with the typical values of observed ratio (body/diet) about 0·25 and observed ratios (urine/plasma) about 3 (ref. 7). Thus an estimate of the strontium-90 to calcium of the total diet would be obtained from the measured strontium-90/calcium of the urine divided by the observed ratio (urine/diet), typical value 0·67 ; an estimate of the strontium-90 to calcium being laid down in the skeleton would be obtained by the strontium-90/calcium of the total diet multiplied by the observed ratio (body/diet), typical value 0·25.

Table 2. STRONTIUM–CALCIUM RELATIONSHIPS BETWEEN DIET AND URINE OF HUMAN BEINGS

Sex	Age	Method	Observed ratio (urine/diet)	Ref.
M	4 yr. 10 m.	(stable strontium and calcium in urine and diet)	1·0	8
F	9 yr. 9 m.		0·83	8
M	13 yr. 1 m.		0·60	8
M	14 yr. 4 m.		0·72	8
M	14 yr. 6 m.		0·78	8
F	29 yr.	(stable strontium and calcium in urine and diet)	0·45	7
F	31 yr.		0·59	7
M	40 yr.		0·50	7
M	45 yr.		0·50	7
M	50 yr.		0·40	7
F	18 yr.	(fall-out strontium-90 and stable calcium in urine and diet)	1·1	10
F	19 yr.		0·43	10
F	19 yr.		0·64	10
M	21 yr.		0·44	10
F	28 yr.		0·93	10
F	29 yr.		0·94	10
F	64 yr.		0·45	10
		Mean ± standard error of mean	0·67 ± 0·058	

Additional work is needed to get better measures of variability and of the effects of unusual diets, age or other factors that might modify the value of observed ratio (urine/diet). For example, it has been shown that new-born babies apparently have considerably higher values of observed ratio (urine/diet)[9]. In any event, it would appear feasible to collect enough urine samples (from the same individual and/or from different individuals) to give results of quite sufficient accuracy for purposes of food monitoring. The lower the contribution of food contamination relative to the total radiation exposure of the population, the lower the accuracy needed for assessment. Since present exposures from strontium-90 are only a few per cent of exposures from natural background, there would seem little need to evaluate food contamination with an accuracy much greater than appears to be possible from urinary surveys.

The advantages of using urinary assays are listed as follows : (*a*) the urinary samples reflect the total amount of strontium-90 and calcium that enters body tissues, and there is no need to guess the contributions of given food items or the availability of the strontium-90 in given food items; (*b*) values are calculated in terms of total strontium-90 and total calcium in the intake, which is probably the most important parameter governing the body burden ; (*c*) any sudden changes in levels of strontium-90 would be noted early, and the results could reflect any day-by-day variations; (*d*) sampling and bulking would be relatively simple and work could be designed to reflect individual intakes as well as group averages classed according to any category of interest ; (*e*) the results could be interpreted to give values for concentrations of strontium-90 and strontium-90/calcium ratios in areas of newly forming and exchangeable bone ; (*f*) from a continued programme of sampling it would be possible to construct the time pattern of radiostrontium-levels in bone.

Monitoring of food supplies by urinary assays would not eliminate the need for the examination of foods themselves, to obtain an understanding of the factors that govern the movement of radio-strontium in the food chain.

This investigation was supported by the U.S. Atomic Energy Commission: it was mentioned at an information meeting of the Commission last autumn.

C. L. COMAR
JAY GEORGI

Department of Physical Biology,
N.Y. State Veterinary College,
Cornell University,
Ithaca, New York.

[1] Comar, C. L., Wasserman, R. H., and Nold, M. M., *Proc. Soc. Exp. Biol. and Med.*, **92**, 859 (1956).

[2] Comar, C. L., and Wasserman, R. H., *Radioisotopes in the Biosphere*, edit. by Caldecott, R. S., and Snyder, L. A., 526 (Centre for Continuation Study, Minn., 1960).

[3] Bryant, F. J., Chamberlain, A. C., Spicer, G. S., and Webb, M. S. W., *Brit. Med. J.*, i, 1371 (1958).

[4] Lough, S. A., Hamada, G. H., and Comar, C. L., *Proc. Soc. Exp. Biol. and Med.*, **104**, 194 (1960).

[5] Hogue, D. E., Pond, W. G., Comar, C. L., Alexander, L. T., and Hardy, E. P., *J. Anim. Sci.* (in the press).

[6] Wasserman, R. H., and Comar, C. L., *Nature*, **185**, 629 (1960).

[7] Harrison, G. E., Raymond, W. H. A., and Tretheway, H. C., *Clinical Sci.*, **14**, 681 (1955).

[8] Bedford, J., Harrison, G. E., Raymond, W. H. A., and Sutton, A., *Brit. Med. J.*, i, 589 (1960).

[9] Widdowson, E. M., Slater, J. E., Harrison, G. E., and Sutton, A., *Lancet*, ii, 941 (1960).

[10] Spencer, H. (unpublished work).

Metabolism of the Sulphur Amino-Acids and of Sulphate in *Blattella germanica*

THE cockroach and the Japanese beetle are able to utilize sulphate-S for the synthesis of methionine and cystine[1]. The German cockroach, *Blattella germanica* (L.), is also able to satisfy its sulphur requirements with homocystine, methionine sulphoxide, cysteic acid, taurine, and β-hydroxyethanesulphonic acid[2]. However, removal of the intracellular symbionts from the cockroach prevents incorporation of sulphate-S into cysteine and methionine[3].

The present communication describes the results obtained when ^{35}S-methionine, ^{35}S-cystine, and their oxidation products were administered to xenic (symbionts and intestinal micro-organisms present) and aposymbiotic *Blattella*. Further developments in the study of the metabolism of ^{35}S-sulphate are also described.

One to five microcuries of the ^{35}S-compound were injected into the insects, which were then maintained at 25° C. for one to seven days. They were then homogenized in 80 per cent v/v ethanol and the distribution of ^{35}S-components in the extracts and in hydrolysates of the residues was investigated. The results are summarized in Table 1.

The approximate distribution of the radioactivity in the known compounds is indicated by the number of plus signs. The results show that: (1) Cystine and cysteine were rapidly converted to glutathione, taurine, and sulphate by both xenic and aposymbiotic *Blattella*, but conversion of cysteine to methionine occurred only in xenic *Blattella*. (2) Cysteic acid was decarboxylated to taurine and oxidized, to a small extent, to sulphate by both xenic and aposymbiotic insects. (3) Most of the taurine is excreted unchanged. However, when intestinal bacteria are present, some of the taurine is degraded to sulphate, which may then be used by the xenic cockroach for the synthesis of cystine. (4) Sulphate sulphur is incorporated into cystine, methionine, taurine, sulphite, and various unidentified compounds by xenic *Blattella* but only sulphite and unidentified substances are formed by the aposymbiotic insect[3]. In contrast to the chick embryo xenic *Blattella* forms only a barely detectable amount of ^{35}S-taurine after $Na_2{}^{35}SO_4$ administration. This suggests that, in the cockroach, taurine is not formed directly from sulphate, but via cysteine. The reduction of sulphate to sulphite, which is widespread in plants and micro-organisms, occurs in the cockroach and, to our knowledge, only in the vitellin sac of the chick embryo[4]. (5) Methionine and methionine sulphoxide appear to be completely interconvertible by both xenic and aposymbiotic *Blattella*. A small quantity of CH_3SH, but no hydrogen sulphide or cysteic acid, was produced by the insects injected with methionine. (6) The sulphur of methionine sulphone was not detected in any other compound after its administration.

Cysteic acid has been proposed by a number of workers as an important intermediate in sulphur metabolism. As cysteic acid was not found in extracts of cockroaches after feeding or injection of sulphate, cystine, cysteine, methionine, or methionine sulphoxide, it does not seem to be a significant intermediate in *Blattaria*.

The results indicate that methionine and cystine are converted in the cockroach to sulphate by at least two pathways: (*a*) methionine sulphoxide ⇌ methionine → (homocysteine) → (cystathionine) → cysteine → (cysteinesulphinic-acid) → β-sulphinyl pyruvic acid → sulphite → sulphate; or (*b*) methionine → (homocysteine) → (cystathionine) → cysteine → (cysteinesulphinic-acid) → (2-aminoethane sulphinic acid) → taurine → ? → sulphate. Pathway *a* for the oxidation of cysteine is characteristic of most organisms and is probably the principal method of sulphate production in the cockroach.

In order to show that the results obtained with the aposymbiotic cockroaches were not due to traces of aureomycin carried over from the maternal diet, normal *Blattella* nymphs were placed on the aureomycin diet several days prior to cystine-^{35}S administration and caged in aureomycin-coated flasks. No inhibition of trans-sulphuration occurred as a result of this treatment.

The hypothesis that the intracellular symbionts are responsible for converting sulphate-S into methionine-S–cystine-S was substantiated by feeding *Blattella* nymphs a diet containing 0·1 per cent aureomycin until they matured. The adult insects, devoid of intestinal bacteria but retaining the intracellular symbionts, incorporated sulphur-35 from $Na_2{}^{35}SO_4$ into methionine, etc.; but their aposymbiotic progeny were unable to utilize sulphate for amino-acid synthesis even after re-establishment of the gut microflora.

Table 1. RADIOACTIVE METABOLITES OBSERVED IN THE ALCOHOLIC EXTRACTS OF *Blattella germanica* AFTER INJECTION OF VARIOUS COMPOUNDS LABELLED WITH SULPHUR-35

Physiological condition of insect	Compound injected	Metabolites into which sulphur-35 was incorporated					
		Cysteine	Glutathione	Methionine + methionine sulphoxide	Sulphate	Taurine	Unidentified compounds
Xenic	Cyst(e)ine	+++	+++	+++	+++	++	Present
Aposymbiotic		+++	+++	–	+++	++	,,
Xenic	Cysteic acid	–	–	–	+	+++	Absent
Aposymbiotic		–	–	–	+	+++	,,
Xenic	Taurine	+	++	+	+	+++	Present
Aposymbiotic		–	–	–	+	+++	,,
Xenic	Sulphate	+++	+++	+++	+++	+	Present
Aposymbiotic		–	–	–	+	–	,,
Xenic	Methionine	+	+	+++	+++	–	Present
Aposymbiotic		+	+	+++	++	–	,,
Xenic	Methionine sulphoxide	+	+	+++	+++	–	Present
Aposymbiotic		+	+	+++	++	–	,,
Xenic	Methionine sulphone	–	–	–	–	–	Absent
Aposymbiotic		–	–	–	–	–	,,

The effect of temperature on sulphate utilization in xenic cockroaches was studied. Two unidentified compounds were produced in greater quantities at 5° C. while a third, which was almost absent at 5° C., accounted for about 10 per cent of the total radioactivity of the ethanol extract when the animals were maintained at 20 to 25° C. A chloroform extract of cockroaches which had been injected with sulphate-^{35}S and held at temperatures above 15° C. contained another unidentified radioactive compound, possibly a sulpholipid. Acid hydrolysis of this compound yields sulphate. Cystine and methionine were not formed at 5° C.

This work was supported in part by Grant-*E*-2608 (*C*-1) of the National Institutes of Health, U.S. Public Health Service.

Richard J. Block
S. Mark Henry

Boyce Thompson Institute
for Plant Research,
Yonkers, New York.

[1] Haines, T. H., Henry, S. M., and Block, R. J., *Contrib. Boyce Thompson Inst.*, **20**, 363 (1960).
[2] Gordon, H. T., *Ann. N.Y. Acad. Sci.*, **77**, 290 (1959).
[3] Henry, S. M., and Block, R. J., *Contrib. Boyce Thompson Inst.*, **20**, 317 (1960).
[4] Chapeville, F., and Fromageot, P., *Biochim. Biophys. Acta*, **26**, 538 (1957).

PHYSIOLOGY

Selective Uptake of Specifically Bound Cobalt-58 Vitamin B_{12} by Human and Mouse Tumour Cells

Many body fluids have been reported to contain substances that combine with vitamin B_{12}. However, research concerning the role of these substances in the absorption of vitamin B_{12} by animal cells has been confined mainly to work involving intrinsic factor preparations or normal human gastric juice. A large amount of evidence has led to general acceptance that vitamin B_{12} bound to intrinsic factor is taken up by intestinal mucosa cells to a greater extent than unbound vitamin B_{12} (ref. 1). Intrinsic factor also has been shown to promote uptake of vitamin B_{12} by rat liver slices[2,3] and liver homogenates[4]. The latter process apparently is not influenced by changes of temperature, and it is not affected by metabolic inhibitors such as 2,4-dinitrophenol[3].

The evidence that human serum may promote uptake of vitamin B_{12} by mammalian tissues remains contradictory. Herbert[3] has reported that the uptake of vitamin B_{12} by rat liver slices is enhanced if the slices are first incubated in the presence of human serum and then incubated in the presence of free vitamin B_{12}. Miller[2], however, was unable to demonstrate a stimulation of uptake of vitamin B_{12} upon incubating rat liver slices simultaneously with human serum and vitamin B_{12}. Several authors have reported that normal human serum may be required for the utilization of vitamin B_{12} by human erythroid cells in tissue culture[5,6], but others have not been able to confirm this finding[7]. We have shown that human serum caused marked stimulation of uptake of vitamin B_{12} by HeLa cells and mouse Ehrlich ascites tumour cells. Enhancement of uptake of vitamin B_{12} by Ehrlich ascites tumour cells was produced also by mouse ascites fluid. These observations led to the further work reported here.

Ehrlich ascites tumour cells were taken from *CF*1 mice seven or eight days after intraperitoneal transplantation. The cells were centrifuged and, after removal of the supernatant ascites fluid, were washed three or four times in cold Krebs–Ringer phosphate solution. The cells then were re-suspended in sufficient Krebs–Ringer solution to yield a 1 : 5 dilution; 0·5 ml. of this suspension per 5 ml. of incubation medium was used. The ascites fluid, filtered through cheesecloth after clotting had taken place, contained 1·8 mμgm., or less, vitamin B_{12} per ml. on assay with *Euglena gracilis*[1].

HeLa cells (generously supplied by Dr. A. M. Masson, Virology Laboratory, McGill University, Montreal) cultured in medium 199 (purchased from Microbiological Associates, Washington, D.C.) containing 20 per cent calf serum were collected six days after sub-culture by shaking in 0·045 *M* ethylenediamine tetraacetate at *p*H 7·4 for 30 min. The cells were washed three times in cold Krebs–Ringer phosphate solution containing 100 mgm. glucose per 100 ml. After re-suspension in Krebs–Ringer solution, approximately 0·2 ml. of packed cells was used per 6 ml. incubation medium. Human serum was obtained from patients with pernicious anæmia in relapse and from patients in the third trimester of pregnancy in order to ensure a low concentration of non-radioactive vitamin B_{12}. The sera used had a vitamin B_{12} content of 0·25 mμgm., or less, per ml., on assay with *Euglena gracilis*[8].

Hog intrinsic-factor concentrate (supplied by Ayerst, McKenna and Harrison, Ltd., Montreal) was optimally active in the Schilling test in a dosage of 100 mgm. per 0·5 μgm. vitamin B_{12}. Normal human gastric juice was obtained, after histamine stimulation, from at least six donors. The juice was filtered

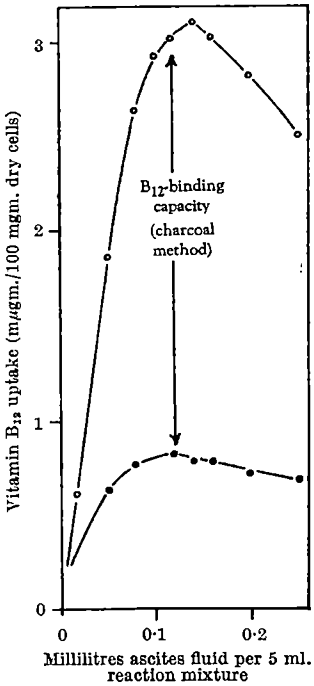


Fig. 1. Uptake of cobalt-58 vitamin B_{12} by Ehrlich ascites tumour cells with varying concentrations of ascites fluid. Vitamin B_{12}-binding capacity of ascites fluid by charcoal adsorption method was 5 mμgm. B_{12}/0·12 ml. ascites fluid. (5 ml. incubation medium contained 5 mμgm. cobalt-58 vitamin B_{12} and approximately 10 mgm. dry weight of cells.) ○—○, Uptake in 60 min. ●—●, uptake in less than 1 min.

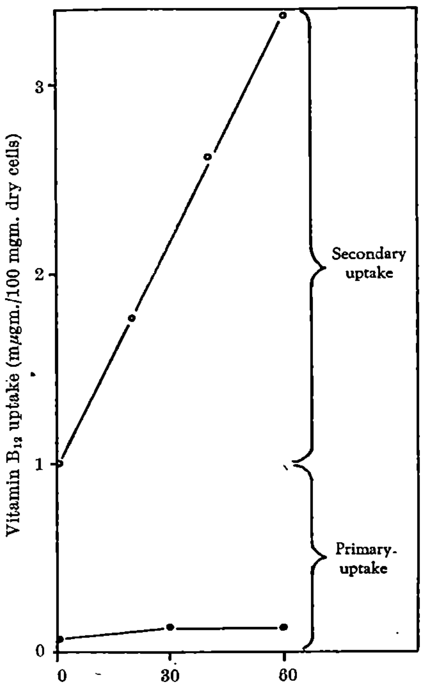


Fig. 2. Rate of uptake of cobalt-58 vitamin B_{12} by Ehrlich ascites tumour cells. (5 ml. incubation medium contained approximately 10 mgm. dry weight of cells.) ○—○, Medium contained 5 mμgm. cobalt-58 vitamin B_{12} and 0·12 ml. ascites fluid per 5 ml.; ●—●, medium contained 5 mμgm. cobalt-58 vitamin B_{12} per 5 ml., but no ascites fluid

through cheesecloth, adjusted to *p*H 10 to destroy pepsin, and neutralized to *p*H 7·0. The human serum, gastric juice, and mouse ascites fluid were stored separately in small aliquots at −20° C. until they were used.

The tumour cells were incubated in flasks containing 2–5 mμgm. cobalt-58 vitamin B_{12} (obtained from Merck of Canada), together with human serum or appropriate quantities of other B_{12}-binding materials, in the presence of Krebs–Ringer phosphate solution. Flasks were incubated at 37° C. in an atmosphere of air in a constant-temperature shaking water-bath. After an appropriate incubation period, the cells were removed by centrifugation and washed three times in cold phosphate-buffered saline. The cells were then resuspended in 1 ml. of water and counted in a well-type scintillation counter.

Ascites cells were incubated with a constant quantity of cobalt-58 vitamin B_{12} and varying amounts of ascites fluid and the amount of vitamin B_{12} taken up by the cells was estimated. The augmentation by ascites fluid of vitamin-B_{12} uptake by the cells was found to be optimal when the B_{12}-binding capacity of the ascites fluid added was equivalent to the quantity of vitamin B_{12} in the medium (Fig. 1). The B_{12}-binding capacity of ascites fluid, as determined by a modification of the charcoal adsorption method of Miller[9], was found to be 41·6 mμgm. vitamin B_{12} per ml. A similar relationship was observed between the charcoal-binding capacity of human serum and the uptake of cobalt-58 vitamin B_{12} by HeLa cells.

The pattern of uptake by ascites cells of bound cobalt-58 vitamin B_{12} is shown in Fig. 2. Negligible uptake values were obtained when ascites cells were incubated in the absence of ascites fluid. However, the addition of ascites fluid to the reaction mixture resulted in a very rapid initial uptake, occurring within the first minute of incubation, followed by a slower uptake directly proportional to time for at least 60 min. Further experiments showed that the initial or 'primary' uptake was almost completely unaffected by changes in temperature or *p*H, whereas the slower, 'secondary' uptake was dependent on temperature and had a *p*H optimum at 7·4.

The effect of increasing concentrations of the ascites fluid–B_{12} on these two phases of uptake is shown in Fig. 3. Both describe exponential curves with increasing concentration. Similar observations were made with HeLa cells incubated in human serum.

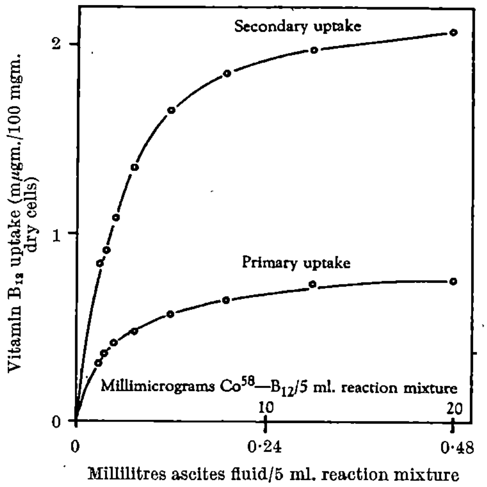


Fig. 3. Effect of varying concentrations of cobalt-58 vitamin B_{12} and ascites fluid on B_{12} uptake by Ehrlich ascites tumour cells. (5 ml. incubation medium contained approximately 10 mgm. dry weight of cells)

Both ascites cells and HeLa cells were found to take up the vitamin B_{12} only in the presence of certain fluids. HeLa cells took up vitamin B_{12} bound only to human serum, whereas vitamin-B_{12} uptake by ascites cells was stimulated by human serum, mouse ascites fluid, and mouse serum. Neither type of cell took up vitamin B_{12} bound to hog intrinsic-factor concentrate, human gastric juice, chick serum or calf serum. However, incubation with hog intrinsic-factor concentrate followed by incubation with cobalt-58 vitamin B_{12}, as described by Herbert[3], was effective in enhancing uptake by these cells: conversely, incubation with human serum or mouse ascites fluid followed by that of cobalt-58 vitamin B_{12} was not effective. This suggests that the phenomenon described here probably differs from that observed with rat liver slices by Miller[2] and Herbert[3].

In summary, mouse ascites fluid and human serum have been found to produce approximately a twenty-fold increase of uptake of vitamin B_{12} by mouse ascites tumour cells and by HeLa cells. Under the same conditions, human gastric juice and hog intrinsic-factor concentrate were ineffective in promoting uptake of vitamin B_{12}. Uptake of vitamin B_{12} occurring in the presence of mouse ascites fluid or human serum was found to be a bi-phasic reaction consisting of a rapid or 'primary' uptake which is probably of a physico-chemical nature, and a slower or 'secondary' uptake differing from the first in depending on temperature and possessing a *p*H optimum at 7·4.

We thank Dr. L. Lowenstein and Prof. J. H. Quastel for valuable suggestions and advice. The work was supported by a Canadian National Health Grant (604–13–21), a Canadian National Research Council Grant (*MA* 802) and a grant from the U.S. Public Health Service (*C*3596); for this assistance we are most grateful.

BERNARD A. COOPER
WILLIAM PARANCHYCH

Hæmatology Service,
Dept. of Medicine,
The Royal Victoria Hospital and the McGill University Clinic;
and the McGill–Montreal General Hospital Research Institute,
Montreal.

---

[1] Castle, W. B., *Gastroenterology*, **37**, 377 (1959).

[2] Miller, O. N., Raney, J. L., and Hunter, F. M., *Fed. Proc.*, **16**, 339 (1957).

[3] Herbert, V., *Amer. J. Clin. Nutr.*, **7**, 433 (1959).

[4] Herbert, V., Castro, Z., and Wasserman, L. R., *Proc. Soc. Exp. Biol. Med.*, **104**, 160 (1960).

[5] Callender, S. T., and Lajtha, L. C., *Blood*, **6**, 1234 (1951).

[6] Pendl, I., and Franz, W., *Nature*, **181**, 488 (1958).

[7] Swan, H. T., Reisner, E. H., and Silverman, M., *Blood*, **10**, 735 (1955).

[8] Mollin, D. L., and Ross, G. I. M., *J. Clin. Path.*, **5**, 129 (1952).

[9] Miller, O. N., *Arch. Biochem. Biophys.*, **68**, 255 (1957).

Outflux of Inorganic and Organic Phosphate during Membrane Depolarization of Excitable Tissues

It was reported in a previous paper[1] that the outflux of phosphorus-32 from an isolated sartorius muscle of the frog into phosphate-free Ringer solution was accelerated markedly during electrical stimulation, or when the external solution was devoid of calcium or contained a high potassium (30 m*M*) concentration. Subsequent findings revealed that not only inorganic phosphate but also many organophosphates such as adenosine triphosphate (ATP) and phosphocreatine were released during membrane depolarization under such experimental conditions. The present communication concerns the outflux of nucleotides and other phosphates from frog sartorius muscle and spinal nerve roots during electrical stimulation and calcium depletion.

Isolated frog sartorius muscle was immersed in frog Ringer solution containing phosphorus-32 (carrier-free orthophosphate, 25 µc./ml.) for 2 hr. at room temperature (25° C.). In general, five paired muscles were used for each control and experimental run. The muscles were then washed in Ringer solution for 20 sec. and the initial soaking procedure continued for 2 hr., during which time the muscles were transferred every 20 min. into a series of beakers containing 10 ml. of phosphate-free Ringer solution. The extracellular phosphorus-32 was virtually washed out by this procedure[1]. The control muscles were then placed in 2 ml. Ringer solution (112 m*M* sodium chloride; 2 m*M* potassium chloride; 1·8 m*M* calcium chloride; and 2 m*M* sodium bicarbonate) and the experimental muscles in calcium-free Ringer solution (112 m*M* sodium chloride; 2 m*M* potassium chloride; 2 m*M* sodium bicarbonate; and 4 m*M* ethylenediamine tetraacetic acid (EDTA)) for a period of 1 hr. For stimulation experiments, the test muscles were immersed in Ringer for 40 min. while supramaximal electrical stimulation (1/3 sec. pulses) was applied through a pair of platinum electrodes dipped in Ringer solution. The final external solution was then run through a 'Dowex 1' anion-exchange column, and the phosphates separated and analysed as described elsewhere[2].

Two pairs of sciatic-spinal root nerve preparations were isolated from a bull-frog, and unilateral pairs used for test and control preparations. The nerve roots (ventral and dorsal) of the preparation were soaked in Ringer solution containing 50 µc./ml. of orthophosphate-^{32}P for 2 hr. (the sciatic nerves and spinal ganglia were kept exposed in a moist chamber during the whole course of the experiment). The nerves were washed and treated in the same manner described for the muscle preparations. The application of stimulation (50 pulses/sec.) and recording of action potential was carried out on the sciatic nerve for 40 min. The incubation medium was then analysed as in the case of muscle. The external solution contained phosphorus-32 originating from within the nerve fibre, while the phosphorus-32 of the interfibre was considered to diffuse out completely during the 2-hr. washing procedure (unpublished work).

Both the total amount and the specific activity of the tissue phosphates were studied in the external solution following incubation. In a calcium-free solution containing EDTA there was a considerable increase in all the phosphates examined, including

Table 1. SPECIFIC ACTIVITY AND AMOUNT OF PHOSPHATES LOST FROM FROG NERVE AND MUSCLE DURING ELECTRICAL STIMULATION AND IN CALCIUM-FREE EDTA

		Inorganic phosphate		Phosphocreatine		ATP		ADP		AMP		CMP		UMP	
		µmoles	*sa*	µmoles	*sa*	µmoles	*sa*	µmoles	*sa*	µmoles	*sa*	µmoles	*sa*	µmoles	*sa*
Calcium-free EDTA muscle	Control	0·04	280	0·01	50	0·02	40	0·005	—	0·008	—	0·005	—	0·00	—
	Exp.	0·15	270	0·13	60	0·04	50	0·01	10	0·02	2	0·01	1	Trace	—
	Change (per cent)	365	−6	770	20	100	25	100	—	250	—	100	—	—	—
Stimulated muscle	Control	0·03	250	0·01	45	0·02	40	0·00	—	0·00	—	0·00	—		
	Exp.	0·15	225	0·02	50	0·03	45	Trace	—	Trace	—	Trace	—		
	Change (per cent)	500	−10	100	11	150	12	—	—	—	—	—	—		
Calcium-free EDTA nerve	Control	0·05	210	0·005	45	0·01	35	0·00	—						
	Exp.	0·15	225	0·008	50	0·03	38	Trace	—						
	Change (per cent)	200	7	160	11	200									
Stimulated nerve	Control	0·04	205	0·005	40	0·01	30	0·00							
	Exp.	0·06	220	0·007	35	0·02	33	Trace							
	Change (per cent)	50													

ADP, adenosine diphosphate; AMP, adenosine monophosphate; CMP, cytidine monophosphate; UMP, uridine monophosphate. Each result is an average of 3 experiments agreeing within 10 per cent. *sa* = counts per min./µmoles phosphate × 10^{-3}

cytidylic and uridylic acid, the increase being greatest in inorganic phosphate and phosphocreatine. A small increase in the specific activity of phosphocreatine and ATP was observed, the activity in the other nucleotides being too low to compare. In the case of the electrically stimulated muscle, a 500 per cent increase occurred in inorganic phosphate, a 100 per cent increase in phosphocreatine, and 150 per cent in ATP. The other nucleotides, though showing a slight increase after stimulation, were present in only trace amounts. When frog spinal nerves were exposed to calcium-free EDTA solutions, a 200 per cent increase was noted in the concentration of inorganic phosphate, phosphocreatine and ATP, while the change in the specific activity of these fractions was insignificant. After electrical stimulation of frog nerves, a 50 per cent increase in the phosphorylated intermediates was observed.

The present results can be interpreted in a number of ways. The most likely interpretation is that there is a loss of calcium in excitable tissues exposed to either calcium-free EDTA solutions or electrical stimulation[3] This loss of calcium may result in a structural re-arrangement of the excitable membrane in such a way as to permit the loss of certain substances, either from the membrane itself or from the internal body of the tissue. The phosphates such as ATP may actually be intimately involved in the structural configuration of the membrane ; and with the breakdown or loss of ATP there is a concomitant change in membrane permeability, as well as membrane depolarization. It is conceivable that the calcium diffuses out of the tissue as a phosphate complex, since, at the *p*H of the cell, calcium would be expected to form a complex readily with such phosphates as orthophosphate and ATP. The loss of phosphocreatine, however, would tend to indicate that some of the phosphates, at least, are not associated with calcium. To some extent, the efflux of phosphates from muscle may be due to the contractile events, but, in view of the results with nerve roots, it appears that to a considerable extent the efflux is associated with membrane depolarization. It has been suggested elsewhere[4] that the high-energy phosphates in excitable tissues may be compartmentalized, and that the important phosphates, so far as excitation is concerned, may be associated with the membrane itself. Preliminary work indicates that the specific activity of the phosphates in the external solution obtained during incubation is greater than that in internal muscle or nerve. It is possible, therefore, that the phosphates leaking out during loss of calcium are probably those primarily associated with the membrane, and that they may be more intimately related to the chemical events associated with excitatory phenomena.

This work was supported by a U.S. Public Health Service grant, *B*–1650, and by the Teagle Foundation.

L. G. ABOOD
K. KOKETSU
I. KOYAMA

Research Laboratories,
Department of Psychiatry,
University of Illinois College
of Medicine,
Chicago.

---

[1] Koketsu, K., Miyamoto, S., and Abood, L. G., *Fed. Proc.*, **20**, 298 (1961).

[2] Abood, L. G., and Goldman, E., *Amer. J. Physiol.*, **184**, 329 (1956).

[3] Koketsu, K., and Miyamoto, S., *Nature*, **189**, 403 (1961).

[4] Abood, L. G., and Koketsu, K. Noda, *Amer. J. Physiol.*, **200**, 431 (1961).

A Hydrostatic Function of the Accessory Respiratory Organs in Air-breathing Fishes

ACCESSORY respiratory organs in fishes have generally been thought to be concerned with breathing atmospheric air[1]. That, like any other gas-filled space, they can also be connected with the perception of hydrostatic pressure has not been reported earlier. In fishes, perhaps the very existence of a swim-bladder, which takes the major role in regulating the density of the fish[2] and functioning as a hydrostatic organ[3], has diverted attention from other gas-filled organs which may have a similar function.

The accessory respiratory organs are supposed to have developed in response to exceptional environmental conditions which made aquatic respiration inefficient[4]. They may be simple secondary pharyngeal folds as in *Ophicephalus*, or sets of complicated and highly evolved opercular branchial chambers as in *Clarias*, *Heteropneustes* and *Trichogaster*[5]. In every case, fishes possessing these organs are known to visit the surface to gulp air[6].

When such air-breathing fishes are subjected to variations in hydrostatic pressure, control of the volume of air contained in the accessory respiratory organs occurs. At increased pressure the air in the accessory respiratory organ is retained, the fishes stay at or near the bottom and postpone their visit to the surface for relatively longer periods. When the pressure is decreased, the fishes begin to float up until they release gas from their accessory respiratory organs. Even in bottom-living fishes such as *Clarias* and *Heteropneustes*, a small reduction in pressure of 6–7 cm. mercury causes gas bubbles to be released. Further reduction brings about more release of gas, and when the pressure is reduced by 60–65 cm. mercury, practically all the gas from the accessory respiratory organs escapes. At such a reduced pressure, fishes like *Ophicephalus* and *Trichogaster*, which have a functional, physolistic type of swim-bladder[7], float helplessly upwards after the gas from the accessory respiratory organs has been expelled. Other fishes such as *Clarias* and *Heteropneustes*, which have a non-functional physostomatous type of swim-bladder[8], when put under reduced pressure, make dashing movements to the surface to gulp air, but being unable to retain it, eventually sink to the bottom. To ensure that in these physostome fishes the release of gas occurs from the accessory respiratory organs and not from the swim-bladder, further tests were made in *Heteropneustes* by puncturing its accessory respiratory organs. As the accessory respiratory organs in this fish are long, paired, sac-like structures situated dorsally, the puncturing operation could be made without causing undue injury to the fish. When such fishes were subjected to a reduction in pressure, the air was partly expelled through the punctured opening and partly from under the operculum.

The above experiments clearly show that there are striking similarities between the accessory respiratory organs and the physostomatous swim-bladder. In both cases the fish can control the volume of gas by swallowing and releasing air, and in this way either of them can act as a hydrostatic organ. It is interesting to note that physostome fishes predominate in fresh-waters[9] and so do the air-breathing fishes. In some forms such as *Clarias* and *Heteropneustes* the swim-bladder has become reduced and non-functional. These fishes possess large and highly developed accessory respiratory organs presumably to compensate for the hydrostatic function of the swim-bladder as well as acting as respiratory organs. Such a correlation does not seem merely an accident, for in many fishes the swim-bladder acts as a respiratory organ[3]. It is therefore less surprising to find the accessory respiratory organ taking a part in the hydrostatic function.

S. Z. Qasim
R. Hasan

Department of Zoology,
Aligarh Muslim University,
Aligarh, U.P., India.

[1] Qasim, S. Z., Qayyum, A., and Garg, R. K., *Proc. Ind. Acad. Sci.*, **52**, 19 (1960). Carter, G. S., *J. Linn. Soc.* (*Zool.*), **34**, 219 (1935).

[2] Jones, F. R. H., *J. Exp. Biol.*, **28**, 553 (1951).

[3] Jones, F. R. H., and Marshall, N. B., *Biol. Rev.*, **28**, 16 (1953).

[4] Carter, G. S., in *The Physiology of Fishes*, **1**, 65 (Acad. Press, Inc., N.Y., 1957).

[5] Das, B. K., *Proc. 27th Ind. Sci. Cong.*, part 2, 215 (1940).

[6] Das, B. K., *Phil. Trans. Roy. Soc.*, **216**, 183 (1927); *Proc. Roy. Soc.*, B, **115**, 422 (1934).

[7] George, M. J., *J. Zool. Soc. India*, **8**, 181 (1956).

[8] Day, F., *Fishes of India*, **1** (William Dawson, London, 1958). Bridge, T. W., and Boulenger, G. A., *Camb. Nat. Hist.*, **7**, 302 (1958).

[9] Jones, F. R. H., in *The Physiology of Fishes*, **2**, 305 (Acad. Press, Inc., N.Y., 1957).

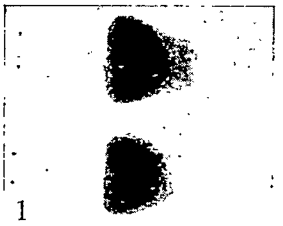
HÆMATOLOGY

Abnormal Fœtal Hæmoglobins

During the past few years, certain human fœtal hæmoglobin (Hb) variants characterized by electrophoretic mobility different from that of fœtal hæmoglobin have been described. They are: the Hb of Fessas and Papaspyrou[1], characterized by mobility at alkaline *p*H, faster than that of Hb *F*; Hb Bart's described by Ager and Lehmann[2], and also characterized by mobility at alkaline *p*H faster than that of Hb *F*; Hb Alexandra, slower than Hb *F*, described at the same time by Fessas *et al.*[3] and by Vella *et al.*[4].

The two fast hæmoglobins (Hb of Fessas and Papaspyrou and Hb Bart's) are most probably the same hæmoglobins. In fact, both these hæmoglobins have electrophoretic mobilities at alkaline *p*H between those of Hb *J* and Hb *H* (refs. 2–5) and at acid *p*H anodic mobility slightly lower than that of Hb *H*; their resistance to alkali denaturation is greater than that of Hb *A* but smaller than that of Hb *F* (refs. 2–5); their ultra-violet spectra are of the fœtal type (refs. 2–5), and in chromatography at *p*H 6 they have greater mobility than Hb *H* and any other type of hæmoglobin[2–5,6].

Hb Alexandra has been found rather frequently[6] in new-born Asiatic infants (Chinese, Malayans, etc.). Its mobility at alkaline *p*H is between that of Hb *S* and that of Hb *E* or A_2; it does not separate from Hb *F* on agar-gel electrophoresis at acid *p*H or on ion-exchange chromatography; its resistance to alkali denaturation and its ultra-violet spectrum are similar to those of Hb *F*; it gradually disappears during the first post-natal months.



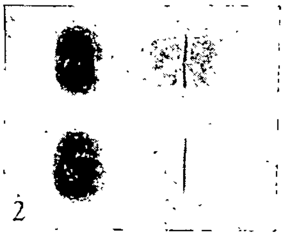


Fig. 1. Paper electrophoresis of Hb Bart's (399 *F*, above) and cord blood control (below). Glycine buffer *p*H 8·8, ionic strength 0·04. Staining with bromophenol blue. Greater mobility of fast fraction than of cord blood hæmoglobin

Fig. 2. Starch gel electrophoresis of Hb *H* (above) and Hb Bart's (390 *F*, below). Phosphate buffer *p*H 6, ionic strength 0·12. Staining with amido-Schwarz. Identical mobility of faster fœtal fraction and Hb H_2 fraction

In a survey conducted among the Italian population on 1,200 samples of the umbilical cord blood of new-born infants, we have found two cases which had a fast fraction and one with a slow fraction.

The fast hæmoglobin has greater electrophoretic mobility at alkaline *p*H than that of the mixture of Hb *A* + Hb *F* (Fig. 1) and is very similar to that of fraction H_2 (the less fast of the two fractions present in carriers of Hb *H*, ref. 7). At acid *p*H, its anodic mobility is identical with that of fraction H_2 of Hb *H* carriers (Fig. 2).

Agar-gel electrophoresis at acid *p*H reveals between fraction *F* and fraction *A* two very small spots near each other which are, however, also found in umbilical cord hæmoglobins without a fast fraction, and which correspond probably to the two small fractions which normally precede Hb *A*.

On 'Amberlite *IRC*-50' column chromatography at *p*H 6, the fast fraction does not appear to be identifiable, probably due to the small quantity present.

Quantitatively (Table 1) the amount of fast Hb is small. The ultra-violet absorption spectrum of the isolated fast fraction is identical with that of Hb *F*; the alkali-resistance of the isolated fast fraction[8] is 4–5 min., which is intermediate between the resistance of Hb *A* and that of Hb *F*.

It is evident that the fast hæmoglobin has all the characteristics of Hb Bart's or Hb of Fessas and Papaspyrou. These characteristics are, however, identical also with those we found for H_2. In a previous survey[7] on 5 cases of Hb *H*-microcythæmia, we have shown the constant presence of two fractions of Hb *H*, one faster, Hb H_1, and one less fast, Hb H_2; and we have shown that Hb H_2 has a fœtal type of ultra-violet spectrum, whereas Hb H_1 has a normal

Table 1. Percentages and Spectrophotometric Index of the Various Hæmoglobin Fractions
Readings made on a Beckman *D.U.* spectrophotometer

Cases	Hb *a–r* per cent on the total Hb*	Values by starch block electrophoresis† (per cent)			$\frac{E\ (3\cdot000\ \text{Å.})}{E\ (2\cdot800\ \text{Å.})}$			$\frac{E\ (2\cdot800\ \text{Å.})}{E\ (4\cdot150\ \text{Å.})}$			$\frac{Ed‡}{E_0/2§}$		
		Fast Hb	Slow Hb	Hb *A* + Hb *F*	Fast Hb	Slow Hb	Hb *A* + Hb *F*	Fast Hb	Slow Hb	Hb *A* + Hb *F*	Fast Hb	Slow Hb	Hb *A* + Hb *F*
399 *F*	65·8	4·01		95·99	46·5		48·3	0·40		0·25	0·82		0·77
033 *F*	66·3	3·05		96·95	48·4		50·0	0·54		0·33	0·82		0·57
0153 *F*	60·9		13·05	86·95		45·9	45·5		0·31	0·25		0·74	0·56

* Hæmoglobin resistant to denaturation by alkali, according to Singer *et al.*
† According to Kunkel and Wallenius.
‡ $Ed = E$ (5,800 Å.) of the solution at the end of denaturation by alkali.
§ $E_0 = E$ (5,800 Å.) of the solution before denaturation by alkali.

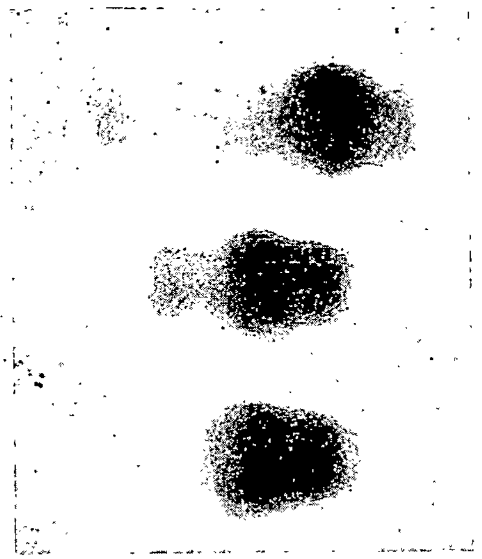


Fig. 3. Starch gel electrophoresis of adult microcythæmic hæmoglobin (above), cord blood hæmoglobin with slow fraction (0153 *F*, at centre), normal cord blood hæmoglobin (below). Borate buffer *p*H 8·4, ionic strength 0·06. Staining with amido-schwarz. The slow fœtal fraction is faster than Hb A_2

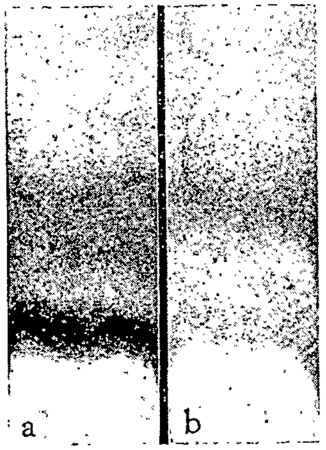


Fig. 4. Chromatography on columns of 'Amberlite *IRC*–50' of cord blood hæmoglobin with (*a*) slow fraction (0153 *F*) and (*b*) normal cord blood hæmoglobin. Citrate buffer *p*H 6, ionic strength 0·05. The slow fœtal hæmoglobin lies between *A* and *F* bands

spectrum. This finding has also been reported in the literature[9].

Hb Bart's and Hb H_2 have therefore the same electrophoretic and spectrophotometric behaviour (Table 1). It is now known[10] that Hb Bart's is a hæmoglobin composed of γ-chains only, that is, a fœtal hæmoglobin without α-chains, and Hb *H* (chromatographically separated)[11] consists of β-chains only, that is, it is an adult hæmoglobin without α-chains. It can therefore be deduced that Hb H_2 also consists of γ-chains only and Hb H_1 (corresponding to Hb *H* of the literature) of β-chains only: this would agree also with the spectrophotometric properties of these fractions.

The Hb of Fessas and Papaspyrou[1] and Hb Bart's[2] were originally described in microcythæmic new-born infants and later found to be present even after infancy; Tuchinda *et al.*[9b] and Boyo *et al.*[12], on the contrary, have reported disappearance of the fast fraction within three months after birth.

In the present two cases, an examination after three months showed that the fast fraction had disappeared. No trace of microcythæmia has been found in the children or their parents. The conclusion must be drawn that Hb Bart's, present at birth in non-microcythæmic individuals, disappears within a few months, whereas Hb Bart's, which appears in carriers of microcythæmic syndromes, persists.

The slow fœtal hæmoglobin, identified in one case during the survey, has a mobility at alkaline *p*H higher than that of Hb A_2 (Fig. 3) and lower than that of Hb *S*. On acid *p*H paper, starch-gel or agar-gel electrophoresis, it does not separate from Hb *F*. Chromatography reveals, in our hands, in tests extending over more than 36 hr., the separation of an intermediate band between that of Hb *F* and that of Hb *A* (Fig. 4) which does not appear in fœtal hæmoglobins without the slow fraction. This differs from the finding described in the literature for Hb Alexandra and requires confirmation.

A small amount of this slow hæmoglobin is also present (Table 1); it is always greater than the amount of fast fœtal Hb. The ultra-violet spectrum of the slow fraction isolated is of the fœtal type, but the alkali-resistance test also gives results for this hæmoglobin intermediate which are between those of Hb *F* and those of Hb *A*.

This investigation has been supported by a grant from the National Council of Research.

E. Silvestroni
I. Bianco

Istituto d'Igiene dell' Università di Roma,
Centro di Studi della Microcitemia e delle Anemie
Microcitemiche, Roma.

[1] Fessas, Ph., and Papaspyrou, A., *Science*, **126**, 1119 (1957).

[2] Ager, J. A. M., and Lehmann, H., *Brit. Med. J.*, i, 929 (1958).

[3] Fessas, Ph., Mastrokalos, N., and Fostiropoulos, G., *Nature*, **183**, 30 (1959).

[4] Vella, F., Ager, J. A. M., and Lehmann, H., *Nature*, **183**, 30 (1959).

[5] Fessas, Ph., *Brit. Med. J.*, ii, 886 (1959).

[6] Vella, F., *Nature*, **184**, 272 (1959).

[7] Silvestroni, E., Bianco, I., and Muzzolini, M., *Policlinico, Sez. Prat.*, **67**, 41 (1960).

[8] Silvestroni, E., Bianco, I., Muzzolini, M., Modiano, G., and Vallisneri, E., *Progr. Med.*, **13**, 705 (1957).

[9] (*a*) Ramot, B., Sheba, Ch., Fisher, S., Ager, J. A. M., and Lehmann, H., *Brit. Med. J.*, ii, 1228 (1959). (*b*) Tuchinda, S., Vareenil, C., Bhanchit, P., and Minnich, V., *Pediatrics*, **24**, 43 (1959). (*c*) Fessas, Ph., *VII Congr. Eur. Soc. Haemat.* (London, 1959).

[10] Hunt, J. A., and Lehmann, H., *Nature*, **184**, 872 (1959).

[11] Jones, R. E., Schroeder, W. A., Balog, J. E., and Vinograd, J. R., *J. Amer. Chem. Soc.*, **81**, 3161 (1959).

[12] Boyo, A. E., and Hendrickse, R. G., *Nature*, **184**, 997 (1959).

Thalassæmia and the Abnormal Human Hæmoglobins

The preponderance of hæmoglobin *A* over hæmoglobin *S* in sickle-cell trait was observed in the course of our initial studies on the hæmoglobin of sickling cells[1]. Later work carried out in collaboration with other laboratories showed that the ratio of these hæmoglobins is inherited[2] and that simultaneous presence of a gene for an abnormal hæmoglobin and a gene for thalassæmia results in partial or complete suppression of synthesis[3,4] of hæmoglobin *A*. An exceptional case in which presence of a thalassæmia gene did not alter the proportion of hæmoglobins *A* and *C* in hæmoglobin *C* trait was observed elsewhere[5]. These findings formed the basic evidence on which we proposed the following concepts about the inheritance of hæmoglobin: (1) alleles may be present that control the synthesis of hæmoglobin *A* at different rates[6]; (2) there may exist more than one locus for thalassæmia[7]; (3) in some cases thalassæmia and abnormal hæmoglobins may be controlled at the same locus[8]; (4) a structurally abnormal but electrophoretically normal hæmoglobin may be synthesized at sub-normal rate in thalassæmia[8,9].

The discovery[10] of a chemical abnormality in hæmoglobin *S* and the discovery[11] of two types of polypeptide chains in hæmoglobin *A* resulted in further additions to the theory of inheritance of hæmoglobin, namely, that control of the structure of hæmoglobin and control of its rate of synthesis are properties of the same allele, and that separate genetic control of synthesis of the two chains would result in four hæmoglobins in double heterozygotes; these postulates about the inheritance of thalassæmia and the abnormal hæmoglobins were given in a review[9] in 1957. The concepts that the alleles controlling the synthesis of myoglobin, adult hæmoglobin, fœtal hæmoglobin and hæmoglobin A_2 evolved from the same primitive allele and that comparative structural studies might reveal similarities in amino-acid sequence or position were proposed in the same review[9]. The presence of four hæmoglobins in double heterozygotes and the existence of structural analogies between hæmoglobin and myoglobin have been corroborated recently[12–16].

That chemical studies reported since 1957 have not substantially altered our inferences is evident from the conclusions expressed by Ingram and his associates in a recent series of papers[17–20]. These papers are remarkable for the extent to which the custom of giving pertinent references to the ideas and findings of others has been ignored.

An experimental test of our thalassæmia hypothesis by comparison with the amino-acid composition of peptides of hæmoglobin from normal human beings and patients with thalassæmia major was proposed by Ingram and Stretton[17]. We point out that thalassæmia minor, in which, according to our hypothesis, a mixture of normal adult and thalassæmia hæmoglobins is present, is a more readily available source of hæmoglobin than thalassæmia major, and might be used for this purpose. The observation of non-integral numbers of two amino-acid residues per molecule of hæmoglobin from a subject with thalassæmia minor would indicate the presence of an inherited variant with a single amino-acid substitution in the hæmoglobin of thalassæmia. The fractional deviation from the proper ratio of integral numbers would be proportional to the relative rates of synthesis of hæmoglobin *A* and thalassæmia hæmoglobin.

Familial differences in the ratio of hæmoglobin *A* to hæmoglobin *S* in sickle-cell trait were ascribed by one of us[9] to different alleles that result in electrophoretically indistinguishable but chemically distinct forms of hæmoglobin *A*. Accurate chemical analyses of the hæmoglobin *A* of sickle-cell-trait subjects with different inherited ratios would provide a test of this hypothesis. Furthermore, non-integral numbers of two amino-acid residues in the hæmoglobin of a normal individual would suggest heterozygosity in two inherited variants of hæmoglobin *A*.

Total amino-acid analyses of hæmoglobin or its constituent chains would fail to detect one type of alteration that has been suggested as a possible basis for electrophoretically identical subtypes, namely, the transposition of amino-acid residues in a chain[21]. Analysis of peptides obtained by tryptic digestion would also fail to detect transposition if the transposed segment did not contain a basic amino-acid. A search for such an alteration by complete sequential analyses of different samples of hæmoglobin *A* would be a formidable task for any laboratory. However, a number of laboratories are studying the amino-acid sequence of hæmoglobin *A*, with use of samples from different sources. If disagreements emerge with regard to the sequence of residues in polypeptide fragments of identical composition, inherited transpositions must be considered as a possible explanation.

HARVEY A. ITANO
National Institutes of Health,
Public Health Service,
Bethesda 14, Maryland.

LINUS PAULING
California Institute of Technology,
Pasadena, California.

[1] Pauling, L., Itano, H. A., Singer, S. J., and Wells, I. C., *Science*, **110**, 543 (1949).

[2] Neel, J. V., Wells, I. C., and Itano, H. A., *J. Clin. Invest.*, **30**, 1120 (1951).

[3] Sturgeon, P., Itano, H. A., and Valentine, W. N., *Blood*, **7**, 350 (1952).

[4] Sturgeon, P., Itano, H. A., and Bergren, W. R., *Blood*, **10**, 389 (1955).

[5] Zuelzer, W. W., and Kaplan, E., *Blood*, **9**, 1047 (1954).

[6] Itano, H. A., *Amer. J. Human Genetics*, **5**, 34 (1953).

[7] Itano, H. A., *Amer. Med. Assoc. Arch. Int. Med.*, **96**, 287 (1955).

[8] Pauling, L., *The Harvey Lectures*, Series 49, 216 (1953–54).

[9] Itano, H. A., *Advances in Protein Chem.*, **12**, 215 (1957).

[10] Ingram, V. M., *Nature*, **180**, 326 (1957).

[11] Rhinesmith, H. S., Schroeder, W. A., and Pauling, L., *J. Amer. Chem. Soc.*, **79**, 4682 (1957).

[12] Atwater, J., Schwartz, R., and Tocantins, L. M., *Blood*, **15**, 901 (1960).

[13] Raper, A. B., Gammack, D. B., Huehns, E. R., and Shooter, M. A., *Brit. Med. J.*, ii, 1257 (1960).

[14] Itano, H. A., and Robinson, E. A., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1492 (1960).

[15] Kendrew, J. C., Dickerson, R. E., Strandberg, B. E., Hart, R. G., Davies, D. R., Phillips, D. C., and Shore, V. C., *Nature*, **185**, 422 (1960).

[16] Perutz, M. F., Rossmann, M. G., Cullis, A. F., Miurhead, H., Will, G., and North, A. C. T., *Nature*, **185**, 416 (1960).

[17] Ingram, V. M., and Stretton, A. O. W., *Nature*, **184**, 1903 (1959).

[18] Baglioni, C., and Ingram, V. M., *Nature*, **189**, 465 (1961).

[19] Baglioni, C., Ingram, V. M., and Sullivan, E., *Nature*, **189**, 467 (1961).

[20] Ingram, V. M., *Nature*, **189**, 704 (1961).

[21] Itano, H. A., in *Conference on Hemoglobin*, 144. Pub. 557, National Academy of Sciences—National Research Council, Washington, D.C., 1958).

PATHOLOGY

Anaphylactoid Reaction in Rats

THE primary injection of dextran or egg white into rats produces hyperæmia, pruritus, and œdema of the face, tongue and paws; symptoms of the so-called anaphylactoid reaction[1]. While studying factors which modify this reaction, it became apparent that a proportion of rats failed to show this response. This problem was therefore examined in detail.

Most of the experiments were performed on groups of either male or female albino Wistar rats obtained from the Agricultural Research Council's Field Station at Compton. Their diet consisted of cubes (No. 41, Associated London Flour Millers, Ltd.) and they were allowed drinking water *ad lib.* The initial dose of dextran was 0·3 ml. per 100 gm. body-weight (180 mgm./kgm.). Fifteen out of 60 adult rats (25 per cent) failed to show the anaphylactoid reaction when this dose of dextran was given intraperitoneally, and a similar proportion did not react when the dextran was given intravenously together with Evan's blue dye. The animals initially resistant to this dose of dextran were resistant to doses of 0·05, 0·2 and 0·8 ml. per 100 gm. when tested at weekly intervals. Similar results were obtained when fresh egg white (10 ml./kgm.) or a second brand of dextran was used. The rats which did not respond to dextran also failed to respond when subjected to stress (withdrawal of food for 24 hr.). They were not diabetic, and the weights of their adrenal glands were similar to those of control rats. There was no deficiency in the 5-hydroxytryptamine content of the skin of rats not responding to dextran, and dextran was ineffective in these rats even when pre-treated with 5-hydroxytryptophan. The intravenous toxicity of histamine was also unchanged.

Procedures which potentiate the anaphylactoid reaction in control rats were then tried in animals not responding to dextran. Pre-treatment of the rats with insulin (4 I.U./kgm.) or chlorpropamide (100 mgm./kgm.) failed to facilitate the response, and so did adrenalectomy when the animals were tested at 6, 13 and 20 days after the operation. In these experiments, up to 50 per cent of the control animals died after showing the anaphylactoid reaction. When insulin and the stress of food withdrawal were used in adrenalectomized rats, the resistant animals again failed to show the anaphylactoid reaction.

In this work, 832 rats have been used, and no fewer than 162 (about 19·5 per cent) have failed to respond to dextran or egg white. When, however, some of the experiments were repeated using Sprague Dawley and hooded Lister rats, the anaphylactoid reaction developed at a fast rate, and no failures were obtained. Further work is now in progress to determine the characteristics of the strain specificity with regard to dextran. The immunological aspects of the problem may be of paramount importance in the study of human allergies.

J. M. HARRIS*
G. B. WEST

Department of Pharmacology,
School of Pharmacy,
University of London,
29–39 Brunswick Square,
London, W.C.1.

* Present address: School of Pharmacy, Brighton Technical College.

[1] Selye, *Endocrinology*, 21, 169 (1937).

Treatment of Thrombocytopænic Hæmorrhage with Œstriol Succinate

THE therapeutic indications for œstriol have, until now, been limited to the treatment of superficial conditions of the cervix, vagina and vulva, and to the relief of menopausal symptoms. At the dosage-level effective in these cases, œstriol has no effect on the endometrium and does not, therefore, lead to irregularities in the menstrual cycle or to uterine hæmorrhage.

I have recently carried out an investigation into the hæmostatic properties of a pure intravenous preparation of œstriol succinate (kindly supplied by N.V. Organon, Oss, Holland) in hæmorrhages of varying pathogenesis. It became clear very early in our investigations that this substance has a rapid effect on all types of thrombocytopænic bleeding, its action being particularly marked in the acute toxic and allergic thrombocytopænias.

The substance was administered to patients of normal weight in intravenous doses of 10 mgm. and provided adequate protection from hæmorrhages for about 48 hr. None of the 100 patients so far treated in this way has shown side-effects.

My investigations into the mechanism of the normal clotting process have demonstrated that œstriol succinate acts on this mechanism in two distinct ways: (1), a direct action on the blood vessels, reducing their permeability; and (2), an effect closely similar to that of thrombocyte factor 3. The latter action can be irrefutably demonstrated using the thrombokinase-formation test. Details of my work on the clotting mechanism will be published elsewhere.

No significant increase in thrombocyte count was observed during the above treatment. The dangerous incidents which occur all too frequently in the course of thrombocytopænia can, nevertheless, be rapidly and economically controlled in this way until such time as a more normal thrombocyte count can be restored by other means. I have had frequent recourse to œstriol succinate in cases with symptomatic thrombocytopænia consequent on leukosis, panmyelopathy and overdosage of cytotoxic agents. In addition, hæmorrhages following overdosage with anticoagulants, as well as post-traumatic bleeding in patients on anticoagulant therapy, can be better controlled when œstriol succinate is administered than on vitamin K therapy alone. This effect must be attributed to the direct vascular action of the preparation. Œstriol succinate, in view of its high therapeutic efficiency and its very good tolerance, has proved to have a wide field of application in this hospital, and one must hope that it will become generally available in the near future.

HUBERT POLIWODA

Medizinische Universitätsklinik,
Göttingen.

Effect of Enzyme Injections on Mitosis in Regenerating Liver

THE addition of deoxyribonuclease to tissue-culture media affected mitosis in tissue cultures[1,2]. In mice bearing Ehrlich ascites tumours, injections of the enzyme decreased the incidence of mitoses in the tumour cells and increased the survival-time of the animals[3]. Similarly, injections of xanthine oxidase and ribonuclease affected the rate of tumour growth and the survival time[4–6].

In view of these facts, it has been thought of interest to study the effect of deoxyribonuclease (DNase), ribonuclease (RNase) and xanthine oxidase on a rapidly dividing normal tissue such as regenerating liver following partial hepatectomy. (Deoxyribonuclease and ribonuclease were purchased from Worthington Biochemical Corporation, Freehold, New Jersey. The xanthine oxidase was a gift from Prof. F. Bergel, Chester Beatty Research Institute, London.)

Wistar rats of average weight 218 gm. (194–242 gm.) were partially hepatectomized (66 per cent) according to the method of Higgins and Anderson[7] between 9.30 and 11.30 a.m. to eliminate the variations due to the periodicity of mitotic activity[8]. The hepatectomized rats were injected intraperitoneally with sodium chloride 0·85 per cent or solutions of albumin, DNase, RNase (5 mgm./ml.), or xanthine oxidase in sodium chloride 0·85 per cent. The original solution of xanthine oxidase prepared in phosphate buffer containing sodium salicylate and ethylenediamine tetraacetic acid as preservatives

Table 1. EFFECT OF ENZYME INJECTIONS ON MITOSIS IN REGENERATING RAT LIVER

Group	No. of rats	Mitoses No.	Prophases No.	Prophases Per cent	Metaphases No.	Metaphases Per cent	Anaphases No.	Anaphases Per cent	Telophases No.	Telophases Per cent
Controls*	6	4·7 ±0·2	1·8 ±0·1	38·4 ±1·7	2·0 ±0·2	41·8 ±2·6	0·4 ±0·1	9·0 ±0·9	0·5 ±0·1	10·8 ±0·6
DNase (*A*)	5	4·3 ±1·4	1·0 ±0·3	24·2† ±1·2	2·5 ±0·8	59·7† ±3·2	0·4 ±0·2	8·2 ±2·7	0·4 ±0·2	7·9 ±1·7
DNase (*B*)	4	4·0 ±0·4	1·0 ±0·1	24·2† ±3·3	2·3 ±0·4	58·0† ±5·2	0·3 ±0·1	6·6 ±1·6	0·4 ±0·1	11·2 ±2·1
Xanthine oxidase (*A*)	5	3·4 ±1·4	0·7 ±0·3	19·4† ±2·2	2·1 ±0·7	61·4† ±2·0	0·3 ±0·1	8·7 ±2·2	0·3 ±0·2	10·5 ±1·7
Xanthine oxidase (*B*)	4	4·1 ±2·5	1·1 ±0·5	26·9† ±1·8	2·1 ±1·0	52·1† ±2·0	0·4 ±0·3	9·1 ±2·6	0·5 ±0·3	11·9 ±2·0
RNase (*A*)	3	4·3 ±1·4	1·9 ±0·7	43·0 ±2·3	1·9 ±0·7	43·5 ±3·9	0·2 ±0·1	6·6 ±3·0	0·3 ±0·1	6·9 ±1·2

* Controls were injected intraperitoneally with 1 ml. sodium chloride 0·85 per cent or albumin (5 mgm./ml.) immediately after the hepatectomy. Groups DNase, xanthine oxidase and RNase (*A*) were injected intraperitoneally with 1 ml. of enzyme (5 mgm./ml.) immediately after the hepatectomy. Groups DNase and xanthine oxidase (*B*) were injected intraperitoneally with 1 ml. of enzyme (5 mgm./ml.) at 6 and 24 hr. after the hepatectomy.

† Statistically significant at a level better than 99·9 per cent.

was dialysed against two litres of sodium chloride 0·85 per cent during seventy-two hours in the cold. After dialysis, the enzyme activity was determined by a standard method[9] and the activity corresponded to the destruction of 1·27 μmole of xanthine per min. per ml. of solution.

Forty-eight hours after hepatectomy, the rats were killed by decapitation and the excised liver fixed in neutral 10 per cent formaldehyde. Histological sections were prepared by the usual method and stained with eosin and hæmatoxylin.

The incidence of mitotic figures in liver parenchyma was determined by differential counts. A reticule delimiting a square field was placed in one of the oculars of a binocular microscope and this field was placed at random over the histological section. The nuclei present in the field were examined under immersion and differential counts were made of resting cells, prophases, metaphases, anaphases and telophases. When all the nuclei present in a field had been examined, the counts were carried out in similar fields taken at random until at least 6,000 nuclei were counted. The results were expressed as the number of mitotic figures per hundred cells.

Table 1 shows the results obtained for the incidence of mitosis and mitotic phases in regenerating liver following the injection of control or enzyme solutions. The proportions of the mitotic phases are also included. From the results, it would seem that the mitotic activity was not much affected by enzyme injections. The largest variation was observed in the group xanthine oxidase (*A*) injected with a single dose immediately after hepatectomy and was of the order of 30 per cent. The incidence of prophases, on the other hand, was much affected in rats injected with DNase or xanthine oxidase. Decreases from 1·8 to 1·0, 1·0, 0·7, and 1·1 respectively were observed. The incidences of metaphases, anaphases and telophases, however, varied only slightly. The important decrease in the incidence of prophases became masked in the values obtained for the incidence of mitosis because it represents only a fraction of the mitotic activity.

The decreases in the number of prophases were reflected by significant decreases in the proportions of prophases relative to the other phases of the mitotic cycle. Significant increases in the proportions of metaphases were consequently observed. The injection of RNase did not affect the number of the various mitotic phases or their proportions.

Table 2 shows the most important cytological abnormalities observed in the mitotic figures. The control rats showed a few pycnotic metaphases as well as a few metaphases with one or two lagging chromosomes. These abnormalities were very frequent in the animals treated with DNase. In these animals it was also frequently noticed that chromosomes were lagging to join the two sets at anaphase and chromosomal bridges were present. In the animals treated with xanthine oxidase, pycnotic metaphases as well as colchicine-like metaphases were frequently observed.

Table 2. CYTOLOGICAL ABNORMALITIES

	Control	DNase
Chromosomes lagging to reach the equatorial plate in metaphase	rare, one or two chromosomes lagging	very frequent, many chromosomes lagging
Pycnotic metaphase	rare,	frequent,
Chromosomes lagging to join the two sets in anaphase	nil	frequent
Bridges of chromosomes in anaphase	nil	present
	Control	Xanthine oxidase
Colchicine-like metaphase	nil	frequent

From the results described it would seem that the duration of prophase has been shortened by the injection of DNase and xanthine oxidase as indicated by the significant lowering of the incidence of prophases. The metaphase and anaphase seemed also to be affected to a certain extent as shown by the abnormalities noted.

We acknowledge the help of Dr. Roger Boothroyd, of McGill University, in the classification of the mitotic abnormalities. Dr. Roger Daoust has been most helpful by his advice on differential counts. The work has been supported by grants to Dr. A. Cantero, director of the Research Laboratories, from the National Cancer Institute of Canada.

GASTON DE LAMIRANDE *

Montreal Cancer Institute,
Research Laboratories,
Notre Dame Hospital and
University of Montreal,
Montreal.

* Research Associate of the National Cancer Institute of Canada.

[1] Chevremont, M., Chevremont-Comhaire, S., and Backeland, E., *C.R. Acad. Sci., Paris*, **247**, 2197 (1958).

[2] Montgomery, P. O'B., and Bonner, W. A., *Proc. Amer. Assoc. Cancer Res.*, **3**, 44 (1959).

[3] de Lamirande, G., *Proc. Amer. Assoc. Cancer Res.*, **3**, 105 (1960).

[4] Haddow, A., *et al.*, *Brit. Empire Cancer Campaign Ann. Rep.*, **31**, 35 (1953); **32**, 50 (1954).

[5] Haddow, A., de Lamirande, G., Bergel, F., Bray, R. C., and Gilbert, D. A., *Nature*, **182**, 1144 (1958).

[6] Ledoux, L., *Nature*, **175**, 258 (1955) ; **176**, 36 (1955).

[7] Higgins, G. M., and Anderson, R. M., *Arch. Path.*, **12**, 186 (1931).

[8] Jaffe, J., *Anat. Rec.*, **120**, 953 (1954).

[9] de Lamirande, G., Allard, C., and Cantero, A., *Cancer Res.*, **18**, 952 (1958).

BIOLOGY

Myopathy affecting the Rottnest Quokka (*Setonix brachyurus*) reversed by α-Tocopherol

THE quokka (*Setonix brachyurus*) is a small marsupial about the size of a fox terrier which inhabits Rottnest, an island lying in the Perth Basin ten miles from the coast of Western Australia. In recent years the species has been extensively studied by the Department of Zoology of the University of Western Australia, as the potential offered by the animal for ecological research is unique.

For several years in Perth and overseas, paralysis of the hind limbs has been observed in the quokka while in captivity. This disease has hitherto ended fatally despite various therapeutic measures including antibiotics and antihelminthics. It is characterized by weakness of the hind limbs which begins insidiously but progresses rapidly to complete paralysis. There is marked wasting of the muscles of the pelvic girdle and the disease invariably terminates in death in a few days to several weeks. At autopsy pallor and atrophy of the pelvic and femoral groups of muscles are found. On histological examination of affected muscles individual fibres are swollen, fragmented and show variability of cytoplasmic staining. Many fibres are without nuclei, and in others there is pycnosis. There are proliferations of sarcolemmal cells and infiltrations of variable cellularity of lymphocytes, plasma cells and polymorphs. Metazoan parasites are not found.

Early in 1960 representative slides of the muscle lesion were sent to Dr. Raymond Adams, Bullard professor of neuropathology, Harvard Medical School, for his opinion. He replied that the cause of the paralysis and of the lesions observed might well be due to deficiency of vitamin E in the diet. Dr. ten Seldam, professor of pathology, University of Western Australia, came to the same conclusion when investigating other similar material for Dr. S. Barker of the Zoology Department. Accordingly experiments were undertaken to test the effects of vitamin E administration to paralysed animals.

Ten quokkas which developed paralysis while in captivity were selected for trial treatment. These animals were from several groups which were maintained on a standard diet of high protein (17–21 per cent) 'sheep cubes' consisting of bran, pollard, oat meal, linseed meal, whale meal, molasses, urea and mineral salts. This diet was supplemented with bread and green vegetables including lucerne, lettuce, spinach and grasses growing in the vicinity. Nine of the treated animals were given the vitamin orally in doses ranging from 200 to 600 mgm. over several days. In all these animals there was rapid and complete clinical recovery in two to three weeks. Only one of the ten animals died. It was moribund at the beginning of the experiment and failed to respond to the vitamin given by injection.

Table 1

Date	Quokka	Urine (vol. 24 hr.)	Creatine (24 hr.)	Creatinine (24 hr.)
27.10.60	6	27 ml.	24 mgm.	0·010 gm.
28.10.60	7	48 ml.	27 mgm.	0·008 gm.
2.11.60	8	92 ml.	58 mgm.	0·011 gm.
13.1.61	6	48 ml.	15·8 mgm.	0·03 gm.
13.1.61	7	67 ml.	36·8 mgm.	0·057 gm.
13.1.61	8	96 ml.	24·0 mgm.	0·079 gm.

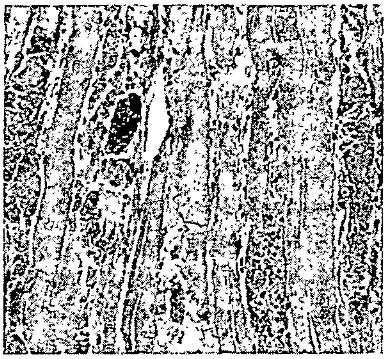


Fig. 1. Muscle biopsy of quokka 5. Original biopsy, October 17, 1960. Pronounced degenerative changes. (Hæmatoxylin and eosin × 78)

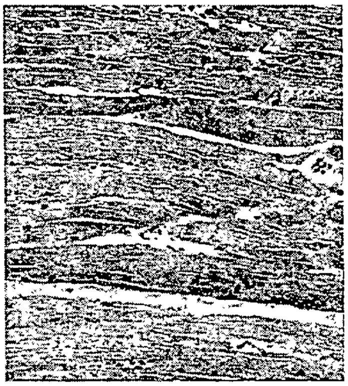


Fig. 2. Muscle biopsy of quokka 5. Second biopsy November 21, 1960. Restoration to normal. (Hæmatoxylin and eosin × 78)

Muscle biopsies were taken on the first or the second day of treatment. In the nine animals which recovered this first biopsy showed histological changes similar to those depicted in Fig. 1. In seven animals subsequent biopsy was performed at a site close to the original. These biopsies were taken from 1–8 weeks after clinical recovery. Two of the second biopsies showed the muscle to have completely recovered. In four others there was still minimal evidence of degeneration (Fig. 2), and in one no improvement of the lesion was observed notwithstanding the clinical recovery. In general, the longer the period was between biopsies the greater the observed improvement was in the histological appearances. 24-hr. samples of urine for creatine and creatinine estimations were taken from three of the animals at the time of the original biopsy and again after full recovery. The results, which support the clinical and biopsy findings, are given in Table 1.

Deprivation of vitamin E in the diet of the quokka is at present being tried in a small series of animals, and the early results suggest that the lesion can be induced in this manner. No control animals have died naturally or have shown evidence of disease.

Grateful acknowledgment is made to Prof. ten Seldam, who suggested this work, and for the advice and technical assistance given by Dr. I. Kaldor (Department of Physiology), and members of the Department of Zoology of the University of Western Australia. Mr. H. Upenieks, medical illustrator,

prepared the photomicrographs. The biochemical estimations were performed through the kindness of Dr. D. Curnow (Royal Perth Hospital). The α-tocopherol was kindly supplied by Roche Products, Ltd.

B. A. KAKULAS

Department of Pathology,
University of Western Australia,
Perth, Western Australia.

Structure and Action of the Sucker of *Echeneis*

FIG. 1 is a diagram of the sucker of *Echeneis* taken after staining it with alizarin red and rendering it transparent in glycerin. This reveals the presence of a chamber below the posterior shield of the sucker, about which no mention has so far been made in the literature. Hora[1], who at first doubted whether the sucker was a real sucker, later was convinced that the outer rim functioned as a sucker and that there were smaller paired suckers as well; Sewell's[3] observations confirmed this.

My observations show: (1) That in addition to the paired chambers there is an unpaired chamber below the posterior shield which has no opening on the dorsal side. (2) While all the other paired chambers are filled with sea water when the fish is swimming about, this chamber also contains some fluid but not sea water as there appears to be no connexion with the exterior. (3) At the posterior end of the chamber there is a pear-shaped hollow bony structure with one or more openings in it and there are two channels one on either side of it suggesting that by its movement forward and backward it can act as a valve regulating the flow of liquid in the chamber. (4) The entire base of the disk is paved with bone consisting of plates overlapping each other and with two pairs of holes in them. (5) The laminæ carry transverse bars against which the bases of the teeth abut, forming effective buttresses. (6) A pair of large hooks from the buttress interlock with the holes in the basal plates. (7) The pear-shaped, hollow, bony structure becomes completely detached when teased in caustic potash.

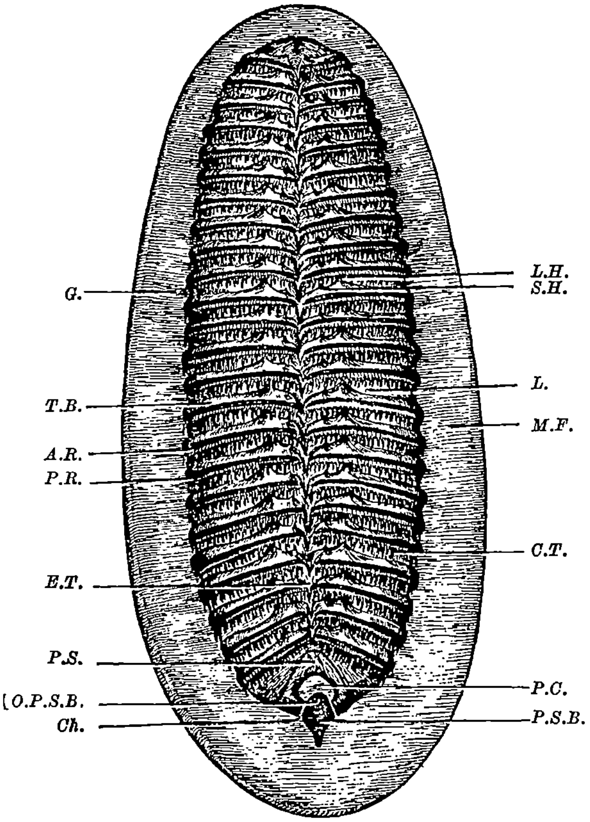


Fig. 1. The sucker of *Echeneis* as seen when stained in alizarin red and rendered transparent in glycerin. *A.R.*, anterior ridge; *Ch.*, channel; *C.T.*, comb-like teeth in three rows; *E.T.*, enlarged tooth pointing backwards; *G.*, glyph or spillway; *L.*, lamella between ridges; *L.H.*, large hook which fits into basal plate below; *M.F.*, marginal flap; *O.P.S.B.*, opening in pear-shaped body; *P.C.*, posterior chamber; *P.R.*, posterior ridge; *P.S.*, posterior shield; *P.S.B.*, pear-shaped body (valve); *S.H.*, small hook which fits into basal plate below; *T.B.*, transverse bar or buttress

The attachment presumably takes place in the following stages: (1) the marginal flap is the first to adhere; (2) the sea water is next sent out of the paired pouches from below the posterior ridge of each lamella; (3) next the interlocking of the lamellæ with the basal plates takes place; (4) any remaining water escapes by the spillways resembling siphonoglyphs; (5) the posterior shield is the last to adhere when the body fluid in the median chamber is withdrawn.

The release of the disk can be brought about by admitting sea water artificially: that is, by inserting sticks, strings or the blade of a penknife, but in Nature the processes mentioned above work in reverse. The posterior shield is the first to be released by the admission of body fluid into the chamber below it. Sea water now enters the space between the shield and the surface of adhesion and is carried to the siphonoglyphs, through which it enters, bringing about the release of the hooks. Water now is able to enter into the paired chambers, producing the "hissing sound" mentioned by Hora[2] and the "slight sucking noise" of Sewell[3]. During the process the water is strained free of all solid particles which might be floating in the sea water by the comb-like teeth. As each chamber is partly overlapped by the next a set of chain reactions occur so that all the paired pouches are progressively filled with sea water and the entire disk released.

Sewell mentions that when *Echeneis* is attached to any object all the movements of the fish except those of the mouth and gills seem to be suspended, and Norman[4], quoting it, says a detailed study of the nervous mechanism involved would be of interest. I venture to suggest that the movement of the body fluid in and out of the posterior chamber is obviously responsible for the setting of the nervous mechanism to work.

A detailed description of the sucker is in preparation, and it is expected to show that the sucker instead of being a partial vacuum is really a mechanism precisely built and as efficient as any that human ingenuity can invent, in which case the sucking power must be 15 lb./sq. in. As some of the suckers cover an area of more than 8 sq. in., the fish, theoretically at least, must be able to suck with a power exceeding 100 lb.

B. BONNELL

"Bonanza,"
Agaram Road,
Tambaram,
Madras.

[1] Hora, *Nature*, 111, 68 (1923).
[2] Hora, *Nature*, 115, 47 (1925).
[3] Sewell, *Nature*, 115, 48 (1925).
[4] Norman, *Nature*, 143, 52 (1939).

The Path of the Giant Cell Axons in *Aplysia depilans*

THE presence of large nerve cells in the abdominal ganglion of *Aplysia* has provided a valuable preparation for the study of the physiology of invertebrate neurones[1-8]. The largest cell is usually referred to as the giant nerve cell[1-3,6-8] and it may be 300–800μ in diameter. It is excited synaptically by pathways entering most, if not all, of the nerves of this ganglion, but its only efferent axon passes anteriorly along the right pleuro-visceral connective. As almost all the work on the abdominal ganglion has been done with it completely isolated from the rest of the nervous system, the further path of this axon and the possible functions of this very large cell have not been understood. Experiments, which included the insertion of micro-electrodes into this giant cell when the abdominal ganglion retained its functional connexions with the rest of the nervous system in a whole animal preparation, have provided evidence for the branching of this axon and have excluded certain possibilities concerning the function of the giant cell.

Fig. 1 summarizes diagrammatically the results of several different types of experiment which have confirmed one another. The axon of the giant cell after passing to the right pleural ganglion divides into branches which enter each of the main nerves innervating the right parapodium and the right side of the foot. Another branch continues forwards in the cerebro-pleural connective and through the cerebral ganglia to the left pleural ganglion. Exactly where the branching occurs is not certain; but, from the evidence available, that shown in the diagram seems the most probable. Electrical stimulation of any of the nerves containing a branch of this axon produces an impulse in each of the other branches. In these conditions, an antidromic spike is recorded in the giant cell following the passage of an impulse down the right pleuro-visceral connective. However, transmission between the different branches of this neurone is not always readily achieved, and repetitive stimulation is sometimes necessary. On the other hand, transmission often fails at relatively low frequencies. When this happens, however, it is not invariably preceded by a gradual lengthening of the delay which might be expected if a synaptic process were involved. Furthermore, delay between the application of the stimulus to one branch of the neurone and its appearance at another point is the same, regardless of the direction of impulse conduction. It may be assumed, therefore, that the failure of transmission in an antidromic direction is due to the low safety factor at the branching, especially as the more distant axonal branches are smaller than the main axon. A failure to conduct an impulse from the main axon to the branches in the orthodromic direction was never observed. Further confirmation was obtained by stimulating the giant cell itself by depolarizing the soma membrane and recording impulses with constant delays in the different nerves to which it sends a branch. In addition it is clear that, in spite of the axon crossing several ganglia, this cell is only excited synaptically in the abdominal ganglion. Different natural stimuli to an intact animal have never elicited a spike from a part of this neurone other than that contained in the abdominal ganglion.

Another giant cell has now been discovered on the left side of the animal. Work similar to that outlined above showed an almost identical pattern of branching (Fig. 1). The main difference is that in this case the cell body lies in the pleural ganglion and not in the abdominal ganglion. However, if the long axon in the right pleuro-visceral connective were shortened a symmetrical arrangement would result.

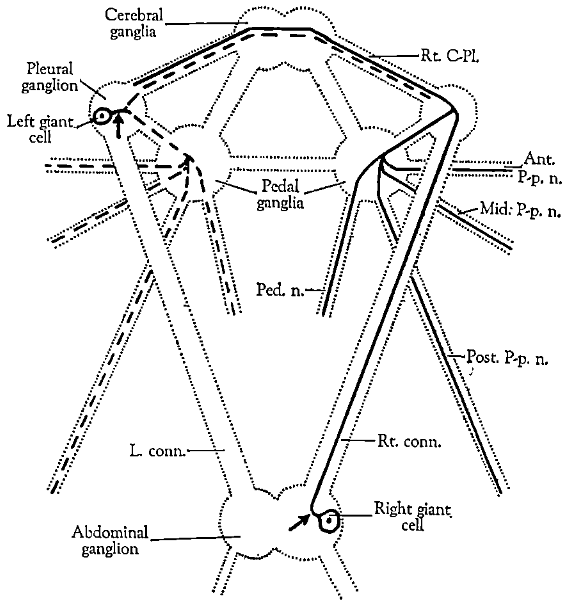


Fig. 1. Diagram of the path of the two giant cell axons in the nervous sytem of *Aplysia depilans*. Right cell, full lines; left cell, dashed lines. Arrows indicate the region where the giant neurones are excited synaptically. *Rt. C-Pl.*, right cerebro-pleural connective; *Rt. conn.*, right 'pleuro-visceral' connective; *Ant.*, *Mid.*, and *Post P-p. n.*, anterior, middle and posterior parapodial nerves; *Ped. n.*, pedal nerve

The precise homologies of the ganglia associated with the visceral loop in *Aplysia* are incompletely understood. This is mainly due to the absence of developmental evidence, and present views are based largely upon considerations of the external morphology of the central nervous system. The discovery that the largest cell on the two sides has basically the same distribution suggests the tempting hypothesis that they are homologous and that the cell bodies reside in different ganglia as a result of the complex morphogenetic changes which occur during torsion. In an idealized gastropod the visceral loop has along its length two parietal ganglia and a single or double visceral ganglion. If, following torsion, the right parietal ganglion became fused with the visceral ganglion, whereas the left ganglion or some part of it had remained fused with the left pleural ganglion, the difference in position of these two cells would be explained. Fusion of the right parietal ganglion (supra-intestinal) with the visceral ganglion is generally accepted, but the fate of the left parietal ganglion has not been established[9].

Little information has been obtained concerning the function of the left giant cell, because of the difficulty of recording from this cell in the intact preparation. Activity of the right giant cell has been recorded intracellularly in many preparations, and a study made of the conditions in which it becomes active. This occurs when mechanical stimulation is applied to any part of the body, with the head and gill region being the most sensitive. Excitation of the cell by either depolarizing currents passed through an intra-cellular micro-electrode or direct mechanical stimulation has not consistently evoked any overt movement of the animal. This disposes of the possibility that this cell is directly involved in the dorsal folding movements of the parapodia which occur when the animal is prodded and seem to represent a form of escape reaction. Nevertheless, during such movements of the parapodia which occur spontaneously in some preparations, the giant cell nearly always fires and it seems to be associated with this response although many other neurones in the abdominal ganglion do the same. It is clear that the giant cell does not have a function similar to the giant efferent neurones of other animals. Recordings were made from single neurones of the right pleural and pedal ganglia during stimulation of the giant cell and the abdominal ganglion. No evidence has been obtained for the existence of synaptic connexions between the giant cell axon and the cells which were sampled in several preparations. The possibility that this cell has an effect on the intensity or threshold of this defence response or other activities in the animal is difficult to investigate and remains a possible function of this very large cell.

This work was carried out in the Marine Biological Institute of Arcachon, France.

G. M. Hughes*
L. Tauc

Centre d'Études de Physiologie nerveuse du C.N.R.S.,
4 Avenue Gordon-Bennett,
Paris 16e.

---

* Department of Zoology, Cambridge, England.

[1] Arvanitaki, A., *Arch. int. Physiol.*, **52**, 381 (1942).
[2] Arvanitaki, A., and Chalazonitis, N., *C.R. Acad. Sci., Paris*, **240**, 349 (1955).
[3] Arvanitaki, A. and Chalazonitis, N., *Arch. Sci. Physiol., Paris*, **10**, 95 (1956).
[4] Tauc, L., *J. Physiol., Paris*, **47**, 769 (1955).
[5] Tauc, L., *Arch. Ital. Biol.*, **96**, 78 (1958).
[6] Tauc, L., *C.R. Acad. Sci., Paris*, **250**, 1560 (1960).
[7] Tauc, L., *J. Physiol.* (London), **152**, 36 (1960).
[8] Tchou-Si-Ho, *Contribution à l'étude de la physiologie des cellules nerveuses chez l'Aplysie* (Bosc et Rion, Lyon, 1942).
[9] Eales, N. B., L.M.B.C. Mem. 24 *Aplysia* (1921).

Maxillary Index in *Anopheles gambiae* Giles

Freshwater *Anopheles gambiae* may be distinguished as two co-existing biological races[1–3], on the basis of their maxillary indices, namely: (i) a paucidentate race, breeding in temporary pools of low organic content, which is anthropophilic and mainly exophilic, and (ii) a multidentate race, breeding in permanent types of water of high organic content, which is zoophilic and endophilic. Others[4–7] hold the opposite view, that the maxillary index is not a precise enough character for demonstrating this distinction. The present observations are in agreement with the reported breeding preferences of paucidentate and multidentate populations, but they also support the conclusion in ref. 6 that the larval environment has a powerful influence on the maxillary dentition.

Observations were made on three populations of *A. gambiae* (I, II and III), in the neighbourhood of Kampala, in Uganda, East Africa. Population I bred in a large temporary pond, on the Kampala-Hoima Road; II, in small, temporary, rain puddles, in an old road passing through Nakazi Swamp, on the Kampala-Bombo Road; and III, in permanent water, in the more exposed parts of Nakazi Swamp. Population I occurred some 10 miles from both II and III; while II and III were only 10–50 yards apart. The maxillary indices of the three populations are summarized in Table 1.

Table 1. Maxillary Indices (M.I.) of the Three Populations

Maxillary Index	Mosquito population and frequency (per cent)		
	I	II	III
9·5	2·5		
10·0	5·5		
10·5	12·5		
11·0	10·5	1·0	
11·5	27·5	3·0	
12·0	13·5	6·5	2·0
12·5	17·0	13·0	1·5
13·0	3·5	19·0	4·5
13·5	6·0	24·0	11·5
14·0	0·5	15·0	17·0
14·5	1·0	13·5	25·0
15·0		5·0	9·5
15·5			11·5
16·0			6·0
16·5			8·5
17·0			2·0
17·5			1·0
No. mosquitoes dissected	200	200	200
Mean M.I.	11·64	13·38	14·66

Table 1 shows that population III had a higher maxillary index than either I or II; thereby supporting the conclusion reached by other workers[1–3] that multidentate populations tend to breed in permanent waters and the paucidentate in temporary waters. In this respect, the distinction between the maxillary dentition of II and III is particularly striking, in view of the close proximity of their breeding places. In such circumstances, genetic distinctiveness could not be maintained, unless there were sexual isolation between the two populations.

The difference in the maxillary dentition between populations I and II is also particularly interesting. The breeding water of I was less temporary, and, presumably, had a higher organic content, than that of II. It would, therefore, be expected that population I should have a higher maxillary index than II. The results (Table 1) show the reverse. The explanation of this anomaly may be found in the spatial relations of the three populations. If, as it has been suggested[1–3], we are dealing here with two co-existing biological races, the close proximity of the breeding places and, consequently, of the adult populations may have resulted in the production of a hybrid population (II) with an intermediate maxillary index. The distance between temporary and permanent breeding places may, therefore, affect significantly the difference between the maxillary indices of the respective mosquito populations.

That the maxillary index may be increased by increasing artificially the temperature of the breeding water is shown in Table 2. These results agree with those in ref. 6. However, the significance of these results, in relation to what happens in the field, is not obvious. The vast majority of *A. gambiae* breeding places are exposed, temporary, shallow pools, in which the water temperatures are often considerably much higher than in the less favourable, permanent-water habitats. Yet, the maxillary index is higher in the latter than in the former. It is probable that this discrepancy is due to the fact that the artificially high laboratory temperatures are made continuous; while those in the field occur for only a short period of the day and thus they fluctuate with the daily rhythm. In this respect, a study of the maxillary index of *A. gambiae* breeding in continuously high temperatures, in tepid water from hot springs[8], would be particularly worth while.

Table 2. MAXILLARY INDEX (M.I.) OF POPULATION I BRED IN FIELD WATER AT CONSTANT HIGH TEMPERATURE (32° C.) IN THE LABORATORY

Maxillary Index	Frequency (per cent)
13·5	1·7
14·0	12·1
14·5	20·7
15·0	15·5
15·5	20·7
16·0	12·1
16·5	8·6
17·0	5·2
17·5	1·7
18·0	0·0
18·5	1·7
No. mosquitoes dissected, 58; mean M.I., 15·31.	

Table 3 shows the results of laboratory experiments in which the organic content of field breeding-water was increased by adding fresh cow-dung to the water. It will be seen that the maxillary index of population I became higher. These results indicate that it is possible for an *A. gambiae* population which would otherwise have developed as paucidentate to change into a multidentate one, by the mere fortuitous dropping of dung by cattle into the breeding place. It has been postulated[9] that a higher maxillary index confers on the mosquito a greater ability to feed on cattle and other thick-skinned domestic animals. Consequently, it has been assumed that the prevalence of multidentate mosquitoes, in an area with a high cattle population, proves that natural selection has acted in favour of such mosquitoes. However, the present observations would seem to indicate that the co-existence of multidentate mosquitoes and high cattle populations, in an area, may simply be the result of a greater proportion of the breeding places being polluted with dung and other organic matter of animal origin; which, in turn, increases the maxillary dentition of the mosquitoes.

Table 3. MAXILLARY INDEX (M.I.) OF POPULATION I BRED IN 'COW-DUNGED' FIELD WATER IN THE LABORATORY (room temperature varied 22–25·5° C.)

Maxillary Index	Frequency (per cent)
11·5	1·2
12·0	4·9
12·5	13·6
13·0	17·3
13·5	21·0
14·0	16·0
14·5	14·8
15·0	7·4
15·5	2·5
16·0	1·2
No. mosquitoes dissected, 81; mean M.I., 13·58.	

This work was carried out under a scheme of research on tropical swamps supported by the Nuffield Foundation and undertaken by the Departments of Botany and Zoology, at Makerere University College, Kampala, Uganda.

L. K. H. GOMA

The Ross Institute,
London School of Hygiene and
Tropical Medicine,
Keppel Street (Gower Street),
London, W.C.1.

[1] Campbell, R. W. H., *Bull. Ent. Res.*, **42**, 647 (1951).

[2] Maillot, L., *Bull. Soc. Path. Exot.*, **46**, 839 (1953).

[3] Holstein, M. H., *Biology of Anopheles gambiae* (W.H.O. Monogr., No. 9, 1954).

[4] Gillies, M. T., *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, 443 (1954).

[5] Gillies, M. T., *Bull. W.H.O.*, **15**, 437 (1956).

[6] Gillies, M. T., and Shute, G. T., *Nature*, **173**, 409 (1954).

[7] Fox, J. W., and Fox, R. M., *Mosq. News*, **15**, 207 (1955).

[8] Goma, L. K. H., *Bull. Ent. Res.*, **51**, 77 (1960).

[9] Roubaud, E., *Bull. Soc. Path. Exot.*, **14**, 577 (1921).

Rectal Glands in Woodlice

THE structure and function of the rectal glands of insects have been investigated by several workers[1]. Later, Wigglesworth[2] studied these glands more thoroughly in a number of species. No report, however, has yet appeared in the literature regarding the presence of rectal glands in woodlice (Crustacea : Isopoda), although Edney[3] stated : "the fæcal pellets are moist, and there appears to be no mechanism comparable with the rectal glands of insects for reabsorption of water contained in the rectal contents". This observation, however, was not based on any experimental or histological studies.

The present report is concerned with two common species of woodlice in Great Britain, *Oniscus asellus* L. and *Porcellio scaber* Latr. Examination of the rectal contents of these animals shows that they are in a much more fluid state than the fæcal pellets, which are

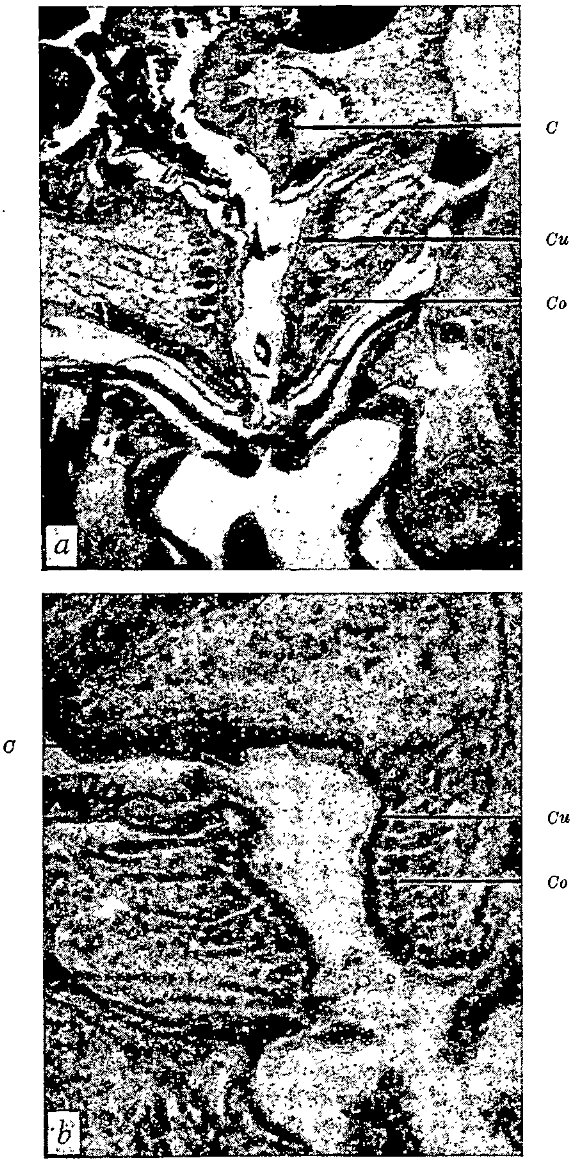


Fig. 1. Sections of the 'rectal gland' of (*a*) *Porcellio scaber* Latr.; (*b*) *Oniscus asellus* L. *C*, Cubical epithelial cell; *Co*, columnar epithelial cell; *Cu*, cuticle. (× 60)

finally discharged from the rectum in the form of rather black, large, solid, and cylindrical pellets. The water content of these pellets has been determined by Kuenen[4].

Considerable difficulty has been experienced by workers in the histological study of woodlice[5]. In the present work a variety of fixatives has been used[6], among which the best results were obtained with Carnoy's fixative. After fixing, the tissue was passed through decreasing strengths of alcohol down to water. In order to provide support, the tissue was embedded in agar and dehydrated. After double embedding, microtome sections were prepared in the usual way and the sections stained by the phloxine–tartrazine method of Lendrum[7].

In both *Oniscus asellus* and *Porcellio scaber* the lower part of the rectum is narrow, with a very thin cuticle covering cubical epithelium thrown into very small longitudinal folds (Fig. 1). In the posterior part of the rectum the cubical epithelial cells are absent and just below the cuticle lie columnar epithelial cells, tracheæ, muscle fibres and connective tissue. This structure is exactly similar to that described by Wigglesworth[2] in insects as rectal glands. These glands completely encircle the posterior part of the rectum.

Experimental evidence has been brought forward by Wigglesworth (ref. 2*a*) that rectal glands are concerned with absorption of water in insects. Water is the most important ecological factor in the habitat of woodlice, owing to the absence of cuticular wax layer. Desiccated woodlice are said to absorb water through the anus[8], and this has been confirmed in the present work. Further work is needed to elaborate the functions of the rectal glands in woodlice. Possibly these glands, too, are concerned with absorption of water from the rectal contents, although some is still lost in the fæcal pellets[4].

My thanks are due to Prof. J. L. Cloudsley-Thompson for his supervision during the work.

MINATI GUPTA

Department of Zoology,
King's College,
Strand,
London, W.C.2.

[1] Tonkov, V., *Rev. Russe d'Entom.*, **18**, 69 (1923). Borri, C., *Atti Soc. Toscana di Sci. Nat.*, **36**, 226 (1925). Chun, C., *Abt. Senckenb. Ges.*, **10**, 27 (1876).

[2] *a*, Wigglesworth, V. B., *J. Exp. Biol.*, **8**, 411 (1931). *b*, *Quart. J. Micro. Sci.*, **75**, 131 (1932).

[3] Edney, E. B., *Biol. Rev.*, **29**, 185 (1954).

[4] Kuenen, D. J., *Ent. Exp. and App.*, **2**, 287 (1959).

[5] Collinge, W. E., *Ann. Mag. Nat. Hist.*, (9), **7**, 212 (1921). Gorvett, H., *Quart. J. Micro. Sci.*, **87**, 209 (1946).

[6] Gatenby, J. B., and Beams, H. W., *The Microtomist's Vade-Mecum* (Churchill, London, 1950). Gray, P., *The Microtomist's Formulary and Guide* (Constable, London, 1954).

[7] Lendrum, A. C., *J. Path. Bact.*, **59**, 399 (1947).

[8] Spencer, J. O., and Edney, E. B., *J. Exp. Biol.*, **31**, 491 (1954).

Mechanism of Differential Staining of Nucleic Acids

WORK in this Department, to be published elsewhere, has shown that several histological methods for the demonstration of mucin depend for their selectivity on the use of cations of large size. Thus alcian blue, with a molecular weight of about 1,341 (ref. 1), is almost specific for acid mucins, being apparently unable to enter other, more dense, basophilic structures. The dialysed iron method[2] similarly depends on the use of large particles (colloidal ferric hydroxide), while in Southgate's mucicarmine the small carmine molecule is probably adsorbed on to colloidal aluminium hydroxide.

Arising from this work, it was thought that size of cation might influence the selectivity of other commonly used basic dyes. In the present work, fourteen basic aniline dyes have been investigated, ranging in molecular weight from 264 to 1,341 (Table 1). A variety of formalin- or Helly-fixed tissues has been used, including frog pancreas, liver, ovary, intestine, stomach and kidney, rat trachea, stomach, pancreas and intestine, and monkey pancreas.

Table 1

Dye and source	Molecular weight	Cationic weight	Percentage solubility	
			Alcohol	Water
Alcian blue 8*GN*150 (I.C.I.)	*c.* 1,341	1,341 (?)	6·0	9·5
Victoria blue 4*R* (National Aniline Co.)	520	485	20·0	3·0
Janus green *B* (National Aniline Co.)	483	448	1·0	5·0
Bismarck brown *R* (National Aniline Co.)	461	388	3.0	1·5
Methyl green *OO* (Grübler)	458	387	3·0	8·0
Hofmann violet (Merck)	422	387	7·5	6·0
Crystal violet (National Aniline Co.)	408	373	8·75	9·0
Nile blue *A* (National Aniline Co.)	733	316	5·0	6·0
Safranin *T* (Merck)	351	316	3·5	4·5
Brillantcresylblau (Grübler)	318	282	2·0	3.0
Toluidine blue (Grübler)	306	271	1·75	3·25
Pyronin (Grübler)	302	267	0·5	9·0
Azur *A* (National Aniline Co.)	292	257	?	?
Thionin (Grübler)	264	229	1·0	1·0

(1) Molecular weights and solubilities in alcohol and water are from ref. 1. Cationic weights are calculated from data from the same source.
(2) One molecule of nile blue *A* gives rise to two cations on ionization.

In initial experiments 0·01 *M* aqueous solutions of the dyes were used, the stained, air-dried sections being mounted directly in '*D.P.X.*' (synthetic mountant, British Drug Houses). It was found that in these conditions sections were markedly overstained by certain dyes (notably victoria blue 4*R*, bismarck brown *R* and hofmann violet). It is noteworthy that most of the dyes concerned are more soluble in alcohol than in water (Table 1), and it is possible that this is a reflexion of some property common to this group of dyes, which is responsible for a loose, non-ionic bonding with tissue components. This is to some extent confirmed by the fact that thorough dehydration in ethyl alcohol (one minute each in 90 per cent, 95 per cent and absolute alcohol) was sufficient to remove the excess dye.

The results about to be described were obtained on sections stained for 15 min. in a 0·005 *N* solution of the dye in veronal-acetate buffer at *p*H 4·25, washed in tap water, dehydrated in alcohol and mounted in '*D.P.X.*'.

Under these conditions alcian blue (weight of cation about 1,341) stained nuclei minimally, and did not stain nucleoli or cytoplasmic ribonucleic acid. Victoria blue 4*R*, janus green, bismarck brown *R*, methyl green *OO*, hofmann violet and crystal violet (with cationic weights from 485 down to 373) stained nuclei more deeply than nucleoli or cytoplasmic ribonucleic acid, while safranin and nile blue (cationic weight 316) stained nuclei, nucleoli and cytoplasmic ribonucleic acid with approximately equal intensity.

Brilliant cresyl blue, toluidine blue, pyronin, azur *A* and thionin (cationic weights from 282 down to 229) stained nucleoli and cytoplasmic chromidial substance more intensely than they stained nuclei.

From these results it appears that dye cations having a combined atomic weight of between about 350 and 500 are able to penetrate into and become attached to nuclear deoxyribonucleic acid (DNA), but do not as readily stain the nucleoli and cytoplasmic granules of ribonucleic acid (RNA), which are presumably denser and better stained by smaller dye cations (of a combined atomic weight of less than about 280).

Differential staining of DNA and RNA by mixtures of two basic dyes (as in the classical methyl green/pyronin method) is now readily explicable. The proportions of the two dyes are chosen in such a way that the larger cation (methyl green) competes under favourable conditions for the DNA, while the smaller cation (pyronin) stains only RNA. If the principal factor in such differential staining is the weight of the dye cations, it should be possible to reproduce the 'methyl green/pyronin effect' (differential staining of DNA and RNA) with other pairs of basic dyes, provided that the dyes chosen are of sufficiently different cationic weights. The dyes must also, of course, be of contrasting colours, be mixed in appropriate proportions and be used at the correct *p*H.

To confirm this prediction, 1 per cent solutions of several basic dyes were prepared. Paraffin sections were stained for 15 min. in a mixture of a pair of such types (combined in various proportions), rinsed in tap water, dehydrated thoroughly in alcohol and mounted in '*D.P.X.*'. The following dye pairs stained DNA and RNA differentially under these conditions, when mixed in correct proportions: bismarck brown *R*/toluidine blue, bismarck brown *R*/azur *A*, victoria blue 4*R*/hofmann violet, victoria blue 4*R*/safranin, victoria blue 4*R*/pyronin, janus green/pyronin and janus green/safranin. In every case, the dye with the larger cation stained the nuclei, while the smaller cation stained nucleoli and cytoplasmic chromidial substance. A few nuclei, especially the small nuclei of frog red blood cells, stained with the smaller cation instead of the larger. This may be due to such nuclei lying wholly within the plane of the section; in such a case the nuclear membrane will be intact, and prevent access of the larger cation[3].

Dye pairs in which there was a large difference between the weights of the cations gave particularly good discrimination between DNA and RNA. Thus victoria blue 4*R*/pyronin or janus green/pyronin (mixed in a proportion of one part by weight of the larger cation to four parts of the smaller) gave results which compared favourably with those obtainable with the classical methyl green/pyronin method. Where the difference between the cationic weights of the two dyes was not large (for example, victoria blue 4*R*/hofmann violet), or where incorrect proportions of the two dyes were used, good differential staining of nuclei and nucleoli was sometimes obtained, but cytoplasmic RNA stained with both dyes. This is taken to indicate that the density of cytoplasmic RNA granules is intermediate between that of DNA and that of the nucleolus.

The use of basic dyes of varying cationic weights can also give some indication of the change in density of a structure under different conditions. Thus the staining of so-called 'depolymerized' DNA with pyronin instead of methyl green in the methyl green/pyronin method[4] probably indicates that the heat, or other treatment to which the DNA has been subjected, has altered its structure in such a way as to increase its density, and render access of the larger cation more difficult.

Dye-pairs in which there was relatively little difference between the cationic weights (for example, crystal violet/pyronin or safranin/thionin) gave little or no differential staining of DNA and RNA, irrespective of the proportions in which the dyes were mixed.

It appears from these results that the chemical explanations which have been advanced to explain the specificity of the methyl green/pyronin method[4] are unnecessary, the chief factor being the weights of the dye cations. The same factor explains the effect of the dye mixtures, malachite green/acridine red, methyl green/toluidine blue and celestine blue/pyronin, which give the same empirical results as methyl green/pyronin[4]; malachite green has a cationic weight of 328, celestine blue 329 and acridine red 240.

It should not be concluded from the above that the weight of the cation is the only relevant factor in staining with basic dyes. Mordants, *p*H, solubilities in various media, etc., may also play an important part in some procedures[5].

D. J. GOLDSTEIN

Department of Anatomy,
University of the Witwatersrand
Medical School,
Hospital St., Johannesburg.

---

[1] Gurr, E., *Encyclopædia of Microscopic Stains* (Leonard Hill (Books), Ltd., London, 1960).

[2] Hale, C. W., *Nature*, **157**, 802 (1946).

[3] Lison, L., *Quart. J. Micro. Sci.*, **96**, 227 (1955).

[4] Kurnick, N. B., *Int. Rev. Cytol.*, **4**, 221 (1955).

[5] Baker, J. R., *Principles of Biological Microtechnique* (Methuen and Co., Ltd., London, 1958).

Dispersal of Tropical Seeds by Ocean Currents

DURING the winter of 1956 and the following summer, farmers living near the Ninety Mile Beach on the west coast of North Auckland (latitude 34–35° S.) noticed that numbers of unknown fruits and seeds, recognized as of tropical origin, were being washed ashore. Specimens were forwarded to the Botany Division, Department of Scientific and Industrial Research, by Mr. H. G. Halliwell, instructor in agriculture, Kaitaia, for identification.

The fruits and seeds were all of kinds known to be carried long distances by ocean currents[1]. They were: fruit of *Barringtonia asiatica*, fruits of a cycad, seeds of *Entada* sp. (nicker bean), *Guilandina crista* (Molucca bean or grey nicker), *Mucuna gigantea*, seeds apparently of two other species of *Mucuna*, and nuts of *Aleurites fordii* (candlenut). There was also a red leguminous seed 17 mm. long and an unidentified fruit, possibly from one of the Myristicaceae. Apparently nicker beans and candlenuts were the most common. According to a newspaper report[2], at times many coconuts were being washed up. Candlenuts from Mangonui, on the east coast at about the same latitude, were also sent in by Dr. A. Couper, of the Geological Survey. They were called in the north 'petrified walnuts'. Some of the material must have been afloat for a considerable time, for the *Barringtonia* fruit, most of the cycad fruits, and many of the nicker beans had remains of a polyzoan on them, and were sometimes quite covered.

Although the number and variety seem to be unusual, this is not the first time that tropical seeds have drifted to New Zealand. It was known in Auckland thirty or so years ago that nicker beans were cast up on the coasts of North Auckland from time to time. The newspaper report refers to present conditions as "a reversion to an earlier pattern of ocean currents" and says "in greater profusion than in past years all sorts of tropical flotsam are being cast up".

Seven out of twenty-two seeds of *Entada* sown at Lincoln, Christchurch, in November 1956, germinated about 21 months later. Two of the plants are now growing in the glasshouses, one a vine 4 ft. long, the other 8 ft. Another plant in the tropical house, Botanic Gardens, Christchurch, is 12 ft. long. In North Auckland plants have been grown successfully outdoors by local farmers, and one plant is reported to have grown to a great height. As *Entada* sp. in Fiji is most characteristically found in the mangrove formation and also grows in the forest[1], it seems unlikely that it will become established of its own accord on the Ninety Mile Beach, an exposed shore backed by sand-dune country.

The candlenuts were either empty or the seed had decayed into a soft oily mass, as reported previously for seeds of this species carried by ocean currents.

R. Mason

Botany Division,
Department of Scientific and
Industrial Research,
Private Bag,
Christchurch, New Zealand.

[1] Ridley, H. N., *The Dispersal of Plants throughout the World* (L. Reeve and Co., 1930).

[2] *The Christchurch Star-Sun*, 12 (April 2, 1957).

Classification of the Tea Plant

Dr. W. Wight, referring to Watt and Mann's categories of leaf-form within the Assam variety of tea plant[1], goes on to say: "Number of leaf veins was emphasized; but this has not proved to be a satisfactory criterion". I may say at once that Watt was solely responsible for the leaf vein idea for separating the varieties of tea. When I wrote, with him, the second edition of the *Pests and Blights of the Tea Plant* I had to include the leaf vein idea, but I never was, myself, able to make anything out of it. I never agreed with Watt on this subject, and it was included in our book in deference to him. Thus, so far as I am concerned, the number of leaf veins was an unsatisfactory criterion fifty-seven years ago.

H. H. Mann

Bivia, Woodside,
Aspley Guise,
Bletchley.

[1] Wight, W., *Nature*, 183, 1726 (1959).

Staining Reaction of the Pit Membrane of Wood Cells

In a recent paper Harada and Wardrop[1] report that the lignin content of the ray parenchyma cell walls of *Cryptomeria japonica* (Linnæus fil.) Don. is about 43·5 per cent and also that the lignin is distributed nearly uniformly through the cell walls. In view of the bearing on their observations and also the possible relation to the movement of materials through wood, the results of the reaction of the pit membrane when wood sections are treated with differential stains are recorded.

Radial longitudinal sections of approximately 10μ were stained with safranin and fast green according to the following schedule: (1) Sections warmed with aqueous safranin for 3–5 min.; (2) washed with several changes of water and then dehydrated in the normal way with ethyl alcohol; (3) stained with fast green in clove oil for approximately 15 sec. (this time can be increased with dilute fast green); (4) washed with clove oil, cleared with xylene and mounted in canada balsam. Washing was continued until the wash liquid was colourless.

In the following species the pit membranes of the ray parenchyma to tracheid pits were stained green in contrast to the red stained unpitted wall:

Pinus radiata D. Don, *P. khaysa* Royle, *Pseudotsuga menziesii* (Mirb.) Franco, *C. japonica*, *Callitris hugelii* (Carr) Franco, *Abies lasiocarpa* (Hooker) Nuttall. The pit membranes of the ray tracheid to tracheid pits were not differentiated to the same degree as the ray parenchyma pit membrane. Both in *P. radiata* and *C. hugelii* the torus of the bordered pits of the tracheids was stained green.

With the following species the membranes of the ray parenchyma to vessel pits and the parenchyma pits generally were differentiated green in contrast to the unpitted cell wall, which was stained red:

Sloanea woollsii F. Muell., *Elaeocarpus grandis* F. Muell., *Cryptocarya glaucescens* R. Br., *Flindersia schottiana* F. Muell., *Toona australis* Harms, *Sterculia acerifolia* A. Cunn., *Eucalyptus viminalis* Labill., *E. pilularis* Sm., *E. microcorys* F. Muell. Due to their small size the pit membranes of the fibres were hard to detect, but in a number of the above species a distinct green coloration was obtained. Of the species examined, only *Nothofagus moorei* J. H. Maiden failed to show differentiation of the pit membrane of the ray-vessel pits. The pit membrane in this species appeared to be masked by abundant polyphenolic materials.

Where possible, sapwood rather than heartwood was used for these observations as it was found that the polyphenolic materials deposited during the transition from sapwood to heartwood obscured the pit membrane, showing instead a red stain with the safranin.

According to Johansen[2], safranin and fast green adequately differentiate lignified and non-lignified tissue, so that this differentiation indicates that the pit membranes in a number of cells are not lignified. This observation is interesting, as it is those layers which make up the pit membrane, namely, the primary wall and middle lamella, which have the highest concentration of lignin in the unpitted cell wall[3]. The pit membrane, therefore, retains the unlignified condition of the newly differentiated cell wall and explains how the remarkable expansion of the pit membrane which occurs during tyloses is possible. The tylosed pit membrane in *Angophora costata* Domin. and *E. microcorys* on the basis of the above staining reaction did not appear to be lignified.

While Liese[4] and Harada *et al.*[5] describe encrusting material present in the pit membrane, it would appear that this material is not lignin. The absence of lignin in the pit membrane has been indicated by means of other techniques by A. B. Wardrop (private communication). The fact that lignin is confined to those parts of the wall where a secondary wall is formed implies very selective deposition, as mentioned by Wardrop and Bland[3]. The foregoing staining reaction serves also as an excellent technique for clearly defining the size and shape of the pit membrane and it has been suggested that this technique should have general application in anatomical work.

R. K. Bamber

Division of Wood Technology,
Forestry Commission of New South Wales,
Sydney.

---

[1] Harada, H., and Wardrop, A. B., *J. Jap. Wood Res. Soc.*, **6**, 34 (1960).

[2] Johansen, D. A., *Plant Microtechnique* (McGraw-Hill, New York, 1940).

[3] Wardrop, A. B., and Bland, D. E., *Fourth International Congress of Biochemistry*, Vol. 2, 92 (Pergamon Press, London, 1959).

[4] Liese, W., *Holz als Roh- und Werkstoff*, **15**, 449 (1957).

[5] Harada, H., Miyazaki, Y., and Wakashima, T., *Bull. Gov. For. Exp. Sta.*, **104**, 115 (1958).

A Sealing Compound for use in Biological Work

In the course of rhizosphere studies, it was desired to measure continuously the respiration of intact sterile roots during the growth and development of a variety of plants. To separate the photosynthetic and respiratory activities of the portions above ground from the respiration of the roots, a material was required to prevent diffusion of gases from one zone to the other. The material had to fulfil several requirements in order to be effective : (1) be impermeable to gases and water ; (2) be non-toxic to plants ; (3) cause no physical damage to plants ; (4) be sufficiently pliable to permit normal enlargement of stems ; (5) withstand a continuous positive pressure of 0·5–1·0 lb./in.^{2}; (6) resist microbial and environmental deterioration; (7) be autoclavable; (8) be easily handled and applied; (9) be relatively inexpensive.

Although a variety of substances was tested, only one compound has been found which fulfilled all these requirements. This material is a viscous silicone fluid which solidifies at room temperatures on the addition of a catalyst. (Material is marketed by the Dow Corning Corporation, Midland, Michigan, under the trade name of 'Silastic *RTV*' (room temperature vulcanizing silicone rubber), and by the General Electric Co., Silicone Products Department, Waterford, New York, under the name of 'Silicone Rubber *RTV*'.) The time for complete vulcanization can be controlled by the type and amount of catalyst used ; working time can be adjusted from 10 min. to several days. The plasticity can be increased by the addition of a silicone thinner (Dow Corning '200 Fluid', 20 centistokes). The base, catalyst, and thinner may be autoclaved separately and mixed aseptically just prior to pouring around the stem.

The rubber-like solid forms an impermeable seal with glass, metal, certain plastics, sand, and with plant roots and stems. Although a continuous positive pressure of only 0·5–1·0 lb./in.^{2} is regularly used in our work, the material is capable of withstanding considerably higher pressures, both positive and negative. A layer 10 mm. thick and 38 mm. in diameter withstood a continuous positive pressure of 7 lb./in.^{2} and a continuous negative pressure of 500 mm. mercury; a layer 10 mm. thick and 70 mm. in diameter withstood a pressure of 2 lb./in.^{2}. At higher pressures, the sealer itself does not leak, but the interface bond between the sealer and either the wall of the glass tube or the plant stem is ruptured.

Although a gas-tight seal is formed around the stem of plants, the seal is sufficiently pliable to permit normal development of the stem as well as of the entire plant. The consistency of the vulcanized product does not change during autoclaving nor during prolonged use, and may be employed at temperatures ranging from -70 to 500° F. Other physical and chemical properties are described by the manufacturers.

In our work, surface sterilized seeds are planted in short glass growth tubes (38 mm. internal diameter) contained in specially designed culture units, which will be described fully elsewhere. The roots grow into a sterile respiration chamber, and the upper portions of the plant are allowed to develop in air. To separate the two zones, the sealer is poured around the plant stem inside the growth tubes and allowed to solidify before a continuous air-stream is passed through the root respiration chamber. Both monocotyledonous and dicotyledonous plants have been successfully cultured in the presence of this material : legumes and corn have produced fruit ; cotton, crucifers, cucurbits, and seeded bananas are at present growing vigorously.

Other compounds tested as sealers were not suitable for several reasons. Various combinations of paraffin, petrolatum and lanolin, as well as some carboxyvinyl polymers, were extruded from the growth tubes when pressure was applied ; this difficulty was accentuated when the temperature of the environment was high. Several materials, for example, latex, poly*iso*butylenes, urethane polymers, polyacrylic and polyvinyl resins, were toxic; some set too rapidly for easy handling ; some, for example, urethane polymers and some microcrystalline waxes, cured to a consistency that constricted the stem and prevented normal development ; some, for example, various paraffin-petrolatum mixtures, butyl-latex emulsions, distilled acetylated monoglycerides, carboxyvinyl polymers, shrank on curing and failed to form a gas-tight interface with either the glass tube or the plant stem ; some did not withstand autoclaving ; and others, such as the polyethylene glycols, being water-soluble, were dissolved by condensation, transpiration and guttation water.

In addition to being used in sterile root cultures, the silicone material is also being employed in these Laboratories to seal surface-sterilized intact roots in tubes containing root-infecting organisms (Halmos, S., and Beckman, C. H., unpublished results) ; to make gas-tight gaskets for attaching respiration and transpiration chambers to leaves[1] ; to make impressions of leaf surfaces for microscopic examination (Brun, W. A., unpublished results ; and Zelitch, I., unpublished results) ; and to make closures for odd-shaped and hard-to-reach openings which pre-manufactured rubber stoppers do not fit. Additional applications are described by the manufacturers.

The non-toxicity of this compound to plants, as well as its other properties, should make it useful in a variety of biological studies.

G. Stotzky
W. Culbreth
L. B. Mish

Central Research Laboratories,
United Fruit Co.,
Norwood, Massachusetts.

---

[1] Brun, W. A., *Plant Physiol.* (in the press).

MICROBIOLOGY

Activation of Amino-Acids in Micro-Organisms which produce Antibiotics

It seems well established that the first steps in the biosynthesis of proteins are the activation of amino-acids in the presence of adenosine triphosphate (ATP) and the transfer of the activated amino-acids to a soluble fraction of ribonucleic acid (RNA)[1]. Such reactions are also involved in the biosynthesis of some peptides, for example, the condensation of glutamylcysteine with glycine to give glutathione[2]. Enzymes activating almost all the naturally occurring L-amino-acids have been isolated from animal, plant, and microbial sources. Only one enzyme active on a D-amino acid, D-alanine, has been demonstrated in cells of *Lactobacillus arabinosus* and other Lactobacillaceae[3].

If the mechanisms for protein synthesis are involved also in the synthesis of peptides, it should be possible to demonstrate activation and transfer of amino-acids present in peptides but absent from proteins, such as many of the D-amino-acids. To test this hypothesis the following micro-organisms were selected, since they produce antibiotics containing one or more of the amino-acids that do not occur in proteins: *Penicillium chrysogenum P*–4 (Lepetit S.p.A.), producing penicillin that contains D-valine ; *Streptomyces antibioticus* 23 (Lepetit S.p.A.) and *Streptomyces parvus N.R.R.L. B*-1455 (both producing antibiotics of the actinomycin group in which D-valine, sarcosine, D-allo*iso*leucine and N-methyl-L-valine are present) ; *Bacillus polymyxa A.T.C.C.* 10401 and *B. polymyxa* 2459 (Ch. Pfizer and Co.) producing, respectively, polymyxin-D and polymyxin-B, containing D-phenylalanine, D-leucine, D-serine, D- and L-α,γ-diaminobutyric acid; *Bacillus circulans A.T.C.C.* 14040, producing circulin containing D-leucine and L-α,γ-diaminobutyric acid. The micro-organisms were grown on complex media capable of supporting antibiotic synthesis and were collected when the production of the antibiotic reached its maximum (2 days for the bacteria, 3 for *P. chrysogenum*, and 6 to 8 for the Streptomycetes). Cell-free extracts were prepared by suspending the centrifuged cells in one volume of 0·066 *M* phosphate buffer and 0·002 *M* reduced glutathione (*p*H 6·9) and disrupting them with glass beads in a refrigerated mechanical disintegrator. Following centrifugation at 20,000*g* for 20 min., the presence of amino-acid-activating enzymes was assayed on the supernatants by measuring the exchange between ATP and radioactive pyrophosphate[4].

The results (Table 1) indicate that, while a fair number of the L-amino-acids is activated, no activation of the D-amino-acids is detectable even if the micro-organism is capable of synthesizing compounds containing amino-acids with such an optical configuration. In addition, sarcosine, which has no optical activity, is not activated by the tested Streptomycetes. L-α,γ-diaminobutyric acid, which has not been found in proteins, is activated by strain 2459 of *B. polymyxa* ; this is the first time that this amino-acid is reported to be activated.

Table 1. ACTIVATION OF AMINO-ACIDS
Micromoles of pyrophosphate-phosphorus-32 exchanged per 15 min. per mgm. of protein

	B. polymyxa 10401	*B. polymyxa* 2459	*B. circulans*	*Str. antibioticus*	*Str. parvus*	*P. chrysogenum*
L-Alanine	0	0	0·077	0	0	0·012
L-Arginine	0·013	0	0	0·115	0·044	0
L-Aspartic acid	0·080	0	0	0	0	0
L-Cysteine	0·243	—	—	0·107	0·059	0
L-α,γ-Diaminobutyric acid	0	0·641	0	—	—	—
L-Glutamic acid	0	0	0	0·062	0	0
Glycine	0	0	0	0·133	0·075	0
L-Histidine	0	0·182	0	0·986	0	0
L-*iso*Leucine	2·001	0·695	0·490	1·129	0·162	0·116
L-Leucine	2·073	1·690	3·623	0·498	0·044	0·124
L-Lysine	0·094	0·140	0	0·098	0	0·020
L-Methionine	0·278	0·416	0·487	0·444	0	0
L-Phenylalanine	0·064	0	0	0	0	0·016
L-Proline	0·144	0	0	0·204	0	0
L-Serine	0	0·055	0	0·302	0	0·039
L-Threonine	0·210	0·050	0·035	0·088	0·044	0
L-Tryptophan	0·083	0	—	0·133	0	0
L-Valine	0·106	1·790	0·986	0·489	0·235	0·076
Sarcosine	—	—	—	0	0	—
D-Alanine	0	0·162	0	0	0	0
D-*iso*Leucine	0	0	—	—	—	—
D-Leucine	0	0	0	0	0	0
D-Phenylalanine	0	0	0	—	—	0
D-Serine	0	0	0	—	—	—
D-Threonine	0	0	0	—	—	—
D-Valine	—	—	—	0	0	0
None (control)	0·133	0·280	0·355	0·507	0·073	0·100

The assay system contained 20 *μM* of *tris* buffer (*p*H 7·9); 5 *μM* of ATP (*p*H 6·5); 2·5 *μM* of $MgCl_2$; 3·2 *μM* of sodium pyrophosphate-phosphorus-32 (corresponding to approximately 340,000 counts per min. on a 'Tracerlab' mica window counter); 10 *μM* of amino-acid; cell-free extracts ranging from 0·068 to 0·49 mgm. of protein; glass distilled water, 0·5 ml.

The values obtained in the controls containing no amino-acids were subtracted from all the experiments. The activation was considered present when the experimental data were at least 10 per cent higher than the controls.

The possibility that the enzymes activating the D-amino-acids and sarcosine through an ATP-pyrophosphate exchange are destroyed during the process of extraction or are present in too small amounts for detection cannot be ruled out, but seems unlikely. It appears more probable that the pathway of biosynthesis of these peptides does not involve the activation of the D-amino-acids. Such a hypothesis is in agreement with the recent findings that exclude a process of amino-acid activation in the biosynthesis of some petides containing D-alanine and other L-amino-acids by *Staphylococcus aureus*[5]. Work under way in this laboratory[6] has shown that while D-leucine-^{14}C is incorporated in the polymyxin-D synthesized by *B. polymyxa*, the amino-acid is not activated by an ATP-pyrophosphate exchange nor transferred to soluble RNA.

We wish to acknowledge the kind release of cultures from Lepetit S.p.A., Milan, and Charles Pfizer and Co., Brooklyn, N.Y. This work has been supported by a grant of the International Institute for Scientific Research.

O. CIFERRI
M. DI GIROLAMO
A. BENDICENTI DI GIROLAMO

Istituto di Genetica,
Università di Pavia,
Pavia.

[1] Lipmann, F., *et al.*, *J. Cell. Comp. Physiol.*, **54**, Supp. 1, 75 (1959). Hoagland, M. B., *Fourth Int. Congr. Biochem.*, *Symp.* 8, 199 (1960). Novelli, G. D., *Ann. Rev. Microbiol.*, **14**, 65 (1960).

[2] Bates, H. M., and Lipmann, F., *J. Biol. Chem.*, **235**, *PC* 22 (1960).

[3] Baddiley, J., and Nehaus, F. C., *Biochem. J.*, **75**, 579 (1960).

[4] DeMoss, J. A., and Novelli, G. D., *Biochim. Biophys. Acta*, **22**, 49 (1956).

[5] Ito, E., and Strominger, J. L., *J. Biol. Chem.*, **235**, *PC* 5, *PC* 7 (1960).

[6] Di Girolamo, M., Ciferri, O., and Bendicenti Di Girolamo, A. (unpublished results).

Enzymic Binding of Hydroxylamine by Fumaric Acid

AN organism similar to *E. coli* isolated in our laboratory was found to grow in a minimal medium containing glucose and ammonium sulphate in the presence of relatively large concentrations of hydroxylamine (up to 100 μgm./ml. of the hydrochloride—amounts which were highly toxic to other organisms). The amine, which was determined by the method of Csaky[1], was found to disappear during growth.

The metabolic conversion of hydroxylamine by the organism was measured using washed cell suspensions, lyophilized cells, or cell-free preparations purified by fractionation with ammonium sulphate and further treatment on a *DEAE*-cellulose column. The reaction mixture (for composition see Table 1) was incubated at a low *p*H of 5·6–5·8 in order to minimize spontaneous decomposition of the hydroxylamine.

When a sonicated cell-free extract of washed lyophilized cells was employed instead of living cells, very little, if any, hydroxylamine disappeared. The activity of the extract could, however, be restored by the addition of the wash fluid of the freshly lyophilized preparation, fumaric or malic acid. While succinate showed only slight activity, maleate and malonate were inactive. Some of the results are shown in Table 1. The rate of disappearance of hydroxylamine was dependent on the amount of fumaric or malic acid present. In fact, for each μmole of added fumarate (or malate) about 0·8–0·9 μmole of hydroxylamine was consumed[2]. Changes in the amounts of fumaric and malic acids were followed chromatographically[3].

Using aged lyophilized preparations (stored in the deep freeze at – 18° C.) disappearance of hydroxylamine could be achieved by the addition of fumaric but not malic acid. Thus, fumaric acid seems to be the active substrate, while the effectiveness of malic acid can be ascribed to the presence of a labile fumarase. This assumption was confirmed by adding citrate or phthalate buffer (50 μmoles–*p*H 5·8)[4] to the reaction mixture. Under these conditions only fumaric acid was active.

Ammonia, when added instead of hydroxylamine, did not react with fumaric acid (ammonia did not disappear), thus no aspartase seems to be involved. The presence of hydroxylamine reductase[5,6] can be ruled out as no ammonia, or very little, was formed. Nitrate, nitrite or gaseous nitrogen compounds were not found.

The reaction carried out with the purified enzyme was not activated by any of the following ions: Mn^{2+}, Mg^{2+}, Co^{2+}, Zn^{2+}, Fe^{2+}, PO_4^{3-}, or adenosine triphosphate (ATP). Diphosphopyridine nucleotide (DPN) (0·3 μmole) activated some purified enzyme preparations but not others. Sodium cyanide ($10^{-2}M$) or hydrazine ($10^{-2}M$) had no inhibiting effect on the reaction.

Identification of the enzymic condensation product of hydroxylamine with fumaric acid was attempted. At the end of the reaction (after 1–2 hr. at 37° C.) the complete system and the respective controls were subjected to hydrogenation in the Parr apparatus as follows: a minute amount of platinum oxide was added to 1 ml. of the reaction mixture acidified with hydrochloric acid to about *p*H 1·0 and shaken for 6 hr. under a pressure of 15 lb./in.². The hydrogenated samples were filtered and chromatographed in order to detect amino-acids. The complete system showed a spot of aspartic acid in contrast to the various controls (without enzyme, fumaric acid or hydroxylamine). The amount of aspartic acid formed by the catalytic hydrogenation was roughly comparable to the amount of hydroxylamine which disappeared. The formation of aspartic acid when most of the hydroxylamine and fumaric acid had disappeared concomitantly suggests that hydroxylamine as such combined with fumaric acid at one of the carbon atoms adjacent to the double bond. The nature of this compound, which seems a likely intermediate, had not as yet been established. Still to be demonstrated is whether or not this compound can be enzymically reduced to aspartic acid by extracts of this micro-organism.

A varying portion of the hydroxylamine which disappeared during the reaction (from insignificant amounts to about 50 per cent) could be recovered by boiling the reaction mixture for 10 min. with 6 *N* hydrochloric acid, and the hydroxylamine determined after neutralization. Thus, the formation of a bound hydroxylamine product, as found by Endres[7] in *Azotobacter* cultures, can be shown with our *E. coli* preparation. The variability in the extent of the hydrolysis may perhaps indicate the presence, in some preparations, of an additional intermediary product and labile enzyme.

Although the enzymic reaction was studied in detail only with organisms similar to *E. coli*, we have

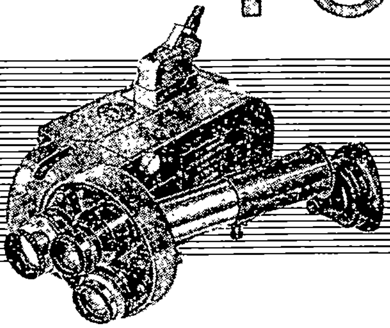
Table 1. EFFECT OF DICARBOXYLIC ACIDS ON THE DISAPPEARANCE OF HYDROXYLAMINE

		1	2	3	4	5	6	7	8
1. Lyophilized cells (from hydroxylamine-containing medium), 100 mgm.		+							
2. Ditto, washed twice by centrifugation			+	+	+	+	+	+	
3. Supernatant from No. 2 concentrated to original volume (wash-fluid)				+					
4. Sonicated, cell-free extract, 15 mgm.									+
5. Fumaric acid, 10 μ*M*					+				+
6. L-Malic acid, 10 μ*M*						+			
7. Maleic acid, 10 μ*M*							+		
8. Malonic acid, 10 μ*M*								+	
9. Succinic acid, 10 μ*M*									
10. Hydroxylamine, 5 μ*M*		+	+	+	+	+	+	+	+
		Optical density at 530 mμ							
Time (min.)	0	0·420	0·400	0·400	0·415	0·430	0·420	0·400	0·415
	30	0·230	0·385	0·140		0·120			
	60	0·095	0·360	0·030	0·010	0·030	0·395	0·370	0·140

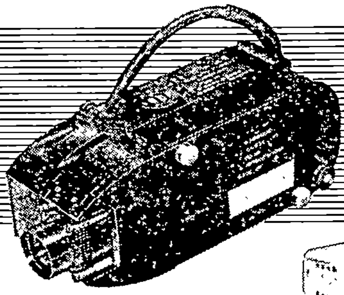
The system contained in addition: pyridoxal phosphate, 5 μgm.; sodium molybdate, 5 μ*M*. Final volume, 2·5 ml.; *p*H 5·6. The reaction mixture was incubated at 37° C. and at intervals aliquots were taken and the remaining hydroxylamine determined by the method of Csaky (ref. 1), using a Coleman Junior spectrophotometer.

Vinten 16 mm

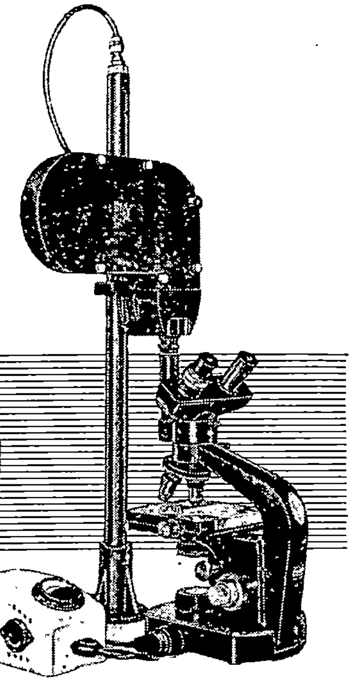
SCIENTIFIC
RESEARCH
AND HIGH
SPEED
CAMERAS



Mk. II Scientific Camera with triple lens turret and rotating mirror reflex shutter. For 8, 16, or 24 f.p.s. or single shot operation.



16 mm. High Speed Camera up to 250 f.p.s. with timing device giving accurate visual time scale on film and event marker.



Mk. I Research Camera with extended exposure facility which enables lengthy microscope observations to be left to the camera. Battery or mains operation. 8, 16, 24 f.p.s.—or single shots by intervalometer.

Information about Vinten scientific cameras and their uses in Research and Industry are contained in two booklets which we should be pleased to send on request.

W. VINTEN LTD., NORTH CIRCULAR ROAD, CRICKLEWOOD, N.W.2. GLADSTONE 6373

MUREX manufacture
ductile
NIOBIUM
ROD, WIRE, SHEET AND TUBE
Write for full technical information

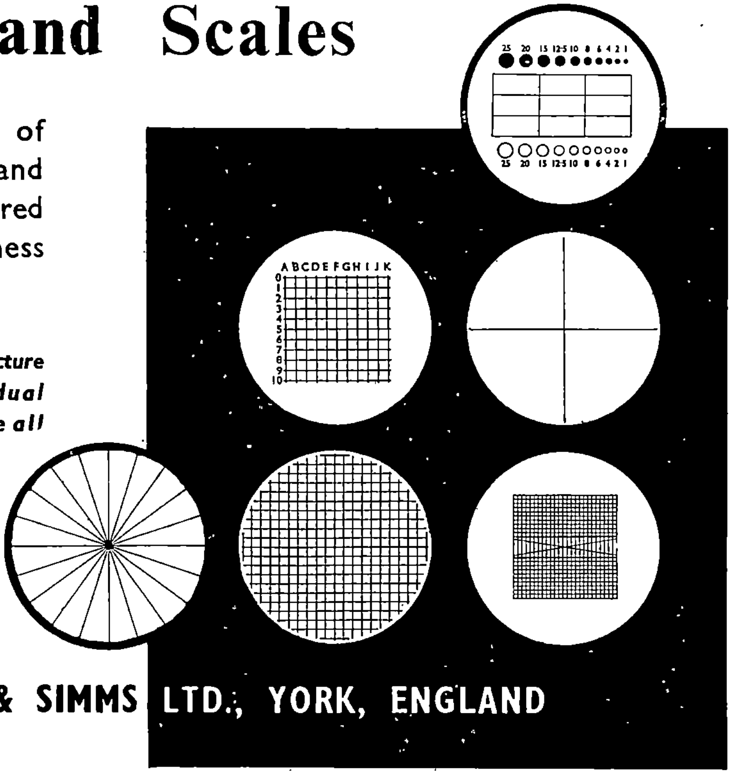
MUREX LTD. (*Powder Metallurgy Division*) RAINHAM • ESSEX
'phone: Rainham, Essex 3322 Telex 28632 'grams: Murex, Rainham-Dagenham Telex
LONDON SALES OFFICE: CENTRAL HOUSE, UPPER WOBURN PLACE, W.C.1

Graticules and Scales

We offer a wide range of eyepiece graticules, scales and graduated circles, manufactured to a fine degree of sharpness and accuracy.

We are also prepared to manufacture graticules and scales to individual specification, and will welcome all enquiries.

Send for our leaflet CM.GRAT. for further information on our present range of graticules.



COOKE, TROUGHTON & SIMMS LTD., YORK, ENGLAND

GLASS

All Glass Requirements for Scientific Instrument Manufacturers and Research Workers

- SHEET & PLATE-GLASS—cut and ground to exact dimensions.
- LENSES, PRISMS & FLATS—to any standard of accuracy.
- MIRRORS—back silvered and front surface aluminised, etc.
- BLOOMING of optical components.

C. J. WHILEMS, LTD.
ILFORD OPTICAL WORKS
FOREST ROAD, BARKINGSIDE, ESSEX

Telephone : HAInault 5454-5

MANSFIELD OIL-GAS PLANT FOR LABORATORIES' GAS SUPPLY

• *Reliable* • *Simple to Operate* • *Safe*

Provides a gas supply at a constant calorific value of 1,350 B.T.U.'s per cu. ft. produced from Light Diesel Oil, Gas Oil, Paraffin, or other suitable oils available at low cost.

Enables the efficient use of a full range of modern gas burning appliances in any part of the world. Ideal for glass blowing and gives perfect oxidizing or reducing flames with ordinary atmospheric burners.

For full particulars send details of estimated number of burners to be used.

MANSFIELD & SONS LTD.
62 HAMILTON SQUARE, BIRKENHEAD

GLASSWORKERS'

STRAIN-VIEWING POLARISCOPE

Full wave retardation analyser for 5800 A gives most sensitive colour indication of strain. Field and sight aperture 1¼" dia. Dimensions 6½" × 5½" × 5". Price : **£11 16s. 6d.**

As above but without colour analyser showing strains against black field. Price : **£5 5s. 0d.**

Immediate Delivery.

H. S. B. MEAKIN, LTD., 36, Victoria Street, S.W.1
SUPPLIERS OF POLAROID PLASTIC and GLASS **Tel.: Abbey 6713**

noted the disappearance of hydroxylamine in the presence of fumaric acid using preparations of other organisms such as *Escherichia coli B* and *Azotobacter vinelandii*.

The assistance of (the late) Mrs. Klara Rifczes and of Edna Rittersporn is gratefully acknowledged.

NATHAN GROSSOWICZ
YEHUDITH LICHTENSTEIN

Department of Bacteriology,
The Hebrew University
Hadassah Medical School,
Jerusalem.

[1] Csaky, Z., *Acta Chem. Scand.*, 2, 450 (1948).
[2] Grossowicz, N., and Lichtenstein, Y., *Proc. Third Intern. Congr. Biochem., Brussels*, 98 (1955).
[3] Lugg, J. W. H., and Overell, B. T., *Austral. J. Sci. Res.*, 1, 98 (1948).
[4] Massey, V., *Biochem. J.*, 55, 173 (1953).
[5] Taniguchi, S., Mitsui, H., Nakamura, K., and Egami, F., *Ann. Acad. Scient. Fennicae, A II*, 60, 200 (1955).
[6] Zucker, M., and Nason, A., *J. Biol. Chem.*, 213, 463 (1955).
[7] Endres, G., *Ann.*, 5, 109 (1935).

Induced Increase in Resistance of *Entamoeba histolytica* to Fumagillin

ALTHOUGH there has been much work on *in vitro* effects of various amœbicidal agents on *Entamoeba histolytica*, I have not found any published data dealing with the induced increase in resistance of this protozoan to amœbicidal agents. The antiamœbic activity of fumagillin was first studied by McCowen *et al.*[1] and was confirmed by Hrenoff and Nakamura[2]. Many workers have since studied the clinical efficacy of fumagillin in amœbiasis[3–7]. The present work was undertaken to determine the activity of fumagillin against a number of newly isolated strains of *E. histolytica* from a variety of clinical infections. Also, an attempt was made to increase resistance of these strains to this highly active amœbacidal antibiotic. The main reason for using fumagillin in these experiments is that it is not antibacterial in activity and therefore this agent can be studied in cultures of amœbæ growing in association with a mixed bacterial flora. The associated bacteria are required because *E. histolytica* cannot be cultivated axenically consistently. Furthermore, use of fumagillin minimizes the indirect activity of the drug on amœbæ since the bacterial flora apparently are unaffected, and the inhibition of the amœbæ can be considered as directly due to the antibiotic.

Six strains of *E. histolytica* isolated approximately four months earlier were used. Two strains were isolated from human cases of intestinal amœbiasis, two strains from human cases of cutaneous amœbiasis, one strain from a case of hepatic amœbiasis, and one strain from a case of appendicitis due to *E. histolytica*. The amœbæ were cultured regularly on a medium consisting of a solid phase of egg and whole blood (a coagulated slant) overlaid with 5 ml. of Ringer solution. Rice powder was added just before inoculation of the media. The amœba cultures were transferred three times a week and incubated at 37° C. Amœbacidal concentrations of fumagillin were determined by growing the amœbæ in culture tubes containing varying concentrations of the agent. Control tubes not containing the antibiotic were also inoculated. The tubes were read after 48 hr. of incubation by removing approximately 0·1 ml. of the sediment from each tube, placing on a microscope slide, covering with a glass cover slip, and counting the numbers of amœbæ in the low-power field of the microscope. Ten different fields were counted and average counts recorded; two samples were taken from each tube. Each experiment was performed in quadruplicate and each experiment was repeated three or four times. The concentration of fumagillin necessary to produce 100 per cent inhibition (no detectable surviving amœbæ) was determined. Resistance to fumagillin was induced by growing the amœbæ in culture media containing concentrations of the antibiotic which produced 50 per cent inhibition. The amœbæ were grown on media containing antibiotic for 2 weeks, then the susceptibility to fumagillin was re-assayed to determine the new amœbacidal end-point. Strains of amœbæ which developed resistance were afterwards cultivated in media not containing the antibiotic for 2 weeks and re-tested for their sensitivity to fumagillin in order to determine the stability of the induced resistance.

Complete inhibition of *E. histolytica* by fumagillin was obtained by concentrations ranging from 0·01 μgm. to 100 μgm./ml., depending on the strains. The two strains obtained from two different cases of cutaneous amœbiasis possessed the highest resistance to the antibiotic; concentrations of 50–100 μgm./ml. were needed to produce 100 per cent inhibition. The strains obtained from cases of intestinal amœbiasis were the least resistant to fumagillin; 0·01–0·1 μgm./ml. of fumagillin produced 100 per cent inhibition. The strains isolated from a case of hepatic amœbiasis and a case of amœbiasis of the appendix were completely inhibited by 1–10 μgm./ml. of fumagillin. The cutaneous strains, which had the highest degree of resistance, were grown in the presence of 1–10 μgm./ml. of fumagillin and re-tested for their susceptibility to fumagillin. No increase in resistance was noted. However, when the two strains from the intestinal cases which possessed the least amount of resistance, were grown in the presence of 0·001–0·01 μgm./ml. of fumagillin, the resultant cultures were highly resistant to fumagillin. These more resistant strains required 75–100 μgm./ml. before 100 per cent inhibition could be obtained. These two strains retained this induced resistance to fumagillin in spite of cultivation in the absence of the antibiotic. Sufficient time was not available to determine the permanence of this resistance. The strains which possessed intermediate sensitivity to fumagillin, namely, those obtained from the liver and the appendix, also developed resistance to the drug. However, none of the strains developed resistance to the degree that would permit growth in the presence of more than 100 μgm./ml. of the antibiotic.

It appears that resistance to fumagillin can be induced more readily in strains which are more sensitive to this antibiotic. Furthermore, the level of resistance produced was high; for example, a 1,000- to 10,000-fold increase in resistance was induced in the strains isolated from the intestine. Although this resistance was maintained for a period of one and a half months, it would be desirable to determine its permanence. No adequate explanation can be given for the failure to induce resistance in the strains which were initially more resistant to the antibiotic. It is interesting that the induced resistance of the most susceptible strains reached a maximum which was approximately the concentration required to kill the most resistant (initially) strains. It is also interesting that the resistance induced in the intestinal strains was not gradual but a sudden maximal resistance. It is conceivable that in the case of fumagillin, at least, strains of *E. histolytica* isolated from different clinical materials have different degrees of susceptibility to this antibiotic. Admittedly, the number of strains studied could have been larger; however, the availability of amœba strains from cutaneous, brain, and extra-intestinal amœbiasis is limited.

This work was supported in part by a research grant (*E*-3129) from the National Institute of Allergy and Infectious Diseases, Public Health Service, to Dr. Francisco Biagi F., Chief, Seccion de Parasitologia, Unidad de Patologia, Escuela de Medicina, University of Mexico, Mexico City, and by a training grant (2*E*-7) from the National Institutes of Health, Public Health Service, to Louisiana State University School of Medicine, under the direction of Dean William W. Frye.

MITSURU NAKAMURA*

Department of Microbiology,
Montana State University,
Missoula, Montana.

---

* Louisiana State University School of Medicine Research Fellow in Tropical Medicine and Parasitology (summer of 1960).

[1] McCowen, M. C., Callender, M. E., and Lawlis, jun., J. F., *Science*, 113, 202 (1951).

[2] Hrenoff, A. K., and Nakamura, M., *Proc. Soc. Exp. Biol. Med.*, 77, 162 (1951).

[3] Anderson, H. H., Hrenoff, A. K., Anderson, J. Van D., Nakamura, M., and Contopoulos, A. N., *Amer. J. Trop. Med. Hyg.*, 1, 552 (1952).

[4] Elsdon-Dew, R., Wilmot, A. J., and Armstrong, T. G., *Lancet*, 1180 (1953).

[5] Jung, R. C., Garcia-Laverde, A., and Katz, F. F., *Amer. J. Trop. Med. Hyg.*, 4, 989 (1955).

[6] Killough, J. H., Magill, G. B., and Smith, R. C., *Science*, 115, 71 (1952).

[7] Killough, J. H., and Magill, G. B., *Amer. J. Trop. Med. Hyg.*, 3, 999 (1954).

VIROLOGY

Effect of *p*-Fluorophenylalanine on the Multiplication of Foot-and-Mouth Disease Virus

MANY amino-acid analogues are known to prevent protein synthesis. *p*-Fluorophenylalanine in particular has been shown to inhibit synthesis by competition with phenylalanine. The growth of several animal viruses is inhibited by the addition of this analogue to the growth medium (for example, the viruses of poliomyelitis[1], influenza[2], fowl plague[3] and Western equine encephalomyelitis[4]). In the case of poliovirus and influenza virus, the effect of *p*-fluorophenylalanine on the infectivity and hæmagglutinating activity, respectively, was studied. A more detailed examination of the effect of the inhibitor on the synthesis of the sub-units of fowl plague virus and Western equine encephalomyelitis virus has shown that information can be obtained on the manner in which the complete virus particles are formed. The present communication describes the effect of *p*-fluorophenylalanine on the synthesis of the infective component and viral ribonucleic acid of foot-and-mouth disease virus.

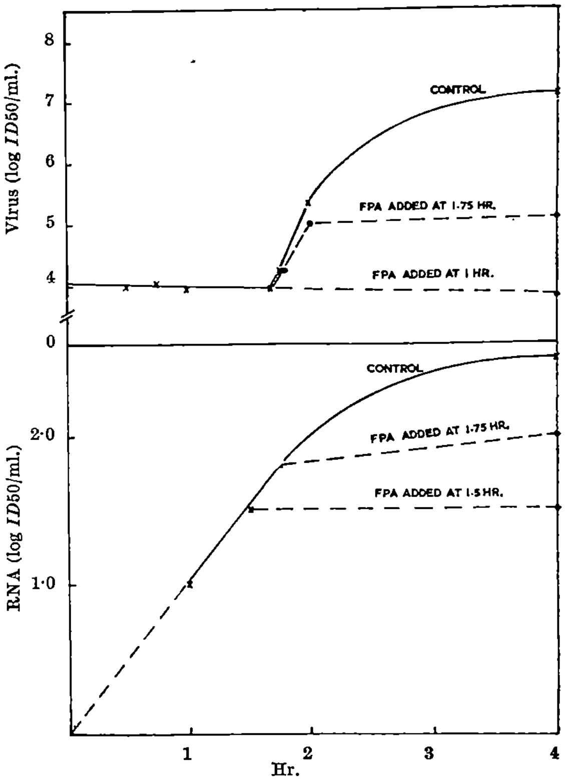


Fig. 1. Effect of adding 1,000 μ*M* *p*-fluorophenylalanine to infected cells at different times during growth cycle (this concentration completely inhibits virus synthesis)

Infection of pig kidney tissue culture cells with the virus (strain 997, type *C*) leads to the rapid production of new virus in the cells (Fig. 1). The formation of the infective viral ribonucleic acid, one of the precursors of the complete virus, precedes the appearance of the virus by 15–30 min. (ref. 5). It has previously been shown[6] that the inclusion of *p*-fluorophenylalanine in the growth medium reduces the yield of virus, the extent of the reduction depending on the concentration of the analogue. Table 1 shows some typical figures for the reduction of virus titre at 4 hr. when the suspending medium was phosphate-buffered saline containing 0·1 per cent glucose. The effect is not due to toxicity of the *p*-fluorophenylalanine, since it can be reversed by the addition of phenylalanine[6].

As the production of foot-and-mouth disease virus depends on the prior synthesis of its infective ribonucleic acid component, it seemed to be of interest to determine the effect of *p*-fluorophenylalanine on the production of the infective nucleic acid. The figures in Table 1 show that the reduction in yield of infective ribonucleic acid obtained from infected cells inhibited by *p*-fluorophenylalanine corresponded with the fall in virus titre. It seems likely, therefore, that the formation of the precursor infective nucleic acid is in some way connected with a protein the synthesis of which is inhibited by the analogue. With phage, the early inhibition of protein synthesis prevents the formation of phage nucleic acid, since specific enzymes[7,8] must be induced to synthesize the hydroxymethyl cytosine base prior to incorporation in the nucleic acid. Once these enzymes are formed, inhibition of protein synthesis does not prevent the production of phage nucleic acid, although complete phage particles are not formed. This does not appear

Table 1. YIELD OF VIRUS AND VIRAL RIBONUCLEIC ACID AT 4 HR. IN PIG KIDNEY TISSUE CULTURE CELLS WITH *p*-FLUOROPHENYLALANINE

Concentration of *p*-fluorophenylalanine (μ*M*)	Virus titre (mouse log *ID*50/ml.)	Ribonucleic acid titre (log *ID*50/ml.)
0	6·9	2·7
50	5·9	2·0
100	5·5	1·0
150	5·4	1·0
500	4·0	—

Titre of infected cells at time 0: $10^{4·3}$ *ID*50/ml.

to be the case with foot-and-mouth disease virus, as the addition of p-fluorophenylalanine (1,000 μM), even immediately before the maturation stage, leads to complete inhibition of infective virus and viral nucleic acid synthesis within 20 min. (Fig. 1). Even for natural amino-acids it has been reported that 15 min. is required for 75 per cent penetration[9], so 20 min. probably represents the time required for p-fluorophenylalanine to reach its site of inhibition.

It appears, therefore, that the synthesis of the infective nucleic acid of foot-and-mouth disease virus, like that of Western equine encephalomyelitis virus[4], is intimately related to the continuous synthesis of protein. This might be due to the need for the continuous synthesis of induced enzymes or to the fact that ribonucleic acid and protein synthesis are intimately coupled, as has often been postulated[10].

F. Brown
D. N. Planterose
Doreen L. Stewart

Research Institute
(Animal Virus Diseases),
Pirbright, Surrey.

[1] Ackermann, W. W., Rabson, A., and Kurtz, H., *J. Exp. Med.*, **100**, 437 (1954).
[2] Ackermann, W. W., and Maassab, H. F., *J. Exp. Med.*, **102**, 393 (1955).
[3] Zimmermann, T., and Schäfer, W., *Virology*, **11**, 676 (1960).
[4] Wecker, E., and Schonne, E., *Proc. U.S. Nat. Acad. Sci.*, **47**, 278 (1961).
[5] Brown, F., and Stewart, D. L., *Virology*, **7**, 408 (1959).
[6] Planterose, D. N., *Biochim. Biophys. Acta* (in the press).
[7] Flaks, J. G., and Cohen, S. S., *J. Biol. Chem.*, **234**, 1501 (1959).
[8] Kozloff, L. M., *Ann. Rev. Biochem.*, **29**, 475 (1960).
[9] Eagle, H., *Harvey Lectures*, Series 54, 156 (1958–59).
[10] Chantrenne, H., *Ann. Rev. Biochem.*, **27**, 35 (1958).

Leaf Curl Virus and the Flavonoid Content of Resistant and Susceptible Strains of Cotton

Massey and Andrews[1] reported finding larger amounts of unidentified 'brown' pigments in aqueous extracts obtained from healthy cotton leaves than in those obtained from leaves infected with cotton leaf curl. By means of the known chemical tests I ascertained that the pigments are in fact anthocyanins. The work described here is a study of the changes in the amounts of various flavonoids in leaves of resistant and susceptible strains of cotton, in the course of infection.

Top leaves from two strains of Domains Sakel were used, *BAR* 14/25 as a highly susceptible strain and *X*17/30 *A* as a strain resistant to leaf curl virus. Both strains were grown in the same plot and were cultivated under identical conditions. It was observed that virus infection appeared in the susceptible strain (*BAR* 14/25) thirty days after sowing and 100 per cent infection had occurred 115 days after sowing. Infection started in the resistant strain (*X*17/30 *A*) 70 days after sowing and only 40 per cent infection was observed 120 days after sowing.

Leaf extraction and methods of determining total phenols, anthocyanins, leuco-anthocyanins, and flavanols were those used by Swain and Hillis[2].

The results (Table 1) show that the percentages of total phenols, leuco-anthocyanins and flavanols are higher in the resistant than in the susceptible strain throughout the season. Infection with the virus caused a rise in the amounts of total phenols, leucoanthocyanins and flavanols in both strains, followed in all cases by a gradual decrease in these amounts.

Table 1. Phenolic Compounds in Cotton Leaves in the Course of Infection with Leaf Curl Virus
(Percentage on dry weight basis)

Days after sowing	Total phenols		Anthocyanins		Leucoanthocyanins		Flavanols	
	H	I	H	I	H	I	H	I
	BAR 14/25 (Susceptible)							
40	7·01	8·74	1·22	1·34	0·03	0·06	0·16	0·20
60	5·14	6·97	0·93	1·24	0·03	0·05	0·17	0·21
80	6·54	4·56	0·68	0·72	0·08	0·05	0·30	0·23
100	5·61	3·32	0·55	0·52	0·07	0·04	0·28	0·21
	X 17/30 *A* (Resistant)							
40	10·06	—	1·66	—	0·08	—	0·24	—
60	9·22	—	1·42	—	0·09	—	0·30	—
80	7·26	10·47	1·23	0·91	0·19	0·75	0·43	1·55
100	7·83	4·26	0·89	0·41	0·27	0·09	0·56	0·22
120	6·56	1·89	0·83	0·23	0·23	0·09	0·43	0·29

H, healthy; I, infected

The anthocyanins behaved in the same manner in the susceptible strain, that is, there was a sharp rise in the amounts following infection, then a gradual decrease, but in the resistant strain these amounts actually decreased rapidly after infection had occurred.

The results obtained in this work suggest that there might be some connexion between the flavonoid content of the leaves and the resistance of cotton plants to leaf curl virus. A study of the flavonoid content may act as a guide in spotting resistant strains in the course of breeding for resistance towards this disease.

This work was carried out in the Faculty of Agriculture, University of Khartoum; I am indebted to Dr. T. Swain of the Low Temperature Research Station, Cambridge, for his help.

Esam M. Moustafa

Plant Chemistry Division,
Department of Scientific and
Industrial Research,
Palmerston North,
New Zealand.

[1] Massey, R. E., and Andrews, F. W., *Emp. Cott. Gr. Rev.*, **9**, 32 (1932).
[2] Swain, T., and Hillis, W. E., *J. Sci. Food Agric.*, **10**, 63 (1959).

SOIL SCIENCE

Isolation of Anthraquinone from Humus

It is now generally accepted that the skeletal part of humic acids consists of heteropolycondensates of various aromatic nuclei. This concept, however, has been deduced rather indirectly from physico-chemical data such as X-ray analysis, and benzene seems to be the sole aromatic ring identified by the method of organic chemistry[1]. Recently we found anthraquinone in the decarboxylation products of the water-soluble acid fraction obtained by the alkaline permanganate oxidation of humic acids.

The humic acids used in this work belonged to so-called *A* type[2], and were obtained from a volcanic ash soil at the foot of Mt. Fuji, and from a muck soil presumably formed under the influence of volcanic ash showers on low-moor peat at Tanemori, Akita Prefecture, both soils being treated with 0·5 per cent sodium hydroxide at 100° C. for 30 min.

The sample, dissolved in N potassium hydroxide, was heated at about 90° C. and oxidized by adding potassium permanganate powder in small portions, until the permanganate to humic acid ratio became 4·0. The mixture was cooled, filtered to remove the precipitated manganese dioxide, and washed thoroughly with water. The filtrate was slightly acidified with sulphuric acid and concentrated under reduced pressure.

After the removal of inorganic salts and acid-insoluble organic acids by filtration, the reddish-brown solution, adjusted to contain about 4 *N* sulphuric acid, was subjected to ether extraction for many days. The extract, composed mainly of polycarboxylic acids, was freed from ether by evaporating to dryness, and the reddish-brown solid was obtained, which was decarboxylated by the copper-quinoline method of Montgomery and Holly[3].

The distillate during decarboxylation was collected in ethyl alcohol, in which the presence of benzene was confirmed by its ultra-violet spectrum ; pyridine was also detected.

The dark-coloured residue from the decarboxylation was mixed with ether, filtered through a filter paper to remove insoluble materials, and washed with hydrochloric acid (1 : 1) and 5 per cent sodium hydroxide successively to remove basic and acid fractions, respectively. The neutral fraction was then freed from ether by evaporating to dryness, and after drying in a desiccator containing silica gel, dissolved in small quantities of carbon tetrachloride.

The carbon tetrachloride solution was chromatographed through an alumina column. By developing it with carbon tetrachloride, a yellow band moved down, giving at first colourless, then yellow filtrates. Development was continued until the yellow band passed through the column and the filtrate became nearly colourless. This filtrate was passed through a silica gel column. The top of the silica gel column (fraction 3) was stained strongly reddish-brown, the other part of the column (fraction 2) colourless, and a colourless filtrate was obtained (fraction 1). Fractions 3 and 2 were scraped separately, and eluted with ether.

Three fractions could be distinguished under ultra-violet light on the alumina column after development ; the upper layer (fraction 6) being dark brown in appearance and showing no fluorescence, the middle layer (fraction 5) yellowish brown and yellowish green fluorescence, and the under layer (fraction 4) grey and partly red fluorescence. These fractions were scraped out separately, and eluted with acetone. Fractions 5 and 6 were reserved for further investigation.

Fractions 1–4 were tested for aromatic compounds by examination of infra-red and ultra-violet spectra, besides routine organic analysis. The results obtained so far may be summarized as follows :

First it must be mentioned that yellow crystals were readily obtained from fractions 3 and 4 by evaporating the eluates. Needle-shaped crystals obtained by recrystallization from ethyl alcohol followed by sublimation were identified as anthraquinone, because its melting point was 285° C. and this was not depressed by mixing with authentic specimen, and the infra-red spectrum was identical with that of anthraquinone.

Fraction 1 was considered to be mainly composed of paraffins, but the ultra-violet spectrum showed small quantities of naphthalene derivatives to be present. In the case of Fuji humic acid, 2-methyl-naphthalene was confirmed by its ultra-violet spectrum and the melting point of its picrate (115–116° C.). The presence of naphthalene derivatives in fraction 2 was also presumed.

From these results, though very qualitative, it is reasonable to suppose that humic acids showing a higher degree of humification contain not only a benzene ring but also anthracene, probably in the form of anthraquinone and naphthalene rings.

Differences in the nature and properties of humic acids obtained from various soils may be explainable by the composition of their aromatic nuclei. Further investigations are proceeding.

KYOICHI KUMADA
AKIO SUZUKI
Department of Agricultural Chemistry,
Nagoya University,
Anjo.

KAZUYUKI AIZAWA
Department of Agricultural Chemistry,
University of Tokyo.

---

[1] Flaig, W., *Landw. Forsch.*, 6 Sonderheft, 94 (1955).
[2] Kumada, K., *Soil and Plant Food, Japan*, 1, 29 (1955).
[3] Montgomery, R. S., and Holly, E. D., *Fuel*, 35, 49, 60 (1956).

PSYCHOLOGY

Measuring Visual Constancy for Stationary or Moving Objects

IF two objects of the same size are viewed so that one is twice as far from the eyes as the other, the retinal image of the farther object will be one-half the size of the image of the nearer object, but it does not appear half the size to the observer. They both appear almost the same size and the effect, which is due to perceptual interpretation of the retinal image, is known as size constancy[1,2].

In this method of measuring constancy of size, the eye is placed close to a small light-source which casts the shadow of an object (such as a flat disk) on a vertical screen. The angle subtended at the eye by the shadow will remain constant for any distance of the screen, but the shadow appears to expand as the screen on which it is projected is moved away from the observer. Other positions of the eye in relation to the source will produce size changes in the retinal image when the screen is moved. With the eye nearer the screen, the retinal image will contract (Fig. 1) ; with the eye behind the source it will expand. The degree of size constancy may be described in terms of the Thouless ratio, which is given by :

$$\log \frac{d_2 l_1}{d_1 l_2} \Big/ \log \frac{d_2}{d_1}$$

where d_1 and d_2 are distances of the screen from the eye when the shadow measures l_1 and l_2 respectively, the position of the eye being such that no size change is observed.

One can also measure constancy of shape, this being the tendency for a flat object to have a perceived

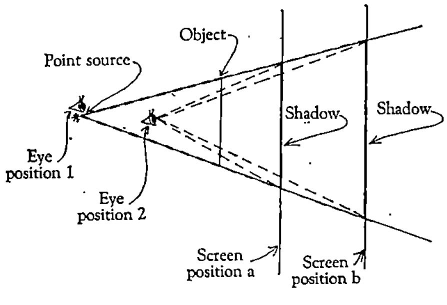


Fig. 1. Angle subtended at eye by shadow with eye in position 1 is constant for varying positions of screen ; with eye in position 2, angle subtended decreases as screen moves from position *a* to position *b*

shape corresponding very nearly to its normal projection ('normal' in the geometrical sense). If the screen is rotated around its vertical axis while the shadow-casting object remains stationary, the shadow appears to enlarge horizontally. Although the shadow on the screen changes shape, the retinal image is unaltered. The shape perceived is nearly that of the shadow viewed normally.

The magnitude of this effect can be measured, again using a null method, by arranging for a compensatory change of shape of the retinal image. This may be done by rotating the (flat) object. The screen and object may be linked to provide simultaneous rotations, their relative angles of rotation being adjustable. The magnitude of the effect may again be stated in terms of the Thouless ratio, which is given by :

$$\frac{\log P - \log S}{\log R - \log S}$$

where R is the ratio of the width of the shadow on the screen to its height, P the ratio of perceived width to perceived height (arranged to be constant and measured when the screen is normal to the observer), and S the ratio of the retinal width of the shadow to the retinal width when the screen is normal. If the angles subtended at the eye are small, the Thouless ratio is given by $\frac{\log \cos p}{\log \cos q}$, where p is an angle turned through by the object and q the corresponding angle turned through by the screen (Fig. 2), the relative angles of rotation being such that no change of shape is observed.

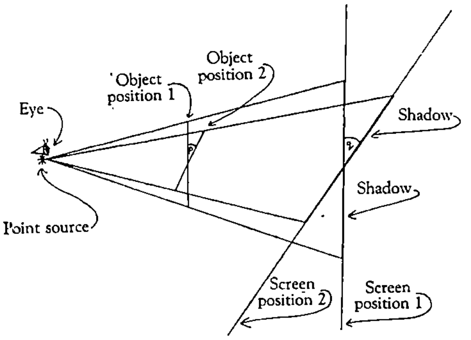


Fig. 2. With eye as close as possible to source, relative angles of rotation are adjusted until no change in shape observed

We are using this shadow method to investigate constancy during movement, which should be important in considering perceptual judgments involved in flying or driving. In particular we have tried to discover whether apparent movement due to constancy can be adapted. It is known that continuous presentation of real movement gives rise to an after-effect of apparent movement in the opposite direction[3]. The question here is: Can apparent expansion or contraction of the shadow produce an after-effect ? By projecting the shadow on a series of screens which move continuously away from the observer and are stopped after 1 min., we find that an after-effect does take place, though the retinal image of the shadow remains unchanged during the movement of the screens. During the movement the shadow appears to expand ; in the after-effect, it appears to contract. An after-effect of movement can thus be produced without shift of the image across the retina. Observation of the moving screens without the shadow does not produce the same after-effect of contraction of the shadow thrown on the screen now stationary, so the after-effect is not merely an induced movement, as described by Duncker[4].

One of us (S. M. A.) is in receipt of a Department of Scientific and Industrial Research studentship, and another (C. D. S.) of a grant from the Medical Research Council, which also provided support for apparatus.

S. M. ANSTIS
C. D. SHOPLAND
R. L. GREGORY

Psychological Laboratory,
University of Cambridge.

[1] Thouless, R. H., *Brit. J. Psychol.*, 21, 339 (1931) ; 22, 1 (1931) ; 22, 216 (1932).

[2] Vernon, M. D., *A Further Study of Visual Perception* (Cambridge Univ. Press, 1954).

[3] Wohlgemuth, A., "On the After-Effect of Seen Movement," *Brit. J. Psychol. Monograph Supplement* (Cambridge Univ. Press, 1911).

[4] Duncker, K., "Induced Motion", in *A Source Book of Gestalt Psychology*, edit. by Ellis, W. (Routledge and Kegan Paul, London, 1938).

ANTHROPOLOGY

The Juvenile Mandible from Olduvai

FURTHER to my report in *Nature* on February 25, p. 649, about the new discoveries of hominid remains in Bed I and Bed II at Olduvai Gorge, additional information can be given on the mandible of the juvenile from the site *FLK.NN*.I, at a horizon older than that of *Zinjanthropus*.

The third lower premolar (often called the first lower premolar by anatomists) is, in all the published Australopithecines, markedly shorter mesio-distally than wide bucco-lingually. The published figures for fully erupted lower third premolars of Australopithecines in South Africa include 14 from Swartkrans with indices $(100 \times L)/B$ ranging from 76·00 to 91·9 and a mean of 83·2. Kromdrai has only one reasonably preserved third lower premolar with an index of 83·2. Sterkfontein has only two good measurable examples published, which give indices of 77·8 and 76·8 respectively. Makapan has two measurable teeth, and the indices are said to be identical, at 88·4.

It is thus clear that, on the basis of the available published data (which are confirmed by my own observations on the original material at Pretoria and Johannesburg, which Prof. R. A. Dart, Prof. P. V. Tobias and Dr. J. T. Robinson kindly allowed me to examine), the third lower premolars of all known Australopithecine specimens are broader bucco-lingually than they are long mesio-distally. The index $(100 \times L)/B$ gives a range of 76·00–91·9.

In the juvenile hominid from the level below *Zinjanthropus* of site *FLK.NN*.I the measurements of the third lower premolars are as follows :

	Length	Breadth	Index
Left	11.00	9·5	115·8
Right	11·00	9·5	115·8

Thus, while all the published length–breadth indices of fully erupted lower third premolars of all *Australopithecus* and *Paranthropus* specimens are much less than 100 (that is, broader than long) and have a range of 76·00–91·9, the indices of the tooth in the juvenile hominid from the pre-*Zinjanthropus* level are greater than 100, being in fact 115·8.

Quite apart from this very clear measurable difference which puts the third lower premolar completely out of the known range of measurable

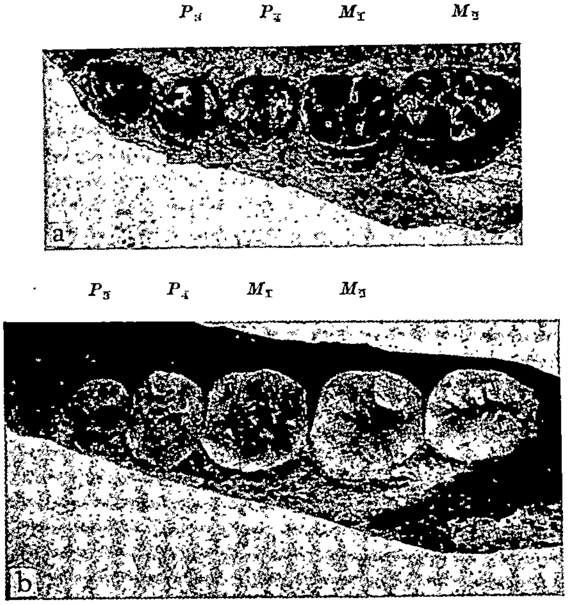


Fig. 1. Comparison between the teeth of the left side of the (*a*) pre-*Zinjanthropus* juvenile mandible with those of (*b*) an adult *Paranthropus* to show the morphological differences. In the Olduvai specimen the third molar is not present. (Not to the same scale, but reduced so that the length $P_{\overline{3}} - M_{\overline{2}}$ inclusive is approximately the same so as to show relative proportions)

variation in the Australopithecines, the morphological picture is also completely different from that of any Australopithecine that I have been able to examine, or of which there are published photographs available to me (Fig. 1).

Turning next to the published data of lower fourth premolars (second premolars of some anatomists) of known Australopithecines, we find that there are 13 measurable specimens from Swartkrans, all of which are much wider bucco-lingually than they are mesio-distally. The index (100 × *L*)/*B* ranges from 61·1 to 91·1 with a mean of 82·3 (very close to the mean for the third lower premolars). Only two specimens from Kromdrai are known and they fall well within the range with indices of 84·4 and 84·7. Only two Sterkfontein lower fourth premolars appear to have been described in the literature, and these give indices of 87·0 and 82·9. I know of no measurable fully erupted fourth lower premolars from Makapan that have been recorded; the known specimens are either not fully erupted or very worn and damaged.

When we turn to the juvenile hominid from the pre-*Zinjanthropus* level at *FLK.NN*.I, we find that the fourth premolars are markedly longer mesio-distally than they are bucco-lingually. The measurements and indices are as follows:

	Length	Breadth	Index
Left	11·0	10	104
Right	11·0	10·50	104·7

Thus we find that whereas in the known Australopithecines the range in the index (100 × *L*)/*B* is from 61·1 to 91·1, in the juvenile from the pre-*Zinjanthropus* level at Olduvai, the position is quite different. The mesio-distal length is greater than the bucco-lingual width, so that the indices are more than 100.

Again, apart from measurable differences, the fourth premolars in the new jaw are morphologically different from those I have seen in the collections of Australopithecines at Johannesburg and Pretoria (Fig. 1).

The published data on the first lower molars of *Australopithecus* shows that the index (100 × *L*)/*B* varies from 114·6 in one of the Sterkfontein specimens to 100·8, also from Sterkfontein, and the indices from other sites fall within this range, both in respect of *Australopithecus* and *Paranthropus*. The first lower molars in the juvenile jaw from the pre-*Zinjanthropus* level show identical measurements of length 14·25 and breadth 12·00, giving an index of 117·00. This is outside the published range of variation of Australopithecine first lower molars. Morphologically, too, there are differences in the crown pattern, which is much simpler and more like that which can be observed in some modern Australian Aborigine teeth.

The second lower molar of the pre-*Zinjanthropus* juvenile is 16·00 mm. long and 14·00 mm. wide. In this case, the index falls within the range of variation of the known Australopithecines. Some morphological differences seem to me to be present, but need further study. I do not recall (nor have I notes on) any really comparable tooth in the collections in Pretoria or Johannesburg, but I say this with reservations.

There is a single upper molar (which appears to be an unerupted upper molar) in which the roots have not yet formed and in which the crown is completely fresh. It seems likely that it represents the third upper molar of the same juvenile as is represented by the mandible, and in which the lower second molar is erupted but scarcely worn.

If this tooth is correctly interpreted as an unerupted third upper left molar, then it confirms my view that the teeth of the juvenile pre-*Zinjanthropus* differ, in most measurable respects, from the Australopithecines. The tooth has a mesio-distal length of 13·5 and a bucco-lingual width of 12·75, giving an index of (100 × *L*)/*B* of 106, which seems to be outside the range of variation published for any Australopithecine upper third molar. Even if it is not a third upper molar but a second, it is still outside a published range, while morphologically it does not seem to be of Australopithecine type.

In conclusion, may I say that when I used the word 'child' with reference to the pre-*Zinjanthropus* juvenile in my report in *Nature* of February 25, I was not intending thereby to imply that the father was a 'man' in the sense of the accepted definition of treating him as the 'maker of tools to a set and regular pattern'. I used the word 'child' simply as meaning a juvenile of the family Hominidae.

There are stone tools and a bone tool in direct association with the juvenile, but it is not yet certain whether his family made these tools, any more than it is quite certain that *Zinjanthropus* made the tools which are in direct association with the skull from *FLK*.I.

If I am right in believing that the juvenile from *FLK.NN*.I is not an Australopithecine, but a very remote and truly primitive ancestor of *Homo*, then it is possible (and I stress the fact here that I only use the word 'possible' at this stage of the inquiry) that it was this branch of the Hominidae that also made the Oldowan tools at the site *FLK*.I where *Zinjanthropus boisei* was found. We do not yet know the final truth of this problem.

L. S. B. Leakey

The Coryndon Museum,
Box 658, Nairobi,
Kenya.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Biochemists (2) (science graduates, preferably with experience in medical biochemistry), for duties involving laboratory work in clinical pathology at Foresterhill or at Woodend Hospital—The Secretary, The University, Aberdeen (July 27).

Animal Physiologist (with research experience), to develop work in the field of adaption of animals to environment—The Secretary, Animal Breeding Research Organization, A.R.C., Glenbourne, South Oswald Road, Edinburgh 9 (July 29).

Lecturer or Assistant Lecturer in Organic Chemistry—The Registrar, The University, Manchester 13 (July 31).

Lecturer in Psychology at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 4).

Lecturer (preferably with research experience in surface chemistry or chemical kinetics) in Pharmacy at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 11).

Lecturer (graduate in science or engineering) in the Department of Building Science—The Registrar, The University, Liverpool (August 12).

Research Assistant (with a higher degree in theoretical physics or mathematics) in Theoretical Physics—The Registrar and Secretary, King's College (University of Durham), Newcastle-upon-Tyne 1 (August 12).

Fellow (specialized in organic chemistry, with special reference to the chemistry of compounds of neuropharmacological significance) in the Department of Physiology, John Curtin School of Medical Research, Australian National University—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 14).

Physical Research Chemist (physical chemist, preferably with several years' postgraduate experience), for work concerned with fundamental studies on gelation—The Director of Research, British Gelatine and Glue Research Association, 2a Dalmeny Avenue, London, N.7 (August 14).

Lecturer or Assistant Lecturer in the Department of Applied Mathematics—The Registrar, The University, Liverpool (August 15).

Lecturer/Senior Lecturer (preferably with research interests in epidemiological or immunological aspects) in Parasitology in the Faculty of Rural Science, University of New England, Armidale, New South Wales, Australia, to teach and carry out research in parasitology with particular reference to livestock aspects—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 15).

Research Assistants (2) (with at least a B.Sc. degree or equivalent, and a special interest in physical chemistry or chemical engineering) in the Department of Chemistry, to continue a study of the equilibrium properties of imperfect gases under the terms of a contract between the University and the D.S.I.R.—The Registrar (Room 22, O.R.B.), The University, Reading (August 15).

Senior Technician or Technician (with the A.I.S.T. or equivalent qualification) in the Department of Zoology, University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 15).

Textile Technologist (man or woman, with a first- or second-class honours degree in textile technology, chemistry or physics, or Associate or Fellow of the Textile Institute, or equivalent qualifications) at the Tropical Products Institute, London, W.C.1, to supervise a group concerned with research into, and laboratory examination of, various aspects of animal and vegetable fibres, pulp, paper and board-making materials, hides, skins, etc., of tropical origin—Civil Service Commission, 17 North Audley Street, London, W.1), quoting Ref. No. S/5333/61 (August 15).

Lecturer or Assistant Lecturer (with experience and research interests in plant physiology) in Botany at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 20).

Lecturer in Mechanical Engineering—The Registrar, Trinity College, Dublin 2 (August 28).

Reader in Aeronautics at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 29).

Director of the Natural History Museum, Adelaide, South Australia—The Agent-General for South Australia, 56 Strand, London, W.C.2 (August 31).

Keeper of Scientific Books (with considerable experience of university or research library administration and a good honours degree preferably in science), to superintend the Radcliffe Science Library—The Librarian, Bodleian Library, Oxford (August 31).

Lecturer in Philosophy at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, August 31).

Lecturers (3) in Chemistry at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

Reader (with a special interest in the properties of materials) in Electrical Engineering at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 31).

Lecturer in the Department of Medical Physics—The Secretary, Royal Free Hospital School of Medicine (University of London), 8 Hunter Street, London, W.C.1 (September 1).

Senior Lecturer in Applied Electronics at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 1).

Lecturer or Assistant Lecturer (medically or non-medically qualified candidate) in the Department of Histology—The Registrar, The University, Liverpool, quoting Ref. CV/N (September 4).

Lecturer or Junior Lecturer in Pure Mathematics—The Registrar, Trinity College, Dublin 2 (September 4).

Lecturer in Social Anthropology at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, September 8).

Lecturer/Senior Lecturer (well qualified in electrical engineering or physics, general experience in electronics and a considerable detailed knowledge of solid state devices and circuits) in Electrical Engineering at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

Senior Lecturer in Physics at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, September 30).

Assistant Lecturer (with an honours degree or an honours diploma in technology in applied physics, and preferably research or industrial experience in some branch of applied physics) in Physics—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

Biochemist (with a first- or second-class honours degree or graduate member of the Royal Institute of Chemistry), for duties in the Hæmatological Laboratory—The House Governor, King's College Hospital, Denmark Hill, London, S.E.5.

Lecturer or Assistant Lecturer (particularly interested in petrology and mineralogy) in Geology—Prof. D. Williams, Geology Department, Imperial College of Science and Technology, London, S.W.7.

Lecturer (preferably with a higher degree and research experience in an industrial environment) in Metallurgy in the Department of Materials—The Recorder, College of Aeronautics, Cranfield, Bletchley, Bucks.

Lecturer (university graduate in physics with industrial and/or research experience in radiation protection or a kindred subject, and preferably some teaching experience) in Physics—The Clerk to the Governing Body, Borough Polytechnic, Borough Road, London, S.E.1.

Physicists or Electrical Engineers (with experience in one or more of the following fields: high vacuum; R.F. techniques (75 Mc./s.); high power rectifier and pulse circuits; or general electronic instrumentation and controls), to assist with the construction and commissioning of a large heavy ion linear accelerator—The Assistant Director, Linear Accelerator Laboratory, Physics Department, The University, Manchester 13.

Postgraduate Student (recent graduate with a good degree in physics or physical chemistry) in the Department of Physics, to research under the supervision of Dr. J. G. Powles on some aspect of magnetic resonance—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1.

Research Assistant (recent graduate in chemistry with an interest in zoology or comparative biochemistry), to join a team investigating chemical constituents of the biles of many species, with a view to a fuller understanding of evolutionary theory and taxonomic principles—Prof. G. A. D. Haslewood, Biochemistry and Chemistry Department, Guy's Hospital Medical School, London Bridge, London, S.E.1.

Research Assistant (recent physics graduate) in the Department of Physics, for track analysis in connexion with the National Bubble Chamber project—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15.

Scientific Officer (with a first- or second-class honours degree in chemical engineering or in chemistry, and postgraduate training or experience in chemistry) at the Humber Laboratory, Kingston-upon-Hull, Yorkshire, to work on problems of both fundamental and applied research concerned with fish-smoking; the transport of fresh and frozen fish; and the manufacture of fish meal—The Director, Torry Research Station, D.S.I.R., Aberdeen, quoting Ref. Torry 8Z.

Senior Lecturer (graduate in pharmacy or chemistry with an established interest in the medicinal field) in Medicinal Chemistry at the Department of Pharmacy—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1.

Senior Lecturer (with a good university degree in agriculture or veterinary science, considerable postgraduate experience in research and/or teaching in a higher institution, and conversant with tropical work in animal husbandry) in Animal Husbandry in the School of Agriculture, Kumasi College of Technology, Ghana—The Assistant Registrar, Kumasi College of Technology, 29 Tavistock Square, London, W.C.1.

Senior Research Assistant (with a Ph.D. or appropriate research experience) in the Department of Physics, The University, Leeds 2.

Senior Research Associates (2) (experienced electrical engineers or physicists) in the Department of Physics, for the development of the proton synchrotron—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15.

Technical Officer (with a degree or National Diploma in Agriculture or a degree in agricultural botany), to assist in work on herbage seed production—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Boiler Availability Committee. Bulletin No. MC/316: Testing Techniques for Determining the Corrosive and Fouling Tendencies of Boiler Flue Gases. Pp. 68. (London: The Boiler Availability Committee, 1961.) [185

British Association for Commercial and Industrial Education. Interviewing in Twenty-six Steps. By John S. Gough. Pp. 16. (London: British Association for Commercial and Industrial Education, 1961.) 3*s*. 6*d*. [185

Royal Institute of Chemistry Monographs for Teachers, No. 4: Principles of Metallic Corrosion. By Dr. J. P. Chilton. Pp. v + 64. (London: Royal Institute of Chemistry, 1961.) 6*s*. [185

Tobacco Manufacturers' Standing Committee. Research Papers, No. 4: Cigarette Smoke Condensate—Preparation and Routine Laboratory Estimation. By H. R. Bentley and J. G. Burgan. Second edition. Pp. 14. (London: Tobacco Manufacturers' Standing Committee, 1961.) [185

Westminster Press Provincial Newspapers, Ltd., Report of the Director and Accounts, year ended 31st December, 1960. Pp. 10. (London: Westminster Press Provincial Newspapers, Ltd., 1961.) [195

Forestry Commission. Forest Record No. 45: Fires in State Forests in the years 1929–1956. By Haldane Charters. Pp. 37. 3*s*. net. Forest Record No. 47: Provisional Yield Tables for *Abies grandis* and *Abies nobilis*. By J. M. Christie and T. E. A. Lewis. Pp. 48. 3*s*. net. (London: H.M. Stationery Office, 1961.) [195

The Royal Observatory, Edinburgh. Report of the Astronomer Royal for Scotland for the year ending 31st March 1961. Pp. 12. (Edinburgh: The Royal Observatory, 1961.) [195

Publications of the Royal Observatory, Edinburgh, Vol. II, No. 6: Spectrophotometric Measurements of Early Type Stars. 7: Results and Discussion for 10 Stars of M.K. Type B_5 and 7 Stars of Miscellaneous Types. By H. E. Butler and G. I. Thompson. Pp. 225–257. (Edinburgh and London: H.M. Stationery Office, 1961.) 9*s*. net. [195

B.B.C. (TV). Saturday Morning Television introduces Science on Saturday. Pp. 16. (London: B.B.C. (TV)). [195

British Scientific Instrument Research Association. A Survey of Instrument Parts No. 4: Instrument Ball Bearings—a Survey for the User. By P. J. Geary. Pp. vii + 73. (Chislehurst: British Scientific Instrument Research Association, 1961.) 21*s*. [245

The Worshipful Society of Apothecaries of London: Faculty of the History of Medicine and Pharmacy. Second Annual Report and List of Members 1960–61. Pp. 20. (London: The Worshipful Society of Apothecaries of London, 1961.) [245

Library Association. Annual Report, 1960. Pp. 28. (London: Library Association, 1961.) [245

The Future of Television Broadcasting in Great Britain. Prepared by the British Institution of Radio Engineers. Pp. 19. (London: The British Institution of Radio Engineers, 1961.) 5*s*. [245

Western Regional Hospital Board, Glasgow. Annual Report of the Regional Physicist for the year 1960. Pp. 50. (Glasgow: Western Regional Hospital Board, Physics Department, 1961.) [245

Other Countries

New Zealand Forest Service. Information Series No. 33: Statistics of the Forests and Forest Industries of New Zealand. Third edition. Pp. 96. (Wellington: Government Printer, 1961.) [175

Museum of Comparative Zoology at Harvard College. Breviora. No. 133 (February 27, 1961): On the Generic Limits in the Family Pilidae (Prosobranchia: Mollusca). By Edward H. Michelson. Pp. 10. No. 134 (February 28, 1961): Enzymatic Constitution of *Alsophis* Saliva and Its Biological Implications. By George Hegeman. Pp. 8. No. 135 (April 7, 1961): Notes on Hispaniolan Herpetology. 2: A Review of the *Anolis semilineatus* Group with the description of *Anolis cochranae*, New Species. By Ernest E. Williams and A. Stanley Rand. Pp. 11. No. 136 (April 8, 1961): Notes on Hispaniolan Herpetology. 3: The Evolution and Relationships of the *Anolis semilineatus* Group. By Ernest E. Williams. Pp. 8. No. 137 (April 10, 1961): Notes on Hispaniolan Herpetology. 4: *Anolis koopmani*, New Species, from the Southwestern Peninsula of Haiti. By A. Stanley Rand. Pp. 4. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1961.) [175

Institut des Parcs Nationaux du Congo et Ruanda-Urundi. Exploration du Parc National Albert: Mission G. F. De Witte (1933-1935). Fascicule 97 (1) Perlidae (Plecoptera). By H. B. Noel Hyne. (2) Bruchidae (Coleoptera Phytophagoidae). Par Jean Decelle. (3) Athalia (Hymenoptera Tenthredinidae). By Robert B. Benson. Pp. 21. Exploration du Parc National de la Garamba: Mission H. de Saeger en collaboration avec P. Baert, G. Demoulin, I. Denisoff, J. Martin, M. Micha, A. Noirfalise, P. Schoemaker, G. Troupin et J. Verschuren, (1949–1952). Fascicule 18. (1) Pentastomidae. Par Jean Doucet. (2) Cixiidae. Par Henri Synave. (3) Bruchidae. Par Jean Decelle. Pp. 75. Fascicule 19. (1) Perli. By H. B. Noel Hyne. (2) Anthribidae. Von Paul Wolfrum. (3) Meloidae. Von Zoltan Kaszab. Pp. 55. Fascicule 22: Languriidae (Coleoptera Clavicornia). Par André Villiers. Pp. 71. Exploration du Parc National de l'Upemba: Mission G. F. de Witte en collaboration avec W. Adam, A. Janssens, L. Van Meel et R. Verheyen (1946–1949). Fascicule 55: Araneae Lycosaeformia II (Lycosidae). Von Carl-Fr. Roewer. Pp. 1040 (2 vols.). Fascicule 59. (1) Pentastomida. Par Jean Doucet. (2) Cicadidae. By Jiri Dlabola. (3) Alticidae. Par Jean Bechyné. (4) Euaesthetinae. By David H. Kistner. (5) Genus *Zyras*. By Horace Last. (6) Bruchidae. Par Jean Decelle. Pp. 144. (Bruxelles: Institut des Parcs Nationaux du Congo et du Ruanda-Urundi, 1959, 1960 et 1961.) [185

Astronomische Abhandlungen der Hamburger Sternwarte. Band 5, Nr. 6: Dreifarben-Photometrie von NGC 7789. Von István Ozsváth. Pp. 127–214. Band 5, Nr. 7: Dreifarben–Photometrie einer Sternhaufengruppe in der Cassiopeia (NGC 103, NGC 129, NGC 136, NGC 146, K 14 und K 16.) Von Johannes Hardorp. Pp. 215–262. Band 5, Nr. 8: Proposal for a Photo-electric Meridian Circle and a New Principle of Instrumental Design. By Erik Høg. Pp. 263–272. Band 5, Nr. 9: Über die Polarisation des Sternlichtes, die Strahlung der Sterne sowie Bemerkungen zur Galaktischen struktur. Von G. Thiessen. Pp. 273–319. Band 6, Nr. 1: Die Veränderlichen im Südteil der Cygnuswolke, Teil 1. Von Arthur Arno Wachmann. Pp. 1–96. Hamburger Sternwarte: Warner and Swasey Observatory. Luminous Stars in the Northern Milky Way, II. By J. Stock, J. J. Nassau and C. B. Stephenson. Pp. 30. (Hamburg–Bergedorf: Hamburger Sternwarte, 1960 und 1961.) [195

Sveriges Geologiska Undersökning. Avhandlingar och Uppsatser. Ser. C. No. 568: Tectonic Conditions on the Front Range of the Swedish Caledonian in Central Norrland. By Torsten du Rietz. Pp. 57+4 plates. 9.50 kr. No. 57: The Miogeosynclinal Rocks of Eastern Central Sweden. Per H. Lundegårdh. Pp. 68+1 plate. 10.00 kr. No. 571: On Tylocidaris Species (Echinoidea) and the Stratigraphy of the Danian of Sweden, with a Bibliography of the Danian and the Paleocene. By Fritz Brotzen. Pp. 81+3 plates. 7.00 kr. No. 572: En Zon av Kaolinlera och Vittrad Blodsten vid Svappavaara, Norrbotten. Av Rudyard Frietsch. Pp. 45. No. 573: Issjöar och Isavasmältning i Östra Jämtland. Av Jan Lundqvist. Pp. 29. 2.00 kr. No. 574: An Interglacial Peat at Ale near Lulea, Northern Sweden. By Erik Fromm. Pp. 14. 2.00 kr. No. 575: The Interglacial Ooze at Porsi in Lapland. By G. Lundqvist. Pp. 26. 3.00 kr. No. 576: Vaggerydssyeniten. Av Percy Quensel. Pp. 38+1 plate. 4.00 kr. Ser. Ba, Nr. 20: Jordartskarta över Götaälvdalen. Karta i tre blad. (Map in thee sheets.) Av Björn Järnefors. 11.00 kr. per blad. Åsberättelse för År 1958. Pp. 15. 0.50 kr. Årsberättelse för År 1959. Pp. 8. 0.50 kr. (Stockholm: Sveriges Geologiska Undersökning, 1960.) [245

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 60–26: Subsurface Stratigraphy of Ordovician Rocks in Southwestern Ontario. By B. V. Sanford. Pp. iv+54. 50 cents. Map 49–1960 (Geology), Prince George, Cariboo District, British Columbia. (Preliminary Series, Advance edition, Sheet 93G). Bulletin No. 75: The History and Geology of Meighen Island, Arctic Archipelago. By R. Thorsteinsson. Pp. xi+19. 75 cents. (Ottawa: Queen's Printer, 1961.) [245

Soils Research and Survey Section of the Regional Research Centre, Imperial College of Tropical Agriculture, University College of the West Indies. Soil and Land-Use Surveys No. 12: Jamaica—Parish of Hanover. By R. W. Price. Pp. 25. (St. Augustine, Trinidad: Imperial College of Tropical Agriculture, 1960.) 14*s*. [245

United States Department of the Interior: Geological Survey. Bulletin No. 1058–1: Tofty Tin Belt, Hot Springs District, Alaska. By Russell G. Wayland. Pp. iv+363–414+plates 40–43. Bulletin 1084–H: An Evaluation of Whole-Order, $\frac{1}{2}$-Order, and $\frac{1}{3}$-Order Reporting in Semiquantitative Spectrochemical Analysis. By Paul R. Barnett. Pp. iii+183–206. 15 cents. Bulletin 1098–A: Geochemical Studies on the Coeur d'Alene District, Shoshone County, Idaho. By Vance C. Kennedy. With a section on Geology by S. Warren Hobbs. Pp. v+55+7 plates. 1.25 dollars. Water-Supply Paper 1498–C: Depth-Discharge Relations of Alluvial Streams—Discontinuous Rating Curves. By David R. Dawdy. Pp. iv+16. 15 cents. Water-Supply Paper No. 1533: Reconnaissance of Ground-Water Resources in the Blue Grass Region, Kentucky. By W. N. Palmquist, Jr. and F. R. Hall. Pp. iv+39+3 plates. Water-Supply Paper 1539–B: Jet Drilling in the Fairbanks Area, Alaska. By D. J. Cederstrom and G. C. Tibbitts, Jr. Pp. iii+28. (Washington, D.C.: Government Printing Office, 1960 and 1961.) [245

United States Department of the Interior: Fish and Wildlife Service. Fishery Bulletin No. 181: Filefishes (Monacanthidae) of the Western North Atlantic. By Frederick H. Berry and Louis E. Vogele. Pp. iv+61–109. (Washington, D.C.: Government Printing Office, 1961.) 40 cents. [245

Geological Survey of Tanganyika. Bulletin No. 32: The Crystalline Rocks of the Igawa Area. By Dr. P. E. Brown. Pp. iii+23+15 plates. Shs. 7. Quarter Degree Sheet 53 S.E.: Kilosa. First edition. (Dar-es-Salaam: Government Printer, 1959 and 1960.) [245

Indian Forest Bulletin (New Series). Timber Mechanics. No. 224: Recent Work on Testing of Wood Poles. By A. C. Sekhar, S. S. Sachar and P. Pal. Pp. 29. No. 227: Comparative Studies on Natural and Plantation Teak. By A. C. Sekhar, R. K. Bhartri and M. S. Rawat. Pp. 10. Entomology. No. 228: Results of Experiments on the Anti-borer Protection of Two Species of Timber, *Salai* and *Bijasal*, by means of Insecticides. By M. L. Roonwal, P. N. Chatterjee and R. S. Thapa. Pp. 4. (Delhi: Manager of Publications, 1960.) [245

United States Department of Health, Education, and Welfare. Public Health Service Publication No. 815: Highlights of Research Progress in General Medical Sciences 1960—Items of Interest on Research Studies and Research Training Programs supported by the Division of General Medical Sciences, National Institutes of Health, Bethesda, Md. Pp. v+45. (Washington, D.C.: Government Printing Office, 1961.) 25 cents. [245

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

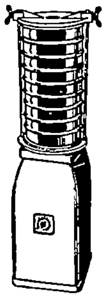
Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

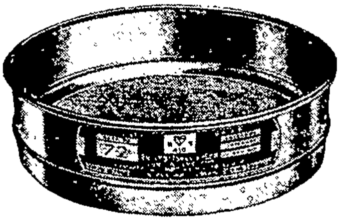
All rights reserved. Registered as a newspaper at the General Post Office

ENDECOTTS
TEST SIEVES
For Accuracy!

POCKET INTERCHANGER SIEVES

'ENDROCK' TEST SIEVE SHAKER





TEST SIEVES
B.S. 410: 43 U.S. Standards and Tyler Equivalents

We operate a Recovering Service

Consistent mechanical action—
saves valuable time of skilled personnel

ENDECOTTS (FILTERS) LTD. *Phone:* **LIBerty 8121/2/3**
Dept. B Lombard Rd., London, S.W.19. *Grams:* **Endfilt, London**

Plan Ahead

by effecting now "the young man's policy" which gives to a man under 35 immediate life assurance cover at low cost and the valuable right to effect within ten years further assurances even though his health has become impaired.

The Equitable has no shareholders and pays no commission for the introduction of business; unusually attractive terms can, therefore, be offered.

Write today for details to

The Equitable Life Assurance Society
(Founded 1762)
Head Office: **19 Coleman Street, London, E.C.2**

Please send me, without obligation, details of the young man's policy.

Name..

Address..

....

SOUTHERN
Analytical

A growing range of instrumentation for the Analytical and Control Chemist.

Pulse Polarograph, Cathode Ray Polarograph, Manual Polarograph, Dissolved Oxygen Meter, Hydrogen Generator, Nilox Gas Scrubber, Potentiostat for studies at controlled anode or cathode potential, static and flow turbidimeter and absorptiometer, phthalic anhydride continuous absorptiometer.

INSTRUMENTS
WITH THE USER IN MIND

Southern Analytical Ltd
FRIMLEY ROAD CAMBERLEY SURREY. TEL: 3401

APPOINTMENTS VACANT

AUSTRALIAN NATIONAL UNIVERSITY

INSTITUTE OF ADVANCED STUDIES

SENIOR FELLOWSHIPS IN MICROBIOLOGY

Applications are invited for two Senior Fellowships in the Department of Microbiology of the John Curtin School of Medical Research, which carries out research in virology. (Head of Department: Professor F. J. Fenner, F.R.S.) An applicant will be expected to have made substantial independent contributions to his field of knowledge and to be capable of supervising research students and post-doctoral fellows. For one post a man is required who has had research experience in studies on the pathology and pathogenesis of viral infections of animals. The other post is designed for work in viral biochemistry. Adequate laboratory facilities exist in the Department and the School for advanced work in both these fields. The salary range of a Senior Fellowship is from £A.3,000 to £A.3,550; increments are £A.90 per annum. A successful applicant will, in the first place be appointed at a salary and incremental limit fixed by reference to his standing and experience. Appointment is made for an initial period of five years; thereafter it may be made permanent. A Senior Fellow is entitled to one year's study leave on full pay, plus a contribution towards travel and other expenses, in every six years. Superannuation is on the F.S.S.U. pattern. Reasonable travel expenses are paid and assistance with housing is provided.

Further details should be obtained from the Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on September 15, 1961. Successful applicants will be notified as soon as possible thereafter.

UNIVERSITY OF ADELAIDE

TECHNICAL OFFICER OR SENIOR TECHNICAL ASSISTANT

Applications are invited for the above-mentioned appointment in the Department of Botany. Salary scales: Technical Officer (male), £A.1,385 by £A.45 to £A.1,745, with a bar at £A.1,565; (female), £A.1,040 by £A.33 15s. to £A.1,310, with a bar at £A.1,175; Senior Technical Assistant (male), £A.1,335 by £A.35 to £A.1,580; (female), £A.1,003 by £A.26 to £A.1,185. Qualifications: Considerable laboratory experience and competence with apparatus and techniques in biology, and preferably with the preparation and collection of botanical teaching materials and histological methods. Duties: Supervision of basic laboratory apparatus and preparation of undergraduate classes. General: (a) No provision is made for travelling expenses, but arrangements could be made to help a candidate from the United Kingdom to secure an assisted passage under the Commonwealth Migration Scheme. (b) Preference will be given to a qualified male applicant, but women also are invited to apply. (c) Any further information desired will be supplied on request to the Registrar.

Applications, giving particulars of qualifications and experience, marital status, the names and addresses of two referees, and the time when the applicant would be free to assume duty, should be sent to the Registrar, The University of Adelaide, Adelaide, South Australia, not later than August 31, 1961.

MAKERERE UNIVERSITY COLLEGE

EAST AFRICA

Applications are invited for the following appointments in the Faculty of Agriculture: (i) Readership or Senior Lectureship in Agricultural Chemistry. (ii) Lectureship in Agricultural Chemistry. (iii) Lectureship in Animal Husbandry. Salary scales: Reader, £1,974 by £75 to £2,724 per annum; Senior Lecturer, £1,950 by £75 to £2,475 per annum; Lecturer, £1,350 by £51 to £1,707 by £72 to £1,851 by £75 to £2,151 per annum. Entry point according to qualifications and experience. F.S.S.U. Child allowance £50 per annum per child (maximum £150 per annum). Passages for appointee and family (up to five adult passages) on appointment, termination and leave (three months every 21 months). Rent according to quarters provided £45 to £84 per annum (including basic furniture).

Detailed applications (six copies), naming three referees, by September 15, 1961, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

METALLURGISTS

and

PHYSICISTS

required by the

CENTRAL ELECTRICITY GENERATING BOARD RESEARCH LABORATORIES

(Materials Division, Leatherhead, Surrey)

Advancing conditions in steam generation necessitate fundamental and applied investigations into the behaviour of ferritic and austenitic steels at high temperatures under complex stress systems. Successful candidates will join teams working on problems associated with creep, fatigue, phase changes, erosion and oxidation. Post-graduate industrial or university experience in one of these fields is desirable but not essential. Excellent facilities for research are available and publication of results is encouraged.

These posts have arisen solely through a decision to increase our activity in these fields.

Applicants (preferably under 26 years of age) should possess a good honours degree or equivalent qualification. There are also a few vacancies for staff who are slightly less qualified academically, but who have particularly relevant experience.

● Salaries up to £1,325 per annum (or higher in exceptional cases) will be fully commensurate with responsibilities, experience, ability and qualifications, and excellent prospects exist for promotion to higher grades.

Applications stating age, qualifications, experience, present position and salary, to the Appointments Officer, 24/30, *Holborn, London, E.C.*1, *by July* 31. *Quote " Ref. N/*315."

UNIVERSITY COLLEGE OF THE WEST INDIES

FACULTY OF AGRICULTURE

(IMPERIAL COLLEGE OF TROPICAL AGRICULTURE)

TRINIDAD, THE WEST INDIES

Applications are invited for (a) Lectureship or (b) Assistant Lectureship in Soil Science. Knowledge of tropical soils desirable. Salary scales: (a) £1,050 by £50 to £1,400 by £75 to £1,850 per annum; (b) £800 by £50 to £950 per annum, plus local allowance of £250 per annum (pensionable). Entry according to qualifications and experience, but in case of Assistant Lecturers and Lecturers below merit bar consideration is given to age in fixing salary. Child allowance. Settling-in allowance. F.S.S.U. Unfurnished accommodation at rental of 10 per cent of basic salary. Up to five full passages on appointment, normal termination, and triennial study leave.

Detailed applications (six copies), naming three referees, by August 14, 1961, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF MANCHESTER

Applications are invited for the post of Lecturer or Assistant Lecturer in Organic Chemistry in the Faculty of Science. Duties to commence as soon as possible. Salary scales as follows: Lecturer, £1,050 to £1,850 per annum; Assistant Lecturer, £800 to £950 per annum. Membership of F.S.S.U. and children's allowance scheme.

Applications should be sent not later than July 31, 1961, to the Registrar, the University, Manchester 13, from whom further particulars and forms of application may be obtained.

UNIVERSITY OF LONDON

READERSHIP IN AERONAUTICS AT IMPERIAL COLLEGE

The Senate invite applications for the Readership in Aeronautics tenable at Imperial College (salary scale £1,675—£1,975—£2,425 a year plus £80 London allowance).

Applications (10 copies) must be received not later than August 29, 1961, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained.

COURTAULDS

Research Chemists

SPONDON, DERBYSHIRE

BRITISH CELANESE LIMITED, part of the COURTAULDS GROUP, have a number of vacancies for Chemists at the Research Laboratories at Spondon, near Derby, which are part of the Courtaulds Research Organisation. These laboratories are concerned with exploratory investigations and process development in the fields of petrochemicals, general organic chemicals, polymers and plastics. Work in these fields covers a diverse range of topics and provides an absorbing challenge for organic or physical chemists.

The Research Laboratories operate a number of pilot plants which can provide valuable experience for anyone wishing to transfer later to production.

Candidates should write for a detailed form of application to the Director of Personnel, Courtaulds Limited, 16 St. Martin's-le-Grand, London, E.C.1, quoting reference number D.12/3.

BOC

SCIENTIFIC DIVISION

A few vacancies occur for:

PHYSICISTS

to investigate high and low temperature processes.

PHYSICAL CHEMISTS

for research in paints, plastics, and low temperature fields.

CHEMICAL ENGINEERS

to undertake low temperature studies concerned with the production and applications of atmospheric gases.

MECHANICAL ENGINEERS

to study low temperature plants and to design pilot plants.

Applicants should have a good honours degree or equivalent and are requested to write to:

The Administration Manager, Scientific Division
THE BRITISH OXYGEN COMPANY LIMITED
Deer Park Road, London, S.W.19

UNIVERSITY COLLEGE OF WALES

WELSH PLANT BREEDING STATION
PLAS GOGERDDAN, NEAR ABERYSTWYTH

Applications are invited for appointment to a vacancy in the Experimental Officer Grade at the Station. The vacancy is in the Cereal and Brassica Breeding Section, and the work is concerned primarily with recording and handling of breeding and genetical material. Applicants should be graduates in agriculture, botany, or agricultural botany. The commencing salary will be determined according to the age and experience of the successful applicant.

Applications, in writing, with the names of two referees, should be sent to the Secretary before August 10.

RESEARCH ASSOCIATION OF BRITISH FLOUR-MILLERS

Applications are invited for the following two posts: 1. Chemist with some research experience in physical or biochemistry to work on certain aspects of the chemistry of wheat protein. Commencing salary between £1,200 and £1,500 with membership of F.S.S.U. 2. Assistant (male) for small animal unit concerned mainly with nutritional research on flour and bread. Must have had some experience in the management of laboratory animals. Salary dependent on age and experience.

Applications, with details of qualifications, etc., to be sent to: The Director of Research, Cereals Research Station, Old London Road, St. Albans, Herts.

AUSTRALIA

THE UNIVERSITY OF NEW SOUTH WALES
CHAIR OF HOSPITAL ADMINISTRATION

The University of New South Wales invites applications for appointment to the Chair of Hospital Administration. The successful applicant will be responsible for the administration and development of the School of Hospital Administration and all courses provided by it, including those of its Department of External Studies. The School provides a course leading to the degree of Master of Hospital Administration and a full-time extension course in Hospital Administration. The Professor will be expected to collaborate in the work of the Faculty and serve on the Professorial Board and other committees. The salary will be £A.4,292 per annum. After passing a medical examination, the successful applicant will be eligible to join the State superannuation scheme, which provides for a maximum pension of £A.2,184 per annum. Professors are eligible for six months' study leave on full salary after three years of service or twelve months after six years of service. Subject to the consent of the Council of the University, Professors may engage in a limited amount of higher consultative work. With the approval of the University and its bankers, married men may be assisted by loans to purchase a home. First-class ship fares to Sydney of appointee and his family will be paid. The University reserves the right to fill the Chair by invitation.

Further details of conditions of appointment may be obtained from the Agent-General for New South Wales from the address given below. Four copies of applications, with the names of three referees and a recent photograph, should be lodged with the Agent-General for New South Wales, 56-57 Strand, London, W.C.2, and a copy forwarded to the Appointments Section, the University of New South Wales, Box 1, Post Office, Kensington, N.S.W., by airmail to arrive before September 8, 1961.

UNIVERSITY COLLEGE OF THE WEST INDIES

Applications are invited for (a) Lectureship or (b) Assistant Lectureship in Chemistry. Applicants should have special interest in Physical Chemistry. Salary scales: (a) £1,050 by £50 to £1,400 by £75 to £1,850 per annum; (b) £800 by £50 to £950 per annum, plus local allowance of £250 per annum (pensionable). Entry according to qualifications and experience, but in case of Assistant Lecturers and Lecturers below merit bar consideration is given to age in fixing salary. F.S.S.U. Child allowance. Settling-in allowance. Unfurnished accommodation at rental of 10 per cent of basic salary. Up to five full passages on appointment, normal termination, and triennial study leave.

Detailed applications (six copies), naming three referees, by August 14, 1961, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

RESEARCH FELLOWSHIPS

Vacancies exist for Research Fellows to work on the Tandem Van de Graaff generator, at A.W.R.E., Aldermaston.

The programme currently includes studies of charged particle reactions using proton, deuteron and triton beams with energies up to 14.5 MeV, heavy ion Coulomb excitation using 45 MeV oxygen ions and measurements of nuclear lifetimes. Plans for the installation of a pulsed injector of novel design to enable the Tandem generator to be used for neutron time of flight experiments are at an advanced stage.

Candidates for Research Fellowships must offer proof of their ability to carry out original research in Nuclear Physics and would normally be expected to be in possession of a Ph.D. degree.

Please write for further details to the Senior Recruitment Officer, A.W.R.E., U.K.A.E.A., Aldermaston, Berks, quoting ref. 2974/34.

MSL *have been retained to advise on this appointment.*

DIVISIONAL MANAGER High Vacuum

to create, within a major engineering group, a new team to work on the design and development of rotary and diffusion pumps with associated pipework and gauges for use in mass production processes and research work in scientific, mechanical and engineering fields.

He will be given assistance in staffing his design, drawing office, administrative and commercial department and all production facilities are already available.

Candidates must be graduate physicists or engineers, or hold equivalent institution membership (minimum A.Inst.P. or A.M.I.Mech.E.), with experience of leading a professional team in the design of high vacuum equipment. Administrative and commercial experience is very desirable.

Preferred age 35 to 40. Initial salary not less than £2,000 with pension and life insurance. Please send brief details in confidence to J. G. Smith quoting reference U.9217.

In no circumstances will a candidate's identity be disclosed to our client unless he gives permission after a confidential interview at which he will be given full details of the appointment.

MANAGEMENT SELECTION LIMITED
17 Stratton Street, London, W.1

UNIVERSITY OF LONDON
READERSHIP IN ELECTRICAL ENGINEERING AT IMPERIAL COLLEGE

The Senate invite applications for the Readership in Electrical Engineering with special interest in the properties of materials tenable at Imperial College. Candidates should be capable of initiating and guiding research into the properties of materials such as are related to future developments in electrical engineering. A large well equipped new laboratory will shortly be available. Salary scale £1,675 to £1,975 to £2,425 a year plus £80 London allowance.

Applications (ten copies), must be received not later than August 31, 1961, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF MANCHESTER
LINEAR ACCELERATOR PROGRAMME

Short-term appointments are available in the Department of Physics of the Faculty of Science for qualified Physicists or Electrical Engineers to assist with the construction and commissioning of a large heavy ion linear accelerator. Experience in one or more of the following fields would be desirable: high vacuum; R.F. techniques (75 Mc./s.); high power rectifier and pulse circuits; general electronic instrumentation and controls. The appointments are for one year in the first instance, and the salary range is £1,000 to £1,500 per annum.

Applications should be addressed to the Assistant Director, Linear Accelerator Laboratory, Physics Department, the University, Manchester 13, from whom further details may be obtained.

Central Electricity Generating Board

PHYSICS DIVISION

BERKELEY NUCLEAR LABORATORIES (GLOUCESTERSHIRE)

Applications are invited for posts in these Laboratories. Those appointed will work initially in the following sections, although subsequent transfer between sections will be possible, depending on the developing interests and abilities of the staff concerned.

A. INSTRUMENTATION

For work developing nuclear radiation detectors and other specialized nuclear instruments. Preference will be given to candidates who are capable of devising and developing new techniques of measurement.

B. GRAPHITE

To study the fundamental mechanisms and assess the extent of irradiation damage in graphite and the corresponding changes of physical properties.

C. THEORETICAL PHYSICS

To study various theoretical problems of interest to the Laboratories, including reactor physics and the theory of radiation damage.

- **Initial salaries up to £1,325 per annum are offered in accordance with experience, ability, and qualifications of persons appointed.**

Candidates must have a good honours degree and should be under 26 years of age.

Applications, stating age, qualifications, experience, present position, salary and quoting type of work interested in, to the Appointments Officer, 24/30 Holborn, London, E.C.1, by August 3. Quote Ref. N/317.

R.N. COLLEGE, GREENWICH, LONDON, S.E.10. Post for Senior Lecturer or Lecturer in Physics and Electrical Engineering available January, 1962. Candidates (male) must be British and possess an appropriate honours degree. Duties include lecturing and conducting laboratory work to degree standard and postgraduate teaching. Special experience in telecommunications an advantage. Excellent research facilities. Appointment as Senior Lecturer (£1,392 to £1,714) or Lecturer £773 to £1,267) according to age, qualifications and experience. Starting salary above minimum may be authorized. Post carries F.S.S.U. benefits. Single accommodation available.—Particulars and application forms from Director (P), Education Department, Admiralty, London, S.W.1. Closing date for applications August 31, 1961.

AUSTRALIAN NATIONAL UNIVERSITY

JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a Readership and a Fellowship in the Department of Biochemistry (Head of Department: Professor A. H. Ennor, D.Sc., F.A.A.). The Department is well equipped to carry out studies involving protein fractionation, enzyme purification, and most aspects of associated biochemical work. It is also provided with modern counting equipment for use with tritium, C^{14} and other radioactive isotopes. Applicants for the Readership will be expected to have had extensive experience in biochemistry with emphasis on enzymology and intermediary metabolism. They will also be expected to have made substantial published contributions to the subject. The salary range for a Reader is £A.3,250 to £A.3,750 per annum, and the successful applicant will be made a permanent member of the University staff. A Reader is entitled to one year's study leave on full pay, plus a contribution towards travel and other expenses, in every six years. Applicants for the Fellowship will be expected to have had considerable experience in enzymology or in intermediary metabolism. The salary range for a Fellow is determined within the limits £A.2,120 to £A.3,000 per annum. Appointment is for an initial period of five years and thereafter may be made permanent. A Fellow is entitled to one year's study leave on full pay, plus a contribution towards travel and other expenses, in every seven years. In both cases commencing salary will depend on qualifications and experience. Superannuation is on the F.S.S.U. pattern. Reasonable travel expenses are paid and assistance with housing is provided.

Further details should be obtained from the Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1. Applications close, in Australia and London, on September 1, 1961.

AUSTRALIAN NATIONAL UNIVERSITY

JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

DEPARTMENT OF PHYSIOLOGY

Applications are invited for a Fellowship in the Department of Physiology. Applicants will be expected to have specialized in organic chemistry, with special reference to the chemistry of compounds of neuropharmacological significance. The salary range for a Fellow is determined within the limits £A.2,120 to £A.3,000. Appointment is for an initial period of five years and thereafter may be made permanent. A Fellow is entitled to one year's study leave on full pay, plus a contribution towards travel and other expenses, in every seven years. The commencing salary will depend on qualifications and experience. Superannuation is on the F.S.S.U. pattern. Reasonable travel expenses are paid and assistance with housing is provided.

Further details should be obtained from the Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1. Applications close, in Australia and London, on August 14, 1961.

UNIVERSITY OF ADELAIDE

WAITE AGRICULTURAL RESEARCH INSTITUTE

The University invites applications for appointment at the Waite Institute as Lecturer in Crop Ecology. Duties: To undertake ecological or physiological studies on the lucerne crop, and to evaluate and develop lucerne ecotypes for grazing. The appointment is financed by a grant from the Wool Research Trust Fund and will be made within the Lecturer salary range of £A.1,675 by A.95 to £A.2,340, with provision for superannuation on the F.S.S.U. basis. The appointment is for three years in the first instance, renewable annually thereafter. The Waite Institute has excellent facilities in field, glasshouse, and laboratory, and the lecturer will be associated with a considerable group of colleagues in his own field of study.

Further information about the University and this appointment and the general conditions of appointment can be obtained from the Registrar of the University or the Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1. Applications, in duplicate, setting out the information specified in the last paragraph of the general conditions, should be lodged with the Registrar, The University of Adelaide, Adelaide, South Australia, not later than August 31, 1961.

A SENIOR PHARMACOLOGIST

To be in charge of the Pharmacology Laboratories which are at present in the course of construction for Reckitt and Sons Ltd.

This Company is a member of the Reckitt and Colman Holdings Group, which has considerable interests in pharmaceutical, foodstuff, household, cosmetic, horticultural and other product groups in Britain and in over 25 countries overseas.

The headquarters of pharmaceutical research and development are in Hull, where the existing Laboratories are being extended to provide new Laboratories for research in pharmacology, biochemistry, pathology and histology.

The Organic Chemistry Laboratory is already established and well-equipped to support the new Pharmacology Laboratory in research directed to the development of medicinal chemicals.

There is a good Library and Information Service and a Statistical Department is available for help in the planning and analysis of experimental work.

The Senior Pharmacologist should have some years' post-doctoral experience, either in an academic institution or in an industrial establishment, and will be encouraged to pursue special research interests. The conditions of work and employment are excellent and the attitude towards research is liberal.

The salary paid will be a matter for individual discussion. There is a good contributory pension fund with provision for widows and dependants, and a Prosperity Sharing Scheme. Assistance will be given in securing housing accommodation.

Inquiries will be treated in strict confidence and should be addressed to the Technical Director, Reckitt and Sons Ltd., Dansom Lane, Hull.

AUSTRALIAN ATOMIC ENERGY COMMISSION

RESEARCH ESTABLISHMENT

REACTOR PHYSICS

Applications are invited for the following senior positions in the Reactor Physics Section at the Research Establishment, Lucas Heights, N.S.W.

Grade: Senior or Principal Research Officer.

Salary: £2,395/£2,720 or £2,980/£3,500 (Aust.).

Duties: (Position R.18.) To take charge of a group working on sub-critical and critical reactor problems and experiments associated with the AAEC high temperature gas-cooled reactor programme. Major equipment includes HIFAR, the 10 MW DIDO-class reactor and MOATA, the 10 KW UTR-10 type reactor.

Duties: (Position R.80.) To carry out neutron physics studies both of a fundamental nature and also associated with the AAEC high temperature gas-cooled reactor programme, particularly neutron energy spectrum, spectrum, conversion ratio and resonance integral measurements, and general neutron reaction studies over a wide energy range.

Qualifications: Honours or Higher Degree in Physics and several years' relevant experience.

Travel: Under specified conditions, first-class air/sea transport for the appointee and dependants (wife and dependent children) will be provided.

Contract Employment: Consideration may be given to a contract agreement if the appointee desires to enter into an agreement for a three (3) year contract of employment in Australia. In this case, the AAEC will bear the cost of the return fares together with assistance for removing the appointee's furniture and effects.

Applications: Forms obtainable from AAEC Liaison Officer, Australia House, Strand, London, W.C.2. OTHER POSITIONS are vacant in these groups in the Research and Senior Research Officer range £1,510 to £2,265 and £2,395 to £2,720 (Aust.). Enquiries to the above address.

PATHOLOGICAL LABORATORY TECHNICIAN

Applications are invited for the position of Pathological Laboratory Technician with a well-known firm of pet food manufacturers.

The successful applicant should be a member of the Institute of Medical Laboratory Technicians, and have passed the intermediate examination of the Institute. Experience should include all branches of this field including Histology.

Starting salary will not be less than £950, rising to a maximum of £1,400 per annum.

In addition the company operates generous non-contributory pension, sickness and life assurance schemes.

Write for an application form giving brief details to the:

Personnel Officer (S),
PETFOODS LIMITED
Melton Mowbray, Leics.

UNIVERSITY OF QUEENSLAND

LECTURER IN MECHANICAL ENGINEERING

The University invites applications for the above-mentioned position. Applicants should possess a Degree, preferably with Honours, in Mechanical Engineering of a recognized university. Some industrial experience is highly desirable. Previous teaching experience, although desirable, is not absolutely essential. The appointee will be required to conduct lectures, laboratory and drawing office work in the fields of Applied Mechanics (including dynamics and stress analysis) and Engineering Drawing and Design. Salary £A.1,830 by £A.70 to £A.2,330 per annum. The Department has a very active research and postgraduate school and has already achieved world standing for its research into the mechanical milling of sugar cane and the application of solar energy to air-conditioning. It possesses well equipped laboratories, including a machine tool laboratory and workshop capable of precision work, and an excellent Metrology Laboratory which is registered by the Australian National Association of Testing Authorities. The successful applicant will be entitled to participate in the benefits available to the academic staff which include F.S.S.U. type superannuation, housing assistance, study leave, and travel grants.

Additional information on the conditions of appointment, staffing, and activities of the Department, together with application forms, will be supplied on request to the Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1. Applications close, in Australia and London, on August 31, 1961.

HUDDERSFIELD COLLEGE OF TECHNOLOGY

Principal: Dr. W. E. Scott, M.B.E.

DEPARTMENT OF PHYSICS

Required September 1, 1961, or earlier if possible, Grade B Assistant to teach Physics. Salary (Burnham Scale) £700 by £27 10s. to £1,150 per annum. There are additions for approved qualifications and training, and the commencing salary may include increments for approved industrial experience.

Forms of application and further particulars obtainable from the Principal, to whom applications should be returned without delay.

H. GRAY,
Clerk to the Governors.

BACTERIOLOGICAL TECHNICIAN

A technician aged 20 upwards is required in the Heinz Bacteriological Laboratory where he will be engaged primarily on heat sterilisation problems.

This opening would be suitable to a young man who has achieved one of the following qualifications:

Intermediate I.M.L.T.
Endorsed Certificate (Inst. of Biol.).
Membership I.S.T.

but practical experience in Bacteriology is the most important consideration.

Conditions of employment are excellent and include a non-contributory pension and life assurance.

Further study is actively encouraged by the Company and an excellent Tuition Aid Scheme is in operation.

Please apply with a brief summary of personal history to date to:

The Personnel Officer,
H. J. Heinz Company Limited,
Waxlow Road, Harlesden,
London, N.W.10.

RESEARCH CHEMIST REQUIRED BY A large London Brewery. Applicants should have a first-class honours degree. Industrial experience not necessary. Interesting work and good remuneration.—Please write, giving full details of education and qualifications, to Box 978, T. G. Scott and Son, Ltd., 1 Clement's Inn, London, W.C.2.

INSTITUTE OF CANCER RESEARCH: Royal Cancer Hospital. Technical Assistant required in Chemical Section of laboratory engaged in radiobiological research at Sutton, Surrey. Varied work involving a wide range of chemical and physical techniques. Salary on M.R.C. scales within range £370 to £860 according to age, qualifications and experience.—Applications, with names of two referees, to the Secretary, 33 Sumner Place, S.W.7, quoting ref. 301/B/59.

UNIVERSITY OF CAPE TOWN

SENIOR LECTURER OR LECTURER IN ZOOLOGY

Applications are invited for the above-mentioned post. The duties attaching to the post will include teaching and laboratory supervision of students and some examining and organizing of courses. The successful applicant will also be expected to undertake research work. A thorough knowledge of Comparative and General Physiology is essential. The appointment will be made at the Lecturer or Senior Lecturer grade according to qualifications and experience. The Senior Lecturer will be required to take charge of degree courses in General Physiology and to give instruction in Comparative Animal Physiology for advanced students in zoology. The lecturer will be required to perform similar duties but will not be in charge of the teaching in General Physiology. The salary scale is R.3,520 by R.120 to R.4.120 (£1,760 by £60 to £2,060) per annum for a Senior Lecturer and R.2,680 by R.120 to R.3,520 (£1,340 by £60 to £1,760) per annum for a Lecturer. Applicants should state age, experience, academic qualifications, research work completed or in progress, and give the names of two referees whom the University may consult.

Two copies of the application and testimonials should reach the Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (from whom memoranda giving the general conditions of appointment, including transport expenses on appointment, and information on the work of the department may be obtained) not later than August 21, 1961. An additional copy should be sent direct by airmail to the Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa, to reach him by the same date. The University reserves the right to appoint a person other than one of the applicants or to make no appointment.

UNIVERSITY OF KHARTOUM

Applications are invited for the appointment of two Senior Technicians or Technicians in the Faculty of Veterinary Science: 1. In the Department of Animal Health. 2. In the Pre-Clinical Department. Minimum qualification Certificate of Membership of the Institute of Laboratory Technicians (M.I.L.T.) or its equivalent, plus for the first post, at least five years experience in bacteriological techniques in a recognized veterinary or medical institution; and for the second post, at least five years experience in various techniques of histological preparations and anatomical museum specimens. Salary scales: Senior Technician, £S.1,250 by £S.50 to £S.1,500 per annum; Technician, £S.950 by £S.50 to £S.1,200 per annum. Entry point according to qualifications and experience. Generous allowances are payable. Passages for appointee and family on appoinment, termination and annual leave. There is a superannuation scheme and arrangements can be made to maintain F.S.S.U. policies. Appointment on contract for five years with possibility of renewal. Unfurnished accommodation provided at rent (excluding rates) up to 7½ per cent of salary.

Applications (six copies), detailing qualifications and experience and naming three referees by August 18, 1961, to Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

UNIVERSITY OF ST. ANDREWS

Applications are invited for the posts of Research Assistant and Research Student in the Wellcome Laboratories of Pharmacology, Gatty Marine Laboratory, St. Andrews. Candidates should be suitably qualified in zoology, physiology, biochemistry or chemistry, for work on the endocrinology of lower vertebrates. Suitable candidates may register for a higher degree. Salary scale for Research Assistant £700 to £800; for Research Student £400 to £500; appointment for three years in the first instance.

Applications, with names of three referees, should be lodged, not later than July 31, 1961, with the Joint Clerk to the University Court, College Gate, St. Andrews.

APPLICATIONS ARE INVITED FOR A post as Research Assistant to take part in a programme designed to study human chromosome abnormalities and associated disorders. Experience in cytology desirable, but not essential. Suitable for candidates graduating this year. There may be opportunity to read for a higher degree. Salary £700 to £900 per annum, according to qualifications and experience.—For further details please apply to the Secretary, Genetics Department, the University, Sheffield, 10.

CONSERVATION OF WATER IN GREAT BRITAIN

WATER has been the key to man's advancement since before the beginning of recorded history. Not only do we need it for personal use, but it is also essential to grow crops, to water stock, to move cargoes, and to dispose of wastes. Many manufacturing processes depend on this precious liquid: it is the universal solvent, the universal coolant. It powers part of industry, and when work is done it is essential to much recreational activity. Without water, life as we know it would cease. To-day, this vital raw material is assuming an ever-increasing importance as the need for it and water-related services and products grow. Inconvenience arises by both an absence or over-abundance of water. The conservation of water therefore becomes of importance whenever extremes are experienced, or when the demand for water by a growing community begins to exceed the minimum supply available. There has been for some years now mounting evidence that we are rapidly approaching a time when the latter situation will arise in Great Britain.

In 1959, as a result of a dry summer, some ten million people (nearly one quarter of the population of England and Wales) were affected by drought orders or restrictions on their water supply. Moreover, although 1959 had a remarkably dry summer the year as a whole was not exceptionally dry—there have been ten drier years in the present century, notably in 1955. These restrictions demonstrated the already known fact that Great Britain's defences against drought have been a diminishing quantity for some time[1]. On the other hand, the above-average rainfall in the autumn of 1960, coming after a wet summer, led to very serious and prolonged flooding in a number of areas. These experiences now seem to have convinced the central Government authorities that there is an increasing need to regard water as a prime natural resource basic to our existence, and to legislate for its conservation and proper use.

The past year or so has seen the welcome appearance of a number of valuable reports and publications, notably the hydrological surveys and the annual year books issued by the Surface Water Survey[2], together with the reports of the Central Advisory Water Committee[3] and the Welsh Advisory Water Committee[4]. The British National Committee for Geodesy and Geophysics has also issued a useful report on current hydrological activity in the United Kingdom[5].

Valuable as these reports are, it must be admitted that they do not match up to the remarkable study of the same problem undertaken in the United States during the past two years by the Senate Select Committee on National Water Resources[6]. The British reports, however, represent a significant step in the right direction, and accompanying them have been welcome indications that the Ministry of Housing and Local Government is now preparing the way for new legislation to meet the future water needs of the country. In June 1960 the Minister stated: "We are approaching the point at which we may very possibly have to take a new look at water conservation. . . . It may be that the time is not far distant when we have to look at all the resources of a river basin as a whole in the widest possible way, to make sure that all the various and sometimes conflicting interests are properly served; that the river is kept clean and the interests of fishing, navigation and so on are safeguarded, while, at the same time, the maximum amount of water is made available to all kinds of users as economically as possible".

Since this announcement, one of the sub-committees of the Central Advisory Water Committee has made an investigation into the complex problems involved in achieving this end, and the Committee is now engaged on the preparation of a report. In view of the public concern and of the wide support that can be expected for a bold approach, the Minister has decided to open confidential discussions with representatives of affected interests, both private and public, in advance of the report. An indication has therefore recently been given by the Ministry of the proposals which it has in mind as a basis for legislation dealing with water conservation.

There has been support in some quarters for a Central Water Authority mainly concerned with policy and planning, but also possessing powers of direction. It has been suggested that such an authority should have a regional organization with executive powers, and ideas on its constitution have varied from a body such as the Central Advisory Water Committee with extended functions, to a newly appointed permanent body of persons with specialist experience and knowledge in water problems. This proposition has been considered, but has been rejected by the Minister on grounds of constitutional and administrative difficulties.

It is proposed instead to set up conservation authorities and to re-define the powers and responsibilities of the Minister. Section 1 of the Water Act, 1945, requires the Minister "to promote the conservation and proper use of water resources and the provision of water supplies in England and Wales and to secure the effective execution by water undertakers under his control and direction, of a national policy relating to water". The present statutory provisions, however, are ill-suited for the execution of this policy: thus the only specific provision to enable conservation to be carried out is to be found in section 14 of the Act, which empowers the Minister to control new abstractions of underground water in those parts of the country designated as conservation areas. So far as surface water is concerned, there is no provision whereby both the development and allocation of supplies can be controlled in the light of an overall plan which has regard to all the various interests involved. It has been clear to many and for some time now that if we are to make the best use of our water resources and due regard is to be paid to future needs, then a completely new comprehensive approach is urgently needed.

The Minister has accepted that any new approach should be based on certain propositions which are beyond argument. These are briefly: (*a*) that the approach to the problem should be by way of the natural hydrological unit of the river basin; (*b*) that surface and underground water are interdependent and any measures for development and control must be related to both; (*c*) that the machinery of conservation must be capable of taking into account all the conflicting interests; (*d*) that regulatory powers alone are insufficient and that a positive policy involving new works will in certain cases be necessary; (*e*) that a need will exist for some central co-ordinating and appellate authority.

It is therefore proposed to establish water conservation authorities (their exact title has yet to be decided) founded on river basins or groups of river basins. The question immediately arises whether the new organizations should be set up in parallel with the existing river boards. In the Minister's view, conservation functions and those at present carried out by river boards are so interdependent that they should be the responsibility of a single authority in each area.

It is thought that the new conservation authorities should be responsible for: (*a*) keeping a complete up-to-date hydrological survey of the area; (*b*) the preparation of an overall plan for the development of the water resources; (*c*) the control and development of the resources; (*d*) the allocation of water between users by means of a licensing system, subject to a right of appeal to the central authority; (*e*) the recharge of underground aquifers, the discharge of compensation water and the transfer of water between conservation areas; (*f*) land drainage, flood prevention and the control of pollution; (*g*) the administration of salmon and freshwater fisheries legislation, and the reconciliation of navigational needs with the above interests.

The new approach is therefore comprehensive, and all water-using interests in a particular river basin would be brought under the one umbrella of the conservation authority. This is, in fact, the logical pattern which has been and is being followed in the development of many new overseas territories. The existing system within England and Wales has grown up piecemeal over the centuries, and we now have a great multiplicity of bodies concerned with all the above aspects, and the boundaries of which rarely reflect the basic hydrological and geographical facts. The transformation will clearly not be achieved without some opposition and will take some time. It is important, however, that progress on these lines should be made in order to ensure adequate supplies of water for future generations.

The financial aspects of the proposals are likely to be complex and will need careful thought. It is proposed to hold discussions with affected interests before any final report is issued. The principle that the cost of the main land-drainage system of an area should be financed by the community it serves has long been established and is not changed by the proposals of the Land Drainage Bill at present before Parliament. Therefore, as land-drainage authorities it is thought that the new conservation bodies should inherit from the river boards those boards' sources of income, that is, precepts on local authorities and on internal drainage boards, the proposed general and special drainage charges and the Exchequer grants payable to the boards at the present time.

As conservation authorities, expenditure would be incurred on administration and control, research, and on the building and maintenance of a variety of works to ensure the best use of the water resources. It is thought that this expenditure should be self-supporting on the basis of income received from water users within the area. The exact method by which this income would be raised has yet to be decided: licence fees, consumption charges, flat rates, etc., will be fully considered. There is, however, likely to be a special feature in the early years in that some major works will be desirable well in advance of the maximum demands which are likely to arise. Present-day users cannot always be expected to meet the full cost of works which will serve the interests of the new generation, and the Minister proposes that authorities should be empowered to levy a small precept (possibly not more than the product of a 1*d*. rate) for conservation works. In time it is thought that the water conservation authorities would be able to accumulate reserves against forthcoming capital expenditure and that the need for precepts would become less.

The financial suggestions are somewhat less definite than the hydrological proposals, and one can foresee a good deal of difficulty arising because of the inequality in the distribution of water resources, population and other economic resources. The intention not to seek powers to make Exchequer grants towards expenditure might well prove to be a pious hope. Some schemes are likely to be beyond the financial capabilities of the parent river basin and if completed might benefit larger areas. It would be desirable to consider the country as a whole as well as the individual parts. Clearly, much discussion and thought will be needed before the financial aspects are presented to Parliament.

The constitution of the water conservation authorities will also need careful thought. The principle underlying the constitution of the river boards is that those interests contributing to their revenues should be represented on them. This principle applied to the proposed conservation authorities would produce a large and unwieldy body. An alternative would be a small nominated group appointed by the Minister in consultation with the interests concerned. There is a constitutional difficulty here in that, apart from the Metropolitan Police, nominated bodies have not the power to precept. However, the water conservation authorities will themselves be unprecedented, and it may be that the interests concerned would be prepared to accept a nominated body. Another alternative would be a body half elected and half nominated: here the

Milford Haven Conservancy Act of 1958 might provide a guide.

It will be clear that some of the Minister's existing functions under the 1945 Act would with the above proposals pass to the water conservation authorities. The Minister's powers and responsibilities with regard to conservation would need re-defining in order to cover the co-ordination of the conservation authorities' assessments of local resources and potentialities; to watch from the national point of view the demand for water for domestic, industrial, agricultural and other purposes; to approve conservation schemes; to authorize and initiate schemes for inter-basin transference of water; to exercise appellate functions arising from decisions of the conservation authorities; and to approve capital investment programmes of the conservation authorities.

A new approach will also be necessary on the legal rights to particular water resources. Parliament has already considered it proper to restrict in some cases the right, arising from ownership of land, to take the water from beneath the land. It is further intended to amend the law so as to restrict the amount of water which can be taken without licence from boreholes sunk before control was applied, as well as to extend control to all underground waters. In the case of right to surface water, more particularly the rights of a riparian owner to use water flowing in a stream, it is proposed to provide that such an owner will be able to obtain as a right, from the conservation authority, a licence entitling him to the ordinary use of the water flowing past his land. "Ordinary" use will not include abstraction for irrigation and industrial purposes, and these uses will involve separate licences.

With water-storage capacity in Britain falling steadily behind rising consumption-rates, insurance against drought diminishes year by year and the need for both action and speed is pressing. The proposals outlined above are a welcome indication that the central Government authorities are at last alive to the necessity for comprehensive measures.

---

[1] Balchin, W. G. V., *Geog. J.*, **124**, Pt. 4 (December 1958).

[2] See *Nature*, **189**, 278 (1961).

[3] See *Nature*, **184**, B.A. 18 (1959).

[4] See *Nature*, **191**, 135 (1961).

[5] "Hydrological Activity in the United Kingdom", The Royal Society, London (1960).

[6] See *Nature*, **187**, 562 (1960); and p. 444 of this issue.

ACHIEVEMENTS IN PHYSICS

The New Age in Physics

By Prof. Sir Harrie Massey. Pp. 342+12 plates. (London: Elek Books, Ltd., 1960.) 42*s*. net.

ABOUT thirty years ago, physical science seemed to many of its students to have reached a peak of perfection. The nuclear atom held an established position and the power of quantum theory in interpreting atomic phenomena had been abundantly confirmed. Observational astronomy and thermodynamics had combined to predict the probable fate of the Sun and stars; only details apparently remained to be fitted into what was considered to be an elegant and satisfactory picture of the physical universe. One of the marks of this stage of achievement was the appearance of books of broad coverage, notably those of Jeans and Eddington, which sought to stimulate the interest of the general reader in the processes of scientific thought and experiment. The significance of these books was not diminished by the fact that the apparent self-sufficiency of physics in 1930 rapidly proved to be illusory. The spectacular discoveries in nuclear physics in the following decade became, as a result of awakening interest, increasingly matters of public concern.

By the end of the Second World War, physics was accepted as one of the basic elements of a scientific civilization, and it is now a commonplace that some knowledge of the subject and of its applications in contemporary affairs should form part of general education. Even more than in the days of Jeans and Eddington, there is a need for sober and balanced, but easily readable, works of exposition. It is to this need that Sir Harrie Massey's book makes a notable contribution.

The New Age in Physics is a book for the educated layman, but it can be read with pleasure and profit by the professional physicist. The mathematics used is of the simplest kind, but is sufficient to show that physics is above all a quantitative subject. Most of the book is devoted to atomic, nuclear and high-energy physics, and this is right, since it is on the atomic and nuclear scale that the new laws of physics exhibit their strangest manifestations. Sir Harrie Massey is known to the general public as an enthusiastic supporter of the space research programme, and it is therefore not surprising to find his chapter on neutrino phenomena ending with an account of hydrogen burning in the Sun, and his next (and final) three chapters devoted to radio astronomy, exploring the upper atmosphere, and artificial satellites and space probes. These chapters, like that on fission and fusion, are admirably lucid and compact accounts of the developments in physics which have most deeply excited the popular imagination. They will probably be more fully appreciated by the general reader than the rest of the book, which is concerned as much with concepts as with facts. Few non-scientific readers, however much they enjoy the adventures of Alice in "Looking-Glass House", will really find themselves greatly moved to learn that mirror symmetry is violated in weak interactions. Nor is the polarization of the vacuum a matter of everyday experience, although the ideas of special relativity are being increasingly discussed as interplanetary travel enters more within the bounds of possibility.

In his introduction the author sets out his intention to "choose a number of outstanding theoretical and experimental developments which illustrate the remarkable position which has been reached and the extraordinary rate of progress". In the event, one may comment that little choice has been necessary, since practically everything of general interest in atomic and nuclear physics, in the physics of the solid state, and in that of the solar system receives some mention. It is a pleasure to have authoritative and informative accounts of the contents of such an extensive field, and to sense an underlying unity of treatment which is often lacking in works of similar scope. The book gains much from well-chosen illustrations, and suffers but little from the finite number of misprints and the occasional errors in dates. It has an air of currency, but even so, it has just missed the Mössbauer effect, and it would be entirely consistent with the author's belief in the inexhaustible bounty of Nature if some outstanding event were to occur before the book reached a second edition. Perhaps one may hope that when the stage is set and the space station exists, mankind may be offered the spectacle of another supernova in his own galaxy.

W. E. BURCHAM

A NEW APPROACH TO GENERAL RELATIVITY

Relativity

The General Theory. By J. L. Synge. (Series in Physics.) Pp. xv+505. (Amsterdam: North-Holland Publishing Company; New York: Interscience Publishers, Inc., 1960.) 110*s*.

THIS massive treatise is no mere conventional text-book of Einstein's theory of gravitation. On the contrary, although by a master of that subject, it is a highly individual work. For, although the author claims that the book is conservative in so far as it deals mainly with Einstein's theory in its original form, leading up to the famous triad of empirical tests, the mathematical treatment is novel. This treatment derives from Synge's conviction that, great as was Einstein's achievement, the true revolution in mental perspective was due to Minkowski, although this has not yet been fully appreciated by most students of the subject.

In accordance with a recent tendency among some students of relativity, Synge will have nothing to do with the principle of equivalence. Instead, he maintains that in Einstein's theory the existence of a gravitational field depends entirely on whether or not the curvature tensor does not or does vanish. This is an absolute property of space-time and does not depend on the observer. Synge accordingly endorses Minkowski's protest against the use of the word 'relativity' to describe a theory based on an 'absolute' (space-time). Indeed, he remarks that if Minkowski had lived to see the general theory of relativity he would have repeated his protest in stronger terms.

Although Synge's outlook is geometrical, in the sense that it is based on a thorough-going acceptance of the space-time way of looking at things with its fixed diagrams which are "easier to deal with" than the kinematical pictures of Newtonian mechanics, the peculiarity of his approach lies in combining this point of view with a deep conviction that time is the only basic measure, distance being essentially a derived concept. Consequently, Synge's version of general relativity is based on what he prefers to call Riemannian 'chronometry' rather than 'geometry'. This leads him to make general relativity more 'operational' (in Bridgman's sense) and thus more closely related to special relativity than has previously been customary. In this respect, I believe that Synge has been indirectly influenced by the pioneer example of kinematic relativity.

In considering the geodesic world-line of a particle in free fall, Synge interprets gravitational acceleration as a consequence of the curvature of the world-line of the observer. He is thus led to develop a new method of gravitational analysis based on a function first introduced in 1931 by H. S. Ruse. This function, which Synge calls the 'world-function,' is, to within a trivial factor, the square of the geodesic distance between two events in space-time, regarded as a function of the eight co-ordinates concerned. His use of this function has the advantage of combining the power-series technique with that of the tensor calculus.

Among the many topics discussed are Fermi transport, the material continuum and field equations, integral conservation laws and equations of motion, special universes, gravitational waves and geometrical optics. There is an extensive bibliography with references to reviews. The print is clear and the formal mathematics is relieved from time to time by the author's irony and wit. Irrespective of whether one accepts his point of view, his book will be found original and stimulating.

G. J. WHITROW

NEW HANDBOOKS FOR LUNAR STUDIES

Structure of the Moon's Surface

By Dr. Gilbert Fielder. Pp. xiii+266. (London and New York: Pergamon Press, 1961.) 50*s*. net.

Surface of the Moon

Its Structure and Origin. By V. A. Firsoff. Pp. 128+10 plates. (London: Hutchinson and Co. (Publishers), Ltd., 1961.) 21*s*. net.

Orthographic Atlas of the Moon

Compiled by D. W. G. Arthur and E. A. Whitaker. Edited by Gerard P. Kuiper. Edition "A" showing the Standard Orthographic Coordinate Grid. Part 1: Central Area. (Contributions, Lunar and Planetary Laboratory, No. 1.) Pp. 3+31 plates. (Tucson: University of Arizona Press, 1960.) n.p.

RESEARCH into the nature of the Moon's surface has become increasingly intensive in recent years. This increase can partly be ascribed to the advances in 'space-science', in particular the Russian achievement in photographing the Moon's 'other face'. However, new ideas had begun to appear before this. Spurr's *Geology Applied to Selenogy* was published between 1944 and 1949; although neither Dr. Fielder nor Mr. Firsoff subscribe to Spurr's speculative theories, they both believe that terrestrial geology is of prime importance in interpreting the appearance of the Moon's surface. They also share the view that the morphology of the surface features is a more rewarding line of research than the recording of ever smaller minutiæ.

Dr. Fielder's book is a complete handbook to the Moon. It is divided into two parts: the first describes the methods used to determine the physical properties of the Moon's surface and discusses the available observations. The second part is devoted to the morphology of the lunar surface, where the author's own researches play a more prominent part. Ray systems, the grid system, rilles and wrinkle ridges, and the distribution and frequency of craters are subjects well fitted to this approach. The book concludes with a chapter on the origin of the Moon's surface. This does not support any particular theory but is confined to what may reasonably be inferred from observation. Dr. Fielder has covered his subject completely and thoroughly—the only faults are occasional lapses into irrelevance.

For many years there have been two rival theories for the formation of lunar craters—the meteoric, which explains them as the result of meteors striking the surface, and the volcanic, which identifies them with extinct volcanoes. Mr. Firsoff's book is an exposition of his own theory, which is derived from the latter. The craters are now identified as collapsed volcanic calderas, the result of the collapse of the surface layers after the magma beneath them had been spewed out in a volcanic eruption. The book is divided into three parts of which the first outlines what is known about the Moon. The second examines the inadequacies of the meteoric theory and puts forward the 'caldera' theory. The third is concerned with reducing our present knowledge of the Moon's surface to a coherent system; it is largely devoted to an examination of the analogies between the surfaces of the Moon and Earth. The chief criticism to be levelled at this clear and closely reasoned book is the reckless use of the word 'random'. In a scientific work this word should be confined to its statistical sense.

In 1960 a team of workers led by G. P. Kuiper published their *Photographic Lunar Atlas*. This covered every part of the Moon's surface four times, at differing angles of illumination, on a scale of one millimetre to every 1·37 km. on the Moon. The group have now published the first of a series of supplements. Twenty-nine of the best prints in the *Atlas* have been chosen to give a single coverage of the central portion of the Moon's disk. These are over-printed with a grid showing the orthographic ξ,η co-ordinates, in other words, rectangular co-ordinates fixed in the Moon with ξ axis northwards and ζ axis towards the Earth. The side of each square is 0·01 of the Moon's radius and positions can easily be read to an accuracy of 0·001. There are two editions: (*A*), on paper as described above and (*B*), on 'Tensalex' plastic, with an additional grid showing selenocentric longitude and latitude at 2° intervals.

D. H. P. JONES

ISSUES IN EVOLUTION

Evolution after Darwin

Edited by Sol Tax and Charles Callender. Vol. 3: Issues in Evolution. Pp. viii + 310. (Chicago: University of Chicago Press; London: Cambridge University Press, 1960.) 60*s*. net.

THIS is the third volume of the Darwinian Centennial Symposium held at Chicago in 1959 (see *Nature*, 189, 340; 1961 for review of previous volumes). It comprises a first section on "Evolution and Religion" presented as essays on "Creation and Evolution in the Far East" by Ilza Veith, "Current Roman Catholic Thought on Evolution" by J. Franklin Ewing, *S. J.*, and "Creation and Causality in the History of Christian Thought" by Jaroslav Pelikan (it is refreshing to note that a Roman Catholic view has been included). The second section is given over to the centennial celebrations, made up chiefly of a television preview panel of five scientists and including Adlai Stevenson, five panels on the origin of life, the evolution of life, man as an organism, the evolution of mind, and social and cultural evolution, with a variety of chairmen and panellists. There is a convocation address by Sir Julian Huxley, a final television postscript panel and a personal conclusion by the general editor, Sol Tax. The index to the three volumes is included in this third volume, and there are also lists and photographs of the participants and panels.

The five panels were essentially a form of glorified 'brains trust' on a high level, and they were all given in public to audiences of about a thousand people. Members of the panels for the most part were generally those who had previously given papers in the first two volumes of this series. Thus the opportunity was given to tie up loose ends, to give the participants a chance to air their agreements and disagreements, and to put forward their ideas. These panels were most interesting, and probably their chief result will be to enable readers of the whole series of essays to sort out facts from speculations and to assess the present state of evolutionary ideas.

It is perhaps significant that the three essays on "Evolution and Religion" together amounted to only 40 pages, less than half the number of pages of some of the essays on specialized topics in the first two volumes. About 90 pages were given to such subjects as the "Origin of Life"—a subject which surely by any serious standard is about 99 per cent speculation and fancy and only 1 per cent fact. The contrast here is illuminating, but, dare I say it without fear of excommunication by my fellow biologists, it also pinpoints the paucity of evolutionary theory as a whole in spite of the brilliant expositions of its magnificent edifice by so many scientists; it also shows the weakness and barrenness of a purely materialistic concept of evolution and indicates without doubt that modern evolutionary theory has only a limited application to human beings. Why must biologists shut their eyes to the spiritual in man, pretend it is not there and thus remain only on the fringes of the study of the nature of man, who is demonstrably infinitely more than a mere animal? It is a tragedy that a wonderful chance to produce a modern synthesis of knowledge of man and evolution has been missed in this great and superbly produced series of books born of a great idea by Sol Tax. But still the three books as a whole stand as probably the best of their kind so far published, and the committee under Sol Tax, the contributors and the University of Chicago, which made it possible, are to be congratulated and thanked.

P. G. FOTHERGILL

HETEROCYCLIC CHEMISTRY

Pyridine and Its Derivatives

Edited by Erwin Klingsberg. (The Chemistry of Heterocyclic Compounds: a Series of Monographs, Vol. 14). Part 1. Pp. x + 613. 367*s*. Part 2. Pp. x + 576. 282*s*. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960 and 1961.)

Heterocyclic Systems with Bridgehead Nitrogen Atoms

Part 1. By William L. Mosby. (The Chemistry of Heterocyclic Compounds: a Series of Monographs, Vol. 15). Pp. viii + 747. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1961.) 360*s*.

WITH two or three exceptions, previous volumes in the Interscience Series, *The Chemistry of Heterocyclic Compounds*, have tended to dwell on the less common heterocyclic systems. This is true of only one of the three volumes under review, and it is interesting to see the treatment accorded to a more familiar topic—*Pyridine and its Derivatives*. The first two volumes discussed here constitute Parts 1 and 2 of a four-volume treatise, which will surely become the standard reference work on pyridine chemistry and a worthy successor to Maier-Bode's *Das Pyridin*.

Volume 1 comprises two chapters, the first of which is devoted to an introductory account of the physical properties and chemical reactions of pyridine and its simple derivatives. This is, in itself, a useful 90-page summary of pyridine chemistry and the author has made an admirable job of correlating and unifying the subject in terms of electronic theory. He has also provided an appropriate introduction to the remaining volumes of the treatise which deal, in detail, with a number of specialized topics. The remaining chapter of the first volume is entitled "Synthetic and Natural Sources of the Pyridine Ring", and is both comprehensive (500 pp.), and systematic. Every known synthetic method appears to have been included, and several intriguing, little-known reactions are brought to light. (Is benzene really transformed into pyridine by carbonyl azide ?) Also included is an interesting table of pyridine derivatives which have been encountered in alkaloid degradation reactions. A feature of this chapter is the inclusion of 240 pages of tables, illustrating the scope of each synthetic method in a style reminiscent of *Organic Reactions*. Mechanisms are mentioned only briefly, and Chapter 2 will be valued chiefly as an exhaustive survey of synthetic methods, for as such it is a notable contribution to organic chemistry.

In Volume 2, the detailed discussion of special topics begins, and within its six chapters this volume covers organo-metallic compounds, quaternary compounds and N-oxides, alkyl, aryl, halo- and nitro-pyridines. Each type is discussed systematically under the sub-headings, preparation, properties and reactions, and there is liberal provision of tables at the end of each chapter; these summarize reaction conditions and products, or alternatively list physical constants. Spectral data are rather scarce, and no curves are reproduced. Likewise, little attention is paid to reaction mechanisms; for example, the chemistry of pyridine-N-oxide is described (30 pp.) with the use of only three curved arrows. In fairness it must be admitted that by following the references provided, the reader will be able to find the spectra he is seeking, and will encounter a more mechanistic treatment of the subject. The editor may have preferred to stick to facts, and for a series which is destined to endure for several decades, this is probably a wise decision. Nothing dates a book more than out-moded theory.

The third volume discussed here is entitled *Heterocyclic Systems with Bridgehead Nitrogen Atoms*, Part I. This deals only with fused ring-systems and begins with the smaller rings. (A second volume is planned to complete the discussion of fused systems and to deal with bridged systems.) This is a fascinating book, which assembles and classifies a group of heterocycles, many of which have never before been reviewed. However, it is no mere collection of curiosities. Many of the claims in the literature reported in this book are highly improbable, and the author has been commendably critical in discussing them. The earlier chapters, in particular, are rich in unsolved problems. Later chapters deal with less-obscure topics (for example, pyrrocolines) and provide tables of derivatives. An exhaustive review of penicillin chemistry would have been impracticable and the author has elected to describe only syntheses of the thiazolidine-β-lactam system, and to provide key references.

This volume will probably attract less attention than those mentioned above, but the author is to be congratulated on his treatment of a difficult subject; this is a valuable contribution to the chemical literature. G. L. BUCHANAN

COASTAL INLETS

Stability of Coastal Inlets

By P. Brunn and F. Gerritsen. Pp. xvii + 123. (Amsterdam : North-Holland Publishing Company, 1960.) 23*s*.

THIS book is concerned with the stability of channels connecting estuaries and inlets with the open sea. The depths in such channels are becoming increasingly critical as the dimensions of ships are enlarged ; whereas 30 ft. was a generally acceptable depth fifty years ago, depths of up to 50 ft. are required to accommodate the vessels of 100,000 tons now being built. As a result many estuaries in which important ports are situated are being closely examined—often with the aid of hydraulic models and theoretical studies—to find out if their depths can be increased and their navigational facilities improved. The estuaries of the Rivers Thames, Mersey and Tees are among those being investigated in Great Britain.

These investigations are much handicapped by the lack of sound theoretical foundations on which the design of stable channels in mobile material can be based, especially when the complications introduced by tidal flow and littoral drift have to be considered. By assembling an extensive collection of data on the régime in coastal inlets, and by developing the theoretical foundations on which calculations can be made, the authors of this book have made very valuable contributions to the solution of the complex problems involved in the design of coastal inlets, and in the improvement of existing estuarine channels. The authors are, respectively, head, and associate professor of the Coastal Engineering Laboratory of the University of Florida, and have very extensive experience in coastal investigation.

The book opens with some general remarks on the origin of coastal inlets ; this is followed by a comprehensive review first of the theoretical relationships which have been developed from the dynamical and continuity equations describing tidal flow in inlets, and second, of the theories governing the stability of bed material—ranging from the empirical equations developed by Lacey (1930) to the rational theories of Lane (1958), Chien (1957) and others. The significant forces involved in inlet stability are then considered and a relationship between stability sheer stress, maximum tidal flow and cross-sectional area is given. An analysis of data taken from existing inlets follows, and graphs are given which show how the maximum tidal flows are related to cross-sections for a number of inlets and estuaries in Europe and the United States ; it is demonstrated that the stability sheer stress has a general value of 0·080 lb./ft.^{2}, although particular inlets may have values ranging from about 0·072 lb./ft.^{2} (where littoral drift and sediment loads are low) to about 0·103 lb./ft.^{2} (where littoral drift and sediment loads are relatively high).

Later in the book the methods by which sand is passed across inlets are discussed, and it is suggested that there are two radically different methods of by-passing. If the ratio of predominant littoral drift (cubic yd./yr.) to maximum spring tidal flow (cubic yd./sec.) is greater than 200–300, sand generally crosses the inlet on a submerged offshore bar: if the ratio is less than 10–20 the transfer of sand across the inlet involves the movement of tidal channels, and there is no stable offshore bar.

The book ends with a short section on the design of tidal inlets, and it is pointed out that, for stable inlet conditions, the tidal prism and the maximum tidal flow must be large in comparison with the littoral drift and that a suitable stability sheer stress must be chosen. A comprehensive bibliography is included.

Stability of Coastal Inlets can be recommended to all those interested in the design of new inlets, or the improvement of existing inlets and estuaries.

W. J. Reid

MATHEMATICAL STUDIES IN THE PSYCHOLOGY OF WAR

Statistics of Deadly Quarrels

By Dr. Lewis F. Richardson. Edited by Quincy Wright and C. C. Lienau. Pp. xlvi + 373. (London: Stevens and Sons, Ltd., 1960.) 63*s*.

Arms and Insecurity

A Mathematical Study of the Causes and Origins of War. By Dr. Lewis F. Richardson. Edited by Nicolas Rashevsky and Ernesto Trucco. Pp. xxvi + 307. (London: Stevens and Sons, Ltd., 1960.) 63*s*.

The Strategy of Conflict

By Prof. Thomas C. Schelling. Pp. vii + 309. (Cambridge, Mass.; Harvard University Press; London: Oxford University Press, 1960.) 50*s*. net.

WARS begin in the minds of men. Hence the study of war is essentially a task for psychology, and as such demands scientific treatment. Specialists in other fields have been ready with explanations based on some cherished concept of their own—the instinct of pugnacity, the growth of population or the expansion of trade, the ambitions of nations or their leaders, a society based on acquisitiveness and aggression, carried away by emotional forces similar to those that generate conflict and disharmony in the mind of the individual. But, until quite recently, no one has specialized in research on war psychology as such, or attempted a systematic analysis of the available data to check and verify these alternative theories by the methods of empirical science.

The pioneer in this newer approach was Lewis Fry Richardson. A Quaker by upbringing and a physicist by training, his guiding principle was to study war and peace by the same quantitative techniques that he developed for the solution of apparently intractable problems in the field of physics; and as early as 1919 he published a short pamphlet on *The Mathematical Psychology of War*. His best-known work was in applied mathematics and meteorology. His highly original paper on "The Approximate Solution by Finite Differences of Physical Problems involving Differential Equations" anticipated the method of 'relaxation'; and his book *Weather Prediction by Numerical Process* has been acclaimed as "the first textbook on dynamic meteorology".

In 1926 he was elected a Fellow of the Royal Society; and shortly afterwards, at the age of forty-eight, took an honours degree in psychology. From 1930 until his death in 1953 he devoted himself almost entirely to what he called "generalized foreign politics". His various investigations were collected and embodied in two major books, which, thanks to the generosity of United States scholars and the Littauer Foundation, have now been edited and published.

The first is concerned primarily with the problems and consequences of an 'arms race'. Richardson's plan is to express the underlying tendencies in the form of two differential equations. From these he deduces a variety of corollaries which he then seeks to verify by comparing the theoretical 'predictions' with the actual data for the periods that ushered in the two World Wars. A further deduction, based on the usual method for determining a position of equilibrium, enables him to formulate the conditions that are most likely to ensure a stable balance of power.

His second book treats war as a "large scale example" of what he terms "deadly quarrels"—a phrase which includes any form of human conflict issuing in the death of one or more of the contestants—murder, gangsterism, and banditry, as well as the wars of history. With amazing industry he has compiled a mass of information relating to the causes of the various outbreaks during the years 1820–1929, and supplements it by the data collected by Prof. Quincy Wright for the "wars of modern civilization". He then attempts to estimate the relative importance of the causes commonly alleged by an exhaustive statistical analysis. His chief conclusion is that wars are due to a wide variety, and usually to a plurality, of predisposing and precipitating factors. The mistake of previous theorists was to single out and over-emphasize just one of many. Of the three main groups he considers geographical conditions more important than economic, and psychological more important than either.

Both volumes, however, are restricted to conditions obtaining before the Second World War, and are therefore of greater interest to the historian than to the student of contemporary politics. Dr. Schelling, who is professor of economics and an associate of the Centre for International Affairs at Harvard University, is concerned with newer problems arising from the development of nuclear bombs and intercontinental missiles. His method is to estimate probabilities rather than to measure causal forces; and his mathematical techniques are those of the economist rather than the physicist. He points out that the present situation is no longer one of "pure or absolute conflict", but of "conflict resting on a basis of certain common interests". On either side the policy that results is a series of competitive manœuvres in which each tries to win the maximum advantage for himself without necessarily inflicting an equal disadvantage on the other. In simplified form such situations are dealt with in the 'theory of games' where the game is of the 'non-zero sum' type. The conclusions to which his arguments lead are often unexpected, sometimes paradoxical, but always instructive. He warns us that the analysis he adopts assumes that both sides argue rationally, whereas in practice the behaviour of rival powers is largely influenced by a variety of irrational factors—personal, emotional, or even wholly accidental. This, however, presents problems in political psychology, varying from moment to moment, rather than in political arithmetic. The moral of it all is that, if you want peace, it is not enough to prepare for war, you must seek to understand it.

Cyril Burt

Magnetic Materials in the Electrical Industry

By P. R. Bardell. Second edition (revised). Pp. 320. (London: Macdonald and Co. (Publishers) Ltd., 1960.) 32*s*. 6*d*. net.

THE scope of this book is precisely indicated by its title: it is about magnetic materials, not about magnetism, and it describes their uses rather than, though not to the exclusion of, their general properties. There is a further restriction: the author's interests are mainly in communications and his book is biased accordingly. He is an engineer and his book is primarily for engineers, although it might also interest a physicist who wants to find out the technical relevance of some of the properties he measures. This prying physicist would occasionally raise his eyebrows; for example, at some diagrams of magnetic domain boundaries of types recognized since about 1950 as impossible; but one or two such slips do not affect the general practical usefulness of the book. The only serious lapse is that the author thinks that manganese-zinc and other double ferrites are used for the sake of increased permeability rather than for increased flux-density.

The text uses trade names freely, and for many readers this will be helpful. The book is generously illustrated, both with diagrams which are good and with photographs which do not really add much to the text. The contents pages and index are good. One gem of over-zealous editing is included: a 10^{11} in the manuscript was presumably rendered by the typist as 10" and now appears as 10 in. Fortunately this is not typical; the proof-reading is good and the book is well produced. It packs a great deal of information into a reasonable space and within its intended limited scope it is to be recommended.

A. C. LYNCH

The Physiology of Crustacea

Edited by Talbot H. Waterman. Vol. 2: Sense Organs, Integration, and Behaviour. Pp. xiv+681. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 23 dollars.

THIS volume completes the survey of physiological processes in Crustacea which was so well begun by the volume published last year. There are thirteen chapters written by separate authors dealing with a wide range of topics, but all related in some way to the means by which a crustacean receives information abouts its environment and the ways in which it responds to this information. The general standard of these chapters is excellent; the two middle chapters by Wiersma on the neuromuscular system and the central nervous system are particularly valuable. They provide a link between the chapters dealing with sensory perception and the chapters on locomotion and complex behaviour. The link is by no means complete yet, but the crustacean nervous system is most economically organized and contains relatively few neurons so that it offers the possibility of a type of analysis which would be much more difficult to achieve in vertebrates. The fourteenth chapter is an attempted synthesis of the two volumes, and might almost be described as a philosophers' guide to comparative physiology.

As in the first volume one is impressed by the amount already known about the physiology of the Malacostraca, and the Decapoda in particular, but one also gains a strong impression that the lower Crustacea are greatly in need of more detailed study. This is one of the great strengths of the book; the gaps in our knowledge are clearly shown.

In a work of this size it is inevitable that a few minor errors should creep in; *Limnadia*, for example, is not a notostracan as might be inferred from Table 1 in the first chapter, and the young stages of *Bosmina coregoni* would not generally be regarded as metanauplii. These errors do not detract from the great achievement of the editor in bringing together these excellent summaries. The information presented and the detailed bibliographies make this book essential to every student of the Crustacea, and provide the general zoologist with a detailed, up-to-date account of the nervous system and behaviour of this large group.

J. GREEN

Sound Production and Sound Reception by Insects

A Bibliography. By Mable Frings and Hubert Frings. Pp. vi+108. (University Park, Pennsylvania: Pennsylvania State University Press, 1960.) 4 dollars.

THIS is a bibliography which will prove useful to the increasing number of students of sound production and hearing in insects. It includes 1,752 titles, and is tolerably complete up to the end of 1957. No annotations are provided but a simple set of symbols indicates the ground covered by each paper or book quoted. The bibliography proper is followed by two indexes which refer to the numbered entries in the bibliography; one is classified under the orders and families of insects, the other under the types of organ used for sound reception. The authors proceed on the assumption that any mechano-receptor may on occasion be brought into service for the detection of waves, vibrations or rapid barometric changes in the surrounding medium, and may serve as an occasional organ of hearing. The bibliography therefore covers a wide field. Modern methods of recording and analysing sounds have attracted more workers into this field in recent years; the present compilation should be widely used.

V. B. WIGGLESWORTH

Doctors and Disease in Tudor Times

By W. S. C. Copeman. Pp. xiv+186+13 plates. (London: Dawson's of Pall Mall, 1960.) 42*s*.

THIS book can be read with pleasure, profit and interest. It is also a book to have by you so that it can be read or referred to again and again.

It begins well with an introduction that is unusual because it is good; it is in itself a concise essay on the importance of, and justification for, presentation of medical history.

The first chapter is especially acceptable because it gives an excellent review of the history not only of medicine but also of Europe as well. The evaluation of the profession is thus presented in its relation to the whole pattern of life and society.

As a whole this book contains in a concise yet clear and readable form valuable information about the historical evaluation of medicine that makes one somewhat disturbed, even ashamed, that it is not generally completely known, so fundamental is it.

In some chapters a certain amount of repetition indicates the origin of the book in separate lectures. While this is noticeable when the book is read as a whole it would not be noticeable when it is used for reference, and in this way the repetition is in fact likely to be helpful.

The author is to be congratulated on such a scholarly, interesting and eminently readable book.

RUSSELL BROCK

SCIENCE IN THE HIMALAYA

By Dr. L. G. C. E. PUGH
National Institute for Medical Research, London

THE Himalayan Scientific and Mountaineering Expedition, 1960–61, led by Sir Edmund Hillary, and which was sponsored by the *World Book Encyclopædia*, recently concluded its operations after a period of nearly ten months in the field. The final stages were marred by an unfortunate series of accidents, due to illness, which led to the withdrawal of the party from its final mountaineering objective—the ascent of Mount Makalu without the use of supplementary oxygen. Nevertheless, the programme of scientific research, both on Mount Makalu and in the Ming Bo region, near Mount Everest, where the party spent the winter, was successfully completed.

The expedition set out from Katmandu at the beginning of September 1960, and returned during May–June 1961. Scientific work was carried on continuously from December to May on the Ming Bo Glacier at an elevation of approximately 19,000 ft. Physiological observations on a smaller scale were made on Mount Makalu at altitudes of 21,000 ft., 24,000 ft. and 26,000 ft.

The winter programme was made possible by the provision of a prefabricated hut designed for the expedition by E. Levin, of the Timber Development Association, Ltd., London. The hut provided complete immunity from the weather, reasonably stable indoor temperatures around 60°–70° F., and excellent lighting. It was a cylindrical structure composed of about 100 sections and weighing approximately 1 ton. The walls consisted of 4-in. thick plywood panels of stressed skin construction, lined with aluminium foil and filled with foamed plastic. Heating was provided by a kerosene stove, designed for operation at 20,000 ft., by the British Petroleum Co.'s Research Centre at Sunbury-on-Thames. Electricity for lighting and scientific apparatus, including a radio transmitter, was provided by a Lucas wind generator and a motor generator, with 24-V. nickel–iron storage batteries. The hut stood on jacks on a snow surface and was held down by steel cables.

The scientific team was organized and led by Dr. L. G. C. E. Pugh, of the Division of Human Physiology, National Institute for Medical Research, and was composed as follows: Mr. B. Bishop, National Geographic Society, Washington (glaciologist); Mr. M. G. Gill, University of Otago, Dunedin, New Zealand (medical student); Prof. S. Lahiri, Presidency College, Calcutta, 12 (physiologist); Dr. J. S. Milledge, University of Birmingham and the Southampton Chest Hospital, Southampton, Hants (physician); Captain T. Nevison, U.S. Air Force Medical School (physiologist); Dr. M. P. Ward, Department of Surgery, London Hospital, Whitechapel, London, E.1 (surgeon); Dr. J. B. West, Department of Medicine, Postgraduate Medical School, Hammersmith Hospital, London, W.12 (physiologist).

Captain B. Motwani, of the Defence Science Laboratory, Delhi, was attached to the Expedition as an observer, and assisted with physiological and other work.

The only non-scientist with the party was W. Romanes, a builder from New Zealand. His knowledge and skill played an important part in the successful erection and maintenance of the laboratory hut and its equipment. He also acted as assistant to B. Bishop in his glaciological and meteorological work.

As may be inferred from the composition of the team, the emphasis in planning the scientific programme had been placed on physiological research. Studies in glaciology and meteorology were also undertaken, but on a more limited scale. A programme of psychometric testing designed by the Medical Research Council's Applied Psychology Unit at Cambridge was carried out by M. Gill, as well as tests of the special senses of hearing and vision.

The principal physiological investigations carried out during the winter were as follows:

(1) *Functions of the heart.* The cardiac output was measured by the acetylene technique in men exercising at three work intensities on a bicycle ergometer. Serial electrocardiograms were taken at intervals throughout the expedition.

(2) *Pulmonary function.* The diffusing capacity of the lungs for carbon monoxide and oxygen was measured at three grades of muscular exercise.

(3) *Respiratory regulation.* The response threshold and sensitivity of the respiratory centres to carbon dioxide and the interaction with oxygen were studied, using a technique worked out by Lloyd and Cunningham, and their associates in the Department of Physiology at the University of Oxford.

(4) *Respiratory exchanges.* Oxygen-consumption, lung-ventilation and heart-rate were measured under basal conditions and during muscular work at 300, 600 and 900 kgm./min. on the bicycle ergometer. Data were obtained on the effect of breathing supplementary oxygen.

(5) *Blood.* Samples of arterialized venous blood from the heated hand at rest and during exercise at three rates of work were analysed for oxygen and carbon dioxide. The arterial oxygen saturation at rest and exercise was also followed, using a Waters Conley ear oximeter. The effect of a prolonged sojourn at altitude on blood volume, hæmatocrit, red cell and hæmoglobin concentration was studied.

(6) *Renal function.* The following tests were carried out: maximum and minimum *p*H of urine after suitable doses of sodium bicarbonate and ammonium chloride, respectively; the maximum specific gravity during water deprivation; maximum water elimination after a standard water load.

On Mount Makalu (27,900 ft.) the respiratory exchanges were measured on subjects working on the bicycle ergometer at 21,000 ft. and 24,000 ft. and electrocardiograms were recorded at both of these camps. Twenty-five samples of alveolar air were taken at 26,000 ft. and brought back to London for analysis.

Results

Preliminary results of some of these investigations are already available, having been worked out before leaving the Himalaya. The principal findings were as follows:

The electrocardiographic records obtained at 19,000 ft. showed marked right axis deviation, which was not abolished by breathing oxygen. The output of the heart for a given work-load on the bicycle ergometer was somewhat lower than the control values at sea-level; the maximum output was limited to 16–18 l./min. compared with 25–30 l./min. at sea-level. Heart-rates at low work intensity were higher than sea-level control values at the same work-rate, but during maximum work the heart-rate was comparable with or lower than the corresponding sea-level value. Maximum heart-rates were limited to 130–150 beats/min. compared with 180–190 beats/min. at sea-level.

The diffusing capacity of the lungs for carbon monoxide and oxygen during exercise was not significantly altered by prolonged residence at altitude, the results being closely comparable with control values obtained at sea-level. Arterial blood-oxygen saturations of 65–70 per cent were observed in resting subjects at 19,000 ft. During severe muscular exercise arterial oxygen saturations fell to 50–55 per cent—values which would not be consistent with consciousness in unacclimatized subjects acutely exposed to lack of oxygen.

It was found possible to work for up to 5 min. at 900 kgm./min. on the bicycle ergometer at 19,000 ft. and 1,200 kgm./min. could be kept up for 2 min. by the fittest subjects. These work intensities were very much higher than those studied by previous investigators. Maximum oxygen intake during exercise at 19,000 ft. was 2·0–2·5 l./min. compared with 3·5–4·0 l./min. at sea-level when the subjects were less fit. Ventilation volumes of 120–150 l./min. body temperature, pressure, saturated were observed during sub-maximal work (900 kgm./min.), but at 1,200 kgm./min. values as high as 200 l./min. were recorded just before the breaking point. These results confirmed previous observations made on Mount Everest in 1953.

The studies of respiratory regulation indicated that the increased breathing at altitude is accounted for partly by a lowering of the threshold of response to carbon dioxide and partly by an increase in sensitivity to carbon dioxide. The interaction between oxygen and carbon dioxide, whereby carbon dioxide sensitivity increases at low oxygen tension, was not apparently affected by acclimatization to altitude.

Blood-volumes measured by the carbon monoxide method showed a small but significant reduction after 4–6 weeks at altitude; after 4–6 months, however, four out of six subjects studied showed major increases in blood-volume amounting to about 1 litre. The remaining two subjects showed no late increase in blood volume, and their hæmoglobin concentration and hæmatocrit values declined. It was thought that this might be associated with high-altitude deterioration.

Meteorology

Fine weather with clear skies prevailed throughout the autumn and winter. Apart from afternoon mist and a few short storms, the fine weather continued until the end of April, when the pre-monsoon period of unsettled weather set in.

The lowest temperature recorded was −27° C. Winds of 45 knots gusting to 60 knots were common during the winter, and it was seldom necessary for the motor generator to be used. In April, calm weather prevailed.

These observations, which are the first to be made at high altitude during the Himalayan winter, suggest that a safer time for climbing operations might be autumn or early spring rather than the traditional pre-monsoon season.

THE CHESTER BEATTY RESEARCH INSTITUTE (OF THE INSTITUTE OF CANCER RESEARCH: ROYAL CANCER HOSPITAL)

By PROF. A. HADDOW, F.R.S.
Director

AN extension of the Chester Beatty Research Institute (see Fig. 1) was opened by Sir Howard Florey, president of the Royal Society, on June 9, in the jubilee year of the formal inauguration of the Research Institute of the then Cancer Hospital (Free), by H.R.H. the Duke of Connaught on May 23, 1911. The main cost of the new construction was defrayed from funds contributed in the past by the charitable public, approximately £250,000 having been made available to the Committee of Management of the Institute of Cancer Research by the Board of Governors of the Royal Marsden Hospital, the latter acting as custodians. In addition, a grant of £40,000 was made by the Wellcome Trust, in recognition whereof a section of the extension has been designated the Wellcome Laboratory, for research in pharmacology and experimental chemotherapy. The new building, of which the architects were Messrs. Lanchester and Lodge and the contractors Messrs. Humphreys, is linked on four floors with the older Chester Beatty Research Institute, and contains extensive animal-house accommodation, laboratories in biology, organic chemistry, biochemistry, physical chemistry, immunology and cytogenetics, and a conference hall to seat approximately 180. The opportunity has also been taken to re-model the older building, and, among other things, to provide increased library facilities. The present scientific staff at the Fulham Road site, together with an experimental station at Pollards Wood, Chalfont St. Giles, Buckinghamshire (directed towards work on biological mutation, tracer biochemistry, nucleic acid chemistry, protein synthesis and virology), numbers about one hundred, with an approximately equal number of technicians.

It may be of interest to summarize the history of the institution over the past fifty years. The Cancer Hospital (Free), of which the Institute was to become

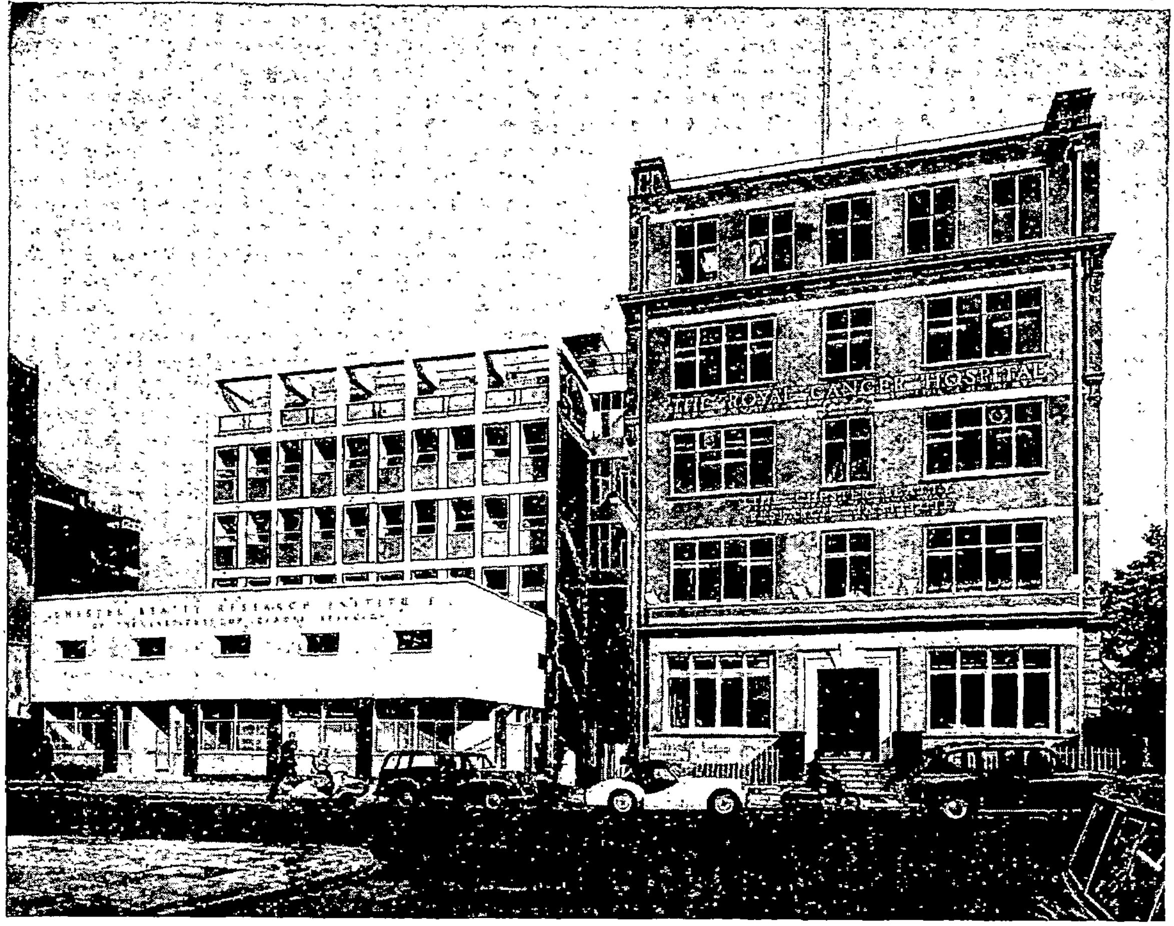


Fig. 1. The Chester Beatty Research Institute and the New Extension

a part, was founded by Dr. William Marsden in the year of the Great Exhibition. At St. Bartholomew's Hospital, Marsden was among the last students of John Abernethy, who had been surgical dresser to Sir Percivall Pott, in turn author in the latter part of the eighteenth century of a celebrated description of cancer of the skin as an occupational disease of chimney sweeps (due to contamination of the skin with soot)—a key observation in direct line with the great advances made by Kennaway and his school, at the Institute, 150 years later.

From its foundation, the Cancer Hospital soon achieved a considerable tradition of clinical research and discovery. However, at the turn of the nineteenth century, increasing recognition of the need for the experimental method led in due course to the establishment of a Research Institute, in 1909. Almost thirty years later its facilities proving inadequate, the Institute was transferred to new quarters in 1939, through the benevolence and vision of Sir Chester Beatty, in recognition of which the Institute was re-named. In the meantime, the Institute, with parts of the Physics and Radiotherapy Departments of the Hospital, had for several years been recognized as a School of the University of London.

With the advent of the National Health Service in 1948, the Institute became the nominal responsibility of the Ministry of Health. Very soon, however, mutual arrangements were made whereby the main financial responsibility was assumed by the Medical Research Council, and the Institute became affiliated to the British Postgraduate Medical Federation. Very necessary additional finance is provided by the British Empire Cancer Campaign, which has supported the Institute increasingly over the past thirty-eight years, and also on a substantial scale from the United States Government through its Public Health Service, and to a lesser, but valued, extent from private American benefactions such as the Jane Coffin Childs Memorial Fund for Medical Research, and the Anna Fuller Fund, of New Haven and Yale.

The first director was Alexander Paine, most noted for his work with Poynton on *Streptococcus rheumaticus*. In its earliest days the Institute rapidly attracted an extraordinary group of men—including Charles Singer, Schryver (its first physiological chemist), Keith Cannan (now of the National Research Council in Washington), Casimir Funk, Jack Drummond, G. W. Nicholson and E. H. Kettle. Casimer Funk, who at that time was studying the etiology of the deficiency diseases (beriberi, polyneuritis, epidemic dropsy, scurvy and pellagra), had coined the name 'vitamine' in 1912 (while at the Lister Institute), and joined the staff in 1913. Drummond himself joined Funk in 1914, and was appointed to succeed him as physiological chemist in 1918. During these years Drummond carried out much remarkable work, in advance of its time, on the amino-acid composition of tumours, and, later, it was he who advocated dropping the final 'e' of 'vitamine', so that the word 'vitamin' should be acceptable under the Chemical Society's scheme of nomenclature.

In 1920, Paine was succeeded by Archibald Leitch. Dogged by ill-health, Leitch nevertheless deserves the utmost credit for giving the Institute its continuing theme—chemical carcinogenesis and its mechanisms—and, during his directorship, which coincided with a period of great stimulus following the discovery by the Japanese, in 1915–18, that cancer could be artificially produced by long-continued application of coal-tar to the skin of the rabbit or mouse, wrote several papers of classical merit.

On Leitch's death, he was succeeded in 1931 by E. L. Kennaway, who had been appointed physiological chemist ten years before. In the 1920's, Bloch and his co-workers in Zurich had already adduced evidence to suggest that the active agent in coal-tar might well be a cyclic hydrocarbon. Of this, Kennaway supplied a virtual proof, through the generation of artificial carcinogenic tars, either by passing isoprene or acetylene with hydrogen through heated tubes, or by the pyrolysis of natural products. Study by Mayneord of the ultra-violet fluorescence properties of carcinogenic tars led to recognition of a (largely) characteristic spectrum, and this essential clue in turn resulted in the detection by Hieger of the same spectral qualities in 1 : 2-benzanthracene, the discovery of the first chemical individual to manifest pronounced carcinogenicity (1 : 2-5 : 6-dibenzanthracene), the isolation by Cook, Hieger and Hewett in 1933 of 3 : 4-benzpyrene from carcinogenic pitch, and the generation (following a prediction by J. W. Cook) of the potent carcinogen 20-methylcholanthrene from deoxycholic acid.

In these earlier days it seemed possible that the cyclic aromatic hydrocarbons might exercise some specific function in carcinogenesis, and it is still not to be excluded that, by their formation in even trace amounts through local abnormalities of steroid metabolism, they could play an important part in the natural causation of cancer in man. However, the experimental findings of the past thirty years have shown beyond question that carcinogenicity is equally a property of many other chemical types, among which may be included various azonaphthalenes studied by Kennaway himself, a series of aminostilbenes described by Haddow, Harris, Ross and George Kon, many instances of endocrine carcinogenesis in the work of Harold Burrows and Eric Horning, and a range of polymers, plastics and metals—to name but a few.

In the past fifteen years, attention has naturally become centred on problems of the mechanisms of action. The mode of action of the hydrocarbons themselves and of the aromatic amines is still obscure, although valuable progress has been made through the investigations by Boyland and his group of the intermediary metabolism of such agents. It is probably true to say that of all the groups of carcinogenic agent more recently recognized, the now very large class of so-called biological alkylating agents is the most important. Of some practical relevance in that they have yielded chemotherapeutic agents such as 'Myleran', 'Chlorambucil' and 'Melphalan' of useful application in the treatment of chronic myelogenous leukæmia, chronic lymphatic leukæmia and myelomatosis respectively (from the work of F. Bergel, G. M. Timmis, W. C. J. Ross, D. A. G. Galton, L. A. Elson, and others), their main importance is, however, fundamental. The father of these modern developments seems to have been no other than Paul Ehrlich, who at the turn of the century (many years before the demonstration of chemical carcinogenesis or of chemical mutation) was convinced of the unique quality of ethyleneimine (among many hundreds of compounds of which he had experience), in its capacity to effect irreversible interactions with receptors in 'protoplasm', of much significance (as he thought) for cellular heredity.

Certain it is that the alkylating agents, from their relative simplicity and chemical reactivity, possess numerous advantages in the elucidation of carcinogenic mechanisms. Within the Institute, detailed studies of their cytological effects (by P. C. Koller and S. H. Revell), and of the dependence of many of the biological properties on a minimum chemical reactivity and a bi- or poly-functional structure, led Goldacre, Loveless and Ross twelve years ago to propose the cross-linkage hypothesis, with implications for the chemical cross-linkage of contiguous linear macromolecules perhaps within the chromosome structure itself. The hypothesis in its early form is now known to be over-simplified, and is not, for example, applicable to biological mutation at large. Yet it proved highly fertile in development, and rapidly led to recognition of new alkylating types of biological interest, a process in which much help was gained from the field of textile technology.

A key problem concerns the relative importance of the reactive groups in proteins, nucleic acids and nucleoproteins, which may be the centres of attack—with special reference to the striking dethiolating properties of 'Myleran' studied by J. J. Roberts and G. P. Warwick, to the esterification of phosphate groups brought about by other alkylating agents (P. Alexander, W. C. J. Ross), and to alkylation of the purine and pyrimidine bases. Involvement of purines was reported by Press and Butler some years ago, and later work by Lawley showed the special importance of *N*-alkylation at the 7-position of guanine. Of special interest is the possibility that esterification of phosphate groups, and alkylation of purine moieties, may represent consecutive steps in a reaction leading to delicate interference with the integrity of the nucleic acid structure. Most recently, Lawley and Brookes have recovered, among other products of the reaction of both sulphur and nitrogen mustards, *bis*-guanyl structures which very likely—although there is no formal proof—reflect cross-linking of the twin spirals of the Crick–Watson helix.

In general, the work of the Institute points towards a conclusion identical with that being reached by many other laboratories using different lines of attack, namely, that malignant transformation may involve biochemical deletion, with permanent release from those enzymic systems responsible for homeostasis of the growth potential of the normal cell. Diverse as are not only chemical but also physical and viral carcinogens, in their nature and modes of operation, and indeed in the tumours they produce, it is still not to be excluded that they may, by varied routes, lead to a key alteration similar or even identical in the various cases; and it is towards some such synthesis that the work of the Institute will continue to be directed.

METABOLIC PRODUCTION OF SUCROSE FROM FAT

By Prof. H. BEEVERS

Department of Biological Sciences, Purdue University, Lafayette, Indiana

THE production of sucrose from reserve fat occurs on a large scale in some germinating seeds. For example, an ungerminated castor bean contains some 260 mgm. of fat (70 per cent of its dry weight) and 15 mgm. of total carbohydrates[1]. During germination at 25° C. in the dark over a period of 8 days the total fat content of the seedling falls to about 50 mgm. while the carbohydrate rises to 230 mgm., and this is in spite of the fact that sugars are being used extensively in the growth and respiration of the seedling proper. Thus there is a production of more than 1 gm. of sugar (principally sucrose) for each gram of fat consumed. This interconversion is strictly confined to the endosperm tissue (respiratory quotient (RQ) about 0·35); sucrose moves into the growing seedling where it supports carbohydrate respiration (RQ = 1·0).

In considering the pathway of this interconversion, we should note first that glycerol is converted quantitatively into sucrose in the endosperm and quite efficiently in the cotyledons also[2]. The labelling pattern in the hexose produced from glycerol-1,3-^{14}C shows that the intact 3-carbon unit is incorporated, presumably after conversion to triose[2]. It should be clear, however, that the synthesis of sucrose from the glycerol moiety of the fat could account for only a small fraction of the conversion actually observed. Thus the problem resolves into the synthesis of sucrose from long-chain fatty acids.

The metabolism of fatty acids in plant tissues has been intensively investigated by Stumpf *et al.*[3]. Although enzymic mechanisms inducing α-oxidation engaged earlier attention, the metabolism of specifically labelled acids by tissue-slices pointed to the operation of a β-oxidation pathway *in vivo*, and such a sequence has indeed been shown to occur in mitochondria from peanuts and other tissues when these were suitably fortified with co-factors. Formidable problems remain in elucidating the details of utilization of the unsaturated acids, but it seems safe to conclude that the breakdown results in the production of acetyl coenzyme A (CoA)[3]. Certainly the efficiency of the overall conversion of fat to carbohydrate is such that the loss of carbon as carbon dioxide during the production of 2-C units must be very small indeed.

Now it has been established that acetate is utilized readily by slices of endosperm tissue[4]. In a 9-hr. experiment in which specifically labelled acetates were provided, it was found that at least 33 per cent of the added carboxyl carbon and 68 per cent of the added methyl carbon were converted to sucrose; the amounts converted to carbon dioxide were 46 per cent and 10 per cent, respectively. This net synthesis of sugar from acetate, with little diversion to other products, posed a problem analogous to that presented by those microbial cells which can grow on acetate as a sole source of carbon. The elucidation of the glyoxylate cycle[5], by which a net synthesis of a 4-C acid such as malate can be achieved from two acetate units, offered a basis for its solution. An examination of castor bean tissues showed that the requisite enzymes, and particularly those newly described from micro-organisms, malate synthetase and isocitratase, were indeed present. A proposal was made, therefore, that the pathway of synthesis involved the production of a dicarboxylic acid by the glyoxylate cycle followed by conversion of malate into hexose[6]. Since this time we have carried out extensive experiments on tissues of fatty seedlings and extracts which have confirmed this suggestion and firmly establish the pathway of sucrose synthesis from acetate in such material. This evidence will now be summarized.

Distribution and Capacity of the Enzyme Systems and Changes during Germination

The demonstration that isocitratase was present in the germinating castor bean raised the possibility that it was widespread in plants and accounted for the general occurrence of glyoxylate and its reduction product, glycollate. An examination of some twenty-five different tissues revealed that this was not so[7]. Far from being widespread, the enzyme was shown to be confined strictly to tissues rich in fat, particularly those of germinating seedlings. Furthermore, it was shown to be localized, in these seedlings, to those tissues in which fat is converted to sugar; it could not be detected in the adjacent parts of the seedling proper. No activity was found in the ungerminated seed or in the maturing seed on the parent plant. During the first few days of germination the enzyme activity rose dramatically and a peak was reached at about the time fat breakdown and sucrose synthesis were most rapid. Later on, as the fat content declined, so did the activity of the isocitratase. It is most significant that this decline in

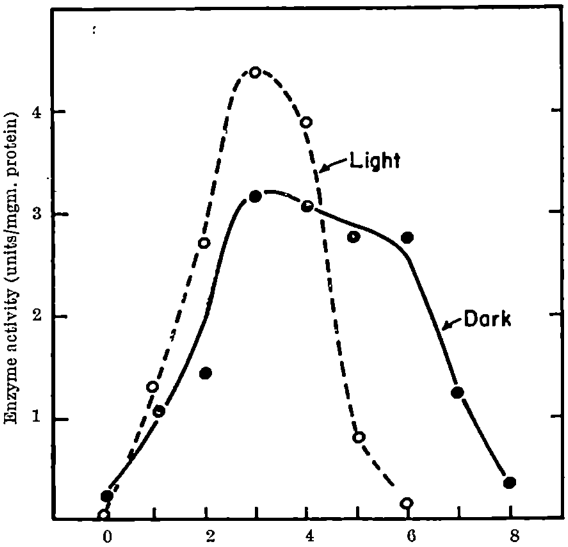


Fig. 1. Changes in isocitratase activity during germination of *Cucurbita pepo* (pumpkin) seeds
One set was exposed to light during the germination period and greening of the cotyledons occurred (ref. 7)

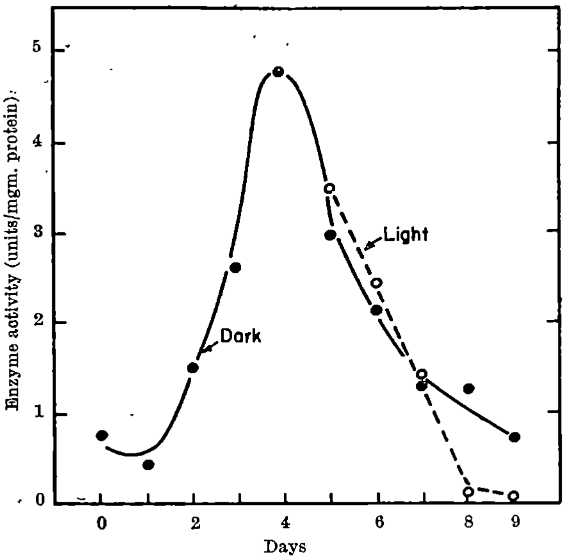


Fig. 2. Changes in malate synthetase activity during germination of *Cucurbita pepo* (water melon) seeds
One set of seedlings was exposed to light during the germination period and greening of the cotyledons occurred (Yamamoto and Beevers, unpublished)

isocitratase activity was not confined to seedlings, such as the castor bean, in which the fat reserves are in tissue which is completely absorbed during germination: it is noticed also in those seedlings the fat of which is originally present in cotyledons which persist as functioning leaves during the later growth[7]. Fig. 1 shows results obtained with seeds of the latter type. The fall in isocitratase activity occurred whether or not the cotyledons were allowed to become functional green leaves.

Malate synthetase was also found in the endosperm or cotyledon tissue of every fatty seedling examined, and it, too, showed changes in activity which paralleled the changing pace of fat breakdown[8]. It is again of interest that malate synthetase activity disappears from the cotyledons of cucurbits after the fat has been utilized (Fig. 2), since this implies a selective breakdown or inactivation of those enzymes constituting the glyoxylate by-pass at a time when other metabolic machinery is operating smoothly. Malate synthetase is not widespread in plants. Although the richest sources are fatty seedlings, it has been detected in a few tissues, for example, corn embryos, which are not primarily engaged in converting fat into sugar[8].

Isocitratase[7] and malate synthetase[9] have been partially purified from castor beans and their properties are similar to enzymes isolated from other sources. Both show an absolute requirement for divalent metal ions and are maximally activated by magnesium ions; in addition, the isocitratase shows a requirement for a compound containing —SH groups (gluthathione, cysteine). The presence of these enzymes in germinating fatty seeds has been confirmed and the amounts which can be extracted are adequate to account for the rate of sucrose production observed *in vivo*[6,8,10–12]. There is evidence that mitochondria prepared from fatty seedlings contain each of the enzymes of the glyoxylate cycle[11], but isocitratase and malate synthetase are found also in the supernatant solutions from such preparations.

Each of the enzymes which would be required to convert malate to sucrose by the following pathway has been shown to be present in the endosperm of germinating castor-oil seeds[13].

malate → oxalacetate → phosphoenolpyruvate → phosphoglycerate → di-phosphoglycerate → triose phosphate →
hexose phosphate → sucrose

The formation of phosphoenolpyruvate and phosphoglycerate from oxalacetate is brought about by phosphoenolpyruvate carboxykinase. Adenosine triphosphate (ATP) is required for this step and for the formation of diphosphoglycerate in the phosphoglyceryl kinase reaction. It is of interest that two triosephosphate dehydrogenases are present, one requiring diphosphopyridine nucleotide (DPN) and the other triphosphopyridine nucleotide (TPN) as a coenzyme[14]. Of the two known systems which might bring about sucrose synthesis from hexoses, only the uridinediphosphoglucose – fructose transglucosylase was found to be present[15]. The 'assimilatory power' (ATP and reduced TPN or DPN) required for driving these reactions *in vivo* most probably is part of that produced during the oxidation of the long-chain fatty acids to acetyl CoA.

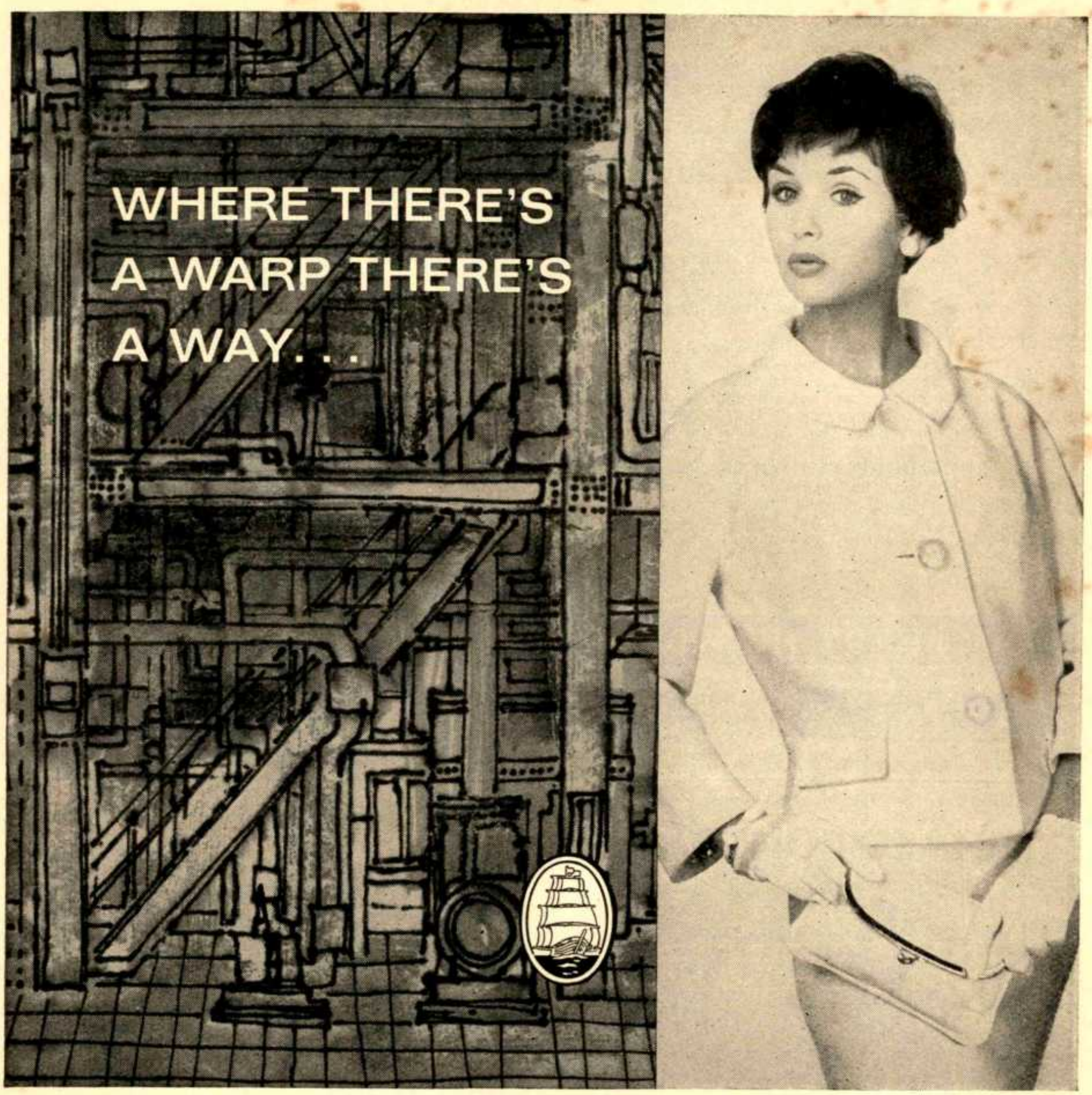
The experiments with cell-free extracts establish, therefore, that the enzymic equipment for carrying out a net synthesis of sucrose from acetate is present in the germinating castor bean. The distinctive distribution pattern and activity changes of malate synthetase and isocitratase in seedlings coincide remarkably well with the occurrence and timing of the fat → sucrose conversion. Experiments with intact tissues, which establish that such a sequence is indeed operative *in vivo*, are outlined in the next section.

Utilization of Acetate and Other Intermediates in Sucrose Synthesis

The earlier results on acetate-utilization[4] are accommodated for the sequence outlined. We have now completed an investigation of the kinetics of acetate-utilization over shorter periods in order to establish what the intermediate products are and the pathway of their interconversion. It will be realized that if the sole fate of acetate is conversion to malate in the glyoxylate cycle and then conversion to sucrose by the sequence above, the methyl carbon atom of acetate would be found only in the centre carbon atoms of malate, none would appear as carbon dioxide and it would contribute equally to carbon atoms 1, 2, 5 and 6 of the hexose units. Half the carboxyls of acetate would be lost in the conversion of oxalacetate to phosphoenolpyruvate and half would appear in carbon atoms 3 and 4 of the hexose. In the recent work with castor bean endosperm slices these predictions have been remarkably well fulfilled. In the meantime, Bradbeer and Stumpf[10] have presented valuable data from experiments with sunflower and peanuts which established that in these tissues also the products isolated after several hours in contact with labelled acetate and labelling patterns in malate and hexose were largely those predicted from the operation of the sequence as outlined.

When acetate-1-^{14}C and acetate-2-^{14}C were supplied to slices of endosperm tissue from castor bean the following results were obtained[16].

(*a*) After 2 min., and before any carbon-14 was present in sugars, virtually all the radioactivity was recovered in organic acids (other than acetate). Support for the dual entry of acetate into the gly-



A PROBLEM that persistently perplexed the linen industry was the excessive friction between warp threads, and between warp and weft, in the weaving process. Production suffered when threads became snarled—and often snapped. The Linen Industry Research Association came to Price's with the problem. Result: Price's have developed Estol 22, a universal size lubricant which has proved to be a complete answer to the industry's problem.

PRICE'S RESOURCES—AND RESOURCEFULNESS—which produced Estol 22 in co-operation with the Linen Industry Research Association—are freely available to assist all divisions of industry with their problems. You are invited to take full advantage of Price's wide experience, and the unique facilities at our Bromborough Pool plant for creative oleochemical research, pilot-scale development, and on-the-spot technical sales service.

PRICE'S (BROMBOROUGH) LTD

OLEOCHEMICALS IN THE SERVICE OF INDUSTRY

BROMBOROUGH POOL • BEBINGTON • WIRRAL • CHESHIRE • Telephone: Rock Ferry 2020 Telex: 62408

PS 78-6749 100

Can you discharge ·01 microlitres from a direct-reading syringe?

With a HAMILTON ONE-MICROLITRE capacity syringe you can—very accurately and very easily! It utilizes the needle bore to contain the liquid (which means no "dead" volume) and the readings are taken from the normal glass syringe barrel—**as robust and easy to read as a 50-microlitre model** (Fig. 1).

HAMILTON SYRINGES are available in **six models from 1 to 500-microlitre capacity**—and all are available with a Chaney adaptor (Fig. 2), an ingenious device limiting the descent of the plunger and ensuring high accuracy and reproducibility in repetitive injections. The 10-microlitre model has a special plunger guide (Fig. 3) making it as sturdy as a 100-microlitre syringe.

HAMILTON SYRINGES are an invaluable aid in any laboratory and indispensable for gas chromatography. Gas tight models are available, too (Fig. 4), from 0.1 to 10 ml. capacity.

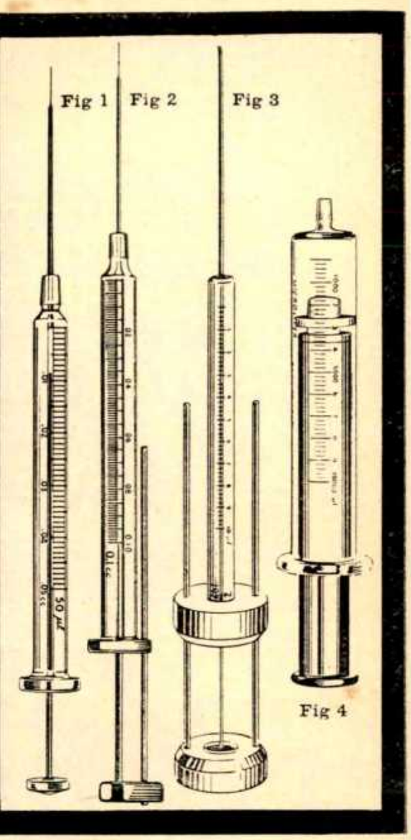
Write your name and address in the margin and send us this advertisement. We will send you our illustrated leaflet by return.

HAMILTON MICROLITRE SYRINGES

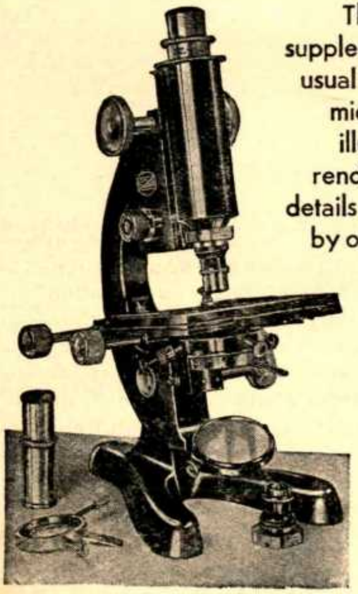
SHANDON

SHANDON SCIENTIFIC CO. LTD.
6 CROMWELL PLACE, LONDON SW7
KNIGHTSBRIDGE 1131

GD 615



PHASE CONTRAST



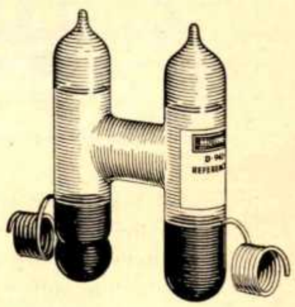
This method supplements the more usual techniques of microscopical illumination, rendering visible details not observable by other methods.

Full particulars on application

R. & J. BECK LTD.
69/71 Mortimer Street, London, W.1

WESTON
CELLS

STANDARD AND INDUSTRIAL REFERENCE TYPES



MUIRHEAD Weston Cells are accurate fundamental standards of voltage. More than twelve Saturated and Unsaturated types available—choose one to suit your application. Ask for Publications 425 and 430.

MUIRHEAD
PRECISION ELECTRICAL INSTRUMENTS

MUIRHEAD & CO. LIMITED, BECKENHAM, KENT, ENGLAND
Telephone: Beckenham 4888

MUIRHEAD INSTRUMENTS LIMITED, STRATFORD, ONTARIO, CANADA
Telephone: 271-3880

MUIRHEAD INSTRUMENTS INC., 441 LEXINGTON AVE., NEW YORK 17, N.Y., U.S.A.
Telephone: Murray Hill 2-8131

514

oxylate cycle is provided by the finding that malate and citrate were the most heavily labelled acids.

(*b*) As shown in Fig. 3 the percentage of carbon-14 in the acid fraction declined steeply during the experiment and extrapolated clearly to 100 per cent at 'zero' time while that in the sugar fraction increased with time. This pattern establishes that a precursor–product relationship holds for the organic acids and the sugars.

(*c*) At 2 hr., when some 60 per cent of the added acetate had been utilized, the contributions of C-1 and C-2 to sucrose and carbon dioxide are those predicted (Table 1). Relatively small amounts of ^{14}C remain in other fractions; the low incorporation into amino-acids, which arise secondarily from the organic acid, should be especially noted.

Table 1. UTILIZATION OF ACETATE CARBONS BY ENDOSPERM SLICES (Data of Canvin and Beevers, ref. 16)

	Per cent of added carbon-14 in components after 2 hr.			
	Sugars	Carbon dioxide	Amino-acids	Organic acids
Acetate-1-^{14}C	40	2	7	10
Acetate-2-^{14}C	22	22	6	13

(*d*) Degradation of malate isolated after supplying acetate-2-^{14}C showed that at all times after 2 min. the label was equally distributed between carbon atoms 2 and 3, and even after 120 min. these carbon atoms contained more than 90 per cent of the total carbon-14 in the malate. When acetate-1-^{14}C was the substrate, the carbon-14 was exclusively confined at all times to C_1 and C_4 and equally distributed between them.

(*e*) ^{14}C-glucose-6-phosphate was detected in the acid fraction during ^{14}C-acetate utilization.

(*f*) The first labelling in the sugar fraction appeared after 5 min. exposure to the labelled acetate. At this time sucrose was labelled equally in its component hexoses but free glucose and fructose were not radioactive. At later times, when the carbon-14 in sucrose was increasing rapidly, the total radioactivity in glucose and fructose was never more than 6 per cent of that in sucrose, and the specific activity (c.p.m. per mgm. carbon) of the component hexoses was quite close to that of sucrose. Evidently, sucrose is the first and major free sugar produced, and the hexoses arise secondarily from this.

(*g*) Degradation of the glucose moiety of the sucrose produced by endosperm slices incubated with labelled acetates is shown in Table 2. At all times the distribution is close to that predicted; the methyl group of acetate is found equally in carbon atoms 1, 2, 5 and 6, while the carboxyl carbon appears equally in carbon atoms 3 and 4. (See also results from 3-hr. experiments with sunflower and peanut tissue by Bradbeer and Stumpf[10].)

Table 2. PER CENT DISTRIBUTION OF CARBON-14 IN GLUCOSE MOIETY OF SUCROSE ISOLATED FROM ENDOSPERM SLICES INCUBATED WITH CARBON-14 ACETATE FOR THE TIMES INDICATED (Data of Canvin and Beevers, ref. 16)

Substrate	Acetate-1-^{14}C				Acetate-2-^{14}C			
Carbon atom of glucose	Min. 15	30	60	120	Min. 15	30	60	120
1	3·2	5·8	4·9	8·0	22·5	21·5	21·9	22·5
2	6·0	4·8	5·3	12·2	23·8	24·5	23·8	24·0
3	32·9	37·1	38·7	35·3	2·5	4·7	6·0	3·6
4	43·5	37·6	41·6	29·2	3·7	5·3	8·8	9·6
5	7·4	8·2	5·4	7·2	23·0	21·1	20·8	16·6
6	7·0	6·4	4·0	8·0	24·5	22·7	18·8	23·4

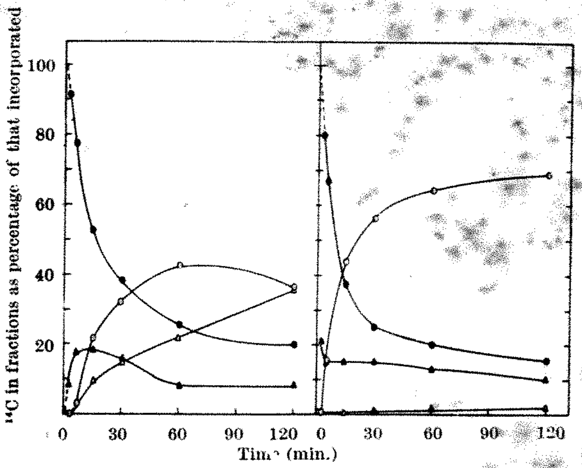


Fig. 3. Changes in carbon-14 content of water-soluble components and carbon dioxide during exposure of slices of castor bean endosperm to acetate-1-^{14}C (left) and acetate-2-^{14}C (right; ref. 16) ⊙, Sugars; *Δ*, carbon dioxide; ●, organic acids; ▲, amino-acids

The distinctive contributions of C-1 and C-2 acetate to the final products, the order of appearance of these carbon atoms in the various components and the degradation data are thus seen to be in good agreement with the proposed sequence.

Experiments with Other Labelled Substrates

(*a*) *Succinate and malate.* In contrast to acetate, which is rapidly absorbed and utilized, the dicarboxylic acids apparently penetrate poorly; the bulk of the added acid (about 80 per cent) was recovered from the medium, even after 8 hr. However, of the acid that did penetrate, a considerable fraction was metabolized to carbon dioxide or sugar. Furthermore, the relative contributions of the carboxyl and centre carbon atoms to these products are those predicted. Thus the carboxyl groups appeared equally in sucrose and carbon dioxide while the centre carbon atoms of succinate made a much larger contribution to sucrose[16] (Table 3).

Table 3. DISTRIBUTION OF RADIOACTIVITY IN CARBON DIOXIDE AND SUGAR FORMED FROM ^{14}C-LABELLED DICARBOXYLIC ACIDS (Data of Canvin and Beevers, ref. 16)

	Time of incubation (hr.)	Radioactivity (c.p.m.)		Ratio Carbon dioxide/sugar
		Carbon dioxide	Sugar	
Succinate-1-^{14}C	4	617	610	1·01
Malate-^{14}C	8	1,189	886	1·34
Succinate-2-^{14}C	4	662	4,593	0·144

Table 4. INCORPORATION OF PYRUVATE CARBON ATOMS INTO ENDOSPERM CONSTITUENTS (Data of Neal and Beevers, ref. 17)

Labelled pyruvate	1-^{14}C	2-^{14}C	3-^{14}C
	Percentage of added radioactivity in component		
Carbon dioxide	49·3	23·8	2·9
Sugars	7·5	15·1	22·8
Organic acids	1·1	8·0	8·5
Amino-acids (alanine)	32·2	29·5	33·2

Equal samples of endosperm slices were incubated with the particular labelled pyruvate for 4 hr.

(*b*) *Pyruvate.* As shown in Table 4, pyruvate is actively converted into sucrose by endosperm slices, but the individual carbon atoms contribute in very different measure[17]. Carbon from positions 2 and 3 accounts for 85 per cent of that appearing in sucrose, with C-3 contributing about twice as much as C-2. The relative contributions to respired carbon dioxide are precisely in the reverse order. About one-third of the added pyruvate is converted to alanine (each of the pyruvate carbons contribute equally) and the acids of the glyoxylate cycle are much more heavily labelled from C-2 and C-3 of pyruvate than from C-1. It is thus seen that C-2 and C-3 of pyruvate behave in a similar manner to C-1 and C-2 of acetate. There is no evidence for a direct conversion of the intact pyruvate skeleton which would require a complete reversal of glycolysis; the conversion of C-1 of pyruvate to sucrose can be ascribed to re-fixation of the carbon dioxide released from this position[18]. The results indicate that pyruvate is converted to acetyl CoA and enters the glyoxylate cycle. Apparently the entry into the glycolytic sequence is indeed at the level of phosphoenolpyruvate, as seemed likely from other considerations[19]. On the basis of the foregoing evidence we may summarize the process of sucrose-formation from fatty acid as follows:

$$\begin{array}{l}
\text{C16 fatty acid} \xrightarrow{\beta\text{-oxidation}} \text{8 acetyl CoA} + 28\ [\text{H}] \\
\text{8 acetyl CoA} \xrightarrow{\text{glyoxylate cycle}} \text{4 oxalacetate} + 24\ [\text{H}] \\
\text{4 oxalacetate} + 8\ \text{H} \xrightarrow{\text{glycolysis reversed}} \text{sucrose} + 4\ \text{CO}_2 \\
\hline
\text{C}_{16}\text{H}_{32}\text{O}_2 + 11\ \text{O}_2 \longrightarrow \text{C}_{12}\text{H}_{22}\text{O}_{11} + 4\ \text{CO}_2 + 5\ \text{H}_2\text{O}
\end{array}$$

The carbon dioxide released during fatty acid utilization is thought to arise exclusively from the oxalacetate → phosphoenolpyruvate step and thus a maximum of 75 per cent of the carbon atoms in the entering acetyl units may be converted to sucrose. Values of more than 50 per cent conversion with C-2 contributing twice as much as C-1 have in fact been observed experimentally. The gm.-for-gm. conversion of fat to sucrose is accounted for, as well as the *RQ* of 0·35 observed with endosperm slices. It should be emphasized that the quantitative agreement between the predictions of the sequence and the physiological features of the fat–carbohydrate conversion indicate that metabolism by way of the glyoxylate cycle is the fate of all of the acetate produced. The overall efficiency is such that very little must pass through the decarboxylative steps of the tricarboxylic acid cycle or be drained off in other reactions. Feeding experiments with pyruvate and acetate show that glutamate (α-ketoglutarate) production accounts for only a very small fraction of the acetate carbon utilized.

The limited distribution of the malate synthetase and isocitratase, the increasing activity and subsequent declines which they show in synchrony with the pace of fat breakdown during germination, indicate that the importance of the glyoxylate cycle in higher plants may well be confined to the part it plays during the early stages of germination of fatty seedlings.

This work was supported by grants (*G*–5072 and *G*–16149) from the National Science Foundation.

[1] Desveaux, R., and Kogane-Charles, M., *Ann. inst. nat. recherch. Agron.*, **3**, 385 (1952).
[2] Beevers, H., *Plant Physiol.*, **31**, 440 (1956).
[3] Stumpf, P. K., and Bradbeer, C., *Ann. Rev. Plant Physiol.*, **10**, 197 (1959).
[4] Beevers, H., *Biochem. J.*, **12**, 114 (1956).
[5] Kornberg, H. L., and Krebs, H. A., *Nature*, **179**, 988 (1957).
[6] Kornberg, H. L., and Beevers, H., *Biochim. Biophys. Acta*, **26**, 531 (1957).
[7] Carpenter, W. D., and Beevers, H., *Plant Physiol.*, **34**, 403 (1959).
[8] Yamamoto, Y., and Beevers, H., *Plant Physiol.*, **35**, 102 (1960).
[9] Yamamoto, Y., and Beevers, H., *Biochim. Biophys. Acta*, **48**, 20 (1961).
[10] Bradbeer, C., and Stumpf, P. K., *J. Biol. Chem.*, **234**, 498 (1959).
[11] Marcus, A., and Velasco, J., *Biochim. Biophys. Acta*, **28**, 365 (1960); *J. Biol. Chem.*, **235**, 563 (1960).
[12] Heydeman, M. T., *Nature*, **181**, 627 (1958).
[13] Benedict, C. R., and Beevers, H. (in the press).
[14] Benedict, C. R., Yamamoto, Y., and Beevers, H. (in the press). Benedict, C. R., and Beevers, H., *Fed. Proc.* (1961).
[15] Canvin, D. T., and Beevers, H., *J. Biol. Chem.*, **236**, 988 (1961).
[16] Benedict, C. R., and Beevers, H. (in preparation).
[17] Neal, G. E., and Beevers, H., *Biochem. J.*, **74**, 409 (1960).
[18] Stiller, M. L., Neal, G. E., and Beevers, H., *Plant. Physiol.*, **33**, xxiv (1958).
[19] Krebs, H. A., and Kornberg, H. L., *Energy Transformation in Living Matter* (Springer-Verlag, Berlin, 1957).

RADIOCÆSIUM AND POTASSIUM IN NORWEGIANS

By JOHAN BAARLI* and KJELL MADSHUS

Norsk Hydro's Institute for Cancer Research, Norwegian Radium Hospital, Oslo, Norway

AND

KURT LIDÉN and RICHARD C. McCALL

Radiation Physics Department, University of Lund, Sweden

As a result of nuclear weapons tests, large amounts of fission products have been introduced into the atmosphere. Up to the end of 1958 more than 250 test explosions had taken place and fission materials equivalent to 92 MT TNT had been released[1].

The fission products, once introduced into the troposphere and stratosphere, have in turn given rise to a world-wide surface deposition of isotopes. The contamination of plants and soil with these isotopes has, either directly or through selective uptake, made these isotopes available in the human diet and in the human body itself.

Among the various isotopes present in fall-out, strontium-90 and cæsium-137 are of special interest due to their long half-lives, their relatively high fission yields and similarities to important body constituents. Strontium-90 is chemically and metabolically related to calcium and must therefore be classified as a bone-seeker, while cæsium-137 behaves similarly to potassium and concentrates mainly in the muscle, liver and spleen.

The introduction of cæsium-137 into the biosphere has received considerable attention, and the mechanism of uptake of this isotope from soil through plants and animals has been discussed in detail in an effort

---

* Present address: CERN, Geneva, 23.

Table 1. RESULTS OF WHOLE-BODY MEASUREMENTS OF A GROUP OF NORWEGIANS FROM OSLO AND BERGEN

Subject	Sex	Date of measurement	Age (years)	Weight (kgm.)	^{137}Cs (nc.)	K (gm.)	^{137}Cs/K pc./gm.)	gm. K/kgm.	Residence	Milk (l./day)	Goat's cheese (gm./day)
K. M.	M	17/1/61	26	75·5	106·0	131	809	1·74	Bergen	1·0	40
E. F.	M	18/1/61	23	78·0	72·0	143	504	1·83	,,	1·0	7
T. S.	M	17/1/61	29	76·5	59·8	132	453	1·73	,,	1·0	7
M. R.	M	18/1/61	26	63·0	49·0	119	415	1·89	,,	0·25	0
E. L.	M	17/1/61	23	83·0	51·2	131	391	1·58	,,	0·50	0
I. L.	F	30/11/60	38	54·0	20·8	71	293	1·32	,,	0·50	0
B. M.	F	16/1/61	23	56·5	27·2	79	344	1·40	Oslo	0·30	40
B. W.	M	25/11/60	48	83·5	31·6	133	238	1·59	,,	0·30	25
U. N.	F	20/1/60	27	63·0	19·4	86	226	1·36	,,	0·20	0
T. M.	M	19/1/61	26	62·0	27·5	122	225	1·97	,,	0·75	0
M. S.	M	18/1/61	20	76·5	23·3	129	181	1·69	,,	1·0	0
G. G.	M	16/1/61	20	91·0	26·5	148	177	1·63	,,	1·0	40
J. B.	M	17/1/61	40	86·5	24·2	137	177	1·58	,,	0·30	0
K. M.	M	4/10/60	35	64·5	21·8	126	173	1·95	,,	0·30	25
I. K.	M	16/1/61	22	77·0	24·3	139	175	1·81	,,	0·75	50
S. S.	F	20/1/61	27	69·0	14·5	93	156	1·35	,,	0·50	0
K. M.	F	19/1/61	23	61·0	12·8	82	156	1·35	,,	0·75	6
K. H.	M	18/1/61	21	63·5	15·7	102	154	1·61	,,	1·0	7
O. S.	M	20/1/61	24	75·5	19·5	128	152	1·70	,,	1·0	0
S. H.	F	16/1/61	23	56·5	10·4	73	142	1·29	,,	0·50	0
T. B.	M	19/1/61	17	73·0	11·3	147	77	2·01	,,	1·0	0

to evaluate the relationship between body burdens in man and deposition of fall-out[2]. Several uncertainties seem to exist, among them the discrimination factor of cæsium-137 relative to potassium through the food chain and the rate of elimination of cæsium from the body.

The presence of cæsium-137 in various foodstuffs and also in human beings has been reported by several investigators[3-6]. Since cæsium-137 is a γ-emitter, its radiation can be detected in bulk materials by ordinary γ-spectroscopic means. Heavily shielded scintillation counters in connexion with multichannel analysers are mainly used for that purpose, and measurements of content of cæsium-137 in man are at present being made in the United States, Sweden, Britain, Germany, Canada and France.

Due to the chemical similarity between cæsium and potassium it is usual to state the concentration of cæsium-137 relative to potassium in the body. This partially eliminates effects due to differences in body-weights and simplifies comparison of body-burdens in different subjects. The results, usually expressed as pc. cæsium-137/gm. potassium to-day show a mean value of approximately 50 for persons without any occupational exposure to cæsium-137 in the countries mentioned. However, reports have been published on special cases where this number has been found to be considerably higher. In connexion with the Windscale accident, a value of 443 pc./gm. potassium was reported with a pre-accident value of 107 (ref. 7). At Hanford, an off-site visitor was found to have a cæsium-137 content of 66 nc. and a cæsium-137/potassium ratio of 370 (ref. 8). This was traced to an extremely unusual diet and milk source.

Comparison between values for the cæsium-137 content of individuals measured at different laboratories might be open to question. Agreement between different laboratories on the standardization of point sources is not always good. Recently[9] comparison of a group of so-called standard sources from several countries showed a spread of about 20 per cent although each of the sources was supposed to be standardized to ± 5 per cent or better. In comparing measurements at various times, it should be kept in mind that the cæsium-137 body-burden was increasing until some time in the middle of 1959 and has been decreasing since.

The content of cæsium-137 in human beings has of course a close relationship to the content of this isotope in the diet, which in turn partially depends on the local fall-out level. It has been established that the distribution of stratospheric fall-out depends on the geographical location, with higher concentrations in the northern hemisphere around 30°–60° N. The fall-out is also greatly influenced by the local rainfall.

Data on the cæsium-137 content of milk, which is a significant part of the diet, have indicated a larger content of cæsium-137 in milk produced in Norway[10] and Finland[11] than in milk from many other countries. This is especially true for milk from the western part of Norway. The reasons for this difference in cæsium content between eastern and western Norway are not well understood. It is probable that the amount and nature of the rainfall is at least one cause. The different grazing habits of cows in these two areas probably also contribute to the difference. Other dairy products used in the daily diet in Norway, among them cheese, have been studied, and pure goat's cheese produced in the western and northern parts of Norway in 1960 have shown values ranging up to 41 nc. cæsium-137/kgm. cheese. A blended goat's cheese is also produced in Norway which is made of 35 per cent goat's milk and 65 per cent cow's milk. This type of cheese is produced in parts of the country having lower cæsium-137-contents. Consequently the blended goat's cheese has only 15–20 per cent of the cæsium-137 content of the pure goat's cheese. In this article and in Table 1 only pure goat's cheese is considered. Table 1 lists the amount of pure goat's cheese eaten, according to the subject's own estimate. However, the difference between the pure and the blended goat's cheese is not popularly understood, and some of these estimates may include blended goat's cheese. Measurements carried out on the cæsium-137 content in other major food products have shown considerably lower values except for reindeer meat, which seems to have activities of the same order as goat's cheese.

Preliminary measurements made at the Radiation Physics Department in Lund of the cæsium-137 content in five Norwegians indicated that Norwegian inhabitants could have higher cæsium-137 body-burdens than those reported for people elsewhere. Due to the fact that no whole-body counter exists in Norway, it was decided to send another group of Norwegians to Lund to have their body radioactivity measured in the whole-body counter there. These people, mostly 20–30 years of age, were arbitrarily selected, with one group of 6 persons from Bergen (at the west coast of Norway 60° N.) and a group of 15 from Oslo (60° N.). None was occupationally exposed to cæsium-137. A few, 3 from Bergen and 5 from Oslo, stated that they regularly used pure goat's cheese in their daily diet.

The measurements were made in an iron room with subject sitting in the 'Argonne chair' position, the detector unit was a 5-in. diameter × 4 in. thick sodium iodide (thallium) crystal and a multichannel pulse-height analyser.

The results of the measurements are shown in Table 1. This gives the measured contents of cæsium-137 in nc. and the number of gm. potassium, together with approximate daily consumption of milk (litres) and pure goat's cheese (gm.).

As can be seen from Table 1, the average cæsium-137/potassium ratio (184 pc./gm. potassium) in the people from Oslo is considerably higher than the 1960 values of ~ 50 pc./gm. potassium reported elsewhere. This can be compared directly with the average cæsium-137/potassium ratio of a group of people from Lund, Sweden (56° N.), of 76 pc./gm. potassium. The average value of the cæsium-137/potassium ratio of 477 pc./gm. potassium found for the Bergen group is even higher. With one exception (I. L.) the values for the Bergen group are well above those of the Oslo people. Two of the subjects, G. G. and I. K., seem to have surprisingly low cæsium-137/potassium ratios in view of their milk and goat's cheese consumption. It has been impossible to determine what type and source of goat's cheese these subjects used; but it is probable that they used the blended type.

It is generally accepted that 55–60 per cent of cæsium-137 in the human body is caused by consumption of dairy products[12,13]. It is also stated[12] that people in the United States contain a substantially constant amount of cæsium-137 due to non-dairy sources and that the remaining fraction of their cæsium-137 burden is directly proportional to their milk input. Due to this fact and because milk constitutes a significant part of children's diet, special interest has been focused on the radioactive content in this material.

Milk samples from different parts of Norway have been analysed for their content of cæsium-137 [10,14]. Beside the usual seasonal variations which are generally established, large variations are found between samples from the western and eastern parts of the country, amounting to almost a factor of 30 (Möre, on the west coast of Norway, about 900 pc./l. Oslo, about 30 pc./l. September 1960). These variations will, on a smaller scale, also show up in the population and to some extent explain the difference between the cæsium-137-level in the Bergen people and the Oslo people. The milk distribution systems for both areas are based on local central dairies; people within the same district having equal daily intake of milk should therefore be exposed to approximately equal amounts of radioactive contaminants and thus show nearly equal body burdens. As can be seen from the measured data, this condition does not hold. A man from Bergen (K. M.) and a woman from Oslo (B. M.) show body-burdens which are almost a factor of 2 higher than the mean values obtained for people in the respective districts. These two people regularly consumed rather large quantities of pure goat's cheese; it is concluded that the excess quantity of cæsium-137 in these two cases, to some extent, can be attributed to the cheese consumption.

The cæsium-137-levels in goat's cheese from different parts of the country show even larger variations than those that have been reported for cow's milk. A calculation of the cæsium-137 excess in people, due to the cheese intake, would require a complete knowledge about place and time for production of the cheese. This has been an impossible task in all cases except for one single person (B. M.), who consumed pure goat's cheese from Hallingdal only. The content of cæsium-137 in this particular cheese has been measured. The calculation of cæsium-137 excess due to cheese consumption will, however, require detailed knowledge about the discrimination factor of cæsium-137 in going from diet to man, and the biological half-time of this isotope in the human body. Studies of the discrimination factor for cæsium-137 have been reported in the literature and values ranging from 1·8 to 3·5 have been given[3]. The biological half-time, which is closely related to the discrimination factor, will show similar variation, and reports indicate values ranging from 143 to 81 days. Any of these figures might be used, yielding an excess amount of cæsium-137 due to cheese intake ranging between rather wide limits. As a compromise calculations were carried out applying a discrimination factor of 2 and a biological half-time of 100 days. The result of this calculation gives a value of 12·2 nc. cæsium-137 for B. M. due to goat's cheese consumption. This value should be compared with 27·2 nc. from Table 1. The difference 15 nc. between the mentioned figures should then be responsible for cæsium-137 intake due to other food products; this value seems to be in reasonable agreement with measured data for females not eating goat's cheese given in Table 1.

The radiation exposure for the extreme case from Bergen was calculated using the formula given by the International Commission on Radiological Protection[16] and turned out to be 0·31 mr./week. This dose is comparable to the radiation dose caused by the potassium-40, which in this case amounts to 16 mr./year or 0·3 mr./week.

It is of interest to estimate the body-burden due to a daily intake of 50 gm. of pure goat's cheese containing 41 nc. cæsium-137/kgm., which actually has been measured. This intake of cheese is considered to be quite normal in Norway. Assuming again a discrimination factor of 2 for cæsium in relation to potassium and an elimination-rate of 0·7 per cent a day (100 days biological half-life), this will in one year accumulate to 135 nc. cæsium-137. The total body irradiation caused by this amount will be of the order of 0·38 mr./week or 20 mr./year.

This investigation was carried out while one of us (R. C. McC.) was holding a Postdoctoral Fellowship of the National Cancer Institute, U.S. Public Health Service.

[1] *Hearings before the Special Subcommittee on Radiation, Congress of the United States*, **3**, 2521 (1959).

[2] Langham, W. H., and Anderson, E. C., *Health Phys.*, **2**, 30 (1959).

[3] Miller, C. E., and Marinelli, L. D., *Science*, **124**, 122 (1956).

[4] Anderson, E. C., Schuch, R. L., Fisher, W. R., and Langham, W. H., *Science*, **125**, 1272 (1957).

[5] Burch, P. R. J., *Nature*, **183**, 515 (1958).

[6] Booker, D. V., *Physics in Medicine and Biology*, **2**, 29 (1957).

[7] Rundo, J., *Nature*, **188**, 703 (1960).

[8] Roesch, W. C., McCall, R. C., and Palmer, H. E., *HW*-67045 (1960).

[9] Liden, K., and McCall, R. C. (unpublished results).

[10] Madshus, Kj., and Baarli, J., *Nature*, **186**, 527 (1960).

[11] Miettinen, J. K., Paakkola, O., Näsänen, R., Vuorinen, A., and Merten, D., *Nature*, **189**, 324 (1961).

[12] *Hearings before the Special Subcommittee on Radiation, Congress of the United States*, **2**, 1133 (1959).

[13] McNeill, K. G., and Johns, H. E., *J. Canad. Assoc. Radiol.*, **11**, 11 (March 1960).

[14] Madshus, Kj., and Baarli, J. (in preparation).

[15] McNeill, K. G., and Green, R. M., *Canad. J. Phys.*, **37**, 528 (1959).

[16] *Health Phys.*, **3**, 15 (1959).

NEWS and VIEWS

Sir Frederick Gowland Hopkins (1861–1947)

To mark the centenary of the birth (June 30, 1861) of Sir Frederick Gowland Hopkins, "the father of British biochemistry", and as a tribute to one "whose services to nutrition enriched the well-being of mankind", Messrs. Van den Berghs, Ltd., manufacturers of vitaminized margarine, have published a 26-page memoir. It has been written by Prof. Ernest Baldwin, a former colleague of Hopkins, and it is a moving account of Hopkins as a great scientist and as a most lovable man. The booklet, which contains a reproduction taken from the well-known portrait of Hopkins owned by the Royal Society, can be obtained from Messrs. Van den Berghs, Ltd., Kildare House, Dorset Rise, London, E.C.4.

Chemistry in University College, Dublin : Prof. Eva M. Philbin

Dr. Eva M. Philbin, senior lecturer in organic chemistry in University College, Dublin, has been appointed, in succession to the late Prof. J. Algar, to the chair of organic chemistry tenable in that College. Prof. Philbin graduated with first-class honours in chemistry at University College, Galway, and afterwards was awarded the degrees of Ph.D. and D.Sc. in the National University of Ireland. After a time in industry, Prof. Philbin joined the staff of University College, Dublin, in 1945, and was later appointed senior lecturer in organic chemistry. Her research interests have been mainly in the field of flavonoids, though recently she has moved on to investigations relative to the stereochemistry of pyrans. She has held visiting and research fellowships in the United States, and at Zurich, with Prof. V. Prelog.

Agriculture at Bangor : Prof. E. J. Roberts

Prof. E. J. Roberts retires this year from the chair of agriculture at Bangor, after sixteen years of distinguished service as a teacher and a prominent figure in the farming world. He comes from the Vale of Clwyd, and graduated with honours in chemistry at Bangor in 1917. After a short period in the Navy as sub-lieutenant, he proceeded to the School of Agriculture at Cambridge, for postgraduate work under the late Prof. T. B. Wood. He was appointed lecturer in agriculture at Bangor in 1922. At the outbreak of the Second World War he was seconded as agricultural executive officer for Denbighshire. In 1942 he was appointed to the chair of agriculture at Aberystwyth, returning to Bangor when that professorship fell vacant in 1945.

Roberts's research interests have covered a wide range of problems in animal husbandry; among these were lactations of various breeds of dairy cows, flushing and fat-lamb production from grassland, and the breeding of polled Friesians and Welsh Blacks. In 1936 he was seconded to the Agricultural Research Council to make a detailed survey of grass-drying, and the monograph he produced was the most comprehensive of its kind on this subject. He has served on numerous bodies concerned with agriculture, for example, the Agricultural Improvement Council, Hill Farming Committee. the Sub-committee on Agriculture of the University Grants Committee, and the Welsh Joint Education Committee. The Arton Wilson Report (1956) on the Provincial and Local Organization and Procedures of the Ministry of Agriculture, Fisheries and Food also bears his name among its signatures. At Bangor, during the past ten years, he has carried out, by the aid of moneys from the University Grants Committee, an extensive scheme of remodelling and adaptation of the College Farm to form an efficient teaching unit.

Prof. I. A. McK. Lucas

Mr. Ian A. McK. Lucas succeeds Prof. Roberts at the comparatively early age of thirty-four. A graduate in agriculture of the University of Reading, he was a prizeman in agriculture. During 1947–49 he held a Ministry of Agriculture postgraduate scholarship in the Nutrition Department of Macdonald College of McGill University. There he took his M.Sc. under Prof. E. W. Crampton. After a short period as assistant lecturer in agricultural chemistry at Harper Adams Agricultural College, Prof. Lucas in 1950 joined the Applied Nutrition Department of the Rowett Research Institute as head of its section on pig nutrition, which post he now holds with distinction and with the rank of principal scientific officer. For a year (1957–58) Lucas, on study leave, held a New Zealand Department of Scientific and Industrial Research Fellowship at Ruakura Animal Research Station, where he broadened his experience by conducting experiments on grazing cattle and sheep. For six weeks thereafter he toured Australia at the invitation of the Australian Meat Board. He is the author of some thirty-two papers on various aspects of animal nutrition, mainly those concerned with the physiology of nutrition of the pig, particularly during early growth. Lucas is at present technical secretary of the Agricultural Research Council's Technical Committee on Nutrient Requirements of Farm Livestock and of its Working Party on Terminology. He is also a member of the Working Party on Pigs. Lucas also serves as a member of the Pig Commission of the European Society of Animal Production.

Presidency of the Mellon Institute: Dr. P. C. Cross

Dr. Paul C. Cross, of the University of Washington (Seattle), has been appointed president and chief executive officer of the Mellon Institute. Dr. Cross, a native of Bruin (Butler County), Pa., has been professor and executive officer of the Department of Chemistry of the University of Washington since 1949. He will assume his new post on September 1. He was born on July 19, 1907, received his bachelor's degree from Geneva College in 1928, and his doctor's degree from the University of Wisconsin in 1932. Afterwards, he held research fellowships at Wisconsin, the California Institute of Technology, and Stanford University. He became an assistant professor at Stanford in 1926, and in 1938 joined Brown University as an associate professor. During 1942–49 he served successively as professor, director of the Metcalf Research Laboratory, and chairman of the Chemistry Department at Brown University.

Department of Technical Co-operation

THE Department of Technical Co-operation came into being on July 24. The Minister in charge is Mr. Dennis Vosper, who will be known as the Secretary for Technical Co-operation. The director-general—the permanent head of the Department—is Sir Andrew Cohen. The Department's headquarters are at Carlton House Terrace, London, S.W.1. The new Department is taking over present work on technical assistance performed by the three overseas departments (the Foreign Office, the Commonwealth Relations Office and the Colonial Office) and the Ministry of Labour, together with their present staffs engaged on this work. The object is to enable the United Kingdom to meet more effectively requests for technical assistance received from the Governments of developing countries inside and outside the Commonwealth. Among the main activities of the Department of Technical Co-operation will be the following: (i) Recruitment of staff for service with overseas Governments and international organizations. (ii) Arrangements for the training of such staff, including staff already in the service of overseas Governments. (iii) Administration of the Overseas Service Aid Scheme which was set out in the White Paper, *Service with Overseas Governments* (Cmnd. 1193). (iv) Responsibility for the work of the Directorate of Overseas (Geodetic and Topographic) Surveys and the Directorate of Overseas Geological Surveys. (v) Aspects of Commonwealth educational co-operation affecting the United Kingdom. (vi) Special technical assistance projects overseas undertaken at the request of the Governments concerned. (vii) The handling of requests from United Kingdom dependent territories for technical assistance (including the financing of such assistance) under the Colonial Development and Welfare Acts, and from Commonwealth and foreign countries and international agencies. (viii) Making arrangements for assistance in the field of research, principally on subjects of importance to United Kingdom dependent territories, or where the United Kingdom has a special contribution to make overseas. (ix) Supplementing the work of other organizations in assistance to overseas Governments in placing students in the United Kingdom. (x) Providing assistance in certain instances to overseas Governments on technical matters relating to air and surface transport, telecommunications and postal services. (xi) Provision to overseas Governments of specialist advice at their request on a wide variety of subjects, such as agriculture, education, fisheries and medicine. Apart from certain educational projects, the new Department will not be responsible for the provision of capital aid, which will remain the responsibility of the overseas Departments.

Research and Development in Scotland

THE Parliamentary Secretary for Science, Mr. D. Freeth, replying to questions in the House of Commons on July 11, said that of 77 applications for grants for special researches received in 1960 from Scotland by the four Research Councils, 61 were accepted. Receipts from Scottish industry during 1960–61 amounted to about 4 per cent of the total receipts from industry by the Department of Scientific and Industrial Research in respect of work done at its establishments. Of 105 postgraduate awards for training in science and technology tenable at universities and colleges in Scotland made by the Department in 1960, 17 were in biology and biochemistry, 6 in chemical engineering and metallurgy, 40 in chemistry, 5 each in electrical engineering and engineering, 3 in geology, 6 in mathematics, 22 in physics and 1 in the human sciences.

Effects of Insecticides on Birds

THE Minister of Agriculture, Fisheries and Food, Mr. C. Soames, stated in reply to a question in the House of Commons on July 3 that the report of the survey of the effects of insecticides on animal life in Britain had been discussed at a meeting on June 29 with all the organizations interested. The report showed that the widespread occurrence of deaths of birds was confined to springtime, and that the main trouble could be obviated by not using dressings containing dieldrin, aldrin and heptachlor for spring sowings. Accordingly, it had been agreed that, as from January 1 next, these dressings will not be used at all for spring-sown grain, and they will only be used for dressing autumn and winter wheat where there is real danger of attack from wheat bulb fly. These arrangements will operate under the terms of the Notification of Pesticides Scheme. It has also been agreed that the Ministry should continue its survey, and that the arrangements should be reviewed at a meeting in June 1962. Pressed on various points, Mr. Soames promised to consider carefully the recommendations of the Estimates Committee regarding the voluntary schemes, but his information was that they had worked.

Farm Buildings in Britain

IN reply to a question in the House of Lords on July 6, the Minister for Science, Lord Hailsham, said that the Government does not think that a case has been established for a Government farm buildings research centre, but the Agricultural Research Council is undertaking a survey of farm buildings in Great Britain and is issuing a bibliography of research publications on the subject. The Council, in co-operation with the farmers concerned, is also organizing experiments with buildings on farms and contributing towards their capital cost. Lord Hailsham said further that with the experimental scheme started last year, by which landowners interested in scientific experiment could receive a 50 per cent grant for genuinely experimental buildings, the owners had to work in co-operation with the Agricultural Research Council. This method, covering the whole of Britain, with its different types of farming, circumstances and climate, appears to offer much better value than a research centre and the scheme has given very promising results. Of 177 applications received, 27 had been accepted as suitable for grants, 8 withdrawn and 90 rejected; 52 are under consideration.

Bibliography of Farm Buildings Research 1945–58

THE Agricultural Research Council is compiling a general bibliography of research publications on farm buildings. This is the first bibliography of the subject to be compiled, and *Buildings for the Drying and Storage of Grain* is the fifth section of the bibliography to appear. It will be followed by a section dealing with buildings for the processing and storage of fodder and a general section which will deal with miscellaneous types of building and with the construction and maintenance of farm buildings. The bibliography regards farm buildings as part of farm equipment and gives references to any matter affecting their design, construction, economics and use. It includes the findings of scientific research, of such systematic but less-precise forms of investigation as surveys, and of field trials and similar developmental phases of new techniques. It includes information from any country where conditions are relevant to Great Britain. Abstracts of the original material and a review summarizing the main conclusions are provided. The present bibliography covers the period January 1, 1945, to April 30, 1958. The Council intends to keep it up to date by publishing supplementary bibliographies from time to time. *Buildings for the Drying and Storage of Grain* (3*s*., postage 3*d*.) is obtainable from the Agricultural Research Council, Cunard Building, 15 Regent Street, London, S.W.1.

Public Health as a World Problem

THE fourth report from the World Health Organization's Expert Committee on Public Health Administration, of which Sir John Charles is chairman, is entitled *The Planning of Public Health Services* (Technical Report Series, No. 215. Pp. 48. Geneva: World Health Organization; London: H.M.S.O., 1961. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars). It reviews broadly the experience in this field of Ceylon, India, Czechoslovakia, the U.S.S.R., the United Arab Republic and the United Kingdom, before considering in detail the general principles of planning such services in countries at varying stages of development and with different social and economic systems, to which any planning must be related. Stress is laid on the use of surveys to secure the information on which planning must be based, the importance of securing full co-ordination and collaboration from the medical and other professions concerned and the interest and understanding of the general public. The determination of priorities and the choice of long-term targets are also necessary, and since planning is a continuous process arrangements should be made for continuous review and assessment, so that the health programme is kept constantly in line with changing conditions; and in this connexion the value of operational research is emphasized. It is no longer satisfactory to plan health services on an empirical basis, and further research in public health service is needed to provide indicators for assessing on a more scientific basis the level of public health, the efficiency of health services, standards, and the economics of disease and disability and their reduction.

National Foundation for Scientific Research, Belgium

THE thirty-third annual report of the Belgian National Foundation for Scientific Research, covering the year 1959–60, comprises chiefly alphabetical lists of recipients of grants and of their publications during the year, in which grants totalling 39 million francs were voted (Fonds National de la Recherche Scientifique. Trente-troisième Rapport Annuel, 1959–60. Pp. 284. Bruxelles: Fonds National de la Recherche Scientifique, 1961). Of this total, 382,500 francs was to the University of Ghent, 240,000 francs to the University of Liège, 730,000 francs to the University of Brussels and 850,000 francs to the University of Louvain. Special grants noted in the report include 1,000,000 francs to the Committee for the Study and Exploitation of Electronic Calculators; 1,875,000 francs for research programmes concerned with the technique of regulation (chemical processes and stochastic phenomena) of the transmission of heat, automobile suspension and the mechanics of flight; 300,000 francs for studies of the effect of *P*, *Q*, *R*, *S*, *ST* and *T* waves of the vectorial electrocardiogram of man, the dog and the rabbit; 2,175,000 francs for a study of dynamic and static deformations, stresses, vibrations and temperatures in construction; 500,000 francs for studies to determine the structure of natural products (sesquiterpenes, steroids, triterpenes and alkaloids); and 300,000 francs for a study of the diffusion of atoms and free radicals in the solid phase at 4° K. as a function of their masses. There is also a note on research during the year at the Jungfraujoch scientific station.

Teachers and Health Education

HEALTH education is not always recognized as a discipline of its own and given its appropriate place in the training of teachers. A new report (Joint WHO/Unesco Expert Committee on Teacher Preparation for Health Education. World Health Org. Tech. Rep. Ser., 1960. No. 193. Pp. 19. 1*s*. 9*d*. Published also in French and Spanish) provides a flexible framework of guiding principles for such training, which should take place not only through courses of instruction but also through the teacher's actual mode of life in his training institution, his contact with student health services, and his experiences when practising teaching. Health education in schools should be based on the health needs and interests of the children, the teacher having many opportunities for imparting it both directly and by the part he can play in the school health services and in planning for a healthy school. To enable him to make the most of his opportunities, he must have some knowledge of growth and development, personal and community health, school health practice, and methods of health education. The report discusses these subjects, the future teacher's life as a student in relation to his opportunities for health work and to student health services, practice teaching, and the education he acquires as a fully-fledged teacher. Other sections deal with planning of training for teachers in health education, the desirability of one person in each training institution being responsible for co-ordination of courses and activities in health education, and possible future national and international developments, studies and research in the subject.

Hospital Efficiency Studies

THE Ministry of Health has issued recently, to hospital authorities, a second set of *Abstracts of Efficiency in the Hospital Service*. The first set, comprising seven investigations, was published in April. The aim of the series is to enable all hospitals to profit from simplification, improvements and money-saving ideas which have been found practicable in the hospital field and to reduce the risk of related measurements and tests being unnecessarily repeated. All the hospital authorities have been asked to contribute to the series by supplying brief factual details from studies they have undertaken. The second set is made up of six studies. These cover: maintenance of grounds and gardens; document copying; medical stenographers' department; medical records (including mobile steel-shelving and addressing machine systems); and, finally, the collection of swill from hospital wards.

How Old are the Angiosperms?

In the first of two recently published papers a fossil nut which looks very like some of the modern Icacinaceae is described ("An Early Cretaceous (Hanterivian) Angiosperm Fruit from California", by M. E. J. Chandler and D. I. Axelrod; "How Old are the Angiosperms?" by D. I. Axelrod. *Amer. J. Sci.*, 259, 441; 1961). This tropical family is regarded as moderately advanced, and if the fossil (which is classified with great caution) is rightly placed it fits very nicely with the thesis of the second paper. In this, Axelrod defends his well-known idea that the flowering plants arose in the 'uplands' during the late Palæozoic (and being upland were very seldom preserved). Here in the uplands they evolved most of their families during the early Mesozoic, and when they appear in force in the Upper Cretaceous this is due to their conquest of the delta habitat rather than to evolution.

Axelrod's arguments had recently been attacked by Scott, Barghoorn and Leopold ("How Old are the Angiosperms?", *Amer. J. Sci.*, 258, A, 284; 1960), and a good deal of the present paper can be crudely summarized thus: lack of evidence can be explained away (and in several different ways); and if certain early fossils supposed to be Angiosperms have not been strictly proved to be Angiosperms, neither have they been strictly proved not to be Angiosperms. To the reviewer it looks as if at least as much thesis has by now been built on the early fossils as they will support, and what is needed is more support; not perhaps by more early fossils but by fuller knowledge of those we have. If, for example, the fossil nut in the first paper was shown by further knowledge to agree with the Icacinaceae and with them exclusively, we would have a very useful basal fact.

Yorkshire Philosophical Society

The report of the Council of the Yorkshire Philosophical Society for 1960 rightly states that it was an important year for the Society. By a mandate given at a special general meeting in December 1959, an agreement was made with the Corporation of York for it to take over the future maintenance of the Yorkshire Museum, its collections and Gardens. They will be managed by a joint committee, one-third of the members of which will be nominated by the Council of the Society. Founded in 1822, the decision to give the Museum and Gardens to the City after 139 years of an existence entirely supported by members is a historic one and the occasion was marked by appropriate ceremony. It is felt that the community at large will benefit greatly by such a gift, and the Society will be free to develop its separate interests. An unexpected discovery in the excavations in the Garden was an apparently late-fourth-century ditch. It is hoped that further work will shed more light on this last phase of Roman York.

International Business Machines, United Kingdom, Ltd.

International Business Machines, United Kingdom, Ltd., have announced the foundation of three research fellowships, one in each of the years 1961, 1962 and 1963, tenable at any university or university college in the United Kingdom, under a continuing scheme (*IBM Research Fellowships.* Pp. 4. London: IBM United Kingdom, Ltd., 1961). The fellowships, which will carry a salary of not less than £1,000 a year, will be in no way restricted as to subject, and will be of sufficient duration to allow research into major problems to be undertaken. The 1961 fellowship has been awarded to the Department of English and General Linguistics at the University of Edinburgh. The facilities of the full range of installed International Business Machine equipment are also to be made available free of charge for approved academic and humanitarian research of a non-profit-making nature. The terms of this Endowed Research Time scheme are set out in a further booklet (*Endowed Research Time.* Pp. 3. London: IBM United Kingdom, Ltd., 1961).

Research Grants in Radiological Health

In view of the rapid expansion which is forecast in the use of nuclear energy, X-rays and other sources of radiation, a greatly expanded programme for research grants in the field of radiological health has been developed by the U.S. Public Health Service. These grants are offered to support research by individuals, universities, hospitals, laboratories and other public or private institutions in the assessment and control of the effects of man-made and natural radiation on the individual. Research proposals should contribute to the determination of the extent and character of the radiation problem as well as the mechanisms by which radiation produces damage. Information and application forms can be obtained from Dr. Paul F. Hahn, chief of the Office of Extramural Grants, Division of Radiological Health, U.S. Public Health Service, Washington 25, D.C.

American Grants for Nuclear Equipment in Argentina, Brazil and Israel

The United States is to make available to the International Atomic Energy Agency approximately 80,000 dollars-worth of nuclear equipment to assist in developing the peaceful uses of atomic energy in Argentina, Brazil and Israel. Argentina will receive a grant for a mass spectrometer ion-source to be used in the electromagnetic separation of short-lived radioactive material, and an electron paramagnetic resonance spectrometer to be used in connexion with solid-state physics work being conducted at the Institute of Physics of San Carlos de Barilocho. The grant to Brazil will provide equipment for research in the use of isotopes in agriculture. Israel will receive equipment for one mobile and two stationary monitoring stations as part of its radiological protection programme to provide routine monitoring of the area surrounding the swimming-pool-type research reactor located near Rehovoth, Israel.

Monograph Prizes

The American Academy of Arts and Sciences is offering three 1,000-dollar prizes, to be awarded annually to the authors of unpublished monographs —one each in the fields of the (1) humanities, (2) social sciences, (3) physical and biological sciences. The final date in 1961 for receipt of manuscripts by the committee on awards is October 2. Details can be obtained from the Committee on Monograph Prizes, American Academy of Arts and Sciences, Little Hall 33, Harvard University, Cambridge 38, Mass.

NATO Advanced Study Institute Programme, 1962

Short courses at which scientific topics are discussed at an advanced level may be eligible for financial support under the NATO Advanced Study Institute Programme. Courses should last for two weeks or longer, and participants should be drawn from several countries. The NATO grant may be used for administrative expenses, publication of the proceedings, and to subsidize the expenses of foreign visitors. Courses in any of the sciences, for example, mathematics, astronomy, physics, chemistry, biology, etc., are eligible for support. The meetings may consist of experimental classes, as well as the more usual arrangement of lectures and discussions. The Programme, which this year is supporting nineteen courses, will continue in 1962, subject to confirmation from the appropriate authorities. It is hoped to raise the budget-level of the Programme. Persons interested in organizing such courses and requiring financial assistance from NATO should write, before December 1, to the Scientific Affairs Division, NATO, Place du Maréchal de Lattre de Tassigny, Paris 16e, France.

Royal Meteorological Society: Officers for 1961–62

THE following have been elected officers and Council for 1961–62 of the Royal Meteorological Society: *President*, Dr. H. L. Penman; *Vice-Presidents*, Dr. A. G. Forsdyke, P. J. Meade, J. S. Sawyer, Dr. J. M. Stagg; *Vice-President for Canada*, Dr. F. K. Hare; *Vice-President for Scotland*, R. Cranna; *Treasurer*, B. C. V. Oddie; *Secretaries*, Dr. R. C. Frith, Dr. R. S. Scorer, Dr. F. Pasquill (*Editor*), G. A. Bull (*Librarian*); *Ordinary Members of Council*, Dr. J. A. Chalmers, T. J. Chandler, R. H. Clements, J. R. D. Francis, W. A. Grinsted, Dr. J. T. Houghton, E. Knighting, A. L. Maidens, Dr. J. L. Monteith, P. R. Owen, Inst. Capt. R. Thorp, Dr. G. B. Tucker.

University News : Edinburgh

THE following have been made readers within their respective faculties, with effect from October 1: (*a*) Faculty of Medicine, Dr. Eric Samuel, honorary senior lecturer in radiodiagnosis (status of honorary reader), Dr. H. M. Adam, senior lecturer in pharmacology, and Dr. J. S. Robson, senior lecturer in therapeutics; (*b*) Faculty of Science, Dr. N. A. Mitchison, lecturer in zoology.

Appointments have been made as follow: Dr. P. P. Brown, lecturer in bacteriology; Dr. W. P. Doyle and Dr. J. L. Holmes, lecturers in chemistry.

The Department of Radiotherapy has received an anonymous gift of £1,000 for research.

Royal College of Science and Technology, Glasgow

DR. DAVID W. A. SHARP has been appointed senior lecturer, and Dr. Graham R. Knox lecturer, in the Department of Chemistry from September 1.

London

THE following have been appointed readers in the Imperial College of Science and Technology : Dr. C. B. Alcock (chemical metallurgy), Dr. J. N. Hunt (physical oceanography), Dr. J. A. Kitchener (science of mineral processing).

Manchester

MR. R. H. KANTOROWICH, formerly lecturer in town planning in the Department of Land Surveying and Civil Engineering at the University of Cape Town, has been appointed professor of town and country planning in succession to the late Prof. Clifford Holliday.

Southampton

THE following appointments have been made: Mr. B. M. Bland, at present deputy librarian in the University of Birmingham, to the University librarianship; Mr. H. J. Trump, at present director of extramural studies in the University of Glasgow, to the directorship of adult education; Dr. J. P. Jones, senior lecturer in the Department of Aeronautics and Astronautics, to the Westland readership in helicopter engineering. The title of reader has been conferred on Dr. I. G. M. Campbell, senior lecturer in the Department of Chemistry.

Announcements

SIR RUDOLPH PETERS, emeritus professor of biochemistry in the University of Oxford, and Dr. J. L. Pawsey, assistant chief of the Division of Radiophysics, Commonwealth Scientific and Industrial Research Organization, Sydney, have been awarded the degree of Hon. D.Sc. of the Australian National University at Canberra.

MR. DAVID OWEN, assistant head of the Department of Operational Research and Cybernetics of the United Steel Companies, Ltd., has been appointed head of the Department in succession to Mr. Stafford Beer, who is to be managing director of a new international firm of operational research consultants based in London.

DR. B. J. A. BARD, vice-chairman of Dracone Developments, Ltd., has been appointed chairman of the company, in succession to Prof. W. R. Hawthorne, professor of applied thermodynamics in the University of Cambridge, the inventor of the Dracone project, who will continue on the board of the company as technical adviser.

MR. N. G. MCCULLOCH has been appointed an honorary life governor of the Cotton, Silk and Man-made Fibres Research Association, Shirley Institute, Manchester, in recognition of his outstanding services to the Association and the industry. Mr. McCulloch has been intimately associated with the Shirley Institute for more than twenty years and played a leading part in the discussions which led to the recent fusion of the British Cotton Industry Research Association and the British Rayon Research Association.

THE Faraday Society has arranged a general discussion on "The Structure and Properties of Ionic Melts", to be held in the Department of Inorganic and Physical Chemistry, University of Liverpool, during September 5–7. Further information can be obtained from the Secretary, Faraday Society, 6 Gray's Inn Square, London, W.C.1.

AN international conference on plasma physics and controlled nuclear fusion research will be held by the International Atomic Energy Agency at Salzburg, Austria, during September 4–9. It is expected that the conference will show what progress has been achieved since the Second United Nations International Conference on the Peaceful Uses of Atomic Energy, held in Geneva in 1958. The proceedings of the conference will appear in special issues of *Nuclear Fusion: Journal of Plasma Physics and Thermonuclear Fusion*, published by the Agency. Further information can be obtained from the Division of Public Information, I.A.E.A., Vienna I, Kärntnorring 11, until August 31, and afterwards at Europahaus, Salzburg, Austria.

WATER RESOURCES OF THE UNITED STATES

As a result of Senate Resolution No. 48 of the Eighty-sixth Congress of the United States, a Select Committee on National Water Resources was set up in 1959 to study the extent and character of water resources activities required to provide the future quantity and quality of water needed for all segments of the economy in the United States.

Under the vigorous leadership of Senator Robert S. Kerr from Oklahoma, this Committee has now completed its assignment and presented its final report and recommendations to the first session of the Eighty-seventh Congress. The final report was preceded by a series of specialist reports which have been appearing steadily over the past two years. A number of these reports has already been reviewed in *Nature* (187, 562; 1960), and have been marked with an asterisk in the now complete list given here. Many of these reports and also the final recommendations will be of interest to those faced with water problems in the United Kingdom and Commonwealth, as well as elsewhere. A brief outline of the salient points of the new reports and the final recommendations therefore follows.

List of Committee Prints issued by the Select Committee on National Water Resources, U.S. Senate, 1959–60, under the title *Water Resources Activities in the United States*. Obtainable from the Government Printing Office, Washington

(*A*) BACKGROUND

1. *Water Facts and Problems*, U.S. Geological Survey.
*2. *Reviews of National Water Resources during the Past Fifty Years*. Legislature Reference Service, Library of Congress.
*3. *National Water Resources and Problems*, U.S. Geological Survey.
*4. *Surface Water Resources of the United States*, U.S. Geological Survey.
*5. *Population Projections and Economic Assumptions*, Census Bureau and Resources for the Future.
6. *Views and Comments of the States*, State reports on their water resources and problems.

(*B*) PROJECTIONS OF FUTURE NEEDS

*7. *Future Water Requirements for Municipal Use*, Public Health Service.
8. *Future Water Requirements of Principal Water-Using Industries*, Business and Defence Services Administration and Bureau of Mines.
*9. *Pollution Abatement*, Public Health Service.
10. *Electric Power in Relation to the Nation's Water Resources*, Federal Power Commission and others.
11. *Future Needs for Navigation*, Corps of Engineers, U.S. Army.
*12. *Land and Water Potentials and Future Requirements for Water*, Department of Agriculture.
*13. *Estimated Water Requirements for Agricultural Purposes and their Effects on Water Supplies*, Department of Agriculture.
14. *Future Needs for Reclamation in the Western States*, Bureau of Reclamation.
15. *Floods and Flood Control*, Corps of Engineers, U.S. Army.
*16. *Flood Problems and Management in the Tennessee River Basin*, Tennessee Valley Authority.
17. *Water Recreation Needs in the United States*, National Park Service.
18. *Fish and Wildlife and Water Resources*, Fish and Wildlife Service.
*19. *Water Resources of Alaska*, U.S. Department of the Interior.
*20. *Water Resources of Hawaii*, U.S. Department of the Interior.

(*C*) TECHNIQUES FOR MEETING NEEDS

*21. *Evapo-Transpiration Reduction*, Department of the Interior and Department of Agriculture.
*22. *Weather Modification*. Weather Bureau and Dean A. M. Eberle.
*23. *Evaporation Reduction and Seepage Control*, Bureau of Reclamation.
*24. *Water Quality Management*, Public Health Service.
*25. *River Forecasting and Hydrometeorological Analysis*, Weather Bureau.
*26. *Saline Water Conversion*, Office of Saline Water, Department of the Interior.
*27. *Application and Effects of Nuclear Energy*, Atomic Energy Commission.
*28. *Water Resources Research Needs*, Department of Agriculture.
29. *Water Requirements for Pollution Abatement*, Prof. George Reid, University of Oklahoma.
30. *Present and Prospective Means for Improved Re-use of Water*, Abel Wolman Associates.
31. *The Impact of New Techniques on Integrated Multiple-Purpose Water Development*, E. A. Ackerman and Associates.
32. *The Supply and Demand of Water in the United States*, Dr. Nathaniel Wollman, Resources for the Future.

(*D*) HEARINGS OF EVIDENCE

Hearings Held by the Select Committee on National Water Resources and published in 23 parts with index. Pp. 3968. (Washington, 1960.)

(*E*) FINAL REPORT AND RECOMMENDATIONS

Report of the Select Committee on National Water Resources, Report No. 29 of the First Session of the Eighty-seventh Congress, 1961.

Committee Print No. 1 consists of copies of the charts prepared by the U.S. Geological Survey on the occasion of the presentation of an account of the water resources and related problems of the United States at a meeting in July 1959. Much of the material in this report is also covered in Print No. 3, which has already been reviewed.

Committee Print No. 5 contains the population projections and economic assumptions on which the studies for the water resources committee are based. This report is of considerable interest and has wider implications beyond that of the water problem. The first part predicts that the population of the United States will reach between 225 and 278 million by 1980, and between 267 and 431 million by the year 2000. Breakdowns of these projections by States and water resource regions, and between urban and rural areas in each water resource region are given. The report covers projections made by the U.S. Census Bureau, the Committee Staff and 'Resources for the Future', a private organization.

Committee Print No. 6 is a voluminous report detailing the views and comments of each State on the water problem. The replies received from the invitation to submit evidence range from brief letters to elaborate treatises from States where water is an acute problem. Taken as a whole the responses provide a valuable background to the national problem.

Report Nos. 8, 10 and 11 form a natural group dealing with industry, electricity and navigation, respectively. Committee Print No. 8 attempts an assessment of the future water requirements of the principal water-using industries. The 1954 census showed a total use of water of more than 12,000 × 10^9 gal. annually in the primary manufacturing industries, exclusive of electric power generation. The Department of Commerce estimates that the 1954 intake of 7,700 × 10^9 gal. for the iron and steel, chemical, pulp and paper, food and beverage, aluminium and copper industries could rise to 26,500 × 10^9 gal. by 1980 and 61,600 × 10^9 gal. by the year 2000. The Bureau of Mines projects water intake in the mining industries from 1,790 × 10^9 gal. in 1954 to 3,765 × 10^9 gal. in 1980 and 4,770 × 10^9 gal. in the year 2000. Taking all industries exclusive of electric power together, a total need of water of up to 36,500 × 10^9 gal. a year by 1980 and double this amount by the year 2000 is estimated. Committee Print No. 10 deals with the needs of the electric power industry, and it is not surprising to see even more astronomical figures. During 1959 about 26,813 × 10^9

gal. of water were circulated in thermal plants of investor-owned systems. By 1980 these will need 106,409 × 10^9 gal. yearly. To this must be added the needs of nuclear power plants and the rural electrification schemes of the Department of Agriculture. Fortunately, from the point of view of national water supplies, most of this water is needed for cooling purposes, and less than 1 per cent is a consumptive use. The same consideration also applies to hydro-electric developments: the report shows that present hydropower capacity represents between one-quarter and one-third of the actual and economic water-power potential, and that the overall resource limitation on hydro development in the United States is not yet within sight. Committee Print No. 11 details the future needs for inland navigation. This report covers briefly the history of navigation on the inland waterways system of the United States (which extends to some 20,000 miles), gives an inventory of the present system, and discusses possible improvements and extensions thereto in the light of the estimates that freight traffic will double by 1980 and quadruple by the year 2000. The improvement of some 10,041 miles of existing waterways and the creation of some 3,070 new waterways might eventually be needed.

Committee Print No. 14 investigates the future water needs for reclamation in seventeen Western States. These States have had in recent years a more rapid growth in population and economic activity than the United States as a whole, and the trend is likely to continue. The Bureau of Reclamation, which has been responsible for much of this development, was asked, therefore, to report on anticipated future developments of reclamation in the Western States between now and the year 2000, including Federal, State and local projects. In preparing its report, the Bureau has inventoried more than 1,000 projects, of which it estimates that some 800 will be realized by the year 2000, bringing in 17 million acres of newly irrigated land and creating 230,000 family-sized farms for an 'on-farm' population of 800,000. This could support a local non-farm population of 2·4 million and provide food and fibre for 25 million people. The full efficient utilization of water resources in the Western States would require significant changes in existing patterns of use of water. Many involved water exchanges would have to be worked out, the State laws with respect to ground water would need modification, a good deal of research on the hydrological cycle would be necessary and many financial problems would need resolution. Appendixes to the report include maps and charts showing the location and construction costs of the 1,085 projects. This report has proved to be one of the most interesting, stimulating and also challenging accounts of the whole series.

Committee Print No. 15 discusses the flood problems of the United States, tabulates past and expected future flood damage by river basins, presents the historical and legislative background of flood control in the United States, and considers possible solutions for flood problems in the light of the fact that overall flood damage is increasing more rapidly than it has been possible to reduce it by the construction of control works. Three alternative methods of reducing damage exist: (*a*) abandonment or evacuation of flood plains; (*b*) regulation of flood plain use to accommodate the use to the flood hazard; (*c*) provision of works and measures for flood protection or control. The report presents an inventory of works and projects needed to prevent flood damage in the foreseeable future until 1980. These works include more than 64 million acre-feet of flood control storage in reservoirs, 4,300 miles of levees and flood walls, and 5,900 miles of channel improvements.

Committee Prints Nos. 17 and 18 serve as salutary reminders that the water problem is not confined to industrial and agricultural interests. The rapidly rising demand for outdoor recreation by an expanding population with a rising standard of living is considered in Report No. 17. There are few outdoor activities from which water is absent, and the report sets forth recommendations from the National Park Service regarding future water recreation needs. It suggests that some 15 per cent of the oceanic and major inland water shoreline should be acquired, that certain free-flowing streams the recreational value of which outweighs other considerations should be preserved, that water reservoirs should where possible be made multi-purpose, that efforts to combat pollution should be increased. Little consumptive water is involved in the report, which is concerned mainly with the need for conservation of water for recreation. Committee Print No. 18 deals with a rather more specialized aspect of outdoor recreation—that of fish and wild life in relation to water resources. The report points out that the problem of maintaining fish and wild life resources are compounded by the combination of the rapidly increasing population and increase in leisure time together with the gradual and increasing encroachment of man's activities on the habitat available for fish and wild life. It is urged that fish and wild-fowl conservation and development should be considered as a co-equal purpose with other demands on water resources development in the United States.

Committee Print No. 29 on pollution abatement supplements Nos. 9 and 24 previously reviewed. One of the principal requirements for water in the future is for the dilution of effluent resulting from the treatment of municipal sewage and industrial wastes which are disposed of into rivers. Prof. Reid, who with the advice of technical experts from the Public Health Service, developed short-cut methods for estimating dilution water requirements, is largely responsible for report No. 29. The study is limited to the rivers in the 22 water resource regions delineated and excludes the problems of tidal estuaries. Waste loadings that would normally be discharged directly into the sea or large lakes were not considered.

Committee Print No. 30 is an important account of present and prospective means for the re-use of water. As demands for water rise, it becomes increasingly necessary for the public to use second-hand water. With all the indications pointing towards a three-fold increase in water withdrawal by 1980, rising to a six-fold increase by the year 2000, the need for improving techniques of water re-use is obviously urgent. The report indicates that already 70 million of the 117 million people now served by public water systems are re-using water that may have passed through a domestic sewage or industrial waste disposal plant at least once. The report is based on a review of several thousand technical papers: it presents methods of reclaiming water from sewage and industrial wastes, outlines the problems encountered in the re-use of water, goes into considerable detail on the restoration and maintenance of underground water supplies, covers ground water recharge, and reclamation of saline waters.

As a fitting companion to Committee Print No. 30 we find No. 31 dealing with the impact of new techniques on integrated multiple-purpose water development. To meet a situation within this century in which water requirements will cause the United States to go beyond the traditional approaches to water development, the report suggests a new type of approach termed "comprehensive development", which is defined as "the application of integrated multiple-purpose design, planning and management which includes joint consideration of ground and surface water, conservational and other measures for engineering of demand, and the treatment and management of water having a substandard quality". This report has been prepared under the direction of Dr. Edward A. Ackerman.

Finally, in Committee Print No. 32, we find a summary report on the estimated supply and demand for Water in the United States for 1980 and 2000 by "Resources for the Future". The basic projections and information supplied by the Federal agencies for each of the 22 regions into which the contiguous parts of the United States were divided for the purposes of the study has enabled tentative balance sheets of water supply and demand to be drawn up. The report also deals with the basic physical problems of providing the necessary facilities for storage and treatment of water. It shows very clearly the great economic advantages that could follow from the more complete treatment of wastes.

These reports, together with the hearings, occupy some 5,800 pages and provide a most detailed picture of the present practice of use of water in the United States, and of impending problems. The final report of the Select Committee on National Water Resources to the Senate (No. 29 of the Eighth-seventh Congress, First Session) summarizes the findings and gives the Committee's conclusions and recommendations. The need for meeting problems before they arise is stressed, and five major categories of effort are thought desirable so as not to inhibit national or regional economic growth: (*a*) regulating stream-flow through the construction of surface reservoirs and watershed management; (*b*) improving the quality of streams through pollution abatement programmes; (*c*) making better use of underground storage; (*d*) increasing the efficiency with which water is used through the elimination of wasteful practices, improved sewage treatment methods, recirculation, increased irrigation efficiency, and substitution of air for water cooling; (*e*) increasing the natural yield of water by desalting, weather modification, and other artificial means.

The Committee's main recommendations to Congress are accordingly:

(1) The Federal Government, in co-operation with the States, should prepare, and keep up to date, plans for comprehensive water development and management for all major river basins in the United States. The Executive Branch should be requested to submit plans to Congress in January 1962, for undertaking and completing such work in all basins by 1970.

(2) The Federal Government should stimulate more active participation by the States in planning and undertaking water development and management activities by setting up a 10-year programme of grants for water resources planning.

(3) The Federal Government should undertake a co-ordinated scientific research programme on water. This should include both research into ways to increase available supplies and ways to increase efficiency in the use of water. The Executive Branch should be requested to submit a programme of research to Congress in January 1962.

(4) The Federal Government should prepare biennially an assessment of the water-supply demand outlook for each of the water resource regions of the United States. The Executive Branch should be requested to submit a programme of research to Congress in January 1962.

(5) The Federal Government, in co-operation with the States, should encourage efficiency in water development and use, by regulating flood plain use, by studying and anticipating water problems, by anticipating storage needs, by increasing public awareness of water problems and by the provision of public hearings where necessary.

Water is to-day assuming an ever-increasing importance as civilization's needs for water and water-related products and services grow. Areas of the globe that will thrive, or even survive, will be determined by the availability of water resources and their wise use to serve man. Growing communities will need increasingly to organize and conserve these resources. Commendable foresight has been shown in the United States in the efforts being made to provide adequate water supplies for the next generation. Most countries, especially the United Kingdom and many members of the Commonwealth, will find the approach, the reports, the findings and the recommendations of the Senate Select Committee which have been considered here of great value and interest to them in dealing with their own water problems.

W. G. V. BALCHIN

FLORA EUROPÆA

THE second symposium of Flora Europæa was held during May 21–28, 1961, in Italy. It was divided into three parts, the first in Genoa, where five days were devoted to various papers and discussions, the second consisting of an excursion to Viareggio and the Apuane Alps, ending in Florence where the final sessions of the symposium were held in the Botanical Institute.

The meeting was attended by participants from Denmark, Switzerland, Sweden, Finland, Iceland, Poland, Portugal, Spain, Rumania, Germany, Yugoslavia, Belgium, France, Austria, Norway, as well as Italy and by all the members of the British Editorial Committee.

In Genoa the symposium was held in the University Botanical Institute, founded by the British philanthropist and merchant, Thomas Hanbury, whose name the Institute still bears. The Institute is situated high above the city, surrounded by its Botanic Garden, and made a very appropriate and comfortable meeting place. The arrangements were made by Prof. R. E. G. Pichi-Sermolli, director of the

Institute, with the co-operation of the University authorities.

The keynote of the meeting was international co-operation in European taxonomy, an appropriate theme for the Flora Europæa organization.

The opening session on Sunday morning included speeches of welcome by Prof. Pichi-Sermolli and University dignitaries, to which Prof. T. G. Tutin (Leicester, chairman of the Editorial Committee) replied in English and Prof. D. A. Webb (Dublin) in Italian. In the afternoon Prof. Tutin outlined the progress made by the Flora Europæa organization since the last meeting in Vienna. He recalled how the Editorial Committee had received a substantial grant from the Department of Scientific and Industrial Research, which enabled the secretariat to be established on a permanent basis and allowed the employment of two research assistants. The circulation of manuscripts of Volume 1 to the regional advisers had proceeded successfully during the past two years, and he discussed the three main points that had been raised by the advisers. These were distributional, ecological and cytological data. There had been great insistence on the importance of giving as detailed ecological information as possible, but in the discussion that followed Prof. Tutin's paper it was made quite clear that although some species have a fairly consistent general ecology there are many which show surprising exceptions or unexpected variation. None the less some advisers, such as Prof. R. Nordhagen (Oslo), felt that even such elementary information as whether a species grew in wet or dry habitats should be given. Prof. W. Lüdi (Zurich) suggested that certain generalized information could be given by using categorized types of ecological preferences which would be useful for non-taxonomists. It was eventually agreed that authors of accounts should attempt to summarize the ecological characteristics of the species in the manuscripts circulated to the regional advisers and these could then be criticized by them.

Dr. V. H. Heywood (Liverpool, secretary of the Editorial Committee) then gave a brief report on the state of preparation of Volume 1 of the *Flora*. The first manuscripts were sent out to regional advisers in December 1958. Since then 150 have been circulated and a further 50 have been prepared and are being revised by the Editorial Committee prior to circulation. Approximately two-thirds of the first volume have been completed and manuscripts of the remaining third are in active preparation. Furthermore, several authors are preparing accounts for the second volume and some have already been submitted. Since the Vienna meeting a supplement to the Green Book (*Guide to Contributors*) had been published[1], the Vienna Symposium report had appeared as a number of *Feddes Repertorium*[2] and the first of the *Flora Europæa Notulæ Systematicæ* were in the press[3].

The second day of the meeting was devoted to a consideration of post-war European taxonomic and floristic literature. Regional advisers were asked before the meeting to prepare reports on the situation in their countries, together with a bibliographical list of the more important papers published since 1945. Reports from 16 countries were received and duplicated copies were distributed to participants as a volume of some 250 pages.

Dr. Heywood gave an introductory analysis of these floristic reports, making the following points: It was interesting to note how frequently it was mentioned that taxonomic and floristic studies had been neglected in several countries since the War for one reason or another. Equally interesting were the reasons for renewal of activity in many cases. For example, phytosociological or topographic-distributional investigations (often undertaken for economic reasons) have given an impetus for much floristic work. There were dangers, however, attendant on this since it often involved cursory identification in the field subject to considerable errors. It was in fact true of certain regions that the only floristic information available had to be drawn from phytosociological lists. Another noteworthy feature was the lack of modern Floras in the Mediterranean region, precisely those areas with the greatest floristic diversity. In more northern countries the tendency was towards biosystematic studies. An unfortunate feature was the small number of monographs or revisions that were being prepared, a tendency which fits in with the world pattern.

The number of journals in which European taxonomic and floristic literature is published is about 500, which in itself presents problems, since few institutions regularly receive more than a proportion of them. As a result there is often considerable ignorance as to the activities of other taxonomists, with consequent duplication of effort in some cases. The solution to this kind of problem lies very largely in improving abstracting services, a subject which was discussed later. It was encouraging to note that several European journals or organizations already produce some sort of annual listing of relevant literature published in their country. Prof. Böcher (Copenhagen) suggested that it would be of great service to taxonomy if these annual reports could be united and circulated together. It was agreed to investigate the possibilities of doing this.

Brief surveys of post-war taxonomic activity were then given by Prof. T. W. Böcher (Copenhagen, for Denmark), Prof. F. Markgraf (Zürich, for Albania), Prof. R. Nordhagen (Oslo, for Norway), Dr. A. Lawalrée (Brussels, for Belgium), Prof. A. Borza (Cluj, for Rumania), Prof. K. H. Rechinger (Vienna, for Austria and Greece), Dr. N. Hylander (Uppsala, for Sweden), Prof. R. E. G. Pichi-Sermolli (Genoa, for Italy), Dr. S. M. Walters (Cambridge, for Great Britain), Prof. D. A. Webb (Dublin, for Ireland), Prof. B. Pawlowski (Cracow, for Poland), Prof. W. Lüdi (Zürich, for Switzerland), Dr. A. Löve (Montreal, for Iceland), Dr. Pinto da Silva (Sacavém, for Portugal), Prof. A. R. Merxmüller (Munich, for West Germany), Prof. W. Rothmaler (Griefswald, for East Germany), Prof. E. Guinea (Madrid, for Spain), Dr. P. Jovet (Paris, for France), and Prof. E. Mayer (Ljubljana, for Yugoslavia).

It is intended to publish revised versions of all these floristic reports in a special number of the journal *Webbia* in 1962.

On May 23, two papers were given on the Italian flora. Dr. G. Moggi (Florence) gave a survey of the state of floristic investigation in the southern half of Italy and in Sicily. It was a revelation to most participants to learn just how poorly large areas of southern Italy had been studied floristically, and it is hoped that Dr. Moggi's map illustrating this will be published with his paper as part of the *Proceedings of the Symposium*. The second paper was by Dr. S. Pignatti (Padua) on the taxonomy and relationship of the genus *Limonium* in Italy and Spain. This was followed by a lively discussion.

The following morning was devoted to considering the problems of abstracting and indexing European taxonomic literature. Dr. S. M. Walters (Cambridge) surveyed the major schemes of indexing and abstracting already in existence, such as the *Index Kewensis, Excerpta Botanica,* and national ones such as the *Plant Breeding Abstracts* published by the Commonwealth Bureau of Plant Breeding and Genetics, Cambridge, and certain private systems. All these have certain limitations such as incompleteness. The second question to be considered was how far they overlapped one another, and the third aspect covered was a consideration of how these facilities could be improved and modified. During the discussion attention was directed to several other abstracting systems that existed, and the regional advisers agreed to submit information on these to the Flora Europæa secretariat. The combined information would then be circulated.

A more long-term possibility of improving the abstracting facilities was discussed by Mr. S. Gould (Connecticut) who attended the meeting as an observer. He is working with a grant from the U.S. National Science Foundation, towards the setting up of an International Plant Index. The details of his methods, which involve the use of a punched card system, were explained and keenly discussed, and many participants agreed that the International Plant Index might form a useful basis which could be utilized for indexing European taxonomic literature. The possibilities of co-ordination would be further investigated and it was hoped that Mr. Gould's scheme would be successfully continued.

The next paper was by Dr. E. Weinert (Halle), who gave, on behalf of Prof. H. Meusel (Halle), who was unable to attend, examples of methods of indicating extra-European distributions for species in the text of *Flora Europœa.* The general principles employed in the examples were accepted, and Dr. Weinert said that the staff of Prof. Meusel's Institute would supply the necessary information for the whole of Volume 1, details in critical cases to be arranged with the authors of the accounts.

A problem related to the use of European taxonomic literature was reported on by Prof. D. A. Webb, who outlined progress that had been made in the preparation of a *Lexicon Polyglottum.* This was essentially a Latin-based list of about 200 terms commonly used in Floras with the equivalents in most European languages. A number of partial lists had already been prepared for immediate use and these had revealed a number of unexpected problems. It was, for example, impossible to find equivalents for certain terms in some languages. Also, terms may be used in different senses in some countries so that considerable problems of interpretation arise in producing the glossary.

The question of terminology was then discussed further by Prof. N. A. Burges (Liverpool), who outlined the problems he had come across in attempts to introduce some sort of standardization of terminology in the manuscripts of the first volume of the *Flora.* As might be expected, this generated a lively controversy over the use and application of technical terms. What several participants regarded as loose British usage of the term 'fruit' came in for considerable criticism and several minutes were devoted to discussing how the fruiting structures of *Ranunculus* and similar genera should be described. The discussion had to be terminated before it extended to detailed consideration of the whole botanical vocabulary. Prof. Burges suggested that the number of complicated terms used was to some extent correlated with the age and experience of the writers of the manuscripts, although there were exceptions, but this provocative issue was not pursued !

Alien plants was the first subject for discussion on May 25. Prof. Webb recalled the various suggestions that had been made at the Vienna Symposium concerning the treatment of aliens, notably by Dr. Hylander (Uppsala) and Prof. Jalas (Helsinki), and outlined the decisions made by the Editorial Committee. Several of the proposals, such as the distinction of casual species by a special sign, had been rejected on the grounds that the number of species involved would be too large, to say nothing of the difficulties of obtaining the necessary information. Similarly the proposal to include archæosynanthropes with native plants and the designation of only neosynanthropes as alien was rejected on the grounds of impracticability. Other proposals were however accepted, such as the inclusion of well-established aliens even if found only in one small district. Prof. Webb then went on to consider the terminology to be applied since there was considerable confusion in the literature. The term 'adventive', for example, is used in various senses. The terms proposed for use in *Flora Europœa* were: (1) cultivated or planted; (2) wild ; (3) native ; (4) alien; (5) established; (or naturalized) alien; (6) casual. Definitions of these were given, and regional advisers were invited to submit agreed equivalents in their own languages. Criteria for the inclusion of aliens in *Flora Europœa* were then discussed. Prof. Webb concluded with a call for information about: (*a*) botanists who take a special interest in alien plants and who could assist in assessing information about them according to the criteria agreed on ; (*b*) literature references published since the appearance of the standard Flora of the country. This information was particularly urgent for a number of countries such as Albania, Corsica, Greece, Spain, Sardinia, Turkey-in-Europe, none of which possesses an adequate Flora less than twenty years old.

The next subject for discussion was again introduced by Prof. Webb. It concerned the need for the preparation of regional check-lists for *Flora Europœa.* In a number of regions the information available in existing Floras was not sufficient to establish the presence even of non-critical species. The compilation of lists for certain regions such as Albania (P. W. Ball, Liverpool), Turkey-in-Europe (D. A. Webb, Dublin), Spain (V. H. Heywood, Liverpool) was now in hand, but lists for such areas as Greek Macedonia and Thrace, and Yugoslav Macedonia were urgently needed. Another such area was Sardinia, and Prof. Pichi-Sermolli thought that it might be possible to organize a list.

The final session in Genoa was devoted to a consideration of hybrids in *Flora Europœa,* and the main paper was given by Prof. D. H. Valentine (Durham) who, after a theoretical introduction, surveyed the type of problems arising in genera such as *Rumex, Salix* and *Saxifraga* which occur in the first volume of the *Flora.* No major departure from the rules set out in the *Guide to Contributors* was proposed, although there were proposals for greater consistency in the designation of hybrids by formulæ and binomials.

In the discussion attention was directed to the necessity for careful investigation in groups where hybridization was known, since the hybrids often resembled one of the parents so closely that they were not detected. The question of intergeneric hybrids was also discussed, particularly with reference to the Gramineae.

In Genoa both the University and the city authorities provided generous hospitality. A reception was held at the Palazzo Tursi by the Mayor of Genoa, and the participants had an opportunity of examining there some of the original correspondence of Christopher Columbus. An extensive tour of Genoa and the Riviera de Levante, including Santa Margherita Ligure and Portofino, was provided by the Tourist Organization. The meeting in Genoa ended with a banquet given by the University at Boccadasse. The festivity of this occasion was enhanced by several speeches, including one by Prof. A. Borza in Latin which received an ovation. Prof. Borza, the doyen of the botanists present, took the opportunity of announcing two new species he had discovered—*Tutinia gravis* and *Heywoodia agilis*. Since the eloquent Latin descriptions he gave were only verbal and were not therefore effectively published according to the *International Code of Nomenclature*, steps are being taken to remedy this through other channels.

The next two days were spent on a profitable and enjoyable field excursion—on May 26 to the nature reserve of Portofino, Passo del Bracco and the celebrated *Pinus pinea* forest at Migliarino, spending the night at Viareggio. On May 27 the excursion went inland to the marble mountains of the Apuane Alps, climbing to the peak of Monte Altissimo. Florence was reached by evening and the final sessions of the symposium were held the next day, May 28, in the Botanical Institute of the University. In the morning members were shown around the various sections of the Institute, including the newly constructed phytotron and the elegant and spacious herbaria and library. A reception was held in the library dominated by the bust of Philip Barker Webb, whose herbarium, together with the other major collections, make Florence one of the most important centres for taxonomic botany in Europe.

The closing session of the symposium was held in the afternoon when the main business was the report of the Nomenclature Sub-committee, given by the chairman, Dr. N. Hylander. Most of the discussion centred round the validity of publication of the species contained in such works as Gandoger's *Flora Europae*. It was decided to make further studies and arrive at an assessment of the number of species involved and the number of changes of name which they might cause before reaching a decision. It was also agreed to circulate lists of *nomina ambigua* to the members of Flora Europæa with the view of reaching agreement on which of these names should be rejected.

The meeting ended with speeches by Prof. Pichi-Sermolli and Prof. Lüdi. Prof. Borza expressed the hope that it would be possible to hold the next meeting in Rumania. The second Flora Europæa symposium was thus successfully concluded, and co-operation for the future firmly established.

V. H. Heywood

---

[1] Heywood, V. H. (compiler), *The Presentation of Taxonomic Information, Supplement* (Alcobaça, 1960).

[2] Heywood, V. H. (edit.), *Feddes Repertorium*, **63**, Heft 2 (1960).

[3] Heywood, V. H. (edit.), *Feddes Repertorium*, **64**, Heft 1, 1 (1961).

EUROPEAN BROADCASTING CONFERENCE, STOCKHOLM

During May 26–June 23, a European conference on "Broadcasting" was held in Stockholm under the auspices of the International Telecommunication Union. The objects of the conference were: first, to revise the 1952 Agreement reached also at Stockholm for the use of the very high-frequency Bands I, II and III for sound and television broadcasting; and secondly, to prepare plans for the future development in the European area of sound broadcasting in Band II, and of television in the ultra-high frequency Bands IV and V. This year the conference was attended by delegates from some forty national administrations, and by representatives of a number of international organizations.

The conference was opened by Dr. Hakan Sterky, director-general of Swedish Telecommunications; and at the first meeting, Dr. E. Esping, technical director of Swedish Telecommunications, was appointed chairman, assisted as vice-chairmen by Captain C. F. Booth and Mr. A. Badalov, leaders of the United Kingdom and U.S.S.R. delegations, respectively.

The technical basis for this recent conference had been formulated in March at a meeting of technical experts of the International Radio Consultative Committee held in Cannes. A detailed report of this meeting provided full information on technical matters, such as the characteristics of transmitters and antenna systems, propagation curves for land and sea conditions, and the signal protection ratios requisite for satisfactory reception of television in the ultra-high-frequency bands from 470 Mc./s. upwards.

The final report adopted by the recent conference at Stockholm will await endorsement by the various national administrations represented. It contains full details of the stations proposed for sound and television broadcasting in the future. In particular, some five thousand stations are listed in the ultra-high-frequency Bands IV and V, together with the frequencies assigned to these stations to avoid mutual interference during operation. A uniform channel-width of 8 Mc./s. was proposed for each station, consistent with the adoption of the 625-line standard for the vision signals. In view of the rapid development of television throughout the world at the present time, it was very opportune that this conference was held so that satisfactory plans could be made on a sound technical basis.

One of the participating bodies at Stockholm was the Inter-Union Committee for the Allocation of Frequencies for Radioastronomy and Space Science, which was established by the International Council of Scientific Unions last year. This committee was represented by Drs. H. Sterky, and J. H. D. von der Toorn, by Prof. B. Linblad, and by Dr. R. L. Smith-Rose, secretary-general of the Committee; and it put forward two recommendations designed to protect observations made by radio astronomers in certain frequency bands. One of these sought to secure the free use of the band 606–614 Mc./s. for the radio astronomy service, and was received very sympathetically by the delegates present at Stockholm, who in a majority of cases agreed to avoid, so far as is practicable, the use of this band for television broadcasting. The second recommendation pointed out the danger of harmonics from certain broadcasting stations in Bands IV and V interfering with radio astronomy observations in the hydrogen band 1,400–1,427 Mc./s. which, in the existing International Radio Regulations, is allocated exclusively for the radioastronomy service. It was recommended that administrations should take all practicable precautions to avoid such interference.

THE BRITISH ELECTRICAL AND ALLIED INDUSTRIES RESEARCH ASSOCIATION

THE annual report of the British Electrical and Allied Industries Research Association opens with a reference to the fact that it records the work of the Association's fortieth year.

Of the work started by the Association in its early days which has contributed substantially to the development of the electrical industry may be mentioned, particularly, research on insulation, on circuit breaking and switchgear, and on the loading capacity of cables. These branches of work still feature largely in the Association's activities.

It was in the third year of the Association's existence that electricity supply undertakings, joining as associate members, initiated the co-operation in research between manufacturers and supply authorities which has continued ever since. Work which is of vital importance to the supply industry and which has been pursued continuously for many years is the determination of the properties of steam at high temperatures and of the characteristics of steel in relation to withstanding high temperatures and stresses.

The present major classifications of the Association's research activities are as follows: insulation and dielectrics; switch and control gear; power plant; insulated cables; overhead lines; surge phenomena; integrating meters; magnetic materials; transformers; flame-proofness and intrinsic safety; earthing; safety and circuit problems; rural electrification; heating, cooking and allied problems; electric equipment of automotive systems and communication interference. In addition, there is a small miscellaneous group designated "Unclassified Researches".

The more important results obtained during the year in these various fields, summarized in individual paragraphs of the report, comprise for the most part technological information which is applicable directly in the electrical industry, but there is in addition a substantial volume of new scientific knowledge.

An investigation of the resistance of industrial insulating materials to surface discharges, a phenomenon known as 'tracking', has shown that the resistance to tracking of a poor material is not increased greatly by a substantial increase in the creepage distance.

Measurements relating the temperature of buried cables to seasonal variations of soil properties have been made, and the possible advantages of using special 'backfills' for cable trenches are being investigated.

In relation to flame-proof equipment it has been shown that for some explosive gases obstructions in close proximity to, but *outside*, the flange gap increase the hazard of explosion, a wrapping of tape to improve dust tightness being particularly dangerous.

Following an investigation of the characteristics of electrical floor-heating for buildings, recommendations have been issued and are now being adopted.

It is also noted that interesting records were obtained last year of the behaviour of the heating system installed for frost protection under the road surface at the Mound in Edinburgh.

Work on vacuum circuit breakers, switches and contactors continues, and it is reported that the vacuum contactor has now reached the stage of commercial development.

In relation to interference arising from industrial radio-frequency equipment, it is stated that all the information has been obtained which is necessary for the preparation of statutory regulations.

In the field of agriculture, reference is made to investigations into methods of drying grain. It appears that for continuous-flow drying the most suitable electrical technique will be infra-red heating.

Among the fundamental researches conducted in university departments under the ægis of the British Electrical and Allied Industries Research Association may be mentioned the work carried on in Liverpool on the theory of conduction in semi-conductors. It is known that the conductivity of germanium increases by several orders of magnitude at fields of 2 V./cm. and at liquid helium temperature. This phenomenon, which is often termed 'breakdown', has been shown to be quite unconnected either with instability or with destruction of the lattice. It can, in fact, be predicted theoretically on the basis of earlier work on 'hot' electrons.

Work on gas-discharge phenomena is also in progress with the Townsend ionization chamber on the measurement of ionization and attachment coefficients, and a comprehensive range of data has been obtained for carbon monoxide.

The velocity distribution of cathode particles originating from the spot of a low-current arc between copper electrodes *in vacuo* has been investigated at Oxford.

At the University of Durham experiments are in progress on collision ionization at ultra-high frequencies.

Perhaps the most noteworthy illustration of the traditional freedom of scientific inquiry occurs under the heading "Astrophysics" in the section of unclassified research work.

Originating in the important technological investigation of arc phenomena in circuit breaking, there has developed the idea of applying fundamental electrical discharge theory to problems on the macroscopic scale of astrophysics. Since 1944 several papers dealing with the development of these hypotheses have been published.

During the past year, observations have become available from astrophysical and space research which confirm predictions of this general theory in some detail.

The application of electrical discharge theory to explain a well-known type of stellar spectrum suggested the existence of spiral nebulæ on a stellar scale. Photographic evidence of the existence of such nebulæ has become available. The theory explains the general form of the planetary nebulæ and of the gas movements observed in them.

Evidence in support of another prediction has resulted from satellite observations by U.S. naval scientists. The observations of 80,000-V. X-rays associated with solar flares implies the existence of temperatures of about 100,000,000° K. in solar atmospheric electrical discharges. The prediction that such temperatures must exist in this region was made from electrical discharge theory applied to magnetic storms.

Another interesting by-product of theoretical work in a different field is a new method of measuring the surface tension of a clean surface in a liquid. This results from the mathematical solution of the problem of the equilibrium shapes of bubbles in electrical and centrifugal fields which was required in a study of the electrical breakdown of liquids, itself part of an investigation on breakdown of highly stressed impregnated dielectrics.

The most notable addition, during the year, to the Association's experimental resources has been the building and equipping of a Creep Testing Laboratory, intended to accelerate the important investigations on the creep behaviour of steels for use at temperatures above 1,050° F. (565° C.).

At the end of the year under review a major reorganization of the Association's committee structure was put into effect. Six divisional committees now guide and superintend the Association's work, replacing twenty-two sectional committees and eighty-one research committees.

An interesting reference is made to the desirability of fostering still closer relations between the Association and the universities and technical colleges. Through its extra-mural programmes the Association already maintains close contact with quite a number of departments in universities and technical colleges. To supplement this, special facilities were instituted to provide for all universities and technical colleges in the Commonwealth obtaining the published reports of the Association. A further step has now been taken in the development of this policy in that professors of electrical engineering and heads of electrical engineering departments in technical colleges have been invited to apply for associateship of the British Electrical and Allied Industries Research Association with full membership benefits.

J. Greig

FOUR DECADES OF STATE FORESTRY IN NEW ZEALAND

The annual report of the Director of Forestry in New Zealand for the year ended March 31, 1960, made to Sir Ernera Tirikatene, the Minister of Forests, is full of interest*. Essentially it consists of 37 appendixes in tabular form, from which progress towards various objectives can be measured, if desired. This will be the last report made by Mr. Entrican, who has just retired after long service.

The report is refreshing for its frankness on some of the mistakes made in the past. It does not disguise the difficulties encountered in the somewhat erratic progress being made towards the building up of a sound forestry practice and the creation of areas of managed forest which are expected to make important contributions to the national economy.

The State service is organized into a number of divisions which deal with management, public and private forestry, development, which comprises training and research, protection forestry, engineering work, operations, industrial operations and, finally, commercial operations.

In the past no more than lip service has been paid to the fundamental principle in forestry of sustaining the yield, and in this report we have a plain confession of neglect to adhere to that principle. "Thus", it says, "on a 2,000-acre area to be managed on a 40-year rotation, 50 acres would be planted annually, with the first 50 acres coming to maturity . . . in the forty-first year, concurrently with the completion of planting over the whole area." New Zealand speaks thus from bitter experience. The Murupara working circle in the largest planted forest there—Kaingaroa—covers 200,000 acres, the oldest portion of which is 37–47 years of age and extends to 10,000 acres only. The next age-class —27–37 years old—covers no less than 150,000 acres, the remaining 40,000 acres being of younger ages. This vast mass of 150,000 acres, composed mainly of Monterey pine (*Pinus radiata* D. Don) is described as "steadily moving towards maturity *en masse*". For various reasons it has been found impossible to cope with the tending of this large area and, in respect of other species in which there are fewer natural deaths, the growing-stock is said to be "accumulating towards stagnation". Here there is a lesson for other countries engaging in large-scale afforestation on fertile land.

New Zealand is anxiously trying to find an outlet for what is frankly called its second-grade timber as a pulp-wood, having produced an excess of certain assortments. An export trade to Australia has been established, but New Zealand openly looks ahead to supplying pulp and paper wood to the rapidly developing populations of the Asiatic countries. A strong plea is made for increasing the area of plantations of exotic conifers, even on good farming land, to produce timber for export, and it is the producer of not enough butter-fat per acre—not of mutton and wool—that is being urged to switch over to forestry. Farmers are asked to plant trees extensively, even to the extent of providing one out of the three million acres of the prescribed afforestation programme, which is expected to increase the area of planted exotic trees to 3,000,000 acres by the year 2025.

Unlike Great Britain, New Zealand still has a large area of indigenous forest. In 1840 the area there covered by these must have amounted to about two-thirds of that of the whole country. Since then they have been considerably reduced. Their treatment has followed the usual Anglo-Saxon pattern of unplanned clearance for farming and of devastation, and exploitation goes steadily on. At one time no thought was given to its conservation as forest, and various arguments were put forward which contributed towards its neglect. Impossibility of regenerating some of the species was one which is now being proved to be without foundation. The main contributing factor was probably the ease and assumed success with which the early exotic plantations were begun. Ironically, one authority has said recently that "the indigenous forest will serve to tide over any crisis arising out of the destruction of the exotic plantations by widespread epidemics of insects and disease".

---

* New Zealand. Report of the Director of Forestry for the year ended 31 March, 1960. Pp. 115. (Wellington: Government Printer, 1960).

Immense areas of the natural forests on the mountainous watersheds of the major rivers have been devastated. Browsing by animals in these areas leads to loss of tree cover followed by erosion and devastating flooding in the rich settled lowland country. In a vast area of more than two million acres of ratakamaki protection forest in the South Island, disastrous results of browsing by opossums, deer and chamois are predicted. In the Waimakariri River catchment area sheep are "slowly making a desert land by close cropping of vegetative cover and exposing the soil to erosion", and threatening the city of Christchurch with possible catastrophe by floods.

The division of protection forestry of the service is responsible for the control of noxious animals, on which nearly £312,000 was spent during the year. In official operations during the year 31,494 deer, 24,830 goats and 4,920 chamois were killed and it is claimed that a bounty was paid in respect of 1,106,000 opossums destroyed.

Not all the new exotic plantations have been made on deep volcanic soils. In one forest in South Island an area of 3,500 acres was blown over by wind on a shallow soil, where, owing to rooting difficulties, the trees appear to be unable to stand after reaching a height of 50 ft. This sort of thing is also very liable to happen in Great Britain.

The New Zealand report faces up to another forestry problem, namely, that of putting timber grown in remote areas on the market. The costs have been found to be so high that they are used as a strong argument that farmers and others should grow trees nearer centres of consumption and should exploit and convert them there. This runs counter, however, to any policy which seeks to establish, or maintain, a healthy rural population.

The future of forestry in New Zealand is looking more hopeful for two main reasons. The first is that forest management, though still very backward when compared with some European countries, is being given more attention. Management plans, which are "forest regulations prescribing the application of certain sylvicultural rules, and the execution of certain works to produce a definite result", now apply to no less than 3,080,000 acres of the indigenous forest. The definition of the objects for which each forest is to be managed is a matter of vital importance and the progress made recently is heartening.

The second reason is that great stress is at last being laid on the need for research in forestry. There is now a large staff of workers so engaged, and the Forest Research Institute at Rotorua publishes its own report, which is rather voluminous, and describes work on a wide variety of subjects. It is gratifying to see that attention since 1956 has been directed to the study of the indigenous forest, which may be a dangerous concept, but is a very significant reality in New Zealand, from which very important lessons can be learnt.

THE CASE FOR A NEW PROFESSION EMBRACING SCIENCE, LIBRARIANSHIP AND FOREIGN LANGUAGES

A SURVEY*, supported by the National Science Foundation and sponsored by the Modern Language Association of America, and carried out under sub-contract with the latter Association as part of a contract with the United States Office of Education, Department of Health, Education and Welfare, argues the case for a new profession concerned with the handling of scientific information, librarianship and foreign languages. Its six chapters delineate first the characteristics of this new profession and the influences which have determined its emergence; the snares and problems of recruitment; undergraduate and graduate education in relation to the supply of such recruits; professional training; and the nature of the professional support which is required, in which connexion the authors recommend establishment of an advisory board to devise an appropriate well co-ordinated programme of courses, seminars, research groups or short-time institutes for training those already engaged in the profession or proposing to enter it. There are numerous detailed recommendations, many of which are applicable in Great Britain also, but although Messrs. L. Cohen and K. Craven lean towards the formation of a professional body, they are not explicit on the point and do not recognize the difficulties inherent in the presence of corporate bodies and individual practitioners which have, in Great Britain, considerably hindered the development of a really strong and representative professional qualifying body.

Some of the recommendations are directed towards management, for example, those relating to salary structure, status and standards. The contribution of the scientist is recognized in recommendations relating to co-operation and mutual understanding between scientists as such and those engaged in information work. Recommendations relating to undergraduate facilities stress the importance of at least two foreign languages, a broad liberal arts education and a major course in a scientific discipline for those entering on scientific information work. Establishment of a graduate school of science information leading to a master's degree is recommended as well as research leading to the establishment of a doctoral programme in this field and the growth of specialized information centres at the universities. They also recommended that technical institutions should explore the needs of industry and government for technicians in special libraries and science information centres and offer appropriate curricula, covering such subjects as machine searching, operation of related communication and retrieval instruments, routine indexing and coding tasks.

---

* Science Information Personnel: The New Profession of Information Combining Science, Librarianship and Foreign Language. By Leonard Cohen and Kenneth Craven. Pp. vi+74. (New York: Science Information, P.O. Box 624, Radio City Station, 1961.) 1.50 dollars.

While the survey anticipates recognition of a more systematic and integrated approach to the organization of scientific information and the according of professional status to the scientific information specialist, the practical difficulties which the implementation of its recommendations are likely to encounter, for example, in regard to teaching staff, at least in the United Kingdom, are scarcely faced. There is also much preliminary work to be done by those already working as information officers and librarians in this field before an appropriate professional body could be formed. Nevertheless, much already exists to form the basis, and the development of the National Lending Library for Science and Technology and of the National Reference Library of Science and Information should stimulate the support and organization of research experiment and development into the nature and techniques of communication, the information requirements of scientists, the nature of technical literature sources, manual and mechanical information systems and devices, and the organization and dynamics of information facilities, which the authors of this survey both urge and expect.

DISTRIBUTION OF BRIGHTNESS IN EXTRAGALACTIC RADIO SOURCES

By A. T. MOFFET and P. MALTBY

California Institute of Technology, Pasadena

As part of a continuing programme of angular size and brightness distribution measurements, we have observed 180 radio sources with the variable spacing interferometer at the Owens Valley Radio Observatory. In this article we report our results for a group of 88 sources which have been observed with both an east–west and a north–south baseline. All observations were made at a wave-length of 31·3 cm. Some preliminary results of the east–west observations were reported by Bolton[1].

The instrument has been described by Read[2], and consists of two equatorially mounted 90-ft. paraboloids, which can be moved along railroad tracks to certain fixed observing stations. When these observations were taken, there were stations at spacings of 200 ft., 400 ft., 800 ft. and 1,600 ft. east and north of a central junction. With one antenna at the central junction and the other at each of these stations successively, a primary set of observations was made, at transit, of the apparent intensities and positions of the sources. From these observations the amplitude and phase of the complex visibility function[3] for each source in the east–west and north–south directions were deduced. A secondary set of intensity measurements was taken off the meridian at each spacing in order to obtain supplementary information about the behaviour of the visibility function at other spacings or directions. The distribution of brightness of a source is related to its visibility function by a Fourier transformation[3]. The primary observations may be considered to be somewhat the same as observing the source with two fan beams, each having dimensions of about 2 × 50 min. of arc.

With a maximum antenna spacing of 1,557 wavelengths, we were able to distinguish details of source structure larger than about 1·5′. For a source with diameter between 0·6′ and 1·5′, an estimate of the diameter was possible, and this was taken to be the width at half-brightness of the circular Gaussian model which fitted best the observed visibility function. The structure of a larger source was determined by comparing the observed portion of the visibility function with artificial visibility functions, calculated for a number of physically reasonable models.

It was found that the majority of the sources greater than 1·5′ in extent could not be interpreted as simple, symmetrical sources in which the brightness decreased monotonically from a single peak. About 10 per cent of all the sources were found to fit a model with two equal centres of emission. Of these, Cygnus *A* is the classic example. These sources are listed in Table 1.

Table 1. Sources consisting of Two Equal Components

3*C*-75
3*C*-111
3*C*-134
3*C*-219
3*C*-270
NGC 5128 (centre source)
3*C*-310
Cygnus *A*
3*C*-445

The results of the interpretation are shown in Table 2. About half the well-resolved sources were asymmetrical and could be interpreted either as consisting of two unequal components or as single asymmetrical distributions. Comparing the east–west and north–south observations, we found that these sources were usually quite elongated, with a ratio of major to minor axes of ⩾ 2 : 1. The remaining one hundred sources, which we have observed only along one baseline, seem to be distributed among the various classifications in more or less the same proportions as those reported in Table 2.

Table 2. Numbers of Sources of Different Types. Diameters refer to a Circular Gaussian Model

Interpretation	No. of sources
Two equal components	9
Halo + core	4
Asymmetrical or two unequal components	26
Diameter between 1·2′ and 2·5′ in one direction; most are elongated ⩾ 2 : 1	10
Diameter < 1·2′	39

We suggest that many of the asymmetrical sources really consist of two unequal components. To obtain further proof of this, it will be necessary to take observations with a smaller effective beam-width than that used in this work. At large antenna spacings, the visibility function of a source with two well-separated, unequal components will differ markedly from that of a source with a single asymmetrical distribution of brightness. Long baseline observations made at Jodrell Bank[4] tend to support our suggestion.

We have considered the possibility that the double radio sources may be connected with double, interacting galaxies, as is the case in Cygnus *A*. A number of identifications of radio sources with optical objects has been reported[1,5,6]. Comparing these results with our own, we find that there is not a clear correlation between double radio sources and double galaxies.

We have suggested that the majority of the discrete radio sources are double, physically linked radio systems; however, it will be necessary to have more identifications with optical objects before conclusions can be drawn about the physical mechanism responsible for this phenomenon.

Details of this work will be published elsewhere. The programme of research in radio astronomy at the California Institute of Technology is supported by the United States Office of Naval Research. One of us (A. T. M.) has held a National Science Foundation pre-doctoral fellowship during the course of this work. The other (P. M.) has a Fulbright travel grant and a grant from the Norwegian Research Council for science and the humanities.

[1] Bolton, J. G., Introductory talk at the session on discrete sources at the U.R.S.I. General Assembly, London (1960).

[2] Read, R. B., *Inst. Rad. Eng. Trans. on Antennas and Propagation*, AP-9, 31 (1961).

[3] Bracewell, R. N., *Proc. Inst. Rad. Eng.*, **46**, 97 (1958).

[4] Palmer, H. P., and Brown, R. H. (private communication).

[5] Dewhirst, D. W., *Paris Symposium on Radio Astronomy*, edit. Bracewell, R. N. (Stanford Univ. Press, 1959).

[6] Mills, B. Y., *Austral. J. Phys.*, **13**, 550 (1960).

COMPARISON OF BERYLLIUM-7 AND CÆSIUM-137 RADIOACTIVITY IN GROUND-LEVEL AIR

By DR. P. F. GUSTAFSON, M. A. KERRIGAN and S. S. BRAR

Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois

SINCE the discovery of carbon-14 produced by cosmic rays by Anderson and Libby in 1947 [1], a number of other radionuclides, also produced by cosmic-ray interactions with atmospheric constituents, have been found in the atmosphere and in the oceans. Several investigators, notably Lal[2] and Peters[3], have suggested that these naturally occurring radionuclides might prove useful in the study of atmospheric mixing and circulation. For this purpose, beryllium-7, first reported by Arnold and Al-Salih in 1956 [4], is probably the most important. The rate of production of beryllium-7 is exceeded only by that of carbon-14 and tritium. The high specific activity resulting from its 53-day half-life and the lack of dilution by stable beryllium make it an attractive choice for such work. The emission of an 0·48-MeV. γ-ray in 11 per cent of the disintegrations[5] make it fairly easy to detect beryllium-7 by means of present-day γ-ray counters. Furthermore, detectable increases in beryllium-7 attributable to nuclear weapon tests have not been found as was the case for sulphur-35 (ref. 3).

The use of isotopic concentration as a means of measuring circulation and mixing stems from the inherently higher concentration prevailing in the stratosphere. The difference in concentration between stratosphere and troposphere is due primarily to the increase in cosmic-ray flux with altitude, and the resultant increase in isotope production via cosmic-ray neutrons as a function of altitude. In addition to lower initial production, the concentration within the troposphere is further reduced by the continual removal of activity by precipitation. Until recently all production-rates were based largely upon theory, but in 1960 Lal *et al.*[6] measured the rate of production of beryllium-7 directly in oxygen (water) exposed on Mount Evans, Colorado, and found the empirical results to be only 10 per cent higher than the calculated rate.

Beryllium-7 has been measured in surface air at Argonne National Laboratory (42° N., 88° W.) since mid-1959, at which time the concentration of γ-emitting fission products in air had diminished sufficiently so that the 0·48-MeV. line from beryllium-7 was readily apparent in the γ-ray spectrum of ashed air filters. The filters consisted of '*HV*-70' (Hollingsworth–Vose) filter paper mounted on 'Sutorbilt' air pumps and operated at flow-rates of 25–30 cubic metres per hr. The '*HV*-70' paper has an efficiency of 96 per cent for particles greater than $0{\cdot}25\mu$ in diameter. Filters from 8–10 air pumps operated continuously were grouped by calendar month and partially ashed at 300° C. (total ashing was not possible due to the presence of an asbestos base) prior to γ-counting. The volume of air from which particulates were removed was of the order of 10^5 cubic metres per month. The ashed residue was compacted into a cylindrical wafer and placed on a 5 × 4 in. sodium iodide (thallium) crystal for γ-ray spectrometric analysis. Both sample and detector (consisting of the crystal and a DuMont 6265 photomultiplier tube) were enclosed in a 9-in. thick steel shield with an inner shield consisting of 1 in. of mercury in a stainless steel annulus to achieve low background conditions. Pulse analysis was implemented using an Argonne type 256 channel analyser. Suitable sources of a number of fission products were used for standardization purposes. The concentrations of cæsium-137, zirconium-95, cerium-144, antimony-125, ruthenium-106, and beryllium-7 were determined by a method utilizing a combination of simultaneous equations and successive approximations.

The concentrations of beryllium-7 and cæsium-137 expressed in curies/cubic metre of air are shown in Fig. 1. The cæsium-137 measurements extend from January 1959 to the end of 1960, whereas those for beryllium-7 start in August 1959 and are corrected for decay to the middle of the month of collection. Both radionuclides exhibit maximum concentrations during the spring of 1960, and cæsium-137 shows a similar maximum in 1959. Such maxima, in the absence of recent nuclear tests, are attributed to the disruption of the northern hemisphere tropopause in middle latitudes during the spring months, resulting in an influx of high-altitude debris, much of it of polar origin, into the lower atmosphere. A repetition of the beryllium-7 maximum in 1959 is not seen owing to lack of data prior to August 1959; however, a similar trend in beryllium-7 concentration in surface air was noted by Cruikshank, Cowper and Grummitt[7] at two sites in Canada in 1954.

Following the period of maximum concentration, the level of cæsium-137 diminishes rapidly, proceeding at a rate in accord with that ascribed to removal of tropospheric debris by precipitation (~ 30 day half-

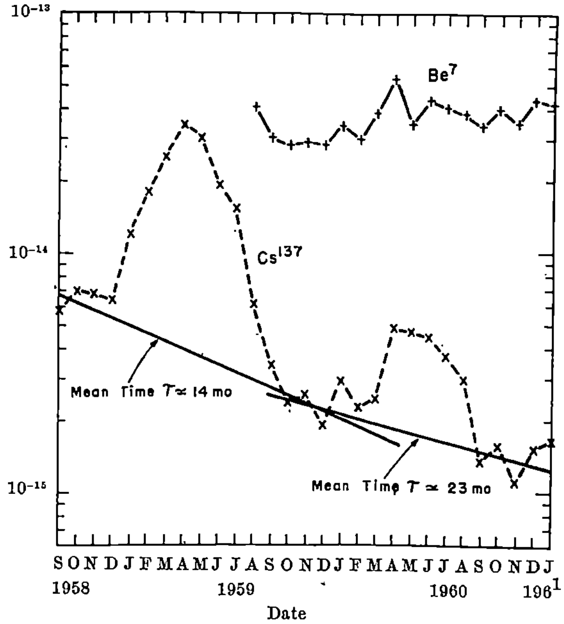


Fig. 1. Concentration of beryllium-7 and cæsium-137 in surface air at Argonne National Laboratory

time). The level of beryllium-7, on the other hand, decreased slightly and then remained at a substantially higher concentration than that observed prior to the maximum until the end of 1960. The difference in behaviour between beryllium-7 formed at a constant (though not homogeneous) rate throughout the entire atmosphere and cæsium-137 coming entirely from the stratosphere is an intriguing one, particularly in that it may provide information regarding transport of debris from the stratosphere into the troposphere. The apparent increase in beryllium-7 during the summer of 1960 might have been due to a decrease in precipitation, resulting in a lower rate of removal, and hence a build-up toward true equilibrium between production and radioactive decay. If precipitation is indeed the controlling factor, then the effect should have been in the opposite direction ; the beryllium-7 in surface air should have decreased since rainfall is more abundant during the summer months in middle and northern latitudes in the United States and Canada.

It appears that there was an actual increase in the concentration of beryllium-7 in the troposphere during the summer of 1960, and by inference during the summer each year. The basis for such a hypothesis is shown in Fig. 2, in which the reciprocal of the average monthly beryllium-7 concentration in surface air ($1/N$) is plotted as a function of the fraction of the annual precipitation which fell during the previous month (R), according to the method of Cruikshank *et al.*[7]. The assumption is made that equilibrium exists between beryllium-7 production and removal in the troposphere. Hence:

$$Q = \Lambda N \qquad (1)$$

where Q is rate of production of beryllium-7 in atoms/litre/day; Λ is rate of removal of beryllium-7 per day $= \lambda + kR$; λ is disintegration constant of beryllium-7 per day; R is fraction of annual precipitation falling in previous month; k is removal by precipitation per day; N is concentration of beryllium-7 in atoms/litre.

Equation (1) may also be written as:

$$1/N = \Lambda/Q = \lambda/Q + kR/Q \qquad (2)$$

Values of $1/N$ show a considerable range for a given value of R, the variation being outside the statistical accuracy of the determinations. For the most part the points appear to belong to two distinct groups as shown by the weighted average of each set. The intercept at $R = 0$ provides a measure of the production rate Q according to the relationship $1/N = \lambda/Q$. It should be noted that a higher concentration occurred during April–August 1960 and in August 1959. The variation in precipitation is similar during the periods of different production rate (that is, different concentration) and would seem to indicate that the difference is not due to this source. Although local precipitation values were used, they are qualitatively representative of those pertaining over a much wider area. The true effect of rainfall was probably seen during the autumn of 1960 when the beryllium-7 in surface air was consistently higher than in 1959 whereas the precipitation was markedly lower than in the autumn of 1959. The fact that the $1/N$ values fall in the low production group during this period lends support to this contention.

The question now arises as to the source of the increase in beryllium-7 concentration during the summer months. Prolonged increases in cosmic-ray intensity may be ruled out as none was observed. An increase would result if the release of beryllium-7 from the stratosphere were greater in summer than in winter. A related parameter, which varies seasonally, is the height of the tropopause in middle and northern latitudes, rising in summer and sinking to lower altitude in winter. As a result, regions of higher production of beryllium-7 will be included in the troposphere during summer and will be located within the stratosphere during the colder season. If this is the physical mechanism involved, it should be possible to verify it, in a crude way at least, by considering the change in production rate as a function of tropopause height. In the vicinity of 40° N. the mean winter tropopause is located at 33,000–35,000 ft. and that in summer at 45,000–48,000 ft. The

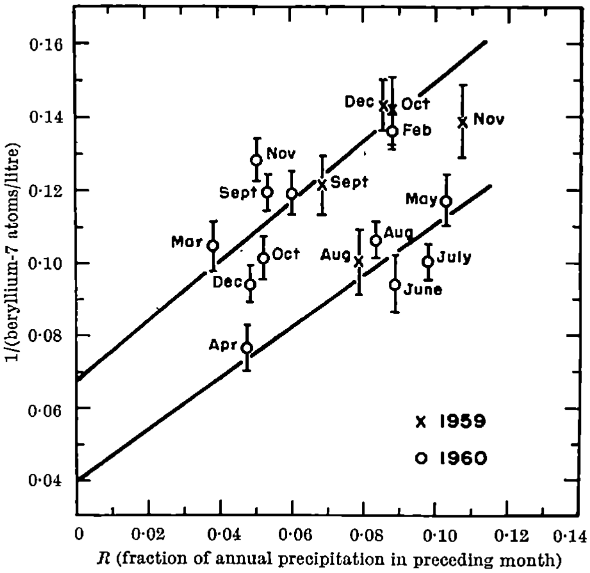


Fig. 2. Concentration of beryllium-7 in air as a function of precipitation

values of Q obtained from Fig. 2 are 0·37 and 0·20 atoms/litre/day or 260 and 130 atoms/cm.^{2}/day for high and low production respectively. These values are a full order of magnitude lower than the value of 2,200 atoms/cm.^{2}/day computed by Lal[2], including the empirical correction, for beryllium-7 production in the troposphere at 55° N. geomagnetic latitude. Because of inhomogeneities within the troposphere caused by incomplete mixing, removal of debris by precipitation preferentially from only the lower levels, as well as loss at the surface owing to dry deposition and impingement, measurements of air-borne debris made at ground-level are not representative of the mean level present throughout the troposphere. The existence of a fairly steep concentration gradient of air-borne debris between ground-level and the tropopause has been noted in aircraft profile studies[8]. Integration of the cæsium-137 and gross fission product concentration as a function of altitude during the spring and summer of 1960 indicated that an average factor of 13·5 existed between mean tropospheric concentration and that found at ground-level in the first half of 1960. The same factor was also found applicable to beryllium-7 concentration[9]. Converting the surface production values found from Fig. 2 into those for the mean troposphere yield values of 1,755 and 3,510 atoms/cm.^{2}/day for winter and summer production, respectively, which are in fair agreement with that of 2,200 atoms/cm.^{2}/day given by Lal[2]. The corresponding stratospheric production rate at this latitude computed by Lal is 9,400 atoms/cm.^{2}/day. The mass of air present between 35,000 and 50,000 ft. is 13 per cent of the total mass in a vertical column extending from the surface to 50,000 ft. The effective mean production within the summer troposphere using this mass distribution and Lal's values for tropospheric and stratospheric beryllium-7 production is found to be :

$$Q_s = 0{\cdot}87 \times 2{,}200 + 0{\cdot}13 \times 9{,}400 = 3{,}130 \text{ atoms/cm.}^2\text{/day}$$

There is reasonably good agreement between this figure and that of 3,510 atoms/cm.^{2}/day obtained from Fig. 2, particularly since no consideration has been given to transverse mixing of air which might further vary the apparent production of beryllium-7 as it is also a function of geomagnetic latitude.

If the concept can be accepted that the increase in concentration of beryllium-7 at the surface is due to the elevated tropopause, then it should be possible to relate the cæsium-137 concentration in the region of 35,000–50,000 ft. during and following the time of the maximum to that observed afterwards in surface air. The cæsium-137 present in the troposphere several months after a nuclear test may be considered to arise from continual leakage through the tropopause plus varying amounts of additional activity periodically injected by the spring break and the rising tropopause. The minima in levels of cæsium-137 in surface air seen in Fig. 1 during the autumn and winter are probably most closely a measure of the continual, 'steady state', removal of stratospheric debris by diffusion and minor fluctuations in the tropopause. Thus the amount of surface activity due to leakage during May–July 1960 should have been intermediate to the levels seen during late 1959 and the autumn of 1960. For May–July a concentration of 2×10^{15} curie/cubic metre was taken for cæsium-137 due to leakage (Fig. 1), which multiplied by 13·5 becomes $2{\cdot}7 \times 10^{-14}$ curie/m.^{3} for the mean concentration in the troposphere. Again using a value of 13 per cent for the fraction of the total air mass in the summer troposphere contained between 35,000 and 50,000 ft., and the observed value of $4{\cdot}5 \times 10^{-13}$ curie/m.^{3} for the cæsium-137 concentration in this region[10], a concentration of $\sim 6 \times 10^{-15}$ curie/m.^{3} is derived for the surface during May–July 1960. The measured concentration during this time varied from 3·8 to $4{\cdot}5 \times 10^{-15}$ curie/m.^{3}. The essential agreement between observed and calculated values tend to support the hypothesis that the rising tropopause causes the mass transfer of debris from the lower stratosphere into the troposphere from whence it is readily removed by means of precipitation and dry deposition.

The relative importance of the rising tropopause and the spring break as they affect the total removal of stratospheric debris may be estimated by integrating the total cæsium-137 found in surface air and that presumed to come only from steady-state removal. Taking a cycle extending between successive autumn minima, it was found that from the autumn of 1958 to the autumn of 1959, 75 per cent of the removal occurred via the spring break and the rising tropopause, whereas only 40 per cent was so removed during the autumn of 1959 to autumn 1960. Of these two mass transfer processes, that of the spring break is probably more important. The greater removal during the autumn of 1958 to the autumn of 1959 is presumably due to the fact that debris from the Soviet tests in October 1958 was essentially all removed by the end of 1959. Further evidence of the importance of these perturbations to steady-state removal may be seen by considering the mean stratospheric residence-time. Since the autumn minima are believed to reflect continual steady-state removal of stratospheric debris, the relationship between successive minima, as indicated in Fig. 1, should yield information relative to the mean stratospheric residence-time. During 1958–59 the mean time so derived was approximately one year, and during 1959–60 it lengthened to more than 20 months. Hence were it not for the seasonal increases in the rate of removal of nuclear debris from the stratosphere the mean residence-time would be approximately twice what it is. The non-uniformity in the rate of stratospheric removal adds to the complexity of fall-out mechanics; however, its proper consideration and better understanding will not only improve the accuracy with which ground contamination from present stratospheric debris may be predicated, but will also further our knowledge of the meteorology of the upper atmosphere.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

We are indebted to Dr. J. Sedlet of the Industrial Hygiene and Safety Division of this Laboratory for the use of air filters from his collection network.

[1] Anderson, E. C., Libby, W. F., Weinhouse, S., Reid, A. F., Kirshenbaum, A. D., and Grosse, A. V., *Science*, **105**, 576 (1947).

[2] Lal, D., Malhotra, P. K., and Peters, B., *J. Atmos. Terr. Phys.*, **12**, 306 (1958).

[3] Peters, B., *J. Atmos. Terr. Phys.*, **13**, 351 (1959).

[4] Arnold, J. R., and Ali Al-Salih, H., *Science*, **121**, 451 (1955).

[5] Strominger, D., Hollander, J. M., and Seaborg, G. T., *Rev. Mod. Phys.*, **30**, No. 2, 585 (1958).

[6] Lal, D., Arnold, J. R., and Honda, M., *Phys. Rev.*, **118**, 1626 (1960).

[7] Cruikshank, A. J., Cowper, G., and Grummitt, W. E., *Canad. J. Chem.*, **34**, 214 (1956).

[8] Peirson, D. N., Crooks, R. N., and Fisher, Miss E. M. R., *AERE–R*3358 (1960).

[9] Telegadas, K., and List, R. J., Health and Safety Laboratory Quarterly Report, January 9, 1961, *HASL*-105, 150 (1961).

[10] List, R. J., and Telegadas, K., Health and Safety Laboratory Quarterly Report, April 1, 1961, *HASL*-111, 186 (1961).

SUSTAINED OSCILLATIONS IN A CATALYTIC CHEMICAL SYSTEM

By R. A. SPANGLER* and F. M. SNELL

Department of Biophysics, University of Buffalo School of Medicine, Buffalo, New York

TO account for some features of periodic phenomena observed in biological organisms, the existence of some autonomous timing mechanism, or 'living clock', has been postulated. Although many different properties of these periodic phenomena have been reported, two characteristics are of particular theoretical interest. The frequency in certain instances is virtually independent of temperature, while in a variety of species the clock appears to be subject to external synchronization and modulation[1-3]. Any theoretical consideration of a timing mechanism must be reconciled with these observations[4].

Based entirely on chemical kinetic considerations, model mechanisms which exhibit periodic behaviour may be formulated. Because of the fundamentally dissipative nature of non-equilibrium chemical processes, however, certain non-linearities of reaction kinetics must be exploited to generate undamped oscillations in a system with a finite number of variables[5,6]. As early as 1920, Lotka[7] demonstrated a theoretical basis for undamped oscillations in a sequential series of autocatalytic reactions, a class of systems which has been treated in general by Moore[8]. More recently, Holmes[9] has discussed another non-linear kinetic mechanism, but has resorted to a discontinuous reaction coupling function to achieve oscillatory behaviour. The relationship to biological reality of both these system classes would appear remote.

Using a steady-state analytical approach as well as digital and analogue computer techniques, we have undertaken a study of the periodic behaviour exhibited by a particular realistic class of catalytic reaction systems. Being a type of relaxation oscillation, the periodicity of this system is non-harmonic in nature, and sustained oscillations may be achieved over a reasonably wide range of parameter values.

Consider a cross-coupled catalytic system formulated as follows:

$$\begin{gathered}\text{source. } A \rightarrow A + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B \xrightarrow{k_2} E + P \xrightarrow{k_3} \text{sink } P \\ \alpha P + E' \underset{k_{-4}'}{\overset{k_4'}{\rightleftarrows}} I' \qquad \alpha P' + E \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} I \qquad (1) \\ \text{source } A' \rightarrow A' + E' \underset{k_{-1}'}{\overset{k_1'}{\rightleftarrows}} B' \xrightarrow{k_2'} E' + P' \xrightarrow{k_3'} \text{sink } P'\end{gathered}$$

The parameter α is the stoichiometric number for the association of the product (P or P') of one reaction sequence with the catalyst (E' or E) of the other to form an inactive complex (I' or I).

Under steady-state conditions, the following implicit relationship may be derived:

$$\left(\frac{k_3P}{k_2E_t - k_3P}\right)\left(1 + K_4\left[\frac{k_1'k_2'E_t'A'}{k_3'(k_2' + k_{-1}')(1 + k_4'P^\alpha) + k_1'k_3'A'}\right]^\alpha\right) - \frac{k_1A}{k_2 + k_{-1}} = 0$$

$$J_{ss} \equiv k_3P \qquad K_4 \equiv \frac{k_4}{k_{-4}} \qquad K_4' \equiv \frac{k_4'}{k_{-4}'} \qquad (2)$$

where the species symbols are used to denote concentrations. J_{ss} is the unprimed steady-state reaction flux, defined as k_3P; E_t and E_t' are the total catalyst concentrations which are conserved.

When $\alpha = 1$, the partial derivative $\left(\frac{\partial J_{ss}}{\partial A}\right)_{A'}$ remains positive and finite; thus J_{ss} is a single-valued function of A for all positive values of A. However, with $\alpha = 2$, the derivative may become negative over a limited range of A-values, passing through infinity at the boundaries of the region. J_{ss} becomes a multi-valued function of A in this region, and the form of the relationship under these conditions with A' constant appears in Fig. 1.

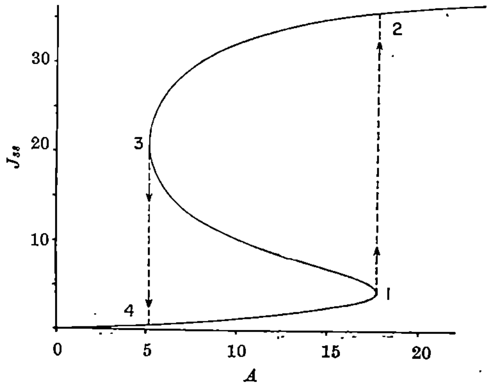


Fig. 1. Steady-state reaction flux J_{ss} plotted against reactant concentration A, as given by equation (2) with constant $A' = 10$. Axes are in arbitrary units of appropriate dimensions. Rate-constants employed for this solution are: $k_1 = k_{-1} = k_2 = k_4 = 1$; $k_3 = 0{\cdot}4$; and $k_{-4} = 0{\cdot}5$, with $k_i' = k_i . E_t = E_t' = 4$. Numbers on the curve are referred to in the text

It may be seen that this steady-state solution bears analogy to certain negative impedance devices, for example, the tunnel diode. Each possesses a region of negative slope, and each exhibits bi-stable, 'flip-flop' characteristics in this region. If, for example, A is varied cyclically over a range including the negative slope region, again with A' held constant, the system will proceed along the trajectory indicated by the dashed lines in Fig. 1, passing directly from point 1 to point 2 for increasing A, and from 3 to 4 as A is decreased. Thus the system exhibits what is analogous to a hysteresis loop in the phase-plot of steady-state conditions.

In the conditions that A is fixed at arbitrary concentrations, the negative slope portion of the continuous curve corresponds to possible stable steady-state solutions, although this segment is accessible only in rather physically unrealizable circumstances. On the other hand, if the influx of reactant A, J_A^{in}, is fixed, making A a dependent variable, this region of negative slope is characterized by instability; any random fluctuation from a given steady state in this region can only lead to

---

* Fellow, U.S. Public Health Service, Institute of Arthritis and Metabolic Diseases.

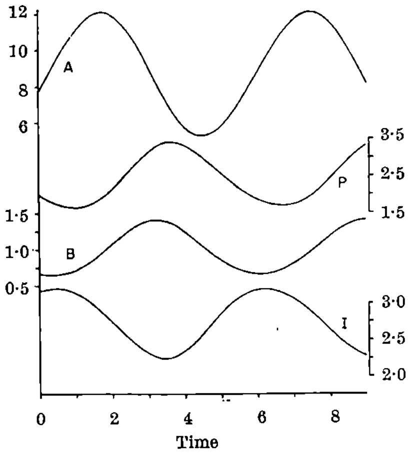


Fig. 2. Digital computer solution of the set of differential equations representing the system (1) with constant $J_A^{\text{in}} = J_{A'}^{\text{in}} = 1$. Chemical concentrations are plotted as a function of time with axes in arbitrary units. Rate-constants and the E_i's are the same as given in Fig. 1

greater deviation in the same direction. In these circumstances, then, with J_A^{in} between points 1 and 3 of Fig. 1, oscillations would in general be expected, since no stable steady-state solution exists which is compatible with the magnitude of flux from the source. Specifically, the system would be expected to trace in an anti-clockwise direction a closed trajectory in the phase-plot, roughly similar to the hysteresis loop, but not coinciding precisely because of departures from steady-state conditions. No general analytical solution for this dynamic situation exists, and it must be repeated that the foregoing analysis is based on the assumption of steady-state conditions and a constant A' concentration. The oscillations predicted by this analysis are evident, however, in the digital and analogue computer solutions of the dynamic system. Furthermore, oscillatory behaviour has been observed in the computer solutions of the fully symmetrical system in which both J_A^{in} and $J_{A'}^{\text{in}}$ are fixed. An example of these oscillations may be seen in Fig. 2.

Further work is in progress on this and other classes of reactions in an effort to delineate the detailed conditions for sustained oscillation. Preliminary observations, however, suggest several general properties of this particular system. It would appear, for example, that the frequency of oscillation of the complete system is less dependent, thermally, than the individual reactions, and it is conceivable that complete temperature compensation may be obtained by having appropriate relationships among temperature coefficients of the rate constants. It also appears that the period of oscillation is one-to-several orders of magnitude larger than the time-constants of the individual uncoupled catalytic reactions, a feature possibly of significance in consideration of the long periods of some biological phenomena. In addition, several means by which external stimuli may modulate the phase or frequency of oscillation suggest themselves, such as by alteration of either the reactant influx or the total catalyst concentration.

In view of these preliminary results and the feasibility of the assumptions involved, it appears that biological periodicity may be based entirely on chemical kinetic systems, without resort to diffusive or phase boundary effects. The model discussed here is proposed as one specific mechanism possibly underlying the biological clock.

We are grateful to Mr. Harold Meese and Dr. John Fleck of the Cornell Aeronautical Laboratories for their assistance and the use of the *IBM* 704 computer facilities. This investigation was supported by a U.S. Public Health Service Research Grant No. *RG*–6730 from the Division of General Medical Sciences.

[1] Bünning, E., *Cold Spring Harbor Symp. on Quantitative Biology*, **25** (1960).

[2] Pittendrigh, C. S., *Cold Spring Harbor Symp. on Quantitative Biology*, **25** (1960).

[3] Brown, F. A., *Science*, **130**, 1535 (1959).

[4] Schmitt, O. H., *Cold Spring Harbor Symp. on Quantitative Biology*, **25** (1960).

[5] Hearon, J. Z., *Bull. Math. Biophys.*, **15**, 121 (1953).

[6] Finn, R. K., *Bull. Math. Biophys.*, **16**, 181 (1954).

[7] Lotka, A. J., *J. Amer. Chem. Soc.*, **42**, 1595 (1920).

[8] Moore, M. J., *Trans. Farad. Soc.*, **45**, 1098 (1949).

[9] Holmes, W. F., Abstr. Fifth Ann. Meeting of the Biophys. Soc., St. Louis, Missouri, TB 13, February 1961.

MINIMUM ENERGY THEOREM FOR FLOW OF DRY GRANULES THROUGH APERTURES

By R. L. BROWN

British Coal Utilization Research Association, Leatherhead, Surrey

ALTHOUGH the motion of granules flowing down a vessel to escape through an aperture is complex, the flow-rate (Q gm./sec.) over small intervals of time is remarkably steady. It seems permissible to represent the average flow by stream-tubes in a continuum of density ρ gm./c.c. Then conservation of number flowing in a stream-tube gives:

$$N_1 \mathrm{d}A_1 = N_2 \mathrm{d}A_2 \tag{1}$$

where N granules per cm.²/sec. are flowing orthogonally to an elementary area $\mathrm{d}A$ (Fig. 1). If v cm./sec. is the velocity of the continuum normal to $\mathrm{d}A$:

$$\rho v = mN \tag{2}$$

where m gm. is the weight of a granule. It is assumed that the granules all have the same weight.

Consider an elementary volume $\mathrm{d}\tau$ c.c. at co-ordinates (R,θ). The mass is $\rho \mathrm{d}\tau$ gm., the height above the origin of co-ordinates is $R \cos\theta$ cm. and the velocity v cm./sec. Writing $T\mathrm{d}\tau$ for the sum of the potential and kinetic energies:

$$2T = \rho(v^2 + 2gR\cos\theta) \tag{3}$$

Now it is well known[1] that the flow-rate Q does not vary with the head of material in the vessel, except perhaps at very small heads. Pressure is not transmitted far in a granular mass, owing to the development of arches and to frictional forces between granules. These considerations suggest that the pressures on the faces $\mathrm{d}A$ of the volume $\mathrm{d}\tau$ do little or no work. Energy is dissipated, however, on the

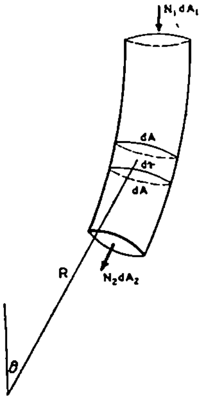


Fig. 1. Stream-tube in continuum

curved surface of dτ and by collisions, rotations and frictional forces within dτ. It is postulated therefore that the energy T per unit volume decreases in the direction of flow along a stream-tube. Remembering that the granules below the aperture are falling freely (apart from collisions in horizontal planes), the vertical pressure just above the aperture cannot be large, and it seems likely that the energy postulate will hold at least in the neighbourhood of the aperture, provided that there is no appreciable cohesion between granules.

Above apertures in a horizontal base plate, a portion of the mass resting on the base is stationary, and the stream-tubes are forced to converge. Visual observation suggests that, to the first order, the flow takes place in a straight-sided wedge or cone with half-angle β (Fig. 2). The simplest way of satisfying the continuity equation (1) is to take the stream-tubes to be radial. Then for plane flow between parallel vertical plates, distant l cm. apart, through a slit of width S cm.:

$$N = \frac{AK(\theta)}{R} \tag{4}$$

whence, taking A independent of (R,θ),

$$Q = 2mAl\int_0^\beta K(\theta)\mathrm{d}\theta \tag{5}$$

For axial flow down a cylinder through a circular aperture of diameter D cm.:

$$N = \frac{BL(\theta)}{R^2} \tag{6}$$

and

$$Q = 2\pi mB\int_0^\beta L(\theta)\sin\theta\mathrm{d}\theta \tag{7}$$

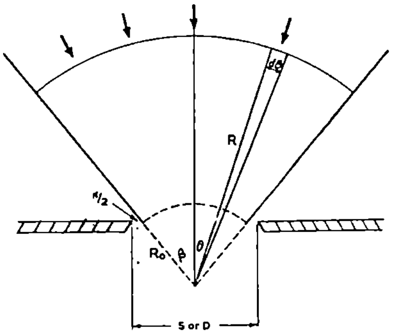


Fig. 2. Flow in a wedge or cone

It has been shown elsewhere[2] that there is an effectively empty annulus, of width $k/2$ cm., adjacent to the edge of the aperture. k is the same for slits and circles and depends *inter alia* on the size of the granules. This annulus can be taken into account (Fig. 2) by locating the vertex of the wedge or cone a distance R_0 cm. from the edge of the annulus, where:

$$2R_0\sin\beta = (S-k) \text{ or } (D-k) \tag{8}$$

In what follows it will be assumed that ρ is constant throughout the wedge or cone, apart from a possible discontinuity at $\theta = \beta$. Thus v is taken to be proportional to N.

Then in plane flow, equations (2), (3) and (4) give:

$$2T = \rho\left[\frac{m^2A^2K^2(\theta)}{\rho^2R^2} + 2gR\cos\theta\right] \tag{9}$$

which has a minimum at R_m where:

$$gR_m^3 = \frac{m^2A^2K^2(\theta)}{\rho^2\cos\theta} \tag{10}$$

Likewise for cylindrical flow, equations (2), (3) and (6) give:

$$gR_m^5 = \frac{2m^2B^2L^2(\theta)}{\rho^2\cos\theta} \tag{11}$$

If the energy postulate that T decreases along a stream-tube is to hold, then the surface of minimum energy (R_m,θ) must lie below the wedge or cone.

The flow-rate Q may be expressed in terms of aperture size and the surface of minimum energy by means of equations (5), (7), (8), (10) and (11), namely:

$$\sigma = \frac{Q}{\rho l(S-k)\{g(S-k)\}^{1/2}} = \frac{\int_0^\beta (R_m/R_0)^{3/2}\cos^{1/2}\theta\mathrm{d}\theta}{2^{1/2}\sin^{3/2}\beta} \tag{12}$$

$$\gamma = \frac{4Q}{\rho\pi(D-k)^2\{g(D-k)\}^{1/2}} = \frac{\int_0^\beta (R_m/R_0)^{5/2}\cos^{1/2}\theta\sin\theta\mathrm{d}\theta}{\sin^{5/2}\beta} \tag{13}$$

It would not be unexpected in flow from wide vessels to have β a constant, determined by material characteristics. If this were the case, equations (12) and (13) show that σ, γ are constants, provided (R_m/R_0) is a function of θ, β only. Experimental evidence has been given elsewhere showing that σ, γ are constants for several materials discharging from wide vessels through a range of aperture sizes[2]. Such discrepancies as have been noted, are small. (This is not the case for narrow vessels.) It would appear, therefore, that (R_m/R_0) is, to the first order, a function of θ, β only.

Now the sides of the wedge or cone at $\theta = \beta$ cease for $R \leqslant R_0$. Thus the surface $R = R_0$ can be regarded as an upper limit to the surface (R_m, θ) whence equations (12) and (13) yield upper limits for σ, γ:

$$\sigma_u = \frac{\int_0^\beta \cos^{1/2}\theta\mathrm{d}\theta}{2^{1/2}\sin^{3/2}\beta} \tag{14}$$

$$\gamma_u = \frac{2(1-\cos^{3/2}\beta)}{3\sin^{5/2}\beta} \tag{15}$$

It is interesting to note that, taking the average velocity around the arc of the wedge as $(Q/2R\beta l\rho)$ and

Table 1. FLOW FROM FLAT-BOTTOMED VESSELS

Material	Symbol	Mean weight diam. (cm.)	Empty annulus (k cm.)	Bulk density† (ρ gm./c.c)	Plane flow		Cylindrical flow	
					β degrees	observed σ	β degrees	observed γ
Glass beads	(1)	0·096	0·13	1·71	65	0·82	31–34	0·69
Glass beads	(2)	0·023	0·041	1·70	66	1·16	40–41	0·83
Rounded sand	*X*	0·062	0·10	1·49	52	0·82	27–30	0·68
Rounded sand	●	—	—	1·50*	56	0·90	—	—
Tapioca	*T*	0·145	0·22	0·72	56	0·82	29–31	0·68
Sharp sand	1	0·054	0·12	1·25	41	0·90	29–30	0·74
Sharp sand	2	0·038	0·079	1·23	45	0·95	22–27	0·75
Sharp sand	3	0·017	0·036	1·18	40	0·87	22–26	0·71
Coal	*C*	0·061	0·14	0·65	45	0·90	32–35	0·72

* Assumed.
† A minimum value measured by fluidizing bed and then slowly reducing air flow to zero; used for ρ in equations 12 and 13.

the average height of the arc as ($R \sin \beta/\beta$), application of the minimum energy theorem at $R_m = R_0$ gives:

$$\sigma_{\text{av.}} = \frac{\beta^{1/2}}{2^{1/2} \sin \beta} \tag{16}$$

the corresponding equation for cylindrical flow being:

$$\gamma_{\text{av.}} = \frac{(1 - \cos \beta)^{1/2}}{2^{1/2} \sin^{3/2} \beta} \tag{17}$$

For values of β ranging to more than 70°, equations (16) and (17) differ by less than 1 per cent from equations (14) and (15), respectively. It would seem, therefore, that the flow-rates may not be very sensitive to the profiles $K(\theta)$, $L(\theta)$. These profiles are associated with the minimum surface (R_m, θ) by equations (10) and (11).

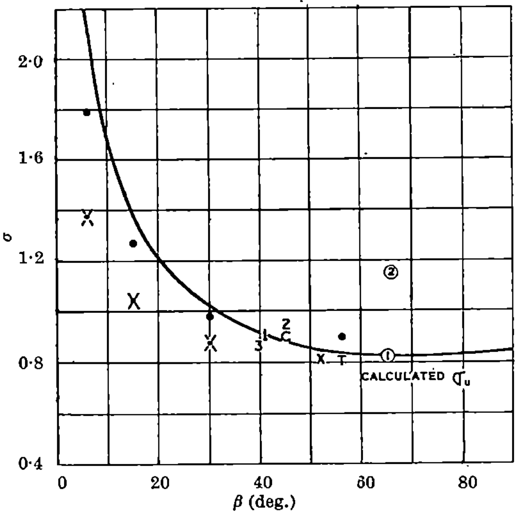


Fig. 3. Plane flow. Symbols are defined in Tables 1 and 2

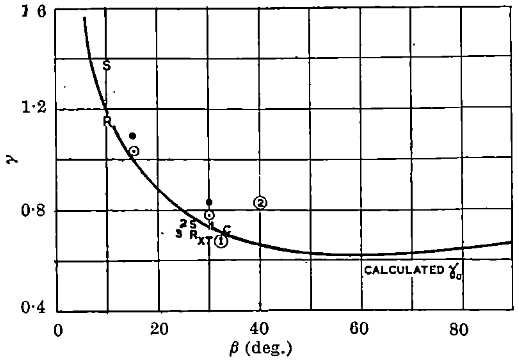


Fig. 4. Cylindrical flow. Symbols are defined in Tables 1 and 2

Data already published on σ,γ can be used to test whether the predicted relation of σ_u,γ_u with β, is valid. This is attempted first for flow from flat-bottomed vessels where the material forms its own wedge or cone. The angle β in plane flow was measured by observation through a 'Perspex' side plate. Marker particles were used in cylindrical flow; those emerging in the first runnings were noted. The estimates of β found by these methods are considered to be reasonably good; details will be given elsewhere. It was found that β for cylindrical flow was appreciably less than for plane flow (Table 1).

The observed values of σ,γ are plotted against β in Figs. 3 and 4. Agreement with the calculated values of σ_u,γ_u is seen to be good and it follows that the angle β of the wedge or cone is a major factor in determining the rate of efflux. It is satisfactory to see that in plane flow the experimental data follow the theoretical curve round to its minimum at about 65°. At larger values of β, flow would be taking place at angles below the angle of rest to the horizontal, which is clearly impossible. The theoretical equation for cylindrical flow exhibits a similar minimum flow-rate, but in practice the cone angle formed by the material is appreciably smaller than 60°.

Discrepancies appear in Figs. 3 and 4 for small glass beads, which are extremely mobile: this might be due to the effective wedge or cone angle being appreciably smaller than those reported in Table 1.

Also shown in Fig. 3 are data (Table 2) for flow of a rounded sand through troughs with half-angles of 30°, 15°, 6°, these being less than the natural wedge-angle of 56°. Two regimes of flow were found with the troughs, separated by a distinct discontinuity. At the higher slit-sizes Q was proportional to $(S - k)^{3/2}$

Table 2. FLOW THROUGH TROUGHS AND CONICAL HOPPERS

Material	Symbol	Mean sieve size (cm.)	Bulk density† (ρ gm./c.c)	Trough or hopper angle β deg.	Observed σ or γ	
					Low	High
		Plane flow (Fig. 3)				
Rounded sand	×, ●	*c.* 0·055	1·50*	30 15 6	0·89 1·06 1·40	0·99 1·29 1·81
		Cylindrical flow (Fig. 4)				
Radish seed (ref. 3)	*R*	0·25	0·73	27 10	0·71 1·15	
Socony beads (ref. 3)	*S*	0·43	0·73	27 10	0·73 1·38	
Potassium nitrate (ref. 4)	⊙	0·043	1·24	30 15	0·78 1·03	
	●	0·025	1·27	30 15	0·83 1·09	

* Assumed.
† As reported, a normal, not a minimum bulk density.

with the same k as for a flat-bottomed vessel; these points (●) follow the theoretical curve.

At the smaller slit-sizes Q still varied with $(S-k)^{3/2}$ but now k varied with trough angle and the flow-rates (×) were lower. Possibly, the 'crowdedness' of the emerging granules is greater.

Results for cylindrical flow through conical hoppers are given in Fig. 4, by recalculating data reported by other workers (Table 2). Agreement with the theory is satisfactory.

The minimum energy theory applied to radial flow from wide vessels shows that σ or γ are determined respectively by the angle of the wedge or cone formed by the material itself, or for flow through a trough or conical hopper, by the angle of the trough or hopper. For flat-bottomed vessels the difference in β for plane and for cylindrical flow is an important fact for elucidation of the observed flow-rates.

Acknowledgment is made to the British Coal Utilization Research Association for permission to publish and to Mr. J. C. Richards and Mrs. J. Tee for assistance with the experiments.

[1] Brown, R. L., and Richards, J. C., *Trans. Inst. Chem. Eng.*, **37**, 108 (1959).

[2] Brown, R. L., and Richards, J. C., *Trans. Inst. Chem. Eng.*, **38**, 243 (1960).

[3] Rausch, J. M., Ph.D. thesis, University of Princeton (1949).

[4] Deming, W. E., and Mehring, A. L., *Indust. Eng. Chem.*, **21**, 661 (1929).

COMPARATIVE ANALYSIS OF INACTIVATING EFFICIENCY OF RADIATION ON DIFFERENT ORGANISMS

By DR. M. TERZI

Istituto di Genetica, Università, Pavia

IN 1953, Epstein[1], and after him other authors, put forward an interpretation of the lethal effect (direct effect) of ionizing radiations based on the idea that the effect itself is exerted upon the nucleic acids. The dose – effect relationship was expressed as the ratio of volume of nucleic acids (calculated by other physical measurements) to 'target' volume. Their theory, however, suffered some limitations, since it gave an unsatisfactory fit when organisms other than small viruses were studied.

This interpretation can now be revised in the light of new structural data on nucleic acids, and extended to more complex organisms.

Available data are summarized in Table 1, which shows the following:

(*a*) The X-ray dose, expressed in roentgens, corresponding to $\exp^{-1}$ individuals surviving (D_0); such a dose is calculated from the logarithmic portion of the survival curve to radiations when the organisms were in a protecting medium or in a dry state. Survival is defined on the basis of infecting ability for viruses, or colony-forming ability for other organisms.

(*b*) The nucleic acid content of several organisms: for ribonucleic acid (RNA) viruses, it gives the RNA content; for all other organisms, the amount of deoxyribonucleic acid (DNA) is given: both are expressed in molecular weight units. Data were selected by choosing the most recent observations available in the literature. I have given two values when two limits are given by the same author, and also when two divergent results were published almost simultaneously. In order to reduce the number of references, I have often referred to reviews rather than to original papers.

(*c*) The lethal efficiency (e) per ion pair produced in the nucleic acids corresponding to the dose D_0.

(*d*) The mean energy (in ergs) absorbed by nucleic acids; this value is termed E and it is inversely proportional to the efficiency of inactivation.

The values for e and E are plotted in Fig. 1 in a randomized order; this procedure (suggested to me by Prof. M. Ageno) enables a non-biased evaluation of the groupings.

One can see that four different levels, expressing inactivation efficiency, correspond to four systematically distinct groups. The different degree of complexity of their genetic structure justifies their reaction to ionizing radiations.

The first level ($\bar{e} = 0{\cdot}64$) corresponds to single-stranded DNA or RNA viruses; such data are in good agreement with those obtained by Hutchinson[2] ($\bar{e} = 0{\cdot}51$) with some forty dried proteins irradiated with electrons, and with the results of Aurisicchio *et al.*[3] on the efficiency of assimilated phosphorus-32 decay of alpha phage.

At the second level one finds animal and bacterial viruses containing double-stranded DNA. In this group $\bar{e} = 0{\cdot}62 \times 10^{-1}$ in agreement with the figure calculated by Hershey *et al.*[4] for the assimilated phosphorus-32 decay of the T-even phages. The vaccinia virus (No. 2) shows an aberrant e value; some of its properties, however, such as its enzyme content[5] and the presence of many 'targets'[6], point independently to an unusual structure. It is not clear, on the other hand, why *Haemophilus influenzae* should belong to this group.

It is worth pointing out that the differences in E values between the first and second group cannot depend on the particle size, because both groups are extremely variable in this respect.

In the third group ($\bar{e} = 1{\cdot}23 \times 10^{-2}$), bacteria and haploid yeast are represented. The finding (Morse *et al.*[7]) that crossing-over in *E. coli* occurs at the four-strand stage indicates that the hereditary structure

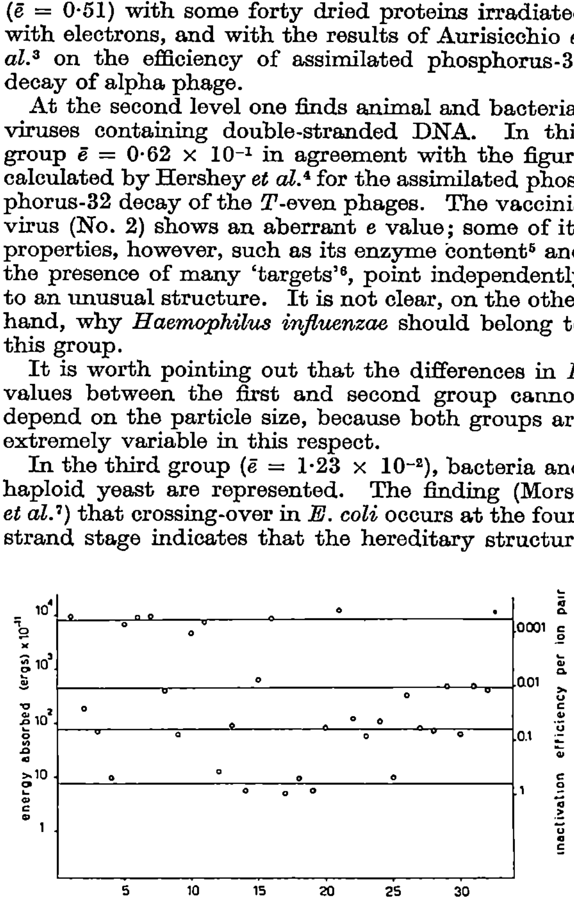


Fig. 1

Table 1

Organisms	D_0	Ref.	Nucleic acid content (mol. wt. units)	Ref.	e	E ($\times 10^{-11}$)
Tobacco mosaic virus	$1·8 \times 10^{5}$	1	$2·0 \times 10^{6}$	2	1·00	5·2
Tomato bushy stunt	$4·5 \times 10^{5}$	3	$1·6 \times 10^{6}$	4	0·51	10·2
Fowl plague	$1·0 \times 10^{5}$	3	$2·2–6·0 \times 10^{6}$	5	1·70–0·62	3·1–8·4
Tobacco ringspot	$4·6 \times 10^{5}$	3	$1·5 \times 10^{6}$	6	0·54	9·7
Tobacco necrosis	$6·7 \times 10^{5}$	3	$1·5 \times 10^{6}$	6	0·38	13·6
Phage alpha	$2·2 \times 10^{4}$	7	$3·1 \times 10^{7}$	7	0·55	9·4
Phage φ *X*174 (*S* 13)	$2·5 \times 10^{5}$	8	$1·7 \times 10^{6}$	9	0·87	6·0
Vaccinia	$8·0 \times 10^{4}$	3	$1·8 \times 10^{8}$	5	$0·26 \times 10^{-1}$	200
Shope papilloma	$4·4 \times 10^{5}$	3	$1·4 \times 10^{7}$*	10	$0·61 \times 10^{-1}$	86
Phage *BM*	$1·9 \times 10^{5}$	11	$2·5 \times 10^{7}$	12	$0·81 \times 10^{-1}$	64
Phage *T*1	$8·5 \times 10^{4}$	13	$5·0 \times 10^{7}$	13	$0·87 \times 10^{-1}$	60
Phage *T*2 (*T*4–*T*6)	$4·0 \times 10^{4}$	13	$1·7 \times 10^{8}$	13	$0·64 \times 10^{-1}$	93
Phage *T*3 (*T*7)	$8·5 \times 10^{4}$	13	$6·5 \times 10^{7}$	13	$0·68 \times 10^{-1}$	77
Phage *T*5	$3·5 \times 10^{4}$	13	$1·3 \times 10^{8}$	13	$0·81 \times 10^{-1}$	64
Phage lambda	$1·1 \times 10^{5}$	13	$7·0 \times 10^{7}$	13	$0·48 \times 10^{-1}$	108
Phage *P*22	$1·1 \times 10^{5}$	13	$5·0 \times 10^{7}$	13	$0·67 \times 10^{-1}$	77
Phage *P*8	$8·3 \times 10^{4}$	13	$7·0–14·0 \times 10^{7}$	13	$0·64–0·32 \times 10^{-1}$	82–160
Hæmophilus influenzae	$5·0 \times 10^{3}$	14	$1·2 \times 10^{9}$	14	$0·62 \times 10^{-1}$	84
Bacterium aertrycke	$4·0 \times 10^{3}$	3	$7·2 \times 10^{9}$	15	$1·3 \times 10^{-2}$	400
Diplococcus pneumoniae	$2·0–3·0 \times 10^{4}$†	16	$1·2 \times 10^{9}$	14	$1·5–1·0 \times 10^{-2}$	347–520
Micrococcus pyogenes aureus	$4·0 \times 10^{3}$	17	$8·5 \times 10^{9}$	15	$1·1 \times 10^{-2}$	477
Aerobacter aerogenes	$1·4 \times 10^{3}$	17	$1·7 \times 10^{10}$	15	$1·5 \times 10^{-2}$‡	337
Escherichia coli	$4·0 \times 10^{3}$	18	$4·8–13·0 \times 10^{9}$	19–20	$1·9–0·74 \times 10^{-2}$	281–702
Saccharomyces cerevisiae (haploid)	$3·0 \times 10^{3}$	21	$1·6 \times 10^{10}$	22	$0·77 \times 10^{-2}$	676
S. cerevisiae (diploid)	$2·1 \times 10^{4}$	21	$3·3 \times 10^{10}$	22	$0·54 \times 10^{-3}$	9,670
(triploid)	$1·3–1·8 \times 10^{4}$	21	$4·5 \times 10^{10}$	22	$0·65–0·46 \times 10^{-3}$	8,000–11,200
(tetraploid)	$1·3 \times 10^{4}$	21	$6·8 \times 10^{10}$	22	$0·42 \times 10^{-3}$	12,400
(pentaploid)	$7·7 \times 10^{3}$	21	$8·0 \times 10^{10}$	22	$0·60 \times 10^{-3}$§	8,680
(hexaploid)	$7·4 \times 10^{3}$	21	$9·6 \times 10^{10}$	22	$0·52 \times 10^{-3}$§	9,980
Guinea pig	$1·0 \times 10^{2}$	23	$5·0 \times 10^{12}$	24	$0·74 \times 10^{-3}$	7,020
Chick	$3·0–4·0 \times 10^{2}$	25	$1·5 \times 10^{12}$	24	$0·81–0·62 \times 10^{-3}$	6,400–8,420
Man	$5·0–6·0 \times 10^{1}$	26	$6·2 \times 10^{12}$	24	$1·20–1·00 \times 10^{-3}$	4,350–5,200

*, the value calculated in 1942 by Taylor *et al.* was 4×10^{6}. †, γ-rays. ‡, assuming 4 per cent dry weight its DNA content. §, assuming that the same relation between ploidy and amount of DNA found up to tetraploid holds up to hexaploid.

[1] Wohlhieter, J. A., Buzzell, A., and Lauffer, M. A., *Biochim. Biophys. Acta*, **43**, 163 (1960). [2] Friesen, B. S., and Sinsheimer, R. L., *J. Mol. Biol.*, **1**, 321 (1959). [3] Lea, D. E., *Action of Radiations on Living Cells* (Camb. Univ. Press, second edit., 1956). [4] Schachman, H. K., and Williams. R. C., on *Viruses*, edit. by Burnet, F. M., and Stanley, W. M., **1**, 316 (Acad. Press, New York, 1959). [5] Schäfer, W., on *Viruses*, edit. by Burnet, F. M., and Stanley, W. M., **1**, 475 (Acad. Press, New York, 1959). [6] *The Nucleic Acids*, **3**, 217; edit. by Chargaff, E., and Davidson, J. (Acad. Press, 1960). [7] Aurisicchio, S., Celano, A., Coppo, A., Donini, P., and Graziosi, F., *Supp. Nuovo Cim.* (in the press). [8] Tessman, I., Tessman, E. S., and Stent, G. S., *Virology*, **4**, 209 (1957). [9] Sinsheimer, R. L., *J. Mol. Biol.*, **1**, 43 (1959). [10] Watson, J. D., and Littlefield, J. M., *J. Mol. Biol.*, **2**, 161 (1960). [11] Castagnoli, C., Donini, P., and Graziosi, F., *Giorn. Microbiol.*, **1**, 86 (1959). [12] Aurisicchio, S., Cortini, G., Emma, V., and Graziosi, F., *Int. J. Rad. Biol.*, **1**, 86 (1959). [13] Stent, G. S., *Adv. Virus Res.*, **5**, 98, edit. by Smith, K. M., and Lauffer, M. A. (Acad. Press, 1958). [14] Stuy, J. H., *J. Bact.*, **79**, 707 (1960). [15] Bacterial volume taken from Bergey's *Manual of Determinative Bacteriology*, edit. Bread, S. B., Murray, E. G. D., and Hitchens, A. P. (Baltimore, William and Wilkins, 1948). DNA content taken from Davidson, J. N., *Biochemistry of Nucleic Acids* (Methuen, 1950). [16] Drew, R. M., *Rad. Res.*, **3**, 116 (1955). [17] Fram, H., Proctor, B. E., and Dunn, C. G., *J. Bact.*, **60**, 263 (1950). [18] Pollard, E., *Amer. Nat.*, **94**, 71 (1960). [19] Gillies, N. E., and Alper, T., *Biochim. Biophys. Acta*, **43**, 182 (1960). [20] Harold, F. M., and Ziporin, Z. Z., *Biochem. Biophys. Acta*, **28**, 482 (1958). [21] Mortimer, R. K., *Rad. Res.*, **9**, 312 (1958). [22] Ogur, M., Minckler, S., Lindegren, G., and Lindegren, C. G., *Arch. Biochem. Biophys.*, **40**, 175 (1952). [23] Marin, G., and Colombo, R., *Atti Congr. A.G.I.* (Roma, in the press). [24] Leslie, I., in the *Nucleic Acids*, **2**, 1, edit. by Chargaff, E., and Davidson, J. N. (Acad. Press, 1955). [25] Rubin, H., and Temin, H. M., *Virology*, **7**, 75 (1959). [26] Puck, T. T., *J. Cell. Comp. Phys.*, **52**, Supp. 1, 287 (1000). [27] Ginoza, W., and Norman, A., *Nature*, **179**, 520 (1957). [28] Goodgal, S. H., Herriott, R. M., *Chemical Bases of Heredity*, edit. by McElroy, W. D., and Glass, B., 336 (Johns Hopkins Press, Baltimore, 1957). [29] Stuy, J. H., *Rad. Res.* (in the press). [30] Lerman, L. S., and Tolmach, L. J., *Biochim. Biophys. Acta*, **33**, 371 (1959). [31] Doty, P., quoted by Shooter, K. V., in *Progr. in Biophys.*, **8**, 310 (1957). [32] de Filippes, F. M., and Guild, W. R., *Rad. Res.*, **11**, 38 (1959).

Table 2

Organisms	D_0	Ref.*	Nucleic acid content (mol. wt. units)	Ref.*	e	E ($\times 10^{-11}$)
Tobacco mosaic virus	$1·8 \times 10^{5}$	1	$2·0 \times 10^{6}$	2	1·0	5·2
RNA	$1–3 \times 10^{5}$	1–27	$2·0 \times 10^{6}$	2	0·9	5·8
Hæmophilus influenzae	$5·0 \times 10^{3}$	14	$1·2 \times 10^{9}$	14	0·062	84
Transforming principle DNA	$1·7 \times 10^{5}$	14	$1·5–3 \times 10^{7}$	28–29	0·100	52
Diplococcus pneumoniae	$2–3 \times 10^{4}$	16	$1·2 \times 10^{9}$	14	0·012	435
Transforming-principle slow	$2–3 \times 10^{6}$	30	$7·7 \times 10^{6}$	31	0·028	18
Transforming-principle fast	$5·0 \times 10^{5}$	32	$7·7 \times 10^{6}$	31	0·100	52

* For references, see footnotes Table 1.

of bacteria is closer to that of yeast (a conventional halpoid organism) than to that of bacteriophages.

It may be mentioned that isolated DNA or RNA of different organisms (tobacco mosaic virus, *Haemophilis influenzae*, *Diplococcus pneumoniae*) show the same e value as the organism from which they are derived (see Table 2). In the case of *Diplococcus pneumoniae*, the transforming principle shows a two-slope survival curve. The value of E calculated from the slowly inactivated part shows no significant departure from E for the whole cell, while the E calculated from the fast inactivated portion of the curve is close to the E of the second group. But it is not known whether the molecular weights of the two fractions—if there are actually two fractions—are equal or not. No conclusion can therefore be reached here.

Cells from vertebrates, including man, and from yeast with ploidy greater than 1, can be placed together in the fourth group ($\bar{e} = 0·69 \times 10^{-3}$).

The fact that the inactivating efficiency for organisms of ploidy greater than 1 is lower than that for haploid cells is shown qualitatively by the different slopes of the exponential portion of the survival curves of haploid and diploid yeast cells[8]. The different efficiencies have been interpreted as due to different killing mechanisms. In haploid cells, recessive lethals (probably point mutations and/or small deficiencies) account for a rather high proportion of total lethality, whereas in polyploid organisms the most efficient type of lethals is the dominant factor (probably chromosomal aberrations or breakages).

It is difficult to offer a physical interpretation of these results. From a biological point of view, it can be said that there are discrete levels of structural organization corresponding to well-defined degrees of radiosensitivity, which seems to decrease with increasing structural complexity from single-stranded DNA to the chromosome of haploid organisms. The further decrease of radiosensitivity from haploid to di- and poly-ploid could be due, however, to a change in the phenogenetic pattern of lethality rather than to increased complexity.

It would be very useful to collect new data and compare them with other parameters related to radiosensitivity. I was limited to the use of those unicellular organisms and those cells behaving like colony-forming organisms, because colony formation appears to be a uniform and reliable way of comparing the efficiency of ionizing radiation. From a physical point of view, it can be suggested that the inactivation of more complicated structures needs perhaps larger ionization clusters, which are obviously rarer. It would also be interesting to compare the above data with others on the mutation frequency in cistrons of known length, to see if gene mutation can also show different degrees of radiosensitivity in organisms of different complexity.

I am indebted to Prof. F. Graziosi, of the University of Rome, for very valuable discussion during the preparation of this communication. The work was supported by a grant from C.N.E.N., Rome.

[1] Epstein, H. T., *Nature*, **171**, 394 (1953).

[2] Hutchinson, F., *Amer. Nat.*, **94**, 59 (1960).

[3] Aurisicchio, S., Celano, A., Coppo, A., Donini, P., and Graziosi, F., Supp. *Nuovo Cim.* (in the press).

[4] Stent, G. S., *Adv. Virus Res.*, **5**, 98, edit. Smith, K. M., and Lauffer, M. A. (Acad. Press, 1958).

[5] Schäfer, W., on *Viruses*, edit. by Burnet, F. M., and Stanley, W. M., **1**, 475 (New York, Acad. Press, 1959).

[6] Lea, D. E., *Action of Radiations on Living Cells*, second edit. (Camb. Univ. Press, 1956).

[7] Morse, M. L., Lederberg, E. M., and Lederberg, J., *Genetics*, **41**, 758 (1956).

[8] Magni, G. E., Supp. to *Nuovo Cim.* (in the press).

FORMATION AND UTILIZATION OF POLY-β-HYDROXYBUTYRIC ACID BY KNALLGAS BACTERIA (*HYDROGENOMONAS*)

By Prof. H. G. SCHLEGEL, G. GOTTSCHALK and R. VON BARTHA

Institut für Mikrobiologie der Universität, Göttingen

THE conclusion that hydrogen-oxidizing bacteria of the *Hydrogenomonas* type are rich in storage material and are able to synthesize it from carbon dioxide and molecular hydrogen has been drawn from three types of experiments: (*a*) the intensity of endogenous respiration remains nearly constant for relatively long periods of aerobic incubation; (*b*) when chemolithotrophically growing cells have reached the stationary growth phase, turbidity continues to increase while the total cell count remains constant; (*c*) washed cells suspended in phosphate buffer oxidize hydrogen in the presence of carbon dioxide three times as fast as they do in its absence. In these conditions, carbon dioxide is assimilated in substrate amounts and incorporated into a cell fraction which amounted to 65 per cent of the dry weight of cells. The solubility in chloroform, the insolubility in ethyl–ether and petroleum ether, and the resistance to potassium hypochlorite solution indicated that the storage material was poly-β-hydroxybutyric acid. This polymeric material is accumulated in the cells when, in the absence of nitrogen or phosphorus, protein synthesis and growth are inhibited, or when the cells have reached the stationary phase of growth.

For identification of the storage material three strains of *Hydrogenomonas* (*H*1, *H*16 and *H*20) were used which had been enriched under chemolithotrophic conditions and isolated independently from each other. The strains differed from one another in several physiological characteristics. They were grown in submerged culture[1]. At the end of the exponential growth phase, the cells were collected, washed and suspended in phosphate buffer. The suspension was stirred (650 r.p.m.) in an atmosphere of hydrogen (60 per cent), oxygen (30 per cent), and carbon dioxide (10 per cent) at 28° C. for 30 hr. In other experiments the nitrogen source in the growth medium was restricted to 300 mgm. ammonium chloride/litre. After having reached a density of 0·75 mgm. dry weight/ml., the cells stopped growing; further increases in dry weight and turbidity were caused by the synthesis of poly-β-hydroxybutyric acid (Table 1).

Table 1. INCREASES IN DRY WEIGHT AND IN POLY-β-HYDROXYBUTYRIC ACID CONTENT IN *Hydrogenomonas H*20 DURING CHEMOLITHOTROPHIC GROWTH FOLLOWING DEPLETION OF THE NITROGEN SOURCE

Incubation time (hr.)	Cell dry weight (mgm./ml.)	Poly-β-hydroxybutyric acid (mgm./ml.)
42	0·75	0·234
111	1·55	1·012
Increase	0·80	0·778

Cells were grown in a mineral medium containing 300 mgm. ammonium chloride/litre in an atmosphere of 60 per cent hydrogen + 30 per cent oxygen + 10 per cent carbon dioxide, *p*H 7·0, 28° C. Cell protein increased up to the thirty-ninth hour and then remained constant.

The accumulation of poly-β-hydroxybutyric acid during the fixation of carbon dioxide in the absence of a source of nitrogen can be demonstrated in a series of micrographs of thin sections (Fig. 1, *a*–*c*.) In log-phase cells no structures other than nuclear material can be detected. After a 12-hr. period of carbon dioxide fixation, several round and oval areas become visible which show a distinct boundary and appear empty. These areas undoubtedly are inclusions of poly-β-hydroxybutyric acid. After a 48-hr. period of carbon dioxide fixation, the whole cell is filled with a few large inclusions of poly-β-hydroxybutyric acid which displace the chromatin bodies and the cytoplasm into a minimum possible area at the periphery and the interstitial areas between the inclusions. Since the density of these areas is even less than the

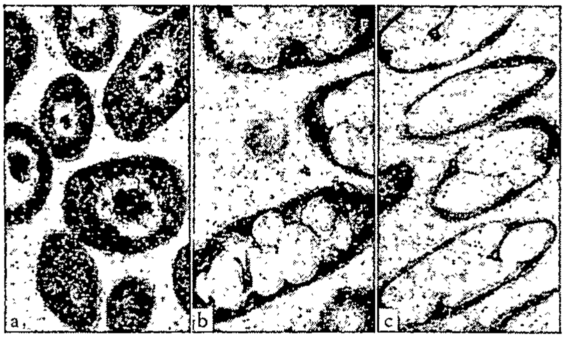


Fig. 1. Electron-micrographs of thin sections of *Hydrogenomonas* *H*16 showing the formation of inclusions of poly-β-hydroxybutyric acid during incubation in phosphate buffer under an atmosphere o 70 per cent hydrogen + 20 per cent oxygen + 10 per cent carbon dioxide for 0 hr. (*a*, log-phase cells), 12 hr. (*b*) and 48 hr. (*c*) Fixation for 2 hr. at 3° C. with 0·5 per cent osmic acid at *p*H 8·0 (veronal acetate buffer); embedded in methacrylate. Electron-microscopical preparations and studies were carried out by Mr. K. Kran. (× 21,500)

density of the surrounding methacrylate of the section, it is concluded that the poly-β-hydroxybutyric acid dilutes the embedding medium.

In order to isolate the storage material, the washed cells were treated with potassium hypochlorite solution (Williamson and Wilkinson's method[2]). The residue, washed with water, ethanol and ethyl-ether, was almost completely dissolved in hot chloroform and after filtration could be re-precipitated with ether. Using this method at a temperature of 0° C., 0·4 vol. of ether will precipitate 7·4 per cent of the storage material, while 0·52 vol. precipitates 33·0 per cent and 1·2 vol. 100 per cent. The melting points and analytical determinations of the purified storage materials derived from *H*1, *H*16 and *H*20 are shown in Table 2. Furthermore, poly-β-hydroxybutyric acid preparations were analysed which had been isolated from *Bacillus megaterium* and *Azotobacter chroococcum*, in both of which poly-β-hydroxybutyric acid had previously been found[3,4]. The different melting-point intervals of the preparations indicated different degrees of polymerization.

Table 2. MELTING POINTS AND ANALYTICAL RESULTS FOR POLY-β-HYDROXYBUTYRIC ACIDS ISOLATED FROM DIFFERENT BACTERIA

Organism	Melting point	C (per cent)*	H (per cent)*
Azotobacter chroococcum	150–155	55·4	7·0
Bacillus megaterium	168–173	55·6	6·9
*Hydrogenomonas H*1	162–166	55·6	7·1
,, *H*16	158–162	55·4	7·0
,, *H*20	167–172	55·6	7·4

* The theoretical values calculated for $(C_4H_6O_2)_n$ are: C = 55·8 per cent; H = 7·0 per cent.

The infra-red spectra of all five substances examined by us were identical. Dry distillation of poly-β-hydroxybutyric acid gave crotonic acid with a yield of 70 per cent. After recrystallization from water, this crotonic acid melted at 70° C. Crotonic acid amide prepared from this acid had a melting point of 160° C. These two derivatives, prepared from extracted poly-β-hydroxybutyric acid, were found to be identical with pure crotonic acid and the crotonic acid amide prepared from it. No depression of the melting points was observed on mixing the substances obtained from extracted poly-β-hydroxybutyric acid with the chemically pure compounds. Free β-hydroxybutyric acid could be prepared by means of alkaline hydrolysis of poly-β-hydroxybutyric acid and ether extraction of the acidified hydrolysate; this was verified by determination of the optical activity and using paper chromatography and reference substances.

In addition to the method for the preparation of poly-β-hydroxybutyric acid described by Williamson and Wilkinson[2], we have had good results with chloroform extraction of lyophilized cells. A rather pure product is prepared with quantitative yield when the dry powder is extracted with chloroform at room temperature, and the poly-β-hydroxybutyric acid precipitated with ether. Methylene chloride is as efficient as chloroform in extracting it.

The efficiency with which poly-β-hydroxybutyric acid is synthesized from carbon dioxide by oxidation of hydrogen was determined by a manometric method in a Warburg apparatus using $NaH^{14}CO_3$. The stoichiometric relationship of the gas mixture consumed to the carbon dioxide taken up was found to be 8–8·5 under the conditions of synthesis of storage material. It does not vary significantly from the corresponding relationship under growth conditions and agrees with the equation:

$$25H_2 + 8O_2 + 4CO_2 \rightarrow (C_4H_6O_2) + 22H_2O$$

During organotrophic metabolism poly-β-hydroxybutyric acid is also stored by *Hydrogenomonas* in considerable quantities (Fig. 2). With β-hydroxybutyric acid or crotonic acid serving as substrate, the speed with which the storage material is synthesized is even greater than in autotrophic conditions. When the cells are incubated with these substrates, it makes practically no difference whether they are in contact with air or with a hydrogen–oxygen mixture. Since

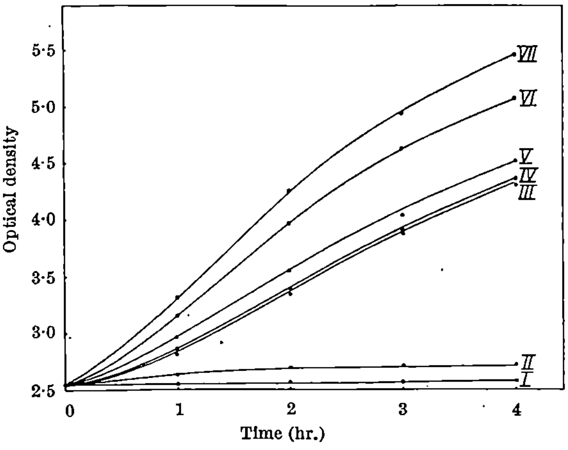


Fig. 2. Synthesis of poly-β-hydrobutyric acid by *Hydrogenomonas* *H*16 in nitrogen-free mineral medium with different sources of energy and carbon

		Increase in poly-β-hydroxybutyric acid (in mgm./mgm. cell protein) in the course of 4 hr. incubation
I.	Under air without carbon source	0·00
II.	Under nitrogen with β-hydroxybutyric acid	—
III.	Under air with crotonic acid	0·41
IV.	Under hydrogen + oxygen with crotonic acid	0·42
V.	Under hydrogen + oxygen + carbon dioxide	0·47
VI.	Under hydrogen + oxygen with β-hydroxybutyric acid	0·54
VII.	Under air with β-hydroxybutyric acid	0·66

20 ml. suspension (1 mgm. cell protein/ml.) in 200 ml. Erlenmeyer flasks were shaken at 30° C. under the conditions indicated (with 1 m*M* β-hydroxybutyric acid or crotonic acid per flask); measurement of turbidity at 436 mμ; determination of poly-β-hydroxybutyric acid gravimetrically after lyophilization of 10 ml. suspension, chloroform extraction and following precipitation with ether

only small amounts of energy seem to be necessary for the polymerization of the monomers taken up by the cell, these may easily be produced by substrate oxidation, with the result that the efficiency of the synthesis cannot be increased by simultaneous hydrogen oxidation. Other organic substrates have not yet been examined.

Cells containing little storage material (less than 10 per cent poly-β-hydroxybutyric acid) and those containing a great deal (more than 50 per cent) differ considerably in their endogenous respiration (Table 3). The former were collected prior to the end of the exponential growth phase, the latter 20 hr. after depletion of the nitrogen source. Oxygen uptake was determined manometrically with cell suspensions in phosphate buffer with and without addition of a nitrogen source (ammonium chloride).

Table 3. ENDOGENOUS RESPIRATION OF CELLS CONTAINING SMALL AND LARGE AMOUNTS OF POLY-β-HYDROXYBUTYRIC ACID
(μl. oxygen/mgm. protein/hr.)

Elapsed time (hr.)	PHBA-poor cells		PHBA-rich cells	
	$-NH_4Cl$	$+NH_4Cl$	$-NH_4Cl$	$+NH_4Cl$
2	12·4	16·0	22·7	37·2
5	4·5	4·7	16·2	30·8
8	3·0	3·2	14·6	25·9

Warburg vessels with 2·5 ml. suspension of *Hydrogenomonas H*20 in 0·06 *M* phosphate buffer at *p*H 7·0; 113·2 *μM* ammonium chloride/vessel; 0·2 ml. 20 per cent potassium hydroxide in central well; atmosphere, air; 30° C.

The endogenous respiration of rich cells is not only greater than that of cells containing little poly-β-hydroxybutyric acid, but is also greatly increased by the introduction of a nitrogen source. This boost in uptake of oxygen over and above endogenous respiration, which is brought about by the ammonium ion and which is not observed with cells poor in poly-β-hydroxybutyric acid, seems to indicate that the accumulated poly-β-hydroxybutyric acid is re-circulated into the metabolism and used for protein synthesis.

This hypothesis was verified by experiments in which the increase and decrease of poly-β-hydroxybutyric acid and cellular nitrogen were measured during endogenous respiration and during hydrogen oxidation, without carbon dioxide, of cells rich in poly-β-hydroxybutyric acid with and without a nitrogen source (Table 4).

Table 4. RE-UTILIZATION OF POLY-β-HYDROXYBUTYRIC ACID FOR PROTEIN SYNTHESIS BY *Hydrogenomonas H*16

Experimental conditions		Cell content		Difference after 12 hr.	
N-source	Atmosphere	Total N (mgm.)	PHBA (mgm.)	Total N (mgm.)	PHBA (mgm.)
Initial suspension		0·358	2·50	—	—
None	Air	0·369	2·30	+0·011	−0·2
Ammon. chloride	Air	0·546	0·67	+0·188	−1·83
None	70 per cent H_2 + 30 per cent O_2	0·367	2·47	+0·009	−0·03
Ammon. chloride	70 per cent H_2 + 30 per cent O_2	0·622	0·61	+0·264	−1·89

Suspension of chemolithotrophically grown cells (50 per cent poly-β-hydroxybutyric acid) in 0·067 *M* phosphate buffer at *p*H 7·0; 75 *μM* ammonium chloride/ml.; 28° C.; atmosphere, air or carbon dioxide-free hydrogen–oxygen mixture (70 per cent hydrogen + 30 per cent oxygen; carbon dioxide absorbed by 20 per cent potassium hydroxide); incubation time 12 hr. (with stirring); Kjeldahl method used to determine cellular nitrogen; poly-β-hydroxybutyric acid determined gravimetrically from lyophilized material after extraction with chloroform and precipitation with ether.

Under air, only a small percentage of the poly-β-hydroxybutyric acid is consumed by endogenous respiration. In the presence of ammonium chloride, the cellular nitrogen increases considerably at the expense of poly-β-hydroxybutyric acid. In an atmosphere of carbon dioxide-free hydrogen–oxygen mixture these effects are even more pronounced: while the poly-β-hydroxybutyric acid decreases, the cellular nitrogen accumulates to a considerable extent. Therefore we must assume that we are dealing with poly-β-hydroxybutyric acid as a genuine storage material, which in the absence of a carbon source can support metabolism, and can be used for protein synthesis in the presence of a nitrogen source. The efficiency of this synthesis is greater during hydrogen oxidation than during endogenous respiration.

The three strains of *Hydrogenomonas* examined up to the present time synthesize poly-β-hydroxybutyric acid chemolithotrophically as well as organotrophically and are able to re-utilize the storage material in their metabolism.

We acknowledge the support of the Deutsche Forschungsgemeinschaft in this work.

[1] Schlegel, H. G., Kaltwasser, H., and Gottschalk, G., *Arch. Mikrobiol.*, **38**, 209 (1961).
[2] Williamson, D. H., and Wilkinson, J. F., *J. Gen. Microbiol.*, **19**, 198 (1958).
[3] Lemoigne, M., *Bull. Soc. Chim. Biol.*, **8**, 770 (1926).
[4] Lemoigne, M., and Girard, H., *C.R. Acad. Sci., Paris*, **217**, 557 (1943).

APPARENT CYTOPLASMIC INHERITANCE OF TYROSINASE COMPETENCE IN *STREPTOMYCES SCABIES*

By PROF. K. F. GREGORY and WEI-JEN SHYU
Department of Microbiology, Ontario Agricultural College, Guelph

STREPTOMYCES scabies, an etiological agent of potato scab, like other members of its genus, resembles the true bacteria in size and many physiological characteristics, but has a mycelial growth habit. The formation of nutritionally balanced heterokaryotic growth between pairs of nutritionally deficient strains has been demonstrated in several species of *Streptomyces*[1–3]. Infrequent genetic recombination, presumably preceded by nuclear fusion, occurs in some of these species, but the great majority of the spores formed by the heterokaryons have the nutritional requirements of one or the other of the two parental strains. Since the two parental-type nuclei share the same cytoplasm within the heterokaryon, any cytoplasmically inherited characteristic should be shared by uni-nucleate spores bearing both types of nuclei. This phenomenon appears to occur with the determinant for tyrosinase production in *Streptomyces scabies*.

Strains of *S. scabies* isolated from potato scab lesions are normally capable of forming a dark brown pigment (presumably melanin) in tyrosine-containing medium in contact with air. Tyrosinase-deficient mutants arise at a relatively high frequency, however, comprising about 0·2 per cent of the total colonies derived from non-irradiated spores and up to 5 per cent of the colonies developing from X-ray treated spores[4]. The tyrosinase-deficient state, by contrast, was found to be highly stable, and no back mutation to tyrosinase competence has been detected, following numerous platings of tyrosinase-deficient mutants during a 5-year period.

Strains of *S. scabies* have been observed cytologically to undergo frequent hyphal fusions[5]. Colonies, concluded to be nutritionally balanced heterokaryons, formed readily on minimal medium, when some pairs of mutants, each bearing two complementary nutritional deficiencies, were plated together. The great majority of the spores derived from these heterokaryons had the nutritional requirements of one or the other of the two parents, but usually in unequal proportions. Thus, in a cross between *S. scabies* strains *A*26–34 (nicotinic acid$^-$, biotin$^-$, tyrosinase$^-$, streptomycin sensitive) and *A*26–37 (arginine$^-$, methionine$^-$, tyrosinase$^+$, streptomycin resistant) the spores formed by the heterokaryon reproduced the two patterns of parental nutritional deficiencies in a ratio of about 50 : 1, respectively, although the ratio varied somewhat from experiment to experiment (Table 1). When platings were made from heterokaryotic colonies on to minimal medium, most of the few colonies which developed were found to be heterokaryons and may have developed from fragments of mycelium or might have arisen *de novo* from the two parental types present. When platings were made from heterokaryons on to nutritionally selective media, essentially all the colonies of both parental nutritional types gave a strong tyrosinase reaction, the incidence of tyrosinase-deficient colonies being no greater than that observed with normal tyrosinase-producing cultures.

Table 1. CYTOPLASMIC INHERITANCE OF TYROSINASE COMPETENCE VIA HETEROKARYON FORMATION

Medium	Counts per ml. of spore suspensions prepared from single colonies of:		
	*A*26–34	*A*26–37	Heterokaryon of *A*26–34 × *A*26–37
Minimal	0	0	$8\cdot8 \times 10^{2}$
Minimal + nicotinic acid + biotin	$4\cdot1 \times 10^{6}$	0	$3\cdot7 \times 10^{6}$
Minimal + arginine + methionine	0	$2\cdot2 \times 10^{6}$	$8\cdot1 \times 10^{4}$
Complete + streptomycin	0	$2\cdot0 \times 10^{6}$	$6\cdot2 \times 10^{4}$
Per cent tyrosinase-producing colonies	0	> 99	> 99

The reverse cross yielded similar results. Thus when strain *A*26–54 (nicotinic acid$^-$, biotin$^-$, tyrosinase$^+$, streptomycin resistant) was plated on minimal medium together with strain *A*26–53 (arginine$^-$, methionine$^-$, tyrosinase$^-$, streptomycin sensitive) and the resulting heterokaryotic colonies plated on nutritionally differential media, once again nearly all the colonies of both parental nutritional types were tyrosinase$^+$ (Fig. 1). The tyrosinase-producing abilities of colonies carrying the nutritional requirements of the formerly tyrosinase-deficient parents were shown to be perpetuated throughout at least six consecutive transfers on complete medium.

Many of the colonies developing on medium supplemented with nicotinic acid and biotin, from both crosses, required only nicotinic acid for growth. This acquisition of independence of biotin was considered to result from the relatively high frequency of back mutation to biotin$^+$ ($1\cdot6 \times 10^{-4}$ per parental spore plated) coupled with the fact that these back mutants made a greater amount of residual growth on the minimal medium than did the diauxotroph. There was thus an increased probability of heterokaryon formation with these biotin$^+$ back mutants. A selection of tyrosinase$^+$ back mutants during heterokaryon formation appears to have been highly unlikely since this back mutation has never been detected. Furthermore, the tyrosinase$^+$ characteristic gave this culture no apparent selective advantage on the media employed.

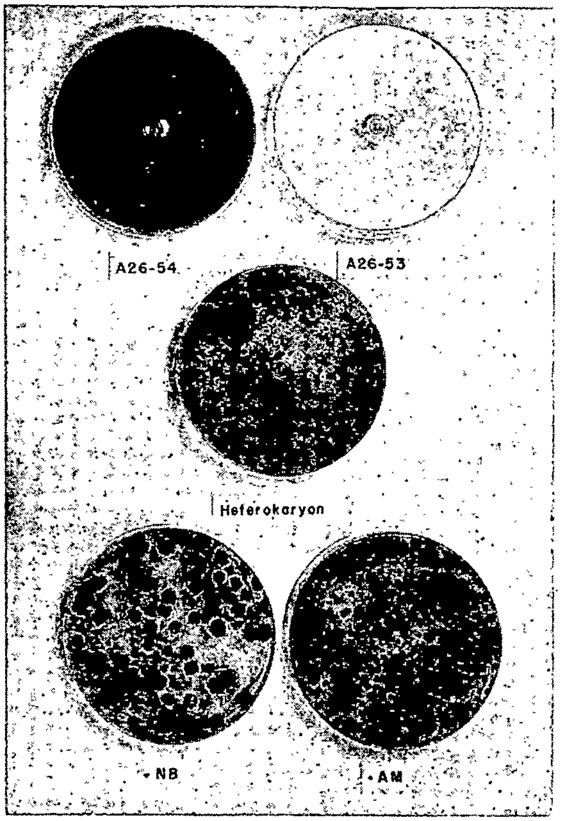


Fig. 1. Appearance of the under side of colonies of parental, heterokaryon and recovered parental nutritional types on tyrosine-containing media. Top: parental strains *A*26–54 (nicotinic acid$^-$, biotin$^-$, tyrosinase$^+$, streptomycin resistant) and *A*26–53 (arginine$^-$, methionine$^-$, tyrosinase$^-$, streptomycin sensitive) on complete medium. Centre: heterokaryons from the above strains on minimal medium. Bottom: colonies developing, from spores formed by heterokaryon, on minimal medium supplemented with nicotinic acid + biotin (+ *NB*) and arginine + methionine (+ *AM*)

Genetic recombination of the determinants for nutritional deficiencies and streptomycin resistance was detected by plating spores from heterokaryons on minimal medium containing various combinations of nutritional supplements and streptomycin. Excluding the nicotinic acid$^-$ biotin$^+$ streptomycin-sensitive class, which was not considered to arise by recombination, recombinants comprised only a small proportion (< 0·1 per cent) of the total spores formed by the heterokaryons. On the assumption that these characteristics were inherited as nuclear genes, it was apparent that the incidence of nuclear genetic recombination in these strains was not sufficient to account for the abundant coupling of tyrosinase-producing capability with the nutritional deficiencies of the formerly tyrosinase-deficient strains.

A selective overgrowth of the heterokaryon with recombinant nuclei containing the tyrosinase$^+$ determinant was improbable in view of the short interval available for such overgrowth (development of one colony), lack of a demonstrable selective growth advantage of this characteristic in the test conditions, and the clear demonstration in *Neurospora* that even nuclei bearing genes with a strong selective advantage did not increase in proportion during the growth of a heterokaryon[6]. Even if the large number of biotin$^+$ colonies produced by the heterokaryons had resulted from the formation, by recombination, of nuclei bearing this characteristic, followed by a selective increase in the proportion of this type of nucleus within the heterokaryon, the high incidence of tyrosinase$^+$ colonies obtained clearly would not have resulted from a close linkage of this determinant to the biotin locus, since most of the biotin$^+$ colonies from *A*26–53 × *A*26–54 heterokaryons would then have been deficient in tyrosinase.

The tyrosinase$^+$ colonies obtained from the heterokaryons were shown not to have been derived from multinucleate or diploid spores, since spores of these cultures showed an exponential rate of killing by X-rays, usually characteristic of a haploid, uninucleate condition, and the other genetic markers could not be recovered afterwards from these cultures. The culture from which these genetically marked strains were derived appeared cytologically to have a single nucleus[5].

Attempts to obtain infective transfer of tyrosinase-producing ability from *A*26–37 to *A*26–34 were unsuccessful. Culturing *A*26–34 in both liquid and solid media containing 10 per cent culture filtrate or a membrane-filtered extract of sonically disrupted mycelium of *A*26–37 did not result in a single tyrosinase-producing colony. The quantitative transfer of tyrosinase-producing ability from a tyrosinase-producing strain of *S. scabies* to a tyrosinase-deficient strain did not, therefore, appear to be mediated by a free virus like the carbon dioxide sensitivity factor in *Drosophila*[7], toxigenicity in *Corynebacterium diphtheriae*[8] or some antigenic changes in *Salmonella* spp.[8].

The phenomenon described here appears to be explained best by postulating the presence of autoreproducing constituents in the cytoplasm, which control the production of tyrosinase in this species. Strong evidence exists for cytoplasmic inheritance of several characteristics in diverse organisms[9]. Horowitz and Fling[10], however, specifically ruled out cytoplasmic inheritance of tyrosinase-producing ability in *Neurospora crassa* with data diametrically opposed to that presented here for *S. scabies*.

The support of this work by a grant from the National Research Council of Canada is gratefully acknowledged.

[1] Bradley, S. G., Anderson, D. L., and Jones, L. A., *Ann. N.Y. Acad. Sci.*, **81**, 811 (1959).

[2] Braendle, D. H., and Szybalski, W., *Ann. N.Y. Acad. Sci.*, **81**, 824 (1959).

[3] Saito, H., and Ikeda, Y., *Ann. N.Y. Acad. Sci.*, **81**, 862 (1959).

[4] Gregory, K. F., and Vaisey, E. B., *Canad. J. Microbiol.*, **2**, 65 (1956).

[5] Gregory, K. F., *Canad. J. Microbiol.*, **2**, 649 (1956).

[6] Pittenger, T. H., and Atwood, K. C., *Genetics*, **41**, 227 (1956).

[7] L'Heritier, P., *Advances in Virus Res.*, **5**, 195 (1958).

[8] Barksdale, L., *Bact. Rev.*, **23**, 202 (1959).

[9] Catcheside, D. G., *Nature*, **184**, 1012 (1959).

[10] Horowitz, N. H., and Fling, M., *Proc. U.S. Nat. Acad. Sci.*, **42**, 498 (1956).

COMPARISON OF PYRIDINE NUCLEOTIDES IN THE LIVER AND IN AN ASCITIC HEPATOMA

By M. WINTZERITH N. KLEIN, L. MANDEL and PROF. P. MANDEL

Centre de Recherches Nucléaires, Applications Biologiques, Strasbourg-Cronenbourg, and Institut de Chimie Biologique, Faculté de Médecine, Strasbourg

THE work of von Euler[1] showed that there was no difference in the diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) contents of the liver, muscle and Jensen's sarcoma in the same animal. Since then, numerous data have accumulated showing that in tumours[2–5], in tumoural mitochondria[6] and in organs in a pre-cancerous state[7] the DPN content is lower than in normal tissues. In animals with a tumour such as sarcoma 37, a lymphoma or a hepatoma, a decrease in DPN of certain cells has been noted[8,9]. However, in most cases, the comparison between normal and neoplasmic tissues concerns cells the morphology of which differs widely. Furthermore, different types of cells contain varying amounts of DPN and TPN whether in normal or neoplasmic tissues.

These reasons prompted us to compare the DPN and TPN contents in normal liver, in the Zajdela hepatoma and in the liver of animals with this tumour. Here we are concerned with homologous types of tissues and with a tumour which has not lost as much of the character of the original tissue as sarcomas or Ehrlich ascitic cells which have been transplanted many times. The hepatoma of Zajdela which we utilize maintains the trabecular structure of liver parenchyma. This is important because hepatomas can originate in various liver cells: biliar-epithelium, parenchymatous cells, reticulo-endothelial cells, and a comparison between the liver and tumour will be valid only if the latter is also of a parenchymatous nature. Pitot and Potter[10] have emphasized recently the different biochemical characters of distinct morphological types of hepatoma. In our comparison of normal and tumorous tissues we have utilized various bases of references besides the wet weight normally used. In particular, we thought it interesting to compare values related to the deoxyribonucleic acid (DNA) content, thus obtaining an indication at the cell-level.

In our comparative experiments on the pyridine nucleotides of liver and hepatoma we used twenty standardized rats, eight being for estimations on the liver and twelve for the tumours. For investigating the effects of nicotinamide we used eighteen rats, ten of which had a tumour. Reduced diphosphopyridine nucleotide (DPNH), DPN and the total TPN +

reduced triphosphopyridine nucleotide (TPNH) were determined by the enzymic method described by Holzer *et al.*[11]. After fractionation according to Schmidt and Thannhauser[12], DNA was determined by the estimation of deoxyribose by the method of Dische[13] as modified by Burton[14]. Our experimental results are recorded in Figs. 1–3.

Fig. 1 shows that the presence of an ascitic hepatoma alters only slightly the DPN and TPN + reduced TPN contents of the liver. Although the values for animals with a tumour vary within narrower limits than in normal subjects and the average results are slightly higher in the controls, the differences are not significant.

Fig. 2 shows the DPN, reduced DPN and TPN contents of liver and ascitic cells. Bearing in mind the difficulty of accurate assessment of the wet weight of ascitic cells, we related our results to accurately determinable quantities, that is, dry weight, proteins and DNA. Our results demonstrate that the values of DPN related to dry weight are higher for the liver than for ascitic cells. The same applies for reduced DPN and the triphosphopyridine nucleotides as a whole. Even though the standard deviation for the results is comparatively high the difference is statistically valid. The average values for the DPN, reduced DPN and TPN + reduced TPN contents of tumorous tissues were, respectively, 61 per cent, 77 per cent and 55·5 per cent of the values for normal liver. The percentages are of the same order, if the results are related to proteins. The differences between normal and ascitic cells are more important and highly significant if the values are related to DNA. Thus, the average values for the DPN, reduced DPN and TPN + reduced TPN of ascitic cells are only 14·4 per cent, 17·6 and 12 per cent, respectively, of the values for normal tissues.

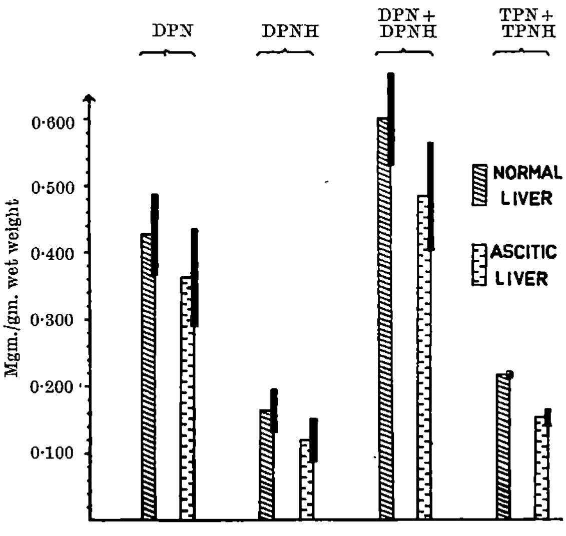


Fig. 1. Comparison of pyridine nucleotide content of normal liver and the liver of an animal with a Zajdela hepatoma

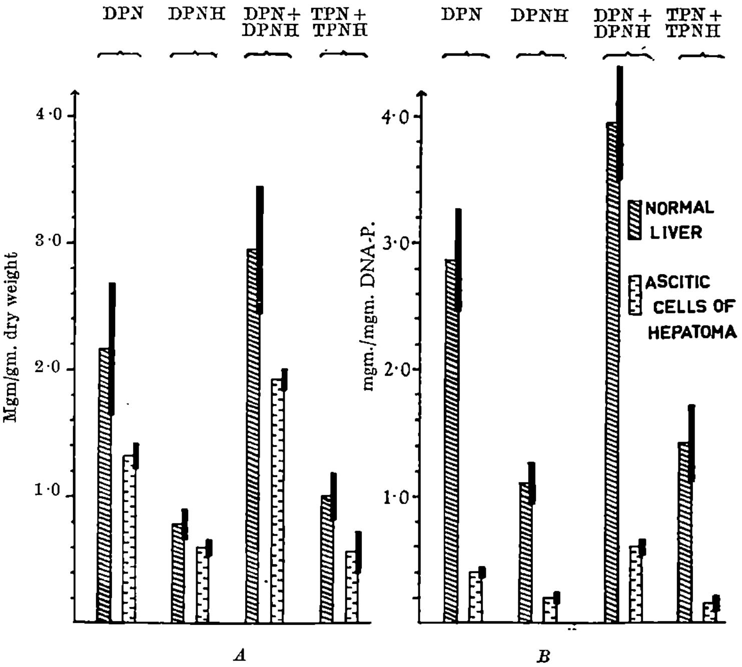


Fig. 2. Comparison of pyridine nucleotide content of normal liver and an ascitic heptoma. *A*, values expressed in mgm. per gm. dry weight; *B*, values expressed in mgm. per mgm. DNA-P. The nuclei of the hepatoma contain on average twice as much DNA as the nuclei of a normal liver

Under the influence of nicotinamide, DPN and reduced DPN increase at about 2–3 times the rate in normal liver as in ascitic cells, while the rate of increase of the triphosphopyridine nucleotides is about twice as great.

The presence of the hepatoma, while affecting the synthesis of ribonucleic acid and DNA of various tissues, as we have shown previously[15,16], does not cause an appreciable fall in the content of di- and triphosphopyridine nucleotides. However, the amounts of pyridine nucleotides in tumour cells retaining the structure of liver trabeculæ are considerably reduced. The most important differences are found at cell level, when referring the values to DNA. Since cell equilibrium can be expressed by the ratio cellular constituent/DNA it is evident that if the quotient, in our case pyridine nucleotide/DNA, is at all modified, it represents a disturbance of this equilibrium.

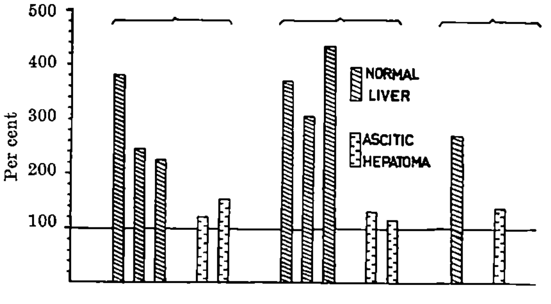


Fig. 3. Effect of injection of 50 mgm. of nicotinamide per 100 gm. wet weight, 8 hr. before killing, on the pyridine nucleotides of a normal liver and the cells of the ascitic hepatoma

The value of 100 was adopted for the controls. The height of the columns which extend above the horizontal line indicates, in per cent, the quantity of pyridine nucleotides in relation to the control

Finally, we find *in vivo* in a homologous tumour a considerable reduction in the net neo-synthesis of di- and tri-phosphopyridine nucleotides after the administration of nicotinamide (Fig. 3).

Summarizing, the reduction of the di- and tri-phosphopyridine nucleotides of an ascitic hepatoma of parenchymatous cells is noticeable when the values are referred to proteins or to the dry weight. The difference is all the more apparent when results are referred to DNA. The synthesis of di- and tri-phosphopyridine nucleotides, after injection of nicotinamide, is much inferior in hepatoma as compared to normal liver. No significant differences in DPN and TPN contents were found between normal liver and the liver of an animal with a hepatoma.

[1] Euler, H., Schlenk, F., Heiwinkel, H., and Högberg, B., *Z. physiol. Chem.*, **256**, 208 (1938).

[2] Bernheim, F., and Felsovanyi, A. V., *Science*, **91**, 76 (1940).

[3] Fischer, A., and Schlenk, F., *Texas Rep. Biol. and Med.*, **6**, 346 (1948).

[4] Carruthers, C., and Suntzeff, V., *Arch. Biochem. Biophys.*, **45**, 140 (1953).

[5] Schneider, W. C., and Hogeboom, G. H., *Cancer Res.*, **11**, 1 (1951).

[6] Wenner, C. E., and Weinhouse, S., *Cancer Res.*, **13**, 21 (1953).

[7] Kensler, C. J., Sugiura, K., and Rhoads, C. P., *Science*, **91**, 623 (1940).

[8] Waravdekar, V. S., Powers, O. H., and Leiter, J., *J. Nat. Cancer Inst.*, **17**, 145 (1956).

[9] Waravdekar, V. S., Powers, O. H., and Leiter, J., *Proc. Soc. Exp. Biol. Med.*, **92**, 797 (1956).

[10] Pitot, H. C., and Potter, V. R., *Biochim. Biophys. Acta*, **40**, 537 (1960).

[11] Holzer, H., Busch, D., and Kröger, H., *Z. physiol. Chem.*, **313**, 184 (1956).

[12] Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, **161**, 83 (1945).

[13] Dische, Z., *Mikrochem.*, **2**, 4, (1930).

[14] Burton, K., *Biochem. J.*, **62**, 315 (1956).

[15] Wintzerith, M., Mandel, L., and Mandel, P., *C.R. Soc. Biol.*, **152**, 2199 (1958).

[16] Wintzerith, M., Klein, N., Mandel, L., and Mandel, P., *Experientia*, **15**, 140 (1959).

BIOCHEMICAL ASPECTS OF RESISTANCE TO 6-MERCAPTOPURINE IN HUMAN EPIDERMOID CARCINOMA CELLS IN CULTURE

By R. W. BROCKMAN, GEORGE G. KELLEY, PATRICIA STUTTS and VIRGINIA COPELAND

Kettering-Meyer Laboratory*, Southern Research Institute, Birmingham, Alabama

THE main pathway by which purines are metabolized to ribonucleotides is by the enzyme-catalysed reactions of the bases with 5-phosphoribosyl-1-pyrophosphate (PRPP) (refs. 1 and 2). Way and Parks[3], and Lukens and Herrington[4] demonstrated that purine analogues react similarly to form nucleotide analogues. A study of the purine ribonucleotide pyrophosphorylase capacity of enzyme preparations from *Streptococcus faecalis* and *L*1210 mouse leukæmia revealed that a specific decrease or loss of inosine 5′-phosphate (IMP) and guanosine 5′-phosphate (GMP) pyrophosphorylase activities accompanied resistance to 6-mercaptopurine (MP) and 8-azaguanine; enzyme preparations from the resistant cells also did not form 8-azaguanosine 5′-phosphate (azaGMP) and MP ribonucleotide in significant amounts[5-7]. Adenosine 5′-phosphate (AMP) pyrophosphorylase activity was not altered in these resistant cell lines.

The present work extends these observations to a human neoplasm in cell culture. The cell line used was the *H.Ep*-2 line derived from a human epidermoid carcinoma[8]; MP-resistant sub-lines of this were developed recently in this Laboratory[9]. The line was grown in static culture on glass and also in suspension culture[10,11]. The resistant sub-lines (*H.Ep*-2/MP) were isolated from clones that developed on glass in the presence of 0·5, 1 and 10 µgm./ml. of MP which, at a concentration of 0·5 µgm./ml., completely inhibited growth of the parent-sensitive line[9]. *H.Ep*-2 cloned in the presence of 1 and 10 µgm./ml. MP grew in suspension cultures in the presence of concentrations of MP as high as 320 µgm./ml. These sub-lines exhibited significant cross-resistance to 8-azaguanine.

The metabolism of purines and analogues was compared in the *H.Ep*-2 cell lines in suspension culture; the cell population per 100 ml. medium at the time of addition of tracer was 40×10^6 for *H.Ep.*-2/*S* and 20–30×10^6 for the MP-resistant sub-lines. The cells were incubated in the presence of the radioactive substrates for 24 hr., and then soluble extracts of the cells were prepared for two-dimensional paper chromatographic–radioautographic analysis by procedures previously described in studies with ascites tumour cells[5,6]. The residues from the alcohol extractions were treated with hot 10 per cent sodium chloride followed by precipitation of the nucleic acid fraction with ethanol[5,6]. The nucleic acid preparations thus obtained were examined spectrophotometrically and the optical density at the absorption maximum, 260 mµ, was used as a measure of the concentration of nucleic acid. The specific activity of the preparations was determined by evaporation of nucleic acid solutions of known concentration on planchets for counting in internal gas-flow proportional counters.

The results summarized in Table 1 show that adenine extensively labelled the nucleic acids of sensitive and resistant *H.Ep*-2 cells. Guanine, 8-azaguanine and hypoxanthine only labelled the nucleic acids of the sensitive cells. Azaguanine was shown to be incorporated into the ribonucleic acid (RNA) of *H.Ep*-2/*S* as azaguanlyic acid by procedures described by Matthews[12]. The *H.Ep*-2/*S* cells incubated in the presence of 6-mercaptopurine labelled with sulphur-35 were too weakly radioactive for the nature of the material containing sulphur-35 to be identified. Results of analysis of the soluble fractions from these cells showed that nucleotides of adenine but not of guanine, 8-azaguanine, hypoxanthine or 6-mercaptopurine were present in the MP-resistant cells. Conversion of adenine, hypoxanthine and 6-mercaptopurine to nucleotides was extensive in *H.Ep*-2/*S*; the soluble pools of nucleotides derived from guanine and 8-azaguanine were small but detectable.

Enzyme preparations were made from sensitive *H.Ep*-2 cells grown in suspension culture and also from suspension cultures derived from clones which grew in the presence of 0·5, 1 and 10 µgm./ml. of MP. Cells were separated from growth medium by

---

* Affiliated with the Sloan-Kettering Institute for Cancer Research, New York.

Table 1. RADIOACTIVITY OF NUCLEIC ACIDS FROM SENSITIVE AND RESISTANT HUMAN EPIDERMOID CARCINOMA CELLS GROWN IN THE PRESENCE OF LABELLED PURINES AND ANALOGUES*

Substrate	Specific activity of nucleic acids (counts/sec./mgm.)		
	H.Ep-2/*S*	*H.Ep*-2/MP †	
		1	10
Adenine-8-^{14}C	14,354	17,401	17,086
Guanine-8-^{14}C	1,551	5	2
8-Azaguanine-2-^{14}C	363	3	3
Hypoxanthine-8-^{14}C	9,991	3	4
6-Mercaptopurine-^{35}S	20	2	2

* *H.Ep*-2 cells were grown in suspension culture and incubated in the presence of radioactive substrates for 24 hr. The nucleic acids were isolated for quantitative determinations of radioactivity.

† The two different sub-lines of *H.Ep*-2/MP were isolated on glass by cloning in the presence of concentrations of 6-mercaptopurine of 1 and 10 μgm./ml., respectively.

centrifugation, washed with physiological saline, and re-suspended in cold 0·05 *M* sodium phosphate buffer, *p*H 7·5. The volume of buffer used was 1 ml. for each 4 × 10^8 cells. The resulting cell suspension was subjected to rapid freezing in an ethanol–dry ice bath followed by gradual thawing; this cycle was repeated twice more to effect disintegration of the cells. The resulting preparation was centrifuged at 95,000*g* for 90 min. at 4° C. in a Spinco centrifuge. The clear supernatant served as the crude enzyme preparation and was frozen and stored at − 15° C. Enzyme solutions thus prepared were analysed for protein by the Oyama–Eagle modification[13] of Lowry's method.

The reaction mixtures used in work on enzyme activities are described in Table 2; they were analysed by methods previously described[5-7]. The results show that enzymes from both the sensitive and the MP-resistant *H.Ep*-2 lines formed nucleotides from adenine, 4-aminopyrazolo(3,4-*d*)pyrimidine, 2,6-diaminopurine, and 4-amino-5-imidazolecarboxamide; in all cell lines the yield of AMP was very much greater than those of these other nucleotides. Conversion of xanthine to xanthylic acid was poor in the sensitive *H.Ep*-2 and was decreased in the MP-resistant lines. Hypoxanthine, MP, guanine, and 8-azaguanine were converted to nucleotides in good yields by enzymes from sensitive cells; yields of these nucleotides with identically prepared enzymes from MP-resistant cells were much lower and in some instances negligible. A decreased capacity for formation of these four nucleotides was evident in all the resistant lines, even in the line cloned in the presence of 0·5 μgm./ml. MP. However, there was some evidence for slight additional loss of capacity in lines cloned at higher concentrations of MP. The cell line cloned in 10 μgm./ml. MP was grown in the absence of MP for 60 generations (*H.Ep*-2/10-MP), after which the loss of enzymic capacity was still essentially complete. This result indicates that resistance to MP probably arose by mutation in the human tumour cell lines, as in other biological systems. The specific loss of IMP and GMP pyrophosphorylase activity in *H.Ep*-2/MP resulted in loss of capacity of the growing cells to form nucleotides of these bases or to incorporate guanine, 8-azaguanine or hypoxanthine into nucleic acids.

The specificity of action of the purine ribonucleotide pyrophosphorylases of *H.Ep*-2 is also of interest. IMP and GMP pyrophosphorylase activities were lost simultaneously, as was the case in MP- and azaguanine-resistant *S. faecalis* and *L*1210[5-7]. This is further evidence that a single enzyme or enzymes closely related genetically[14] may be responsible for this conversion. As in other systems[6,7] MP and azaguanine are evidently metabolized to nucleotides in *H.Ep*-2 by the IMP–GMP pyrophosphorylase enzyme system. Flaks, Erwin and Buchanan[15] observed that 4-amino-5-imidazolecarboxamide and adenine were converted to ribonucleotides by the same pyrophosphorylase. Results of the study of Roy, Haavik and Parks[16] showed that 4-aminopyrazolo(3,4-*d*)pyrimidine and adenine were also converted to ribonucleotides by the same pyrophosphorylase. (These workers found that 20-fold purified AMP pyrophosphorylase from beef liver was active in catalysing reactions of PRPP with 4-aminopyrazolo(3,4-*d*)pyrimidine, 2-fluoro-adenine, 2-aza-adenine and 8-aza-adenine.) Results of the present work are compatible with these results and suggest that in *H.Ep*-2 cells adenine, 4-aminopyrazolo(3,4-*d*)pyrimidine and 4-amino-5-imidazolecarboxamide are acted on by AMP pyrophosphorylase which is clearly distinct from IMP–GMP pyrophosphorylases. (Growth of MP-resistant *H.Ep*-2 cells was inhibited by 4-aminopyrazolo(3,4-*d*)-pyrimidine. Similarly, Skipper *et al.*[17], and Venditti, Frei and Goldin[18] observed that growth of 8-azaguanine-resistant *L*1210 tumours was inhibited by 4-aminopyrazolo(3,4-*d*)pyrimidine; this resistant neoplasm also retained AMP pyrophosphorylase activity while showing significant decreases in IMP–GMP pyrophosphorylase activities[5,6].) Similarly, Lieberman and Ove[19] studied resistance to purine analogues in mammalian cells in culture and observed that a 2,6-diaminopurine-resistant sub-line showed specific

Table 2. CONVERSION OF BASES TO RIBONUCLEOTIDES BY PYROPHOSPHORYLASES FROM 6-MERCAPTOPURINE-SENSITIVE AND -RESISTANT HUMAN EPIDERMOID CARCINOMA (*H.Ep*-2) IN CELL CULTURE

Ribonucleotide formed	mμmoles ribonucleotide formed per mgm. protein in 1 hr. *				
	H.Ep-2 (sensitive)	MP-resistant *H.Ep*-2 †			
		0·5	1·0	10	10-MP ‡
Adenylic acid	414	576	552	429	359
4-Aminopyrazolo-3,4-*d*)-pyrimidine ribotide	67	21	14	71	76
2,6-Diaminopurine ribotide	72	10	22	58	43
5-Amino-4-imidazolecarboxamide ribotide	72	58	32	60	67
Xanthylic acid	21	3	7	1	1
Inosinic acid	839	18	<1	<1	<1
6-Mercaptopurine ribotide	766	16	2	<1	<1
Guanylic acid	383	13	14	4	1
8-Azagunaylic acid	243	5	7	6	1

* The reaction mixture consisted of base (0·0005 *M*; 0·2 μc. per ml.), magnesium PRPP (0·002 *M*), and enzyme (approximately 200 μgm. of protein per ml. incubation mixture) in 0·1 *M tris* buffer, *p*H 7·6. Incubation time was 1 hr. at 37° C.; enzyme action was stopped and reaction mixtures deproteinized by heating for 5 min. at 100° C. Aliquots (0·1 ml.) were analysed by descending chromatography on Whatman 3 *MM* paper using 70 per cent *iso*propanol in an ammonia atmosphere to separate bases from the corresponding ribonucleotides. Radioactive areas of chromatograms located by radioautography were eluted with water; the eluates were evaporated on steel planchets and quantitative determinations of radioactivity were made by counting in gas-flow proportional counters. For identification purposes bases, ribonucleosides and ribonucleotides were separated and identified by two-dimensional chromatography and by paper electrophoresis using techniques already described (refs. 5–7).

† Concentration of MP (μgm./ml.) in which cell lines were originally cloned and isolated on glass. The clones developing in 0·5, 1·0 and 10 μgm./ml. of MP were subcultured in the presence of these concentrations of MP, forming confluent monolayers of cells. These resistant cell lines were then grown in suspension culture to provide material for enzyme preparations.

‡ *H.Ep*-2/MP cloned in 10 μgm./ml. of MP was resistant to concentrations of MP approaching the solubility limit of the inhibitor (320 μgm./ml.). This line was then grown in the absence of MP for 60 generations, after which enzyme preparations were then assayed for purine ribonucleotide pyrophosphorylase activity with the results shown in this column.

loss of AMP pyrophosphorylase activity; they suggested that 2,6-diaminopurine was anabolized by AMP pyrophosphorylase. This would also be consistent with the results obtained here with *H.Ep*-2.

Decreased activity of enzymes involved in the formation of nucleotides has now been observed to accompany the development of resistance to purine analogues in a variety of biological systems. Similar observations have been made in studies of resistance to pyrimidine analogues in both mammalian cells[20-23] and in micro-organisms[24,25]. Such alterations of enzyme activities may be of genetic origin since in many cases these losses have been demonstrated to be stable heritable characteristics of the resistant cells. The development of resistance to metabolite analogues is a serious problem in the therapy of neoplastic diseases[26], and an understanding of the nature and mechanism of the biochemical changes leading to resistance to metabolite analogues poses fundamental questions in areas of enzymology and biochemical genetics.

The work was supported by grants from the Charles F. Kettering Foundation and the Alfred P. Sloan Foundation, and by contract No. *SA*–43–*ph*–2433, Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health.

[1] Kornberg, A., Lieberman, I., and Simms, E. S., *J. Biol. Chem.*, **215**, 417 (1955).
[2] Korn, E. D., Remy, C. N., Wasilejko, H. C., and Buchanan, J. M., *J. Biol. Chem.*, **217**, 875 (1955).
[3] Way, J. L., and Parks, jun., R. E., *J. Biol. Chem.*, **231**, 467 (1958).
[4] Lukens, L. N., and Herrington, K. A., *Biochim. Biophys. Acta*, **24**, 432 (1957).
[5] Brockman, R. W., Bennett, jun., L. L., Simpson, M. S., Wilson, A. R., Thomson, J. R., and Skipper, H. E., *Cancer Res.*, **19**, 856 (1959).
[6] Brockman, R. W., *Cancer Res.*, **20**, 643 (1960).
[7] Brockman, R. W., Debavadi, C. S., Stutts, P., and Hutchison, D. J., *J. Biol. Chem.*, **236**, 1471 (1961).
[8] Moore, A. E., Sabachewsky, L., and Toolan, H. W., *Cancer Res.*, **15**, 598 (1955).
[9] Kelley, G. G., Vail, M. H., Adamson, D. J., and Palmer, E. H., *Amer. J. Hyg.*, **73**, 231 (1961).
[10] Kelley, G. G., Adamson, D. J., and Oliver, K. L., *Amer. J. Hyg.*, **71**, 9 (1960).
[11] Kelley, G. G., Adamson, D. J., and Vail, M. H., *Amer. J. Hyg.*, **72**, 275 (1960).
[12] Matthews, R. E. F., *J. Gen. Microbiol.*, **10**, 521 (1954).
[13] Oyama, V. I., and Eagle, H., *Proc. Soc. Exp. Biol. Med.*, **91**, 305 (1956).
[14] Anderson, E. P., and Law, L. W., *Ann. Rev. Biochem.*, **29**, 577 (1960).
[15] Flaks, J. G., Erwin, M. J., and Buchanan, J. M., *J. Biol. Chem.*, **228**, 201 (1958).
[16] Roy, J. K., Haavik, C. A., and Parks, jun., R. E., *Proc. Amer. Assoc. Cancer Res.*, **3**, 146 (1960).
[17] Skipper, H. E., Montgomery, J. A., Thomson, J. R., and Schabel, jun., F. M., *Cancer Res.*, **19**, 425 (1959).
[18] Venditti, J. M., Frei, E., and Goldin, A., *Cancer*, **13**, 959 (1960).
[19] Lieberman, I., and Ove, P., *J. Biol. Chem.*, **235**, 1765 (1960).
[20] Reichard, P., Sköld, O., and Klein, G., *Nature*, **183**, 939 (1959).
[21] Heidelberger, C., Ghobar, A., Baker, R. K., and Mukherjee, K. L., *Cancer Res.*, **20**, 897 (1960).
[22] Brockman, R. W., and Law, L. W., *Proc. Amer. Assoc. Cancer Res.*, **3**, 98 (1960).
[23] Pasternak, C. A., Fischer, G. A., and Handschumacher, R. E., *Cancer Res.*, **21**, 110 (1961).
[24] Handschumacher, R. E., and Pasternak, C. A., *Biochim. Biophys. Acta*, **30**, 451 (1958).
[25] Brockman, R. W., Davis, J. M., and Stutts, P., *Biochim. Biophys. Acta*, **40**, 22 (1960).
[26] Skipper, H. E., Heidelberger, C., and Welch, A. D., *Nature*, **179**, 1159 (1957).

LIMITS OF HUMAN VISION

By Dr. R. A. WEALE

Department of Physiological Optics, Institute of Ophthalmology, Judd Street, London, W.C.1

FUNDUS reflectometry provides a means for studying the properties of visual pigments in the living eye. Whereas the detection of cone pigments is easy[1,2], the study of the human extra-foveal regions is complicated by their mixed receptor population. A detailed study of these regions[3] has shown that the unequivocal separation of rod and cone effects from each other is unlikely to be achieved without a close examination of difference spectra[4]. However, in the green region of the spectrum (510–520 mμ), where bleaching the dark-adapted eye with white light produces a maximum density difference, $\Delta D_{\lambda\,\mathrm{max.}}$, the effect of rod visual purple may be presumed to be dominant. If the bleaching intensity is expressed in the same units in which the absolute threshold is measured in stated conditions, an attempt to correlate the two types of measurement becomes feasible, all the more so as one is dealing with one species only, namely, man.

The latest model of the fundus reflectometer[2] was used, two retinæ being examined at locations shown in Table 1. Dark-adaptation preceding bleaching measurements always continued for 20 min., but 30 min. in the absolute threshold measurements. The bleaching exposure lasted 30 sec., the intensity being varied as shown along the abscissa (Fig. 1). The threshold measurements involved a criterion of 50 per cent 'seen' of the bleaching light, exposed for 0·036 sec. and reduced from 3 to 1·7° angular diameter.

The changes in density, $\Delta D_{\lambda\,\mathrm{max.}}$, are plotted on a percentage basis (Fig. 1) as a function of bleaching intensity. The maximum values corresponding to each subject and the retinal positions are shown in Table 1 and represent values for double transit through the retina. $\Delta D_{\lambda\,\mathrm{max.}}$ rises with intensity to a maximum at log scotopic trolands = 6·5.

A comparison of the bleaching and threshold data with work of Aguilar and Stiles[5] is of interest. These workers measured accurately the incremental threshold of rods (ordinate) as a function of adapting field luminance (abscissa). Their mean curve is reproduced in Fig. 1. The average of the present threshold measurements agrees with theirs as well as can be expected in view of the difference between the experimental conditions. But the following is the focal point of interest in the comparison. Aguilar and Stiles estimate that, in the region indicated with the arrowed bracket, human rods are saturated. This is to say, no matter how great the field intensity, the increment required to make the test visible approaches infinity. They calculate that this saturation is unlikely to be

Table 1. Maximum Values Corresponding to each Subject and Retinal Positions*

	A $\Delta D_{\mathrm{max.}}$		*B* $\Delta D_{\mathrm{max.}}$
8° *N* ret.	0·093	◑	0·116
10° *T* ret.	0·095	◨	0·150
13° *T* ret.	0·125	▽	0·130
17° *T* ret.	0·085	▲	0·157

* This refers to Fig. 1.

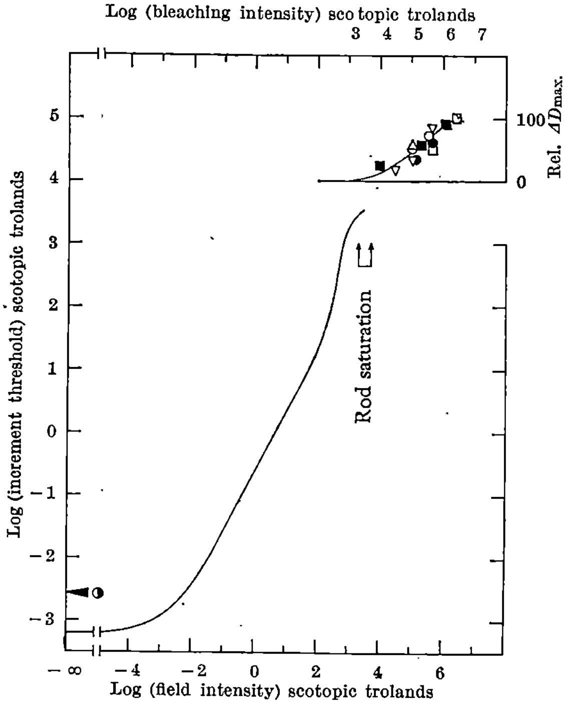


Fig. 1

due to the exhaustion of visual purple. The present results bear out this expectation: in the region of rod saturation the amount of pigment bleached becomes just measurable and is exhausted only at intensities nearly one thousand times as high.

The present results can be related to the absolute threshold in another way. Pirenne has shown in a very interesting paper[6] that visual purple would be expected to bleach at the rate of 1 per cent/sec. when the bleaching intensity is 10^{10} threshold. At first sight the results shown in Fig. 1 appear to contradict this prediction, such a bleaching rate occurring at about 10^7 threshold. But the following argument shows that the present results do, in fact, come close to Pirenne's prediction that the threshold and the rate of bleaching can be related along the lines which he suggested.

Let Q be the number of quanta absorbed at the absolute threshold; t, the time of exposure of the threshold test-light; A, its angular area in square degrees; N, the number of rods per square degree[6]; ν, the number of chromophores per rod[6]; α, the effective percentage absorption of the visual pigment[6], p, the fraction of pigment bleached in T, the bleaching time; R, the ratio (the energy flux per unit time bleaching p per cent of pigment) ÷ (the flux producing the threshold response); γ, the quantum efficiency (number of chromophores bleached ÷ quanta absorbed); D, the orientation factor[8]; then:

$$p = \frac{RTQ\gamma D}{\alpha ANt\nu}$$

The following are among important assumptions in the derivation of this expression: absorption leads to bleaching; the amount bleached is such as not to change the absorption to a very marked extent (a proviso obeyed approximately in present conditions when $p = 0 \cdot 2$); in the conditions of the experiment, the quantum efficiency is approximately 0·5 (refs. 4, 7); blinking[9] is not very important. Denton[8] has shown that the absorbing molecules are so positioned as to render absorption maximal when the light travels along the axis of the rod. It is doubtful whether this can apply in all rigour to the human eye, the rods pointing approximately at the posterior nodal point, and it will apply with even less certainty to light re-traversing the retina after reflexion from the fundus. But accepting a value of $D = 1 \cdot 5$ for this effect and a quantum efficiency of $\gamma = 0 \cdot 5$, the number of chromophores ν has to be multiplied by 1·33. Substitution of experimental values in the equation leads to $Q = 7–14$ if some uncertainty in the results is allowed for. The calculated value yields a result of the order of magnitude obtained by other methods[6]. Although ν, the number of chromophores per rod, was calculated on the basis of frog visual purple data[6], it is unlikely that comparably accurate data for human visual purple would affect the situation very much[10].

An increase in bleaching intensity beyond that shown in Fig. 1 leads to an unexpected result. The change in density recorded for the wave-length of maximum change is smaller at the highest intensity used (cf. Fig. 2) than it is at the top intensity shown in Fig. 1. This finding appears to hold for both extra-foveal and foveal parts of the retina. Control of the pupil diameter[3,11] reduces the likelihood of miosis of the strongly mydriaticized iris being responsible for this effect in intense illumination. An extension of the recording of changes of density to the whole of the visible spectrum shows that the apparent reduction in $\Delta D_{\lambda\max.}$ is due to an upward move of the whole base-line, a finding which can be pictured by the hypothesis that, in very intense illumination, a grey filter is formed in the eye. The effect disappears after dark-adaptation for 3 min. An early stage in thermal lesions, known to lead to retinal œdema, is manifested by a greying of the fundus oculi. The view that the rise in the zero line mentioned above is an even earlier manifestation of such a process is supported by radiometric measurements of the intensity of the bleaching light (top of Fig. 2). These

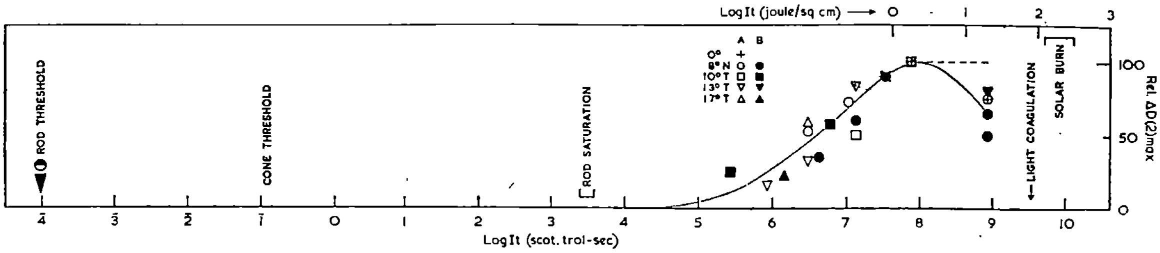


Fig. 2. Ordinate: Relative values of maximum change of density (double transit through retina) in various locations of two dark-adapted human retinæ A and B (see inset). Abscissa: bleaching intensity × exposure time in scotopic trolands × sec. (bottom); in joules/sq. cm. on the retina (top). Free-hand curve. Effects due to blinking and absorption in pre-retinal media are neglected. The rod threshold was measured on subjects A and B (cf. Fig. 1). The broken line represents the function of $\Delta D(2)_{\max.}$ when the œdema effect is compensated

relate the present results to exposures causing solar burns[12], just as an extension of the photometric scale (bottom of Fig. 2) relates them to exposures leading to light-coagulation[13]. It may be suggested tentatively that the constitution of human retinal light-sensitive material is such that the lower limit of the visual range is governed by quantum physics, whereas the upper limit coincides with the safety-level of ocular tissues exposed to thermal hazards.

[1] Weale, R. A., *J. Physiol.*, 127, 587 (1955).
[2] Weale, R. A., *Optica Acta*, 6, 158 (1959).
[3] Weale, R. A., *Vision Res.* (in the press).
[4] Dartnall, H. J. A., *The Visual Pigments* (Methuen and Co., London, 1957).
[5] Stiles, W. S., and Aguilar, M., *Optica Acta*, 1, 59 (1954).
[6] Pirenne, M. H., *Biol. Rev.*, 31, 194 (1956).
[7] Hagins, W. A., *J. Physiol.*, 129, 22*P* (1955).
[8] Denton, E. J., *J. Physiol.*, 124, 16*P* (1954).
[9] Lawson, R. W., *Nature*, 162, 531, 970 (1948).
[10] Dartnall, H. J. A., *N.P.L. Symposium on Visual Problems of Colour*, Paper No. 3 (H.M.S.O., 1958).
[11] Weale, R. A., *Amer. J. Ophthal.* (in the press).
[12] Duke-Elder, W. S., *Text-book of Ophthalmology*, 6 (Henry Kimpton, London, 1954).
[13] Meyer-Schwickerath, G., *Arch. Ophthal.*, 156, 2 (1955).

PHOSPHATASES IN *SCHISTOSOMA MANSONI*

By Dr. D. L. H. ROBINSON
Medical Research Council Group for Research in Bilharzia, Winches Farm, St. Albans

Distribution of Alkaline Phosphatase

DUSANIC[1] made a histochemical study of alkaline phosphatase in larval and adult *Schistosoma mansoni* and tissues associated with these stages in both molluscan and final hosts. In adult flukes the enzyme was found in the subcuticular cells, the mesenchyme, the ovaries, testes and the intestinal cells. High concentrations of the enzyme were found in the intestinal cells, and in the ovaries and testes alkaline phosphatase activity was considerably greater than in the mesenchymal cells.

During an examination of the methods of carbohydrate uptake in schistosomes, I investigated the distribution of alkaline phosphatase in *S. mansoni*. Mature flukes were obtained from the portal and mesenteric veins of hamsters 8–10 weeks after infection with 150 cercariæ of an Egyptian strain of the schistosome maintained in a Puerto Rican strain of *Australorbis glabratus*. After a quick rinse in cold saline, the worms were transferred as rapidly as possible to ice-cold acetone. Alkaline phosphatase activity was determined by Glick's[2] modified Gomori method using a sodium glycerophosphate substrate. Sections cut in both longitudinal and transverse planes at 10μ were incubated at 37° C. for 1 hr., this time having been arrived at by the method of Martin and Jacoby[3]. Control slides, incubated without substrate, were negative.

Fig. 1 shows part of a transverse section of a male *S. mansoni* in the region of the testis. High alkaline phosphatase activity is present in the subcuticular cells and in the mesenchyme, but considerably less in the testis. The black deposit in one of the cæcæ in Fig. 1 is due to pigment in the lumen, but the intestinal cells show little activity except for the nuclei. Similar results were obtained in the intestinal cells of female worms and the ovaries exhibited lower activity than the mesenchyme.

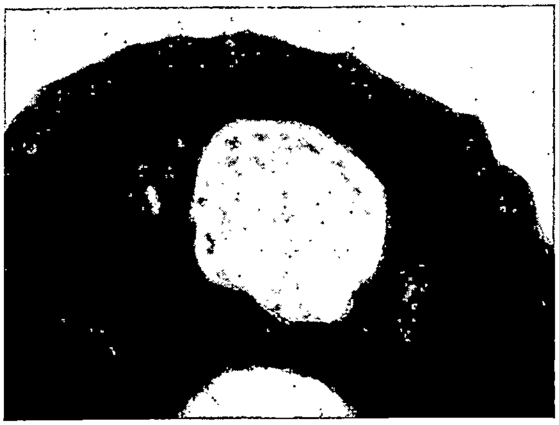


Fig. 1. Portion of a transverse section of a male *Schistosoma mansoni* showing distribution of alkaline phosphatase

There appears to be abundant evidence available to confirm the view of Rogers[4] that regions exhibiting high alkaline phosphatase activity might be associated with carbohydrate absorption. Based on this hypothesis, the results given above, in contrast to those of Dusanic[1], suggest that the major region of carbohydrate uptake from the environment in *S. mansoni* is the cuticle, with the intestinal cells playing a secondary, if not a minor part.

Quantitative Estimation of Phosphatases

HISTOCHEMICAL examination shows that high alkaline phosphatase activity is present in *Schistosoma mansoni* at *p*H 9·4. Dusanic[1] observed that the alkaline phosphatase had a *p*H optimum of 9·0–9·5, and exhibited only slight activity at *p*H 8·6. At *p*H 8·0 no activity could be detected by histochemical methods.

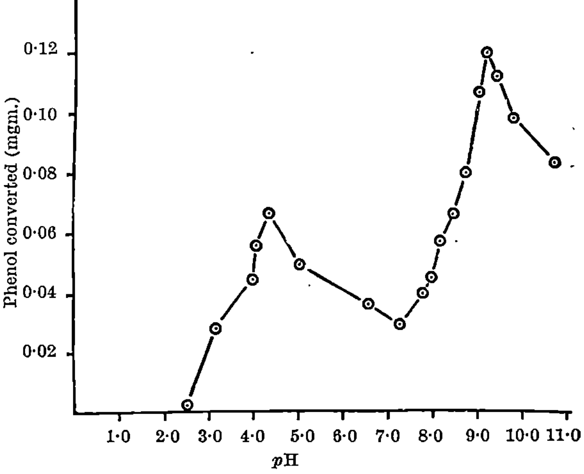


Fig. 2. Phosphatase activity of extract of male *S. mansoni* at different hydrogen ion concentrations. 130 worms, extract made up to 25 ml. Incubation time, 18 hr.

The quantitative experiments reported here were performed: (a) to determine the *p*H optima of the alkaline phosphatase of *S. mansoni*; (b) to demonstrate an acid phosphatase if one were present; (c) to compare the activity of these enzymes, at different hydrogen ion concentrations.

Mature schistosomes were removed from the portal veins of hamsters infected 8 weeks previously with *S. mansoni*. Pairs were separated and male and female worms counted and ground separately in a glass tube containing distilled water. Toluene was then added and the mixture allowed to stand on the bench for 4–5 hr. After centrifugation the supernatant was stored overnight in the refrigerator. The solution was diluted immediately before use.

Incubation mixtures consisted of 1 ml. substrate, 1 ml. extract, 1 ml. buffer and 0·5 ml. toluene. The buffers used were acetate/barbiturate/hydrochloric acid (Michaelis 1931) and glycine/sodium hydroxide (Sorensen Walbum, 1920), and the substrate was 0·01 *M* disodium monophenyl phosphate. The *p*H of each mixture was determined, before and after incubation, on a Pye *p*H meter. After incubation, aliquots were removed for estimation of the phenol liberated, by the method of King and Armstrong[5], using the reagent of Folin and Ciocalteau. Controls consisted of unincubated mixtures, and the results were deducted from those of the test.

An alkaline and an acid phosphatase were present in male and female worms. No sex differences were observed in their characteristics. The alkaline phosphatase had its optimum in the region of *p*H 9·3 and the acid phosphatase had an optimum in the region of *p*H 4·4. As can be seen from Fig. 2, the alkaline phosphatase exhibited the higher relative activity.

Bueding[6] found that carbohydrate uptake in *S. mansoni* amounted to the equivalent of one-fifth of its dry weight per hour. The high alkaline phosphatase activity reported above may therefore be linked with this high carbohydrate uptake and adds further support to the view of Rogers[4] that regions of high alkaline phosphatase activity are associated with carbohydrate absorption.

---

[1] Dusanic, D. G., *J. Infect. Dis.*, **105**, 1 (1959).

[2] Glick, D., *Techniques of Histo- and Cytochemistry* (Interscience, New York, 1949).

[3] Martin, B., and Jacoby, F., *J. Anat.*, **83**, 351 (1949).

[4] Rogers, W. P., *Nature*, **159**, 374 (1947).

[5] King, E. J., and Armstrong, A. R., in *Chemical Methods in Clinical Medicine*, edit. Harrison, G. A. (J. and A. Churchill, London, 1947).

[6] Bueding, E., "Metabolism of Helminths", from *Parasitic Infections in Man*, edit. Most, H., 150 (Columbia Univ. Press, New York, (1951).

SOCIAL DOMINANCE IN INBRED MOUSE STRAINS

By PROF. G. LINDZEY, H. WINSTON and M. MANOSEVITZ

Department of Psychology, University of Minnesota, Minneapolis 14

ORIGINS of the experimental study of temperament in lower animals[1–3] are approximately as remote as origins of the experimental study of learning in lower animals[4,5]. In spite of this historical similarity the two domains to-day are dramatically different in their respective achievements. By almost any reasonable criterion the area of learning has attained a state of maturity far surpassing that of research in animal temperament. While there are undoubtedly many factors that have contributed to this relative deficiency in investigation of animal temperament, one of the most important of these is the absence of a set of sensitive and reliable measures that can be used to assess variables commonly viewed as significant or interesting.

This article is presented as a very small corrective to this deficiency. It describes a measure of social dominance and presents tentative results, based on the comparison of three inbred strains of mice, suggesting that the test possesses reasonable sensitivity and stability. For present purposes we may define social dominance simply as the tendency of an animal to make a goal response when this response involves overcoming the competing response of another animal similarly motivated.

The subjects in this work consisted of 44 animals in each of three inbred mouse strains. Breeding stock for each of the strains was obtained from the colony maintained by Prof. John Bittner in the Department of Cancer Biology at the University of Minnesota. Mice within each strain have been bred by brother–sister mating well beyond the twenty generations that are conventionally believed to lead to offspring that are approximately homozygous. The three strains and the number of generations of inbreeding are as follows: *A*/alb (57), *C3H* (97), *DBA*/8 (28). The subjects were at least 60 days old, and animals that were opposed to each other were matched in age and sex. All animals were individually housed after weaning at 22 days of age.

The dominance test consists of a 'Lucite' tube, 1 in. inside diameter and 17 in. long. The tube is connected at each end to a goal box that is 4 in. × 4 in. × 2 in. and covered by a wire cloth top. Guillotine doors are located at the entrance to each goal box and a small 'Lucite' divider can be inserted midway in the tube. The diameter of the tube is just sufficient to permit a mature mouse to pass through it readily, but not sufficient to permit two mice to pass one another in the tube. It should be mentioned that although this technique was devised in our laboratory, after we had worked with it for a period of time, we learned that Prof. Tsai of Tulane University had used a similar apparatus with rats. Each animal was given a series of five training trials on each of three successive days. The animals were run, deprived of food (85 per cent of normal weight) with the reward of wet mash in the goal box after they had passed through the tube. During the training trials the mice were alternated in the direction that they ran through the tube and the experimenter recorded the time required to reach the goal box on each trial. Following this, there was a period of five dominance test days on each of which the animals received individually three further training trials before the test trials.

On a dominance trial the mice were placed at the same time in two goal boxes at the entrances to the tube and when they had both reached the centre of the 'Lucite' tube the divider was removed and the issue was joined. The trial ended when one mouse had forced the other to retreat with all four feet into the goal box from which it had commenced. The order of the various oppositions within each group, and the goal box in which each mouse began, were systematically varied on successive days. The animals were deprived of food during all the test trials.

Mice were run in groups of four. Each group included two animals from two of the three strains under investigation and each mouse opposed every other mouse in his group on each of the five test days. Thus, there were eleven groups involved in each of the three strain comparisons (*A*/alb versus *C3H*, *A*/alb versus *DBA*/8, *C3H* versus *DBA*/8). We were interested in differences of strain and for each mouse there was a dominance opposition to two mice from the other strain on each test day, or a total of ten such oppositions per mouse by the end of the test series.

The simplest and most meaningful indexes of *stability* consist of the proportion of mice that achieved dominance by winning all five of their trials, the proportion that achieved dominance by winning four out of five trials, and the proportion that won three out of five trials. In the opposition between *A*/alb and *C3H* mice, 57 per cent of the dominant mice won all five trials, 27 per cent won four trials, and 16 per cent won three trials. In the opposition between *A*/alb and *DBA*/8 mice, 75 per cent of the dominant animals won all five trials, 20 per cent won four trials, and 5 per cent won three trials. In the opposition between *C3H* and *DBA*/8 mice, 64 per cent of the dominant animals won all five trials, 18 per cent won four trials, and 18 per cent won three trials. If we combine the results for all strain comparisons, we find that in approximately two-thirds of the cases, there was complete consistency, with the same animal winning all five of the trials. In slightly more than one-fifth of the oppositions the dominant animal won four out of the five trials, and in less than one-eighth of the cases the dominant animal won only three out of the five trials. In general, the consistency across repeated trials of the test was so marked that, knowing the performance of a particular pair of mice for a single trial, provides an excellent basis for predicting the outcome of the four subsequent trials.

The matter of *transitivity* is somewhat more difficult to represent although the results for strain comparisons are obvious. There is complete transitivity between strains, for example, *A*/alb > *C3H*, *C3H* > *DBA*/8, *A*/alb > *DBA*/8; moreover, the fact that the superiority of the *A*/alb over the *DBA*/8 is greater than the superiority of the *A*/alb over the *C3H* is reflected in the dominance of the *C3H* strain over the *DBA*/8 strain. Nevertheless, there are so few strain comparisons possible in our work that this is not altogether a satisfactory measure.

An index of transitivity between individual animals was devised that could be applied to each group of four animals. A group was declared transitive if it arranged itself in an hierarchical manner, so that the most dominant subject dominated all other animals, the second most dominant animal dominated all animals except the most dominant, and so on. Using this procedure there were a total of 33 such groups and all but two met this stringent criterion of transitivity. Thus, our evidence suggests a high degree of transitivity in the dominance relations of individual animals as well as in the relations between strains.

Table 1. STRAIN DIFFERENCES IN DOMINANCE

	A/alb versus *C3H*		*A*/alb versus *DBA*/8		*C3H* versus *DBA*/8	
Per cent of total trials dominant *n* (animals) = 22 *n* (observations) = 220	78	22	94	6	78	22
Per cent of total pairings dominant *n* (animals) = 22 *n* (observations) = 44	84	16	98	2	82	18
Per cent of animals winning more than one-half of the oppositions with other strain *n* (animals) = 22 *n* (observations) = 22	86	14	100	0	86	14

Previous investigators who have been concerned with measuring dominance in animals have not, for the most part, concerned themselves with the reliability or transitivity of their measures. Those investigators who have inquired into those questions[6-8] have reported results indicating much less stability than we have observed.

The results summarized in Table 1 leave no doubt about the existence of *differences between strains* in performance on this test. The results of the strain oppositions are summarized by means of three correlated indexes that are completely consistent in the direction and the magnitude of the results they depict. Perhaps the most revealing of these indexes is the third, which summarizes the number of animals within each strain that won more than half of their trials against animals from the comparison strain. The results indicate that 86 per cent of the *A*/alb mice won more than half of their oppositions against the *C3H* mice, 100 per cent of the *A*/alb mice won more than half their oppositions against the *DBA*/8 mice, and 86 per cent of the *C3H* mice won more than half of their oppositions against the *DBA*/8 mice. Thus, animals in the *A*/alb strain are dominant over animals in the *C3H* and *DBA*/8 strains and animals in the *C3H* strain are dominant over animals in the *DBA*/8 strain. Moreover, even given the genetic diversity of these strains, the magnitude of these differences is unusually large when compared with results obtained with other behavioural measures.

Results of this nature obviously suggest the importance of gene factors in determining dominance behaviour as we have measured it. There seems little doubt that a considerable proportion of the variance in our measure can be attributed to variation in genetic factors. These results are consistent with those reported by Ginsburg and Allee[9] on the basis of observing fighting behaviour in different strains. Uyeno[10], using a food competition measure of dominance, in conjunction with a single generation of selective breeding, also found evidence for the contribution of gene factors to dominance behaviour.

A further result of such a clear-cut differentiation between strains is some increase in our confidence in the measure we are employing. In other words, when subjects known to be quite distinctive in behaviour[11,12] are compared in terms of a particular measure, our interest in this measure is considerably dampened if it proves unable to discriminate between these distinctive organisms. Conversely, when the test can discriminate among subjects with unusual effectiveness, our confidence that the test is measuring something significant is increased.

Given that we have a measure that is rationally linked to dominance, and that it differentiates successfully between subjects that are known to be different in behaviour, there still remain many alternative explanations to be explored before we can be content with the label we have attached to the measure. For example, one might reason that the differences we have observed between strains are attributable to some simple, extraneous factor that may be genetically controlled and at the same time serves to influence performance on this test. It may be, for example, that the strains differ widely in weight so that even when animals are matched in age and deprivation experience certain strains will have an appreciable advantage in weight, and this difference will determine the result of the test procedures. This possibility was explored both within strains and between strains and we found no evidence for an association between weight and dominance. A second alternative hypothesis suggests that the strains may differ in the effectiveness with which they learn the original task involving passage through the tube and, consequently, they may be differentially motivated when placed in opposition in the tube. This possibility was examined by relating performance during the training trials (average time per trial) to performance on the dominance measure, but again we found no evidence of any association between the two variables. Thus, two of the most evident means of weakening the inferential link between the concept of dominance and our measure were found to be inconsistent with available evidence.

It is obvious that future investigations exploring the relation between the present measure of dominance and other measures of the same variable are very much needed. Our preliminary results indicate important differences in the results obtained with our measure and with the food competition measure employed by Rosen[13] and Uyeno[10]. It is also important that the relation between dominance and such related temperament variables as aggression and fearfulness should be explored. Given the results of such research, in addition to more detailed information concerning the determinants of performance on our measure, it should be possible to arrive at a meaningful estimate of the significance of this device for the student of animal temperament.

This work was supported by a grant from the Ford Foundation and Research Grant *M*-3536 from the National Institute of Mental Health of the National Institutes of Health, U.S. Public Health Service.

[1] Berry, C. S., *J. Comp. Neur. Psychol.*, **16**, 333 (1906).
[2] Stewart, C. C., *Amer. J. Physiol.*, **1**, 40 (1898).
[3] Yerkes, R. M., *J. Anim. Behav.*, **3**, 286 (1913).
[4] Small, W. S., *Amer. J. Psychol.*, **11**, 80 (1900).
[5] Thorndike, E. L., *Psychol. Monogr.*, **2**, No. 8, 109 (1898).
[6] Ross, S., and Berg, J., *J. Mammal.*, **37**, 129 (1956).
[7] Ross, S., and Scott, J. P., *J. Comp. Physiol. Psychol.*, **42**, 75 (1949).
[8] Seward, J. P., *J. Comp. Psychol.*, **38**, 213 (1945).
[9] Ginsburg, B., and Allee, W. C., *Physiol. Zool.*, **15**, 485 (1942).
[10] Uyeno, E. T., *J. Comp. Physiol. Psychol.*, **53**, 138 (1960).
[11] Lindzey, G., *J. Comp. Physiol. Psychol.*, **44**, 389 (1951).
[12] Lindzey, G., Lykken, D. T., and Winston, H. D., *J. Abn. Soc. Psychol.*, **61**, 7 (1960).
[13] Rosen, J., *Canad. J. Psychol.*, **12**, 229 (1958).

SIGNIFICANCE OF THE ANTI-SPORULATION EFFECT OF COPPER FUNGICIDES IN THE CONTROL OF SIGATOKA DISEASE OF BANANAS CAUSED BY *MYCOSPHÆRELLA MUSICOLA* LEACH

By E. EVANS, H. L. S. BEVINGTON and E. P. WHITAKER

Fisons Pest Control, Ltd., Chesterford Park Research Station, Nr. Saffron Walden

It is now a well-established belief that all copper fungicides are protective in function when used for the control of plant diseases. As such, an attempt is always made to cover the most susceptible parts of the host plant with a layer of chemical prior to the onset of the disease epidemic. This was the standard practice for the control of Sigatoka disease of bananas in most parts of the world until the introduction of eradicant oil spraying in 1956[1]. Bordeaux mixture is still being used in many parts of the world, but this practice is gradually becoming less economic. Accordingly, attempts have been made to introduce the ultra-low-volume application of copper fungicides from the air and from ground machines.

For the past six years we have been closely associated with the aerial application of copper fungicides to a number of crop plants, more particularly to potatoes and bananas. During visits to the field in West Africa and Central America, as well as from greenhouse studies in the United Kingdom, it has become obvious that the banana plant has unusual physical characteristics from the point of view of the retention of spray droplets. Leach[2] has shown that the spike, first and second open leaves are those most susceptible to leaf spot, yet a simple survey of any air-sprayed area of bananas shows quite clearly that these are rarely adequately covered with chemical. One of the main reasons for this phenomenon appears to be the highly reflective characteristics of these leaf surfaces as well as the exceptionally rapid development of the spike leaf during the main growing season.

From observations such as these it seems reasonable to suggest that any aerial application of a protective fungicide would not be particularly successful in controlling the disease unless such a chemical also acted by some other mechanism. One obvious alternative would be an anti-sporulation effect[2].

By courtesy of the United Fruit Co., laboratory facilities were made available in 1960 to two of us at Palmar Sur in Costa Rica (E. E., H. L. S. B.). During this visit an attempt was made to evaluate a number of standard fungicides as anti-sporulants, using a modification of the method recommended by R. W. Fulton (personal communication). Basically, this consisted of collecting leaf segments from the field, with Sigatoka lesions at the correct stage of

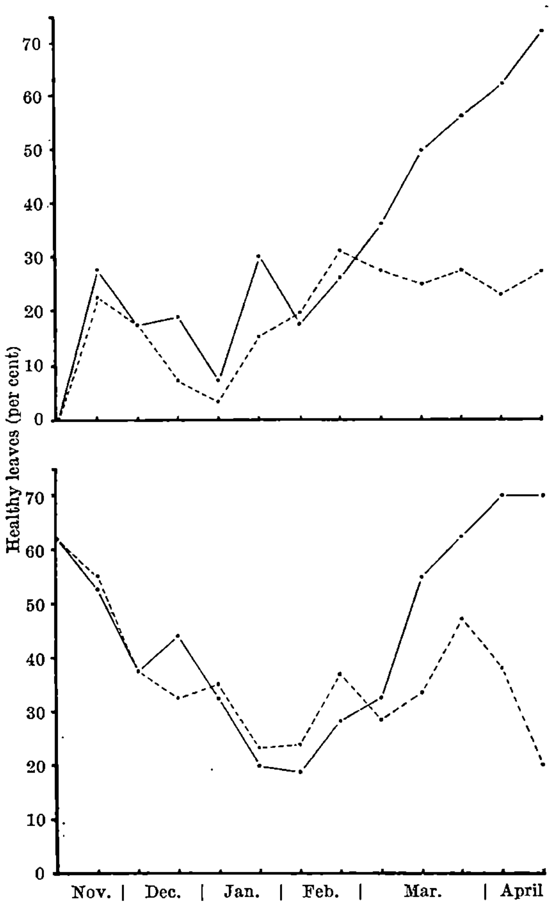


Fig. 1. Percentage of clean and lightly infected leaves in treated and untreated plots on parent (above) and follower (below) plants in a mature stand of bananas. ——, copper oxychloride; – – –, untreated control

development, spraying these lesions with the test chemicals and incubating for 24 hr. at 26° C. prior to examination under a stereoscopic microscope. The evaluations were made by comparing the number of sporulating lesions in the treated samples with those in the untreated controls. Sample data obtained in this manner are given in Table 1.

Table 1 shows quite clearly that standard fungicides such as copper oxychloride, zineb and a mixture of these two preparations have a marked effect on conidial production of lesions of *Mycosphærella musicola*. Since any lesion which produced a single recognizable spore was classified as a sporulating lesion, the results vastly under-estimate the total reduction of spores produced by the treated over the untreated lesions.

At the same time as these experiments were being carried out in Costa Rica, one of us (E. P. W.) was carrying out small-scale low-volume field trials on Gros Michel bananas in the Southern Cameroons. Here two experiments were attempted: site 1 on heavily infected mature banana plants; site 2 on a banana abandonment which was completely cut down then sprayed regularly during regrowth.

Table 1. ANTI-SPORULATION EFFECTS OF CERTAIN STANDARD PROTECTIVE FUNGICIDES

Chemical	Rate of application equivalent of four cycles/treatment at	Mean sporulating lesions after treatment (per cent)
Copper oxychloride	3½ lb. in 1–2 gal./acre	28
Zineb	2 lb. in 1–2 gal./acre	17
3:1 mixture of copper oxychloride and 'Zineb'	3 lb. in 1–2 gal./acre	5
Untreated control	—	79

Application of chemicals to both sites was made at 14-day intervals with a Kiekins Deckar Motoblo machine applying 3½ lb. of copper oxychloride per acre in 1 gallon of water containing 4 per cent v/v amine/stearate formulant[5]. The experiments were commenced in November 1959 and disease recordings were made at two-weekly intervals on one site until the end of April. The technique of recording used was a modification of that suggested by Leach[2]. On site 1 a sample of ten parent plants and ten followers were recorded on each occasion. On site 2, however, two separate samples of 15 plants each were recorded during the final examination, and the results obtained in this manner are summarized in Fig. 1 and Table 2 respectively.

Table 2. PERCENTAGE OF LEAVES RECORDED IN EACH DISEASE CATEGORY ON TREATED AND UNTREATED PLANTS DEVELOPING ON AN AREA THAT HAD PREVIOUSLY BEEN 'CUT DOWN' (SITE 2)

Treatment	Disease category	Samples 1	Samples 2
3½ lb. copper oxychloride in 1 gal./acre*	Nil	56·4	56·4
	Light	15·4	21·4
	Medium	26·5	16·2
	Heavy	1·7	6·0
Untreated control	Nil	24·1	28·5
	Light	12·1	12·3
	Medium	19·3	23·1
	Heavy	44·5	36·1

* This site was sprayed four times between March 10, 1960, and May 1, 1960, when the final assessment was made.

Fig. 1 shows quite clearly that there is a very marked delay in the appearance of any significant difference between the control and the treated areas in mature plantations. This experiment was carried out during the dry season, and as such the incidence of disease was not at its maximum. D. Price (late of the Cameroons Development Corporation: personal communication) has confirmed that similar delays were obtained by the low-volume application of other copper fungicides in his trials which were commenced in July 1958. One possible explanation of this delayed effect is that the copper fungicides are acting primarily as anti-sporulating agents. If this interpretation is correct, then in order to obtain a more rapid control of the disease it would possibly be necessary to have much more cover than is normally given by a single aircraft application of chemical at 1–2 gal./acre, but provided a given fungicide deposit is sufficiently persistent then repeated applications should lead to a build-up of chemical on the older leaves equal to that given by any high-volume ground application. The upper leaves of the banana plant always appear free from disease, and mature lesions only appear on lower leaves in the plant. From a microscopic examination of approximately 6,000 lesions it appears that the 'black lesions' sporulate much more uniformly than any other type of lesion, although spores are also found on the older lesions. These black lesions are most commonly recorded about half-way down the mature plant, although again they may occasionally appear on very old or young leaves. Most of these observations suggest that the main conidia-producing areas of an infected plant are middle and lower leaves, and not until these leaves are adequately covered with an anti-sporulating chemical will any marked effect of spraying from the air become apparent.

Eradicant oil applications from the air would certainly reduce the number of lesions developing to the 'black' stage, but does not prevent sporulation of any lesions that were already present[3,4], and thus a single oil application would not have any long-term effect in that the high inoculum potential present would remain. This suggests that provided climatic conditions remain conducive to the fungus, it would be necessary to apply oil to a given plant until the youngest leaf present during the first application had developed to complete maturity before the maximum effect on the incidence of disease was obtained.

Table 2 demonstrates that under the cutting-out routine, an almost immediate response to spraying was obtained, which suggests that under conditions of low inoculum potential brought about by the removal of the older infected leaves, the low-volume application of a copper fungicide also imparts some protection to the crop. This is the normally accepted explanation of the mode of action of these chemicals, but we would like to suggest that the anti-sporulation effect of such treatments as are mentioned in this report are possibly much more significant in determining field results than was previously realized. If this interpretation is correct, then the aerial application of protective and anti-sporulating fungicide could not be expected to show its maximum effects until (at least) 6–7 months after the commencement of that treatment. Furthermore, the deposits most resistant to weathering should have a marked advantage over less persistent materials.

We wish to express our thanks to the Directors of the Cameroons Development Corporation, United Fruit Co., and Fisons Pest Control, Ltd., for permission to publish these results. More particularly we would like to express our appreciation of the assistance given to us by various members of these organizations, including Drs. Forsyth, Fulton, Klein and Stover of the United Fruit Co., and Mr. Etherington and Mr. Price of the Cameroons Development Corporation.

[1] Guyot, H., and Cuille, J., *Fruit d'autre mer*, **10**, 101 (1955).

[2] Leach, R., *Banana Leaf Spot on the Gros Michel Variety of Jamaica* (Government Printers, Kingston, 1946).

[3] Calpouzos, L., Theis, R., Rivera, C. M., and Colberg, C., *Phytopath.*, **49**, 119 (1959).

[4] Klein, H. H., *Phytopath.*, **50**, 488 (1960).

[5] *Financial Times*, May 12, 1961.

AGE OF BED I, OLDUVAI GORGE, TANGANYIKA

By DR. L. S. B. LEAKEY
Coryndon Museum, Nairobi
AND
DR. J. F. EVERNDEN and DR. G. H. CURTIS
Department of Geology, University of California, Berkeley

OLDUVAI GORGE is justly famous because of its unique geological sequence of Pleistocene deposits, which are exceedingly rich in fossil fauna, as well as a long sequence of stages of evolution of the earlier Stone Age cultures[1].

In the monograph[1] published in 1951 the view was expressed that although Bed I differed from Bed II in faunal content, both belonged to the lower part of the Middle Pleistocene. This view was revised by one of us in 1959 as a result of reviewing the fauna collected in the series of detailed excavations during the period 1952–59 inclusive.

Leakey claimed that it was now apparent that the time-interval between Bed I and Bed II was greater than had been previously supposed, and he reverted to the view which he had published in 1935[2] that Bed I was of Lower Pleistocene age. In view of the extraordinary wealth of fossil and cultural material in the Olduvai deposits, it was not surprising when in 1959 a most important fossil hominid skull—*Zinjanthropus boisei*—was found in Bed I, at site *FLK* I, in association with faunal remains and Stone Age culture material of the Olduwan culture[3]. In 1960, thanks to the Research Committee of the National Geographic Society, the Wenner Gren Foundation and the Wilkie Trust, very extensive further work was carried out, resulting in the discovery of pre-*Zinjanthropus*[4] fossil hominid material at a lower geological level in Bed I.

Fortunately, many of the deposits at Olduvai Gorge are pure volcanic deposits, containing no derived material, and are therefore usable for the potassium–argon dating method. A preliminary examination of deposits in the Gorge and collecting of material were carried out by two of us, Leakey and Evernden, in 1958 and additional material was collected at intervals and sent to Berkeley by Leakey in 1959 and 1960. In 1961, Curtis visited Olduvai Gorge with Leakey and Richard Leakey for further collecting and study. Although many of the samples collected have not yet been studied, we all three feel that a report on those specimens which have a direct bearing on the age of the fossil hominid remains in Bed I should be published, in view of the scientific importance of these early hominids.

The three sites which have yielded hominid remains in Bed I are: (*a*) *FLK* I, the site which yielded the skull of *Zinjanthropus boisei*; (*b*) *FLKNN* I, the site which yielded the remains of a 'child', representing a pre-*Zinjanthropus* hominid; and (*c*) *MK* I, which has not been referred to in the preliminary reports but which has yielded some teeth and parts of a skull of a hominid. All three sites have yielded an archaic fauna and tools of the Olduwan culture, and are regarded as of Lower Pleistocene (Villafranchian) age.

The Bed I samples which have been dated are as follows:

KA 412 437 Basal bed in the tuff and sediment sequence of Olduvai Gorge. Sample collected immediately west of third fault on south side of gorge at knife-edge descent. This horizon can be correlated with the series

Table 1

			Top of Bed I			
KA 664	Biotite	1·39 gm.	6·96% K	1·77 × 10^{-11} mole $A_{40}{}^{r}$	93% $A_{40}{}^{at}$	1·02 × 10^{6} yr.
664*R*	Biotite	5·99	6·96	8·36	82	1·13
KA 861	Oligoclase	6·4	1·17	1·85	93	1·38
			Bottom of Bed I (hominid-containing series of tuffs)			
KA 412	Anorthoclase	29·6	3·29	28·3	67	1·63
KA 437	Anorthoclase	6·68	3·06	6·62	70	1·74
KA 846	Anorthoclase	7·91	2·52	5·55	52	1·57
KA 847	Anorthoclase	5·90	3·18	6·16	70	1·85
KA 849	Anorthoclase	6·50	2·04	4·44	77	1·89
KA 850	Anorthoclase	8·17	3·94	10·20	41	1·78
KA 851	Anorthoclase	7·79	3·69	8·37	61	1·64

of tuffs in which hominid remains have been found by Leakey.

KA 846 Site *MK*, 18 in. below hominid remains. Buff coarse-grained tuff 13 in.–14 in. thick.

KA 847 Site *MK*, just above hominid layer. Light grey tuff, 20 ft. thick. Appears to be Pelean type ash. On every criterion, this must be considered an uncontaminated volcanic deposit.

KA 849 Site *FLK* I. 11-in. crystal tuff overlying *Zinjanthropus* layer.

KA 850 Site *FLKNN* I. 2-in. ash above very fine-grained 1-in. ash resting on pre-*Zinjanthropus* floor.

KA 851 Site *FLK* I. 12-in. tuff immediately under *Zinjanthropus* floor. Sample is rather weathered and soft with numerous Zinj-time root holes.

KA 664 Sample collected near top of Bed I, some distance from the hominid sites. At time
664*R* of collection, it was thought that this horizon was older than the hominid sites and it is so described in a paper submitted for publication by the INQUA Congress, Warsaw, 1961. However, more careful field work has established unequivocally that this horizon is definitely younger than any of the hominid sites.

KA 861 19-in. soft lapilli tuff, near top of Bed I and definitely younger than any of the hominid sites.

In the area of the three hominid sites, between 25 and 30 individual tuffs and tuffaceous beds can be distinguished in Bed I. At these sites these aggregate 39 ft.–43 ft. in thickness. Most tuffs are approximately a foot in thickness, but at the *MK* site the tuff immediately overlying the hominid floor is 129 in. In order of abundance, the tuffs fall into the following categories: anorthoclase-bearing vitric-crystal tuffs; anorthoclase-bearing crystal vitric tuffs; oligoclase bearing lapilli tuffs; and tuffaceous sandstones.

Within individual beds faint lines of stratification and evidence of size sorting may sometimes be seen. Some beds, however, show little or no sorting, vary tremendously in grain and lapilli size and exhibit characteristics of nuée ardente deposits. This is true of the tuff overlying the hominid remains at *MK* site. Some of the crystal vitric tuffs show marked crossbedding and have probably been re-worked. Most beds, however, appear to be primary ash falls.

Vitric shards in most of the tuffs have been more or less altered to clay, but the crystals appear fresh. Dark and strongly altered tops of some tuffs probably marked old soil zones. Numerous root casts below such zones strengthen this interpretation. Angular quartz and quartzite fragments are obvious in only a few of the tuffs. The nearest source of basement complex contaminants of this type is an inselberg of quartzite and gneiss approximately 1½ miles north of the hominid sites. More common visible contaminants are bone fragments. These are usually confined to thin strongly weathered layers; but in some cases, as in the tuff below *Zinjanthropus*, occur throughout the tuff.

Reck believed that the source of these tuffs was to the east, and the rapid thickening and coarsening of the strata in that direction is convincing proof that this is so.

At the *FLK* and *FLKNN* sites the hominid remains occur approximately 15 ft.–16 ft. above the basalt flow at the base of Bed I, while at the *MK* site, the hominid remains occur 9 ft. above the basalt. The samples dated, with the exception of *KA* 851 below the *Zinjanthropus boisei* floor which contained numerous bone fragments, showed no visible contaminants. In four of the six samples, the glass shards had been almost completely altered to clay. In one of these, *KA* 849, about 20 per cent of the glass shards were fresh, while in *KA* 847, from just above the hominid remains at *MK* site, most of the glass shards were fresh. Whether the more intense alteration of the four samples is the cause of their somewhat younger ages is not known, but it appears reasonable.

The run data and derived ages are given in Table 1.

The decay constants used in the computation of ages are $\lambda_{\beta} = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$, $\lambda_{k} = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$. The standard deviation of all of these runs is 0·1 × 10 yr. or less. This figure is, of course, simply a measure of the reproducibility of our results and does not attempt to deal with geological factors which might cause some scattering of the derived ages.

These samples were critically selected and examined and there seems to be no chance of their being contaminated by older eroded debris. It is to be noted that the hominid-site dates fail to fall in the proper relative order but all are between 1·6 and 1·9 million years. At the present time, the best estimate that we can make of the age of these sites is the average of the several ages, that is, 1·75 million years. This figure is in excellent agreement with *KA* 412, which is at the same stratigraphic level, but which was sampled a few miles from the hominid sites. The two samples from the top of Bed I give an average age of 1·23 million years, that is, 0·5 million years younger than the hominid sites. It may be of interest to note that we have obtained[5] an age of 360,000 years on a post-Chellean II tuff in Bed II, Olduvai. The conclusion is inescapable that Olduwan culture and Villafranchian fauna are synchronous in time and that both are approximately 1·75 million years old.

---

[1] Leakey, L. S. B., *Olduvai Gorge* (Camb. Univ. Press, 1951).

[2] Leakey, L. S. B., *Stone Age Races of Kenya* (Oxf. Univ. Press, 1935).

[3] Leakey, L. S. B., *Nature*, **184**, 491 (1959).

[4] Leakey, L. S. B., *Nature*, **189**, 649 (1961).

[5] Evernden, J. F., and Curtis, G. H., *Proc. INQUA Cong.*, *Warsaw* (1961).

LETTERS TO THE EDITORS

SPACE SCIENCE

Interpretation of Cosmic Noise Measurements at 3·8 Mc./s. from Satellite 1960 η1

THE results of measurements of cosmic noise at 3·8 Mc./s. from the satellite 1960 η1 have been reported by Molozzi, Franklin and Tyas[1]. A puzzling feature was the variation in field-strength at different locations. These results have now been analysed, on the assumptions that variations in signal strength are due to three causes: (*a*) the variation of radiation resistance of the antenna due to the ionosphere surrounding of the satellite; (*b*) the restriction in angular aperture of the satellite antennæ due to the ionosphere; and (*c*) the variation of cosmic noise power over the sky. The results indicate that these are the most important factors affecting the cosmic radio signals.

The variation of cosmic noise across the sky can be removed from the field-strength data by plotting noise-field contours along the orbit, using one of the sky maps such as that computed by Steiger and Warwick at 18 Mc./s. (ref. 2). The field-strengths can then be normalized to a standard value of the cosmic-noise field. In this communication the field-strength is normalized with respect to a region of the sky remote from the centre of the Galaxy, where the variation was small and the sky temperature low. Variation in antenna gain over the region is neglected.

The remaining variation in cosmic-noise levels is predominantly due to the effect of the ionosphere, neglecting for the moment the effect of the restriction in angular aperture. It can be shown that the radiation resistance of loop antennæ, such as those used in the experiment, varies as $\varepsilon^{3/2}$, ε being the dielectric constant of the medium. Therefore, as the antenna is immersed in ionosphere of increasing electron density, the resistance due to radiation will decrease, and hence the cosmic-noise power output from the receiver will also decrease.

The largest values of the electric field-strength are assumed to be representative of the noise field outside of the ionosphere, where the dielectric constant is unity. It follows that the ratio of the noise field-strength measured in the ionosphere to that outside the ionosphere, is proportional to $\varepsilon^{3/4}$, when absorption in the upper F region of the ionosphere is assumed to be negligible.

Since several modes, particularly the ordinary and extraordinary modes, can be propagated independently in the ionosphere, each one can contribute to the total noise-power at the satellite. The assumption was made that the energy was evenly divided between the two components above the ionosphere, that their signals added incoherently at the satellite and that the gyro-frequency was of the order of 1·4 Mc./s. at an altitude of 800 km.

Based on the hypotheses in the preceding two paragraphs, first estimates of the values of the dielectric constant and the electron density at the satellite were computed for each of the 22 observations reported by Molozzi *et al.* Since the largest values of the field-strength were observed over Woomera, these values were taken to represent the field-strength outside the ionosphere.

In order to arrive at more precise values of ε, it is necessary to allow for the angular aperture θ of the antenna, by a method of successive approximations. In the limiting case, $\sin\theta = \sqrt{\varepsilon}$.

Using the first value of ε calculated above, the angular aperture can be determined, and a more precise value computed for the noise-field received at the satellite, averaged over the region of the sky being viewed. Following the same procedure as before, a second approximation of the dielectric constant can be arrived at. The procedure can then be repeated and mean values of the electron density found by this means for the North American telemetry stations, Ottawa, Blossom Point and Fort Myers.

Date	Local time (approx.)	Mean height of satellite	Electron density
June 24–26, 1960	2200 hr.	870 km.	$9{\cdot}2 \times 10^4$ per c.c.
June 27, 1960	2200 hr.	855 km.	$10{\cdot}4 \times 10^4$ per c.c.
June 24–26, 1960	1400 hr.	1,050 km.	$7{\cdot}5 \times 10^4$ per c.c.

The difference in electron density between the measurements on June 24–26 and June 27, at the same local time, is approximately 10 per cent. Since a magnetic storm began with a sudden commencement at 0146 G.M.T. on June 27, it is surmised that the increase is due to the effects of the magnetic storm. The observations on June 27 were all made within 6 hr. of the beginning of the storm, during the initial positive phase of the variation of magnetic field.

The sky brightness temperature T computed from the results obtained over Woomera was found to be $8{\cdot}1 \times 10^6$ °K. in the vicinity of R.A. 5·5 hr., Dec. $-30°$, the region used for normalization of the values of field-strength.

A more detailed report will be given elsewhere.

J. H. CHAPMAN
A. R. MOLOZZI

Defence Research Telecommunications
Establishment,
Ottawa.

---

[1] Molozzi, A. R., Franklin, C. A., and Tyas, J. P. I., *Nature*, **190**, 616 (1961).

[2] Steiger, W. R., and Warwick, J. W., *J. Geophys. Res.*, **66**, 57 (1961).

ASTROPHYSICS

Apparent Recession of Nebulæ and the Nature of Radio Stars

IN a recent communication[1m], a brief summary was given of the quantitative evidence in favour of the whirl theory of light and matter, according to which the whole physical universe is the manifestation of Maxwell's electromagnetic waves, wherein the fundamental particles of matter are represented as integral solutions of Maxwell's equations *in vacuo*, in cylindrical co-ordinates, extended from a low limit of the variable parameter of the integrant solution to infinity (the 'compound whirl'), while the photons are represented by the same kind of solution but with a finite upper limit (the 'fragment'). The system of electromagnetic waves represented by the integrant solution was called the 'simple whirl'[1b,1j]. The energy of a fully realized compound whirl is finite, notwithstanding its infinite extension in space, and therefore the fundamental particles of matter are stable[1b,1j]. On the other hand, the total energy of a fully realized fragment would be infinite and therefore it can only be partially realized at the emission and then spread transversally asymptotically tending to its full realization and, at the same time, it must move in the axial direction asymptotically (though with great rapidity) tending to the theoretical 'velocity of light'[1j]. Thus the photon can be regarded as a fragment of a fundamental particle of matter. Its emission, however, does not affect the stability of the emitting fundamental particle, because the upper limit of the integral solution that represents it remains infinite after the emission and only the lower limit becomes slightly higher; its total energy remains finite. The quantum relationship at the emission is determined by Maxwell's electrodynamics[1j,f-l,a].

The spreading of the photon also implies a continuous increase of the wave-length λ, which very rapidly, though asymptotically, tends to proportionality to the distance from the source of emission according to the equation:

$$\frac{\Delta\lambda}{ct} = \frac{\sqrt{2\pi^3\chi_1}}{(q_0\ r_0)^4} \tag{1}$$

(derived by simple transformation from formula (7·9), ref. 1*j*), where t is the time elapsed since the emission of the photon, c is the velocity of light, $\chi_1 = 0{\cdot}0575$ (*ibid.*, formula 8·5) is the average value of the above-mentioned dimensionless lower limit of integration of the integral solution representing the electron or the proton; $q_0 = mc/\hbar$ (where m is the mass of the emitting particle, $\hbar \equiv h/2\pi$ is the Planck constant); r_0 is the radius of the photon at the moment of emission.

This formula permits the determination of r_0 from the generally accepted results of observation. According to the data given by Sir Arthur Eddington[2]:

$$\frac{\Delta\lambda}{{}^0\lambda} = 5{\cdot}75 \times 10^{-24}ct \tag{2}$$

where ${}^0\lambda$ is the wave-length of the photon at the emission.

It can be found from (1) and (2), on substituting the constants, that, for example, for ${}^0\lambda = 4 \times 10^{-5}$ cm. (violet light) $r_0 = 7{\cdot}6 \times 10^{-5}$ cm., whence $2\pi r_0/{}^0\lambda = 3{\cdot}8\pi$. From this it can be found (formula 9·8, ref. 1*b*) that the prevailing part (more than 93 per cent) of the energy of the emitting electron is contained within this radius and hence that the pattern of the emitting particle and of the emitted photon should not be substantially distorted at the emission.

Thus the shifting toward the red of the spectral lines of nebulæ, proportional to their distance from the point of observation, does not require for its explanation the hypothesis of the 'expanding universe' and hence it can scarcely justify the cosmic speculations generated from this hypothesis.

This is equally true in relation to similar phenomena in the radio stars. From the point of view of the whirl theory the radio stars should be regarded, at least for the time being, in the absence of more direct information about their nature, as consisting of atom and molecule-like formations of particle-like compound whirls of comparatively large dimensions ('macroparticles'), which should emit photons of correspondingly large dimensions ('macrophotons'). To evaluate the order of magnitude of macroparticles, we may assume that the scale relationship between the macrophotons and the emitting macroparticles is of the same order as between the photons and the emitting electrons. Taking again the violet light as an example, this ratio $h/(mc\,{}^0\lambda)$ is about 6×10^{-6}. The average order of wave-length of radio-astronomical signals, which we can regard as macrophotons, is about 10 cm. and hence the wave-length h/mc of macroparticles should be of the order of 5×10^{-4} cm. Thus, assuming that the Planck constant of the macroparticles is the same as of the fundamental particles of matter and of the photons, the mass of the macro-electron must be of the order of $3{\cdot}6 \times 10^{-33}$ gm. and that of the macroproton of the order of $6{\cdot}65 \times 10^{-30}$ gm.

It seems likely that macroparticles can, at least occasionally, be formed from the vortices (that is, revolving clouds) of particles of matter. As was pointed out on a number of occasions[1b,j,m], de Broglie undulations are implied in the solutions of Maxwell's equations representing the whirls. In an assembly of whirls (and hence also in an assembly of particles) they tend to become distributed in such a way that the pattern of de Broglie undulations by themselves should satisfy, or at least be near to, a solution of Maxwell's equations, superimposed upon the set of solutions representing the constituent whirls, so that the latter should form a fine structure of the former. No rigorous proof of this proposition was attempted, but it was applied tentatively to gravitation[1j,k,m], and it led to the correct determination of the period of rotation, and of the sidereal motion, and of the distance from the Sun, of Mercury, and of the period of rotation of Venus[1j,k,m].

It seems, therefore, likely that a system of de Broglie waves of protons and electrons revolving together in a vortex can occasionally emit a macroparticle the mass of which is very small in comparison with the mass of the vortex itself. The following consideration should increase the plausibility of this approach. The revolving particles in a vortex generally tend to acquire equal moments of momentum in relation to its axis. Hence their linear velocities tend to become inversely proportional, and the de Broglie wave-lengths tend to become proportional, to the distance from the axis, or, in other words, the angular wave-length of de Broglie waves tends to become constant. This answers the general description of electromagnetic whirl.

The macroparticles can form the atoms and molecules in the same way as the electrons and protons, and where their mass ratio equals the mass ratio between the proton and electron, they will form macroatoms and macromolecules geometrically similar to the atoms and molecules of matter.

N. S. JAPOLSKY

Department of Electrical
Engineering,
King's College,
London, W.C.2.

---

[1] Japolsky, N. S., *a*, *Phil. Mag.*, **19**, 934 (1931); *b*, **20**, 641 (1935); *c*, **30**, 641 (1935); *d*, **22**, 537 (1936); *e*, **22**, 1003 (1936); *f*, *Proc. Nat. Acad. Sci. India*, **9** (4), 213 (1939); **10** (1), 19 (1940); *g*, *Nature*, **137**, 582 (1936); *h*, **154**, 20 (1944); *i*, **159**, 580 (1947); *j*, *Pakistan J. Sci. Res.*, 111 (Oct. 1949); *k*, 1 (Jan. 1950); *l*, *Bull. Soc. Belge de Phys.* (Jan. 1958); *m*, *Nature*, **189**, 651 (1961).

[2] Eddington, Sir Arthur, *The Expanding Universe*, 67 (1933).

GEOPHYSICS

The Frequency of Meteorite Falls throughout the Ages

IN a recent article[1] Prof. H. Pettersson describes his investigations of samples of deep-sea deposits in which were found metallic spherules, mostly less than 250μ in diameter, to which it is reasonable to ascribe a meteoric origin. These occurred in several geographical regions and in layers of various geological ages, and among the conclusions reached is the following: "In general, the number of spherules is greatest in the more recent sediments. Nevertheless, substantial numbers of spherules were found in layers of Tertiary age: this contradicts the assertion made by some authorities that no meteors fell on the Earth during that time". A reference identifies the "authorities" mentioned as the late Prof. F. A. Paneth, who, in a paper in *Vistas in Astronomy*[2], wrote: "The result of our discussion is that *glass meteorites fell on our Earth only during the late Tertiary and early Quaternary; that iron and stone meteorites did not fall before the late Quaternary; that centuries ago the fall of iron meteorites was a much more common phenomenon than to-day; and that even since 1800 meteorite falls seem to have become noticeably rarer*".

It is clear from Paneth's paper that he is discussing meteorites only, and the evidence which he gives for his statement can refer only to those bodies. He consistently emphasized the lack of evidence that meteors and meteorites have a common origin[3], and there is nothing in his conclusions that is inconsistent with Pettersson's findings. Pettersson uses the counts from his samples to generalize for the whole Earth, and concludes that the present rate of accretion is 1,000–5,000 tons per annum. Such a generalization from any sample of meteorite falls would clearly be quite invalid. It follows that Paneth's conclusions regarding the fall of meteorites in past times are unaffected by Pettersson's important observations.

In view of the recent revived interest in the analysis of meteorites[4], it might be timely to point out that Paneth's considerable collection of meteorites is now, by his wish, available for experimental study. The secretary of the "Paneth Meteorite Trust", who would welcome applications from qualified investigators, is Dr. H. Wänke, Max-Planck-Institut für Chemie, Mainz, Germany.

HERBERT DINGLE

104 Downs Court Road,
Purley,
Surrey.

---

[1] *Endeavour*, **19**, 142 (1960).

[2] *Vistas in Astronomy*, **2**, 1686 (1956).

[3] See, for example, *The Origin of Meteorites* (Halley Lecture, 1940, Clarendon Press, Oxford).

[4] See, for example, Bernal, J. D., *Nature*, **190**, 129 (1961).

Murray's and Renard's interpretation of the "cosmic spherules" found in deep-sea deposits[1] that they are of cosmic origin, and were probably derived from iron meteors heated to superficial melting during flight through the atmosphere, seems to be generally accepted at present. Having myself studied hundreds of such spherules, extracted from deep-sea cores taken by the Swedish *Albatross* Expedition (1947–48), I have found Murray's description of their external structure confirmed. In addition, analyses of the composition of individual spherules by the Castaign microanalyser[2] has confirmed that they contain, besides iron, also nickel and cobalt, which supports their extra-terrestrial origin as well as Murray's hypothesis that they are derived from meteors of the nickel-iron type.

The resemblance between their surfaces and that of the iron meteorites seems to support the view that they may be derived from meteorites as well as from meteors which have not reached the surface of the Earth. The presence of cosmic spherules in deposits of Tertiary age (recently confirmed by Germans investigating the "Buntsandstein" in north-west Germany) certainly supports the view that they indicate meteoric activity long before the Quaternary.

Whether the origin of meteorites and ordinary meteors is different seems to be an open question. Whipple assumes most meteors to be of cometary origin, whereas meteorites are usually assumed to be derived from planetary fragments like the asteroids. It seems to me that the origin of the meteorites and, still more, of the tektites is still under debate, whereas the origin of the cosmic spherules is that announced by Murray.

My calculation that the accretion of cosmic spherules to the Earth is 1,000–5,000 tons annually is, of course, due to extrapolation from the counts made by myself and my co-workers in a limited number of cores. I hope to obtain a more dependable figure from the much longer cores to be raised by the Moho-Project.

HANS PETTERSSON

Oceanografiska Institutet,
Göteborg.

---

[1] *Proc. Roy. Soc. Edin.*, **9**, 258 (1876–77). *Challenger Rep.*, **4**, *Deep Sea Deposits*, 327.

[2] Castaign, R., and Fredriksson, K., *Geochim. Cosmochim. Acta*, **4**, 114 (1958).

RADIOPHYSICS

Scintillations and the Latitude Distribution of Ionospheric Irregularities

KENT[1] has reported a series of observations on the 40 Mc./s. transmissions from *Sputnik* 1 made at Cambridge during October 1957. He found that the transmissions always underwent rapid fading (scintillation) when the satellite was north of Cambridge but only occasionally when south of Cambridge. He concluded that the scintillations were due to ionospheric irregularities which are north of the observing station but not south of it. Further, since the southward extent of the scintillation region was greater for satellite passes to the west of Cambridge than to the east, he postulated that the density of the irregularities is controlled by the magnetic rather than geographic latitude (Cambridge's declination is about −9°). Mawdsley[2] objected to Kent's seemingly arbitrary assumption that the irregularities do not occur south of the observer, on the grounds that radio star scintillations are observed at lower latitudes than that of Cambridge. He suggested an alternative interpretation of Kent's results in terms of preferential forward scattering by the irregularities, assumed to be field aligned, when the line of sight from the observer to the source is normal to the field lines. Afterwards, Bain[3] and Frihagen and Tröim[4], working at Slough and Kjeller respectively, observed scintillations when the satellite under observation was south of the observing station. However, they both found that the scintillation activity increased for geomagnetic latitudes greater than that of Cambridge, that is, 55°. This led Frihagen and Tröin to support Kent's interpretation. It is the purpose of this communication to point out the existence of further pieces of evidence which support Kent's point of view rather than Mawdsley's.

It has long been known that the scintillation of radio stars correlates strongly with the occurrence of spread-F (for example, Booker[5]), and recent work[6,7] has shown this correlation applies to scintillation of satellite signals also. The distribution of scintillation activity with latitude may therefore be expected to be similar to that of spread-F. Singleton[8] has shown that the distribution of spread-F depends on geomagnetic latitude and, further, that between 60° and 70° the percentage occurrence rises from about 10–80 per cent or more. Simple calculation shows that the north–south tangent to the Earth's surface at an observing point cuts the 300-km. level at points the latitudes of which differ from that of the observing point by ±17°. For Cambridge (geom. lat. 55°) the range of geomagnetic latitudes over which the 300-km. level is observable is thus 38° N.–72° N. For the 400-km. level this range is 35° N.–75° N. It is clear, therefore, that if the irregularities responsible for the spread-F and scintillations are in the 300–400 km. region, as is believed to be the case, the observed marked increase in scintillation activity when the satellite is north of Cambridge is to be expected. This explanation does not preclude the possibility of observing scintillations at geomagnetic latitudes south of 55° since the incidence of spread-F nowhere falls to zero, and indeed, since spread-F activity increases again at the geomagnetic equator, an increase in scintillation activity would be expected here. This is observed[9].

The departure of the zenith angle dependence of the annual mean radio star scintillation index from the simple secant law, as pointed out by Hewish[10] in Cambridge and Little and Maxwell[11] in Manchester, can possibly be explained in a similar way. This secant law, which takes into account the variation of layer thickness with changing zenith angle, tacitly assumes a constant distribution of irregularities over the region of observation. The Cambridge and Manchester data are obtained mainly from observation of Cygnus and Cassiopeia, the lower culmination of each of which is near the northern horizon of these stations. Thus the larger the zenith angle, the further will the point of intersection of the radiation with the F_2 layer penetrate into the region of incidence of high irregularity to the north, and the larger will be the departure from the secant law. It can be shown that the latitude of the point which is at a height of 300 km. on a ray making a zenith angle of 60° at the observer in the meridian plane differs from the latitude of the observer by 4°. Since Cambridge and Manchester have geomagnetic latitudes of about 55° and 56° respectively and the increase in irregularity activity commences at geomagnetic latitude 60°, the departure from the secant law would be expected to commence at zenith angles of about 60°, as is observed.

An increase in scintillation activity with zenith angle has also been observed at middle latitudes[12], where the latitude distribution of irregularities is flat. However, the crude method of analysis used in this case makes it impossible to draw any conclusion as to whether the secant law can explain the variation or not.

Mawdsley's contention that the most favourable condition for forward scattering by field-aligned irregularities is when the incident radiation is normal to the field lines is contrary to experiment. Singleton, Lynch and Thomas[13], working with a field of view (geom. lat. 15° S.–55° S.) over which the incidence of spread-F is substantially flat, have shown that an enhancement in scintillation activity is experienced for propagation along the field lines, there being no enhancement for propagation normal to the field lines.

It would appear, therefore, that Kent's original interpretation of his results is in accord with the spread-F findings and that the scattering mechanism envisaged in Mawdsley's alternative explanation is not borne out by experiment. As a result it is asserted that Kent's explanations must be accepted.

D. G. SINGLETON

Physics Department,
University of Queensland.

[1] Kent, G. S., *J. Atmos. Terr. Phys.*, **16**, 10 (1959).
[2] Mawdsley, J., *J. Atmos. Terr. Phys.*, **18**, 344 (1960).
[3] Bain, W. C., *Nature*, **189**, 129 (1961).
[4] Frihagen, J., and Tröin, J., *J. Atmos. Terr. Phys.*, **20**, 215 (1961).
[5] Booker, H. G., *Proc. Inst. Radio Eng.*, **46**, 6363 (1958).
[6] Yeh, K. C., and Swenson, G. W., *J. Geophys. Res.*, **64**, 2281 (1959).
[7] de Mendonca, F., Villard, O. G., and Garriot, O. K., *Proc. Inst. Radio Eng.*, **48**, 2028 (1960).
[8] Singleton, D. G., *J. Geophys. Res.*, **65**, 3615 (1960).
[9] Koster, J. R., *J. Atmos. Terr. Phys.*, **12**, 100 (1958).
[10] Hewish, A., *Proc. Roy. Soc.*, A, **214**, 494 (1952).
[11] Little, C. G., and Maxwell, A., *Phil. Mag.*, **42**, 267 (1951).
[12] Bolton, J. G., Slee, O. B., and Stanley, G. G., *Austral. J. Phys.*, **6**, 434 (1958).
[13] Singleton, D. G., Lynch, G. J. E., and Thomas, J. A., *Nature*, **189**, 30 (1961).

PHYSICS

Pulse-shortening in Electron Linear Accelerators

Kelliher and Beadle[1] have published some preliminary observations of the phenomenon of pulse shortening in electron linear accelerators.

Since the problem was first noted[2] investigations have also been in progress in our laboratories. It would now appear that a satisfactory theory has been evolved and experimentally verified as a result of which various methods of avoiding the problem can be devised. Kelliher and Beadle suggested that the effect could be due either to spurious radio-frequency modes or to a 'field effect' associated with power level. It has in the past been difficult to see how a spurious mode could attain sufficient power-level to produce the very large forces required to affect a high-energy beam.

This difficulty has now been resolved by the realization of a significant difference between the action of forward and backward waves. The expression for the tranverse forces associated with a forward travelling-wave acting on a beam contains a term of the form $(1/\beta - \dot{z}/c)$, where β is the ratio of the phase velocity to light velocity, and $\dot{z}$ and c represent beam and light velocity respectively. For a forward wave this term approaches zero as β and $\dot{z}/c$ approach unity, indicating a balance of transverse electric and magnetic forces.

Considering a backward wave, however, it can be seen that in order that energy be transmitted in a direction opposed to the phase velocity the sign of the Poynting vector must be reversed, that is, the H vector must show a phase reversal in its relationship to the E vector as compared with the situation in a forward wave. From this, it follows that the sign in the expression given above will be changed and the electric and magnetic terms will now add and thus any disruptive forces will, in a backward wave, be very much higher than in an otherwise similar forward mode. Consideration of the higher modes which exist in a corrugated wave-guide structure does reveal the existence of backward waves. It can thus easily be seen that there is, in the normal form of linear accelerator containing long uniform sections of corrugated wave-guide, the possibility of action as a backward wave oscillator and, even if only modest power be produced, transverse forces could be large.

An experimental investigation of possible modes in a corrugated wave-guide structure having $a/\lambda = 0 \cdot 11$ and for which the normal E_{01} mode has $\beta = 1$ at 2,998 Mc./s. has been carried out by I. White of this laboratory. The measurements show that there is (among others) an E_{11} mode which is a backward wave and that this wave has a phase velocity equal to light velocity at 4,436 Mc./s., under which conditions its group velocity is 0·038 c. Perturbation measurements of the series impedance (E^2/P) confirm that mode powers of the order of kW. would cause transverse forces sufficient to deflect a high-energy beam. One might therefore expect that a uniform accelerator section of 1½ m. length of this guide fed by a bunched beam travelling at near light velocity would oscillate at a frequency of 4,432–4,433 Mc./s., the slight shift in frequency being necessary to allow for the phase-change between beam and wave from end to end of the section. A bunched beam of energy 8 MeV. and indeed 16 MeV. has been injected into such a section of accelerator and pulse-shortening effects were exhibited both when energized at a power-level of 5 MW. from an S-band klystron and when completely unenergized. Measurements were made at the input end of this section and power was detected at a frequency of 4,433 Mc./s. Examination of the variation of this power with time indicated a rapid increase at the end of the shortened electron pulse. When beam current was reduced until pulse-shortening no longer took place, no power at 4,433 Mc./s. could be detected. There seems no doubt, therefore, that pulse-shortening in this case was associated with backward wave oscillation in the E_{11} mode. Means were taken as mentioned below to reduce the possibility of E_{11} wave build-up and no pulse-shortening could then be detected.

These findings confirm the suggestion that the E_{11} mode is responsible made in the paper by Nygard and Post[3], although they did refer to an E_{110} mode of oscillation. The significance of the 0 is a little obscure as this would with normal cavity nomenclature imply a phase velocity of infinity, that is, that the wave was operating at cut-off. With the type of oscillation we are considering it is, of course, not necessary that the guide should act as a resonant cavity. The E_{11} mode near $\beta = 1$ is such that to a first approximation the fields in the axial region may be regarded as those of a plane wave with, of course, the significant difference that the E and H vectors are phased so as to give the backward group velocity, and the oscillatory motion of the beam will, therefore, be purely transverse. For this reason the longitudinal bunching of the beam at the E_{01} frequency has little or no influence on the build-up of oscillation. The theory has been carried to the extent of fitting known facts qualitatively and rough quantitative agreement has also been obtained. It is hoped that a complete theory will be published in the near future.

Having determined the causes of trouble it is relatively simple to devise means for avoiding them. On the above theory it is quite clear that the constant field guide suggested by Kelliher and Beadle with its essential non-uniformity would avoid the difficulty of pulse shortening. Similarly, arrangements using combinations of shorter uniform sections of different a/λ as used in some of our own accelerators would again avoid the difficulty. More elegant methods of avoidance are possible; for example, new accelerating structures may be used which are incapable of propagating the mode at velocities near that of light, such as that mentioned by Nygard and Post (possibly having $2\pi/3$ phase change per corrugation). Techniques using short sections which cause phase shift between the E_{11} mode and the beam have been used in our own laboratories. It is perhaps worth noting, however, that there are other higher modes which may cause similar troubles although it seems probable that higher starting currents for the oscillation would be required. Although, at present, difficulties seem to have been removed, similar troubles may occur due to these higher modes if and when beam currents are pushed an order of magnitude higher. In this connexion, it is perhaps significant that in the experiment with the accelerator section, mentioned above, a small amount of power has been found to exist under pulse-shortening conditions, at other frequencies, particularly at approximately 7,970 Mc./s. This mode has not yet definitely been identified, but may possibly be the E_{31}. Although it has been reported that pulse-shortening has not been observed on L-band accelerators, it is quite clear that such high mode problems could exist and it is probable that they would be found if the beam current were increased sufficiently.

As regards performance of S-band accelerators, we have in our laboratories a single-section accelerator operating from a 5-MW. klystron and have achieved beam current in excess of 1 amp. at 2 μsec. pulse-length and at an energy of 4 MeV., that is, almost complete radio frequency power absorption without any signs of pulse-shortening.

M. C. Crowley-Milling
T. R. Jarvis
C. W. Miller
G. Saxon

Research Department,
Associated Electrical Industries
(Manchester), Ltd.,
Trafford Park,
Manchester, 17.

[1] Kelliher, M. G., and Beadle, R., *Nature*, **187**, 1099 (1960).

[2] Boag, J. W., and Miller, C. W., *Proc. Second Conf. Geneva*, Paper *P*/62 U.K. (1958).

[3] Nygard, J. C., and Post, R. F., *Nuclear Instrum. and Methods*, **2**, No. 1, 126 (1961).

Displacement Potentials and Bending of Rod-like Polyelectrolytes

In 1954, Jensen *et al.*[1,2] discovered an electromechanical property of solutions of potassium hyaluronate. They named the effect 'flow-potential', but agreed later on to change the name into 'displacement-potential'. The original measurements were performed with techniques including amplification. Experiments in 1959 by us[3] (C. E. Jensen died in November 1959) showed that the classical potentiometric technique without amplification was quite sufficient to measure the potentials. The new measurements corroborated the original findings. Beyond this Jensen and Vilstrup discovered that the potentials were independent of the distance between the electrodes[4].

It seems that the observed phenomena can be derived from the properties of a crude model of the hyaluronate molecule: the molecule may be described as a long cylinder near the core of which a great number, for example 1,000, of negative elementary charges are distributed evenly. The core is surrounded by a sheath of positive electricity evenly distributed on a cylindrical surface. This assumption is based on the fact that the potassium ions are prevented by elastic and electric forces from moving in directions perpendicular to the surface while they are (nearly) free to move in the other two directions. We now inquire what will happen if we bend the cylinder. From the geometry of the toroid, it is known that a cylinder can be bent without any change in surface. What happens is that part of the surface is moved from the inside to the outside. Since the radii of curvature in question are large compared with the diameter of the cylinder, we must assume that the bending cannot cause significant changes in the even distribution on the surface. Therefore, the electrical effect of the bending will be the following: (*a*) if a number of molecules are bent simultaneously the same way, a certain, not negligibly small, amount of electricity will be displaced from the inside to the outside of the molecules under bending; (*b*) when the bending has ceased and the bending stress has disappeared, the bent molecules will tend to unbend, that is, they will tend to chase electricity in the opposite direction, against their centres of curvature. In other words, the entire solution, after bending, is in a state of strain which manifests itself as an electromotive force, tending to chase electricity in the direction from the molecules to their centres of curvature.

A typical experiment is performed as follows: a glass capillary is partly filled with a dilute solution of potassium hyaluronate, for example, with a 1 per cent solution. Two electrodes enter the solution from the sides of the tube. The discoverers of the effect observed two phenomena: when the solution is displaced from left to right relatively to the capillary, the measuring instrument shows that the right electrode is negative during the displacement and when the displacement has ceased its sign is reversed. Thus, the right electrode becomes positive and remains so, as long as the solution remains in the displaced position, or rather, the potential decays slowly, by some 10 per cent in the course of a day.

It is evident that, on account of the shearing forces existing in the solution during its displacement in the capillary, the long molecules must be bent so that most of them will have their insides pointing in the direction of the movement of the liquid. It will, therefore, be seen that predictions based on the model described here are in complete qualitative agreement with the observations. This agreement is corroborated by a third observation: when the capillary is emptied after an experiment and refilled with water without rinsing it, a displacement potential about ten times as large as before is observed[3]. In these circumstances, most of the molecules adhere to the glass and their bendings will be more acute, which must result in an increase of potential. Filling the capillary (internal diameter about 0·7 mm.) with thin glass rods has a similar effect[5].

The order of magnitude of the potentials is 5–10 mV. for experiments of the ordinary type, and for those of the kind described about 100 mV. Preliminary estimates based on the model in question yield results agreeing with these values. It follows from the estimate that the potential must be inversely proportional to the length of radius of curvature.

More detailed papers on the theory of the effect and its applications to the mechanism of hearing will be published elsewhere.

J. A. CHRISTIANSEN
C. E. JENSEN
TH. VILSTRUP

Sundvænget 9,
Hellerup, Denmark.

[1] Jensen, C. E., Koefoed, J., and Vilstrup, Th., *Nature*, **174**, 1101 (1954).

[2] Vilstrup, Th., Jensen, C. E., and Koefoed, J., *Ann. Otol., Rhinol. Laryngol.*, **64**, 406 (1955).

[3] Jensen, C. E., Christiansen, J. A., and Vilstrup, Th., *Symposium über Makromoleküle in Wiesbaden, Kurzmitteilungen*, **5**, A, 11 (1959).

[4] Jensen, C. E., Christiansen, J. A., and Vilstrup, Th. (Lecture delivered by Th. Vilstrup at Colloquium Oto-Laryngologicum in Padua, 1960).

[5] Carlsen, F., Dansgaard, W., and Jensen, C. E., *Acta Chem. Scand.*, **13**, 1851 (1959).

Electrical Effects observed in Polycrystalline Boron

THE conductivity of vacuum-fused polycrystalline specimens of boron is observed to increase in a magnetic field instead of decreasing as expected theoretically. Different samples exhibit the effect to different extents, but all show saturation at a field-strength of about 3,000 gauss. Increases of up to 1 part in 10^3 at saturation have been observed.

Preliminary measurements indicate that the effect persists at low temperatures but decreases above room temperature, falling below the noise-level at about 100° C. in the most sensitive specimen.

The effect shows no temperature hysteresis and is unaffected by changing the ambient atmosphere from air to argon. It is independent of the orientation of the magnetic field relative to the current vector.

All specimens examined were *p*-type by thermoelectric measurement, and contained a variety of traps as evidenced by the long time-constants observed in other measurements. The 'magnetoconductance' effect itself, however, could be measured at frequencies up to 10 kc./s., the limit set by the measuring system used. No reliable zero-frequency measurements have been made owing to the difficulty of maintaining the boron temperature constant to 0·01° C., rendered necessary by the strong dependence of conductivity on temperature.

Samples of polycrystalline boron also exhibit a 'charge storage' effect. To show this, current is passed through a piece of boron for a few seconds and then the source of current is replaced with a current meter. A charge passes through the meter in the same direction as would result from charging and discharging a capacitor in the same way.

Typically, a 100-V. battery is used to charge a 2-mm. thick slice cut from a 6-mm. diameter rod of boron and 10^{-8} coulomb flows through the meter. The discharge curve can be resolved approximately into two decreasing exponentials, with time-constants of 6 sec. and 180 sec.

In a further experiment, the boron is lowered into liquid nitrogen with the battery still connected, thereby 'freezing in' the charge. On substituting the meter for the battery and allowing the boron to warm up, charge is released at certain temperatures which are presumably related to the depths of specific kinds of traps in the boron. The experiment is thus analogous to the production of a 'glow curve' in the examination of a luminescent material.

Some of the boron used in these investigations was prepared in this laboratory by an electron beam zone melting process and the remainder purchased as zone-refined material prepared by the Eagle-Picher Research Laboratories, Miami, Oklahoma.

Electrical connexion to the boron was through baked-on silver paste contacts which were substantially ohmic.

The observations described differ from, but may be related to, the 'magnetic pulse' and 'colour memory' effects studied at the U.S. Army Signal Research and Development Laboratory, Fort Monmouth, New Jersey[1].

C. H. Carmichael
M. Dore

Signals Research and Development Establishment,
Ministry of Aviation,
Somerford,
Christchurch, Hants.

---

[1] Gaulé, G. K., Breslin, J. T., Pastore, J. R., and Shuttleworth, R. A., *Boron*, edit. by Kohn, J. A., Nye, W. F., and Gaulé, G. K., 159 (Plenum Press, Inc., New York, 1960); and private communication to P. H. R. Scholefield of this laboratory.

GEOLOGY

Age of the Orogeny and Granites in South-West England

Mr. Butcher's valuable contribution[1] to this puzzling problem raises a number of points which I would like to answer.

First, there is no evidence that the deformation of the pre-Permian rocks of south-west England was delayed until Stephanian or post-Stephanian times. On the other hand, there is some evidence[2] that metamorphosed, schalstein and spilite areas (and perhaps even the Delabole Slates) were being eroded somewhere south of Wales in Pennant Series (that is, Morganian) times. Hints of early Morganian instability occur in the Bristol Coalfield and in the south-eastern portion of the South Wales Coalfield. Dr. Kelling has recently been studying Pennant rocks in South Wales and is finding that over considerable southern and central areas of that coalfield a mainly southerly source (not too far away either) and a parent mass of rocks like those of Devon and Cornwall are indicated.

Secondly, similarity of fold system[3] does not necessarily prove contemporaneity of deformation. The folded and thrust south-west England sediments (the age of which has not been proved beyond about the Modiolaris Zone) are separated from the Somerset area by the powerful Cannington Park thrust-belt (which could have brought into juxtaposition rocks deformed at different times of the Carboniferous).

Thirdly, no one has ever suggested that the granites were being eroded during Pennant Sandstone (or even later Carboniferous) times. I agree with Mr. Butcher that these granites, when intruded, would be deeply buried. I also agree that "at the present level of erosion, the youngest sediments so far recognized by fossils are referable to the Upper Gastrioceras Zone[1]". There could still, however, have been an appreciable thickness of later Ammanian (for example, Similis-Pulchra Zone) deposits above this "present level of erosion" when the granites were intruded. In other coalfields of England and Wales, the thickness of the Ammanian succession above the Amman Marine Band is at least equal to (and in some cases almost double) that of the underlying Coal Measures. This thickness in the Somerset Coalfield (the one nearest Devon) is about 2,000 ft. If the south-west England area was experiencing a renewal of thick greywacke-type deposition in later Ammanian times, then that higher succession could have attained a thickness of many thousands of feet. Prentice (ref. 4, p. 408) has suggested that quite thick greywackes could have been deposited in a relatively short space of time. The granites could then have been intruded during early Morganian times and yet be buried under a considerable thickness of earlier Coal Measure rocks.

I would (with caution) suggest another possibility to account for the depth of burial in Morganian times of the granites. Great thrusts occur in the Armorican fold belt of Cornwall and south Devon. Such thrusts may have carried masses of Devonian, or older, rocks (removed by later erosion) well north over areas of deformed Culm. There could then have been thrust-masses of pre-Carboniferous rocks on top of the Culm, (*a*) when the granites were being intruded and (*b*) when the Pennant Series was being deposited farther north. Such an interpretation might even explain the presence of Devonian limestone pebbles (of southern aspect) in the New Red Sandstone near North Tawton, Devonshire[5]. The high percentage of Devonian fragments in the Permo-Triassic breccias of south-west England[6] might also be explained in this way.

Lastly, while derived Dartmoor granite is unknown in these breccias, a considerable percentage includes porphyries which could have been connected with the granite intrusions[6].

I therefore maintain that the case for a very late Carboniferous orogeny in south-west England, and also for a Permian granitic injection, has not been proved. On the contrary, available evidence supports an earlier (probably early Morganian) age for the main deformation of the Devonian and Culm rocks of Devon and Cornwall. The granite intrusions probably accompanied this deformation.

T. R. Owen

Geology Department,
University College of Swansea.

---

[1] Butcher, N. E., *Nature*, **190**, 253 (1961).

[2] Heard, A., *Geol. Mag.*, **59**, 83 (1922).

[3] Kulp, J. L., Long, L. E., Giffin, C. E., Mills, A. A., Lambert, R. St. J., Giletti, B. J., and Webster, R. K., *Nature*, **185**, 495 (1960).

[4] Prentice, J. E., *Quart. J. Geol. Soc. Lond.*, **116**, 397 (1960).

[5] Hutchins, P. F., *Geol. Mag.*, **95**, 119 (1958).

[6] Wells, A. K., and Kirkaldy, J. F., *Outline of Historical Geology* (London, 1958).

I feel that the evidence cited by Mr. T. R. Owen does not necessarily support an Upper Westphalian (Morganian) age for the orogeny and granites. An intra-Westphalian orogeny could only be satisfactorily demonstrated by the discovery of younger unconformable Carboniferous sediments, as, for example, in northern Spain[1]. Such post-orogenic sediments are not known in Devon and Cornwall. However, our knowledge of this region is still very meagre, and it may be that they will yet be found. Meanwhile, it seems to me preferable on the present evidence to correlate the main orogeny in Devon and Cornwall with that in adjacent regions to the north. In this, I agree with Kulp *et al.*[2] and Lambert and Mills[3]; but also with Mr. Owen that the case has not been proved.

I imagine that a major orogeny ending with a granite batholith and associated mineralization, such as we now see exposed for more than 100 miles in south-west England, would require a considerable time (perhaps 20 million years) for its development. The whole of the Carboniferous period is thought to be 65 (ref. 4) or 80 (ref. 5) million years. Therefore, with pre-orogenic sedimentation known to be still going on in Lower Westphalian times, I suspect that the granites (and there is more than one time of granite intrusion) could not have been emplaced in the Upper Westphalian. It is of interest that the most recent age determinations of the Dartmoor granites[6] give a slightly *younger* age compared with previous values. The new date of 265 ± 5 million years falls nicely within the Permian period on Kulp's time-scale[4].

N. E. Butcher

Geology Department,
University of Reading.

[1] Wagner, R. H., *Estudios Geologicos*, **16**, 55 (1960).

[2] Kulp, J. L., Long, L. E., Giffin, C. E., Mills, A. A., Lambert, R. St. J., Giletti, B. J., and Webster, R. K., *Nature*, **185**, 495 (1960).

[3] Lambert, R. St. J., and Mills, A. A., *Ann. N.Y. Acad. Sci.*, **91**, 378 (1961).

[4] Kulp, J. L., *Science*, **133**, 1105 (1961).

[5] Holmes, A., *Trans. Edin. Geol. Soc.*, **17**, 183 (1959).

[6] Dodson, M. H., Miller, J. A., and York, D., *Nature*, **190**, 800 (1961).

CHEMISTRY

Evaporated Carbon Films of Large Area for Adsorption Studies by Electron Bombardment

The use of evaporated carbon films for adsorption studies is desirable in view of the difficulty of outgassing carbon powder. By analogy with the comparative properties of metal surfaces produced by evaporation and by reduction of powders, it seems likely that evaporated carbon should be significantly cleaner and more reproducible.

Following common practice with metal films, Sykes and Thomas[1] used a carbon filament mounted on tungsten leads with 'Aquadag'. The extent of evaporation was limited to a few per cent of the total carbon by electrical failure due to 'hot spots'. As a result the film thicknesses were generally of the order of 500 Å., corresponding to weights of the order of 2 mgm. Krypton adsorption isotherms showed that the residual filament had an area several times greater than that of the film, hence it was necessary to remove the remaining filament through a constriction and to seal off. This is inconvenient, and an undesirable feature if cleanliness is to be maintained.

The new technique avoids this difficulty by allowing almost 100 per cent evaporation of the carbon used. A second improvement is that only a light electrical contact to the carbon is required so that difficulties of mounting the carbon are reduced, 'Aquadag' can be dispensed with, and the outgassing of the supports can be made negligible. The film vessel is shown in Fig. 1. A small cylindrical piece of pure carbon (*A*) is supported on a tungsten rod (*B*) so that its tip is within a one-turn tungsten coil cathode (*C*). The cathode is heated to a controlled temperature by means of a 'Variac' and step-down transformer, and a positive potential of 500–2,000 V. is used to accelerate the emitted electrons to the carbon anode. The tungsten support is shielded from bombardment at high anode potentials by a repelling guard electrode (*D*). Depending on the size of the carbon anode a power of about 40 W. is sufficient for evaporation, after prolonged outgassing with a slightly reduced power. As a consequence of the small size of the anode and its almost complete evaporation the power dissipated within the silica film vessel during evaporation is much less than before while film thicknesses are considerably greater. Films as heavy as 50 mgm. have been obtained, but there is a tendency for such thick films to peel from the walls of the vessel.

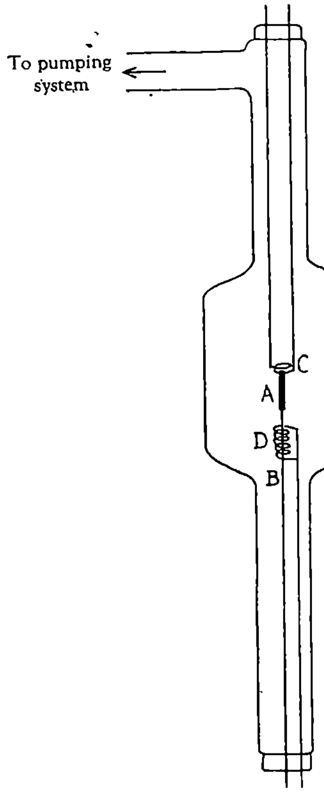


Fig. 1. Diagram of vessel used for carbon evaporation

A krypton isotherm, obtained at −196° C., on a film weighing 11·8 mgm. deposited without cooling the silica bulb, is shown in Fig. 2 with the corresponding B.E.T. plot. An almost equally straight line is

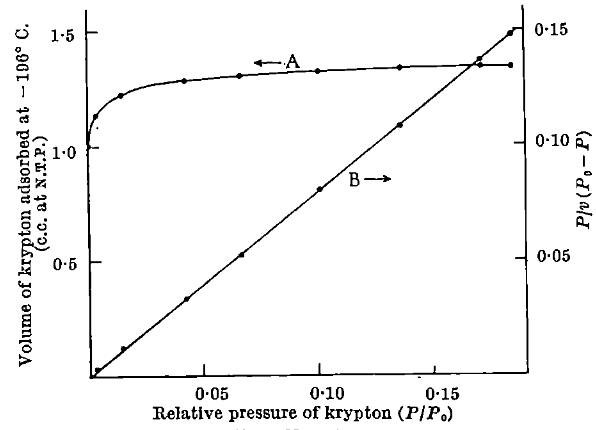


Fig. 2. Absorption of krypton on a carbon film

obtained from a Langmuir plot, as reported for graphite[2]. The B.E.T. monolayer volume is 1·24 c.c. at N.T.P., Taking 20 Å.[2] for the effective cross-sectional area of a krypton atom, the film area is 6·67 m.^{2} or 565 m.^{2}gm.$^{-1}$. Films of this area, which is such that contamination hazards are much less severe than before, are readily prepared by the new method. The specific surface area is some ten times greater than that of the thinner films prepared by Sykes and Thomas, and is only slightly affected by baking at 550° C. A study of the relation of film area to film thickness is under way.

A. E. Bell
J. Pritchard
K. W. Sykes

Chemistry Department,
Queen Mary College
(University of London),
Mile End Road,
London, E.1.

---

[1] Sykes, K. W., and Thomas, J. M., *Proc. Fourth Carbon Conf.*, 29 (Pergamon Press, 1960).

[2] Ross, S., and Winkler, W., *J. Coll. Sci.*, **10**, 330 (1955).

The Marangoni Effect and Liquid/Liquid Coalescence

The rate of coalescence of liquid drops (phase 1) suspended in an immiscible liquid (phase 2) has been shown to increase when a third component, soluble in both phases, diffuses out of the drop[1–5], and vice versa. This behaviour has been attributed to the Marangoni effect[6], whereby interfacial flow is established by gradients in interfacial tension causing an increase in one case, and a decrease in the other, of the rate of thinning of the phase-2 film separating the coalescing interfaces.

We have studied the buoyant rise of a drop of water (phase 1) through cinnamaldehyde (phase 2) to a flat phase 1/phase 2 interface, using an interferometric method[8] to measure the rate of thinning $-(\mathrm{d}h/\mathrm{d}t)$ of the intervening phase-2 film. For drops 650μ diam., the thinning followed the relation[3]:

$$-\frac{\mathrm{d}h}{\mathrm{d}t} = kh^3$$

Table 1 lists experimental values of k and the corresponding values of the mean rest time $\bar{\tau}$ (ref. 3), which offer strong support of the foregoing hypothesis. Similar, but less complete, results were obtained from the system water/diphenyl sulphide.

Table 1

	$\bar{\tau}$ sec.	$10^8 k$ cm.$^{-2}$ sec.$^{-1}$
1 per cent acetone in phase 1	5·3	$\geqslant 3$
Pure system	8·1	3·0
1 per cent acetone in phase 2	12·0	2·0

The film drainage was uniform and symmetrical in each case, and the minimum thickness of the film at rupture was < 500 Å. Lateral oscillations, or 'kicks'[9–11], were observed during the rise of a drop of water through cinnamaldehyde containing 1 per cent acetone. The oscillation became more marked as the drop approached the interface and continued during the early period of film thinning. Despite the fact that kicks were observed to propel the drop distances of several diameters on the interface, the circular patterns of interference colours and rings were undisturbed, indicating that film thinning continued at a uniform rate.

This work was conducted with financial assistance from the Defence Research Board of Canada (*DRB* grant 9510–05).

G. D. M. MacKay
S. G. Mason

Pulp and Paper Research Institute of Canada,
and
Department of Chemistry,
McGill University,
Montreal.

---

[1] Johnson, H. F., and Bliss, H., *Amer. Inst. Chem. Eng. Trans.*, **42**, 331 (1946).

[2] Allan, R. S., and Mason, S. G., *J. Colloid Sci.* (in the press).

[3] Charles, G. E., and Mason, S. G., *J. Colloid Sci.*, **15**, 236 (1960).

[4] Groothius, H., and Zuiderweg, F. S., *Chem. Eng. Sci.*, **12**, 288 (1960).

[5] Sawistowski, H. (private communication).

[6] Scriven, L. E., and Sternling, C. V., *Nature*, **187**, 186 (1960).

[7] Zuiderweg, F. S., and Harmes, A., *Chem. Eng. Sci.*, **9**, 89 (1958).

[8] Allan, R. S., Charles, G. E., and Mason, S. G., *J. Colloid Sci.*, **16**, 150 (1961).

[9] Lewis, J. B., and Pratt, H. R. C., *Nature*, **171**, 1155 (1953).

[10] Haydon, D. A., *Proc. Roy. Soc.*, **243**, 483 (1958).

[11] Davies, T. V., and Haydon, D. A., *Proc. Roy. Soc.*, A, **243**, 492 (1958).

Formation of Calcium Chloraluminate by Interaction of Portland Cement with Sea-water and Chloride Solutions

The effect of chloride-containing solutions on Portland cement has received relatively little attention. Batta[1] and Thorvaldson[2] noted that addition of chloride ions to solutions containing sulphate reduced or inhibited the expansion of concrete and mortar bars. Batta attributed this effect to the increased solubility of gypsum and calcium sulphoaluminate in chloride solutions.

In the present investigation prisms of dilute pastes of Portland cement I and V and of mortar were exposed to various attacking solutions, including Mediterranean sea-water, solutions containing 3 per cent sodium chloride + 0·4 per cent magnesium sulphate (artificial sea-water), 3 per cent sodium chloride + 0·4 per cent sodium sulphate, 0·4 per cent magnesium sulphate, 0·4 per cent sodium sulphate, 3 per cent sodium chloride and 3 per cent magnesium chloride respectively and water from the Dead Sea. The products after different reaction times were identified by X-ray powder methods and simultaneously the expansion of duplicate prisms was measured.

Contrary to observations of Batta and Thorvaldson, addition of chloride ions to solutions containing sulphate was found to increase expansion of cement and mortar bars in all the present experiments. Expansion in sodium chloride solution only slightly exceeded that in distilled water. A considerable increase of volume was observed in magnesium chloride solution but can probably be attributed to the accelerated hydration caused by the reaction of magnesium ions with calcium hydroxide and hydrated calcium silicates of the cement.

X-ray patterns of all samples exposed to chloride-containing solutions showed, besides many other lines, a strong one at 7·90–7·97 Å. It was invariably observed independent of any other components present and was attributed to $3CaO.Al_2O_3.CaCl_2.10H_2O$ (A.S.T.M. card No. 2–0081). This was confirmed by direct comparison of the X-ray patterns with that of synthetic material.

The amount of chloraluminate formed is appreciable, increasing steadily with time of exposure of the bars to chloride solutions. The variations observed in the longest spacing, though small, are genuine and probably correspond to minor changes in composition.

The effect of the formation of chloraluminate on the expansion of cement is uncertain. The specific gravity of $3CaO.Al_2O_3.CaCl_2.10H_2O$ (1·89) is lower than that of gypsum or hydrated calcium silicates, but higher than that of $3CaO.Al_2O_3.3CaSO_4.31H_2O$. Moderately increased expansion in chloride-containing solutions might thus be anticipated, in agreement with the majority of the present experiments. However, the possible effect of chloride ions on the solubility of calcium sulphoaluminate, which was detected on all the photographs, and on its further course of reaction (to be discussed elsewhere) cannot be neglected.

The discrepancy between our results and those of Batta and Thorvaldson may perhaps be interpreted in terms of two competing factors : (*a*) the crystallization of chloraluminate; (*b*) the effect of chloride ions on the stability of other components, particularly sulphoaluminate. Their relative importance depends on the experimental conditions and remains to be investigated.

The very small expansion of cement bars in sodium chloride solution indicates that the increase of volume accompanying chloraluminate crystallization alone was largely absorbed by the pores. Only in the presence of sulphate solutions was excessive expansion observed, due to the combined effect of the formation of calcium chloraluminate and calcium sulphoaluminate.

Failure of previous investigators to detect chloraluminate in the course of their many studies of attack of cement by sea water may perhaps be attributed to the lack of X-ray data in relevant cases.

We wish to express our thanks to the Ford Foundation for a grant which made this research possible.

L. Heller
M. Ben-Yair

Standards Institution of Israel,
Tel Aviv,
and Geological Survey of Israel,
Jerusalem.

---

[1] Batta, G., *Ann. Trav. Pub. Belg.* (August 1948).

[2] Thorvaldson, T., *Proc. Third Intern. Symp. Chem. Cement* (London, 1952).

Chromium, Uranium, Tungsten and Nickel Complexes of Thioglycolic Acid

The most commonly available of the thiol-acids is thioglycolic acid, also known as mercapto-acetic acid. This reagent is favoured by analytical chemists in research and industry[1–3] for the colorimetric estimation of iron in micro-quantities because of certain advantages it offers[4,5]. In this Laboratory this reagent had been used in the annulus method for separation and detection of chromium and titanium on a filter paper[6].

Chromium has been usually determined colorimetrically, for example, with diphenyl carbazide, after oxidizing it to the hexavalent state, but the use of thioglycolic acid has the distinct advantage that chromium can be present in the original solution in the hexa- or tri-valent state. The use of this complexing agent has been extended for the colorimetric determination of the other elements of Group VI *A*, namely, tungsten and uranium, and also to nickel which frequently occurs in admixture with these. This reagent has already been used for the estimation of molybdenum[7] alone or in the presence of

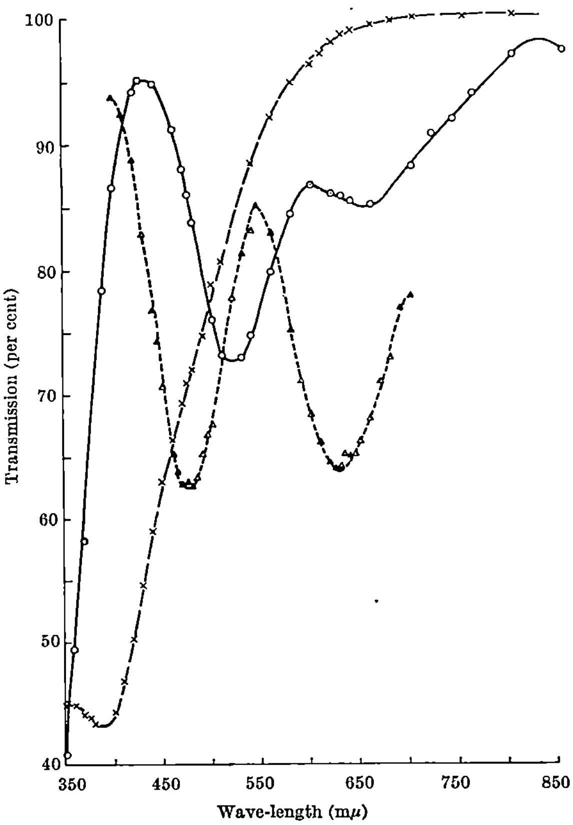


Fig. 1. Transmission curves of the thioglycolic acid complexes. ⍋, chromium, 30 μgm. Cr/ml. at *p*H 9, light-path 40 mm.; ×, uranium, 10 μgm. U/ml. at *p*H 7, light-path 40 mm.; ○, nickel, 23·5 μgm. Ni/ml. at *p*H 7, light-path 40 mm.

Table 1

	Chromium	Uranium	Tungsten	Nickel
Colour of complex in acidic medium	Violet	Colourless	Yellowish	Yellowish-green
Absorption maxima	350 mμ (*p*H 2)	—	(10^{-3} *M* soln.)	(10^{-2} *M* soln.) (*p*H 2)
Colour of complex in alkaline medium	Green	Orange	Colourless	Pink
Working range of *p*H	(6–9)	(6·5–7·5)	(2·5–5·5)	(7–9)
Absorption maxima	*630 mμ and 475 mμ	388 mμ	305 mμ	522 mμ
Molar absorbency index (A_M)	$1·95 \times 10^2$ (on either max.)	23×10^2	53×10^2	$0·98 \times 10^2$
Approximate ratio of A_M	2	25	55	1
Limits of determination (absorptiometrically)	$(1–75) \times 10^{-4}$ *M*	$(2–75) \times 10^{-5}$ *M*	$(4–40) \times 10^{-6}$ *M*	$(2–20) \times 10^{-4}$ *M*
Polarographic $E_{1/2}$ versus S.C.E.	—	—	—	−0·68 V.
Limits of determination (polarographically)	—	—	—	$(0·2–2·0) \times 10^{-4}$ *M*

* In industrial analysis one could use a Spekker absorptiometer and a combined filter (ref. 12) consisting of one filter from Chance's glass-filter set *H*455 and one from Ilford gelatin-filter, namely (OB_2 + 602) and (OY_2 + 607) respectively for 475 mμ and 630 mμ over the same range of concentration with a 40-mm. absorption cell.

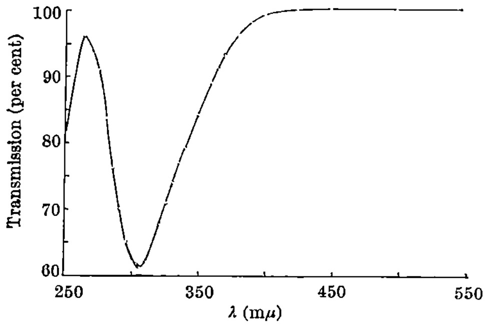


Fig. 2. Transmission curve of thioglycolic acid complex of tungsten. ~ 15 μgm. W/ml. at *p*H 3·5, light-path 10 mm.

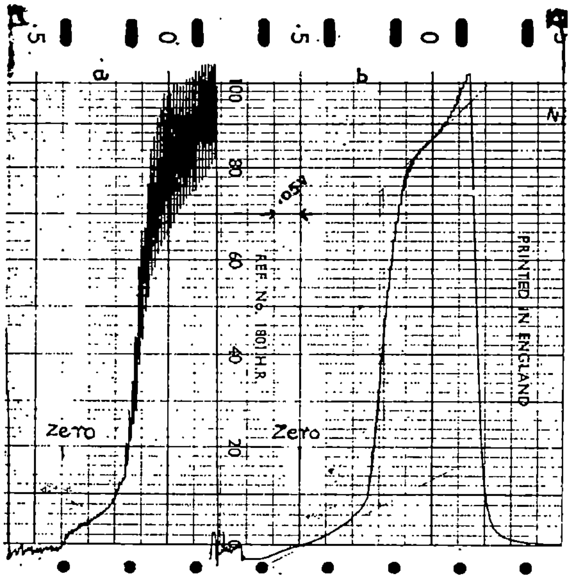


Fig. 3. Cathodic polarographic wave of nickel (12·8 p.p.m.) in ammonium thioglycolate supporting electrolyte at *p*H 8. Sensitivity 2 × 1 μamp./50 division (*a*) without damping, (*b*) at damping position (3) of the Tinsley polarograph mark 19

tungsten[8]. Thioglycolic acid was used[9] as a complexing agent for uranium, but the effect of variation of *p*H has been critically investigated only recently and an absorption maximum reported[10]. The nickel complex has also been investigated polarographically[11] and since the reduction wave is favourably placed at −0·18 V. against a mercury pool, interference from other elements in the polarographic determination of nickel is excluded. The reaction at the dropping mercury electrode involves a two-electron reduction. The wave-height is diffusion-controlled and the temperature coefficient of the wave-height is about 2 per cent per degree rise of temperature. The effect of variation of *p*H and the interference of other ions has been determined in the colorimetric and polarographic determinations of chromium, tungsten, uranium and nickel. Of these, the complexes of thioglycolic acid with chromium, tungsten and nickel have been investigated for the first time.

In a typical determination the solution is treated with excess of reagent on a hot plate and the *p*H of the solution is then adjusted to the required value. After re-heating, the *p*H is checked again. The solution of the complex is then analysed for the respective element. The results of some typical determinations are shown in Table 1. The transmission spectra of different complexes and the reduction wave of the nickel complex[11] with thioglycolic acid are shown in Figs. 1–3.

The complexes of chromium, tungsten, uranium and nickel can be used for the micro-determination of these metals. They are quite stable (the tungsten complex is stable for two weeks), and even a large excess of the thioglycolic acid can be used without any detrimental effect, which is not the case with thiocyanate complexes of tungsten, uranium, etc. Nickel also complexes with *o*-mercaptobenzoic acid, a homologue of thioglycolic acid, which has similar absorption characteristics to nickel thioglycolate. The reagent has been found to be a promising complexing agent for other metals, for example, rhenium, indium, etc. Complete results of these investigations will be communicated later.

I wish to acknowledge the collaboration of Dr. J. A. W. Dalziel, of the Imperial College of Science and Technology, London, in the polarographic investigation of nickel.

V. M. Bhuchar

National Physical Laboratory,
New Delhi-12, India.

[1] Welcher, F. J., *Organic Analytical Reagents*, **4**, 159 (D. Van Nostrand, New York, 1948).

[2] Fiegl, F., and Caldas, A., *Anal. Chem.*, **23**, 8 (1951).

[3] *The Analysis of Titanium and its Alloys*, 47 (Imperial Chemical Industries, Ltd., London, 1959).

[4] Swank, H. W., and Mellon, M. G., *Indust. Eng. Chem.*, Anal. Ed., **10**, 7 (1938).

[5] Sandell, E. B., *Colorimetric Determination of Traces of Metals*, second ed., 364 (Interscience, 1950).

[6] Bhuchar, V. M., and Verma, M. R., *Mikrochim. Acta*, 342 (1958).

[7] Will, Fritz, III, and Yoe, J. H., *Anal. Chem.*, **25**, 1363 (1953).

[8] Otterson, D. A., and Graab, J. W., *Anal. Chem.*, **30**, 1282 (1958).

[9] Davenport, W. H., and Thomason, P. F., *Anal. Chem.*, **21**, 1093 (1949).

[10] Bhuchar, V. M., see *Adv. in Polarography*, **2**, 537; *Proc. Second Intern. Cong. Polarography*, Cambridge (1959), edit. by I. S. Longmuir (Pergamon Press, Oxford, 1960).

[11] Bhuchar, V. M., and Dalziel, J. A. W. (presented to the Second Seminar on Electrochemistry, Central Electrochemical Research Institute, Karaikudi, India, 1960).

[12] Haywood, F. W., and Wood, A. A. R., *Metallurgical Analysis with Spekker Absorptiometer*, 20 (Hilger and Watts, London, 1957).

BIOCHEMISTRY

Interaction of Hydrocortisone, the Sulphydryl Group, and the Catechol Amines

Hydrocortisone potentiates the physiological effects of epinephrine and norepinephrine[1]. The mechanism of this action remains obscure. Recently, reaction of oxidation products of the catechol amines with the sulphydryl group has been demonstrated *in vitro*[2,3]. Further *in vitro* studies suggest that the physiological interrelationship between hydrocortisone and the catechol amines may involve the sulphydryl group.

The norepinephrine, cysteine and manganese dioxide used were previously described[2,4]. Steroids were obtained from the Upjohn Co., the Schering Corporation and the Nutritional Biochemical Corporation. After solution of approximately 10 mgm. of hydrocortisone in 1·0 ml. of absolute ethyl alcohol, the concentration was decreased to 500γ per ml. by addition of water. The other steroids studied were used in solution in absolute alcohol only. Other reagents included 2/3 *N* sulphuric acid and 1 *N* sodium hydroxide. Buffers were 0·1 *M* sodium phosphate of *p*H 7·4 and 8·0. Absorbencies were measured in the Beckman *DU* spectrophotometer with tungsten lamp.

The experimental procedure began with the preparation of a solution containing $0·15 \times 10^{-3}$ *M* cysteine and the indicated concentration of hydrocortisone immediately before its utilization. At zero time, 0·5 ml. of 1 *N* sodium hydroxide was added to separate test-tubes containing 2·0-ml. aliquots of this solution. One-half of these tubes were used for the development of noradrenochrome, the other half for the simultaneous measurement of the residual sulphydryl group. At 5-min. intervals after the alkalinization, 0·70 ml. of 2/3 *N* sulphuric acid, 1·0 ml. of 0·1 *M* sodium phosphate buffer of *p*H 7·4, 0·1 ml. of $3·0 \times 10^{-3}$ *M* norepinephrine, and 0·1 gm. of manganese dioxide were added rapidly to an additional tube and oxygen was bubbled vigorously through the solution for 40 sec. 6 min. later, the absorbency of the noradrenochrome formed was measured at 480 mμ. Simultaneously, every 5 min., 0·68 ml. of 2/3 *N* sulphuric acid and 1·0 ml. of 0·1 *M* sodium phosphate buffer of *p*H 8·0 were added to a separate tube. The sulphydryl group was then determined from the absorbency at 412 mμ of the compound formed by Ellman's technique[5]. Parallel tubes with cysteine but without hydrocortisone were used as controls in each case.

Table 1. PERCENTAGE OF THE SULPHYDRYL GROUP OF CYSTEINE REMAINING IN THE FREE STATE AFTER VARIOUS PERIODS OF TIME

Molarity of hydrocortisone			
Molarity of cysteine	6 min.	15 min.	30 min.
0 (auto-oxidation))	91	84	74
½	84	69	47
1	77	58	26
3	60	25	0

Reagent solutions containing 500γ of hydrocortisone per ml. of alcohol alone or per ml. of alcohol and water as described affected the sulphydryl group identically, the effect of the alcohol being negligible. The effects of various concentrations of hydrocortisone upon the sulphydryl group of cysteine in alkaline solution are presented in Table 1. In contrast to hydrocortisone such steroids as progesterone, testosterone and deoxycorticosterone acetate had no effect, while corticosterone was only slightly effective. On the other hand, 17α-hydroxy-11-deoxycorticosterone, cortisone and hydrocortisone acetate had essentially the same effect as hydrocortisone. This action of the steroids was present only in highly alkaline solution. The results for glutathione were similar in direction but considerably less in magnitude than those for cysteine. The amount of noradrenochrome developed from norepinephrine at *p*H 7·4 increased as the free —SH group decreased. The intensity of the pink colour and the absorbency at 480 mμ reflected the disappearance of the sulphydryl group, just as did the Ellman technique.

The experimental evidence suggests that the sulphydryl group of cysteine reacts in alkaline solution on an equimolar basis with many steroids which have a C_{17} side-chain. The reactivity of hydrocortisone acetate indicates that the C_{21} hydroxyl group is not essential. Conversely, the ineffectiveness of progesterone and deoxycorticosterone acetate shows the inadequacy of the C_{20} oxygen alone. The presence of the keto-group on C_{20} and the α-hydroxyl group on the C_{17} position is characteristic of the steroids which react with the sulphydryl group. It is interesting to note that this high reactivity of cortisone and hydrocortisone *in vitro* parallels their strong potentiation of catechol amine action *in vivo*[1].

The increased development of noradrenochrome at *p*H 7·4 as the cysteine disappears is important because it illustrates a possible mechanism of hydrocortisone action *in vivo*. The norepinephrine–cysteine and norepinephrine–glutathione compounds have no effect upon the blood pressure of the dog, according to recent work done at the University of Louisville in conjunction with Dr. K. C. Huang of the Department of Pharmacology. Hence, by preventing combination of cysteine and glutathione with derivatives of the catechol amines, hydrocortisone might direct the metabolic pathways of epinephrine and norepinephrine toward reactions which would intensify and prolong their physiological effects. An enzyme which would facilitate the reaction of the sulphydryl group and hydrocortisone at *p*H 7·4 is not available as yet. The existence of such an enzyme appears to be quite feasible, and search for it *in vivo* is under way.

This investigation was supported by grant No. *M*-2795 (*C*1) from the National Institutes of Health of the U.S. Public Health Service.

SIDNEY ROSTON

Department of Medicine,
University of Louisville,
School of Medicine, Kentucky.

[1] Ramey, E. R., and Goldstein, M. S., *Physiol. Rev.*, **37**, 155 (1957).

[2] Roston, S., *Arch. Biochem. Biophys.*, **85**, 74 (1959).

[3] Bouchilloux, S., and Kodja, A., *C.R. Acad. Sci.*, **251**, 1920 (1960).

[4] Roston, S., *J. Biol. Chem.*, **235**, 3315 (1960).

[5] Ellman, G. L., *Arch. Biochem. Biophys.*, **74**, 443 (1958).

Effects of Injections of Some Intermediary Metabolites on the 'Insulin Response' of Normal Rabbits

CONTINUED daily injections of β-hydroxy butyrate in gradually increasing doses has been found to increase initially the insulin content of the pancreases of guinea pigs and then bring about a gradual decrease of active insulin/gm. of pancreas tissue[1]. Similar experiments by Schwartz *et al.*[2] have recently confirmed these findings in both guinea pigs and albino rats. The present communication indicates the effects of some members of the trichloroacetic acid cycle, compared to β-hydroxy butyrate, on the insulin response of normal rabbits.

Pure sodium salts of the metabolites concerned and crystalline insulin solution (Lily) were used in these experiments on healthy normal rabbits. Five rabbits were used in each case and each experiment was repeated twice as in cross-over tests in insulin assay[3]. Each rabbit was allowed one week's rest between experiments.

In one set of experiments sodium salts of the metabolites concerned (dissolved in 5 c.c. of normal saline) were injected in fasting (18 hr.) normal rabbits and the blood sugar-level studied at hourly intervals for a period of 3 hr. In a second set of experiments the metabolites concerned were mixed *in vitro* with 2 units of insulin (diluted to 5 c.c. with normal saline), the mixture incubated at 37° C. for 30 min., and the clear solution injected in one leg of the rabbits. The blood sugar-level was then examined as in the previous case. In a third set of experiments the metabolites were injected simultaneously but separately with 2 units of insulin in the two legs of the rabbits to study the *in vivo* effect on the insulin response. Control experiments were carried out simultaneously with each set of the above experiments to study the blood sugar response on injection of 2 units of insulin alone. The micro-method of Folin and Malmros[4] was used in estimating blood sugar in samples of blood drawn from the marginal ear veins of the experimental rabbits. The results of these experiments are given in Table 1.

Table 1. EFFECTS OF INJECTIONS OF METABOLITES ON THE INSULIN RESPONSE OF NORMAL RABBITS

S. No.	Substance injected	Average percentage of reductions in blood sugar			
		2 units insulin only	100 mgm. of substance	100 mgm. and 2 units insulin *in vivo*	100 mgm. and 2 units insulin *in vitro*
1	Insulin (control)	56·9 ± 3·46	—	—	—
2	Sodium pyruvate	—	8·98 ± 1·1	66·78 ± 3·2	50·8 ± 3·4
3	Sodium β-hydroxybutyrate	—	12·84 ± 1·5	66·75 ± 3·5	53·43 ± 2·1
4	Sodium isocitrate	—	17·12 ± 2·0	57·51 ± 3·3	49·0 ± 2·8
5	Sodium succinate	—	22·54 ± 1·8	56·14 ± 3·2	46·0 ± 3·0
6	Sodium citrate	—	30·34 ± 3·0	51·98 ± 3·1	40·83 ± 2·8

From Table 1 it is apparent that all these metabolites when injected alone in normal fasting rabbits produce a hypoglycæmic response which may be due to their stimulating effect, direct or indirect, on the *B*-cells of the islets of Langerhans, initiating a secretion of insulin. This stimulation for the same amount of substance injected is greatest in case of citrate and is progressively lesser in the following order: citrate–succinate–isocitrate–β-hydroxy butyrate–pyruvate.

The results of *in vivo* experiments suggest that while the stimulating power of β-hydroxy butyrate and pyruvate are independent of the presence of exogenous insulin in the system, the other three metabolites, namely, citrate, succinate and isocitrate, seem to lose their stimulating power in presence of exogenous insulin in the system. The results of *in vitro* experiments suggest that while β-hydroxy butyrate and pyruvate have no effect on the biological potency of exogenous insulin, the other three metabolites, namely, citrate, succinate and isocitrate, bring about a decrease in the biological potency of exogenous insulin in similar circumstances, namely, *in vitro* mixing and incubation at 37° C. for 30 min. before injection.

H. D. BRAHMACHARI
G. RAGHUPATHY SARMA

Biochemistry Laboratory,
Birla College,
Pilani (Rajasthan).

[1] Brahmachari, H. D., and Mahendra Kumar, *Nature*, **183**, 51 (1959).
[2] Schwartz *et al.*, *Nature*, **185**, 771 (1960).
[3] Marks, *League of Nations Rep. Insulin Standardization*, C.H. 396 (1928).
[4] Folin and Malmros, *J. Biol. Chem.*, **83**, 115 (1929).

Estimation of Protein in Cellular Material

PROTEIN content is a widely used reference value for microbial growth yield, enzyme activity, and other biochemical quantities. The assay of dissolved protein is relatively easy, being accomplished by measuring the absorbence at 280 mμ or by use of the biuret reagent, etc. The assay of protein in cellular material involves a time-consuming digestion (Kjeldahl method). In addition to being slow, this method is non-specific and does not immediately distinguish between proteins and other nitrogen compounds.

The method reported here is based on the fact that most solid protein samples, including precipitates made with trichloroacetic acid or by heat coagulation, are soluble in a dilute base. By using half-strength biuret reagent (containing 0·4 *M* sodium hydroxide) it is possible to extract and afterwards measure rapidly.

Cell materials seem to fit three categories with regard to method: (1) Require no previous treatment (most bacteria). (2) Chlorophyllous cells (algae and leaf tissue) require extraction with 70–80 per cent ethanol to remove pigments. (3) Cells that resist base extraction of their protein (yeast and algae). These require pre-treatment with 0·5 *M* perchloric acid. The reason for this is not clear but may be related to cell wall thickness or to covalent bonds existing between the protein and structural polymers. Nevertheless, pre-treatment with perchloric acid does make reproducible protein extraction possible. (The bean leaf tissue does not require pre-treatment with perchloric acid but was so treated for other reasons.)

Wet cell masses are collected by centrifugation and washed, if necessary, to remove any large amount of reducing sugar which may be present in the medium. (The cupric ions in the biuret reagent are susceptible to reduction and precipitation as cuprous oxide.) The centrifuged solids are suspended in at least 10 vol. of water to which is added an equal volume of biuret reagent[1]. This mixture is allowed to stand at room temperature for one hour with occasional stirring. The insoluble residue is removed by centrifugation, leaving a water-clear, purple solution. Readings are made in tubes (16 mm. int. diam.) against a reagent blank in a Coleman spectrophotometer at 540 mμ.

Proteins from different sources give somewhat different colour yields. Thus, an absolute protein measurement must be made if it is necessary to calibrate the method. (Such calibration is not necessary when comparisons are to be made only between samples of the same biological origin.) In the examples presented here the protein was first read as a biuret colour intensity and then precipitated with trichloroacetic acid. The precipitate is analysed via Kjeldahl technique and the usual factor of 6·25 mgm. protein/mgm. N is applied. It is assumed that trichloroacetic acid brings down all the protein. This is apparently borne out by analyses which show that only a small amount of nitrogen (attributable to compounds of low molecular weight) remains in the frac-

Table 1. COLOUR YIELDS AND EXTRACTION EFFICIENCY OF CELLULAR PROTEIN WITH BIURET REAGENT

Cell material	Colour yield O.D. × ml./mgm. protein	Percentage of TCA-insoluble nitrogen in extractable nitrogen	Percentage of non-extractable nitrogen (based on extractable plus non-extractable nitrogen)
Bean leaf* *Phaseolus vulgaris*	0·59	96	3
Algae* *Chlorella vulgaris*	0·55	83	29
Yeast† *Saccharomyces cerevisiae*	0·54	91	24
Bacteria *Azotobacter vinelandii*	0·48	84	10
Bacteria *Escherichia coli*	0·39	85	22

* Pre-treated with ethanol and then perchloric acid.
† Pre-treated with perchloric acid.

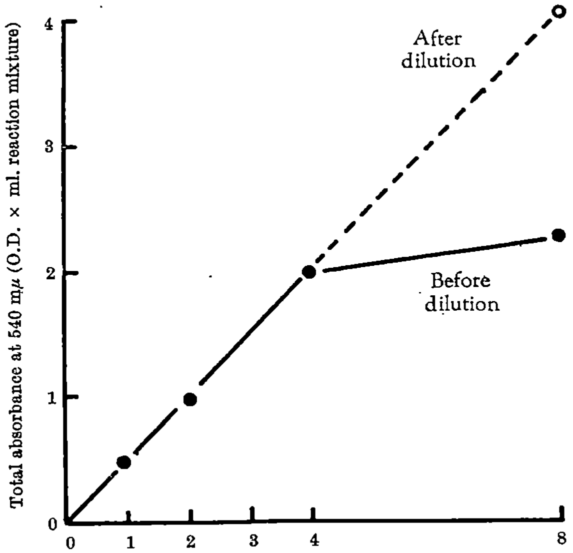


Fig. 1. Linearity of biuret colour intensity versus protein concentration (*A. vinelandii*)

tion soluble in trichloroacetic acid. Table 1 illustrates some representative results.

The biuret reagent does not have a wide range of linearity. Furthermore, the solubilized protein from different sources appears to have individual ranges of linearity. For both these reasons it is well to establish first a colour-concentration curve for each type of material. Fortunately, it is possible to dilute non-linear samples with a 1 : 1 mixture of water and reagent. After an additional incubation of 30 min. the colour is found to be proportional to protein (Fig. 1).

Extraction of protein may not be quantitative for all materials (Table 1). This may not be a serious problem to persons concerned with only one type of cell, especially when repeated extractions yield results within ±5 per cent. In the case of yeast cells the unextracted nitrogen may be a 'fungal chitin'. In the case of *E. coli* at least some of the unextracted nitrogen is protein, as judged by the biuret-purple colour of the remaining solids. This particular extraction might be made more quantitative by pre-treatment with 0·5 *M* perchloric acid.

Chlorophyll-containing materials are extracted in boiling 70 or 80 per cent ethanol until colourless. The solids are then extracted with perchloric acid and/or biuret reagent.

The centrifuged material is suspended in at least 10 vol. 0·5 *M* perchloric acid and incubated, with occasional stirring, at 80° for 90 min. (This is essentially the method used by Basler and Commoner for extraction of ribonucleic acid[2].)

The combined loss of protein due to the treatments with dilute biuret reagent and 0·5 *M* perchloric acid is less than 10 per cent (ref. 3).

DAVID RACUSEN
D. B. JOHNSTONE

Department of
Agricultural Biochemistry,
University of Vermont,
Burlington.

---

[1] Cornell, A., Bardawill, C., and David, M., *J. Biol. Chem.*, **177**, 751 (1949).

[2] Basler, E., and Commoner, B., *Virology*, **2**, 15 (1956).

[3] Racusen, D., and Foote, M., *Arch. Biochem. Biophys.*, **90**, 90 (1960).

1-(Indole-3-acetyl)-β-D-Glucose, a New Compound in the Metabolism of Indole-3-acetic Acid in Plants

STUDYING the fate of exogenously applied indole-3-acetic acid (IAA) in pea epicotyls, we observed, besides indoleacetylaspartic acid[1] (IAAsp), a trace amount of a compound which showed definitely indole reactive properties and which was split on alkaline hydrolysis into IAA. The amount of this metabolite proved too small for identification, therefore we carried out a rough survey on one species from 20 plant families using 2-^{14}C IAA in the hope of finding an accumulation of this metabolite. This unknown compound was found in the leaves of the monocotyledonous plant *Colchicum neapolitanum* Ten. to about 65 per cent of the total amount of IAA taken up, the rest being unchanged IAA (20 per cent) and minor components (Fig. 1, top). IAAsp was detected, if at all, only in very small quantities. In order to isolate a larger amount of this compound, 70 gm. of mature *Colchicum* leaves were cut into pieces and incubated with 1 litre of a 4×10^{-4} *M* solution of IAA for 24 hr. with good aeration. The compound was extracted from the leaves with boiling 80 per cent aqueous ethanol; the extract was then concentrated to a small volume and made up to a powder with 'Hyflo supercel' and then extracted in a Soxhlet-type extractor for 6 hr. with ether. The ether containing only the IAA was discarded, and extraction continued with ethyl acetate for another 6 hr. This solution was taken to dryness and the residue containing the compound chromatographed as streaks on Whatman No. 3 paper in *n*-butanol / glacial acetic acid / water (5 : 1 : 2·2, R_F 0·56) and in *iso*propanol / benzine / water (55 : 30 : 11, R_F 0·39). By elution and re-chromatography between the two solvents, the derivative could be purified and resulted finally in 6·05 mgm. of a highly purified, non-crystalline material. The compound was located on the paper by spraying with *p*-dimethylaminobenzaldehyde.

Hydrolysis of the material with 1 *N* potassium hydroxide at room temperature led to the hydrolysis products IAA and a carbohydrate chromatographically identical with glucose. Acid hydrolysis with 2 *N* hydrochloric acid at 100° C. gave the same result. The compound was split with hydroxylamine to form indoleacetyl hydroxamic acid, chromatographically identical with synthetic hydroxamate, thus showing an ester linkage.

The ultra-violet absorption spectrum of the compound showed a minimum at 248 mμ, a maximum at 278 mμ and a shoulder at 288 mμ. Equimolar solutions of IAA and the unknown had the same extinction coefficient at 280 mμ while the differences in the spectra of the two compounds showed a maximum at 240 mμ ($E = 2·9 \times 10^3$). This spectrum closely resembles that of indoleacetyl-thioesters[2], thus again suggesting an ester linkage between IAA and glucose. On treatment with the Salkowski reagent[3], the resulting colour of the compound was shifted from the IAA peak ($\lambda_{max.}$ 535) towards the longer wave-length ($\lambda_{max.}$ 545); indolethylacetate shows $\lambda_{max.}$ 555 and indoleacetyl-thioesters, $\lambda_{max.}$ 575. Maximal colour intensity was attained in each case 11 min. after the addition of the reagent. Aliquots of the compound were analysed for functional groups and the ratio of the assays was IAA / ester linkage / glucose = 1·00 : 1·01 : 1·00. The IAA concentration was based on the absorption at 280 mμ, glucose was determined with the anthrone reagent[4] and the ester linkage with

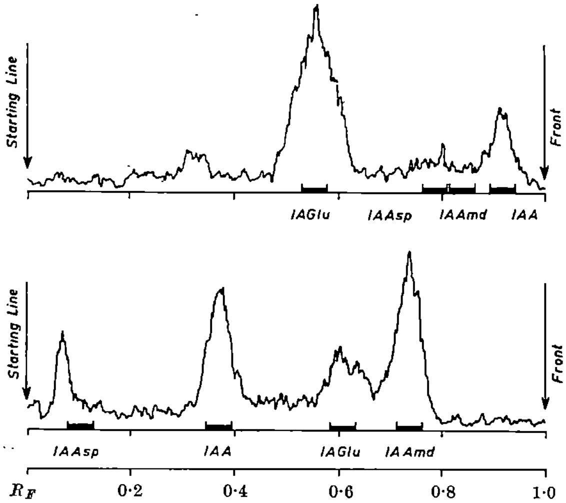


Fig. 1. (Top): Scanning record of a chromatogram of an alcoholic leaf-extract after 24 hr. treatment with 10^{-4} *M* 2-^{14}C IAA developed in *n*-butanol / acetic acid / water (5 : 1 : 2·2). (Bottom): Scanning record of a chromatogram with the same extract developed in *iso*propanol / NH_3 (25 per cent) / water (8 : 1 : 1)

the hydroxamic acid test[5]. In the absence of free IAA, 1 μM of indoleacetyl hydroxamate in 5 ml. test solution gives an extinction of 0·230 Beckman unit at 540 mμ. On the basis of this analysis the compound represents a glucose ester of IAA. To determine which carbon atom of glucose is esterified, the compound was subjected to the action of β-glucosidase (K^+, acetate buffer, *p*H 5·0) and was split quantitatively by this enzyme. The compound lacks reducing properties. These properties are characteristic for an ester linkage in the 1-position of glucose in β-configuration. I am therefore able to assign to the ester the structure of 1-(indole-3-acetyl)-β-D-glucose (IAGlu). (During the preparation of this manuscript a tentative identification of this ester was made by Klämbt, H. A. (*Planta*, 56, 618 ; 1961). A critical discussion of this presentation will be left to a further publication.)

The ester is labile towards ammonia. Using ammoniacal *iso*propanol for chromatography resulted in the ammonolysis of 57 per cent and in the hydrolysis of 13 per cent of the ester, a total of 70 per cent of the ester being destroyed. The predominant reaction product was indoleacetamide (IAAmd) (Fig. 1, bottom). IAGlu is also split in bicarbonate solution containing ammonia salts to yield IAA and IAAmd. I therefore assume that the reports in literature on the occurrence of IAAmd in plants are erroneous and probably due to chromatographic and isolation artefacts. This behaviour of IAGlu is in accordance with the ammonolysis of indoleacetylglucosiduronic acid[6].

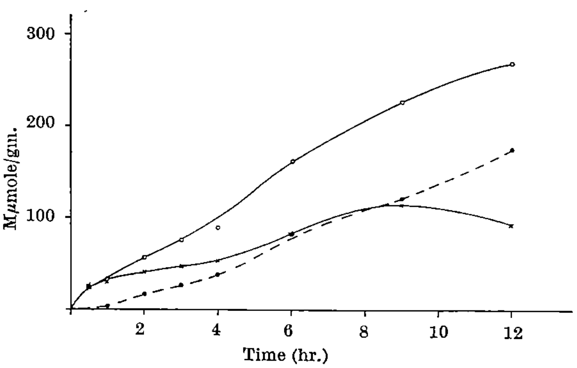


Fig. 2. The uptake of IAA from solution and accumulation of IAA and IAGlu in leaf-disks of *C. neapolitanum* during 12 hr. of incubation in a 5×10^{-5} *M* 2-^{14}C IAA solution. ○, Total uptake of IAA from external solution; ●, IAGlu, and ×, IAA, accumulating in leaf-disks

The uptake and metabolism of IAA by *Colchicum* leaf-disks is shown in Fig. 2. The uptake of IAA during 12 hr. was fairly constant. The quantity of IAA in the free form rises during the first 9 hr. and then falls off again. In contrast to experiments with pea epicotyls[7], the IAA concentration in this leaf tissue exceeded the concentration in solution by a factor of more than 2, a fact which suggests a metabolic uptake of the acid. Unlike the conjugation of IAA with aspartic acid there was no lag-period in the conjugation of IAA with glucose. The synthesis of IAGlu certainly involves a transference of glucose from uridinediphosphoglucose on to IAA.

Besides the conjugation with aspartic acid, I therefore assume a second de-toxification mechanism of aromatic acids in plants, the conjugation with glucose. Both types of de-toxification may operate simultaneously or one or the other may predominate in different plant species.

IAGlu is a member of the recently discovered group of naturally occurring sugar esters[8], and is active in the *Avena* cylinder-test.

This work was supported by the Bundesministerium für Atomkernenergie und Wasserwirtschaft.

2-^{14}C IAA was a gift from Dr. H. Simon, for which we are very grateful.

M. H. Zenk

Botanical Institute,
University of Munich,
Munich 19.

[1] Andreae, W. A., and Good, N. E., *Plant Phys.*, **30**, 380 (1955).
[2] Zenk, M. H., *Naturwiss.* (in the press).
[3] Tang, Y. W., and Bonner, J., *Arch. Biochem. Biophys.*, **13**, 11 (1947).
[4] Aronoff, S., *Techniques of Radiobiochemistry* (Iowa State College Press, 1957).
[5] Lipmann, F., and Tuttle, L. C., *J. Biol. Chem.*, **159**, 21 (1945).
[6] Jepson, J. B.. *Biochem. J.*, **69**, 22*P* (1958).
[7] Andreae, W. A., and van Ysselstein, M. W., *Plant Phys.*, **35**, 225 (1960).
[8] Birkofer, L., Kaiser, C., and Kosmol, H., *Naturwiss.*, **47** 469 (1960).

Effect of Amino-Acid Esters on the Growth of Fibroblast Cultures

Perri *et al.*[1] demonstrated that amino-acid esters inhibit the growth of bacteria. Simultaneously with this finding, Horváth *et al.* described the inhibition of certain amino-acid esters on the adaptive amylase production of *Penicillium chrysogenum*[2]. Fehér *et al.* demonstrated that DL-methionine-ethyl ester caused liver lesions[3].

The mechanism of this inhibition has not yet been examined, but it is supposed that because of the bound carboxyl group the amino group becomes more reactive and thus the activated amino-acid reacts with the ester instead of the intact amino-acid.

The inhibition of ethionine on growth and protein synthesis is well known. We supposed that this effect is unable to diminish even after the esterification of ethionine. To prove this supposition, we attempted to compare the inhibition of growth by ethionine and ethionine ethyl ester on cell cultures.

The pure fibroblast culture used in our experiments was cultivated in tubes of 3 ml. volume described elsewhere[4]. An ocular micrometer was used to measure the radii of the cell cultures. The basis of comparison was the total of radii measured in four directions. The basic growth of the cultures was measured always on the third day after the inoculation of the cells and the substance investigated was added in a volume of 0·1 ml. to the culture medium. The difference between the basic growth and that on the third day of the experimental period characterized the activity of the substances examined.

In the first experiment we investigated whether certain amino-acid esters inhibit the growth of tissue cultures similarly to bacteria described previously[4,5]. 1·5 m.mole/l. methionine ethyl ester was added to the culture medium. An inhibition of more than 50 per cent was observed (Table 1). The inhibition of methionine anti-metabolites, as is well known, can be prevented by methionine or glutamine[3,6]. Next we added 3 m.mole/l. methionine or glutamine to the culture medium, which prevented the inhibition of methionine ethyl ester about 50 per cent. Methionine ethyl ester behaves thus like other methionine antagonists and its growth inhibition may be prevented by addition of methionine to fibroblast cultures.

Table 1. EFFECT OF AMINO-ACID ESTERS ON THE GROWTH OF FIBROBLAST CULTURES

Added substance	No. of cultures	Basic growth	Growth after addition of substance	Growth in percentage compared with the control
Control	520	24·0 ± 5·5	45·0 ± 9·2	100
DL-Methionine-ethyl ester, 0·15 m*M*	440	24·3 ± 5·3	34·0 ± 7·1	45·7
DL-Methionine-ethyl ester, 0·15 m*M* + DL-Methionine, 0·3	385	22·1 ± 4·0	37·4 ± 6·2	72·5
Control	114	29·4 ± 4·4	52·2 ± 5·3	100
DL-Methionine-ethyl ester, 0·15 m*M*	120	29·8 ± 3·8	37·2 ± 5·0	32·0
DL-Methionine-ethyl ester, 0·15 m*M* + DL-Glutamine, 0·3 m*M*	126	28·7 ± 5·7	43·4 ± 6·9	64·5
Control	343	20·4 ± 3·6	36·0 ± 6·9	100
DL-Ethionine-ethyl ester, 1·5 m*M*	163	19·5 ± 4·1	33·7 ± 6·2	89·0
DL-Ethionine, 1·5 m*M*	202	19·6 ± 3·9	25·9 ± 5·4	39·4
DL-Ethionine, 1·5 m*M* + DL-Ethionine-ethyl ester, 1·5 m*M*	209	18·9 ± 3·7	31·8 ± 6·7	80·5

Further, we studied the ethionine sensitivity of the culture. As shown in Table 1, 1·5 m.mole/l. ethionine causes a significant inhibition of growth.

On the basis of the hypothesis discussed here, it might have been expected that ethionine ethyl ester added in the same concentration of ethionine produces inhibition of growth similar to ethionine or methionine ethyl ester. In the next experimental series we therefore added ethionine ethyl ester in a concentration of 1·5 m.mole/l. to the culture medium. The ester was practically ineffective, the inhibition being only 11 per cent.

Due to simultaneous addition of a double amount of ethionine ethyl ester (3 m.mole/l.) and of 1·5 m.mole/l. of ethionine, the inhibition of growth by ethionine decreased about 50 per cent.

It seems that the inhibition of methionine ethyl ester was of a competitive character, because it could be prevented by addition of methionine in greater amounts. In contrast with expectation, however, the inhibition of the ethionine and ethionine ethyl ester added at the same time is not additive, but the ester nearly prevents the inhibition. The quantitative conditions point to competitive inhibition, too, although in this case it is the ester that prevents the effect of the amino-acid.

Our experiments do not explain the mechanism of inhibition of amino-acid ester, but it seems that the hypothesis that inhibition was induced only by the increased reactivity of the amino-group is dubious.

I. FEHÉR*
A. DÖKLEN
V. SELMECI

Institute of Pathophysiology,
University Medical School,
Budapest.

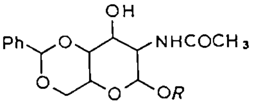
---

* Present address: Inst. for Radiobiol. Res., Budafok.

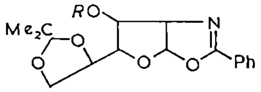
[1] Perri, C. C., Kaplan, L., and Stock, C. C., *Nature*, **183**, 116 (1959).
[2] Horváth, I., Bajusz, S., and Szentirmai, A., *Nature*, **183**, 477 (1959).
[3] Fehér, I., Selmeci, F. V., and Kemény, T., *Nature*, **187**, 422 (1960).
[4] Döklen, A., Fehér, I., and Sós, J., *Acta Physiol. Hung.* (in the press).
[5] Fehér, I., Lengyel, A., and Nász, I., *Acta Physiol. Hung.*, **15**, 275 (1958).
[6] Rabinovitz, M., Olson, M. E., and Greenberg, D. N., *J. Biol. Chem.*, **222**, 217 (1957).

A Convenient Synthesis of Muramic Acid and Other 3-O-Ethers of D-Glucosamine

3-O-ETHERS of D-glucosamine, including muramic acid (3-O-carboxyethyl-D-glucosamine, a component of bacterial cells walls), have been prepared[1-4] from the intermediate I (*R* = Me or Et)[5]. We have applied the Williamson ether synthesis to the intermediate II (*R* = H)[6] and have prepared muramic acid, 3-O-methyl- and 3-O-carboxymethyl-D-glucosamines by this procedure. The intermediate (II) is readily prepared in good yield by a two-stage process, whereas the preparation of substance I requires three steps and a separation of the α and β anomers[7,8].



(I)



(II)

Compound II (*R* = H) was prepared by the method of Konstas, Photaki and Zervas[6] except that the crude N-benzoyl-D-glucosamine which separates from the reaction mixture was used (after thorough drying over phosphorus pentoxide *in vacuo*) without further purification. The overall yield of crystalline II (*R* = H, m.p. 158–160°) from glucosamine hydrochloride was 55 per cent. After recrystallization from ethyl acetate, the compound had the following characteristics: m.p. 160–162°; $[\alpha]_D^{19} + 6°$ (*c*, 2 in chloroform); infra-red absorption (potassium chloride disk) 1,648 cm.$^{-1}$ (C=N stretching vibration).

For the synthesis of muramic acid, intermediate II (*R* = H) (35 gm.) was treated with sodium hydride (2·9 gm.) in refluxing tetrahydrofuran (450 ml., sodium-dried, peroxide-free) for 2 hr. To the cooled solution of the sodio derivative was added dried, redistilled, ethyl DL-α-bromopropionate (35 gm.). There was an immediate precipitation of sodium bromide, and after the initial exothermic reaction had subsided, refluxing was continued for another hour. After cooling, the solution was filtered and the tetrahydrofuran and excess bromo-ester were removed by evaporation under reduced pressure. The crude product was saponified by warming for 5 min. at 50° in a mixture of water (35 ml.), methanol (35 ml.) and sodium hydroxide (8 gm.). This solution was then diluted with water (350 ml.), saturated with potassium chloride and extracted with ether to remove any unreacted intermediate. The aqueous solution containing the sodium salts of the D- and L-ethers II ($R = \text{—CHMe.COOH}$) was carefully acidified to *p*H 4 with normal hydrochloric acid and the free acids (35 gm., 81 per cent) which were precipitated were filtered, washed with water and dried.

The mixed acids (32 gm.) were heated in refluxing benzene (200 ml.) for 15 min., the insoluble material removed by filtration and the filtrate allowed to stand at room temperature overnight before refiltering. The combined benzene-insoluble materials (10·6 gm.) were recrystallized from hot benzene, giving crystals, m.p. 208–210°, $[\alpha]^{20}_D - 26°$ (*c*, 2 in chloroform); infra-red absorption 1,635 cm.$^{-1}$ (C = N stretching vibration) and 1,705 cm.$^{-1}$ (C = O vibration), and analytical figures agreeing with those for the ether II ($R = \text{—CHMe—COOH}$). Acid hydrolysis in refluxing 2·7 *N* hydrochloric acid for 4 hr. followed by chromatography in phenol–water, 3 : 1 (w/w), revealed a ninhydrin-positive spot with R_F = 0·46 corresponding to the isomer of muramic acid[2].

The benzene solution of the soluble acids (21·4 gm.) was evaporated to 100 ml. and on standing at room temperature needle crystals (13 gm., m.p. approx. 160°) separated. Most of this material dissolved readily in warm ethyl acetate (5 ml./gm.) and filtration removed some of the isomer of m.p. 208–210°. On cooling and seeding, large needle crystals (5 gm.) separated. These had m.p. 163–164°; $[\alpha]^{20}_D + 65°$ (*c*, 2 in chloroform); infra-red absorption 1,635 cm.$^{-1}$ (C = N stretching) and 1,725 cm.$^{-1}$ (C = O vibration) and analytical figures agreeing with those for the ether II ($R = \text{—CHMe—COOH}$). Hydrolysis and chromatography as above, gave one spot R_F = 0·55 corresponding to muramic acid obtained from natural sources.

The acids remaining in the mother liquors were converted to the S-benzylthiouronium salts and recrystallization of the product from 20 per cent methanol in water gave 8 gm. of salt, m.p. 134–136°. On decomposition this gave 5·1 gm. of the acid m.p. 163–164°. (The S-benzylthiouronium salt of the acid m.p. 208–210° has not been obtained in the crystalline state.)

The acid of m.p. 163–164° (10 gm.) was hydrolysed in refluxing 2·7 *N* hydrochloric acid for 4 hr., the benzoic acid removed from the cooled hydrolysate by ether extraction and the hydrochloric acid removed by evaporation under reduced pressure. The residue was dissolved in water and 'Amberlite *IR*–*4B*' (OH form) added until the solution was at *p*H 6. After filtration, the aqueous solution was evaporated to dryness and the crystalline product (6·4 gm., 90 per cent based on muramic acid monohydrate) was recrystallized from water/ethanol. The product m.p. 150–151° (decomp.); $[\alpha]^{22}_D + 144°$ (4 min.) falling to + 112° (23 hr.) (*c*, 2 in water) gave satisfactory analytical figures for anhydrous muramic acid after drying for 6 hr. at 60° *in vacuo* over phosphorus pentoxide.

By a similar procedure, the carboxymethyl ether II ($R = \text{—CH}_2\text{.COOH}$) was prepared by the action of ethyl bromoacetate on the sodio derivative of the intermediate II (R = H). This had the following characteristics: m.p. 185–186° (from ethyl acetate); $[\alpha]^{19}_D - 22°$ (*c*, 2 in chloroform); infra-red absorption 1,635 cm.$^{-1}$ (C = N) stretching), and 1,725 cm.$^{-1}$ (C = O). The compound was hydrolysed and the product worked up as for muramic acid. The 3-O-carboxymethyl-D-glucosamine was recrystallized from water/ethanol, giving needle crystals m.p. 163–164° (decomp.); $[\alpha]^{22}_D + 130°$ (6 min.) falling to + 102° (24 hr.) (*c*, 2 in water). After drying for 6 hr. at 60° *in vacuo* over phosphorus pentoxide satisfactory analytical figures for the anhydrous material were obtained.

The reaction of methyl iodide with the sodio derivative of the intermediate gave the methyl ether II ($R = \text{—CH}_3$) in 90 per cent yield; m.p. 117–119° (from methanol); $[\alpha]^{20}_D - 15{\cdot}5°$ (*c*, 2 in chloroform); infra-red absorption 1,647 cm.$^{-1}$ (C = N vibration). This compound was hydrolysed as above and the 3-O-methyl-D-glucosamine hydrochloride recrystallized twice from methanol/acetone; $[\alpha]^{22}_D$ + 112° (4 min.) falling to + 90° (18 hr.) (*c*, 2 in water).

Satisfactory analytical figures were obtained for all compounds described.

ROY GIGG

National Institute for Medical Research,
Mill Hill, N.W.7.

PATRICIA M. CARROLL

Human Nutrition Research Unit,
Mill Hill, N.W.7.

[1] Jeanloz, R. W., *Adv. in Carb. Chem.*, **13**, 189 (1958).
[2] Strange, R. E., and Kent, L. H., *Biochem. J.*, **71**, 333 (1959).
[3] Zilliken, F., *Fed. Proc.*, **18**, 966 (1959).
[4] Lambert, R., and Zilliken, F., *Chem. Ber.*, **93**, 2915 (1960).
[5] Neuberger, A., *J. Chem. Soc.*, 50 (1941).
[6] Konstas, S., Photaki, I., and Zervas, L., *Chem. Ber.*, **92**, 1288 (1959).
[7] Kuhn, R., Zilliken, F., and Gauhe, A., *Chem. Ber.*, **86**, 466 (1953).
[8] Roth, W., and Pigman, W., *J. Amer. Chem. Soc.*, **82**, 4608 (1960).

Differential Inhibition of Human Serum Cholinesterase with Fluoride: Recognition of Two New Phenotypes

IT has been shown that individuals excessively sensitive to the drug suxamethonium have a reduced level of serum cholinesterase activity, and that this peculiarity is genetically determined[1]. It has also been found that the cholinesterase present in the serum of such suxamethonium-sensitive individuals is qualitatively different from that occurring in most normal people[2]. This difference can be most simply demonstrated by determining the degree of inhibition produced by certain anticholinesterase substances. For example, using 5×10^{-5} M benzoylcholine as substrate and 10^{-5} M dibucaine as inhibitor under standard conditions, the enzyme in most normal sera shows about 80 per cent inhibition, while that in sera from suxamethonium-sensitive individuals shows only about 20 per cent inhibition. The percentage inhibition obtained under these conditions has been called the dibucaine number (D.N.) and this appears to be a constant characteristic for any one individual[3].

If sera from a large random sample of individuals in the general population are examined, the distribution of D.N. obtained is sharply trimodal, and people can be classified into three phenotypes on this basis[4]. About 97 per cent of the population have the so-called 'usual' phenotype with D.N. clustering around 80, about 3 per cent are of the so-called 'intermediate' phenotype with D.N. clustering around 62, and about

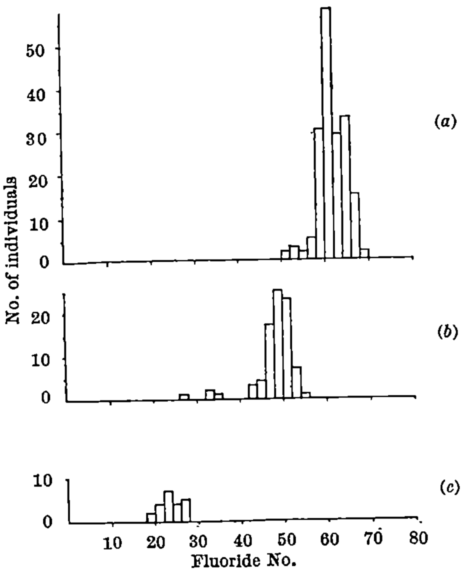


Fig. 1. Distributions of 'fluoride numbers' in individuals of the (a) 'usual', (b) 'intermediate' and (c) 'atypical' phenotypes as defined by dibucaine number determination

1 in 4,000 have the so-called 'atypical' phenotype with D.N. around 20. Suxamethonium-sensitive individuals belong to the 'atypical' phenotype. The three phenotypes are genetically determined. Two allelic genes are thought to be concerned, the 'usual' or normal phenotype being homozygous for one of these, the 'atypical' phenotype being homozygous for the other, and the 'intermediate' phenotype representing the heterozygotes[4,5]. It is likely that in the heterozygotes where both genes are present, both the 'usual' and the 'atypical' forms of the enzyme are synthesized in roughly equal amounts.

Kalow and Davies[6] have shown that a wide variety of inhibitors, but each containing a quaternary ammonium grouping, can be used to demonstrate this differential inhibition of the two enzymes. On the other hand, the differential inhibition was not obtained with the organophosphate inhibitors such as tetraethylpyrophosphonate (TEPP) or di*iso*-propylfluorophosphonate (DFP). They concluded that the esteratic site on the enzyme, where the latter inhibitors are believed to act, was probably the same in the two enzymes, while the anionic site where the positive charged quaternary ammonium inhibitors are thought to be attached is probably different.

We have now found, however, that differential inhibition of the enzymes present in the three phenotypes can also be obtained using sodium fluoride as inhibitor. Furthermore, the use of this inhibitor has enabled us to demonstrate the occurrence of at least two new phenotypes.

Sera from a series of individuals already classified by dibucaine number determinations were examined using fluoride as inhibitor. Inhibition with fluoride and determination of the so-called fluoride number (F.N.) were carried out in exactly the same way as dibucaine number determination except that 5×10^{-5} *M* fluoride was substituted for 10^{-5} *M* dibucaine in the test system. In all, sera from 179 individuals with the 'usual' phenotype, 84 individuals with the 'intermediate' phenotype, and 22 individuals with the 'atypical' phenotype were examined.

Fig. 1 shows the distribution of the fluoride numbers for the three classes. The means and standard deviations are given in Table 1. The F.N. obtained for the 'atypical' phenotype as defined by the D.N. determination are much lower than those for the 'usual' phenotype, and the phenotype 'intermediate' by D.N. determination is also intermediate in F.N. The discrimination between the three phenotypes using fluoride is nearly as clear cut as with dibucaine, and it is apparent that with few exceptions the classification of individuals obtained with fluoride is much the same as that obtained with dibucaine.

Table 1

Phenotype	No. of individuals	'Dibucaine number'		'Fluoride number'	
		Mean	Standard deviation	Mean	Standard deviation
'Usual'	179	80·06	1·56	61·35	3·21
'Intermediate'	84	61·93	4·17	47·79	4·48
'Atypical'	22	21·82	2·92	23·14	2·23

Table 2

Parental types	No. of families	Children			
		U	U_1	I	I_1
$U \times I$	20	36	—	32	—
$U \times I_1$	2	—	5	5	—

The exceptions, however, turn out to be of special interest. A plot of dibucaine number against fluoride number in the 285 individuals examined (Fig. 2) revealed that there was apparently a small sub-group of the 'intermediate' phenotype (D.N. 52–54, F.N. 26–34) in which the fluoride inhibition was relatively less marked than the dibucaine inhibition, and that a similar sub-group of the 'usual' phenotype also existed (D.N. 73–78, F.N. 50–55). The reality of these sub-groups has been confirmed by family studies. Thus, if we call the main groups of the 'usual' and 'intermediate' phenotypes U and I respectively, and the corresponding sub-groups U_1 and I_1, we have been able to compare the offspring from a series of matings $U \times I$ with those from $U \times I_1$ matings. The results are given in Table 2, and they indicate that U_1 and I_1 are genetically related.

These findings, and those from other types of mating, suggest that there may be a third gene which can influence the character of the cholinesterase

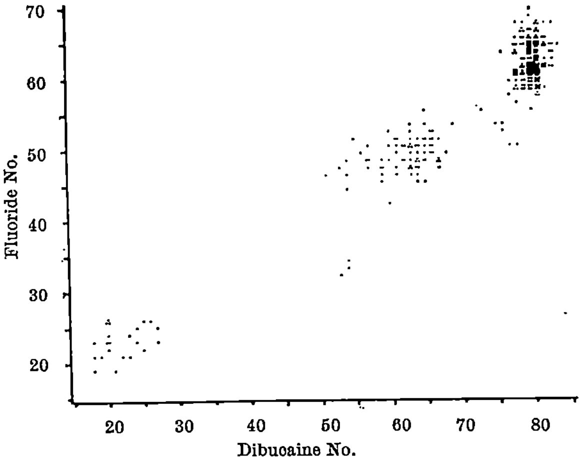


Fig. 2. Correlation between dibucaine numbers and fluoride numbers in the individuals shown in Fig. 1

synthesized. If so, the phenotype sub-groups, U_1 and I_1, would be determined by the presence of this gene in heterozygous combination, with the normal and abnormal genes previously identified. Possibly, this new gene determines yet a further variant of the enzyme which is qualitatively distinct, but much more work is required before this hypothesis can be substantiated.

We wish to thank Dr. H. Lehmann for many of the serum samples. We also wish to acknowledge a grant from the Central Research Fund of the University of London for the purchase of a spectrophotometer.

H. Harris

Department of Biochemistry,
King's College, University of London,
Strand, W.C.2.

Mary Whittaker

Department of Biochemistry,
The London Hospital Medical College,
London, E.1.

[1] Lehmann, H., and Ryan, E., *Lancet*, ii, 124 (1956).

[2] Kalow, W., *CIBA Foundation Symposium on Biochemistry of Human Genetics* (1959).

[3] Kalow, W., and Genest, K., *Canad. J. Biochem. Physiol.*, **35**, 339 (1957).

[4] Kalow, W., and Staron, N., *Canad. J. Biochem. Physiol.*, **35**, 1305 (1957).

[5] Harris, H., Whittaker, M., Lehmann, H., and Silk, E., *Acta Genet.*, **10**, 1 (1960).

[6] Kalow, W., and Davies, R. O., *Biochem. Pharmacol.*, **1**, 183 (1958).

The First Anthocyanins appearing during the Ripening of Blueberries

Although a great deal is known of the anthocyanins of berries and other fruit, this knowledge is still quite superficial and fragmentary[1]. How and in what order the anthocyanins develop in the maturing berries has only been clarified in one case: the first anthocyanin compound to appear in Californian 'Cabernet' grapes was found to be the malvidin-3,5-diglucoside; with advanced ripening of the grapes, malvidin-3-glucoside and delphinidin-3,5-diglucoside develop at almost the same time, while petunidin-3,5-diglucoside is the last to be formed[2]. Free malvidin was not observed in the grape skins until one week after the berries had developed the purple-red colour of complete maturity. These observations are noteworthy because they show that anthocyanins and not anthocyanidins are the first to appear during maturation. Furthermore, the first anthocyanin formed, malvidin-3,5-diglucoside, has quite a complicated structure. This anthocyanin is considered to be the dominant pigment in these grapes.

In order to gain further insight into the development of anthocyanins in maturing fruit, we decided to investigate the blueberry, *Vaccinium myrtillus* L., the characteristic berry of the northern forests of Europe. It is known that the ripe blueberry contains at least seven anthocyanins, derivatives of delphinidin, malvidin, petunidin and cyanidin[1]. Collecting the pigment from raw, reddish berries in sufficient amounts for identification was found to be too laborious a task. The pigments separated from raw berries were therefore compared with pigment fractions from ripe blueberries, which thereafter were used for the purpose of identification.

The pigments were extracted from the skins of raw, reddish blueberries with methanol containing 1 per cent hydrochloric acid. Two anthocyanins were found by paper chromatographic analysis of the methanolic extract (*n*-butanol/acetic acid/water, 4 : 1 : 5), one in amounts twice as large as the other. After acid hydrolysis and paper chromatographic analysis with 'Forestal' solution both these anthocyanins were identified as cyanidin derivatives. Free anthocyanidins were not found in the raw berries. Both anthocyanins were later examined by paper chromatography, with *n*-butanol/acetic acid/water (4 : 1 : 5), water/acetic acid/hydrochloric acid (82 : 15 : 3) and *n*-butanol/2 *N* hydrochloric acid (1 : 1) as solvent systems. The less-abundant compound was found to be identical with the third fraction, the more abundant pigment being identical with the fourth fraction obtained by column chromatography from ripe blueberries (Fig. 1).

The anthocyanins were extracted from the skins of ripe blueberries with the acidified methanol, precipitated as lead salts, dried, and again treated with the above solvent. From the regenerated solution a crude preparation of the pigment was obtained by addition of ether[2,3]. About 200 mgm. of the preparation was examined chromatographically

Table 1. *RF* Values of the Aglycones of the Two Most Dominant Anthocyanin Fractions of Blueberries and of Some Cyanidin Preparations, in Different Solvent Systems

Anthocyanidin	AcOH/HCl/H_2O (30 : 3 : 10)	12 *N* AcOH/6 *N* HCl (2 : 1)	BuOH/2 *N* HCl (1 : 1)	BuOH/HCl/H_2O (7 : 2 : 5)	*m*-Cresol-12 *N* AcOH/6 *N* HCl (2 : 1/1)
Fraction 3	0·50	0·37	0·62	0·81	0·42
Fraction 4	0·50	0·37	0·62	0·81	0·42
Cyanidin (from red currants, *Ribes rubrum* L.)	0·50	0·35	0·60	0·81	0·42
Cyanidin (from cyanidin-3,5-diglucoside)	0·50	—	0·62	—	—

Table 2. *RF* Values of the Two Dominant Anthocyanins of Blueberries and of Some Anthocyanin Preparations, in Different Solvent Systems

Preparation	BuOH/AcOH/H_2O (4 : 1 : 5)	BuOH/2 *N* HCl (1 : 1)	H_2O/AcOH/HCl (82 : 15 : 3)	BuOH/HCl/H_2O (7 : 2 : 5)
Fraction 3	0·38	0·26	0·22	0·43
Fraction 4	0·30	0·17	0·23	0·32
Cyanidin-3,5-diglucoside	0·15	0·05	0·38	0·19
Cyanidin-3-monoglucoside	0·30	0·17	0·24	0·33
Cyanidin-5-monoglucoside	colour unstable	0·38	0·07	0·57
Cyanidin-3-galactoside (from apples, *Pyrus malus* L.)	0·30	0·18	0·23	0·30
Cyaridin-3-galactoside (from rowanberries, *Sorbus aucuparia* L.)	0·32	0·16	0·23	0·30
Cyanidin-3-gentiobioside (from raspberries, *Rubus idaeus* L.)	0·24	0·16	0·59	0·39

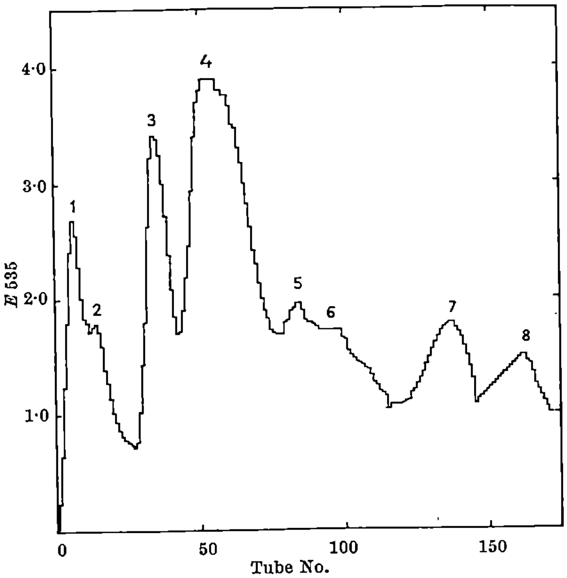


Fig. 1. Fractionation of the anthocyanins of the blueberry (*Vaccinium myrtillus* L.) using a cellulose column filled with Whatman cellulose powder and *n*-butanol/2 *N* hydrochloric acid (1 : 1) as developing solvent

in a column (5 cm. × 110 cm.) filled with cellulose powder (Whatman cellulose powder, standard grade or 'Linterspulver' No. 124, Schleicher and Schüll), *n*-butanol/2 *N* hydrochloric acid (1 : 1) being used as the solvent system[4]. The eluate was collected in 10-ml. fractions and the colour was measured at 535 mμ. From a graph with plots of extinction values (Fig. 1), it was found that the anthocyanins of the blueberry separate out into 8 peaks.

Samples of the eluates of peaks 3 and 4, which correspond to the anthocyanins of reddish blueberries, were hydrolysed by heating. The R_F values obtained by paper chromatography of the liberated anthocyanidins are shown in Table 1. The results establish that the aglycones of both anthocyanin fractions can be identified as cyanidin. This was confirmed by the distribution and oxidation tests and tests with the cyanidin reagent, sodium acetate and ferric chloride (Robinson's method).

The sugars were determined from the hydrolysed fractions 3 and 4. The water extracts were concentrated to about 10 ml., the hydrochloric acid was eliminated by extracting with 10 per cent di-*n*-octylmethylamine containing chloroform, and the solution was further concentrated to 1 ml. The sugars were identified by paper chromatography (*n*-butanol/acetic acid/water, 4 : 1 : 5), and it was observed that fraction 3 contained glucose and arabinose, fraction 4 glucose only. Control experiments proved that the arabinose was not an artefact produced either from the Whatman cellulose powder or from the paper (see ref. 5). Aniline–citric acid reagent was used in the quantitative determinations of the sugars. Comparison of the separately determined concentrations of the aglycones with the sugar concentrations proved that the concentration of glucose in both fractions was in almost equimolar proportion to the concentration of the anthocyanidin. The amount of arabinose, however, was found to be only half the equivalent amount, possibly owing to disintegration or partial splitting.

Table 2 shows the values obtained by paper chromatographic analysis of the anthocyanins. The R_F values of the anthocyanin in fraction 4, the largest fraction, in different solvent systems was found to correspond to the R_F values of cyanidin-3-glucoside. Considering the equivalent proportions of the aglycone and the sugar, the anthocyanin must be cyanidin-3-glucoside. In fraction 3 the R_F values of the arabinose-containing cyanidin derivative do not—as expected—agree with the R_F values of the reference preparations. According to the literature, however, the R_F values of this fraction closely approach the migration of cyanidin-3-glucopentosides.

In conclusion, the two anthocyanins initially appearing during the maturation process of blueberries are both found to be cyanidin derivatives. These two cyanidin derivatives, the first to appear being cyanidin-3-glucoside, and the arabinose-containing cyanidin derivative (possibly cyanidin arabinoglucoside), are the most abundant pigments of ripe blueberries, although it is known that the ripe berries also contain glycosides of malvidin, delphinidin and petunidin[1]. The order of appearance of these pigments during the ripening process bears out the earlier concept of Robinson[6] that biogenetically cyanidin should be regarded as the simplest of the anthocyanidins.

We thank Dr. C. G. Nordström for the preparation of cyanidin-3,5-diglucoside which he kindly placed at our disposal.

Heikki Suomalainen
A. J. A. Keränen

Research Laboratories of the
State Alcohol Monopoly (Alko),
Helsinki.

[1] Suomalainen, H., and Eriksson, Chr., *Lebensmittel-Untersuch. u. Forsch.*, **112**, 197 (1960).

[2] Bockian, A. H., Kepner, R. E., and Webb, A. D., *J. Agric. Food Chem.*, **3**, 695 (1955).

[3] Li, K. C., and Wagenknecht, A. C., *J. Amer. Chem. Soc.*, **78**, 979 (1956).

[4] Chandler, B. V., and Harper, K. A., *Nature*, **181**, 131 (1958).

[5] Harborne, J. B., *J. Chromatography*, **1**, 473 (1958).

[6] Robinson, R., *Nature*, **137**, 172 (1936).

PHYSIOLOGY

Effect of Administration of Lysergic Acid Diethylamide on Serotonin-Levels in the Body

In a previous publication[1], we have suggested that some of the effects of lysergic acid diethylamide (LSD) may be mediated through its effect on the serotonin content of brain. Brodie and his associates[2] have previously reported that LSD—and also some other drugs—do not release serotonin as reserpine does. However, Freedman[3] has recently reported slightly raised levels of serotonin in the whole brain of rats treated with LSD. Using animals which had been given reserpine, they found a twofold increase of brain serotonin in rats treated with LSD over the controls receiving reserpine alone. The whole brain serotonin may not be a true indication of the action of a psychopharmacological agent. Thus Vogt and her associates[4] have reported different effects of LSD and serotonin in different areas of the brain after intraventricular injection.

We have investigated the effect of LSD on the serotonin content of the different parts of the rabbit brain. The brain was divided into four major parts : (1) cerebral hemispheres, excluding midbrain, pons, thalamus, hypothalamus, etc., and mostly consisting of the grey and white matter; (2) cerebellum; (3) stem; and (4) rest of the brain.

The animals were given intravenously 500 µgm. of LSD and were killed 15 min. later. In a second series of investigations, the rabbits were given intravenously 0·92 mgm., equal to 10 µc., of 5-hydroxytryptophan-3-^{14}C (5-HTP) followed by 500 µgm. of LSD 45 min. later. The experimental animals were killed 15 min. after the administration of LSD, while the controls received no LSD and were killed 1 hr. after the administration of the 5-HTP. Since the nature of the results as to the effect of LSD on the serotonin content was similar in both series of investigations, the results of the second series only are reported here. Table 1 presents the results as an average of four to six animals for each group. The first column represents the amount of total radioactivity per gm. of tissue and consists of the radioactivity due to 5-HTP, serotonin and their metabolites. The second column presents the content of serotonin determined spectrophotofluorimetrically, while the third one shows the specific radioactivity of the serotonin. The experimental details will be presented in detail elsewhere.

Table 1. EFFECT OF ADMINISTRATION OF LYSERGIC ACID DIETHYLAMIDE ON THE LEVELS OF SEROTONIN IN THE BODY

Tissue	Total radioactivity (counts per min. per gm. tissue)		Serotonin (µgm. per gm. tissue)		Specific radioactivity of serotonin (counts/min./µgm.)	
	Control	LSD	Control	LSD	Control	LSD
Liver	884	665	1·74	2·62	14·3	15·0
Lung	545	439	1·02	1·46	7·0	3·0
Kidney	1,992	3,089	1·04	1·78	17·0	25·0
Spleen	1,753	1,713	42·14	55·14	1·8	2·3
Heart	277	346	1·34	3·86	—	3·3
Cerebrum	76	83	0·96	0·90	1·2	5·7
Cerebellum	112	450	1·24	1·72	0·7	2·1
Stem	108	273	2·12	2·94	—	6·8
Rest of brain	132	469	2·08	2·68	1·4	4·6

The effect of the administration of small quantities of 5-HTP was a small rise in the serotonin content of the tissues. The spleen is an extremely rich source of serotonin. Several interesting facets of serotonin metabolism are revealed by this work. The specific radioactivity of the serotonin is an index of the metabolic rate of serotonin in the tissue. Thus, kidney and liver are the most active sites, while the serotonin of the spleen is not in such an active state. It could be seen that the total radioactivity of the kidney is enhanced very much subsequent to the administration of LSD. This suggests that a considerably larger amount of the 5-HTP is transported to the kidney in the animal treated with LSD. The increase in the specific activity of serotonin is highest in the kidney, while on a percentage basis it is next highest to the brain tissues. This indicates an increased turnover of serotonin in the kidney and also a general increase in the serotonin content of the different parts of the viscera.

In the cerebrum there is no increase in the serotonin content in the animal treated with LSD. This is presumably due to the rapid destruction of the serotonin by oxidation, as is indicated by the higher specific activity of the serotonin. In other parts of the brain also, whereas the serotonin content increases only by approximately 40 per cent, the specific activity increases by about 350 per cent.

Thus these results indicate an overall increased metabolism of serotonin and an increase of the content of serotonin itself in all parts of the body examined, except in the cerebrum, subsequent to the administration of LSD. Whether the effects of LSD are specific or are shown by its non-hallucinogenic analogue, bromo lysergic acid diethylamide, and whether the action of LSD is limited to serotonin only without significant effects on other neurohormones such as noradrenaline is being investigated.

D. V. SIVA SANKAR
D. BARBARA SANKAR
EDWARD PHIPPS
ELEANOR GOLD

Biochemical Research Laboratory,
Children's Unit,
Creedmoor State Hospital,
Jamaica 27, New York.

---

[1] Siva Sankar, D. V., and Bender, L., in *Recent Progress in Biological Psychiatry*, edit. by Wortis, J., 363 (Grune and Stratton, New York, 1960).

[2] Carlsson, A., Shore, P. A., and Brodie, B., *J. Pharmacol. and Exp. Therap.*, **120**, 334 (1957).

[3] Freedman, D. X., *Fed. Proc.*, **19**, 266 (1960).

[4] Vogt, M., Gunn, C. G., and Sawyer, C. H., *Neurology*, **7**, 559 (1957).

Effect of the Bilateral Section of the Splanchnic Nerve on Erythropoiesis

DESPITE much recent work on erythropoietin which has established the important part played by the humoral factor in erythropoiesis, participation of the nervous system in the regulation of erythropoiesis has not been established. In view of the report of Jacobson *et al.*[1] suggesting the renal production of erythropoietin, as well as the work of Nakayama[2] that showed the effect of the bilateral section of splanchnic nerve in abolishing the decrease of renal blood-flow on loss of blood or anoxic anoxia, we performed a bilateral section of splanchnic nerve in rats to observe the effect on erythropoiesis.

The splanchnic nerve was sectioned bilaterally in the Wistar female rats (180–200 gm.) through the

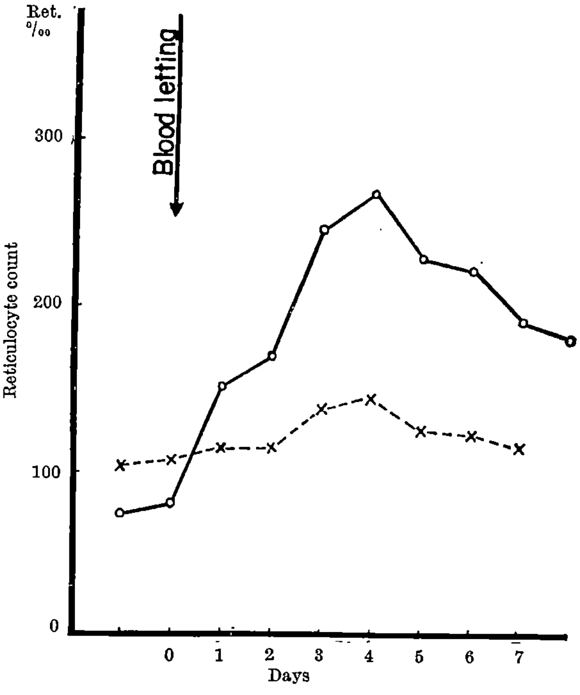


Fig. 1. Effect of bilateral section of splanchnic nerve on reticulocytosis after blood-letting (30 per cent of total blood volume). Each point represents the mean value for five rats. o, normal; ×, operated

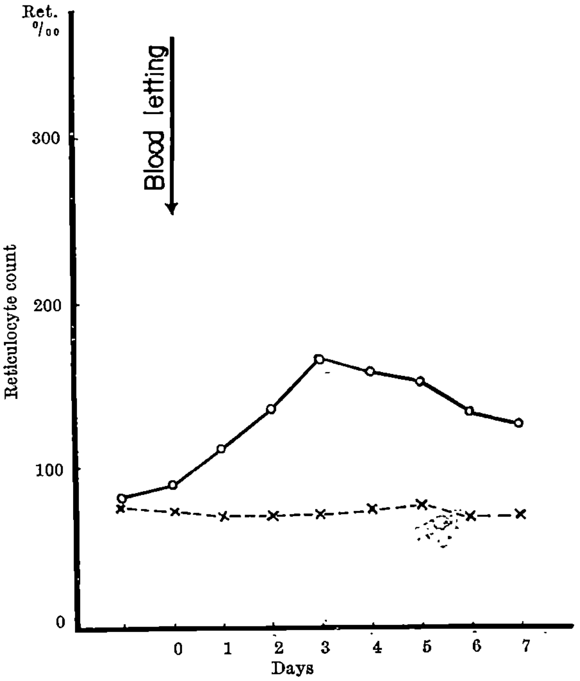


Fig. 2. Effect of bilateral section of splanchnic nerve on reticulocytosis after blood-letting (20 per cent of total blood volume). Each point represents the mean value of five rats. o, normal; ×, operated

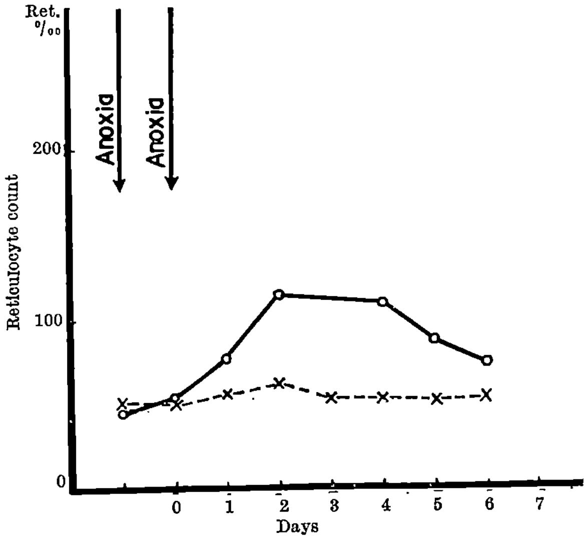


Fig. 3. Effect of bilateral section of splanchnic nerve on reticulocytosis after anoxic anoxia. Each point represents the mean value of five rats. o, normal; ×, operated

abdominal approach. After being bled as much as 1·0–1·5 per cent of their body-weight representing a loss of 20–30 per cent of the total blood volume[3] by cardiac puncture, the changes in the reticulocyte counts were followed in normal and operated rats. The results of experiments in Figs. 1 and 2 show that the loss of 30 per cent and 20 per cent of their total blood volume caused only a slight or no increase in reticulocytes in rats with a sectioned splanchnic nerve in contrast to the marked response to the same procedures for normal rats. When subjected to a mild anoxic anoxia, the rats with a sectioned splanchnic nerve showed no increase in their reticulocyte counts (Fig. 3). Such loss of the reticulocytic response in rats with sectioned splanchnic nerve suggests that the reduction of renal blood-flow which is regulated through the splanchnic nerve plays an important part in the production of erythropoietin when there is loss of blood or anoxic anoxia.

The reduction of the flow of arterial blood to the abdominal organs, including the kidney, in case of loss of blood has been explained as a mechanism for protecting the brain or heart from anoxia. By the experiments performed in our laboratory, it is suggested that the reduction of flow of arterial blood through the kidney in case of loss of blood occurs not only to protect the brain or heart but also to stimulate the erythropoietin production to facilitate the recovery from anæmia.

FUMIMARO TAKAKU
KUNITAKE HIRASHIMA
SHIGEO OKINAKA

Third Department of Internal Medicine,
School of Medicine,
University of Tokyo,
Bunkyo-ku, Hongo, Tokyo.

[1] Jacobson, L. O., Goldwasser, E., Fried, W., and Plzak, L., *Nature*, **179**, 633 (1957).

[2] Nakayama, S., *Respiration and Circulation*, **7**, 191 (1959) (in Japanese).

[3] Gray, S., and Sterling, K., *J. Clin. Invest.*, **29**, 1614 (1950).

Dependence on Substrate of the Electrical Potential across the Isolated Gut

PUBLISHED reports[1,2] have indicated that fluid and salt transport across the isolated small intestine is very low in the absence of an external supply of substrate. Chemical measurements of net transport have shown that, of the many substrates tested, only glucose is able to support optimal absorption[1]. With such measurements, the tissue must be allowed to equilibrate for 20–30 min. before sufficient fluid or salt has been transported to allow accurate chemical measurements. Thus a considerable uncertainty exists as to how rapidly the absorptive process changes when glucose is added to or removed from the bathing media. A clarification of this point would indicate the extent to which utilizable energy is stored in the tissue.

By use of electrical techniques, rapid changes in salt absorption across the epithelia can be followed[3]. In this investigation, it was decided to monitor changes in the electrical potential difference established across the tissue as a consequence of the active absorption of sodium chloride[4,5]. Electrical potentials across isolated segments of rat small intestine (Rochester strain of Wistar) were measured by a technique described before[5], in which the small intestine could be transferred rapidly (within 3 sec.) from one solution bathing the mucosal side to another. Since the potential was found to be unaffected by the glucose concentration of the serosal solution, only the results of glucose changes in the mucosal solution will be presented.

Fig. 1 records the effects of glucose on the potential developed across the mid-jejunal segments. When the tissue was exposed to 200 mgm. per cent glucose in the presence of oxygen/carbon dioxide, the potential was maintained for at least 60 min. However, when the segment was transferred to a glucose-free medium a 60 per cent drop in potential occurred in 2 min. On returning to glucose medium, the potential was restored within 3–5 sec. These data clearly indicate that there is very little utilizable stored energy.

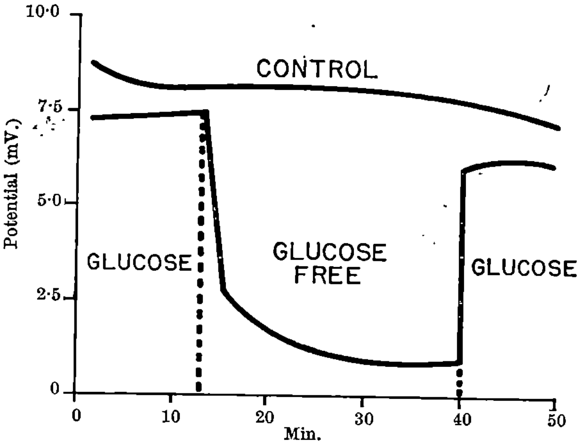


Fig. 1. Effect of glucose on the electrical potential across isolated segments of rat small intestine. Everted mid-jejunal segments were exposed to Krebs–Ringer bicarbonate buffer containing 200 mgm. per cent glucose and to a similar buffer containing no glucose at 37° C. in the presence of 95 per cent oxygen/5 per cent carbon dioxide. Ileal segments behaved identically except that their potentials were, on the average, 50 per cent less. The positive recording electrode was in contact with the serosal solution

The rapid recovery of the potential on addition of glucose deserves further comment. Chinard *et al.*[6] have reported that the mean transit time for passage of glucose across the mucosal cell of the proximal tubules of the kidney is 10 ± 3 sec. Since both cells have a similar structure and are able to transport glucose actively, one would conclude that the mean time of entry for glucose moving into the intestinal cell is about 5 sec., corresponding to the recovery time of the potential as noted in Fig. 1. Thus it is seen that the active transport of salt is stimulated immediately on the entry of glucose into the mucosal cell.

Investigations on the relationship of energy metabolism to active transport have been reported for frog skin[7] and toad bladder[8]. These epithelia have sufficient endogenous substrate to support active transport for many hours. On the other hand, the intestine, because of its complete dependence on external substrate, allows the person performing experiments a greater degree of control of the energy supply. For this reason the isolated small intestine presents an excellent preparation for the study of transfer of energy within the cell.

This investigation was carried out under contract with the United States Atomic Energy at the University of Rochester Atomic Energy Project, Rochester, New York.

T. W. Clarkson
A. C. Cross
S. Toole

University of Rochester School
of Medicine,
Rochester 20, New York.

[1] Smyth, D. H., and Taylor, C. B., *J. Physiol.*, **136**, 632 (1957).

[2] Lifson, N., and Parsons, D. S., *Proc. Soc. Exp. Biol. Med.*, **95**, 532 (1957).

[3] Ussing, H. H., *Fourth International Congress of Biochemistry*, Symp. 3, 1 (Pergamon Press, London, 1959).

[4] Curran, P. F., and Soloman, A. K., *J. Gen. Physiol.*, **41**, 143 (1957).

[5] Clarkson, T. W., and Rothstein, A., *Amer. J. Physiol.*, **199**, 12 (1960).

[6] Chinard, F. P., Taylor, W. R., Nolan, M. F., and Enns, T., *Amer. J. Physiol.*, **196**, 535 (1959).

[7] Leaf, A., and Renshaw, A., *Biochem. J.*, **65**, 90 (1957).

[8] Leaf, A., Page, L. B., and Anderson, J., *J. Biol. Chem.*, **234**, 1625 (1959).

Soluble Material in the Gastrointestinal Tract of Rats under Normal Feeding Conditions

Work on intestinal absorption has almost always been carried out under non-physiological conditions—on fasted animals fed by stomach tube, on anæsthetized animals or animals with fistulæ and on isolated loops. Moreover, single nutrients have usually been administered. It seemed desirable to obtain information under more nearly physiological conditions and to examine the medium from which absorption was proceeding at different levels of the gastrointestinal tract.

Adult male rats were kept in an automatically lighted room arranged so that the period of darkness during which they do most of their feeding was 5 a.m.–2 p.m. They were provided with Thomson cube diet containing 14 per cent dried milk and water *ad libitum*. The animals were killed between 9·0 and 10·0 a.m. and immediately taken to a cold room (4° C.) where the stomach and small intestine were removed. The stomach (S) was tied off and the intestine divided into four arbitrary segments of approximately equal length labelled I_1–I_4, beginning at the pylorus. The contents of the stomach and intestinal segments were removed and spun in a refrigerated centrifuge at 4,000 r.p.m. for 20 min. This usually gave 0·05–0·5 ml. fluid for analysis.

In all animals the stomach contained 3–5 ml. of thick pasty material while the most proximal intestinal segment (I_1) was almost empty, containing only a small amount of thick mucous fluid with few insoluble food residues. The other intestinal segments (I_2–I_4) contained large amounts of material looking like homogenized rat cubes.

As shown in Table 1, there was a large reduction in the concentration of potassium between (S) and (I_2), the concentration in I_3 and I_4 remaining steady. The fall in the concentration of sodium was less pronounced. Chloride estimations were carried out by the Conway micro-diffusion method but the results were not included in the table since they varied widely from animal to animal. But while concentrations of more than 200 m.equiv./l. were sometimes found in the stomach the values for I_3 and I_4 never exceeded 10 m.equiv./l. The nature of the anions present in the lower half of the small intestine, where the concentration of $Na^+ + K^+$ amounted to 80–100 m.equiv./l., is not clear. Bicarbonate may have been present but was not estimated; yet supposing it was present at a level similar to that of plasma, that is, 27 m.equiv./l., this would leave more than 40 m.equiv./l. anions not accounted for. Some of the base may have been bound by the products of protein digestion since the amino nitrogen present in I_3 and I_4 was about 1 gm./l. or 70 m.equiv./l.

The concentration of total nitrogen in the centrifugates was highest in I_1, exceeding that in the stomach, and it fell progressively in the lower segments. The bulk of the nitrogen-containing material was not precipitable by sodium tungstate, that is, was non-protein nitrogen. The pattern for total nitrogen concentration was roughly paralleled by the non-protein levels.

Reducing and total sugar concentrations are expressed as glucose. There was a marked fall in the concentration of free and total reducing sugar between S and I_1 and this did not appear to be due to simple dilution since in all cases only small quantities of material were present in I_1, reflecting the rapid

Table 1. CONCENTRATION OF VARIOUS CONSTITUENTS OF THE SOLUBLE MATERIAL PRESENT AT DIFFERENT LEVELS OF THE GASTRO-INTESTINAL TRACT OF RATS UNDER NORMAL FEEDING CONDITIONS

Segment	S	I_1	I_2	I_3	I_4
Potassium (m.equiv./l.)	135 (± 9·3)	51·5 (± 6·6)	29·0 (± 3·0)	18·5 (± 3·5)	18·6 (± 1·9)
Sodium (m.equiv./l.)	136·6 (± 9·9)	109·4 (± 14)	85·1 (± 18)	80·1 (± 8·7)	68·3 (± 10·0)
Total nitrogen (gm./l.)	6·6 (± 1·0)	9·1 (± 3·1)	6·3 (± 1·3)	4·77 (± 0·45)	3·85 (± 0·26)
Non-protein nitrogen (gm./l.)	4·16 (± 1·3)	6·56 (± 3·1)	5·8 (± 1·5)	4·02 (± 0·6)	3·44 (± 0·28)
Free reducing sugar (as gm. glucose/l.)	68·4 (± 5·8)	10·9 (± 3·6)	21·2 (± 3·8)	21·5 (± 2·9)	20·8 (± 4·1)
Total reducing sugar (as gm. glucose/l.)	93·4 (± 15·4)*	See footnote †	29·3 (± 5·6)*	33·6 (± 4·7)*	35·1 (± 6·2)*

Figures in brackets show the standard deviation for five observations except where indicated by *, when only four observations were made.
† Three observations only. In these, the increase in reducing power on hydrolysis was 1·61 (± 0·29) gm./l.

passage of food through the upper part of the small intestine. The slight difference between the free and total reducing sugar in this segment suggested that monosaccharides constituted the major carbohydrate fraction.

The free reducing sugar was remarkably constant between segments I_2, I_3 and I_4, where it was present at a concentration of about 2 per cent. This constancy was unexpected since it is generally believed that all digestible carbohydrate is absorbed before the chyme leaves the small intestine, and some decrease in reducing power in the last segment might therefore have been expected. The results are of interest in view of Fisher's[1] finding that absorption of water only proceeds from the isolated small intestine of the rat when glucose is present in the lumen.

It is emphasized that these are preliminary results with a small number of animals (5) and that certain improvements of technique are considered to be necessary, for example, a different method of killing instead of the ether anæsthesia and bleeding used here; nevertheless the results are sufficiently consistent to suggest that this more physiological approach to the study of gastro-intestinal function may be of value.

ANNE S. COLE

Department of Physiology,
University of Bristol.

---

[1] Fisher, R. B., *J. Physiol.*, 130, 655 (1955).

HÆMATOLOGY

Stability of Freeze-dried Anti-hæmophilic Globulin

THE stability of anti-hæmophilic globulin in solution has been the subject of many investigations. Hitherto, no systematic work on the factors affecting the activity of dried material has been reported.

In order to obtain comparable results in the present investigation, the period necessary for the loss of 50 per cent of the activity of anti-hæmophilic globulin[1], subjected to the necessary conditions of each experiment, was estimated. Bovine, porcine and human anti-hæmophilic globulins were utilized; no appreciable difference was observed in their behaviour.

Type of container: Anti-hæmophilic globulin stored at room temperature in stoppered M.R.C. bottles or universal containers lost half the activity in two months. Storage in darkness had no significant effect. On the other hand, sealed ampoules preserved the same activity for four months.

Effect of temperature: Sealed ampoules were used in this test. Deterioration was rapid at 37° C.; the standard loss occurred after 5–8 days. At 4° C. the same loss was observed after storage for six months.

Storage under vacuum over phosphorus pentoxide: This had practically the same effect as that produced by storage in sealed ampoules.

Humidity: When samples of anti-hæmophilic globulin in loosely stoppered universal bottles were kept in a humid atmosphere, two weeks were sufficient to lower the activity to 50 per cent.

Time of packing: The potencies indicated on commercial ampoules were invariably found to be higher than my estimates. This led to inquiries which showed that in certain cases this was most probably due to a time-lag between the completion of the drying process (when the activity was determined) and packing. When this delay was minimized, not only did our figures coincide but also the preservation of the activity was prolonged; the subsequent loss of activity of samples treated in this way was almost halved.

It would now appear that in order to obtain and preserve the maximal activity of anti-hæmophilic globulin, delay should be avoided in drying and sealing the material in evacuated or nitrogen-filled ampoules, which should be stored at 4° C.

This work was supported by a grant from the United Manchester Hospitals. I thank Dr. J. F. Wilkinson for laboratory facilities.

F. NOUR-ELDIN

Department of Pathology,
Southmead Hospital,
Bristol.

---

[1] Nour-Eldin, F., *Brit. Med. J.*, ii, 502 (1960). Nour-Eldin, F., and Wilkinson, J. F., *Brit. J. Hæmat.*, 4, 292 (1958).

New *Rh* Phenotype, $Dcce^ie^if$, found in a Chibcha Indian Tribe

IN the course of serological work on various Chibcha-Indian tribes of Colombia it was found that the blood of 6 out of 113 Ica Indians, inhabitants of the Sierra Nevada of Santa Marta, reacted with anti-*D* and anti-*c* sera but failed to react with anti-*C*, anti-*E*, and anti-*e* sera. Further laboratory work carried out at the Caracas Blood Bank as well as in the Blood Group Research Unit of the Lister Institute of London and at the Mount Sinai Hospital in New York has demonstrated that the six samples previously mentioned reacted with anti-*f* and with some anti-*e* sera as well.

Considering these results, we may call the new phenotype $Dcce^ie^if$ and the chromosome $Dce^if(R_i)$, using the *i* as indicative of the tribal denomination of the Ica among whom the phenotype was found for the first time. Genetic studies of a family carrying this chromosome, together with the serological evidence for the differentiation of this phenotype from other *Rh* gene complexes like *Dc-* (ref. 1), will be published elsewhere (Layrisse, M., Layrisse, Z., and Wilbert, J.).

Table 1 shows the frequencies of the *Rh* phenotypes and chromosomes found in 113 samples. For the calculation of the chromosomes we assumed that the phenotype *Dcce* is the expression of dce/Dce^i, since the chromosome *Dce* is rare in pure Indians and since no Negro genes were found in this population: *Js*(*a*–), hæmoglobins *S* and *C* were negative, and the gene *B* (0·44 per cent) was lower than A_1 (1·34 per cent).

Table 1. *Rh* FREQUENCY IN ICA TRIBE USING ANTI-*D*, ANTI-*C*, ANTI-*c*, ANTI-*E* AND ANTI-*e*

Phenotypes	Genotypes	No. observed	Frequency observed	Frequency expected	χ^2 $\left(\frac{\text{Obs.}-\text{exp.}}{\text{Exp.}}\right)^2$
DCCEE	*DCE/DCE*	0·00	0·00	0·00	—
DCCEe	*DCE/DCe*	0·00	0·00	0·00	—
DCCee	*DCe/DCe*	46·00	40·71	36·75	0·43
DCcE	DCE/Dce^i *DCE/DcE*	0·00	0·00	0·00	—
DCcEe	*DCe/DcE*	10·00	8·85	13·39	1·53
DCce	*DCe/dce* DCe/Dce^i	35·00	30·97	34·35	0·33
DccE	*DcE/DcE* DcE/Dce^i	5·00	4·42	5·66	0·25
DccEe	*DcE/Dce*	5·00	4·42	1·81	
Dcce	dce/Dce^i	5·00	4·42	3·31	0·44
ddccee	*dce/dce*	1·00	0·89	0·68	
$Dcce^ie^i$	Dce^i/Dce^i	6·00	5·31	4·04	0·40
Total		113·00	99·99	99·99	3·38 $0{\cdot}70 > P > 0{\cdot}50^{n=5}$

Chromosomes (per cent)	
DCe	60·62
DcE	11·05
dce	8·22
Dce^i	20·11

The peculiar distribution of the chromosome Dce^if among the Chibcha tribes studied is rather surprising. Whereas it occurs in a relatively high frequency among the Ica (20 per cent), the phenotype which identified this chromosome was absent in the two other tribes (Tunebo and Paez) of the same linguistic affiliation (Layrisse, M., Layrisse, Z., and Wilbert, J.).

Since not more than 10 South American Indian tribes have been tested with both anti-*E* and anti-*e*, we shall have to wait for further results before any statement concerning the frequency of the new chromosome in the autochthonous population of the New World can be advanced.

We thank Drs. R. Sanger and R. R. Race for assisting in the study of the family carrying the chromosome; Dr. R. E. Rosenfeld, who provided anti-*f* serum and tested one case of the phenotype $Dcce^if$ shown in Table 1, and Dr. Levine, who provided another serum used in this work. The Creole Foundation, Caracas, sponsored the project and the Instituto Colombiano de Antropología rendered most useful assistance.

MIGUEL LAYRISSE
ZULAY LAYRISSE
ESPERANZA GARCÍA

Instituto Venezolano,
Investigaciones Cientificas,
Centro de Investigaciones,
Banco Municipal de Sangre,
Caracas, Venezuela.

JOHANNES WILBERT

Fundación La Salle de
Ciencias Naturales,
Caracas, Venezuela.

JOAQUÍN PARRA R.

Instituto Colombiano de
Antropología,
Bogotá, Colombia.

---

[1] Tate, H., Cuningham, C., McDade, M. C., Tipett, P. A., and Sanger, R., *Vox Sanguinis*, 5, 398 (1960).

HISTOCHEMISTRY

Direct Relationship of Phosphorylase and Mitochondrial α-Glycerophosphate Dehydrogenase Activity in Skeletal Muscle

PREVIOUS work[1] showed that in mammalian and avian muscle fibres there was a reciprocal relationship between phosphorylase and the oxidative enzymes, as shown by histochemical techniques. Two groups of fibres were described. In the histologically larger group, there was high phosphorylase activity and low activity of oxidative enzymes. (These included succinate, lactate, malate, isocitrate, β-hydroxybutyrate, alcohol and glutamate dehydrogenases, and also the DPN-linked α-glycerophosphate dehydrogenase.) In the group of histologically smaller fibres the converse activities were present.

With the development of a histochemical technique capable of demonstrating the purely mitochondrial α-glycerophosphate dehydrogenase[2], which is not linked with coenzyme, its localization in muscle has become possible. The enzyme is found to be strongly concentrated in the group of larger fibres. Activity in the smaller fibres is weak or absent. Mitochondrial α-glycerophosphate dehydrogenase activity thus runs parallel with phosphorylase activity, unlike all other oxidative enzyme systems so far tested.

Since the non-mitochondrial α-glycerophosphate dehydrogenase is strongest in the group of smaller fibres, the present findings do not fully support the concept that an α-glycerophosphate cycle[3] is an important metabolic pathway in the larger fibres. They do, however, fit the supposition that dihydroxyacetone phosphate, produced by α-glycerophosphate dehydrogenase activity, could enter the Embden–Meyerhof pathway at the level of triosephosphate isomerase. They provide further support for the thesis that the two types of muscle fibre rely substantially on different metabolic pathways in obtaining their supplies of energy for muscular contraction.

A. G. E. PEARSE

Postgraduate Medical School,
London, W.12.

---

[1] Dubowitz, V., and Pearse, A. G. E., *Nature*, 185, 701 (1960); *Histochemie*, 2, 105 (1960).

[2] Wattenberg, L. W., and Leong, J. L., *J. Histochem. Cytochem.*, 8, 296 (1960). Hess, R., and Pearse, A. G. E., *Nature* (in the press).

[3] Bücher, Th., and Klingenberg, M., *Angew. Chem.*, 70, 552 (1958). Zebe, E., Delbrück, A., and Bücher, Th., *Biochem. Z.*, 331, 254 (1959).

HISTOLOGY

Deoxyribonucleic Acid Content of Nuclei dividing amitotically

THE presence of a rather high percentage of binucleated cells in the rat epididymis, especially in the body and tail, is well known[1–3]. The two nuclei, which are approximately equal in volume, arise by amitotic division, as judged by the complete series of morphological stages which can be traced from a small radial fold in the nucleus to its complete subdivision by a furrow. The nuclei remain in close contact with one another, usually with their apposed surfaces flattened.

It is now known that cells about to divide mitotically have twice the deoxyribonucleic acid (DNA) content of resting, undividing cells and that binucleate cells, at least in the liver, have twice the normal diploid amount of DNA[4-6]. It seemed of interest to determine whether the same phenomena would be observed in cells dividing amitotically.

Small portions of the neck of the rat epididymis were dissociated by trypsin digestion and squashed in isotonic sucrose. Fixation for 15 min. in formalin vapour was followed by extraction of plasmalogenic lipids in alcohol and boiling alcohol-ether. After rehydration the slides were stained by the Feulgen procedure. The nuclei were homogeneous and well suited to photometry.

The optical density of the nuclei of mononucleate and binucleate cells was determined in a modified Lison-type histophotometer. With the densities encountered glare could be completely neglected. The projected area of each nucleus was sampled representatively (10 readings) and the resulting mean density multiplied by the area (planimetrically determined) to give DNA content in arbitrary units. Since there was some variation in the measured mean DNA contents in the five specimens of epididymis examined, the values were recalculated to a common mean on the basis of the DNA content of the mononucleate cells. These always formed a single population of nuclei as judged by inspection of the frequency histograms.

Histograms showing the DNA contents of both mononucleate and binucleate cells are shown in Fig. 1. It will be seen that the nuclei of mononucleate cells form a single population. Inspection shows no evidence of any skewness of the frequency distribution, as might be expected if there were a doubling of DNA content before the amitosis.

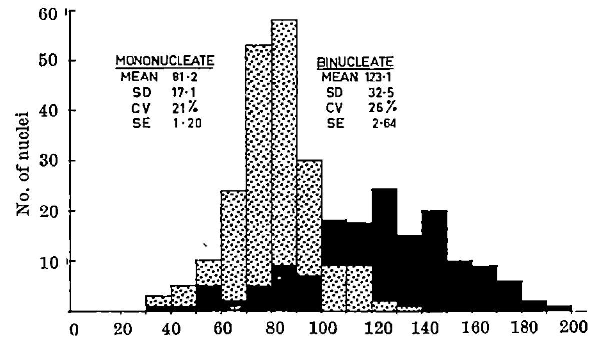


Fig. 1

The binucleate cells also form a single population of cells with respect to DNA content, the mean value being almost exactly half as much again as that of the mononucleate cells (123·1 ± 2·64 as against 81·2 ± 1·20).

It is clear that there is no doubling of the DNA content of the nuclei either before or after amitotis but that, when the amitosis has occurred, there is an increase in DNA content to a value which is 150 per cent of that of the mononucleate cells.

These results can be explained by the following theory: in a nucleus like a rat epididymal nucleus which is not subject to mitosis or endomitosis (endopolyploidy) the presence of two homologous chromosomes normally inhibits duplication of either one. When, however, the homologous chromosomes are separated from one another by the development of the nuclear partition which arises during amitotic division, the control mechanism no longer operates and each duplicates itself (polysomaty or polynemy).

When the chromosome number is large (as in the rat), and in the absence of any somatic pairing, the distribution of paired and unpaired chromosomes in the two nuclei arising by amitosis closely approximates a binomial distribution in which $p = q = 0 \cdot 5$ and $n = 21$ (the number of chromosome pairs). The mean value is 10·5 paired and 21 unpaired chromosomes. Duplication of these unpaired chromosomes would lead to an increase of 50 per cent in the DNA content of the two nuclei.

The total variability in the binucleate cells is the result of normal biological and experimental variation and of the variation in the number of paired chromosomes. The first source may be calculated from the variance found in mononucleate cells and the second calculated from the above binomial expression. On this basis the expected standard deviation of the DNA content of the binucleate cells is 32, which is very similar to the value actually obtained (32·5).

The work here described appears to conflict with that of Vendrely[7], who measured the DNA content of about 90 nuclei chosen randomly from serial sections of mouse epididymis. Normal values were found except for some intermediate between diploid and tetraploid levels; these were ascribed to the presence of mitotic activity in the epididymis. Vendrely does not say how many of the nuclei measured were double.

I am indebted to Prof. H. O. Lancaster for help with the statistics. The work has been supported by the National Health and Medical Research Council and the Normal Haire and Cancer Research Funds of the University of Sydney.

K. W. Cleland

Department of Histology and Embryology,
University of Sydney.

[1] Regaud, C., *C.R. Soc. Biol., Paris*, **53**, 616 (1901).
[2] Benoit, J., *Arch. Anat. Strasbourg*, **5**, 174 (1926).
[3] Reid, B. L., and Cleland, K. W., *Austral. J. Zool.*, **5**, 223 (1957).
[4] Swift, H., *Internat. Rev. Cytol.*, **2**, 1 (1953).
[5] Vendrely, R., and Vendrely, C., *Internat. Rev. Cytol.*, **5**, 171 (1956).
[6] Leuchtenberger, C., in *Gen. Cytochem. Methods*, **1**, 220 (1958).
[7] Vendrely, C., *C.R. Soc. Biol., Paris*, **152**, 652 (1958).

Photo-Sensitivity of Thiazine Leucobases

During the course of experiments designed to investigate the staining of nucleic acid in histological sections, I happened to gather together on one bench a number of solutions of basic dyes reduced to their colourless sulphite leucobase. These included methyl green, basic fuchsin and a series of thiazines, namely, thionin, azure *A*, *B* and *C*, and methylene blue (for a discussion of the structure of these thiazine dyes, see Baker[1]).

The basic fuchsin leucobase (Schiff reagent) was prepared according to the directions of de Tomasi[2]. The other leucobases were prepared by dissolving 8 gm. of sodium thiosulphate in 100 c.c. of a 0·1 per cent solution of the dye in question. Once solution was effected, 0·5 c.c. concentrated hydrochloric acid was added, and the mixture heated over a bunsen flame for 30 sec. The result was a decolorized solution with a grey, curdy precipitate, best left unfiltered, as it appeared to stabilize the solution.

An accidental shaft of sunlight from a point source produced an intriguing result. Each of the thiazine leucobases developed a blue spot in the solution in relation to the point source of sunlight. The blue spot disappeared a few seconds after the solution was shielded from light. The leucobases of methyl green and basic fuchsin showed no such phenomenon. Further examination showed it was not elicited by the light from an ordinary tungsten filament bulb, although the tungsten light source in my microscope, distinctly blue at top intensity, was effective. Daylight indoors had very little result, but daylight outside even with a cloudy sky produced the blue colour almost immediately.

Ultra-violet light seemed the most likely excitant, so the leucobase solutions were placed in front of a Wood's lamp (mercury discharge). Blue-green fluorescence was immediately observed, maximal in the case of methylene blue and the azures, moderate in the case of thionin, absent in methyl green and basic fuchsin leucobases. On subsequent examination, all the leucobases which had fluoresced were also showing photo-oxidation, as proved by the return of the original dye colour, and its subsequent disappearance in the dark.

It appears clear that leuco-thiazines show various effects when irradiated with ultra-violet and possibly visible blue light. Effects of this kind may be responsible for the reputation which these leucobases have for 'instability'. In fact, thiosulphate preparations of thiazines will remain stable for months if kept in a dark, well-stoppered bottle. Even in Coplin jars with loosely fitting lids, reduction will be maintained for almost two weeks, if care is taken to exclude light.

All the dyes described in this communication were supplied by 'Chroma' of West Germany.

D. W. Menzies

Department of Pathology,
University of Melbourne.

---

[1] Baker, J. R., *Principles of Biological Micro-Technique*, 180, 268 (Methuen and Co., London, 1958).

[2] de Tomasi, J. A., *Stain Technol.*, 11, 137 (1936).

PATHOLOGY

Effects of *Grewia populifolia* Extracts on the Development of Chick Embryos

In the course of screening work with various plant extracts for their carcinolytic activities, *Grewia populifolia* extract was found to have anti-tumour activities when injected in rats bearing rhabdomyosarcoma (Kundu *et al.*, unpublished work). It was decided to inject this extract into fertile eggs to study its effects on the organogeny of early chick embryos in a way similar to that of Jurand[1].

Four different concentrations, namely, 16×10^{-5}, 20×10^{-5}, 24×10^{-5} and 32×10^{-5} gm. per c.c., were used. For each concentration, individual eggs from different groups received 1 c.c. of the solution; the *p*H of these varied from 6 to 7. It was found difficult to inject 1 c.c. of the solution into the egg. To overcome this, 1 c.c. of albumen was taken out with a syringe and replaced by the same volume of normal saline solution containing the required concentration. The injection was made quite near the blastoderm through a small hole that was resealed and covered with 'Cellotape'.

Before the injection, each group was incubated at 38° C. for nearly 18 hr. After injection, this was kept at 25° C. overnight to allow the extract to mix with the albumen, and then re-incubated for periods of 24 hr. and 48 hr. respectively. The controls were treated similarly and they received 1 c.c. of normal saline after the removal of 1 c.c. of albumen.

Fixation was carried out with acetic acid-alcohol. Sections were cut at 10μ thickness and stained with celestine blue. In some cases eosin was used as the counter stain.

Of the four concentrations tested, 16×10^{-5}, 20×10^{-5} gm. per c.c. had no effect. This was revealed when the sections were examined under a microscope. A concentration of 32×10^{-5} gm. per c.c. appeared to be very toxic, as in all the eggs receiving this concentration the blastoderm at the time of inspection was disintegrated.

With 24×10^{-5} gm. per c.c. concentration, 60 per cent of the embryos were affected. The abnormalities observed were more or less similar in all the affected embryos. The most noticeable changes, observed after 48 hr. incubation, were the reductions in size of the embryo proper and the blastoderm. Examination of the sections revealed that the neural system was most severely affected throughout. The brain and the neural tube were enlarged, and their lumina were partially filled up with degenerated cell debris. In the brain particularly, the inner and the outer walls were broken, and the cells became loose with pycnotic nuclei (Fig. 1). Mitosis was rare. A section through the brain region from a control specimen clearly revealed the difference (Fig. 2).

In the neural tube, the outer wall was intact but the cell damage was similar to the brain tissue. The notochord revealed the same picture, but the sheath was intact. There was an increase in the mesenchymal cells and the dorsal aortæ were gorged with blood cells. The somites were affected only slightly in the posterior region of the embryo, and there was a clear differentiation into sclerotome, dermatome and myotome.

Examination of sections from embryos fixed after 24 hr. incubation showed that the neural tissue was enlarged. The inner wall of the brain and the neural tube were broken at places and cellular material

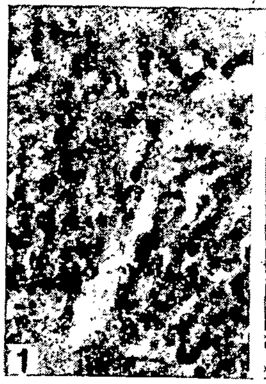
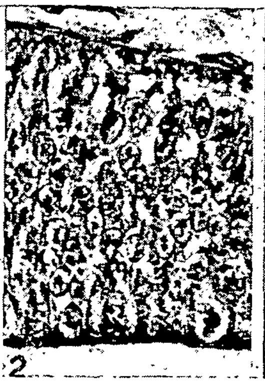
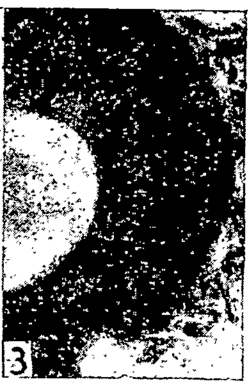




Fig. 1. Transverse section through the brain region of a treated embryo after 48 hr. of incubation. (× 200)

Fig. 2. Transverse section through the brain region of a control specimen after 48 hr. of incubation. (× 200)

Fig. 3. Transverse section through the brain region of a treated embryo after 24 hr. of incubation. (× 100)

was found scattered in the lumen, which appeared to have come from the cells lining it (Fig. 3). Mitosis could be observed, though less than the control. Other tissues which were observed to be affected after 48 hr. were more or less normal.

It appears from the present series of experiments that with concentrations of 24×10^{-5} gm. per c.c. of extracts of *G. populifolia* some organs of the developing embryos are affected. In the neural tissue there is an antero-posterior gradient in the degree of susceptibility, the brain being more affected than the neural tube, thereby showing that regions having a high rate of mitosis are more susceptible. This might also account for the lower degree of susceptibility of the somite where the rate of mitosis is not so great as in the two former tissues.

For the expansion of blastoderm tension is necessary, as reported by New[2]. This tension might have been reduced by breaking the intercellular connexions, resulting in a reduction of its size. A similar explanation has been given by Overton[3].

Engorgement of the blood vessels and an increase in the mesenchymal cells in the embryo proper might be due to the reasons suggested by Overton[3] and Waddington *et al.*[4].

The presence of mitosis after 24 hr. treatment and more or less its complete absence after 48 hr. with cellular derangement suggest that the extract of *G. populifolia* might have acted as a cytotoxic agent.

We are grateful to Dr. Subodh Mitra, director of this Centre, for his interest in the work, and to Dr. P. De for valuable discussions.

S. K. Brahma
N. Kundu

Department of Cell Research and Chemotherapy,
Chittaranjan National Cancer Research Centre,
37 S.P. Mookerji Road,
Calcutta 26.

[1] Jurand, A., *J. Embryol. Exp. Morph.*, 6, 357 (1958).
[2] New, D. A. T., *J. Embryol. Exp. Morph.*, 7, 146 (1959).
[3] Overton, J., *J. Exp. Zool.*, 139, 329 (1958).
[4] Waddington, C. H., Feldman, M., and Perry, M. M., *Exp. Cell Res.*, Supp., 3, 366 (1955).

Pathological Parthenogenesis in a Viviparous Toothcarp

In viviparous toothcarps parthenogenesis has been described in *Lebistes reticulatus*[1,2] and *Xiphophorus helleri*[2], while in this species also some cases of development of ovarial teratomas, caused by pathological parthenogenesis, are known[3]. In addition to these cases we observed a new one, namely in *Heterandria formosa*. It concerns a female isolated immediately after birth and consequently free from any contact with the male sex. After reaching a length of 27 mm. the virginal animal dropped 4 young fishes, all female. After this the animal became pregnant twice again, resulting in the birth of 6 and 10 young fishes, respectively, also exclusively female. For microscopic confirmation of the virginity the ovarial sperm chambers were examined, no spermia being observed. This finding ruled out self-fertilization, a phenomenon incidentally found in viviparous Cyprinodonts and explained by the presence of a bisexual gonad producing oocytes as well as spermia. As in the cases already described[2,3], an infection process caused by the Phycomycete *Ichthyophonus hoferi* was present in the cranial part of the ovary and in the adjacent regions. If an extensive process had been localized in the caudal parts of the ovary and near the junction between ovary and oviduct, birth would undoubtedly have been prevented. The young females appeared without an exception to be haploid.

The coincidence of the *Ichthyophonus* infection on one hand and the parthenogenetic development on the other justifies the assumption that the pathological process in this case had caused the parthenogenetic development. It could be assumed that the *Ichthyophonus* toxin provided the artificial stimulus for the development of the unfertilized oocytes.

Summarizing, we may conclude that this case forms an argument for my theory of pathological parthenogenesis[2,3]. It would certainly be advisable in any new cases of parthenogenesis to carry out a systematic examination for the possible presence of a pathological process in the ovary or in the immediate surroundings of this organ.

A. Stolk

Department of Histology,
Free University,
Amsterdam.

[1] Spurway, H., *Nature*, 171, 437 (1953).
[2] Stolk, A., *Nature*, 181, 1660 (1958).
[3] Stolk, A., thesis, Utrecht (1950); *Proc. Kon. Ned. Akad. Wet. Amsterdam*, *C* 56, 28 (1953); *Nature*, 183, 763 (1959).

Diagnostic Significance of Heterogeneous Lactic Dehydrogenases in Malignant Effusions

There is no laboratory test available for the differentiation of benign effusions from malignant exudates. Wroblewski and Wroblewski[1] reported in 1958 that, in malignancy, concentration of lactic dehydrogenase is frequently higher in effusions than in the corresponding sera. Nevertheless, the diagnostic value of these determinations is restricted since in about one-third of all cases investigated the amount of the lactic dehydrogenase in serum and effusion was the same[1]. Recent work on lactic dehydrogenase of tissues[2] and serum[3-5] indicates that this enzyme occurs in a number of molecular species which can be distinguished on account of their kinetics, antigenicity, inhibition by diphosphopyridine nucleotide analogues and electrophoretic mobility. In serum, starch block[3,4] and agar gel[5] electrophoresis and chromatography on diethylaminoethyl (*DEAE*) cellulose[6] showed the presence of at least four dehydrogenases, which are best labelled according to their electrophoretic mobility, namely, α_1, α_2, β and γ-lactic dehydrogenase. A specific increase in particular fractions has been observed in the following diseases: α_1 in myocardial infarction[3-6], $\alpha_1 + \alpha_2$ in muscular dystrophy[4] and γ in liver disease[5,6].

No information about the heterogeneity of lactic dehydrogenase in effusions is available. Since differences between malignant and benign effusions might occur, some thirty effusions were investigated by starch block electrophoretic separation[7] and determination of lactic dehydrogenase of each fraction using the optical test[8]. Effusions were centrifuged and the sediment discarded. 0·2 ml. serum or effusion was separated on starch block electrophoresis into four main fractions: α_1, α_2, β and γ. Lactic

Table 1. Lactic Dehydrogenase Iso-enzymes in Effusions (Starch Block Electrophoresis)

No.	Age	Sex	Diagnosis	Lactic dehydrogenase in serum Total	α_1	α_2	β	γ	Lactic dehydrogenase in effusions Total	α_1	α_2	β	γ
H-F, *H-D*	72	M	Carcinoma of œsophagus	4·0	1·2	2·1	0·5	0·6	8·0	0·5	1·9	0·8	4·8
H-25, *H*-26	32	M	Lymphosarcoma	42·5	8·0	22·5	6·0	6·0	57·9	6·0	21·7	14·4	18·3
H-9	55	M	Bronchial carcinoma						10·7	2·9	3·7	0·6	4·3
H-74, *H*-75	68	M	Tuberculosis	8·7	2·7	4·6	1·8	1·9	26·1	5·6	10·2	5·5	4·8
H-67, *H*-63	66	M	Tuberculosis	11·1	3·1	4.4	1·8	1·8	12·3	4·5	2·8	2·7	2·3
H-62, *H*-66	65	M	Cardiac failure	5·2	0·9	2·7	0·7	0·9	6·5	1·8	2·7	1·1	0·9
H-81	67	M	Cardiac failure						13·1	5·5	2·4	2·9	2·4
H-84	65	M	Cardiac failure						5·4	1·1	0·8	0·8	2·7

dehydrogenase activity was determined in the starting material as well as the individual fractions. Enzyme concentration was expressed as μmoles diphosphopyridine nucleotide oxidized per hr. per ml. Recoveries, that is, the sum of the concentrations of the individual fractions, were within 70 and 110 per cent of the starting material. To eliminate differences in recoveries, all results were adjusted to total serum lactic dehydrogenase activity (Table 1).

The results may be summarized as follows:

(1) Higher lactic dehydrogenase concentration in effusions as compared to the corresponding sera is frequently encountered in cancer and local infections, whereas in congestion, enzyme concentration is equal or lower in the transudate[1]. Due to overlap, little diagnostic significance can be attributed to these differences in total enzyme activity.

(2) In non-infected non-malignant effusions, heterogeneity of lactic dehydrogenase mirrors the serum pattern as shown in Fig. 1*a*.

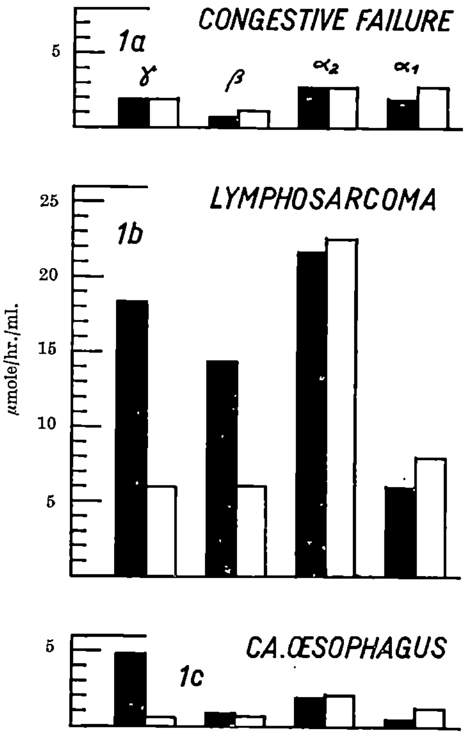


Fig. 1. Heterogeneity of lactic dehydrogenase. Black columns, effusions; white columns, serum. (*a*) 58-year-old male with congestive heart failure (serum lactic dehydrogenase 5·2 units; effusion lactic dehydrogenase 6·5 units). (*b*) 31-year-old male with generalized lymphosarcoma (serum lactic dehydrogenase 43 units; effusion lactic dehydrogenase 58 units). (*c*) 71-year-old male with metastasizing œsophageal carcinoma (serum lactic dehydrogenase 4·0 units; effusion lactic dehydrogenase 8·0 units)

(3) In malignant effusions, γ-lactic dehydrogenase is markedly increased in absolute value as compared with benign effusions, as well as relatively in comparison to the corresponding serum. The α_1 and α_2 enzymes reflect the serum pattern. The increase in the γ-fraction is independent of total lactic dehydrogenase, which may be very high, as in Fig. 1*b* (lymphosarcoma) or 'normal' (œsophageal carcinoma) as in Fig. 1*c*.

(4) If hæmolysis occurs, the heterogeneity of the pattern is distorted in comparison to serum too, but the increase is restricted to the α_1 fraction. In hæmolysed erythrocytes only an α_1 and α_2 enzyme can be demonstrated[3,6].

These findings indicate that in effusions the presence of an increased amount of lactic dehydrogenase migrating as a γ-globulin is suggestive of cancer. The number of samples investigated is too small to justify a definite statement, but the importance of the demonstration of a quantitative and possibly qualitative difference between a normal and a malignant body fluid is obvious. Warburg and Christian[9] first demonstrated abnormal leakage of glycolytic enzymes from cancer cells. Our observations suggest that, independent of the type of cancer, the leaking enzyme is primarily a γ-lactic dehydrogenase, which may be due either to selective leakage or to a distortion of the cellular lactic dehydrogenase pattern in cancer. Wieland and Pfleiderer[2] reported an increase in the slow-migrating lactic dehydrogenases ('γ-enzyme') in one bronchial carcinoma as compared with normal lung tissue, but further investigations will be necessary to settle this question. The discrepancy between effusion and serum pattern in malignancy is puzzling too. The lack of an increase in γ-lactic dehydrogenase in serum may be due to dilution, to local destruction by peptidases or to denaturation.

This work was supported by the Schweizerischer Nationalfond zur Förderung der Wissenschaftlichen Forschung (No. 1968).

R. Richterich
K. Zuppinger
E. Rossi

Medizinisch-chemisches Institut und Kinderklinik,
Universität, Berne.

---

[1] Wroblewski, F., and Wroblewski, R., *Ann. Int. Med.*, **48**, 813 (1958).

[2] Wieland, T., and Pfleiderer, G., *Biochem. Z.*, **329**, 112 (1957).

[3] Vesell, E. S., and Bearn, A. G., *Proc. Soc. Exp. Biol. Med.*, **94**, 96 (1957).

[4] Richterich, R., Gautier, E., Zuppinger, K., Egli, H., and Rossi, E., *Klin. Wschr.*, **39**, 346 (1961).

[5] Wieme, R. J., *Clin. Chim. Acta.*, **4**, 46 (1959).

[6] Hess, B., and Walter, S. I., *Klin. Wschr.*, **38**, 1080 (1960).

[7] Kunkel, H. G., *Methods of Biochem. Anal.*, **1**, 141 (1954).

[8] Kubowitz, F., and Ott, P., *Biochem. Z.*, **314**, 94 (1943).

[9] Warburg, O., and Christian, W., *Biochem. Z.*, **314**, 399 (1943).

An Antigen in Lesions of Molluscum Contagiosum

OUCHTERLONY's technique of agar diffusion[1] has made it possible to demonstrate the presence of certain viral antigens and antibodies not identifiable by other serological means. Datt and Orleans[2], in 1958, found an antigenic substance common to vaccinia and pig pox; Taylor-Robinson and Rundle[3] have demonstrated a common antigen in chicken pox and herpes zoster. These successes prompted a re-investigation of the antigenicity of molluscum contagiosum, a large virus that produces benign skin tumours in man.

The method of Taylor-Robinson and Rundle[3] was used. Skin curettings of molluscum lesions were pooled, ground and lyophilized to give a crude antigen. Four different antigen samples were prepared; 50–150 lesions from two to five patients comprised each sample. The donors were used only once; no anæsthesia was required. The antigen suspended in saline, 3–4 mgm./ml. or 30–40 mgm./ml., was put in the central reservoir of agar plates prepared for immunological study (1 per cent (w/v) Difco agar dissolved in isotonic saline and phosphate buffer pH 7·3 and containing merthiolate 1: 10,000; wells 11 mm. apart and 5 mm. deep). Sera from (1) patients infected with molluscum contagiosum; (2) adults without the disease, and (3) uninfected children were put in surrounding wells. The plates were examined daily for 2–3 weeks.

A single precipitation band (Fig. 1) appeared in 9 of 21 patients (43 per cent), 8 of 73 adults (11 per cent), and 0 of 11 children, and was usually evident within 48 hr. Weak concentrations of antigen (4 mgm./ml.) gave positive reactions only with sera from patients having numerous (more than 50) lesions of molluscum contagiosum. All antigens displayed equal activity. Comparable concentrations of lyophilized normal skin curetted from the same donors or from normal subjects gave consistently negative results. Ground, lyophilized human warts showed no antigenicity in this system. Of the positive reactors in the adult group, four had markedly raised serum globulin-levels related to multiple myeloma or internal cancers. A fifth subject with a high serum γ-globulin had a history of infectious hepatitis. One additional subject had a false-positive serological test for syphilis without a significant electrophoretic abnormality. These alterations were absent in sera from patients with molluscum contagiosum.

These findings indicate the presence of an antigen capable of inducing antibody formation in the lesions of molluscum contagiosum. This may conform to the heat-labile antigen–antibody system reported by Mitchell[4]; he noted weak complement fixation reactions of crude molluscum antigen with sera from 3 of 14 patients infected with the virus. About half our patients with the disease had demonstrable antibodies; they were more prevalent in those patients with extensive lesions. While the positive reactions in adults can be explained on the basis of prior unrecognized infection[5], it seems curious that 50 per cent of the positive reactors had high serum globulin associated with internal malignancy; the possibility exists that a cross-reacting tumour antibody gave the observed precipitation bands. The non-infected children had been confined to an isolated ward and presumably had never been exposed to molluscum contagiosum.

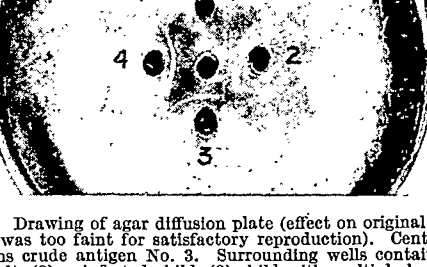


Fig. 1. Drawing of agar diffusion plate (effect on original photograph was too faint for satisfactory reproduction). Centre well contains crude antigen No. 3. Surrounding wells contain sera: (1) adult, (2) uninfected child, (3) child with multiple lesions of molluscum contagiosum, (4) adult with less than 25 lesions

Work is in progress to determine whether the crude antigen is a purely viral substance or an altered tissue antigen.

This work was supported in part by a grant from the Committee on Research, University of California School of Medicine.

WILLIAM L. EPSTEIN
IRENE P. SENECAL
ALAN M. MASSING

Division of Dermatology, Department of Medicine,
University of California School of Medicine,
San Francisco.

[1] Ouchterlony, O., *Acta Path. Microbiol. Scand.*, **25**, 186 (1948).

[2] Datt, N. S., and Orleans, E. S., *Immunol.*, **1**, 81 (1958).

[3] Taylor-Robinson, D., and Rundle, C. J. M., *Brit. J. Exp. Path.*, **40** (5), 517 (1959). Crumpton, M. J., and Davies, D. A. L., *Proc. Roy. Soc.*, B, **145**, 109 (1956).

[4] Mitchell, J. C., *Brit. J. Exp. Path.*, **34**, 44 (1953).

[5] Blank, H., and Rake, G., *Viral and Rickettsial Diseases of the Skin, Eye, and Mucous Membranes of Man* (Boston: Little, Brown and Co., 1955).

Leukæmia in the Progeny of Mice inoculated with a Leukæmogenic Virus

TEN years ago Gross first reported a raised incidence of leukæmia in mice of a low-leukæmic strain (*C3H*) inoculated when newly born with a cell-free extract of leukæmic tissue from a high-leukæmic strain (*AK*)[1]. He also observed a raised incidence in the progeny of the inoculated mice[2]. Since then, the first observation has been several times confirmed, but the second has not been reported from any other laboratory.

In the course of recent work in this Department on another leukæmogenic agent, the virus which Moloney isolated from the sarcoma 37 [3], several groups of stock albino mice ('Schofields') were injected when newly born with cell-free filtrates prepared from tissues of leukæmic mice of various breeds which had received a tissue culture-passaged sample of Moloney's virus[4]. In one experiment a new-born litter of 7 stock albinos (2♂, 5♀) were injected with such a filtrate. Leukæmia began to appear at 12 weeks of age, and 6 mice had developed leukæmia by 20 weeks. Before the appearance of the first leukæmia the mice were brother-sister mated; of their progeny of 18 (9♂ and 9♀) born when the parents were approximately 9 weeks old, 11 have become leukæmic between the 11th and 20th week of age. In the second experiment 7 new-born litters of stock albinos (a total of 46 mice) were inoculated with another filtrate. Leukæmia began to appear at the 11th week, and the incidence reached 50 per cent at 21 weeks. Among the mixed progeny (total 27) of these 7 litters, also born when their parents were about 9 weeks old, 9 leukæmias have appeared between the 11th and 20th week of age.

In a long experience of this breed of mice we have never seen spontaneous leukæmia as early as this. It occurs occasionally between 6 and 10 months of age: the cumulative incidence for the first 12 months is about 3 per cent.

Three contemporary control groups of stock albino mice of the same breed may be directly compared with the inoculated groups and their progeny:

(1) 32 untreated controls, 22 weeks old. No leukæmias.

(2) 46 mice inoculated with the same Moloney filtrate heated for ½ hr. at 80° C. These are now 24 weeks old. One leukæmia appeared at 22 weeks.

(3) 12 progeny of (2), now 16 weeks old. No leukæmias.

It would be premature to speculate at present about the mechanism of this phenomenon. Various possible modes of transmission are being investigated.

M. H. SALAMAN
J. J. HARVEY

Cancer Research Department,
London Hospital Medical College,
Whitechapel,
London, E.1.

[1] Gross, L., *Proc. Soc. Exp. Biol. Med.*, **76**, 27 (1951).
[2] Gross, L., *Proc. Soc. Exp. Biol. Med.*, **78**, 342 (1951).
[3] Moloney, J. B., *J. Nat. Cancer Inst.*, **24**, 933 (1960).
[4] Salaman, M. H., Rowson, K. E. K., and Harvey, J. J. (in the press).

IMMUNOLOGY

Antibodies to Trophoblasts during the Post-Partum Period

RECENT investigation of homograft tolerance has stimulated speculation about the immunological relationship between mother and fœtus during pregnancy[1,2]. Since the fœtus with its placenta is genetically different from the maternal host, it can be looked upon as a homograft tolerated by the mother for nine months. Fundamental to the support of such a concept is the demonstration of an immune response by the mother to her products of conception at some time during or after pregnancy. In human beings, the syncytiotrophoblasts are the fœtal cells in direct contact with the maternal bloodstream. They invade the bloodstream and disappear[1]. It would seem, therefore, that an immune response by the mother would most likely be directed towards trophoblasts. Although experimental homograft immunity is mediated primarily through a cellular response by the host[3], the direct contact between trophoblasts and maternal blood might stimulate a humoral response as well. The large mass of trophoblastic tissue in the placenta could absorb circulating antibody effectively. This might explain previous reports of failure to detect antibodies to placenta during pregnancy[4,5]. After delivery of the fœtus and placenta, the absence of this absorptive surface might allow any anti-trophoblastic antibody to remain in the bloodstream, where it could be detected by appropriate methods.

Based on these considerations, attempts were made to demonstrate anti-placental antibody in the sera of post-partum patients. Portions of placentas obtained from normal deliveries were immediately washed in EDTA-buffered saline (0·01 M EDTA adjusted to pH 7·2) until largely free of blood. A water-soluble extract of the homogenized tissue was prepared and lyophilized for storage. Smaller portions of the fresh placenta were quick-frozen in a dry-ice-ethanol bath and stored in a dry-ice chest. The patient's blood was drawn on the fourth post-partum day and the serum tested against her own placenta in every case.

Attempts to detect a precipitating antigen-antibody system with various concentrations of lyophilized placental extracts and post-partum sera were unsuccessful. In these experiments, the direct precipitin, Ouchterlony agar diffusion and agar immunoelectrophoresis techniques were employed. Concentrated globulin fractions of the sera also failed to give a precipitin reaction. Inconclusive hæmagglutination patterns have been obtained so far, using tanned sheep red blood cells sensitized[6] with various dilutions of placental extract and tested with doubling dilutions of post-partum serum.

Positive results were obtained, however, using the fluorescent antibody method of Coons on tissue sections[7]. The globulin fraction of post-partum serum was obtained by precipitation with 20·4 per cent sodium sulphate, tagged with fluorescein *iso*thiocyanate, and absorbed with mouse and rat liver powders. Globulin from the serum of a non-pregnant normal woman was treated similarly as a control. The frozen placental samples were sectioned at 4μ in a cryostat, dehydrated in alcohol for 30 sec., and stained at room temperature. Treatment of the sections with tagged post-partum globulin for 30 min. resulted in the localization of fluorescence in all cellular elements of the villi as viewed by ultra-violet microscopy. When the section was first flooded with untagged post-partum globulin for 30 min., sites of fixation on the tissue were blocked and subsequent treatment with the tagged material failed to result in fluorescence.

Several factors unrelated to an immune response by the mother to the placenta could play a part in the fixation of globulin by placental tissue. Globulin was fixed non-specifically despite prior absorption with rat and mouse liver powders, as illustrated by the presence of some fluorescence after staining with tagged globulin from a normal non-pregnant woman. Another factor was the known ability of the placenta to transport globulin from the maternal blood to the fœtal circulation[8]. The active sites of such a transport mechanism in the section might bind tagged globulin independently of any antigen-antibody system. A third factor was the possible presence of blood-group substances in the placental tissues. These could also play a part in the fixation of post-partum serum.

These possible explanations were eliminated by flooding the sections with untagged globulin from a non-pregnant normal woman of blood group *O*. Sites of non-specific binding of protein would thus be blocked. Sites involved in the mechanism of globulin transport would also be blocked. Any *A* or *B* blood-group substance on the placental section would combine with the anti-*A* and anti-*B* antibody present in the type *O* globulin and would be unavailable for further reaction. When such a pre-treated section was treated with tagged globulin from a non-pregnant woman, no fluorescence was seen, but treatment with tagged globulin from a post-partum woman resulted in fluorescence which was limited to the cytoplasm of the syncytiotrophoblasts (Fig. 1). This reaction has been observed in each of five patients of differing parities and blood-groups thus far studied.

The evidence favours the interpretation of the binding of tagged globulin from post-partum sera by

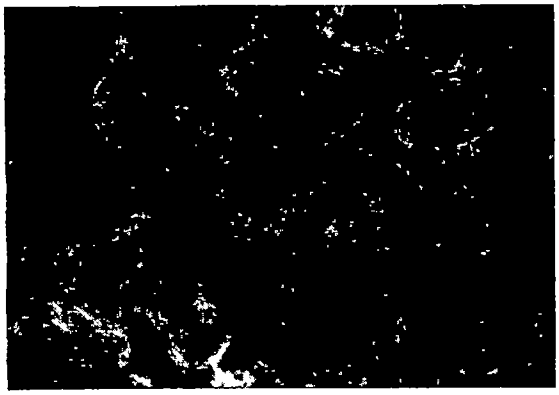


Fig. 1. Frozen section of human placenta treated with globulin from serum of a non-pregnant woman of blood group *O*, then stained with fluorescein-tagged globulin from the serum of the woman who delivered the placenta. Under ultra-violet excitation, only syncytiotrophoblastic cytoplasm fluoresces. (× 90)

the syncytiotrophoblastic cytoplasm as an immune phenomenon. An investigation designed to delineate the time of origin of this reaction with respect to possibly related events such as labour, pre-eclampsia and abortion is under way.

This work was partially supported by a grant from the Population Council, Inc.

J. F. HULKA*
K. C. HSU
S. M. BEISER

Department of Microbiology and the
Department of Obstetrics and Gynæcology,
Columbia University College of
Physicians and Surgeons,
New York, 32.

* Josiah Macy, Foundation Fellow.

[1] Douglas, G. W., Thomas, L., Carr, M., Cullen, N. M., and Morris, R., *Amer. J. Obstet. and Gynec.*, **78**, 960 (1959).
[2] Bardawil, W. A., and Toy, B. L., *Ann. N.Y. Acad. Sci.*, **80**, 197 (1959).
[3] Hildeman, W. H., and Medawar, P. B., *Immunology*, **2**, 44 (1959).
[4] Thornes, D. R., thesis, University of Dublin (1958).
[5] Olivelli, F., and Ruggieri, P., *Minerva Gin Tor*, **10**, 953 (1958).
[6] Stavitsky, A. B., *J. Immunol.*, **72**, 360, 368 (1954).
[7] Coons, A. H., and Kaplan, M. H., *J. Exp. Med.*, **91**, 1 (1950).
[8] Edsall, G., *Ann. N.Y. Acad. Sci.*, **66**, 32 (1956).

Specific Inhibition of Anti-*D* Antibody by Colominic Acid

IN ref. 1 it is reported that certain nucleotides specifically inhibited anti-*Rh* antibodies, and in ref. 2 that there is specific inhibition by certain monosaccharides and substances such as rutinose and streptomycin. Although some differences in the effect of these substances on anti-*C*, anti-*D* and anti-*E* were observed by both groups of workers, in general a substance that inhibited one anti-*Rh* serum also inhibited the others. More recently, Dodd, Bigley and Geyer[3] have reported that N-acetyl neuraminic acid and certain related compounds inhibit anti-*D* but not anti-*C* or anti-*E* ; this is a greater degree of specificity than that claimed in the first two reports.

We have confirmed the work of Dodd, Bigley and Geyer. In addition to the compounds tested by them, we have found that colominic acid, a substance produced by certain strains of *Escherichia coli* and shown probably to be a polymer of N-acetyl neuraminic acid[4,5], inhibits anti-*D*, but not anti-*C* or anti-*E*, in relatively low concentration (0·006 *M*).

In addition to the interesting implications of these observations for the structure of the *Rh* antigens, we believe our last observation is of possible practical importance. Colominic acid is stated[5] to be non-toxic and non-antigenic, and could undoubtedly be produced in quantity if necessary. We are therefore proposing to try it clinically to neutralize the anti-*D* in *Rh*-sensitized pregnant women and thus possibly prevent erythroblastosis in the unborn child.

This work was supported by research grants (*H*–1076 (*C*8), *RG*–4704 (*C*4) and *RG*–6939) from the National Institutes of Health, and a research grant (*NSF* G–5131) from the National Science Foundation. We are indebted to Dr. W. F. Goebel for a sample of colominic acid and a culture of *E. coli* K_{235}.

WILLIAM C. BOYD
EGLI REEVES

Department of Immunochemistry,
Boston University School of Medicine,
Boston 18, Massachusetts.

[1] Hackel, E., Smolker, R. E., and Fenske, S. A., *Vox Sang.*, **3**, 402 (1958).
[2] Boyd, W. C., McMaster, M. H., and Waszczenko-Zacharczenko, E., *Nature*, **184**, 989 (1959).
[3] Dodd, M. C., Bigley, N. J., and Geyer, V. B., *Science*, **132**, 1398 (1960).
[4] Barry, G. T., and Goebel, W. F., *Nature*, **179**, 206 (1957).
[5] Barry, G. T., *J. Exp. Med.*, **107**, 507 (1958).

BIOLOGY

Hydrogen Ion Concentration, Plankton and Fish in Fresh-water Eutrophic Lakes of India

DAS *et al.*[1] have recently published results on the quantitative and qualitative aspects of plankton of some eutrophic fresh-water lakes of Uttar Pradesh, India ; but no satisfactory information is available regarding the physico-chemical factors bearing upon them. Some work on related fields has been done by Chacko *et al.*[2] and Alikunhi[3], but their results deal only with part of the season in fish-hatching tanks and small bodies of water. In order to get detailed information regarding *p*H and plankton, regular weekly quantitative hauls were made and water samples obtained in 'Pyrex' glass-stoppered bottles. Hydrogen ion concentration was determined for each sample colorimetrically by using Helige comparators and Helige indicator solutions in daylight so far as possible in the field. Carbon dioxide was estimated by making use of phenolphthalein as indicator and the titration was done in Nessler jars in the usual way.

The investigations show that *p*H values range from 7·2 to 9·2 during the year. A high *p*H is observed in the months of July and August, when the average is about 8·6. But the values decrease during September and October and reach 7·4 in the last two weeks of October. November and December maintain rather constant values, but January shows a sudden increase in *p*H, reaching 8·8 in the third week; finally, a further rise occurs and reaches a maximum of 9·2 in the first week of February. In March *p*H values are still as high as 9·0, but show a fall to 8·0 in the last week of April. Finally, in the months of May and June, there is sharp decline in *p*H to 7·2.

The plankton records for the same period show that high *p*H values in the months of July and August coincide with the first monsoon phytoplankton peak. Then the decrease in *p*H during September–December can be attributed mainly to the zooplankton peak, along with the decomposition of organic matter caused by death and decay of zooplankton. Again a high *p*H in the months of February and March was observed to be accompanied by the blooming of Myxophyceae, causing an increase in phytoplankton. The lowest *p*H values were obtained during May and June, when there was an abundance of zooplankton and lack of phytoplankton.

It has also been observed that high *p*H values of water coincide with low values for carbon dioxide, and with the phytoplankton peaks, while low *p*H values coincide with high concentration of carbon dioxide in water during the zooplankton peak periods. This appears to be due to the fact that increase in phytoplankton causes photosynthetic activity which uses up dissolved carbon dioxide, and thus the *p*H values are raised in February and March. In the summer months, due to the concentration of aquatic animals (fishes, molluscs, etc.) in the decreased amount of water, combined with the abundance of zooplankton, the concentration of carbon dioxide increases and *p*H values reach a minimum. It is thus clear that, generally, high *p*H means high phytoplankton and low zooplankton; whereas low *p*H means low phytoplankton and high zooplankton in ordinary circumstances.

It has recently been observed (Das, unpublished work) that more fishes are present in natural lakes with a *p*H range of 7·2–8·2, but decline rapidly in highly alkaline waters which remain above *p*H 9. As no Indian eutrophic lakes have been found with a *p*H less than 7·2, fish fertility appears to be indirectly dependent on the *p*H of such bodies of water. Thus a change of environment due to change of *p*H may affect the productivity of fish in fresh-water lakes and tanks. This aspect is now being studied.

S. M. Das

Department of Zoology,
University of Lucknow.

[1] Das, S. M., *et al.*, *Proc. Nat. Acad. Sci.* (India), **26** (1); **26** (4); **27** (3); **29** (4); **29** (4) (1956–59).

[2] Chacko, P. I., *et al.*, *Symp. Marine and Freshwater Plankton in the Indo-Pacific, Bangkok* (Unesco, 1954).

[3] Alikunhi, K. H., *et al.*, *Ind. J. Fisheries*, **2** (1955).

Littoral Interstitial Species from Anglesey representing Three Families new to Britain

In 1956 a brief investigation by Dr. B. Swedmark of beaches in the Anglesey area revealed a well-developed microfauna. A more intensive survey is now in progress, and many typical interstitial species have been found.

Some of the most interesting recent discoveries have been made in the low-water neap zone at Traeth Bychan, a gently sloping beach of coarse sand on the east coast of Anglesey. Three small molluscs belonging to the Pseudovermidae, a nudibranch family new to Britain, have been found in this habitat. They resemble *Pseudovermis schulzi* Marcus and Marcus more closely than any other species described. All three measured 3–3·5 mm. in length when extended, but were unfortunately immature. The shape of the radular plates together with the presence of eyes seem to confirm the identification as *P. schulzi*, but there were as many as fifteen body papillæ and an equal number of cnidosacs (Fig. 1). A maximum of five papillæ was given as a diagnostic feature in the original description. The cnidoblasts were all of one type, penetrants 5·5μ long and 4μ broad with a thread up to 70μ long when discharged. They were found to be identical in size and structure with those of the hydrozoan *Halammohydra vermiformis* Swedmark and Teissier, which has been found in the same area of beach. It is thus highly probable that this *Pseudovermis* species preys upon *H. vermiformis*. Apart from the suggestion by Dr. E. Marcus that the Brazilian *Pseudovermis salamandrops* Marcus derives its nematocysts from *Psammohydra nanna*, Schulz[1], no previous information concerning the food of pseudovermids is available.

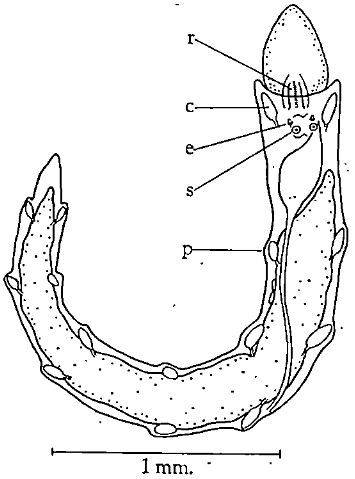


Fig. 1. *Pseudovermis* sp. from Anglesey. Dorsal view. *c*, cnidosac; *e*, eye; *p*, body papilla; *r*, radula; *s*, statocyst

A second species of *Halammohydra*, *H. schulzei* Remane, has been found in the Menai Straits among coarse shell gravel exposed at low water on spring tides. The family Halammohydridae had not previously been recorded from Britain, but an unidentified species has since been reported from the Isles of Scilly[2].

Last August a sample of sand from Traeth Bychan which contained several young *H. vermiformis* yielded a curious interstitial annelid. Further examples of this animal recovered recently prove it to be the sedentary polychæte *Psammodrilus balanoglossoides* Swedmark belonging to the Psammodrilidae Swedmark. This constitutes the third new family from this one habitat to be added to the British fauna list.

P. J. S. Boaden

Marine Biology Station,
Menai Bridge,
Anglesey.

[1] Marcus, E. du B.-R., *Bol. Fac. Filos. Cienc., Zool.*, **18** (1953).

[2] Harvey, L. A., and Wells, J. B. J., *Nature*, **189**, 944 (1961).

Response to Series of Subliminal Stimuli; an Ethological Method for investigating the Properties of Nervous Systems

Queen ants of genus *Formica* react to mechanical disturbance of the nest by running downwards to the deepest chambers. In laboratory colonies a series of shocks could result in a reaction, although one similar shock was too small to produce any visible effect.

To investigate this further, three separate queens of *F. fusca* L., without workers, were subjected at the same time to series of nearby taps on the bench with constant periodicity throughout each series and constant strength throughout this work. The periodicity was changed for different series and the

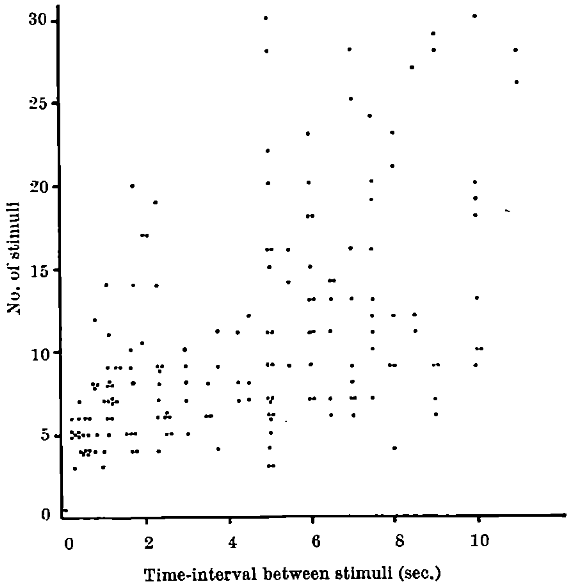


Fig. 1. The relation between the number of mechanical shocks necessary to produce an escape reaction by queen *Formica fusca* L., and the time-interval between the shocks

range of time-interval (t) between taps was 0·2–11·0 sec. Fig. 1 shows the number of taps (n) needed for a reaction plotted against t (regression analysis of log n on t; $P < 0\cdot001$).

There are several causes of the wide variance of these results, including: (i) differences in the internal state of the animals; (ii) differences in their stance; (iii) changes in temperature (range 21·3–24·5° C., mean 23·0° C.); (iv) other mechanical shocks in the same building cannot be completely discounted, but were small compared with the deliberate stimulus, and the reaction always occurred immediately after a deliberate stimulus. These causes are not expected to bias the results and are, for the present, ignored, although the method may provide opportunities for measuring the effect of important factors.

Various difficulties in observation and interpretation were encountered. The reaction varied from a change of posture (a raising of the head and thorax with extension of the antennæ) to rapid running, and in one in eight experiments a queen did not respond in spite of prolonged stimulation. This queen was ignored in this particular experiment, if it had not responded to three times as many taps as taken for the last of the others to react. Only one response, out of 172, was given to the first stimulus and the other queens in this experiment took 6 and 7 taps to react. Habituation did not appear to be an important factor, but at least 30 min. was allowed to elapse between experiments. Isolation of the queens from workers and brood was not relevant either, since a colony exposed to the same conditions at the same time showed similar behaviour with the queen usually reacting before the workers.

Evidently, there is a threshold of some nervous change which has to be reached before reaction occurs. This change shows temporal summation and can be retained for more than 10 sec. although it is gradually lost after stimulation. Exponential decay fits the results better than linear decay and, if it is exponential, its average half-life is estimated to be at least 30 sec. (since, if when $t = 0$, $n = N$, there is a vertical asymptote at the value of t during which $1/N$th of the change decays).

Unless facilitation of sense organs to subliminal stimuli can contribute significantly, these long periods of activation are due to summation and decay of activity in a nervous centre controlling escape behaviour. This centre may be an 'instinctive centre' in the sense of Tinbergen[1] and the activity may be related to the 'stored energy' of Lorenz[2] which is also required to reach a threshold before setting off behaviour.

A. J. Pontin

Bedford College,
Regent's Park, N.W.1.

[1] Tinbergen, N., *Symp. Soc. Exp. Biol.*, Cambridge, **4**, 305 (1950).

[2] Lorenz, K. Z., *Symp. Soc. Exp. Biol.*, Cambridge, **4**, 221 (1950).

Enhanced Plant-Growth with Two Systemic Insecticides

In the search for better methods of control of the red-legged earth-mite, *Halotydeus destructor* (Tuck.) (Acarina : Eupodidae), and the lucerne flea, *Sminthurus viridis* (L.) (Collembola : Sminthuridae), seeds of Dwalganup subterranean clover, *Trifolium subterraneum* L., were treated with the systemic insecticides demeton and methyl demeton before sowing. The seed, dampened with milk, was tumbled in a container with 8 per cent of its weight of a charcoal-based dust containing 50 per cent of the insecticide. Seeds were sown, at the rate of 50 per 8-in. earthenware pot, in sand fertilized with potato 'E' manure at the rate of 1 cwt./acre. (Potato 'E' manure contains 3·5 per cent N, 15·5 per cent P_2O_5 and 8 per cent K_2O.) The pots, of which there were three for each treatment, were kept in a glasshouse and watered at least every second day.

The emergence was 96 per cent in untreated seed; 93 per cent in the demeton treatment and 59 per cent in the methyl demeton treatment. Seedlings removed from the demeton treatment were toxic to both pests for up to 11 weeks after sowing, whereas toxicity was lost in the methyl demeton treatment after about 7 weeks. Demeton-treated seeds produced pale cotyledons and stems, whereas those treated with methyl demeton produced plants which were more heavily pigmented than normal. In both insecticide treatments the early growth of the seedlings was slightly retarded. Despite this, the plants arising from the treated seed later grew more vigorously than those in the control pots.

Three months after sowing, all plants were carefully removed from the pots and the weights of the green tops and of the roots recorded. The results are shown in Table 1. The increased yield in the pots sown with seed treated with demeton and methyl demeton amounted to an additional 39 per cent and 24 per cent respectively ($P < 0\cdot05$). There was a highly significant increase in the weights of the individual plants ($P < 0\cdot001$), but no significant differences in weight of roots. In the methyl demeton treatment it is possible that part at least of the increase in individual plant weight may have been a response to reduced seedling competition.

There was virtually no insect or mite attack on the plants in the glasshouse, and certainly insufficient to account for such large differences of yield. Small increases in growth have since been observed in outdoor pot tests with phorate ('Thimet') and *PP*175 ('Sayfos'), although no precise measurements were

Table 1. EFFECT OF DEMETON AND METHYL DEMETON APPLIED AS A SEED TREATMENT ON THE YIELD IN POTS OF THE GREEN TOPS AND ROOTS OF SUBTERRANEAN CLOVER

Treatment	No. plants at harvest	Yields (gm.)			
		Total tops	Mean tops per plant	Total roots	Mean roots per plant
Control	127	180·8*	1·42†	137·1	1·09
Demeton	125	250·4*	2·04†	148·0	1·18
Methyl demeton	86	223·7*	2·60†	126·1	1·47

* $P < 0·05$ † $P < 0·001$

taken. The phorate used was a 44 per cent charcoal-based dust whereas the *PP*175 was an 80 per cent wettable powder. Similar unexplained increases in growth following seed treatment with phorate and demeton were reported[1,2].

Clearly, these insecticide seed-treatments had some marked beneficial effect other than the direct killing of insect or mite pests. Perhaps there was some influence on the rhizosphere microfauna or flora, or possibly the transmission of virus diseases was inhibited. Whatever the explanation, there appears to be some real response worthy of further investigation.

M. M. H. WALLACE

Commonwealth Scientific and Industrial
Research Organization,
Division of Entomology,
Western Australian Regional
Laboratory,
Nedlands, Western Australia.

---

[1] Guyer, G. E., Brown, H. M., and Wells, A., *Quart. Bull. Mich. Agric. Exp. Stat.*, **40** (1958).

[2] Brown, H. E., *FAO Plant Protection Bull.*, **5** (1957).

Effects of Gibberellic Acid and Allogibberic Acid on Flowering and Internode-Length in *Pisum sativum*

SEVERAL workers[1,2] have shown that gibberellic acid causes an increase in internode-length in peas, especially in the dwarf (*le*) varieties where the increase may be as great as 500 per cent. In addition we have shown that gibberellic acid delays flowering by 1–2 nodes in tall (*Le*) or dwarf (*le*) and late (*Sn*) or early (*sn*) varieties[2]. Segregation of alleles at the *Le* and *Sn* loci has a number of overlapping effects[3]. It thus appears that genes at both loci may be working by way of some aspect of gibberellin metabolism. Accordingly we have tested the effect on flowering and stem-length of peas of a number of compounds related in structure to gibberellic acid. We are indebted to Dr. P. W. Brian (Imperial Chemical Industries, Ltd.) for the gift of the compounds used.

The compounds were applied as a solution in absolute ethanol to the dry seed of the early, dwarf variety, Massey, at a concentration of 0·4 μgm. per μl. Doses of 1, 2, 8, 16 and 32 μgm. were regulated by varying the volume of the solution applied by means of a micropipette. The ethanol rapidly evaporates leaving the compound on the testa of the seed. The seeds were germinated in moist vermiculite and transplanted as seedlings into soil. Comparable doses of pure ethanol had no effect on the subsequent growth of the plant.

Compounds tested include methyl gibberellate, gibberellin *C*, gibberellenic acid, gibberic acid and allogibberic acid. Methyl gibberellate produces similar responses on both flowering and stem-length to gibberellic acid, but is not so active. Gibberellin *C* and gibberellenic acid were inactive at doses of 1 and 2 μgm. per plant. At 32 μgm. they have an effect equivalent to a dose of less than 1 μgm. of gibberellic acid. The effects of these two substances are most probably attributable to contamination with gibberellic acid (Dr. Brian has informed us that it is very difficult to prepare pure samples of these compounds). Gibberic acid had no effect on either growth or flowering at any of the concentrations tested. Allogibberic acid had no effect on stem-length but appeared to cause a small delay (0·5 node) in flowering.

In a more detailed study of the effect of allogibberic acid, four chemical treatments (0, 1, 10 and 100 μgm. of allogibberic acid per plant) were combined factorially with two photoperiods (short days of 8 hr. and long days of 18 hr.). A randomized block design of 20 plants per treatment was used. Experimental procedure was the same as in the previous experiment except that the different doses were applied in each case in the same volume (10 μl.) of ethanol.

Flowering is delayed to a higher node by allogibberic acid. The delay shows a steady increase from zero at a dose of 1 μgm. to a maximum of 0·65 node (statistically significant at the 1 per cent level) at the highest dose of 100 μgm. per plant. In contrast, allogibberic acid does not alter internode-length at any of the concentrations tested. The photoperiod treatment and the photoperiod-chemical interaction are not significant for either node of first flower or internode length. The long- and short-day results have been combined and plotted in Figs. 1 and 2 together with results from a previous experiment on the effect of seed treatment with gibberellic acid on the same pea variety, Massey.

Fig. 1 illustrates that gibberellic acid and allogibberic acid both cause a delay in flowering. The effect of gibberellic acid is approaching a maximum

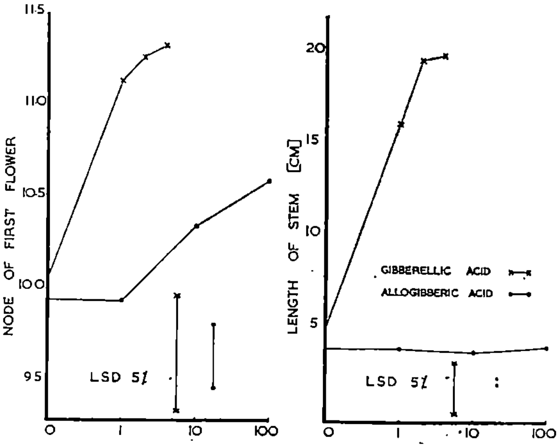


Fig. 1 Fig. 2

Figs. 1 and 2. The node of first flower and the length of stem in cm. between nodes 1 and 4 are shown for various dose-levels of gibberellic acid and allogibberic acid. The compounds were applied in ethanolic solution to the dry seed of an early dwarf pea variety, Massey. The cotyledonary node is taken as zero at a dose of 4 µgm. per plant, whereas the delay caused by allogibberic acid may still be increasing at the maximum dose of 100 µgm. The delay achieved with this highest dose is only about half the maximum delay achieved with gibberellic acid. Fig. 2 contrasts the effect of the two compounds on stem-growth. Two µgm. of gibberellic acid causes about a 400 per cent increase in stem-length whereas 100 µgm. of allogibberic acid has no effect. These results on stem-growth are in agreement with those of Cross[4] and Brian *et al.*[1,5].

The chemical structures of gibberellic acid, allogibberic acid and gibberic acid have been established by Cross *et al.*[6,7]. Chemically, allogibberic acid is a primary degradation product from gibberellic acid and differs from it only in ring *A*. Biologically, allogibberic acid has lost the power to stimulate elongation in dwarf peas but is still capable of delaying flowering. Gibberic acid, which is another degradation product derived from allogibberic acid by a Wagner–Meerwein rearrangement, seems to have lost both activities. The delay in flowering induced by gibberellic acid or allogibberic acid is not altered under different photoperiods and it is unlikely, therefore, that either substance is the flower inhibitor (colysanthin) which we have suggested is produced by the dominant *Sn* gene of late varieties of peas[3].

One of us (I. C. M.) acknowledges the assistance of a Commonwealth Scientific and Industrial Research Organization research studentship. The Rural Credits Division of the Reserve Bank of Australia has helped us by providing funds for technical assistance, apparatus and glasshouses.

I. C. MURFET
H. N. BARBER

Botany Department,
University of Tasmania, Hobart.

[1] Brian, P. W., and Hemming, H. G., *Physiol. Plant.*, **8**, 669 (1955).

[2] Barber, H. N., Jackson, W. D., Murfet, I. C., and Sprent, J. I., *Nature*, **182**, 1321 (1958).

[3] Barber, H. N., *Heredity*, **13**, 33 (1959).

[4] Cross, B. E., *J. Chem. Soc.*, 4670 (1954).

[5] Brian, P. W., Grove, J. F., Hemming, H. G., Mulholland, T. P. C., and Radley, M., *Plant Physiol.*, **33**, 329 (1958).

[6] Cross, B. E., Grove, J. F., MacMillan, J., Mulholland, T. P. C., and Sheppard, N., *Proc. Chem. Soc.*, 221 (1958).

[7] Cross, B. E., Grove, J. F., MacMillan, J., and Mulholland, T. P. C., *Chem. and Indust.*, 954 (1956).

Susceptibility to Leaf Blight, caused by *Cochliobolus heterostrophus*, in Nigerian Varieties of Maize

LEAF blights on maize are prevalent, particularly in the forest areas of Southern Nigeria. One of the commonest of these pathogens is *Cochliobolus heterostrophus* (Dreschl.) Dreschl., already recorded in West Africa in 1928 in Ghana[1] and in 1933 in Nigeria[2]. The lesions develop in abundance on the leaves, from the lower ones upwards. A typically infected maize plant was taken at tasselling time and the area of the various leaves attacked by the fungus determined (Table 1).

Table 1. LEAF AREA AND PERCENTAGE OF THE AREA ATTACKED BY *C. heterostrophus* OF A TYPICALLY INFECTED MAIZE PLANT. LEAVES NUMBERED FROM BASE TO TOP

Leaf No.	Leaf area (cm.^{2})	Percentage of leaf area attacked
1	487	20·1
2	619	11·4
3	818	9·8
4	902	10·9
5	938	8·0
6	993	10·4
7	1,037	8·8
8	988	4·2
9	905	5·6
10	825	2·6
11	758	3·1
12	741	0·6
13	561	0·3
14	431	1·1
15	239	0·8
Total	11,242	6·3

The fungus causes a premature dying back of the leaves from the base onwards, around the time of tasselling. Heavily infected plants lose their lowest leaves earlier and have no more green leaf area within approximately 3 wk. from tasselling, that is, before the grains on the ears are properly formed.

Most of the varieties introduced as breeding material, mainly from the Americas, proved to be very susceptible to this fungus. The most susceptible variety was Coahuila-8 from Mexico. The lowest scores were obtained on the synthetic variety *ES*2, a local selection from the Mexican variety Rocamex 520–*C*. However, even this selection has a considerable reduction in leaf area, in seasons favourable to the pathogen.

For the purpose of a survey, a collection was made of West African maize varieties[3] of which approximately 650 are maintained at Moor Plantation, Ibadan. The main part of this collection consists of Nigerian varieties. Those local varieties of maize were grown at Ibadan during the early season of 1959 (March–July) with 30 plants per variety. Each plant was assessed quantitatively on the occurrence of lesions caused by *C. heterostrophus* with the aid of an arbitrary key. Three leaves were assessed per plant and a mean calculated. Three levels of attack were recognized, roughly corresponding with 0–1 per cent (intensity 1), 2–5 per cent (intensity 2) and 6–10 per cent or more (intensity 3) of the leaf area attacked by the pathogen. This was done in order to obtain information on possible sources for resistance.

The early season of 1959 was favourable for the development of the fungus and provided an excellent opportunity to screen the collection on resistance. The results have been mapped by indicating the average intensity score of *C. heterostrophus* on the various maizes on the place of their origin (Fig. 1).

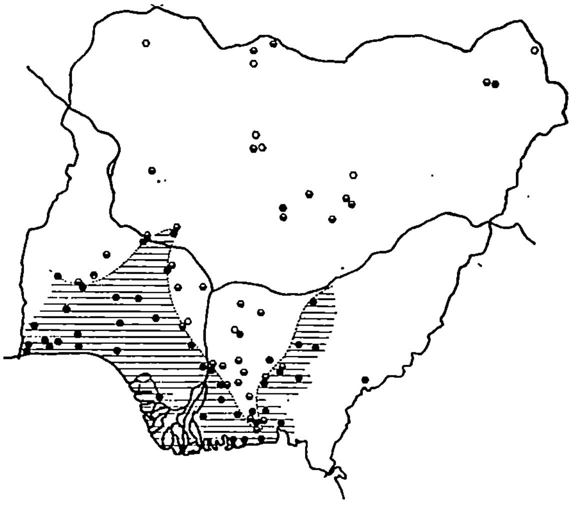


Fig. 1. Map of Nigeria showing the distribution of susceptibility and resistance to *Cochliobolus heterostrophus* in local maize varieties. ●, Intensity 1; ⊖, intensity 2; ○, intensity 3 (scores are mapped on the place of origin of the varieties)

It is noteworthy that, apart from a few exceptions, the more resistant varieties (intensity score 1) have their origin in the forest belt of the south (hatched area on Fig. 1). This area comprises the main maize-producing area of Nigeria in the drier parts of the rain forest and derived savannah of the western region[4]. East of the River Niger, however, a broad front of less-resistant varieties invades the densely populated areas of the eastern region and reaches very far south. Within this triangle the varieties have white or yellow, floury (soft endosperm) or flinty (hard endosperm) grains. The majority of the western region varieties, however, are white and soft-grained. The same is true for varieties in the south and south-east of the eastern region. The northern maize varieties, however, are typically yellow and hard-grained.

Trade and accidental movements of maize grains could be responsible for the phenomenon of maize varieties susceptible to leaf blight in a zone where natural selection would have eliminated those and actually did to the west, south and south-east of the area. In several places just north of this wedge (Keffi, Lafia) 'Southern' floury varieties are cultivated for sale on markets where 'Southerners' have caused a demand for those grains, although the local population often dislikes this type. It is also known that a trade of maize occurs within the area from north to south[5], from the less to the more densely populated areas (for example, from Idoma and Igala divisions towards Onitsha).

It is suggested that the resistance to the Nigerian strains of *C. heterostrophus* is a recessive character, probably based on several genes. The fact that the western region varieties all had a relatively high resistance to this leaf blight would tally with the fact that there is little trade of maize into the area. The influx of seed into the eastern region could then explain the higher susceptibility of the maize varieties in the wedge, and the low number of cases of 'resistant' varieties found in Northern Nigeria, in spite of the fact that the numerous 'Southern' inhabitants of the northern region have, doubtlessly, taken southern maizes to the north, would then be understandable.

C. L. M. VAN EIJNATTEN

Botany Division,
Federal Agricultural Research,
Moor Plantation,
Ibadan, Nigeria.

[1] Bunting, R. H., *Gold Coast Dept. Agric. Bull.* 10 (Govt. Printer, Accra, 1928).

[2] West, J., *Kew Bull.*, 1 (1938).

[3] Stanton, W. R., West African Maize Research Unit, Second Ann. Rep. (Moor Plantation, Ibadan, Nigeria, 1954).

[4] *Report on the Sample Census of Agriculture 1950/51* (Department of Statistics, Survey Department, Lagos, 1952).

[5] Prest, A. R., and Stewart, I. G., *The National Income of Nigeria 1950/51* (Univ. of Cambridge, Department of Applied Economics, 1951)

Diurnal Variation in Transaminase Activity in the Cactus Plant

THE rhythmic acidification and deacidification occurring in cactus and other succulent plants may be expected to be associated with rhythmic alteration in the activity of enzymes involved in the formation and transformation of the organic acids. A change in the activity of an enzyme may be envisaged as being brought about by altering the total amount of catalytic site available, that is, by the synthesis or destruction of enzyme protein, or by the elaboration or removal of activator or inhibitor molecules from the site of enzyme action, or by the accumulation of products of reaction. It has been reported from this laboratory[1] that cactus plants growing in garden soil in the open showed a marked diurnal variation in aldolase activity and it was suggested that the peak of its activity, which was reached in the dark, was linked with the breakdown of carbohydrate to the 3-carbon atom acceptor of carbon dioxide. Continuing the above investigations, we have found that the phylloclades of *Nopalea dejecta* grown in the open under natural conditions of light and darkness show a remarkable diurnal variation in glutamic oxaloacetic transaminase activity.

The transaminase activity was determined by the colorimetric method of Tonhazy *et al.*[2] on homogenates of pooled samples of phylloclades collected at 4-hourly intervals over a 24-hr. period in February and in March of this year. A unit of enzyme was defined as the amount which gave rise to 1 μgm. of pyruvic acid at 30° C. in 30 min. at *p*H 7·4. Specific activity of the enzyme was calculated as units per mgm. dry weight of tissue. Protein was estimated by the biuret method of Gornall *et al.*[3] after precipitation with trichloracetic acid, and total nitrogen by the micro-Kjeldahl method. In some experiments the non-protein nitrogen was determined by Kjeldahl digestion of the centrifuged supernatant from the precipitate formed with trichloracetic acid.

The results reported in Table 1 show that the transaminase activity was maximum at 4 p.m. and was markedly reduced at 8 p.m., to be followed by a further abrupt drop. The enzyme was thereafter maintained at an extremely low level, until noon, when the activity began to increase. When equal volumes of the homogenates from material collected at 4 p.m. and at 8 a.m. were mixed, it was found that the enzymic activity of the mixed homogenate was almost exactly the arithmetic mean of the individual activities of the two samples. This indicated that the observed diurnal alteration in enzymic activity was due essentially to changes in the quantity of active enzyme and not to the presence of activator or inhibitor. There was a rhythmic alteration also in the protein content, which, however, was much less pronounced than that of transaminase activity. Calculated on the basis of dry weight of tissue, the protein content was high in the day-time and low during the night.

Saltman *et al.*[4] demonstrated that during the exposure of leaves of *Bryophyllum calycinum* to

Table 1. DIURNAL VARIATION IN TRANSAMINASE ACTIVITY IN *Nopalea dejecta*

Time of collection of samples	Temperature (°C.)		Dry weight, mgm./ml. 40 per cent homogenate	Specific activity (units/mgm. dry weight)	Acidity, ml. *N*/10 sodium hydroxide/gm. dry weight of tissue	Protein, mgm./gm. dry weight of tissue
	Ambient	Inside phylloclade				
February 25, 1961						
8 a.m.	16·5	11·5	18·4	0·48	26·1	80·5
Noon	26·0	29·5	20·5	8·48	14·6	104·8
4 p.m.	26·0	26·5	17·9	25·84	5·6	88·9(?)
8 p.m.	18·0	13·5	19·4	12·76	8·3	105·0
12 p.m.	16·0	12·0	19·2	10·59	11·5	92·6
4 a.m.	12·5	10·0	22·8	3·62	13·2	66·6
March 11, 1961						
8 a.m.	23·0	17·5	17·4	0·51	23·0	85·1
Noon	34·0	40·0	21·7	2·99	12·9	102·4
4 p.m.	35·0	30·5	20·8	20·83	5·8	122·8
8 p.m.	20·0	20·0	20·6	13·45	7·8	106·1
12 p.m.	21·0	18·5	20·0	3·68	12·0	85·2
4 a.m.	17·5	16·5	18·5	1·12	18·4	62·1

labelled carbon dioxide in the dark for 1 min.–2 hr., radioactivity was incorporated into the acids of the Krebs tricarboxylic acid cycle and amino-acids such as glutamic acid, aspartic acid and alanine. The authors considered that the amino-acids arose from the α-keto acids of the cycle by transamination reactions. The extremely low activity of transaminase observed by us at night may be indicative of predominance of organic acid production during dark fixation of carbon dioxide in cactus, with minimal transformation of α-keto acids to amino-acids. Conversely, the high activity of the enzyme commencing at noon and reaching an abrupt peak at 4 p.m. may be associated with increased amino-acid synthesis in the day-time. Our work on non-protein nitrogen of cactus tissue has been of a preliminary nature, but estimations of total protein reveal a rhythmic increase in the day-time.

One of us (S. K. M.) thanks the Ministry of Scientific Research and Cultural Affairs, Government of India, for the award of a Senior Research Training Scholarship.

S. K. Mukerji
G. G. Sanwal
P. S. Krishnan

Division of Biochemistry,
University of Lucknow,
Lucknow, U.P.

---

[1] Sanwal, G. G., and Krishnan, P. S., *Nature*, **188**, 664 (1960).

[2] Tonhazy, N. E., White, N. G., and Umbreit, W. W., *Arch. Biochem.*, **28**, 36 (1950).

[3] Gornall, A. G., Bardawill, C. S., and David, M. M., *J. Biol. Chem.*, **177**, 751 (1949).

[4] Saltman, P., Lynch, V. H., Kunitake, G. M., Stitt, C., and Spolter, H., *Plant Physiol.*, **32**, 197 (1957).

Shortening the Juvenile Phase of Apple Seedlings

Apple seedlings usually take 7–14 yr. to come into bearing. Many attempts have been made by plant breeders and others to reduce this juvenile phase, in order to obtain an earlier assessment of new seedlings.

Variations in pruning, nutrition and cultural practices have failed to shorten this juvenile period. By working seedlings on the dwarfing rootstock *MIX*, Tydeman[1] was able to shorten the juvenile phase by approximately one year. This practice has the disadvantage that many clones of this rootstock carry virus diseases and will consequently infect the new seedlings. It is therefore necessary to maintain the unworked seedling as well as its progeny worked on the rootstock, a system which doubles the land required by the plant breeder.

It has been found that apple seedlings worked on apomictic seedling rootstocks from *Malus sikkimensis* have a shorter juvenile phase than when worked on *MIX*; and since the apomictic seedlings are virus-free it should not be necessary to maintain the original seedling.

In an experiment begun in 1956, three crosses were made from which twenty-five seedlings were raised. These were multiplied vegetatively during their first year by budding on to one-year-old seedlings of *M. sikkimensis*; the young trees were later transplanted into the orchard.

It can be seen (Table 1) that 15 per cent of the trees were flowering 3 yr. after budding and 53 per cent after 4 yr. Since about one-third of the trees were fruiting by the end of their fourth year, and were carrying an average of seven fruits, it was possible to obtain an early assessment of the value of these new seedlings. None of the original twenty-five seedlings planted alongside for comparison has fruited during the same period, although both sets of trees have been treated similarly and no pruning has been carried out.

The possibility that other apomictic species may have a similar effect on the juvenile phase of apple seedlings worked on them is being investigated.

A. I. Campbell

Department of Agriculture and
Horticulture,
University of Bristol,
Research Station, Long Ashton.

---

[1] Tydeman, H. M., *Ann. Rep. East Malling Res. Sta. for 1927*, 51 (1928).

Table 1. Apple Seedlings worked on *Malus sikkimensis*

Family	No. of trees	Third year				Fourth year			
		Per cent flowering	Per cent fruiting	Av. No. per tree		Per cent flowering	Per cent fruiting	Av. No. per tree	
				Flower clusters	Fruits			Flower clusters	Fruits
Newton Wonder × Emneth Early	88	23	6	4	7	57	25	17	18
Newton Wonder × Woolbrook Pippin	86	5	2	6	1	45	37	32	7
Newton Wonder × Mr. Prothero	34	20	19	4	2	38	29	41	

Cactus Virus in the United States

Since the first report by Molisch[1] of the presence of proteinaceous spindles in the cells of certain cacti, a series of researches has been made demonstrating the association of virus with the spindles and with amorphous inclusions[2].

The immediate source material for these investigations has been exclusively from European greenhouses and botanical gardens. We can now report the presence of virus in cacti cultivated in the United States.

Characteristic cigar-shaped spindles, usually one, but occasionally two to the cell, have been observed, mainly in the hypodermal cells of free-hand sections of pads of *Opuntia monacantha f. variegata* and of four other kinds of flat-padded opuntias cultivated in Montana and California. We examined one field sample of *O. lindheimeri* shortly after it was collected in Texas. No inclusions associated with virus were found. Such inclusions were reported previously to be present in a cultivated sample of this species[2]. No spindles have been found in pads of twenty cactus species examined shortly after field collection in Arizona, California and Montana.

In all cases, virus transmission was successful, as judged by the development of spindles, when sap from spindle-containing specimens was injected into or rubbed on the previously spindle-free pads of *Opuntia* species growing in the Montana State University greenhouse and the University of California at Los Angeles Botanical Garden.

No external symptoms appeared when representatives from the families Leguminosae, Solanaceae, Chenopodiaceae, Cruciferae, Tropaeolaceae or Cucurbitaceae were inoculated. Electron micrographs made from spindle-containing pads by the quick-dip method[3] showed two groups of elongated particles, with one length peak at 515 mμ (similar to cactus virus 1 (ref. 4)) and another at 300 mμ.

We wish to thank H. O. Agrawal and R. A. Solberg for help with some experiments, and Dr. L. Bos for the electron microscopy. This work was supported by the U.S. Public Health Service Grant No. *E*-596(*c*).

(The late) Irene M. Sammons
M. Chessin

Department of Botany,
Montana State University,
Missoula, Montana.

---

[1] Molisch, H., *Ber. Deutsch. Bot. Ges.*, **3**, 195 (1885).

[2] Amelunxen, F., *Protoplasma*, **49**, 140 (1958).

[3] Brandes, J., *Nachrbl. deut. Pflanzenschutzdienst* (Braunschweig), **9**, 151 (1957).

[4] Brandes, J., and Wetter, C., *Virology*, **8**, 99 (1959).

Antimicrobial Substances from Ferns

It has been almost twenty years since Osborn[1] reported his now classical work on antimicrobial substances from green plants. Since that time the literature accumulated on this subject has been voluminous[2]. The vascular plants tested, however, have been essentially spermatophytes, with little attention to the pteridophytes. This work is concerned with the extraction of antimicrobial substances from thirty ferns collected at the Brooklyn Botanical Gardens. The specimens were selected at random, and the cut fronds were allowed to dry in air for at least one week before testing. Four additional dried ferns were obtained from other sources and likewise tested.

Extracts of the plants were prepared in the following manner: 15 gm. of pulverized material were added to 100 ml. of methanol and macerated in a Waring blendor for 3 min. and filtered. Filter-paper disks (6·35 mm. diam.) were saturated with the extract and allowed to dry for 3 hr. The surface of nutrient agar dishes was seeded with 0·5 ml. of a 48 hr. bacterial broth culture while 0·5 ml. of a one-week-old fungal culture was used to seed Sabauraud maltose agar dishes. The prepared disks were placed on the surface of the seeded dishes and incubated. All micro-organisms were incubated for 48 hr., the bacteria at 37° C., the fungi and *Erwinia caratovora* at 22° C. The zones of inhibition were measured from the disk edge to the zone edge and recorded in mm.

In Table 1 is listed the ferns and their zones of inhibition against 8 bacteria. No zones of inhibition were produced against *Aspergillus niger* ATCC 6277, *Candida albicans* ATCC 10231, *Gibberella fugikuroi* NRRL 2284, and *Helminthosporium trucicum* ATCC 11535. The results indicate that antibacterial substances were extracted in the methanol-soluble fraction from 33 of the 34 specimens tested and that plant pathogenic bacteria are more susceptible than human pathogens. This may perhaps explain the relative resistance of ferns to bacterial invaders. Additional extracts are being prepared in this Laboratory and will be the subject of future reports.

Table 1. Antibacterial Properties of Ferns

	11766	8061	10692	9637	9491	9372	8245	9592*
Cyatheaceae								
Alsophila cooperi	+	−	+	−	−	−	−	−
Cibotium glaucum	+	−	+	−	−	+	−	−
Cibotium menziesii	+	+	+	+	+	+	−	−
Cibotium schiedei	+	+	+	+	+	+	+	+
Marrattiaceae								
Angiopteris evicta	+	−	+	+	+	+	+	−
Polypodiaceae								
Acrostichum aureum	−	−	−	−	−	−	−	−
Adiantum capillus-veneris	+	+	−	−	−	+	−	+
Adiantum trapeziforme	+	−	+	+	−	−	−	−
Asplenium nidus	+	−	+	+	−	+	−	+
Blechnum spicant	+	−	+	−	−	+	−	−
Cyrtomium falcatum	+	−	+	−	−	−	−	−
Davallia pentaphylla	−	+	+	−	−	−	−	+
Dennstaedtia punctilobula	+	−	+	+	−	+	+	−
Doodia media	+	−	+	−	−	−	−	−
Dryopteris dentata	+	−	−	−	+	+	+	−
Dryopteris filix-mas†	+ + +	−	+ +	−	+ +	+ +	+ +	+
Microlepia strigosa	+	+	+	−	−	−	−	−
Nephrolepis ensifolia	+	−	+	+	+	−	−	−
Nephrolepis exaltata, a variety	+	−	−	−	−	−	−	−
Polypodium aureus	+	−	−	−	−	+	−	−
Polypodium Meyenianum	+	−	−	−	−	+	−	−
Polypodium punctatum	+	−	+	+	+	+	−	−
Polypodium queroifolia	+	−	+	−	−	−	−	−
Polystichum tsus-simense	+	−	−	−	+	+	−	−
Pteridium aquilinum	−	+	−	−	−	−	−	−
Pteridium aquilinum†	−	+	−	−	−	−	−	−
Pteris tremula	−	+	+	−	+	+	+	−
Pteris vittata	+	−	+	−	+	−	−	−
Pyrrosia lingua	+	−	−	−	−	−	−	−
Stenochlaena tenuifolia	+	−	−	−	−	−	−	−
Tectaria cicutaria	+	−	+	−	+	−	−	−
Tectaria incisa	+	−	+	−	−	−	−	−
Thelyperis setigeria	+	+	−	−	+	−	−	−
Schizaeaceae								
Lygodium japonicum	+	−	+	−	+	+	+	−

* Bacterial test organisms: *Xanthomonas phaseoli* var. *sojensis* ATCC 11766, *Erwinia caratovora* ATCC 8061, *Pseudomonas solonacaerum* ATCC 10692, *Escherichia coli* ATCC 9637, *Staphylococcus aureus* ATCC 9491, *Bacillus subtilis* var. *niger* ATCC 9372, *Bacillus megaterium* ATCC 8245, *Bacillus cereus* ATCC 9592.

† Rhizones and roots.

+, Zones of inhibition 1–5 mm.; + +, zones 6–10 mm.; + + +, zones 11–15 mm.; −, zone of inhibition absent.

Each extract was tested at least in triplicate.

Jasper C. Maruzzella

Biology Department,
Long Island University,
Brooklyn, New York.

---

[1] Osborn, E. M., *Brit. J. Exp. Pathol.*, **24**, 227 (1943).

[2] Nickell, G. L., *Econ. Bot.*, **13**, 281 (1959).

Introduction of the Potato into Western and Central Europe

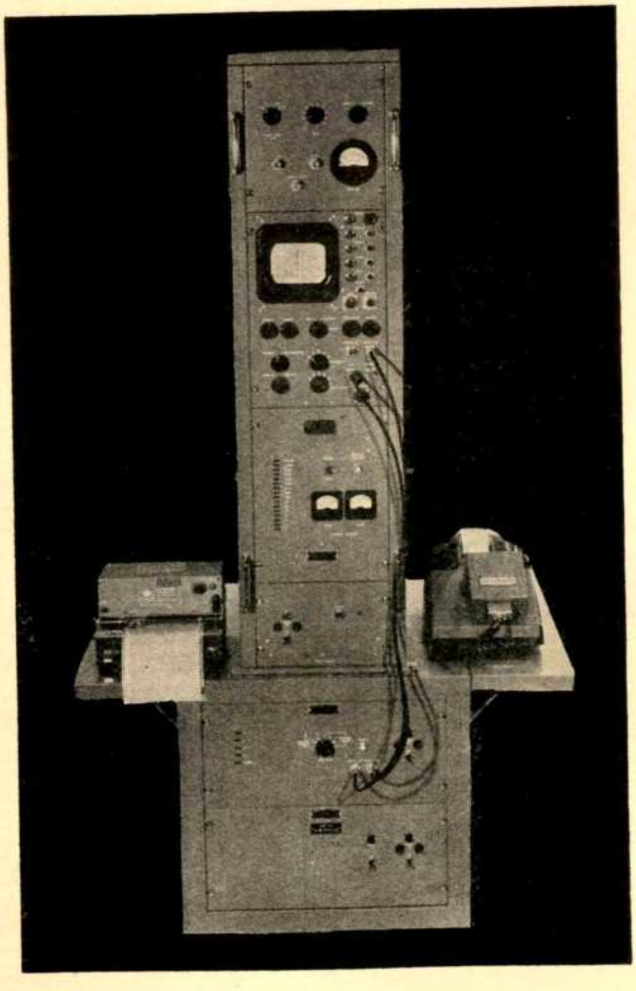
The botanist Clusius, who prepared one of the first descriptions of the potato plant[1], indicated not only the time (the beginning of 1588) of his receiving from Philippe de Sivry (Prefect of the City of Mons, Belgium) two potato tubers and a berry, but also when Philippe de Sivry himself obtained the tubers. Clusius wrote that Philippe de Sivry had received it in the preceding year from a certain friend of the Papal Legate in Belgium under the name of 'Taratouffli'. ("Is a familiari quodam Legati Pontifici in Belgio se

CDC's PROVED PERFORMANCE

100-CHANNEL PULSE HEIGHT ANALYZER

CHALK RIVER KICKSORTER

Manufactured by Computing Devices of Canada, Limited, from specifications prepared by Atomic Energy of Canada, Limited.



The convenience and speed of automatic printout can be obtained by adding a Decimal Scaler, a Print Control Unit, and an Electric Printer as accessories, shown with the Kicksorter. Provision is also made in the circuitry for the addition of a Pen Recorder for more accurate analog output.

88% TRANSISTORIZED

- Printed Circuitry
- Low Maintenance—High Reliability
- Low Power Consumption—120 Watts
- No Ventilation or Cooling Problems

VERSATILE DATA PRESENTATION

- Oscilloscope Display
- Binary Manual count readout allows the count on any channel to be displayed in digital form.
- Magnetic Core Memory—65,535 counts can be stored per channel.
- Permanent Count Storage—sixteen magnetic cores for each of the 100 channels.
- Highly Versatile—can be modified to accommodate most applications which require additional features.

The Chalk River "Kicksorter" is designed to span random pulses of various amplitudes and shapes, sort them according to amplitude into 100 channels, and store a record of the counted pulses in a magnetic core memory. This computer, with its magnetic core memory and versatile data display system, makes it possible to observe the pulse amplitude spectrum as it accumulates or after it has accumulated.

In addition to its wide use in gamma spectroscopy, the "kicksorter" has many applications in medical research, neutron time-of-flight studies, radioactive fallout studies, shielding studies, oil well and uranium logging, neutron activation analyses, measuring of radioactivity in humans, and other areas.

The analyzer is designed so that information accumulated may be displayed or read out in various ways. Data may be observed on the oscilloscope, or information already accumulated in the memory core may be displayed on the screen, plotted on a strip chart recorder, or printed out in decimal form on an automatic printer.

For additional information, wire or write to:

Bendix International
DIVISION OF THE BENDIX CORPORATION
205 East 42nd Street
New York 17, N. Y., U.S.A.
Cable Address: "Bendixint"

Sales Representative for Great Britain
NUCLEAR ENTERPRISES (G.B.) LTD.
BANKHEAD MEDWAY, SIGHTHILL,
EDINBURGH 11, SCOTLAND

SALES AND SERVICE AVAILABLE THROUGHOUT THE WORLD.

LIL

One of the Laporte operations producing Fluorspar

One of the Laporte operations producing the Raw Materials of Progress

Laporte Industries Limited London England

LAPORTE

acceptisse scribebat, anno praecedente Taratouffli nomine"). Roze, the author of *Histoire de la Pomme de Terre*[2], translating into French Clusius's description, erroneously omitted the words "in the preceding year". The same mistake was repeated by Redcliffe Salaman in his monograph *The History and Social Influence of the Potato*[3]. Fuess[4] in Germany, as a result of Roze's fault and evidently having no original text by Clusius, erroneously supposed the time of potato introduction into Mons to be the year 1566. Roze admitted the second mistake in the French translation of Clusius's potato diagnosis. Clusius describes the shape of his potato berries as "orbiculata". That strictly means not globose or rounded but disk-shaped, and his description is very true to the picture. In Salaman's English translation of Clusius's diagnosis, the same lack of punctiliousness is observed.

Disk-shaped berries are rare to-day among cultivated potatoes, and this morphological feature, as well as some other ones (namely, numerous stolons and tubers, very different tuber size, rather late tuberization, probably deep eyes, weakly dissected leaves with short leaflet stalks, leaflets without elongated apices, numerous blossoms, calyx sharply limited from the top of the pedicels, according to the picture probably spear-leaved lobes of the calyx, deeply dissected calyx and corolla, green rays of stella, evidently short anthers (in broad cone ?) and odoriferous flowers), lead me to suppose that at least one tuber of two, or a plant from the berry which Clusius received in Vienna from Philippe de Sivry, belongs to the circle of forms *S. andigenum* Juz. et Buk. convarietas curtibaccata Lechnovitch, from South Peru. The present-day German potato (var. Jubel) has the same type of berries, anthers, and leaves and is, perhaps, connected with Clusius's material. The date of at least one, probably the earliest and possibly the chief, introduction of the potato into Western and Central Europe is quite clear.

V. S. Lekhnovitch

All-Union Crop Husbandry
Research Institute,
44 Herzen Street,
Leningrad, 1.

---

[1] Clusius, Carolus, *Rariorum plantarum historia* (Antverpiae, 1601).

[2] Roze, E., *Histoire de la Pomme de Terre* (Paris, 1898).

[3] Salaman, R. N., *The History and Social Influence of the Potato*, p. 89 (Cambridge, 1949).

[4] Fuess, W., *Die Urheimat der Kartoffel, ihre Einführung und Ausbreitung in Europa. Ernähr. d. Pflanze*, **31** (17), 288 (1935).

ENTOMOLOGY

Air-Swallowing : a Mechanism in Photic Reversal of the Beetle *Trypodendron*

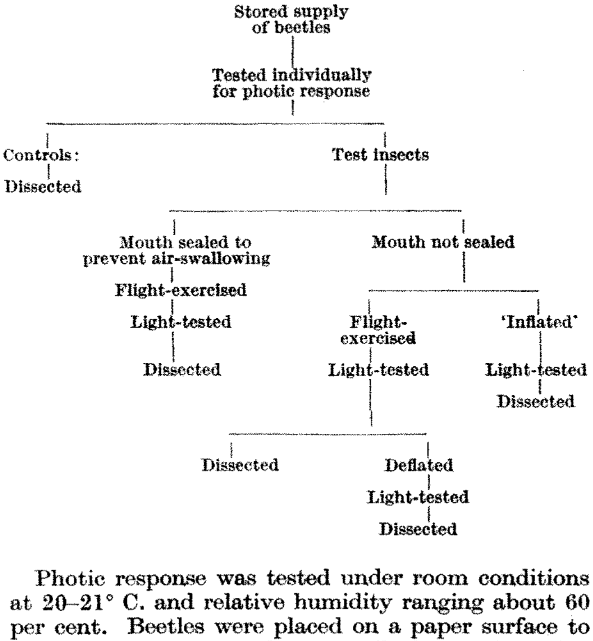
A previous communication[1] reported the effectiveness of flight exercise in abolishing the overruling response to light in overwintered adults of the ambrosia beetle *Trypodendron* (*Xyloterus*) *lineatum* Olivier. This observation is ecologically significant in explaining the behaviour of the insect in terms of one of its normal experiences. Evidently the photopositive response which initially compels it, advantageously, to dispersive flight indirectly sponsors termination of flight and unmasks a latent responsiveness to host factors, allowing it to settle down.

The change seemed to occur only after a period of flight, never in its absence. Nevertheless, belief in the role of flight could be strengthened if a possible mechanism could be demonstrated. Furthermore, a demonstrable mechanism might aid in the interpretation of anomalous behaviour in other work with this insect, or be used for experimental purposes.

Some of the more obvious facts, drawn from existing knowledge, were brought to bear on the question. Chapman[2] had reported, for the same species, that the ventriculus, and often the œsophagus, of beetles taken in flight are distended by a large gas bubble. The significance of the discovery was not clear at the time.

The coincidence of the two phenomena, namely, photic reversal and gas accumulation, associated with a common experience, namely, flight, could not be ignored, but how could gas in the alimentary tract be assumed to affect behaviour ? The first and simplest possibility was that it exerts pressure and creates a signal that is relayed to the central nervous system. Photic reversal associated with pressure in the body of spruce budworm larvæ had been observed by Wellington[3]. The reversal occurred regardless of how the pressure was applied, whether from food, injected fluid or externally applied pressure of a ligature. The influence of pressure is relayed to the higher neural centres through the 5th abdominal ganglion, which can be 'blocked' by local anæsthetizing with methylal (dimethoxymethane).

A schedule of tests was drawn up to compare responses of *Trypodendron* after various treatments contrived to introduce gas pressure.



Photic response was tested under room conditions at 20–21° C. and relative humidity ranging about 60 per cent. Beetles were placed on a paper surface to observe and trace with a pencil their orientation towards a window source of light. Each beetle was given five trials beginning each time from a different direction of point relative to the light.

Beetles of both sexes fresh from overwintering, and thus non-flown, were photopositive in varying degrees. The rate of turn towards light, rate of crawling, and frequency of attempts to fly varied with individuals. Some individuals took short (5–10 cm.) backward flights before taking the typical laterally oscillating course, which advanced them at about 2 m./sec.

Unflown beetles generally had no gas bubble in the ventriculus, and rarely had a small one.

Beetles with a viscous seal over the mouth did not change behaviour after flight, and dissection revealed no gas bubble.

Beetles suspended by the pronotum responded variously to the opportunity for anchored flight, some maintaining almost uninterrupted wing movement for hours, others being intermittent, and still others 'flew' only occasionally. Prolonged flight was generally followed by a decisive reversal of photic response, and distension of the gut with gas was always associated with photic reversal. Deflation by piercing through the elytra and soft dorsum into the alimentary tract with a finely drawn glass capillary (when not accompanied by surgical damage and paralysis) restored the photically reversed beetles to light-positive behaviour.

Unflown beetles, artificially inflated by blowing through a fine drawn capillary sealed to the mouth of the beetle with heavy grease, became photically reversed. Dissection confirmed the successful inflation.

The evidence is thus : that the unflown beetles are strongly photopositive ; that through prolonged flight, but not without it, they become indifferent or negative to light ; that accumulation of gas in the gut accompanies the change, and the change is always associated with the gas ; that deflation restores the photopositive response ; and that artificial inflation is effective in abolishing the photopositive response.

The evidence is thus reasonably conclusive that the release of responses other than photic in *Trypodendron* is brought about by air swallowed during flight, acting presumably by its pressure.

KENNETH GRAHAM

Department of Zoology,
University of British Columbia,
Vancouver 8.

[1] Graham, K., *Nature*, **184**, 283 (1959).
[2] Chapman, J., *Proc. Tenth Intern. Congr. Ent.*, **4**, 375 (1956, 1958).
[3] Wellington, W. G., *Canad. Entomol.*, **80**, 56 (1948).

MICROBIOLOGY

Oxygen Inhibition of Respiration in *Azotobacter*

THE effect of oxygen partial pressure on *Azotobacter* has been studied by Meyerhof and Burk[1], Burk[2] and by Parker and Scutt[3], with essentially similar findings. Respiration in this organism reaches a clearly defined maximum at 0·15–0·30 atm. oxygen, Burk's results tending to the lower values and Parker and Scutt's to the higher. Respiration was measured with either sucrose or glucose as the substrate, and a similar observation was made by Tschapek and Giambiagi[4], who followed glucose dissimilation rather than oxygen uptake. Inhibition of some enzyme system(s) in the respiratory mechanism appears to be the most likely explanation of this effect.

The oxygen effect seems to be peculiar to *Azotobacter*, since we were unable to find such a well-defined inhibition when the respiration of *Bacillus subtilis* (N.C.T.C. 518), *Saccharomyces cerevisiae* and *Rhizobium trifolii* (Australian strain *NA* 30) was measured on sucrose as substrate under different oxygen partial pressures ranging from 0·05 atm. to 1·00 atm. oxygen (Fig. 1).

An attempt has been made to identify a stage sensitive to oxygen of the respiratory system using various substrates as the carbon source and testing for the presence of the inhibition.

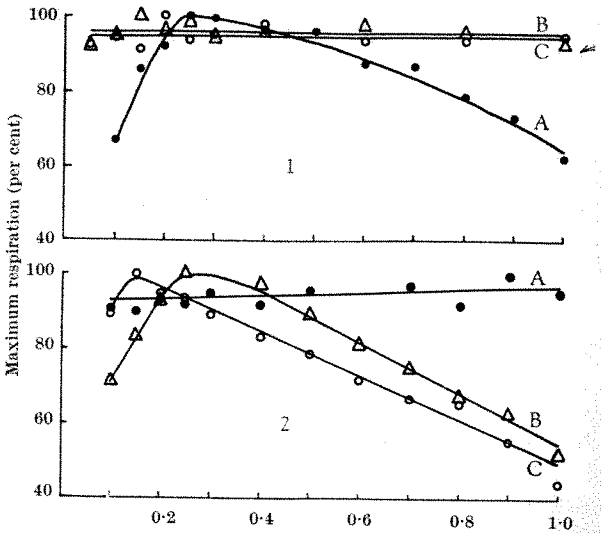


Fig. 1. Plot of oxygen uptake against oxygen partial pressure for three different organisms. Values indicate the percentage of the maximum uptake observed. Curve *A*, *Azotobacter vinelandii*, strain Wisconsin *O*. Curve *B*, *Rhizobium trifolii*, strain *NA* 30. Curve *C*, *Bacillus subtilis*, N.C.T.C. 518

Fig. 2. Plot of oxygen uptake against oxygen partial pressure for *Azotobacter vinelandii* on different substrates. Curve *A*, glycerol. Curve *B*, pyruvate. Curve *C*, succinate

Cells of *A. vinelandii* (strain *O*, kindly supplied by Prof. P. W. Wilson) were grown overnight on synthetic media, using sucrose and urea as the carbon and nitrogen sources, respectively. The cells were recovered by centrifugation, re-suspended in a complete mineral salts medium containing the desired carbon source, and aerated for 4 hr. in the presence of urea as a source of nitrogen. This period sufficed for adaptation of the cells[5]. Oxygen uptake was then measured by the conventional Warburg technique in short-term experiments ($\frac{3}{4}$ hr.), using a known density of organisms under oxygen–argon mixtures containing 0·1–1·0 atm. oxygen.

Comparable results to those given with sucrose were found for glucose, pyruvate, acetate, succinate and malate, although the inhibition was more severe with the organic acids than with the sugars. Sample curves for three of these substrates are shown in Fig. 2. Glycerol is the obvious exception, no inhibitory effects of oxygen being detectable in this case.

Addition of —SH group stabilizing reagents (cysteine, glutathione) at concentrations up to 0·01 *M* failed to protect the organisms from the toxic effects of oxygen. That they are permeable to cysteine was shown by the toxic effect of the higher levels of cysteine used.

The failure of the organic acids to 'short-circuit' the inhibition suggests that the oxygen-sensitive system must be external to glycolysis, lying either in a tricarboxylic acid cycle known for these organisms[6], or in the terminal respiratory pathway. Inhibition by oxygen in the former cycle has been reported for pyruvic acid oxidation in *Staphylococcus aureus*[7] and rat brain[8], and for a step in the reactions between citrate and α-oxoglutarate in potato tubers[9]. Oxygen inhibition of an enzyme in the latter pathway has been shown recently for cytochrome *c* reductase[10].

It is postulated that the pattern seen in Fig. 1 for *A. vinelandii* occurs as a result of two opposing systems : (*a*) a terminal oxidation system which reaches a maximum value about 0·25 atm. oxygen and then maintains this value at least up to 1·0 atm.; and (*b*) an oxygen-sensitive system the inactivation of which increases with increasing partial pressure of oxygen and which determines whether the potential activity of system (*a*) is reached.

The rising part of the experimental curve could then be explained as a rapid rise in the activity of (*a*) compared to which inactivation of (*b*) is negligible. At higher partial pressure of oxygen, the declining activity is due to inactivation of (*b*).

Examples known for system (*b*) have already been mentioned. A cytochrome oxidase system with characteristics of system (*a*) is known from *Pseudomonas*[11].

We are unable to offer an explanation of the apparent lack of inhibitory effect when glycerol is used as the substrate. It suggests the possibility that a permease system is being poisoned, but this seems unlikely as the oxygen effect is similar with substrates of neutral sugars or anionic organic acids. Further work on oxygen poisoning is in progress.

This work was carried out while one of us (M. J. D.) was in receipt of a Commonwealth Scientific and Industrial Research Organization Studentship, and later an I.C.I. (Australia and New Zealand) Research Fellowship. Grateful acknowledgment is made to these sources and to the University of Western Australia for research grants.

M. J. DILWORTH
C. A. PARKER

Soil Microbiology Section,
Institute of Agriculture,
University of Western Australia,
Nedlands.

[1] Meyerhof, O., and Burk, D., *Z. physik. Chem.*, A, **139**, 117 (1928).

[2] Burk, D., *J. Phys. Chem.*, **34**, 1195 (1930).

[3] Parker, C. A., and Scutt, P. B., *Biochim. Biophys. Acta*, **38**, 230 (1960).

[4] Tschapek, M., and Giambiagi, N., *Arch. Mikrobiol.*, **21**, 376 (1955).

[5] Williams, A. M., and Wilson, P. W., *J. Bact.*, **67**, 353 (1954).

[6] Stone, R. W., and Wilson, P. W., *J. Bact.*, **63**, 605 (1952).

[7] Wolin, M. J., Evans, J. B., and Niven, C. F., *Arch. Biochem. Biophys.*, **58**, 356 (1955).

[8] Mann, P. J. G., and Quastel, J. H., *Biochem. J.*, **40**, 139 (1946).

[9] Barker, J., and Mapson, L. W., *Proc. Roy. Soc.*, B, **143**, 523 (1955).

[10] Dixon, M., Maynard, J. M., and Morrow, P. F. W., *Nature*, **186**, 1032 (1960).

[11] Horio, T., Higashi, T., Matsubara, H., Kusai, K., Nakai, M., and Okunuki, K., *Biochim. Biophys. Acta*, **29**, 297 (1958).

A Micro-method for performing the Phenylalanine Test on Cultures from Multi-carbohydrate Media

THE phenylalanine test is of great value for investigating cultures of organisms in the family Enterobacteriaceae. Organisms in the *Proteus* group are able to de-aminate the amino-acid, phenylalanine, to form phenylpyruvic acid, whereas members of the other groups in the family are unable to do so. Buttiaux *et al.*[1] claim that this test is more reliable than the urease test for the identification of *Proteus* strains.

In a previous communication[2] a technique was described in which the bacterial growth from Kligler's iron agar, or other multiple carbohydrate media, could be tested for its ability to de-aminate phenylalanine. Although this technique gives reliable results, it has several disadvantages: (1) the removal of the cells from the Kligler's iron agar slope by washing with phenylalanine solution leaves no cells for further biochemical or serological examination; (2) a shaker and bijou bottles (which need to be sterilized and cleaned before re-use) are needed; (3) it is time-consuming to test a large number of cultures; (4) the cost of the phenylalanine reagent and of the glassware preparation is not inconsiderable.

With these points in mind, a much simpler technique was evolved, whereby the original bacterial culture on Kligler's medium was retained, neither a shaker nor bijou bottles were required and the cost of performing the test was reduced by more than 90 per cent.

In the micro-method now proposed, ordinary 3 in. × 1 in. glass slides have six squares ($\frac{3}{8}$ in. × $\frac{3}{8}$ in. or 1 cm. × 1 cm.) etched on to them on one side, with a diamond glass cutter. The slides are then warmed in a bunsen burner flame, and a black wax pencil is rubbed along the etched lines, leaving these clearly marked in black, when the excess wax is rubbed off with a soft cloth.

From a Pasteur pipette, 1 drop of a 0·4 per cent β,L-phenylalanine solution prepared in 0·01 *M* phosphate buffer at *p*H 7·4 is placed in the centre of each of the etched squares on the slides. A heavy inoculum of bacterial culture is removed from the slope of the Kligler's medium, using a loop, and this growth is emulsified in one of the drops of phenylalanine. This is best done holding the slide against a black background, as it is essential to obtain as heavy a cell suspension as possible. The waxed, etched lines prevent the drop from spreading beyond its square (Fig. 1).

The slide is now placed on a circle of damp filter paper in the bottom of a Petri dish, and, after the cover has been replaced, the assembly placed for 20 min. at 37° C. in an incubator. At the end of this period, 1 drop of an 8 per cent ferric chloride solution (aqueous) is added to each of the drops of bacterial suspension on the slide, and the Petri dish is tapped gently on the side to aid the mixing of the drops. An immediate dark green colour develops if phenylpyruvic acid has been formed. This green colour fades within a minute, therefore the reactions should be noted at once.

In a comparison made between this micro-method and the method previously described[2], thirty cultures

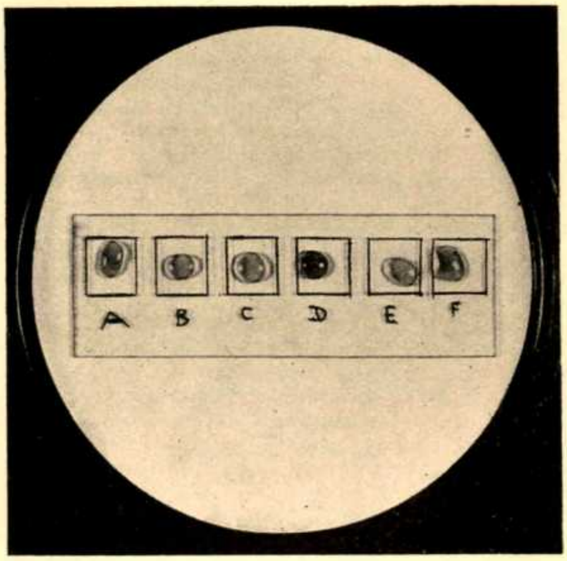


Fig. 1. Slide showing etched, waxed squares, and phenylalanine tests with cultures marked *A*, *B*, *C*, *D*, *E* and *F*. Only *D* is showing a positive reaction

of organisms in the Enterobacteriaceae, including twenty *Proteus* strains, were inoculated on to Kligler's iron agar, incubated for 18–24 hr. at 37° C., and both methods of performing the phenylalanine test were used on the bacterial growth from the slopes. In all cases the micro-method gave the same results as the other method; all the *Proteus* strains were strongly positive, all the other strains were negative (yielding yellow or brown colorations).

Similarly, using another multiple carbohydrate medium: phenol red agar base (Difco) 3·1 per cent, lactose 1 per cent, sucrose 1 per cent and mannitol 0·1 per cent, identical results were obtained.

After completing the micro-test, the slides may be placed in a disinfectant solution until required again, when all they need is to be rinsed with running water and dried.

This simple micro-technique would seem to allow the rapid examination of large numbers of cultures such as might be encountered in the routine and in the research laboratory.

D. J. Stewart

Ministry of Agriculture
for Northern Ireland and
The Queen's University of Belfast.

---

[1] Buttiaux, R., Moriamez, J., and Papavassiliou, J., *Ann. Inst. Pasteur*, **90**, 133 (1956).

[2] Stewart, D. J., *Nature*, **183**, 1537 (1959).

Absence of Lysogeny in *Pasteurella pestis*

A survey of the literature suggests that most bacteria are lysogenic for one or more phages. This communication reports failure to detect or establish lysogeny among strains of *Pasteurella pestis*. The object of the work was to obtain a temperate phage suitable for genetic study by means of transduction[1] of factors determining virulence in this organism[2]. Fifty-four different strains of *P. pestis* (Table 1) were screened for lysogeny and attempts were made to establish lysogeny with phages lytic for *P. pestis*.

In the screening experiments, a method based on that of Fisk[3] was used. Each of the 54 strains was tested both for lysogeny and as a potential phage indicator. Organisms derived from overnight cultures in tryptic digest meat broth grown at 28° C. in gently rotated tubes were used throughout. Indicator plates were prepared by spreading about 2×10^{8} organisms as lawns on broth agar plates containing 0·075 per cent sodium metabisulphite and dried. Approximately 0·01-ml. drops of each culture were applied to each lawn using a multiple inoculator[4]. The plates were examined after incubation for 40 hr. at 28° C. No evidence of lysogeny was seen. Known lysogenic strains of *Salmonella typhimurium* gave clear evidence of their lysogeny when tested by this method, using a suitable indicator strain.

Ultra-violet induction was attempted by irradiation of cultures of each strain in phosphate buffer at 9 ergs/mm.^{2}/sec. for 20 sec. using a Hanovia low-pressure lamp. None showed a reduction in turbidity on subsequent incubation in broth at 28° C. over a 10-hr. period. No phage activity was detected when drops of these cultures were plated on lawns of the 54 strains of *P. pestis*, 5 strains of *P. septica*, 5 of *P. haemolytica* or 6 of *P. pseudotuberculosis*.

Table 1. Sources of Strains of *Pasteurella pestis* Investigated

Microbiological Research Establishment, Porton, England	National Collection Type Cultures, Colindale, England	Dr. R. Devignat, Elisabethville, Congo
*WCDC Pp*1	*EV*(*M*)	*A*16
*WCDC Pp*6	*P.F.*2	*A*28
*WCDC Pp*54	Parel	*A*29
*WCDC Pp*5*A* 2258	*T.S.*	*A*32
New Mexico 499559	Bombay 1	*A*35
New Mexico 499668	Bombay 2	*A*36
I.72		*M*
14		*M*1*d*
Shasta		*M*38
*EV*76		*M*39
139*L*		*M*40
*F*7793/10		*O*12
*F*9581		*O*13
5341		*O*14
*L*27		*O*15
*L*36		*O*18
TRU		*O*19
Harbin		*O*20
Yokohama		*O*23
Java		*O*24
Tjiwidej *S* (*TS*)		*O*25
*A*1122		*O*26
Soemedang		*O*27
Elisabethville		
*MP*6		

In case failure to detect lysogeny had been due to the spontaneous liberation of small numbers of phage particles only by a strain of *P. pestis*, two mixed cultures consisting of about 5×10^{7} organisms of all strains were shaken at 28° C. and 37° C. respectively for 6 hr. to permit such phage, if present, to propagate on a sensitive strain and so increase the chance of its detection. The mixed cultures were centrifuged and the supernatants sterilized with chloroform. None of the *P. pestis* strains was lysed by these culture fluids. Since it was possible that strains of *P. pestis* failed to act as indicators because they were all lysogenic for the same or related phage, attempts were made to 'cure' a strain by repeated heavy ultra-violet irradiation[5]. However, derivatives of strains of *TS* which had been subjected to one, two, three or four serial irradiations in buffer at 11 ergs/mm.^{2}/sec. for 3 min. used as potential phage indicators also failed to reveal phage in the same culture fluids.

Of 17 phages which were lytic for strains of *P. pestis* at 28° C. or 37° C. on sodium metabisulphite medium (8 isolated from local sewage and 9 donated preparations) one, *P*1*k* (ref. 6), gave completely turbid plaques and three others plaques with wide turbid edges. Isolates from the former gave only non-lysogenic colonies resistant to *P*1*k*: those from the latter gave sensitive bacteria and virulent phage which could apparently be 'carried' through at least one single-colony isolation. Microcolonies appearing on lysed lawns of *P. pestis* strains after incubation for 5–7 days at 28° C. followed by 14 days at room temperature also yielded only sensitive bacteria and 'carried' phage. On the basis of host range and efficiency of plating, at least 7 of the 17 phages appeared to be different. Had any of them arisen from the same temperate phage by different mutations to virulence, multiple infection with pairs of them could have resulted in the establishment of lysogeny[7]. This was attempted by inoculating separate lawns of strain *TS* on sodium metabisulphite medium infected with each of 15 of the phages with drops of suspensions of each of them using the multiple inoculator. The multiplicity of infection for both phages within each area covered by a drop was greater than one and the whole process took only 25 min. On prolonged incubation only slow-growing microcolonies appeared which were again shown to consist of sensitive bacteria and virulent phage.

If any strains of *P. pestis* are lysogenic they, or suitable indicator strains, or both, are rare. There are a few reports of failure to detect lysogeny in other species of bacteria[8]. Of these, only Williams Smith's failure to detect it among 62 strains of *Salmonella pullorum* involved experiments on a similar scale to those with *P. pestis*. It is possible that all *P. pestis* strains tested are lysogenic for the same non-inducible phage. The occurrence of colonies comprising sensitive *P. pestis* organisms and virulent phage is thought to be a further example of 'pseudolysogeny'[9], although less stable than that previously reported for this organism[10].

I am indebted to Dr. R. Devignat of Elisabethville, Congo, for strains of *P. pestis*, and Drs. N. Boulgakov, G. Girard and F. Jacob, of Paris, and Dr. D. Eisler, of California, for phage preparations. Discussion with Dr. T. W. Burrows and the technical assistance of Miss R. M. King are gratefully acknowledged.

D. A. Smith*

Microbiological Research Establishment,
Porton,
Salisbury,
Wilts.

---

* Present address: Department of Microbiology, University of Birmingham, 15.

[1] Zinder, N. D., and Lederberg, J., *J. Bact.*, **64**, 679 (1952).

[2] Burrows, T. W., *Symp. Haffkine Inst., Bombay*, 14 (1960).

[3] Fisk, R. T., *J. Inf. Dis.*, **71**, 153 (1942).

[4] Smith, D. A., *J. App. Bact.* (in the press).

[5] Boyd, J. S. K., *Biol. Rev.*, **31**, 71 (1956). Zinder, N. D., *Virology*, **5**, 291 (1958).

[6] Lennox, E. S., *Virology*, **1**, 190 (1955).

[7] Levine, M., *Virology*, **3**, 22 (1957). Kaiser, A. D., *ibid.*, **3**, 42 (1957).

[8] McCloy, E. W., *J. Gen. Microbiol.*, **18**, 198 (1958). Russell, R. L., Jann, G. T., and Froman, S., *Bact. Proc.*, 39 (1958). Williams Smith, H., *J. Gen. Microbiol.*, **5**, 458 (1951).

[9] Jacob, F., Lwoff, A., Siminovitch, A., and Wollman, E., *Ann. Inst. Pasteur*, **84**, 222 (1953).

[10] Girard, G., *C.R. Soc. Biol., Paris*, **151**, 1068 (1957).

GENETICS

Effect of Hybridization on the Primary Polydipsic Trait of an Inbred Strain of Mice

A non-obligatory polydipsia which is reflected in the excretion of large quantities of urine of low specific gravity has been found in more than 90 per cent of an inbred strain of mice (*STR/N*)[1–3]. It appears to be due to an innate thirst, the satisfaction of which is entirely unnecessary for survival. It is present in mild degree at the earliest ages studied (4 weeks), and is greatly accentuated at about 6 months of age. There is an associated high mortality in males due to the combined presence of polyuria and a urethral plug. The latter was found in almost all male mice of all strains examined, and is probably derived from seminal fluid.

To attempt to obtain information on the mode of inheritance of the trait, the intake of water and urine specific gravity[4] of F_1 and F_2 hybrids resulting from the mating of the inbred polydipsic strain and a non-polydipsic strain and back-crosses were studied at 1–5 months of age in a total of 250 animals. The mean urine specific gravities and intake of water of the hybrid mice tended to be intermediate between those for the polydipsic and non-polydipsic strains, but resembled much more closely the non-polydipsic strain values (Table 1). There was no consistent difference between F_1 and F_2 hybrids. For reasons which are not apparent, back-crosses with both polydipsic and non-polydipsic strains tended to resemble the polydipsic strain. The inheritance of the trait does not appear to be as a simple recessive. The precise mode of inheritance and the number of genes involved will require examination of considerably larger numbers of animals.

Table 1. Urine Specific Gravity and Water Intake of *STR/N* and *DBA/2JN* Mice and their F_1 Hybrids*

Strain	*STR/N*		*DBA/2JN*		(*STR/N* × *DBA/2JN*)F_1	
Sex	M	F	M	F	M	F
Number of animals	6	6	6	6	14	11
Body-wt. (gm.)	15 ± 2‡	15 ± 2	14 ± 1	12 ± 1	14 ± 1	14 ± 1
Urine specific gravity	1·032 ± 0·006‡	1·042 ± 0·004	1·072 ± 0·006	1·065 ± 0·008	1·065 ± 0·003	1·059 ± 0·005
	(1·024–1·044)§	(1·023–1·068)	(1·061–1·081)	(1·026–1·100)	(1·027–1·105)	(1·032–1·093)
Water intake†	9·2 ± 0·5‡	9·4 ± 1	7·2 ± 0·5	4·7 ± 0·6	5·4 ± 0·3	5·9 ± 0·3

* Animals 29–44 days old.

† Four daily water measurements made 20–28 days after urine specific gravity measurements, ml. per animal per 24 hr.

‡ *S.E.* § Range.

Emanuel Silverstein*

Laboratory of Pathology and
Histochemistry,
National Institute of Arthritis
and Metabolic Diseases,
National Institutes of Health,
Bethesda, Md.

---

* Present address: Department of Physiological Chemistry, University of Minnesota Medical School, Minneapolis 14, Minnesota.

[1] Silverstein, E., Sokoloff, L., Mickelsen, O., and Jay, jun., G. E., *Fed. Proc.*, **17**, 457 (1958).

[2] Silverstein, E., First International Congress of Endocrinology, Copenhagen, July 1960, *Periodica, Abstr.*, No. 61.

[3] Silverstein, E., Sokoloff, L., Mickelsen, O., and Jay, jun., G. E., *Amer. J. Path.*, **38**, 143 (1961).

[4] Silverstein, E., *J. App. Physiol.*, **16**, 194 (1961).

Chromosome Rearrangements induced by X-Rays in Immature Germ Cells of *Drosophila*

Many investigations have indicated that few chromosomal aberrations or losses have been produced in the immature germ cells of *Drosophila*[1–3]. The present work deals with factors influencing the production of chromosome rearrangements by X-rays in early oocytes and oogonia of *Drosophila melanogaster*.

Attached-*X* females aged 2–3½ and 8–9½ days of the genetic constitution $sc\ ct^n\ oc\ car.y\ In\ 49\ sn$ and an $sc^{V1}.Y^S$ chromosome were irradiated with 2,400 r. X-rays. The dose was given either in 2 min. or in two 1-min. fractions separated by an hour interval in air (*AAA*); or in two 1-min. fractions separated by an hour interval with a 53-minute nitrogen flushing of the flies (*ANA*). After treatment the females were mated with $y\ sc^{S1}\ B\ f\ In\ 49\ v$ males and periodically transferred for 32 days. The progeny were scored for detachments of the attached-*X*. Detachments involve translocations between the *X* and one of the following: the *Y*, the IVth or the major autosomes[4,5].

Table 1. FREQUENCY OF DETACHMENTS OF ATTACHED-*X* CHROMOSOMES PRODUCED BY DIFFERENT MODES OF TREATMENT

	2–3½-day-old females				8–9½-day-old females			
Days after irradiation	2,400 r. No. except./total females	*AAA** No. except./total females	*ANA*† No. except./total females	Control No. except./total females	2,400 r. No. except./total females	*AAA** No. except./total females	*ANA*† No. except./total females	Control No. except./total females
11–13	50/2,348 = 0·021	20/1,528 = 0·013	24/665 = 0·036	0/979	43/1,829 = 0·024	21/1,278 = 0·016	19/793 = 0·024	1/1,101
18–21	68/3,578 = 0·019	38/2,533 = 0·015	37/1,088 = 0·034	0/1,160	67/2,814 = 0·024	35/2,227 = 0·016	30/1,218 = 0·025	0/911
23–26	56/2,501 = 0·022	44/2,363 = 0·019	20/709 = 0·028	0/1,071	45/1,866 = 0·024	39/1,840 = 0·021	16/702 = 0·023	0/700
26–28	47/2,168 = 0·022	25/1,843 = 0·014	9/378 = 0·024	0/634	24/1,228 = 0·020	35/1,248 = 0·028	6/519 = 0·012	0/524
28–32	60/3,429 = 0·017	40/2,808 = 0·014	17/488 = 0·035	0/1,261	47/2,733 = 0·017	36/2,203 = 0·016	25/943 = 0·027	0/979
HARMONICALLY WEIGHTED FREQUENCIES OF EXCEPTIONS FOR EACH TREATMENT								
Days 11–32	0·020 ± 0·0012	0·015 ± 0·0012	0·032 ± 0·0031	0/5,105	0·022 ± 0·0014	0·019 ± 0·0015	0·023 ± 0·0018	1/4,215
0–4	0·049 ± 0·0030	0·040 ± 0·0027	0·072 ± 0·0068	0/4,927	0·050 ± 0·0038	0·052 ± 0·0040	0·086 ± 0·011	0/3,872

* *AAA* = air present during irradiations and air present during 1 hr. interval.

† *ANA* = air present during irradiations and nitrogen present during 53 min. of 1 hr. interval.

The results are presented in Table 1. The frequencies of detachments in all treatments are presented as the observed exceptions relative to the total number of females. The frequency of occurrence of detachments is more complex due to non-random disjunction and different viabilities of the sexes, to mention just two factors. Rearrangements have been obtained from every treatment and sample of germ cells. In young females fractionation of treatment results in significantly fewer rearrangements ($P < 0\cdot01$) than the concentrated treatment. Anoxia during the interval results in significantly higher rearrangement frequencies ($P < 0\cdot001$) than the uninterrupted treatment.

In old females there appears to be no fractionation effect, which is, unexpectedly, the same as the results obtained in eggs laid shortly after irradiation, but the *ANA* series does not show the expected increase in rearrangements. The results for eggs laid 11–32 days are substantially in agreement with the results obtained for rearrangements produced in eggs laid 0–4 days after irradiation[6]. For purposes of comparison, a portion of the 0–4 days results are reproduced in Table 1.

The results for immature oocytes and oogonia are consistent with the notion that, for young females, these rearrangements are formed completely very shortly after irradiation, and show that they depend on oxygen for completion, and that anoxia blocks restitution and usually alters the relationship of rejoining. Furthermore, the results indicate that the age of the female at irradiation appears to influence the restitution process in her various germinal cells.

Genetic analyses demonstrated that detachments of the attached-*X* chromosomes were indeed in many cases translocations involving the major autosomes as well as the *Y* and the smaller IVth chromosome of *Drosophila*[4,5]. Other workers, for example Neuhaus[7], considered the induced detachments to be cross-overs between the *X* and *Y*.

Induced 'crossing-over' is confined to heterochromatic regions[8]. Detachments also occur there. Induced 'cross-overs' are greater in frequency in eggs laid during 6–15 days after irradiation than days 0–4. In the case of detachments more of the changes are found in eggs oviposited in the first days, as is also the case for other induced mutational events, for example, recessive lethals, dominant lethals and chromosome loss[3].

The notable dearth of chromosome translocations found induced in early oocytes and oogonia, as well as in spermatogonia, is partly due to the sorting out, in perhaps half the cases, of complementary parts of the rearrangement into different daughter cells (including a mechanism suggested by Novitski[1] that oogonial sex cells lacking an *X* chromosome may be unable to survive and do not reach the meiotic stage); but it has long been known that this loss of cases is far from enough to explain the lack of translocations obtained from these stages. The present results show that separation of the attached-*X* does not necessarily cause a lethal aneuploidy, and would permit the survival of those cells in which the detachment is rearranged in a euploid order.

The frequencies of gross chromosome rearrangements (if we may so consider them) reported here for immature germ cells in females are the highest observed, but the genetic technique is more sensitive than those used by previous workers.

This research was supported by grants from the National Science Foundation and the Rutgers University Research Council.

SEYMOUR ABRAHAMSON*
Department of Biology,
Rutgers University,
Newark, New Jersey.

* Present address: Department of Zoology, University of Wisconsin, Madison, Wisconsin.

[1] Novitski, E., *Dros. Inform. Serv.*, **27**, 108 (1953).
[2] Muller, H. J., and Herskowitz, I. H., *Amer. Naturalist*, **88**, 177 (1954).
[3] King, R. C., Darrow, J. B., and Kaye, N. W., *Genetics*, **41**, 890 (1956).
[4] Abrahamson, S., Herskowitz, I. H., and Muller, H. J., *Genetics*, **41**, 410 (1956).
[5] Parker, D. R., and McCrone, J., *Genetics*, **43**, 172 (1958).
[6] Abrahamson, S., *Intern. J. Rad. Biol.* (in the press).
[7] Neuhaus, M. J., *Z. Indukt. Abstamm.-u. VererbLehre*, **71**, 265 (1936).
[8] Muller, H. J., *Genetics*, **10**, 470 (1926).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Experimental Officer (with a degree in science or horticulture, or equivalent qualification) in the Physiology Section, for growth and development studies on vegetable crops—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (August 5).

Scientific Officer (with a good honours degree in chemistry, or equivalent qualification, and preferably with some experience of, and interest in, food technology), for investigations into the problems involved in various aspects of biscuit manufacture—The Deputy Director of Research, Baking Industries Research Station, Chorleywood, Herts (August 5).

Lecturer or Assistant Lecturer in Zoology (Entomology) at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 7).

Experimental Officer (graduate in agriculture, botany or agricultural botany) in the Cereal and Brassica Breeding Station, for work on the recording and handling of breeding and genetical material—The Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth (August 10).

Lecturer (preferably with research experience in surface chemistry or chemical kinetics) in Pharmacy at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 11).

Lecturer/Senior Lecturer in Classical Archæology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 11).

Lecturer (graduate in science or engineering) in the Department of Building Science—The Registrar, The University, Liverpool (August 12).

Senior Research Assistant (Ph.D. in theoretical physics or equivalent research qualifications) to Prof. N. H. March, Department of Physics, for work concerned with the study of impurities and imperfections in metals and semi-conductors using density matrix methods—The Registrar, The University, Sheffield 10 (August 12).

Fellow (specialized in organic chemistry, with special reference to the chemistry of compounds of neuropharmacological significance) in the Department of Physiology, John Curtin School of Medical Research, Australian National University—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 14).

Lecturer or Assistant Lecturer (with special interest in physical chemistry) in Chemistry at the University College of the West Indies—Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 14).

Lecturer or Assistant Lecturer (preferably with a knowledge of tropical soils) in Soil Science in the Faculty of Agriculture, University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 14).

Research Assistant (with a degree in chemistry, biochemistry, agricultural chemistry or a related subject) in the Department of Physiological Chemistry, for a research programme supported by the Agricultural Research Council on bone metabolism and egg shell formation in the fowl—The Professor of Physiological Chemistry, The University, Reading (August 14).

Lecturer (with a higher degree in science with specialization in some branch of physical chemistry, with evidence of research ability and preferably with university teaching experience) in Physical Chemistry at the University of Queensland—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 15).

Research Assistants (2) (with at least a B.Sc. degree, or equivalent qualification, and with special interest in physical chemistry or chemical engineering), to continue a study of the equilibrium properties of imperfect gases—The Registrar (Room 22, O.R.B.), The University, Reading, Berks (August 15).

Senior Lecturer or Lecturer (preferably with research interests in epidemiological or immunological aspects) in Parasitology in the Faculty of Rural Science, University of New England, Armidale, New South Wales, Australia, to teach and carry out research in parasitology with particular reference to livestock aspects—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 15).

Textile Technologist (with a first- or second-class honours degree in textile technology, chemistry or physics, or Associate or Fellow of the Textile Institute, or equivalent qualifications) at the Tropical Products Institute, London, to supervise a group concerned with research into, and laboratory examination of, various aspects of animal and vegetable fibres, pulp, paper and board-making materials, hides, skins, etc., of tropical origin—Civil Service Commission, 17 North Audley Street, London, W.1, quoting Ref. No. S/5333/61 (August 15).

Lecturer or Assistant Lecturer in the Department of Anatomy—The Registrar, University College, Cathays Park, Cardiff (August 18).

Assistant Lecturer in Physiology—The Registrar, The University, Sheffield (August 19).

Chair of Pathology in Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 20).

Lecturer or Assistant Lecturer (with experience and research interests in plant physiology) in Botany at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 20).

Reader in Electric Traction at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 21).

Senior Lecturer or Lecturer (with a thorough knowledge of comparative and general physiology) in Zoology at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 21).

Fellow (Rees Jefreys Road Fund) (with an honours degree in mathematics, geography or town planning, or equivalent qualification) at the Road Research Laboratory, Traffic and Safety Division, Langley, Bucks, to work either on the quantitative aspects of urban transport needs or traffic conditions in new towns—The Director of Road Research, Road Research Laboratory, Harmondsworth, Middlesex (August 25).

Reader in Aeronautics at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 29).

Lecturer or Senior Lecturer in Botany at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 30).

Keeper of Scientific Books (with considerable experience of university or research library administration and a good honours degree, preferably in science), to superintend the Radcliffe Science Library—The Librarian, Bodleian Library, Oxford (August 31).

Lecturer (with an honours or higher degree in chemical engineering or equivalent qualifications, and preferably with an interest in mass transfer or nuclear chemical engineering) in Chemical Engineering at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

Lecturer or Assistant Lecturer (preferably with qualifications in inorganic chemistry) in the Department of Chemistry—The Secretary, Chemistry Department, University College, Science Buildings, Merrion Street, Dublin (August 31).

Lecturer in Crop Ecology at the Waite Agricultural Research Institute, University of Adelaide, South Australia, to undertake ecological or physiological studies on the lucerne crop, and to evaluate and develop lucerne ecotypes for grazing—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 31).

Lecturer (with a degree, preferably an honours degree and some industrial and teaching experience) in Mechanical Engineering at the University of Queensland, to conduct lectures, laboratory and drawing office work in the fields of applied mechanics (including dynamics and stress analysis) and engineering drawing and design—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 31).

Reader (with special interest in the properties of materials and able to initiate and guide research into the properties of materials such as are related to future developments in electrical engineering) in Electrical Engineering at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 31).

Scientific Officer (honours graduate with a special interest in plant breeding and genetics), for work on tree fruits under experienced leadership, as one of a team of fruit breeders—The Secretary, East Malling Research Station, Maidstone, Kent (August 31).

Senior Lecturer or Lecturer (male, British, with an appropriate honours degree) in Physics and Electrical Engineering at the Royal Naval College, Greenwich, London, S.E.10—The Director (P), Education Department, Admiralty, London, S.W.1 (August 31).

Technical Officer or Senior Technical Assistant (with considerable laboratory experience and competence with apparatus and techniques in biology, and preferably with the preparation and collection of botanical teaching materials and histological methods) at the University of Adelaide, South Australia, to supervise basic laboratory apparatus and preparation of undergraduate classes—The Registrar, The University of Adelaide, Adelaide, South Australia (August 31).

Assistant Lecturer in Mathematics (Pure or Applied)—The Principal, Royal Holloway College, Englefield Green, Surrey (September 1).

Lecturer in the Department of Medical Physics—The Secretary, Royal Free Hospital School of Medicine, 8 Hunter Street, London, W.C.1 (September 1).

Senior Lecturer in Applied Electronics at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 1).

Lecturer or Assistant Lecturer (medically or non-medically qualified candidate) in the Department of Histology—The Registrar, The University, Liverpool, quoting Ref. CV/N (September 4).

Lecturer or Junior Lecturer in Pure Mathematics—The Registrar, Trinity College, Dublin (September 4).

Lecturer (with experience of modern physical methods of structure determination, and especially of those involving electric birefringence, dipole moments, etc.) in Physical-Organic Chemistry at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 8).

Lecturer in Social Anthropology at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, September 8).

Assistant Director of the British Institute of History and Archæology, Dar es Salaam, Tanganyika, to combine research with the supervision of the Institute's library and to share in the direction of students—Secretary, British Institute of History and Archæology in East Africa, c/o British Academy, Burlington Gardens, London, W.1 (September 15).

Chair of Philosophy in the University of Ceylon—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Ceylon and London, September 15).

Lecturer or Assistant Lecturer in Parasitology (Medical Entomology) at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 15).

READER or SENIOR LECTURER IN AGRICULTURAL CHEMISTRY; LECTURER IN AGRICULTURAL CHEMISTRY; and a LECTURER IN ANIMAL HUSBANDRY in the Faculty of Agriculture, Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 15).

SENIOR LECTURER or LECTURER (well qualified in electrical engineering or physics, with general experience in electronics and a considerable detailed knowledge of solid-state devices and circuits) IN ELECTRICAL ENGINEERING at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (September 15).

CHAIR OF STATISTICS tenable in the Newcastle Division of the University—The Registrar, Durham University Office, 1A Kensington Terrace, Newcastle-upon-Tyne 2 (September 25).

HISTOCHEMIST, Scientific Officer grade (with a first- or good second-class honours degree and preferably with some research experience in a related field), for research into mechanisms of insecticidal action using histochemical methods—The Director, Pest Infestation Laboratory, London Road, Slough, Bucks (September 30).

SCIENTIFIC OFFICER (honours graduate), for research on problems arising out of the vegetative propagation of fruit plants—The Secretary, East Malling Research Station, Maidstone, Kent (September 30).

SENIOR LECTURER IN PHYSICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

ASSISTANT, Grade B (qualified to teach to G.C.E. advanced level), IN BIOLOGY—The Principal, Percival Whitley College of Further Education, Francis Street, Halifax.

ASSISTANT, Grade B, IN THE DEPARTMENT OF PHYSICS—The Principal, College of Technology, Huddersfield.

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER (with previous experience in virological techniques, preferably including tissue culture), for work connected with production of attenuated strains of foot-and-mouth disease vaccines—The Secretary, Research Institute (Animal Virus Diseases), Pirbright, Surrey.

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree in physics or physical chemistry or H.N.C., or equivalent qualification), to work on physical problems on the processing of foodstuffs—The Director, Low Temperature Research Station, Downing Street, Cambridge.

BIOCHEMIST or BIOCHEMICAL ASSISTANT, to assist with metabolic research—The Director, Medical Unit, King's College Hospital Medical School, Denmark Hill, London, S.E.5.

CHEMIST (with research experience in physical or bio-chemistry), to work on certain aspects of the chemistry of wheat protein—The Director of Research, Cereals Research Station, Old London Road, St. Albans, Herts.

LECTURER (preferably with a higher degree and industrial research experience) IN METALLURGY in the Department of Materials, to lecture at postgraduate level in his own special subjects; to supervise student research work, and to assist in initiating and directing long-term programmes of research, some of which may be externally sponsored—The Recorder, College of Aeronautics, Cranfield, Bletchley, Bucks.

METALLURGIST, Scientific Officer grade (with an honours degree), at the Royal Mint, to assist in supervision of production, investigation of problems of materials and manufacture, assaying and general research in the fields of physical metallurgy and metallography—Establishment Officer, Royal Mint, London, E.C.3.

POSTDOCTORAL RESEARCH FELLOW IN THE THEORETICAL PHYSICS INSTITUTE—L. E. H. Trainor, Physics Department, University of Alberta, Edmonton, Alberta, Canada.

RESEARCH ASSISTANT (preferably with experience in cytology), to take part in a programme designed to study human chromosome abnormalities and associated disorders—The Secretary, Genetics Department, The University, Sheffield 10.

RESEARCH ASSISTANT (graduate in physics, with research or industrial experience) IN LOW-TEMPERATURE PHYSICS, to work under the direction of Dr. Yates, on the determination of the thermal constants of materials at liquid-hydrogen temperatures—The Registrar, Bradford Institute of Technology, Bradford 7.

RESEARCH ASSISTANT (recent graduate in physics) IN THE DEPARTMENT OF PHYSICS, for work on track analysis in connexion with the National Bubble Chambers project—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15.

SENIOR LECTURER (with a good honours degree and appropriate teaching and/or industrial experience) IN HIGH POLYMER CHEMISTRY—The Principal, Rutherford College of Technology, Northumberland Road, Newcastle-upon-Tyne 1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Scientific and Industrial Research. Report of the Research Council for the year 1960. Pp. 65. (Cmnd. 1365.) (London: H.M. Stationery Office, 1961.) 4*s*. net. [295

The International Council for Bird Preservation, British Section. Annual Report for 1960. Pp. 38+4 plates. (London: International Committee for Bird Preservation, British Section, c/o British Museum (Natural History), 1961.) 3*s*. [305

Department of Scientific and Industrial Research. Report of the National Chemical Laboratory 1960: Report of the Steering Committee and Report of the Director of the National Chemical Laboratory for 1960. Pp. iv+70+4 plates. (London: H.M. Stationery Office, 1961.) 4*s*. 6*d*. net. [305

British Drug Houses, Ltd. Moisture Determination by the Karl Fischer Reagent. Second edition. Pp. 15. (Poole, Dorset: British Drug Houses, Ltd., 1961.) [305

University of Glasgow Appointments Committee. Report for the year ended 31st December, 1960. Pp. 19. (Glasgow: The University, 1961.) [305

Ministry of Education: Science Museum. Books on the Chemical and Allied Industries: a subject Catalogue of Books in the Science Library—Short-title List of Books published 1930 and onwards on the Chemical Industry, with Books on Mining and Metallurgy, and the Paper, Leather and Textile Industries. Pp. ix+118. (London: H.M. Stationery Office, 1961.) 12*s*. 6*d*. net. [305

Science Museum, London. Science Library Bibliographical Series No. 781: Books on the Chemical and Allied Industries. Pp. 10. (London: Science Museum, 1961.) [305

Other Countries

International Atomic Energy Agency, Vienna. Services and Technical Assistance Available from the I.A.E.A. Pp. 42. (Vienna: International Atomic Energy Agency, 1961.) [245

Food and Agriculture Organization of the United Nations. F.A.O. Picture Sheet Story: Why a Freedom from Hunger Campaign? Pp. 2. (Rome: Food and Agriculture Organizations of the United Nations, 1961.) [245

Mauritius Sugar Industry Research Institute. Annual Report, 1960. Pp. 106+xxxviii. (Reduit: Mauritius Sugar Industry Research Institute, 1961.) [245

Annals of the New York Academy of Sciences. Vol. 93, Article 1: Vertical Take-off and Landing (VTOL) Rotorless Aircraft with Inherent Stability. By L. B. Laskowitz. Pp. 1–24. (New York: New York Academy of Sciences, 1961.) [245

The Year Book of the International Council of Scientific Unions, 1961. Pp. 110. (The Hague: International Council of Scientific Unions, Paleis Noordeinde, 1961.) [245

Akrifter fra Danmarks Fiskeri–og Havundersøgelser, Nr. 21: Fiskriumdersøgelser 1 1960 ved Danmark, Faerøerne og Grønland. Ved E. Bertelsen og Paul M. Hansen. Pp. 114. (København: J. Jørgensen & Co., 1961.) [245

Museum of Applied Science of Victoria. Report of Activities for the year ended 30th June, 1960. Pp. 23. (Melbourne: Museum of Applied Science of Victoria, 1961.) [245

Western Australia: Government Chemical Laboratories. Report for the year 1959. Pp. 27. (Perth: Government Printer, 1960.) [245

Hémostase: Revue Internationale de l'Hémostase, de la Coagulation, des Maladies Hémorragiques et des Thromboses, Vol. 1, No. 1. Pp. 102+xlvii. Revue trimestrielle. Subscription (12 months): 65 N.F.; Students' Tariff 40 N.F. Single issues: 20 N.F.; Students' Tariff 14 N.F. (Paris: S.P.E.I., 14 Rue Drouot, 1961.) [245

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 60–62: Belcher Islands, Northwest Territories, 33M, 34D,E. By G. D. Jackson. Pp. ii+13+map. (Ottawa: Queen's Printer, 1960.) 50 cents. [305

National Academy of Sciences—National Research Council. Nuclear Science Series. Report No. 34: Radioactive Contamination of Materials used in Scientific Research. By James R. DeVoe. Pp. vii+142. (Publication 895.) (Washington, D.C.: National Academy of Sciences—National Research Council, 1961.) [305

United States Department of the Interior: Geological Survey. Bulletin 1089–A: Geology of the Alvord Mountain Quadrangle, San Bernardino County, California. By F. M. Byers, Jr. Pp. iv+71+6 plates. Bulletin 1111–E: Stratigraphy of the Moose River Synclinorium, Maine. By Arthur J. Boucot. Pp. iii+153–188+plate 34. (Washington, D.C.: Government Printing Office, 1960 and 1961.) [305

Durban Museum Novitates. Vol. 6, Part 6 (20th March 1961): Miscellaneous Taxonomic Notes on African Birds, XVI. By P. A. Clancey. Pp. 79–104. 5*s*. net. Vol. 6, Part 7 (15th April, 1961): Notes on South African Forms of the Collared Sunbird *Anthreptes collaris* (Vieillot), with the description of a New Race. By Michael P. Stuart Irwin. Pp. 105–111. 20 cents. (Durban: Durban Museum, 1961.) [305

Deutschen Wetterdienstes. Mitteilungen des Deutschen Wetterdienstes. Nr. 24 (Band 4): Die Kraftwirkung Elektrisch Geladener Eiskristalle auf Aerosolteilchen. Von Paul Hess und Johannes Tauscher. Pp. 24. Nr. 25 (Band 4): Über Zusammenhänge Zwischen Parametern des Luftdruck- und Temperatur-Feldes und dem Niederschlag. Von Hans Mollwo. Pp. 36. Berichte des Deutschen Wetterdienstes. Nr. 73 (Band 10): Beiträge zum Internationalen Geophysikalischen Jahr, 1957/58. 1: Mess-Ergebnisse und Berechnungen zum Wärmehaushalt der Erdoberfläche. Von Ernst Frankenberger. Pp. 41+10 tabellen im Anhang. 10.50 D.M. Nr. 74 (Band 10): Der Einfluss der Bodenbedeckung auf das Klima des Bodens und der Bodennahen Luft. Von Franz Fuss. Pp. 38. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1960 und 1961.) [305

Académie Royale de Belgique. Classe des Sciences. Mémoires, Tome 32, Fascicule 5: Le Problème Naturel aux Valeurs Propres. Par Jean Kestens. Pp. xiv+122. (Bruxelles: Académie Royale de Belgique, 1961.) 120 francs. [305

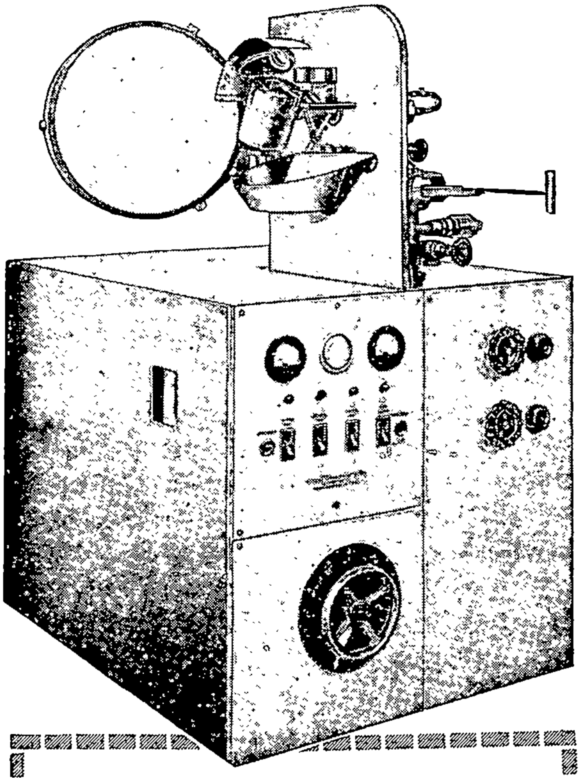
Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office

WILD BARFIELD NRC*

Laboratory Vacuum Furnace



Model 2904

2 lb. to 8 lb. capacity

(FOR OPERATION UP TO 2,000°C)

Made in Britain to the designs of the National Research Corporation of Mass., U.S.A. who have built more high vacuum furnaces now in successful operation than any other firm in the world. The Model 2904 Vacuum Furnace is a self-contained unit for high-temperature operation at high vacuum or under controlled atmosphere. It is heated by a tungsten resistance heating element with molybdenum heat shields in a water-cooled stainless steel sleeve.

This permits:

- Close control of temperature
- Good heat distribution
- Long life
- No outgassing or contamination from refractories

FOR MELTING • POURING • SINTERING
ALLOYING • HEAT-TREATING • ANNEALING

Vacuum Arc and Vacuum Induction Furnaces
Vacuum Analysis Equipment • Vacuum Diffusion Pumps

STANDARD EQUIPMENT

- High vacuum pumping system with Wild-Barfield-NRC oil diffusion pump and Gas Ballast mechanical pump.
- Metal cabinet base, overall dimensions 35″ x 43″ x 34″ high.
- Pyrex bell jar, 18″ diameter x 12″ long, protected with a wire mesh guard, or optionally a stainless steel bell jar.
- Water-cooled basin to shield the bell jar and to catch spilled metal.
- Feed-through for thermocouple wires or heater leads for special work.
- 15 kW. power supply, controlled with a variable auto-transformer. Power is supplied to the split tungsten sleeve through water-cooled coaxial leads.

* NRC *is the trade-mark of the National Research Corporation, registered in the United States Patent Office.*

WILD BARFIELD

ELECTRIC FURNACES
FOR *ALL* HEAT-TREATMENT PURPOSES

WILD-BARFIELD ELECTRIC FURNACES LIMITED

ELECFURN WORKS, OTTERSPOOL WAY, WATFORD BY-PASS, WATFORD, HERTS. TELEPHONE : WATFORD 26091 (8 LINES)

NRC

APPOINTMENTS VACANT

Central Electricity Generating Board

PHYSICS DIVISION

BERKELEY NUCLEAR LABORATORIES

(GLOUCESTERSHIRE)

Applications are invited for posts in these Laboratories. Those appointed will work initially in the following sections, although subsequent transfer between sections will be possible, depending on the developing interests and abilities of the staff concerned.

A. INSTRUMENTATION

For work developing nuclear radiation detectors and other specialized nuclear instruments. Preference will be given to candidates who are capable of devising and developing new techniques of measurement.

B. GRAPHITE

To study the fundamental mechanisms and assess the extent of irradiation damage in graphite and the corresponding changes of physical properties.

C. THEORETICAL PHYSICS

To study various theoretical problems of interest to the Laboratories, including reactor physics and the theory of radiation damage.

● **Initial salaries up to £1,325 per annum are offered in accordance with experience, ability, and qualifications of persons appointed.**

Candidates must have a good honours degree and should be under 26 years of age.

Applications, stating age, qualifications, experience, present position, salary and quoting type of work interested in, to the Appointments Officer, 24/30 Holborn, London, E.C.1, by August 3. Quote Ref. N/317.

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

(UNIVERSITY OF READING)

Assistant Experimental Officer required in the Physiology Department to assist with histological investigations related to the endocrine research programme of the department. Applicants should have a pass degree in biology (including zoology), F.I.M.L.T. or equivalent qualifications, and some experience of histological techniques would be an advantage. Starting salary £450 10s. to £740, according to age in scale to £983. Superannuation.

Application form from Secretary, N.I.R.D., Shinfield, Reading. Quote ref. 61/22.

WHESSOE LIMITED

require a

CHEMIST

for Reinforced Plastic Tank Development

Assistant, age 25 to 40, with degree or other recognized qualification, required to join a team working on development of reinforced plastic vessel design and production. His particular responsibility will be to investigate the properties of plastic materials but he will also have the opportunity of taking part in all aspects of this work.

Applications, quoting reference 37/61, and mentioning this paper, should be sent to the Staff Officer, Whessoe Limited, Darlington.

UNIVERSITY OF MANCHESTER

LINEAR ACCELERATOR PROGRAMME

Short-term appointments are available in the Department of Physics of the Faculty of Science for qualified Physicists or Electrical Engineers to assist with the construction and commissioning of a large heavy ion linear accelerator. Experience in one or more of the following fields would be desirable: high vacuum; R.F. techniques (75 Mc./s.); high power rectifier and pulse circuits; general electronic instrumentation and controls. The appointments are for one year in the first instance, and the salary range is £1,000 to £1,500 per annum.

Applications should be addressed to the Assistant Director, Linear Accelerator Laboratory, Physics Department, the University, Manchester 13, from whom further details may be obtained.

ANTARCTICA

GEOLOGIST required by FALKLAND ISLANDS DEPENDENCIES SURVEY for service in the ANTARCTIC for two years. Salary £550 a year with extra allowances for experience and qualifications. Whilst in the Antarctic everything is provided free of charge including clothing, cigarettes etc. Free passages. Liberal leave on full salary.

Candidates should hold a minimum qualification of a first- or upper second-class honours degree in geology and should preferably have specialized in petrology. The person selected will be required to undergo a course of training before leaving the U.K. at the end of the year. While on duty in U.K. an extra allowance of £150 a year is paid before departure and £200 a year on return.

Apply to CROWN AGENTS, 4 Millbank, London, S.W.1, for application form and further particulars, stating age, name, brief details of qualifications and experience and quoting reference M3C/53437/NF.

LONDON COUNTY COUNCIL

NORWOOD TECHNICAL COLLEGE
KNIGHT'S HILL, S.E.27
PHYSICS AND MATHEMATICS DEPARTMENT

(1) Required Lecturer to teach Physics to General Degree and G.C.E. Advanced Level courses. Applicants should have good honours degree, teaching and/or research experience. Salary within the range £1,408 to £1,601.

(2) Two Grade B Assistants to teach (a) Physics and (b) Mathematics. Teaching and/or research experience an advantage. Salary within the range £738 to £1,201 with additions for qualifications and training to a possible maximum of £1,486. Point of entry dependent on experience. These appointments will be temporary in the first instance, but permanency available if satisfactory.

The work in the department comprises principally teaching physics and mathematics to full-time G.C.E. " A " level students, telecommunications courses (C. and G. and Brit.I.R.E.), Sandwich General Degree students, and part-time courses of similar standard.

Application forms obtainable from Principal, and returnable within fourteen days. Enclose self-addressed foolscap envelope. Please quote (FE.3a/N/1926/7).

AUSTRALIA

THE UNIVERSITY OF NEW SOUTH WALES
BROKEN HILL DIVISION
SENIOR LECTURER
MATHEMATICS

The University of New South Wales wishes to appoint a Senior Lecturer in Mathematics to its Broken Hill Division. Applicants should have a higher degree or equivalent qualifications and be interested in promoting and leading research. Salary: £A.2,592 range £A.3,042 per annum. Commencing salary is determined by qualifications and experience. Climatic and disability allowances of £A.104 per annum for a single man, £A.156 for a married man, will also be paid. After passing a medical examination, the successful applicant will be eligible to join the State superannuation scheme. Senior Lecturers may apply for twelve months' study leave on full salary after six years of service. With the approval of the University and its bankers, married men may be assisted by loans to purchase a home. First-class fares to Sydney of appointee and family will be paid. Further details of conditions of appointment may be obtained from the undermentioned.

Four copies of applications, including names of two referees, should be lodged with the Agent-General for New South Wales, 56-57 Strand, London, W.C.2, and a copy forwarded to the Appointments Section, the University of New South Wales, Box 1, Post Office, Kensington, N.S.W., by airmail to reach there before September 8, 1961.

UNIVERSITY OF LEEDS

DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited for the post of Analyst in the Department of Organic Chemistry. The main duties will be the operation and care of optical equipment including infra-red and nuclear magnetic resonance spectrometers. B.Sc. (Chemistry), and G.R.I.C., or equivalent qualifications desirable. Salary on scale £710 to £1,000 bar to £1,100 with provision for merit award up to £100, according to qualifications and experience.

Apply in writing to Mr. W. A. Wightman, the School of Chemistry, the University, Leeds, 2.

UNIVERSITY OF SHEFFIELD

Applications are invited for a post Junior Research Fellow in Pure Mathematics to begin duties on October 1, 1961. Salary £625 per annum. The appointment will be for one year in the first instance and will be renewable.

Applications (three copies), with the names and addresses of two referees, should reach the Registrar, from whom further particulars may be obtained, by August 19, 1961.

VETERINARY OFFICERS, RESEARCH Officers, Assistant Veterinary Investigation Officers: Ministry of Agriculture, Fisheries and Food. Pensionable posts for men and women aged at least 23. M.R.C.V.S. normally required, and postgraduate experience desirable. Present vacancies for Research Officers in virology, pathology, bacteriology and biochemistry. National salary scales £926 (at 23) to £1,269 (at 32 or over) rising to £1,420, then to £1,778. Promotion prospects.—Write Civil Service Commission, Burlington Gardens, London, W.1, for application form, quoting **130-173-265/61**. Early application advised.

MICROBIOLOGIST OR BIOCHEMIST, recently graduated, required for one year's sponsored research on antibiotics. Salary up to £900 per annum according to experience.—Applications to Dr. J. G. Davis, 9 Gerrard Street, London, W.1.

NEW VETERINARY RESEARCH UNIT DIRECTOR

CIBA is an international chemical undertaking with a world-wide organisation based on substantial research manufacturing and marketing pharmaceuticals, dyestuffs and plastics. In Switzerland and in the U.S.A. CIBA has very well-established pharmaceutical research units and throughout the world a reputation for work of high scientific quality.

It is now proposed to set up veterinary research with the main centre in England. A Director is required for this important development. He will first assist in the design of the new laboratories and build up his team and so bring the new unit to life.

The post will be very well paid by academic standards with pension and other benefits.

Further particulars from Managing Director,

CIBA Laboratories Limited,
Horsham, Sussex.

AUSTRALIA

UNIVERSITY OF NEW SOUTH WALES

The University wishes to appoint Senior Lecturers and Lecturers in the following Faculty:

FACULTY OF SCIENCE

Applied psychology (psychometrics and experimental psychology). Botany. Mathematics, Physics.

Salary: Senior Lecturer, £A.2,586 range £A.3,036 per annum; Lecturer, £A.1,766 range £A.2,471 per annum. Commencing salary is determined by qualifications and experience. Subject to passing medical examination appointees will be eligible to contribute to the State Superannuation Fund. First-class ship fares to Sydney of appointees and family will be paid.

Four copies of applications, including the names of two referees, should be lodged with the Agent-General for New South Wales, 56-57 Strand, London, W.C.2, England, and a copy forwarded to the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Marked SA1.

RADIOCHEMIST

National Physical Laboratory, Teddington, Middlesex, requires a Senior Scientific Officer or Scientific Officer in the Radioactivity Standards group to investigate the optimum chemical composition of radioactive solutions used as standards and to initiate research into the production of thin uniform sources for 4π counting. Opportunities later for investigations using a 3 MeV. Van de Graaff neutron generator for cross-section measurements and the production of radioactive nucleides. Qualifications: first- or second-class honours degree in chemistry or equivalent. At least three years' postgraduate research experience is required for Senior Scientific Officer. Interim salary ranges: Scientific Officer, £773 to £1,267; Senior Scientific Officer, £1,392 to £1,714. Appointments unestablished with possibility of establishment through the Civil Service Commission whilst remaining under 29 (Scientific Officers) and under 32 (Senior Scientific Officers).

Forms from Director, address above, quoting 65ZY.

STATES OF GUERNSEY HORTICULTURAL ADVISORY SERVICE

Applications are invited for the following posts: Plant Pathologist; Horticultural Advisory Officer. Applicants must possess a university degree or equivalent qualification. Final-year students may apply. Both officers will be required to assist in general horticultural advisory work with the emphasis on tomatoes and flower crops under glass, and should be prepared to assist in the development of experimental work. Salaries will be equivalent to the National Agricultural Advisory Service salary scales, starting point according to age and experience.

Applications, giving details of qualifications, experience, relevant personal data, and naming three referees, should be forwarded to the President, States Board of Administration, States Office, P.O. Box No. 43, Guernsey, not later than August 8, 1961. Further particulars can be obtained from the same address.

SK&F

wish to appoint

a

BIOCHEMIST

in their

PHARMACOLOGY DEPARTMENT

The post is newly created and the successful applicant will be responsible for the control of all bio-chemical investigations within the department.

He will be particularly concerned with studies of the actions of new synthetic drugs on metabolic processes with a view to elucidating their mechanisms of action and developing new screening methods for pharmacological activity.

Participation in meetings of scientific societies and publication of results are encouraged.

Previous research experience is essential and the preferred age range is 25-35 years.

An attractive starting salary with annual merit reviews is offered and congenial working conditions in new and well-equipped laboratories will be provided.

Other Company benefits include a non-contributory Pension and Life Assurance Scheme, Staff Cafeteria and an active Sports and Social Club.

Under certain circumstances a married man may be eligible for rented unfurnished accommodation and full payment of removal expenses will be made.

Letters of application, giving fullest possible details considered relevant, should be addressed to:

The Staff Manager,
SMITH KLINE & FRENCH LABORATORIES LTD.,
Mundells, Welwyn Garden City, Herts.
Please quote Reference No. L1491.

Virologist/ Bacteriologist

Extension of activities has created an important senior position in the Vaccine Unit of Pfizer Limited, which is located at Sandwich, Kent.

The post calls for a man generally experienced in biological fields, particularly in the production of bacterial and viral vaccines for human and veterinary use. The man we seek must be a good administrator and able to control and co-ordinate the activities of a large staff engaged in all phases of vaccine operation. He should be qualified to Ph.D. standard, or equivalent, and the preferred age range is 30 to 40.

The appointment will carry an attractive salary, together with other first-class conditions. These include generous non-contributory Pension and Life Assurance Schemes and assistance with housing

Applications are invited: they should be addressed to:

THE CHIEF PERSONNEL OFFICER,
PFIZER LIMITED, SANDWICH, KENT.

THE Pfizer GROUP
CHEMICALS PHARMACEUTICALS VACCINES AGRICULTURAL & VETERINARY PRODUCTS

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION OF FISHERIES AND OCEANOGRAPHY

Applications are invited from Honours graduates in Science, or equivalent qualifications, with postgraduate research experience for appointment to the undermentioned positions at the Division's headquarters, the Marine Laboratory located at Cronulla, Sydney, N.S.W.

BACTERIOLOGIST

(No. 320/209)

The present research programme is concerned with fluctuations in the number of marine heterotrophs in the sea and with the cell-wall composition of a marine pseudomonad. These investigations are part of a general project on biological production in Australian waters. Although the appointee would not be required to follow these lines of research he would be expected to initiate research within the very wide framework of the programme on production. Some of this work would be done at sea, and therefore ability to work at sea is an essential requirement for appointment.

Applicants should have postgraduate research experience in bacteriology.

PHYSICAL OCEANOGRAPHER

(No. 320/210)

The present research programme is concerned with studies on circulation, instrumentation, optical properties and fisheries problems, and is carried out in coastal and oceanic waters around Australia, extending into the south-west Pacific Ocean and the south-east Indian Ocean. The appointee could join in these lines of research or could initiate research of his own in theoretical or field problems. Because the appointee would be expected to act as a cruise-leader for some part of each year, ability to work at sea is essential.

Applicants should have postgraduate research experience in oceanography.

Salary: Dependent on qualifications and experience within the ranges £A.1,510 to £A.2,265 per annum or £A.2,395 to £A.2,720 per annum.

Promotion within C.S.I.R.O. is by merit and may ultimately go beyond the upper limit of the scale within which the original appointment is made. By mutual agreement appointment may be for an indefinite period or for a fixed term of three years.

Fares paid, and for a short-term contract return fares paid. Further particulars supplied on application to:

Mr. E. J. Drake, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,
to whom applications (quoting relevant Appointment number) should be addressed by October 21, 1961.

PHYSICISTS

required by

UNITED KINGDOM ATOMIC ENERGY AUTHORITY

for the Physics Group of the Fast Reactor Project at the Dounreay Experimental Reactor Establishment.

This Group is primarily concerned with experiments on the Dounreay Fast Reactor (D.F.R.) but its responsibilities go further and many of the experiments are designed to increase our general knowledge of fast reactor physics and, in particular, to anticipate problems concerning the design of future fast reactors.

A wide variety of calculations are made of the neutron flux and energy distributions, for a range of core compositions including possible and actual fuel loadings in D.F.R. The scope of the calculations is wide: they may involve simple methods or, for complex solutions, the use of high-speed computers.

Theoretical methods are developed and applied to the investigation of the dynamic behaviour of fast reactors generally. The transient neutron flux is determined and used to evaluate thermal transients and finally reactivity transients. The response of the fuel, coolant and structure is computed and finally, the overall reactor characteristic.

D.F.R., previously run at only 1 MW, will shortly become the world's highest flux fast reactor, presenting an opportunity to check the accuracy of neutron and gamma shielding calculations, especially for thick shields, and also the effect of the many possible streaming paths for radiation caused by coolant ducts, stepped plugs, etc.

Each of these theoretical programmes is complemented by experiment; e.g., flux distributions are measured in the reactor using fission counters and activation samples. The reactivity oscillator is used to examine the reactor dynamics and this apparatus will require development to improve its accuracy. Special shield plugs incorporating established and recently developed methods for monitoring neutron and gamma fluxes are being constructed.

To support this programme an analogue computer is available and a data link is provided to a "Mercury" computer. Access to a large analogue computer (PACE) and other digital computers is also available.

Appointments will be made, and starting salary assessed, according to age, qualifications and experience within the grade of

SENIOR SCIENTIFIC OFFICER

£1,440 to £1,770

OR SCIENTIFIC OFFICER

£790 to £1,310

Applicants should have at least a second-class honours degree in physics or mathematics, and for the senior grade at least three years approved research experience.

Rented housing or house purchase assistance available for married officers living beyond daily travelling distance.

Hostel accommodation available.

Contributory superannuation.

Send postcard for application form to:

Personnel Manager,
U.K.A.E.A., Reactor Group,
E.R.E., Dounreay,
Thurso, Caithness,
quoting reference 253/34.

Reed
PAPER GROUP

Statisticians

required by the Statistical Applications Unit to take a leading part in the development and application of statistical techniques to Operational Analyses, Experimental Design and Quality Control.

Graduates with several years' experience in these fields are wanted, although new or recent honours graduates in mathematics or statistics will be considered.

We have a turnover of £100 million, factories throughout the United Kingdom, and rapidly expanding interests overseas.

Please write, quoting reference S/SAU/37, to :
The Group Personnel Officer,
Albert E. Reed and Co., Ltd.,
Larkfield, near Maidstone,
Kent.

Ferranti computers

Applications are invited for

SENIOR APPOINTMENTS IN THE FERRANTI ATLAS AND ORION PROJECTS

from

PROGRAMMERS	**ENGINEERS**
SYSTEMS ANALYSTS	**CHEMISTS**
PHYSICISTS	**ECONOMISTS, etc.**

who possess good previous responsible experience in their particular field and correspondingly good academic qualifications.

These appointments will, in all cases, carry salaries fully commensurate with qualifications and experience with the benefit of Staff Pension and Dependants Insurance Schemes.

Applicants should write, giving full details of qualifications and experience (which would be treated in strict confidence) to T. J. Lunt, Staff Manager, Ferranti Limited, Hollinwood, Lancs. Please quote reference BBS.

Walls
ICE CREAM

CHEMIST

If you are under 30, have an honours degree in Chemistry, or equivalent, and would like to apply your knowledge in solving the analytical problems that occur in the expanding ice-cream industry, you are invited to apply for a position in the modern laboratories at Acton.

Previous experience of food analysis will be an advantage. Starting salary will be in accordance with experience, and the conditions are those of a progressive company (in the Unilever Group).

Write, stating age, qualifications and experience to:

The Regional Personnel Manager,
T. Wall & Sons (Ice Cream) Ltd.,
The Friary,
Acton, W.3.

DIRECTOR OF RESEARCH

BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION

LEATHERHEAD, SURREY

The post of Director of Research with The B.F.M.I.R.A. will become vacant on December 1st, 1961. The Association employs a staff of 70 and has modern laboratories situated at Leatherhead. Its activities cover the wide field of pure and applied science in relation to the raw materials of the food industry and their conversion into the finished product.

Scientists who are suitably qualified for directing research work in this field are invited to apply for the post.

The salary will be in the region of £4,000 per annum in accordance with qualifications and experience. A contributory superannuation fund is in operation.

Applications should be addressed to The President, Professor A. C. Frazer, M.D., D.Sc., F.R.C.P., at the Laboratories of The B.F.M.I.R.A., Randalls Road, Leatherhead, Surrey, and should not arrive later than Friday, September 1, 1961.

3M COMPANY

MINNESOTA 3M RESEARCH LIMITED

In connection with the impending building of **New Research Laboratories near London** additional Graduate Chemists, Physicists and non-graduate Technicians will be required. Those who have replied to previous advertisements will be considered for the vacant posts without the necessity of re-application.

In the meantime a number of specialist posts are available with the parent Company in the U.S.A. for the following:

(a) Photographic Emulsion Research Chemists.

(b) Physicist/Engineers with experience in the design of modern photographic emulsion coating plants.

(c) Organic Research Chemists with experience in the synthesis of cyanine dyes.

Employment in the U.S.A. may be on a permanent or temporary basis with the option of return after a few years to the new laboratories in England.

Applications, giving details of age, qualifications and experience, which will be treated in strict confidence, should be made in writing to:

Dr. J. D. Kendall, Managing Director, Minnesota 3M Research Limited, 3M House, Wigmore Street, London, W.1.

APPLICATIONS ARE INVITED FOR THE post of Assistant Librarian. Candidates must have passed at least two parts of the Registration Examination and should preferably have had some industrial or college library experience. Duties will include responsibility for book-ordering, classification, and display work. Hours: 38 per week (including some evening duty during term-time). Salary: APTD I (£645 to £815).—Application forms from the Clerk to the Governors, Mid-Essex Technical College, Market Road, Chelmsford, Essex.

A GRADUATE IS REQUIRED TO PARTICIPATE in biochemical research on coagulation in normal human and human cancer tissue. A suitable candidate could register for a higher degree of the University of Dublin. Stipend from the Sarah Purser Medical Research Fund up to £600 per annum for two years from October, 1961, will be according to qualifications and research experience.—Applications, with relevant information and two references, should be forwarded to Dr. W. A. Boggust, Department of Biochemistry, Trinity College, Dublin.

D.S.I.R.

Requires Scientific Officers/Senior Scientific Officers at the Water Pollution Research Laboratory, Elder Way, Stevenage, Herts, for the following posts: (i) Physical Chemists, preferably with good mathematical ability for research on physico chemical problems connected with treatment of industrial and domestic wastes and the effects of pollution on rivers and estuaries. Work includes laboratory studies and pilot plant investigations on unit processes, e.g., neutralization of acid or alkaline wastes and separation of solids from liquids in flocculating and non-flocculating suspensions (reference W.P.R.L.8 ZY). (ii) Physicist to work as a member of a team engaged in the development of indicating, recording and controlling instruments for use in the laboratory and field work in the study of rivers and the treatment of industrial and domestic wastes. The work is varied and covers a wide range of applications in physics, physical chemistry and nucleonics. The applicant should have a basic qualification in physics or physical chemistry, and an interest in electronics. Practical experience in instrument development or electronics, though desirable, is not essential (reference W.P.R.L.9 Y). Qualifications: First- or second-class honours degree in physics or chemistry. Salary ranges: Scientific Officer, £717 to £1,186. Senior Scientific Officer, with at least three years postgraduate experience, £1,302 to £1,604. Housing available in certain cases.

Application forms from Director at the above address quoting appropriate reference number. Closing date September 15, 1961.

DALHOUSIE UNIVERSITY

INSTITUTE OF OCEANOGRAPHY

Applications are invited for an academic post in experimental biology within which the appointee will conduct research on marine problems and undertake a limited amount of formal teaching. Appointments will be made at a rank and salary appropriate to qualifications. For a recent Ph.D. the beginning salary is $6,500.

Address all correspondence and recommendations to: The Director, Institute of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada.

UNIVERSITY OF ABERDEEN

Applications are invited for the post of Assistant in Animal Husbandry with special interest in Pig Husbandry. Salary on scale £800 to £950 with appropriate placing. Superannuation (F.S.S.U.), child allowance and removal allowance.

Further particulars from the Secretary, the University, Aberdeen, with whom applications (two copies), should be lodged not later than August 19, 1961.

PURPOSE IN PUBLICATION

UNTIL the middle of the seventeenth century practically all the major contributions to science had been made by men who possessed exceptional talents in other disciplines apart from scientific method. Aristotle, Bacon and da Vinci were not scientists in the way we think of them to-day: they were artists, moralists, and philosophers as well, and in those days of the non-specialist they had opportunities to pursue wide interests. To be a scientist to-day does not necessarily mean being a philosopher and artist too; but paradoxically, to be a 'good' scientist *alone* these days—a 'good' scientist being one who knows his 'literature' as well as his laboratory—is beyond the capabilities of many. This is not the fault of the scientist, nor is it due to any decline in intellectual grasp, but simply to the overwhelming mass of scientific literature which must be read to keep abreast of the subject.

It should not be deduced from this that, his scientific training and proclivities apart, the good scientist of to-day is ignorant about or, even worse, unaware of, other branches of man's culture. In fact, the contrary is true; indeed, so far as the community of scientists is concerned, the so-called 'two-cultures' (fashionably ascribed to C. P. Snow) scarcely exist. Many scientists are well read outside their own discipline, sometimes still within the ambit of science, but more often well beyond it—in philosophy, history, art, music, the theatre, literature, in fact, in the humanities generally. (Good scientists seldom make good politicians, which is probably understandable.) Some scientists of to-day have written authoritative treatises on music and musicians, and many are first-class musicians themselves; others have published poetry (in certain cases revealing that they are no mere poetasters); some have written highly successful novels; and, as used to be demonstrated at the annual meetings of the British Association for the Advancement of Science, a host of scientists are excellent pictorial artists and sculptors. T. R. Henn, lecturer in poetry and drama in the University of Cambridge, has said: "I am clear that all the great scientists that I myself have known have sought and invariably achieved some balance, some counterpoise, through a form of art, practised or enjoyed, which has taken a prominent place in their lives" (p. 537 of this issue).

In short, it is high time that the general opinion, still very extant, that the man of science is so wrapped up in his scientific literature and so confined to his laboratory that, apart from his calling, he is culturally unbalanced, be challenged. Indeed, the shoe is on the other foot; it is the student of, and savant among, the humanities, art, music and non-scientific literature (especially fiction) who are—and are often proud to admit they are—quite ignorant of science and its now 'jet-propelled' progress.

This unbalanced state of affairs in education and consequently attitudes must have contributed largely toward the movement which is fast culminating in science and technology being used for diplomatic intrigue, political propaganda, international threats and the consequent universal unrest.

It is relevant here to point out that the "Platform Party" at the opening of the magnificently posed Soviet Industrial Exhibition in Earls Court, London, on July 7 was comprised solely of Soviet and British statesmen (not including the Minister for Science) and a few others—but no scientists. Yet a very high percentage of the Exhibition was based on recent advances in science and technology. One can scarcely conceive of an exhibition of Soviet art in Britain or elsewhere being opened without the President of the Royal Academy (or his counterpart in another country) being 'officially' present.

This Exhibition clearly had a purpose other than trade. Yet, impressive though it was, it was not so revealing or informative as most trade exhibitions, and certainly not to be compared to its own advantage with, for example, the annual exhibition of the Physical Society. But advantage was obviously taken to publish a considerable amount of material, which was presumably read by many during, and after, the Exhibition. Many and varied handsomely produced brochures were made freely available. It should be borne in mind that these pamphlets will be read by some thousands of people, most of them not professional scientists, many not particularly interested in trade, and thousands of them very young and very impressionable. The background was mainly science and technology; so those responsible for the Exhibition left it to the Academy of Sciences of the U.S.S.R. to prepare some of the pamphlets. Excellently produced, some well illustrated and all issued between attractive covers, they are bound to command attention. Yet the text (in English) in each one gives a wrong impression of the *background* science which it sets out to describe. The result is that the layman will be left with the impression that nearly all the work (even back to the original) was carried out by Russians. It is true that the Exhibition was devised to show what is going on in the U.S.S.R.; but this is no excuse for confining the background, descriptive brochures to Russian work. For example, that on "Vegetative Hybridization in Plants" briefly mentions Darwin, Daniel and Burbank, then proceeds to devote the rest of its pages to Michurin and Lysenko. Of course, Vavilov is not mentioned. It is an old story, not improved by its re-telling, and significant in that it puts forward no new conceptions since the claims made by Lysenko about two decades ago. Geneticists will appreciate how misleading, therefore, this brochure is. That on "Physical Methods in Structural Analysis" states on p. 1 that: "Valuable results were achieved by G. V. Vulf, who, independently of W. L. Bragg, derived the formula underlying the X-ray diffraction analysis"; it then devotes nearly all the rest of its pages to present-day Soviet scientists. The brochure on "High Molecular Chemistry" adopts a similar approach.

In spite of the fact that these pamphlets were handed out at an Exhibition devoted to Soviet science and industry, it remains clear that they have been and are being read by many people, the majority of whom have scant scientific training. It can be safely assumed that few of these people will ask what contributions (original and more recent) have been made by scientists of other countries; so, to say the least of it, they will end their reading with a very distorted view of the world advancement of science.

In all these background brochures, produced under the auspices of such a distinguished scientific body as the Academy of Sciences of the U.S.S.R., very few (if any) scientists outside the U.S.S.R. are mentioned—and these only gratuitously. These leave anyone who has even an inkling of the subject in no doubt concerning the purpose of each brochure; and that is not to give a true picture of the history and present-day position of the subject. It is doubtful if many non-scientists will understand them, anyhow: they will just be overwhelmed with claims concerning Soviet achievements, and, thanks to their unbalanced education, perhaps, subconsciously, assume that nobody outside the U.S.S.R. has contributed or is contributing to the subject. The ill-informed make good material for insidious propaganda.

Coming now to the scientists in all countries, for they are not entirely blameless, they might well, with advantage to themselves and to world opinion, consider setting their own house in order. We hear much to-day about 'scientific jargon', 'scientese' and 'scientific Towers of Babel'; yet up to a point this is inescapable. The scientist must have his means of communication with his colleagues. The musician has his means of writing his scores (technically a 'language' which must be learned), and the artist also has his methods of presentation—quite often cryptic and sometimes even disturbing to the layman. It is understandable, therefore, that the non-scientist cannot comprehend much of the scientist's professional language unless the latter sets himself the difficult problem of interpreting his work in a more 'popular' fashion. (Writers of the Soviet brochures do not appear to have realized even this.)

But the 'language' of science has now got itself into such a state that sometimes even one scientist cannot understand another.

When, in 1665, the *Philosophical Transactions of the Royal Society of London* were first published, the stage was set for the scientific boom. There were 10 scientific journals in 1750, 1,000 in 1850, 10,000 in 1900, and to-day there are about 100,000. The number of journals has doubled itself every fifteen years or so since 1665 and would seem to indicate that by the year 2000 we shall have reached the million mark. Not only has the number of journals increased, but so also have the sizes of existing journals.

However, there are reasons for believing that the creation of new journals may not be as balanced a process as it might be. To take a specific example, the increase in importance of research in biochemistry, physiology and fundamental and applied biology has been very rapid during the past sixty years. (*The Biochemical Journal* was first published in 1906 and contained 495 pages; in 1926 the number of pages had risen to 1,376, and by 1960 to 2,510.) There is no reason for believing that the volume of publishable research work will decrease in the future, and one wonders whether, despite the startling rise in the number of scientific publications in general, there are enough journals to cope with the amount of material in pure and applied biology.

In the fields of mathematics, fundamental and applied chemistry and physics, and in technology, there are more journals and they are produced more frequently; as a result there usually is a shorter time-lapse between submission of the manuscript and its publication. This is not the case with the biological journals. There is none which deals exclusively with biological or biochemical research published more frequently than once a month ; indeed, most are produced quarterly or even less often than that. Consequently, papers accumulate in numbers which cannot be dealt with in current issues, and in a short time there is a long waiting list.

Nature itself now has a waiting list of biochemical, physiological and biological material in spite of the fact that the journal is becoming unbalanced because it is forced to contain so much on these subjects.

Nature is a weekly journal of science and as such produces items on most scientific topics. A glance at a recent issue shows, however, that in the "Letters to the Editors" section, about 70 per cent of the contributions are on topics of pure and applied biology. Of fifty-one letters, ten are in the biochemistry section and another ten under the heading of biology. It would seem that authors, not prepared to wait for their papers to appear in full elsewhere, will submit them to *Nature* first, with the intention of writing a detailed account for a more specialized journal later. The phrase, "Further details will be published elsewhere", is becoming a familiar sight to the Editors. The phrase is justified if the communication is confined to the gist of the research work; but frequently it turns out that the paper could not contain much more detail if the author(s) tried. Moreover, complaints have been received that the indefinite word "elsewhere" is tantalizing to those sufficiently interested and who wish to examine the details ; and, worse still, sometimes such "details" never see the light of day anyhow.

What is needed is either the creation of a new journal specializing in applied biology, biochemistry and physiology, to be published more frequently than the present ones, and/or for applied biologists and biochemists to use pre- and already- existing journals which have, for some reason or another, become 'unfashionable'. There is no doubt that there will be room for both these ideas as the amount of biological and biochemical research continues to increase.

The idea of a journal like *Nature* is to keep its readers informed about what is going on in *all* fields of science. Therefore, biological, physiological, and biochemical papers in particular, should be more brief and concise.

From another very serious point of view, the time-lag in publication of papers and even short communications is a blessing in disguise. It sometimes prevents authors from publishing erroneous results or unjustifiable conclusions. This, no doubt, is due not only to the enormous increase in scientific research throughout the world to-day but also to the tendency among far too many scientists to rush into print—a tendency all too prevalent and one to which the attention of the scientific world has been directed on several occasions in these columns. While waiting several weeks (sometimes even months) for publication after submission, research workers have found thrust on themselves time for reflexion, time for correction (sometimes necessary) and time for continuation of their work. It is no unusual thing nowadays for contributors to *Nature* to ask for their contributions to be withdrawn entirely or, in any event, returned to them because they have realized either errors in experimental method or in reading results or in coming to unjustifiable conclusions. Recently, on *one* day, letters for publication from three different groups of research workers were received asking for quite important changes to be made in their manuscripts already submitted, but (fortunately for them) not already published. Were it not for a certain amount of delay in publication, especially in the life sciences, the number of errata notes appearing in *Nature* would be deplorably high.

Another comparatively recent innovation, which is now becoming intense, is the establishment of so-called 'press relations' and the appointment of 'press officers'. This is, of course, not confined to science; in fact, scientific research centres are just following a general trend in this connexion. This will be all to the good for scientific advancement and information, provided it is carried out with tact, care and skill.

Nature has developed a close liaison with other journals, the newpaper Press and broadcasting organizations. Indeed, we consider this essential for both scientist and layman. Moreover, *Nature* has frequently advised scientists not to be too aloof and to do their utmost to assist the lay Press whenever their help can be of some specific value.

To-day, however, the tendency in this connexion among some scientific institutions and laboratories—academic, State and industrial—should be viewed with some concern. The number of so-called 'press hand-outs' and 'press releases' which reach *Nature* editorial office each and every day far exceeds the number of articles and communications submitted directly for publication. This might well undermine the general development and enhancement of science. For example, some press releases contain scientific results or claims that are quite fresh in that they have hitherto not been submitted to a learned journal or society for evaluation and possible criticism. Similarly, press conferences are so many and varied that they might well eventually defeat their own purpose. In any event, such 'exclusive' press releases often mean that some potential readers are missed, for it can be assumed that many such releases soon find their way into the editorial waste paper basket. The number received at *Nature* office each week would, if published, make a sizeable book. Press releases also result in duplication of publication. This may be all to the good where the lay Press is concerned (provided the material thus published in several newspapers is scientifically sound), for different people read different newspapers and magazines; but duplication in the scientific Press is highly undesirable, for undue prominence might ensue, and space is too valuable.

Nevertheless, in our own experience, press releases can sometimes be a nuisance (apart from the time-consuming job of reading them) and sometimes even dangerous. Too many of them exhibit the craze for priority or attempts to obtain the kind of publicity which is not always desirable. For example, recently a research publication was submitted to, and accepted for publication in, *Nature*. Shortly afterwards, a press release dealing with the same topic was received at *Nature* office before the original had been published. In order to keep as authoritative as possible, *Nature* very seldom publishes press releases in the form in which they are received; but on occasions they inspire the Editors to obtain an authoritative account of the subject. In the case cited, if the Editors had not noticed that the press release was a mere repetition (in different style) of what had already been submitted, double publication might have resulted in one and the same journal. The time seems ripe when editors of science journals must have a superhuman memory or possess some form of computer which will deal adequately with press releases.

Perhaps from the point of view of scientific advancement the most dangerous form of press release is that obviously written by a press officer who is not specifically acquainted with the subject under review but is doing his best to interpret some scientific finding to the lay Press. A further recent experience in *Nature* office will illustrate this. A fairly important discovery was made outside Britain by a scientist well known for his excellent work and publications. Yet a press release of his most recent discovery, presumably written by a press officer (this time in Britain), was received at *Nature* office. It was obviously meant for the lay Press, and some newspapers published it. Yet, when the Editors of *Nature* approached the scientist himself he disclaimed all knowledge of his discovery having been released to the Press.

Publication is one of the most important facets of the advancement of science. This applies not only to the scientific journal written by scientists for scientists but also to the lay journals and newspapers which are the forum for scientific exposition and discussion on the impact of science on society. In the past, far too many scientists have tended to remain aloof from the layman, and most have scorned any form of publicity. To-day, however, scientists are becoming increasingly aware of the effect of their calling on world society; this is no doubt due mainly to the tremendous strides science (and especially its applications) have made during the past few decades. Now, it is to be hoped that the pendulum is not swinging too far the other way. It is certain that claims for priority are uppermost in too many minds, and there is far too much inter- and intra-national competition and still not enough collaboration and team-work among laboratories and even nations.

Perhaps the most objectionable activity is that of the scientist who is determined that his light shall not be hidden under a bushel and therefore acts as his own press officer. Fortunately, in our experience such men are rare; but they are with us to-day, sometimes going so far as to notify the lay Press that they are about to publish in a scientific journal a communication in which the newspapers may be interested—even before they know whether the communication to the scientific journal has been accepted. Frequently succeeding in their endeavours, such scientific activities achieve publicity out of all proportion to their importance. There may be risks that ill-based claims are read and accepted by many readers.

Perhaps the day is not far off when scientists, through their means of publication, may be divided into the tough and the tender by a future William James, or the idealists and the realists by a future Schiller, or the "extraverts" and the introverts by a future Jung. It is certain that a future editor of *Nature* will be able to distinguish between the sincere scientist and the self-centred or personally ambitious scientist; but, alas, that editor may not live long enough to satisfy himself that the former is the more valuable member of any scientific team or community.

The scientist most desirable to science and society is he who leaves the evaluation of his work to his fellow scientists, presenting his findings with that modesty and dignity which one should always be able to associate with the word 'science'.

WHAT IS BASIC SCIENCE?

Disease and the Advancement of Basic Science
Edited by Dr. Henry K. Beecher. Pp. xi+416. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1960.) 100*s*. net.

THIS collection of articles illustrates the thesis that a study of disease can advance basic science. This thesis might seem self-evident, but it has not turned out to be so. During this century, and particularly in Great Britain, to try to understand Nature's experiments, as exemplified by disease, has tended to become scientifically disreputable. Thirty years ago Sir Thomas Lewis carried out a brief campaign for the recognition of clinical science as a discipline comparable in scope and status to those of other recognized branches of biology. He quoted the words of Sir James Paget of many years before: "I feel sure that Clinical Science has as good a claim to the name and rights and self subsistence of a science as any other department of biology; and that in it are the safest and best means of increasing the knowledge of diseases and their treatment. . . . Receiving thankfully all the help that physiology or chemistry or any other sciences more advanced than our own can give us, and pursuing all our studies with the precision and circumspection that we may best learn from them, let us still hold that, within our range of study, that alone is true which is proved clinically, and that which is clinically proved needs no other evidence". Lewis's advocacy produced an adverse response from medical scientists whose allegiance was primarily to the laboratory. In his presidential address to the Royal Society in 1934, Hopkins expressed the suspicion that the fields of clinical science so brilliantly exploited by Lewis and Mellanby were probably very few. He said: "I seem to have sensed, however, the beginnings of a definite movement in this country, and indeed elsewhere, not, of course, to ignore the laboratory; but in the distribution of funds provided for medical research to endow the Clinic on a scale which might endanger the future of research in fundamental biological science. The tenor of my remarks has been due to a conviction that in the long run such a policy would sterilize advance".

No detached observer would claim that the study of disease has made contributions to technique or to detail comparable with those achieved in the laboratory. But laboratory science has one major danger: it tends to be canalized into those lines that are, for the time being, most productive; the contemporary massive concentration of physiological effort into the behaviour of excitable tissues is an example. The unique contribution made by a study of disease is the prevention of too narrow an outlook and interest. How this has been and is being done is illustrated by the twelve articles in this book. Three may be quoted.

The first is unique, in that Linus Pauling conceived the idea that there might be molecular disease, that is to say, a disease the features of which were the direct outcome of the composition of a specific molecule. From what he was told of it, he wondered if sickle-celled anæmia could be due to a different form of hæmoglobin that crystallized out in the reduced form. This he was able to demonstrate. This story has recently culminated in Ingram's demonstration that normal and sickle-cell hæmoglobins differ by the presence of two amino-acid residues of valine in hæmoglobin S corresponding to amino-acid residues of glutamic acid in hæmoglobin A.

Bearn relates the story of biochemical genetics; how the story really began with Archibald Garrod, with his studies on the "Inborn Errors of Metabolism", albinism, alkaptonuria, cystinuria and porphyria. Garrod's work led him to consider that the abnormal gene resulted in specific biochemical abnormality which, in turn, gave rise to disordered metabolism and disease. This idea was revived, sharpened and more widely illuminated by the brilliant work of Beadle and Tatum on the eye colour of *Drosophila* and the nutritional requirements of *Neurospora* resulting in the one-gene/one-enzyme hypothesis. Bearn goes on to discuss the remarkable advances in our knowledge of copper metabolism produced by study of Wilson's disease, hepatolenticular degeneration, a disease transmitted by a Mendelian recessive gene and characterized by an inability to synthesize the copper-containing protein of serum, which, because of its colour, has been called ceruloplasmin. This is one of the more recent examples of how a genetically transmitted disease can illuminate biochemical mechanisms.

Perhaps the most enthralling story told in these pages is that of pernicious anæmia, first described by Addison in 1855. The break-through in this disease occurred when in 1926 Minot and Murphy showed that a diet of half a pound of liver a day would cure the disease, using as their index of response the percentage of immature cells, or reticulocytes, among the red cells of the blood. Minot and Murphy's trial of liver was based on a false analogy with experiments by Whipple, in which he had shown that liver was one of the most potent dietetic factors stimulating blood regeneration after bleeding in the dog. The analogy was false because the efficacy of liver in the dog was later shown to be due to its iron content, while in man post-hæmorrhagic anæmia has not been shown to respond to liver. There was an old series of observations suggesting that the primary defect in pernicious anæmia lay in the stomach, for Handfield Jones in 1854 had described atrophy of the glandular portion of the stomach in what was probably pernicious anæmia, and in 1870 Fenwick had shown that scrapings of the gastric mucosa, to which diluted hydrochloric acid had been added, failed to diminish the weight of a cube of egg white on incubation. The stomach defect had also been shown to precede the onset of anæmia. These two series of observations were brilliantly synthesized by Castle, who showed that if a normal subject was fed one pound of lean beef and made to vomit it at the end of an hour, and if the vomit was then fed to a patient with pernicious anæmia (as Castle remarked, *honi soit qui mal y pense*), the patient responded with a reticulocyte response and cure of the anæmia. Evidently, two factors were involved in the genesis of pernicious anæmia, an extrinsic factor present in lean beef, and an instrinsic factor present in the normal human stomach but absent from that in pernicious anæmia. The story is now nearly complete. The extrinsic factor is known to be the complex molecule of vitamin B_{12}, cyanocobalamin, the structure of which was so brilliantly identified by Todd and Hodgkin. The intrinsic factor still awaits precise definition, but there is no doubt that it is produced by the stomach and that its function is to facilitate the absorption of vitamin B_{12}.

These essays prompt the question: What is meant by basic or fundamental research? Listening to applicants for research grants, I would have very little doubt that the two adjectives have a substantial snob, prestige or prig value, as you care to look at it. All scientific workers wish to claim that what they do is basic or fundamental. The terms have also been applied to laboratory work as opposed to work outside it. Another usage equates basic science with pure science, the rest is applied. I have to confess that the intention to divide the pursuit of knowledge into fundamental and applied, into useless and useful, into respectable and unrespectable, has always been, and is, utterly repugnant to me. Knowledge is a unity, and an experiment devised and carried out in the laboratory is not necessarily more interesting or more illuminating than one designed, as it were, by the hand of God. It is at once conceded that it is often more difficult to apply the experimental method to the study of disease in man, and that to unravel the experiments of Nature is more difficult than to unravel those designed by man. But the present tendency to laud the laboratory and decry field work of whatever kind is not in the best interests of scientific knowledge as a whole. As Roger Bacon wrote, "All sciences are connected; they lend each other material aid as parts of one great whole. . . . As an eye torn out or a foot cut off, as it is with the different departments of knowledge; none can attain its proper result separately, since all are part of one and the same complete wisdom".

George Pickering

THE OCEAN FLOOR

The Floors of the Oceans

1: The North Atlantic. Text to Accompany the Physiographic Diagram of the North Atlantic. By Bruce C. Heezen, Marie Tharp, and Maurice Ewing. (Geological Society of America Special Paper 65.) Pp. xii + 122 + 30 plates. (New York: Geological Society of America, 1959.) 4.50 dollars. Physiographic Diagram 1.50 dollars.

Until very recently very little was known of the geology of the oceans, and rapid growth of interest and improvement of methods and instruments during the past fifteen years have led to striking advances. Improved echo-sounders, deep-sea corers, underwater cameras, towed magnetometers, shipborne gravimeters and other techniques have disclosed a new field for scientific exploration and geologists are rapidly taking advantage of it. Expanding interest in the history of the oceans, the nature and structure of the sediments and igneous rocks on the sea floor, and the processes at work, was one of the most striking features of the first International Oceanographic Congress held two years ago. The new idea of a borehole in the ocean floor was mentioned, and already an American team operating through water two miles deep has taken continuous samples from a borehole through 500 ft. of sediment and some 50 ft. of underlying rock.

The Lamont Geological Observatory, founded within Columbia University in 1949, has been outstanding in pioneering underwater geology, and this account of the floor of the North Atlantic Ocean by Heezen, Tharp and Ewing is based largely on first-hand experience. They show how they have been able to produce a "Physiographic Diagram of the North Atlantic Ocean" and outline its usefulness. The diagram is published separately for wall mounting, and it is the first of a series planned to cover the oceans of the world. It gives a striking portrayal of the general topography and roughness of the ocean floor. The horizontal scale is about 1 to 5 million, and the vertical scale has to be exaggerated by about 20 to 1 to bring out relief. It might be argued that this exaggeration over-emphasizes the slopes and the sharpness of features, but it is probably about the best compromise. There are nothing like enough soundings to base all the detail on fact, but the interpolation and extrapolation undoubtedly give as realistic a picture as we are likely to get for a long time.

The authors make some systematic classification by dividing the topographical features into three main types: continental margin, ocean-basin floor, and mid-ocean ridge, and subdividing these, sometimes tentatively, into component elements. The top part of the mid-ocean ridge, for example, is divided into rift valley, rift mountains, and high fractured plateau.

The distinctions they make are illustrated with the help of sounding records, bottom profiles, charts and underwater photographs, and, where possible, with the results of coring and dredging, magnetic and gravity anomalies, seismic soundings and heat-flow measurements.

Among the most remarkable features are the abyssal plains, where the underlying topography has been smoothed over by sediment-laden currents so that only the highest hills and seamounts stick out like islands from the surface of a lake. Mid-ocean canyons are steep-walled, flat-floored channels, 1–5 miles wide and perhaps 100 fathoms deep, cut in abyssal plains, generally in the approaches to steps to neighbouring, lower, plains. The mid-ocean ridge is about 1,200 miles wide with varied and irregular topography, and having a median rift, the rift valley, which, so far as we can see, is continuous down the length of the ridge.

Their new ideas are presented carefully, and, while giving much new information and many new ideas, the authors show where they need further support, and what we ought to do next. The book is a special paper of the Geological Society of America. Like several of the Society's publications, it will do much to further the study of the sea. It should be useful to cable companies, because of the new light it throws on some of the natural hazards and destructive processes that occur on the sea-floor. It has an excellent bibliography. G. E. R. Deacon

SECTOR-FOCUSED CYCLOTRONS

Sector-Focused Cyclotrons

Proceedings of an Informal Conference, Sea Island, Georgia, February 2–4, 1959. Edited by F. T. Howard. (Nuclear Science Series—Report No. 26. Publication No. 656.) Pp. xii + 291. (Washington, D.C.: National Academy of Sciences—National Research Council, 1959.) 2.50 dollars.

ACCELERATOR development has progressed with two main objectives in view: the first, to generate higher and higher energies, and the second, to obtain greater intensities. It was in the interests of this latter aim that the Sea Island Conference was held. Scientists from laboratories in America and Europe met to exchange information and ideas on the ways of increasing the intensities of medium- and high-energy cyclotrons by employing sector-focusing. It was fitting that L. H. Thomas, the first to expound this principle in 1938, should be among those present. Having been interested meanwhile in other subjects he could be forgiven his surprise at the delayed-action effect of his work.

The application of frequency modulation to cyclotrons in 1946 effectively solved the problem of obtaining higher energies but only at the expense of intensity, the continuous beam of the conventional cyclotron being sacrificed for a pulsed beam having a duty cycle unattractive for many nuclear experiments. For a time the attractions of the higher energies quenched the desire for greater intensity until of recent years its pursuit has been revived in several laboratories.

The *Proceedings* of the Conference record the papers and discussions at six sessions, the first of which was devoted to theoretical studies and orbit calculations. In this work, the use of large computers had proved invaluable. The achieving of the required magnetic field, however, was generally by way of successive approximations on an experiment model initially and later at full scale. Various methods of magnetic field measurement were described, many of which were mechanized to reduce the amount of repetitive work.

In the session devoted to radio-frequency problems novel approaches had been made in order to obtain a wide tuning range to give variable energy facilities. Then followed a session on quality of the beam, when interesting results were reported from several laboratories. This led naturally to problems of beam-extraction in which resonance phenomena, either deliberately induced or inherent in ridge-focusing, were discussed. The final session included a variety of topics relevant to experiments on cyclotrons, not the least important of which was that of shielding.

Approximately fifty papers were presented and the attendance of more than 80 is indicative of the wide interest in this field of work. Since the Conference several of the accelerators mentioned have come into operation. Perhaps these successes were in some measure helped forward by useful information exchanged there and certainly future workers will benefit from its recording in this series.

M. Snowden

THE PROBLEM OF RETIREMENT

Growing Old in a Mechanized World

The Human Problem of a Technical Revolution. By F. Le Gros Clark. (Studies of Ageing within the Conditions of Modern Industry.) Pp. 147. (London: The Nuffield Foundation, 1960.) 7*s*. 6*d*. net.

MR. F. Le GROS CLARK, with the same clear insight into the urgent practical problems confronting the older worker, restates his theme and provides us with additional data. What is happening to and what is likely to happen to the working man between the ages of 60 and 75, if he remains in good general health, wishes to continue in employment, but because of declining powers finds it more and more difficult to hold his own in the conditions of modern industry ? What effect is the progressive trend towards mechanization and automation likely to have on the prospects of the ageing man ? To provide some data towards an answer to these questions, Mr. Le Gros Clark takes eight technologically advanced industries (motor-cars, rayon yarn, plate-glass, etc.) and examines the position of the older worker in sample factories.

His conclusions are fairly pessimistic—more pessimistic than those of his earlier reports, also published by the Nuffield Foundation. The accelerating progress of mechanization and automation is beginning to affect the prospects of the older employee. Not only is automatized work more demanding in itself (in the need for vigilance, paced performance, etc.) but also the range of alternative 'light' jobs to which older workers might have been transferred is becoming progressively limited. A rigidly imposed retiring age is making it increasingly difficult for the fit man of 65 or more to remain in employment. We must expect to find more and more men retired from manufacturing industry while they are still reasonably healthy. Are these men likely to find alternative work, if they wish to, in other fields of employment ? In a long appendix Mr. Le Gros Clark considers the older man's prospects in other sorts of work (road maintenance, gardening, public services, caretaking) and comes to the conclusion that most of these jobs cannot provide alternative employment for many manual workers who apply well beyond their middle lives.

So what can be done ? Mr. Le Gros Clark has two main suggestions : That we should call into existence new categories of light work (he mentions 'paid social work' but does not define what he means by this) : and that we should work out a new social theory of retirement. In other words Mr. Le Gros Clark is now (reluctantly ?) coming to the conclusion that the period of retirement is "the inescapable third period of life" even for those who are fit and well. "We have spent much of our time studying what chances we have of preserving the health of older men and of adapting factory conditions to their changing physique. We have spent even more of our time studying the needs of the infirm and aged. It is now our business to add to these a third and equally important field of research—that into the full meaning and content of retired leisure". This may well mean a re-casting of social values.

L. S. Hearnshaw

DALTON'S IRISH RIVAL

The Life and Work of William Higgins, Chemist (1763–1825)

Including reprints of "A Comparative View of the Phlogistic and Antiphlogistic Theories" and "Observations on the Atomic Theory and Electrical Phenomena" by William Higgins. By Prof. T. S. Wheeler and Prof. J. R. Partington. Pp. viii + 173 + xiv + 496. (London and New York: Pergamon Press, 1960.) 60*s.* net.

The mere sight of the title of this work will give historians of chemistry (and especially of atomism) great satisfaction. The biographical and critical matter which makes up the modern third of the volume could probably only have been produced by this collaboration of these particular authors. The publishers are to be congratulated on reprinting in facsimile the works of William Higgins, which are discussed in every history of atomism but can in fact have been seen by only few readers. It is an appropriate tribute to one of the many Irishmen who did so much to advance science in the eighteenth century.

The history of the early chemical atomic theory is not synonymous with the work of John Dalton, although he holds the centre of the stage. It seems to us now as if some re-statement of qualitative atomism in quantitative terms were inevitable at the close of the eighteenth century. However, this is historical wisdom after the event. We are not yet certain how Dalton's mind and those of his contemporaries worked. Did Higgins anticipate Dalton ? Did he influence Dalton ? Davy was prepared to say that Higgins's work had been important, but his authority has little value. False notions about the historical origins of their beliefs have often been held, and are often held now, by leading authorities in experimental science. The work which brings a man scientific eminence does not necessarily make him a reliable historian, even of his own field.

The controversies in which Higgins's name was prominent began soon after Dalton's theory was put forward, which was, however, long after Higgins's first book had been published and passed over. Higgins's *Comparative View of the Phlogistic and Antiphlogistic Theories* was printed in 1789 in London. It was a detailed refutation of Kirwan's *Essay on Phlogiston* of 1787 and is remarkable in being "the first English work to support Lavoisier".

At that time the phlogistonist controversy seemed overridingly important. Twenty years later, the newer system expressed in the theoretical language of Lavoisier was supreme. Higgins's advocacy was no longer relevant and the aspect of the book which became, and remains, interesting is Higgins's use of an atomic theory in expounding his antiphlogistic views. There is no doubt that he had a notable insight into the quantitative basis of chemistry, had grasped Lavoisier's teaching well and showed some originality in combining it with atomic ideas.

In some ways he was in advance of his time (as in his views on reaction mechanisms) and might have helped to advance chemical theory if he had possessed two qualities which in fact he lacked. One was the capacity to see wherein lay the fundamental novelty of a quantitative atomic system. The other was a capacity to express himself with clarity and self-evident consistency. Dalton possessed both these qualities, and although he may have failed to see all the consequences of his own theory, he certainly opened the eyes of his contemporaries quickly and effectively.

Dalton was recognized and honoured: in 1814 Higgins felt impelled to advance his own claims to priority by publishing his *Experiments and Observations on the Atomic Theory* reproduced in facsimile in this same volume. It could not give Higgins the high place he claimed, and which Wheeler and Partington conclude he could not justly claim. "Higgins has an honourable place in chemistry, but he does not stand on the same level as Dalton."

The notes, appendixes, and two sets of correlations between *Comparative View of the Phlogistic and Antiphlogistic Theories* and critical text are models of scholarly thoroughness. The biographical text is the first life of Higgins of any completeness.

This is not a biography for the beginner, who would in fact find much of it aridly dull. The authors have evidently not thought it necessary to extend their material by much connecting narrative or exposition intended to make its digestion easier. As an aid to study and as a record of ascertained fact, however, its faults are few.

It is not clear why the facsimile of the *Comparative View of the Phlogistic and Antiphlogistic Theories* is of the second edition, dated, so far as can be deciphered, 1791, when the earlier 1789 edition could have been used. So far as the text goes, however, this is unimportant, because comparison of facsimile with the first edition shows that the two are identical, errata and all. The errata have been corrected by hand in the original copy, but not in the original *Atomic Theory* copy. This is all of no real significance, but it is regrettable that, in my specimen at any rate, the *Comparative View of the Phlogistic and Antiphlogistic Theories* has lost the first page of the list of contents. The student will have to fill this small deficiency as best he may.

For the historian, yet to appear, who will write a worthy history of the early atomists, and will re-interpret them to a generation which consistently misrepresents them, this book will be an indispensable tool.

Frank Greenaway

SCIENCE AND POETRY*

By T. R. HENN, C.B.E.

President, St. Catharine's College, Cambridge, and University Lecturer in Poetry and Drama

I

THE attack on poetry, not by the scientist but rather by the man of common sense, is nothing new in history. Plato (who was, as Sir Philip Sidney pointed out, the most poetical of philosophers, teaching by means of the very myths and parables that his dialectic drove him to condemn) had banished the poets from his ideal "Republic". Some of his leading indictments are still current to-day; in our popular press, in the mouth of the common man, and in those jarring sects and faculties that compose a university. Poets are liars; *they say the thing which is not*. They encourage their listeners to give way to undesirable and womanish emotions, which should be repressed or governed by the intelligence. They tell lies about the gods. They give us examples of men and women doing what they should not; and thus cause others to commit, or at least to contemplate, the practice of evil things.

Now this general indictment is repeated, with differing variations, stresses and tones, from the beginnings of literature; most forcibly, perhaps, by the Tudor Puritans, who found, no doubt, some social justification in the contemporary ballads, and in the condition of the theatres as breeding places of both sin and plague. We may note the heavy cross-fire, from a slightly different angle, in Peacock's attack not only on his fellow-writers of the early nineteenth century but also on the general status and office of the poet. Whatever the function of poetry in the past ages may have been, he says, it was wholly out of place in his own "Iron Age of Industrialism". It is worth quoting a passage:

> "The descriptive poetry of the present age has been called by its cultivators a return to nature. Nothing is more impertinent than this pretension. Poetry cannot travel out of the regions of its birth, the uncultivated lands of semi-civilized man. Mr. Wordsworth, the great leader of the returners to nature, cannot describe a scene under his own eyes without putting into it the shadow of a Danish boy or the living ghost of Lucy Grey, or some similar fantastical parturition of the moods of his own mind. . . .
>
> "In the origin and perfection of poetry, all the associations of life were composed of poetical materials. With us it is decidedly the reverse. We know too that there are no Dryads in Hyde Park, nor Naiads in the Regent's Canal. But barbaric manners and supernatural interventions are necessary to poetry. Either in the scene, or in the time, or in both, it must be remote from our ordinary perceptions. While the historian and the philosopher are advancing in, and accelerating, the progress of knowledge, the poet is wallowing in the rubbish of departed ignorance, and raking up the ashes of dead savages to find gew-gews and rattles for the grown babies of the age. . . .
>
> "A poet in our times is a semi-barbarian in a civilized community. He lives in the days that are past. His ideas, thoughts, feelings, associations, are all with barbarous manners, obsolete customs, and exploded superstitions. The march of his intellect is like that of a crab, backward. The brighter the light diffused around him by the progress of reason, the thicker is the darkness of antiquated barbarism in which he buries himself like a mole, to throw up the barren hillocks of his Cimmerian labours. The philosophic mental tranquillity which looks round with an equal eye on all external things, collects a store of ideas, discriminates their relative value, assigns to all their proper place . . . is diametrically the reverse of that frame of mind which poetry inspires, or from which poetry can emanate".

II

I have quoted Peacock at some length because, with slight changes of emphasis and tone, it represents a considerable body of opinion to-day over the science-poetry controversy. We have swept away the myths, superstitions, "old furniture of the mind", the "Hebrew old clothes" on which are founded the "Magical View of Life" (the phrase is from I. A. Richards)[1] that once provided not only the materials but the very life-blood of poetry. It was a celebrated critic of a hundred years ago who advised Matthew Arnold to "leave the exhausted past". Science is the living example of reason in all things. It brings light, if not sweetness, to mankind. The history of science is that of the march of the intellect, a series of slow triumphs over ecclesiastical or political obscurantism, superstition and human stupidity. Science alone deals with *fact*, "susceptible, at every stage, of verification"; and exploiting, clearly and unequivocally, its practical 'goods'. It leads to the practical 'goods' of an ever-mounting standard of living, of medical care, of comfort, of speed, safety. It conduces steadily towards lighter and shorter hours of work. It gives man power over all things material. To such beliefs we are also inexorably driven, every day of our lives, by the assertions of commercial interests of every conceivable kind, whether overt or subliminal; and these assertions are not disinterested.

This is a common picture of a common belief: and at this stage of our discussion I wish to do no more than interject a quotation from Sir Charles Sherrington:

> "It was Joseph Priestley, writing . . . twenty years before the French Revolution, who foresaw issuing from Science a future 'glorious and paradisiacal beyond our imagination'. 'Nature . . . will be more at our command; men will make their situation in the world abundantly more easy and comfortable; they will probably prolong their existence in it, and will daily grow more happy'. The prediction was correct, save for its concluding sequitur, which as we hear it now rings as a non-sequitur. Priestley's enthusiasm had thought greater knowledge must mean supremacy of 'the values'. It might but it did not. In all this man can look for no help from any mind on his planet but his own. . . . He is the only recognizer of 'values' ".

III

Let us take the present disrepute of what Sidney called 'poor poetry' a little further. The artist or poet has, since the late eighteenth century, tended to appear to the public as a lonely, perhaps eccentric figure. Theseus's implied equation of him with lunatic and the lover dies hard in the mind of the common reader. He remains separate, in a real or imagined attempt to assert his own personality, his sense of his self-importance. He often sees himself as the one against the many. A suggestion of the *vates*, the prophet, the seër, has clung to him from the beginnings of history, and he is reluctant to jettison it. He has been in the past the inspired, even the afflicted (and therefore the venerated) of God. Many of Peacock's gibes still hold in popular estimation. If his audience believe neither in prophecy, nor in the world of the supranatural, nor in the existence of Spirit, his claim to attention may well be a thin one. As reverence for him diminishes, he is apt to clutch, even more tightly, the cloak of his real or assumed personality. The unpicturesque disorder of the 'beatnik' poet in Los Angeles or Greenwich Village is as much a badge of office as the long hair, the flowing tie, of the 'decadent', whether alcoholic or homosexual, of the 'nineties. There is more than a suspicion that his morality, particularly in sex, drink and drugs, will never stand the scrutiny of biographers, or even of the courts: the lives of the poets can be as unedifying to-day as they were, on occasion, to Johnson or Arnold.

---

* Substance of a lecture delivered at Hatfield Technical College on May 12.

With these things go a traditional inability or unwillingness to engage with any notable success in the world of action. We remember that Aeschylus recorded on his tombstone the fact that he had fought at Marathon, and left no word of his dramas. Sophocles commanded a squadron, and:

"Got laughed at, and went back to making plays,
Having allowed us our experiment
Respecting the fit use of faculty".

Chaucer seems to have been an efficient Civil servant and diplomat; Spenser did his job as what we should now call a military assistant to Lord Grey on his Irish Expedition. Marvell was a tolerable member of Parliament for Hull; Milton became First Secretary to the Commonwealth because he was the first Latinist in Europe. Byron had a military-political career that was both dramatically satisfying and reasonably efficient. But for the rest it is a depressing record of temporary and rather futile incursions into the active life—Gibbon, Coleridge, Wordsworth, Shelley, Landor are witnesses. In our time, in this century, I know of no major poet (with the possible exception of Wilfred Owen, who was killed at the crossing of the Sambre Canal on November 4, 1918, and of Sidney Keys in the Second World War) who was engaged in action; though I have known two generals who were minor poets themselves, and we may recall Roy Campbell, Brooks, Masefield and a few others. It is little wonder that our common man is suspicious of the poet's claims to supreme importance, to be the potential saviour of civilization, even to be the provider of a substitute for religion. It is very clear to the common man that poets are not, and never have been, Shelley's "unacknowledged legislators of the world". He can make no sense of Wordsworth's claim that "poetry is the breath and finer spirit of all knowledge". He observes with uneasiness that the poets, so far from facing up to their own particular problems, seem to be talking to their own small circle in a language of unexampled and ever-increasing complexity; and when, with a sense of relief and even pleasure, he turns to recognize his own sensible nostalgia in the work of Mr. Betjeman, he is told by the experts that this is not poetry. In the present literary scene the common man or scientist will note that only two literary figures of importance have taken degrees in science or mathematics, and he will reflect sadly that one has returned from defending poetry to a divided loyalty between the Coal Board and the B.B.C., and that the other is apt to write poetry which is comprehensible with difficulty even to the mathematicians themselves. (Curiously enough, there seems to be some tendency for biologists to be artists—perhaps because of much study of illustrative drawings—and for mathematicians and physicists to be musicians. Perhaps there is something of psychological interest here.)

IV

Let us start again. Very few poets have attempted any kind of 'liaison of vocabulary' between science and poetry. It is true that when science was younger it could and did lend to poetry material for metaphors; some elementary geometry for Marvell; optics, metallurgy, chemistry, a few medical terms, to John Donne; much alchemy, a little astronomy, and a vast quantity of astrology. It is, I think, true to say that no poet to-day would dare to take sides in verse in the Ryle–Hoyle controversy over the origin of the universe. "Never write a poem about the solar system" is a sound maxim; though I notice that this year at Cambridge the prescribed topic, "for a poem conducive to the honour of the Supreme Being and the recommendation of virtue", is "The Spacious Firmament". (But it is only fair to the University authorities to say that this was prescribed before the appearance of the space man.) There is a fair amount of contemporary science reflected in Shelley and Coleridge (in the main, chemistry and physics) and in Tennyson, who is strongly affected by Darwinism. The attempts to find, at the heart of the nucleus, an "Element of Purpose", suggesting the existence of creative mind, do not appear to me to have been very successful; nor is there (as Schroedinger has pointed out) any ascertainable relationship between the quantum theory and the problem of free will. It is true that certain possibilities of science in our time have stirred momentarily the poetic imagination. So D. H. Lawrence's *Relativity:*

"I like relativity and quantum theories
because I don't understand them
and they make me feel as if space shifted about
like a swan that can't settle,
refusing to sit still and be measured;
and as if the atom were an impulsive thing
always changing its mind."

or the attempt to find free-will restored by Einstein:

"But to one muddled mind it seems
A curvilinear universe
Involves free-will, and so redeems
God and the world from Calvin's curse."

But on the whole it seems as if modern poets distrust technological achievements; as in William Empson's poem "To an Old Lady" (which is the Moon):

"Project her no projectile, plan nor man it . . .
Landing, you break some palace and seem odd . . .
No, to your telescope, spy out the land :
Watch, while your ritual is still to see."

The poets seem, on the whole, to look backward with some longing to the period before the break-up of Hellenic and Hebrew mythological knowledge; consider Ronald Bottrall's *Eppur Si Muove:*

"there must be horse-sense, horse-strength, to spare
For building up into new stars
The molecules that the great machine
Has broken up into minute particulars
Remote from all that's viable and keen."

It will not work; any more than did Erasmus Darwin's *Botanic Garden*, or the *Loves of the Plants*. And although I have sometimes thought that some aspects of electronics might lend themselves to images in love-poetry—we might, for example, ponder on the possibilities of 'transistorized' and 'superheterodyne', or even of the experience of love as a 'reactor' rather than the classic 'crucible' or 'alembic' —it is clear that the 'multitudinous twittering polysyllables' of the average scientific paper do not lend themselves readily to rhythmic or musical utterance. Let us abandon all thought of these pseudo-marriages between the two sides, and start afresh with our argument. Poetry and science are separate, as human activities; and here I want to quote from Matthew Arnold:

> "It is the task of science to ascertain the facts of life ; and the task of poetry to relate them to human experience".

But what are the "facts of life" to-day; and how does poetry claim to relate them "to human experience" ?

V

Let me attempt to continue my case by postulating a scientist of high intelligence, who has studied (and on the whole assents to) the familiar case for the denigration of poetry, or at least the relegation of it—following Freud—to an auxiliary but peripheral activity. I should, I think, argue with him in some such terms as these.

In poetry we are concerned with statements of peculiar variety, strength and subtlety; and the operation of these statements we cannot pretend to understand without some agreement as to the nature and operation of the human mind; and in particular to the proposition that, whatever our claims to act under the stimulus of reason alone, the Emotional or E component, whether drawn from the conscious or subconscious, is of great importance. (There are, incidentally, grounds for believing that, for various reasons—which include those of the rapidity of modern communications—this E component is increasing rather than diminishing in the human situation.) Our thoughts and actions are conditioned and governed by a peculiar and varying blend of the R, or rational, and E components; and it is clear that, whether we "follow Priapus or Paul", Freud or Jung or Marx or Sartre, the single most important determinant factor is the past: whether in heredity, environment or upbringing. And this past is in itself a factor of incredible complexity. It is, inevitably, beyond and outside any accurate analysis or assessment by ourselves. It can only be reckoned with by a technique which makes use of a comparable delicacy and complexity in dealing with its effect. I am now hinting at a profound and difficult statement by Shelley, to which we shall return later:

> "Poetry administers to the *effect* by acting upon the cause."

Poetry is making statements about the nature of experience; experience that the poet perceives, organizes, refracts, and casts into moulds of the kind appropriate to his art. It is an art that is both more difficult and perhaps more satisfying than that of the painter, or sculptor, or musician; largely because the poet is using language as a multiple instrument. The formal technique of poetry must be recognized, studied, and, in a particular sense 'understood', if we are to receive what it has to communicate. The poet is making words perform peculiar yet precise functions; setting to work a complex selector-mechanism which can sometimes be shown analogically (as I have tried to do in a little book called *The Apple and the Spectroscope*) in mathematical and electrical terms; and if I were re-writing it, I should, I think, want to attempt to extend my illustrations into elementary chemistry as well. The selection, interaction and continuous modification of the meaning of words is made possible by setting them in complicated matrices of rhythm, tone, accent, pitch; themselves part of a relationship in terms of music which still further defines, amplifies and extends the meaning which the poet attempts to communicate. Further, he can and does use the method of perceiving correspondences between experiences (of events, people and objects) to indicate similarity and/or dissimilarity in that which he perceives, and hence can indicate judgments as regards value—whether positive or negative—regarding his perceptions. We may again refer to Shelley:

> "Reason is the enumeration of qualities already known ; imagination is the perception of the value of those qualities, both separately and as a whole. . . . Reason respects the differences, and imagination the similitudes between things."

But what is this poetic statement about? How can we explain its significance and what is the use of it, anyway ? What relevance has it to our problem of "Science and Poetry" to-day ?

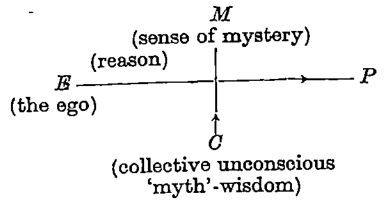
To some of these questions we can give brief but provisional answers. Poetry is 'about' the human situation, in its broadest terms. It is 'about' the highly complicated amalgam of intellect and emotion, of head and heart, of conscious and unconscious habits (but habits which grow and change continuously) that govern our normal lives. To the amalgam of the R and E components we are committed, inescapably, by the human situation. From the beginnings men have attempted to hack through the barrier, to behave in marriage, politics, war, as if humanity were (or might, one day, be) wholly 'rational' in its operations, as in the cold world of Swift's horses. It was in relation to love and marriage and woman's intuition that Meredith complained so bitterly:

> "Their sense is with their senses all mixed in ;
> Destroyed with subtleties these women are.
> More brain, O Lord, more brain, or we shall mar
> Utterly this fair garden we might win. . . ."

But it has not happened like that, and it will not: public and private history, religion, love, philosophy, all proclaim through time the irrationality of man. We must recognize that the greatest single danger to man's integrity and perhaps to our whole civilization is the subjugation of judgment by mass-media, before which even the perennial 'crooked questions' seem to be simplified for commercial or missionary ends; I mean those of love, death, pain, decay; of the essential dichotomy in the marriage relationship; of the existence of evil and tragedy; of the whole paradox of man's nature, the conflict between his 'erected wit' and his 'infected will', or St. Paul's great paradox (*Rom.* 7, 10). It is, I think, probable that we are to-day experiencing an induration or atrophy of the basic spiritual faculties; among those of sympathy, responsibility, and real (as opposed to fictitious or factitious) pleasure. And to this problem of sensitivity we must return.

VI

Now at this point I must attempt a brief excursion into metaphysics. Let us represent human experience by means of a tentative diagram, thus:



showing two poles or dimensions. The 'x' axis may be thought of as empirical in its nature; that is, it deals with the relationships and ideas that make up our normal common-sense world. Let *P* stand for the phenomena that compose our physical world. We may think of these as interrelated by laws of causality, themselves the proper objects of scientific inquiry. Let *E* be the ego which seeks to know the phenomena, which investigates and masters them. This horizontal axis of thought is Cartesian in origin. Along it moves pure reason which, rightly used, can solve all the riddles of the physical universe. Along this line the solution of the problem of the interrelations of all phenomena is possible. This solution is perceived as being in itself a 'good'.

Across this axis cuts the other[2]. Along it moves the 'community mind', *C*: which is not the 'mass mind' of war hysteria, or of popular riots. It is a mind which includes Jung's "collective unconscious", but is not limited to that concept, or to the analogous one of "The Great Memory" (as used, say, by W. B. Yeats). It grows slowly through the ages; it carries with it a store of collective wisdom in its myths. It moves on the vertical axis towards *M*, the sense of mystery in the world: and by mystery I mean those elements in the human situation which lend themselves only to intermittent apprehension, to which the approach cannot be made through rational or logical mechanisms; but which are of infinite importance in the total commitment of men.

I have very much in mind Jung's *Modern Man in Search of a Soul*; as well as the work of Joseph Wheelwright, Maud Bodkin, Austin Farrer and others. I believe passionately that the completed man needs the psychic satisfaction that is given by poetry, mythology, æsthetic experience: which, in many forms, can provide us with what Synge called "the nourishment, not very easy to define, on which our souls live". That this life of the imagination demands continually such nourishment, that it grows atrophied or indurated without it, I am certain. So long as there was a common ground of religious mythology (I prefer to use—following Jung and Kerényi—the word 'mythologem')—whether Biblical or Homeric—there was at least a common core of values, implicit or explicit, to be perceived in relation to them. The break-up of the common area of experience may perhaps be dated from about a hundred years ago, though something of it remained, I think, until 1914. We are familiar with *The Waste Land* of the 'twenties, the 'Pylon poets' of the 'thirties, the recurrent chorus that announced disintegration; and while the very act of perceiving that there is a disintegration is not without its value, we must clearly look for something more positive in character.

Now what this positive may be will depend, in the final analysis, on our own reading of the human situation. It seems to me improbable that poetry has much to give us if we accept wholly any of the empiricist philosophies, among them behaviourism that equates mind with the physical constitution of the body; instrumenticism which "reduces the meaning of any concept to that set of experimental operations by which the denotation of the concept can be objectively shown", and the fashionable semantic positivism which aims at a one-to-one correspondence between units of language and the sets or types of objects which such language units denote. Existentialism alone can point to a bitter ironical poetry of its own; since, while it jettisons many of the primary and traditional values, it holds fast to the status of the individual in his right of choice, in his need for integrity, nobility and courage; even though, to the existentialist, "life begins on the other side of despair".

Poetry does and must deal with the penumbral; and its penumbra may be the twilight world which reason and instinct, memory and present action, the conscious and the unconscious, merge imperceptively into each other. We read poetry because the inmost character of this penumbral world is only perceptible in the poetic statement, in the poem, in its own self-subsisting images, and not in any exegesis of it; because the fusion which it involves is its own highly complex justification; because its relationship to the totality of life is not perceptible without a study of its methods and techniques which can so easily atrophy, and which so often, in our present-day civilization, appears to give rise to a widespread aridity of consciousness.

It is at this point that I return to Shelley's

> "Poetry administers to the *effect* by acting upon the *cause*."

—that is, one operation of poetry is to produce, by exercising the creative imagination, the sense of awareness or sensibility which provides us with an extension of the individual towards unity with others and with the created world. Shelley in his time was arguing against the all-pervading rationalism, the *laissez-faire* economics of his time; and against that reason, unmitigated by pity, which had made "the rich become richer, and the poor become poorer". As he saw it, evil would in theory become impossible if all men were wholly sensitized and sensitive to the suffering of others; you do not do evil to others if your own imagination tortures you at the thought of their suffering. Perhaps this is what St. Paul meant by "charity", and some of us believe that various complex causes—among them the effects of two wars, the rise of the Welfare State, the rapid and violent re-orientation of communal values—have resulted in a diminished sense of pity and love, and perhaps of other virtues that are allied to these.

We can extend this thought a little towards the special problems of the scientist, whose work, habits of thought, pre-occupations with the *fact*, can and does distort or submerge, on occasion, his capacity for purely human relationships, whether in the family, in business, in the State or in international settings. I am clear that all the great scientists that I myself have known have sought and invariably achieved some balance, some counterpoise, through a form of art, practised or enjoyed, which has taken a prominent place in their lives: which seems to be the "nourishment, not very easy to define, on which our souls live". But one of them has suggested to me a further consideration: the possibility that some of the 'great' discoveries may now be going unmade precisely because so many students of science to-day allow their imaginative powers to atrophy. I have used the terms 'nourishment', 'feeding', 'exercise'; for I believe in its need for these things the imagination does not differ in kind from any other mental power. But unless it is exercised it does die quickly away; and with it that mysterious power of making the creative 'leaps in the dark', the habits of synthesizing, finding relationships between disparate events, ideas, and above all, perceiving values through comparison. How far this may be true, none of us, are, I think, competent to say. I can only judge from case-histories within my knowledge; in which the tremendous and ever-increasing pressures on the natural sciences student seem often to make it impossible for him to expand his interests in a manner which would ultimately permit him to bring a trained and flexible mind to the forming of value-judgments, and which, perhaps, diminish the probability that his creative imagination will contribute to his discoveries.

I do not, myself, wish to suggest that there is any difference in kind in the potential or the sensibility of, let us say, the mathematician as contrasted with the arts man in forming these value-judgments. What divergence there is seems to me to arise because of the manner in which the arts student is brought into continual contact with historical, political, philosophical and religious problems (however tangential or partial they may appear) that are refracted through the material which he studies. He thus becomes aware that such problems are highly complex, involving a number of indeterminate factors, and admitting of no quick or easy answer. Some experience of European diplomacy in time of war seemed to suggest the dangers of seeking for solutions that should in theory have been clear-cut, rapid, rational; when they were in fact the products of a slow maturation in time, and of immensely complex human and national factors that were themselves rooted in history. The search for the quick, the black-and-white answer seems to be an eternal temptation of the immature, the man or nation that is in too much of a hurry: and this (though I do not propose to enter into that discussion) is not without relevance to the problem of policy-making, and of taking central or seminal decisions, in industry and in international affairs.

VII

At this stage it will be seen that I am making no special claim for poetry as opposed to any other form of art that serves to feed this creative imagination; remarking only on poetry's greater complexity and 'impurity' of statement as compared with the statement of, say, music or sculpture. I myself believe that poetry has suffered from the zeal, and sometimes the arrogance, of its fanatical supporters. I had some early training as a mathematician, and I went to Cambridge as a modern linguist. I yield to no one in my love of poetry, and I carried much of it in my head during the Second World War, in many countries and in many crises both of personality, diplomacy and war. But I do not believe in the possibility that it should ever become a significant formative force over any considerable area of the national consciousness. It has always been, and I think it will remain, of limited though immensely important appeal and incidence. For poetry demands, like any other art, effort and study: whether it be Shakespeare, or Donne, or 'difficult' modern poetry. In theory all art should offer a delight that is easy, quick and having some considerable degree of universality. No art since the sixteenth century (perhaps the fourteenth) has fulfilled those conditions.

VIII

My own brief summary would, I think, run something like this:

Poetry can communicate to us, in a very special manner, the collective wisdom of generations of men and women. These experiences are (this is the price of their survival) archetypal and perennial; since they are rooted deeply in the human situation, in the conscious and unconscious, in the past and present.

By the peculiar (and still largely unexplored) methods of communication which embody a combination of the semantic and phonetic aspects of language, and by its devices of rhythm, which are strictly related to the total statement made, it can achieve an acceptance of vicarious or stated experience in such a way as to secure its integration into the human personality. It may and does become one 'experienced' aspect of the 'armour against fate'; and this over an unimaginably wide range.

By its presentation of integrated, shaped and organized experience (a play is a convenient example of this rhythmic structure) it satisfies our need for the intermittent perception of a complete experience in a world which, without art, we feel to be fundamentally chaotic, inchoate.

By its feeding, exercising of the imagination—which I would define as that faculty which enables us to be aware of the immaterial in its infinite forms—poetry can fill that perennial human need for a life in which the mythic element can obtain the psychic satisfaction which it requires. I do not hold, with Matthew Arnold, that "the greater part of our religion to-day is its unconscious poetry"; for I do not believe that poetry can ever be a substitute for philosophy or religion; even to those who may believe, as good agnostics, that they have swept and garnished the last room of their minds, and are merely waiting . . . for what ? I believe that poetry may prepare us for our journey into religion or philosophy, by just this process of sensitization, of increasing awareness, of which I spoke; I do not believe that it is a substitute. Nor do I believe (in contradiction to some of my colleagues) that a complete and implicit body of morality can or does arise out of poetry, since I believe that a body of moral belief, which must be won (at whatever cost) by the individual, is a prior necessity. I would argue that many of our problems to-day arise because of our attempts, in popular religions or in popular science, to short-circuit the long and painful processes of gaining knowledge and of adjusting that knowledge to the human situation. As for myself, I am certain that, if we are to allow the impact of any great art on us, we must be prepared to make certain assumptions (I am aware that many would give only a pragmatic assent, "the willing suspension of disbelief that constitutes poetic faith"), which I would set out as follows: (1) that there is a mind, or spirit, or soul, or psyche, which is not susceptible of explanation, as regards its powers and operations, on any known physio-chemical-biological hypothesis; (2) that the human situation involves the apprehension, intuitive in character, of a vast body of mystery,

Appearing in August

Advances in Aeronautical Sciences — Volumes 3 & 4

Editorial Committee Chairman : TH. VON KÁRMÁN

This 2 volume set contains the Proceedings of the 2nd International Congress in the Aeronautical Sciences, Zurich, including the 57 papers submitted and a full author and subject index.

Some Papers Published in Volume 3

The Role of Entropy in the Aero-space Sciences—DANIEL AND FLORENCE GUGGENHEIM MEMORIAL LECTURE—JAKOB ACKERET ; Possibilités et limites actuelles de la théorie des écoulements hypersoniques—J. P. GUIRAUD ; On the Use of Shock Tunnels for Research on Hypersonic Flow—D. W. HOLDER and D. L. SCHULTZ; and On the Theory of Hypersonic Flow over Blunt-nosed Slender Bodies—V. V. SYCHEV.

Some Papers Published in Volume 4

The Aerodynamics of Jet Flaps—JOHN WILLIAMS, S. F. J. BUTLER and M. N. WOOD ; Sub- and Super Alfvenic Flows Past Bodies—W. R. SEARS and E. L. RESLER ; and Einfluss des Start-Lende-Lastwechsels auf die Lebensdauer der Boenbeanspruchten Flugel von Verkehrsflugzeugen—ERNST GASSNER and KARL F. HORTSMANN.

1,300 pages *numerous illustrations* *£15 net* ($45.00) *per set of 2 vols.*

Biological Waste Treatment

W. WESLEY ECKENFELDER, Jr., *and* **DONALD J. O'CONNOR,** *Manhattan College, New York*

Deals with the physical and biological principles of waste treatment and their engineering application to stream pollution analysis and waste treatment plant design. The book has a complete description of pertinent laboratory procedures and pilot plant design and operation. The most recent advances in research are applied to the design of treatment facilities, with emphasis on the mathematical quantitative approach.

All chemists, biologists, civil, design and construction engineers will find this comprehensive book extremely useful. It will be an essential reference work for all sewage boards, water boards and fisheries.

288 *pages* *illustrated* 65*s. net* ($10.00)

Progress in Microscopy

M. FRANÇON, *Professor of Physics, The Sorbonne, Paris University and Institute of Optics*

Describes the recent advances in methods used in microscopy. The first chapter is devoted to image formation, since this theory is essential in understanding the new techniques presented in the following chapters. The main subjects covered thereafter are geometrical measurements, interference microscopy, phase contrast, microscopy by reflection, observations inside the invisible spectrum (ultraviolet and infra-red), microspectrophotometry and microchemistry.

304 *pages* *numerous illustrations* 60*s. net* ($9.00)

Oogenesis — The Storage of Developmental Information

Chr. P. RAVEN, *Professor of Zoology, University of Utrecht*

This absorbing book gives an analysis of the formation of egg cells in the ovary, in which the synthetic and metabolic activities of these cells and their relationships with the surrounding elements are especially taken into consideration. These facts are discussed with reference to the problems of the transmission of information from one generation to the next through the germ cells. Conclusions are drawn on the possible mechanisms serving for the storage of this developmental information in the fertilized egg cell, and an attempt is made to derive reasonable estimates of the amounts of information involved.

310 pages *numerous illustrations* 50*s. net* ($8.50)

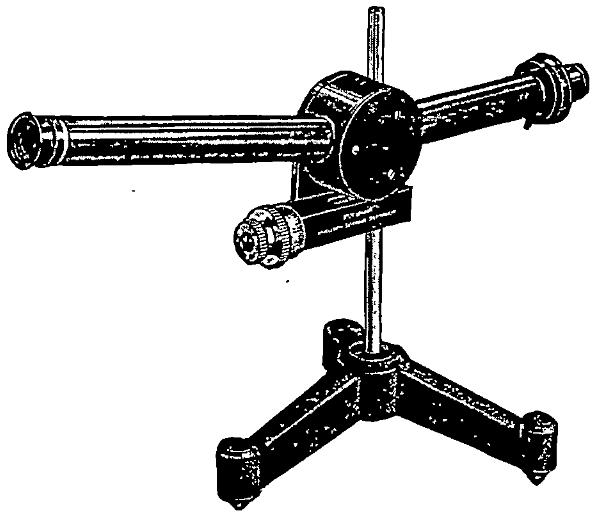
All books gladly sent on 14 days' approval

PERGAMON PRESS

OXFORD LONDON NEW YORK PARIS

Headington Hill Hall, Oxford. 4 *&* 5 *Fitzroy Sq., London, W.*1. 122 *East* 55*th St., New York* 22, *N.Y.*

REVERSION SPECTROSCOPE



This model (No. 3505) is constructed on the reversion principle originated by Prof. H. Hartridge, F.R.S. It enables readings to be made with ease and great accuracy.

Catalogue of Spectroscopes, post free from:—

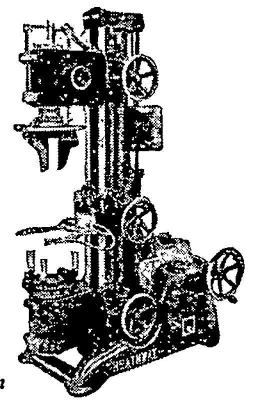
R & J BECK LTD • 69 MORTIMER ST
LONDON W.1

Heathway

Glass Working Lathes

are known throughout the world

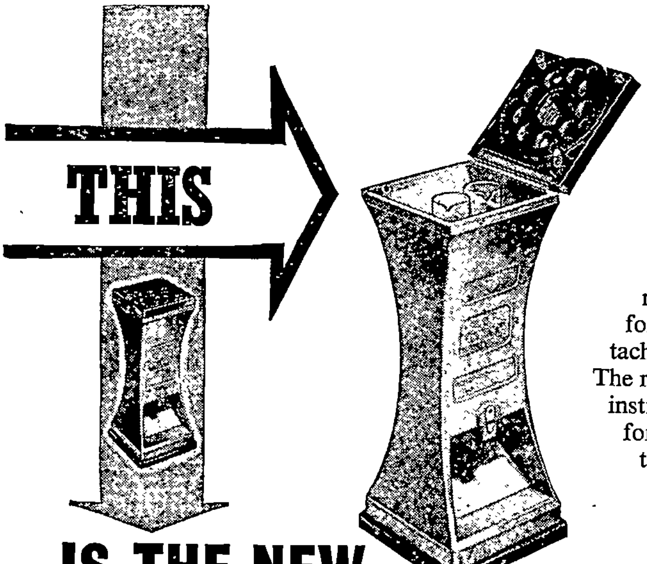
- 5 types available
- May we send you leaflets?



The new universal model in its vertical position

HEATHWAY MACHINERY CO. LTD.
UXBRIDGE ROAD, HILLINGDON, MIDDLESEX
TELEPHONE: UXBRIDGE 36345

Agents in the U.S.A. and France
Richoux Co. Inc., 1133 Broadway, New York 10, N.Y.
Ets. L. Richoux, 22 Cité Trévise, Paris 9e



Apart from its appearance, which we venture to think is considerably improved, there are no doors to open, the Nessleriser glasses can be more easily inserted and removed, and the design gives built-in provision for blue glass filters for use with white light attachments. Most metal parts have been eliminated. The range of interchangeable discs for use with the instrument provides permanent colour standards for some thirty analytical procedures, in addition to seventeen discs for pH determinations. The price, complete with any nine-standard disc and a pair of matched Nessleriser glasses, is £12.10s.0d.

IS THE NEW

B.D.H. LOVIBOND NESSLERISER

THE BRITISH DRUG HOUSES LTD.
B.D.H. LABORATORY CHEMICALS DIVISION
POOLE DORSET

which can be apprehended only by an intermittent and uncertain process of illumination, which grows, I think, less intermittent as we ourselves become older; (3) that poetry gives us one method of progress towards these apprehensions, and in particular of those that concern the penumbral—but not unfamiliar—worlds of which I have spoken; and which we exclude from our lives at our peril.

A quotation from Browning will serve:

> "Just when we are safest, there's a sunset-touch,
> A fancy from a flower-bell, someone's death,
> A chorus-ending from Euripides,—
> And that's enough for fifty hopes and fears
> As old and new at once as Nature's self,
> To rap and knock and enter in our soul,
> Take hands and dance there, a fantastic ring,
> Round the ancient idol, on his base again,—
> The grand Perhaps ! we look on helplessly—
> There the old misgivings, crooked questions are— . . ."

IX

No more than this, you say ? There is indeed; but my final judgment must be empirical, the result of watching many generations in their progress through literature and life. I am certain that the average undergraduate will one day be a better parent, lover, statesman, leader, for having read Shakespeare, Donne, Blake, Keats, Meredith. When he comes to his death he will die the better for having read, say, Dunbar, Donne, Herbert, Wordsworth. He will know better the essence of 'country'—and we neglect those roots at our peril—for having read Traherne, or Wordsworth, or Hopkins, or for having understood Constable's landscapes.

That is not to say that it would be better never to have been in love than never to have read Shakespeare. No literature of war can convey its full horror and exaltation and sense of futility until one has wasted years of life on it, or killed a man. Poetry, like all art, must demand and secure the perpetual opportunity to balance itself against physical life, experience; to test mutually the values thus discovered and embodied. Much of its disrepute is because of its claim to become an end in itself, the plaything of æstheticism, the spell-bound Lady of Shalott, the Sleeping Beauty in the jewelled sensual cave, or far worse than these, the esoteric speech of "the professors whose mouths are gorged with sawdust". Perhaps all that I am trying to say is summed up in an adaptation of a text that you will recognize. Its function is to give life, the strange and vitally energizing life of the psyche; and to give it more abundantly.

---

[1] Richards, I. A., *Science and Poetry*.

[2] I am indebted to *The Language of Poetry*, edit. by Tate (J. Wheelwright's article).

A STRATEGY FOR THE WAR ON HUNGER

By Sir JOSEPH HUTCHINSON, C.M.G., F.R.S.
University of Cambridge
and
Prof. A. H. BUNTING
University of Reading

THE Rockefeller Foundation instituted a public health programme in Mexico in 1919. By 1943, rising health standards there had produced one of the great modern human problems—the problem of feeding the rising population produced by scientific death control. In that year the Foundation and the Mexican Government inaugurated a co-operative programme in agricultural research which has served as a model for similar operations in other countries. Aided by this programme, an average annual population increase, during the past ten years, of about 3 per cent has been over-matched by an average annual increase of 7 per cent in food production. Seven per cent is one of the highest rates of increase of agricultural production recorded anywhere in the world in this period, but it is perhaps relevant to point out that while from 1948–52 to 1957 rice production in Asia increased at about 3·7 per cent per annum, in mainland China the figure was 5·7 per cent (calculated from *F.A.O. Mon. Bull. Statistics*, Dec. 1959).

Co-operation between the Foundation and the Mexican Government was based on a jointly supported and staffed operating unit, the Office of Special Studies, which was set up in 1943 within the Mexican Ministry of Agriculture. The Office of Special Studies has operated a number of research stations and has directed the scientific work in others. In the first instance the Foundation provided senior staff. From the beginning, they undertook the training of Mexican graduates, first on the stations in Mexico, and later in American universities. In this way fully trained Mexican staff became available to replace Americans in senior posts. In fact the development of the work has led to a continuing demand for senior American staff; but the proportion of the senior staff who are Mexican has steadily increased. In 1959–60, of a staff of 147, more than 130 were qualified Mexicans, and in 1960 it was possible to plan the unification of the Office of Special Studies with the Mexican national organization for agricultural research.

This general pattern of activity was repeated in Colombia from 1950, in Chile from 1955 and in India from 1956. Two international programmes have evolved from these experiences. The Inter-American Food Crop Improvement Program has been established to apply the collective experience of the Americas to food-crop research and production (specializing initially on wheat and maize), particularly in those areas of Latin America which do not have operating units of the Foundation. The International Rice Institute in the Philippines, which has also been aided by the Ford Foundation, is a joint enterprise of several rice-growing countries of south-east Asia.

The Rockefeller Foundation has now issued the first report of these activities*. Though nominally confined to a single year, it gives a clear account of the development of the programme and of its methods of work.

The programme is primarily agronomic in the broad American sense, covering the selection and breeding of food crops, forage plants, vegetables and stock, work on plant and animal pests and disease, on soils, and on weed control. If we add to this list agricultural economics and extension methods, we can discern the usual, and very effective, pattern of agricultural activity at State-level in the United States, where research is closely linked with the educational system and with the Federal agencies for more general or more fundamental studies. The programme clearly benefits greatly from the experience of the United States in handling the problems of areas of great geographical diversity, and from the great plant collections assembled by the United States Department of Agriculture. For example, the Inter-American Food Crop Improvement Program uses standard sets of wheat varieties, prepared by the U.S. Department of Agriculture, in different areas to determine the best-adapted types for different localities, in relation both to yield and rust resistance. A standard set of United States maize hybrids has been of great use, in the early stages of maize improvement work in various countries, in indicating the general varietal types most likely to succeed.

It is impossible here to summarize in detail the mass of useful work reported from so many regions; but a few of the points which seemed especially noteworthy may be quoted.

The largest single activity is the improvement of maize, followed closely by that of wheat and sorghum —three crops of which the United States has unique experience. Hybrid maize naturally occupies a central place. A hybrid maize programme has made remarkable progress in India, where double hybrids were to be released in 1960 for most of the maize-growing regions. In Mexico, a series of crosses between Mexican tropical varieties of maize and other Central American and U.S. material has initiated the modern exploitation of the vast genetic reservoir in the Latin American races of this crop. Work on the isolation of superior material by the testing of S_1 lines holds promise for the speedy improvement of indigenous maize. Remarkable progress, based on the American genetic collections, has been made in the improvement of sorghum and wheat. From the same country it is reported that "minute quantities" of potassium gibberellate (a spray of 1 p.p.m. at an unspecified rate) increased the seed yield of beans (*Phaseolus vulgaris*), while high-yielding bean hybrids will probably be released in 1962. A naked-seeded squash for oil extraction has been isolated. A blight-resistance breeding programme in potatoes has been based on *Solanum demissum*.

In Colombia, at Medellin, nearly 6,000 indigenous South American strains of maize, mostly from the Andes, are being preserved, partly in race composites (panmixis bulks), for breeding and research. Accounts of the races of maize in Chile, Venezuela and Ecuador will shortly be published, so that the indigenous maize of the greater part of South and Central America will then have been recorded by various hands. A new source of male sterility has been found in Colombian maize, and the development of broad-based synthetics for areas of less-advanced agriculture is foreshadowed. Important work on potato breeding using species other than *S. tuberosum* is also reported.

The most striking feature of the programme in India, where the Foundation has only six resident staff members, is the inauguration, in October 1958, of the postgraduate school at the Indian Agricultural Research Institute. In 1959–60 there were 186 M.Sc. and 113 Ph.D. students, following typically North American courses combining class-work and research training. The future intake is to be at the rate of 100 M.Sc. and 50 Ph.D. students a year. To write off this great educational effort as merely factory production of technologists would be to miss one of the most important features of such an enterprise in a country where there are many graduates but few trained and experienced research workers. The gap between a first degree and a fully trained research worker is extremely difficult to bridge in such circumstances, and the difficulties of the situation are enhanced by the problem of varying standards in the local universities. Such a postgraduate school is required not only to provide the advanced training that is necessary but also to give that degree of individual attention that is necessary if men of very different initial standards are to be turned out with a fair degree of comparability in experience and attainment. There is no doubt that the Indian Agricultural Research Institute postgraduate school will go far to meet a very real need.

The achievements of the Rockefeller programme must pose many questions for British readers. On a much smaller scale, the Empire Cotton Growing Corporation (also an unofficial organization co-operating with Governments) is in many ways comparable. Based on the genetic improvement of the cotton crop, its activities cover a wide agronomic range in several tropical territories, and in at least one it provides the backbone of agricultural research. Yet in territory after territory with which Britain has historical and still friendly links, it is proving extremely difficult to meet the demand for agricultural research workers and teachers. The Sudan Agricultural Research Division, once the finest organization of its type in tropical Africa, is at present seeking senior staff. For some years past western Nigeria has been attempting to recruit senior staff to build up agricultural research. At least two chairs of agriculture are vacant in English-medium tropical faculties, others have not been easy to fill, and several more are shortly to be created. How is it, that after sixty years of effective and successful agricultural research in all regions of the world, and a leading position in the related basic sciences, it is so hard to find British nationals for this work? Why, in India of all countries, was it necessary for an American organization to develop a postgraduate school in agronomy?

There are perhaps three groups of reasons. One is historic: the ending of the imperial regime usually means a feeling of insecurity for British employees, while the demand for improved educational facilities for the children of expatriates and for greater amenities for their wives often presents real difficulties.

The second and far more serious reason is that we are not producing enough of the right people. In England and Wales we have now virtually completed the deliberate and formal separation from each other of agricultural advice, education and research, the unity of which provides one of the great strengths of the American system. In no English university is there a field of real-life activity within which an agronomist in the broad sense can be trained. None of the university faculties of agriculture is large enough to provide the necessary range, especially since the advisory service, with its husbandry farms, has been separated from the universities. In some university departments, the trend of research interest, which inevitably comes to dominate teaching, is consequently forced away from the primary analysis and solution of practical problems towards narrow specialization and academic fundamentalism. In many agricultural research institutes it is very difficult for young research workers to qualify for higher degrees. Whatever one may think of the relative values of published papers and paper qualifications, it is undeniable that the discipline and criticism of a well-conducted higher degree course can play an important part in a research apprenticeship. Few British universities and research institutes have an active interest in tropical countries; neither the Agricultural Research Council nor any British university has a tropical field station for research on crops and soils; and no reciprocal arrangements for teaching or research exist between tropical centres and their British counterparts.

---

* The Rockefeller Foundation, 1961. *Program in the Agricultural Sciences*. Annual Report 1959–1960. Pp. ix+292+many plates and diagrams. (New York: Office of Publications, The Rockefeller Foundation, 1961.) n.p.

Thirdly, there are administrative reasons of which one or two may be mentioned. Secondment arrangements for tropical service and experience are rare, though secondment would often be possible without cost to home institutions, and secondment contracts of a suitable type could be a powerful aid to recruitment for overseas service. The formation of the Overseas Research Service does not yet appear to have increased the stability or sense of career security of overseas staffs, nor is the Overseas Research Council playing any effective part. The new Technical Assistance Department has an inspiring task and good leadership; if it can steer a way through the jagged reefs of its terms of reference it could have a great future—provided its course is outwards, to the deep blue waters of the stormy ocean, and not inwards to the sheltered calm of the lagoon.

This report of the Rockefeller Foundation under review can be recommended as wholesome and salutary reading for all who are concerned with the British effort in developing countries. United States participation in this field is increasing; and so powerful a reinforcement must be sincerely welcomed, particularly if it leads to a clear-sighted re-appraisal of the British position, and provides a stimulus to Britain's future efforts.

OBITUARIES

Sir K. S. Krishnan, F.R.S.

The sudden death on June 14 of Sir K. S. Krishnan, director of the National Physical Laboratory of India, at the age of sixty-two, is a grievous loss to Indian science in which he has been a leading figure for many years. Following his training at the American College, Madura, the Christian College, Madras, and the University College of Science, Calcutta, and a short period as demonstrator in chemistry at Madras, he joined the Indian Association for the Cultivation of Science, Calcutta, as a research associate in 1923. In association with C. V. Raman (later Sir C. V. Raman) he undertook a variety of researches in optics culminating in the discovery of the Raman effect in 1928, which was not only of immediate significance in itself, but also proved later to be an important tool for the study of molecular structure.

Shortly after, he became reader in physics in the University of Dacca and began a systematic study of the magnetic properties of dia- and para-magnetic crystals. This work continued and indeed bore its main fruit when he returned to the Indian Association as Mahendralal Sircar research professor in 1933. It is described in a series of papers, published in the *Philosophical Transactions of the Royal Society*, by Krishnan and his students during the 1930's. These are distinguished by the elegant simplicity of the experimental methods described (mostly devised by himself), and by the ingenuity of interpretation and the clarity of exposition. The results threw much new light on questions lying on the border between chemistry and physics, such as the arrangement of molecules in organic crystals, the structure of molecules, and the influence of crystalline fields on the paramagnetism of ions. During 1942–47 he was professor of physics in the University of Allahabad, and while still continuing experimental work on optics and magnetism began to interest himself more in theoretical problems such as the resistivity of solid and liquid metals. His election as Fellow of the Royal Society in 1940 and his knighthood in 1946 were very well-deserved recognitions of his scientific achievements.

In 1947 he was appointed director of the newly created National Physical Laboratory of India and with vigorous support from the late Sir S. S. Bhatnagar nursed it through its teething troubles to make it one of the largest and best-equipped scientific institutions of India. The award of the first Bhatnagar Medal to Krishnan shortly before his death was particularly appropriate and gave him much pleasure. During this time Krishnan gradually became one of the elder statesmen of Indian science: no official committee was complete without him, and he was much in demand for opening ceremonies and took an increasing part in international scientific affairs. He was, for example, vice-president of the International Council of Scientific Unions from 1955 and of the International Union of Pure and Applied Physics during 1951–57, and chairman of the Unesco Scientific Advisory Committee in 1955.

However, it must be admitted that though he felt keenly his responsibility as a public figure, administration was not really to his taste. In spite of all the calls on his time, he managed to keep going some active theoretical research of his own, to cultivate his mathematical talents and to read widely in many fields, by no means confined to science. I had the good fortune to spend a year at the National Physical Laboratory a few years ago, and the informal chats I had with Krishnan, particularly over lunch in the canteen, provide some of my most vivid recollections of that time. He never seemed to forget anything he had once read or heard, and had an encyclopædic knowledge in all sorts of unexpected fields. To illustrate an argument he was fond of quoting anecdotes about the great classical figures of physics, and this he could do in the most entertaining way. But he was equally at home in the latest developments of science and was a shrewd commentator on people and politics.

All this erudition was combined with great charm, good humour and kindness. His kindness, however, had its drawbacks when it came to administration, for he could not bring himself to say anything which would disappoint his hearer, and preferred to defer a difficult decision indefinitely rather than cause anyone disappointment. Another idiosyncrasy was that he could scarcely ever be persuaded to write a letter; thus a visit from him usually came as a surprise, preceded at the best by a telegram, but more often introduced by an apologetic chuckle as he explained that he had meant to write. His genial presence both at home and abroad will be sadly missed by a host of friends. He leaves a widow, two sons, four daughters and three grandchildren.

D. SHOENBERG

Dr. N. S. Shatsky

DR. NIKOLAI SERGEEVICH SHATSKY, member of the Academy of Sciences of the U.S.S.R. and director of the Institute of Geology, died in Moscow in November 1960. He was born there on August 28, 1895. He began his geological career as a stratigrapher, carrying out a detailed geological survey in the Donetz basin, and in the Caucasus and Volga regions. This work led him to the study of geological formations as stratigraphical units. At the same time, Shatsky was writing of oil and coal-bearing strata, iron ores, manganese ores and phosphorites. It was, however, in the field of tectonics that Shatsky excelled, and it is not without reason that he is considered to be the founder of a new tectonic school in the U.S.S.R.

His tectonic and palæogeological investigations in the European and Asiatic parts of the U.S.S.R. led him to establish a new Upper Proterozoic system, which he named the Rhiphæan system, and a new orogenetic epoch, coeval with this system, which he named the "Baikalian" epoch. However, his greatest achievement, shared with numerous collaborators, was a tectonic survey of the whole territory of the U.S.S.R., leading to the publication in 1956 of a most original, and in a sense most beautiful, tectonic map of the U.S.S.R. on the scale of 1 : 5,000,000. This map was the first of its kind. In it different colours were used for the major orogenic epochs and a number of tectonic structures were indicated by special signs. The Rhiphæan system and the tectonic map were briefly reviewed in Britain (*Proc. Geol. Soc., Lond.*, No. 1501, 108; 1953; and No. 1557, 29; 1958). Shatsky was also interested in the history of geology, and in 1941 he published, in Russian, a book on the life and work of the famous British geologist, R. I. Murchison, who greatly advanced the progress of geology in Russia. In March 1960, Shatsky was elected an Honorary Fellow of the Geological Society of London.

S. I. TOMKEIEFF

Mr. B. Welbourn

MR. B. WELBOURN, formerly director and chief engineer of British Insulated Cables, died on July 1 at the age of eighty-five. He retired in 1945 on the formation of British Insulated Callender's Cables, Ltd., through the amalgamation of the British Insulated Co. and Callender's Cable and Construction Co.

Mr. Welbourn graduated from King's College, London, in 1896. After a period of service with the Electrochemical Co. of St. Helens, he joined, in 1897, the British Insulated Wire Co., of Prescot, becoming contracts manager in 1902, in which capacity he travelled widely. In 1927 came his appointment as chief engineer, and in 1942 he was elected to the board of the British Insulated Co.

In a life-time spent in the electrical industry, in which he had such a notable career, Mr. Welbourn had served as a member of the Council of the Institution of Electrical Engineers, was a vice-president of the Institution and past chairman of the Manchester and Liverpool centres.

NEWS and VIEWS

Canadian Scientific Attaché in Washington: Dr. E. O. Hughes

DR. E. O. HUGHES, of the National Research Council of Canada, has been appointed as scientific attaché to the Canadian Embassy in Washington. While in Washington, Dr. Hughes will also serve as Canadian liaison officer at the British Commonwealth Scientific Office. Born in Wales, in 1916, Dr. Hughes received his initial education in Winnipeg, Manitoba, and Halifax, Nova Scotia. After undergraduate training in biology at McGill and Dalhousie Universities, he received an M.A. degree in 1939 from the University of Western Ontario. In 1942 he was granted a Ph.D. degree at the Ohio State University. During 1942–45 he was engaged in the administration of medical research for the National Research Council of Canada and the Royal Canadian Army Medical Corps. In 1945, Dr. Hughes became assistant professor of plant sciences in the University of Oklahoma, Norman, Oklahoma. In 1952 he returned to Canada as a research biologist and editor in the Division of Applied Biology of the National Research Council. His work was primarily concerned with the classification and physiology of freshwater algae. During 1956–60 he was assistant to the director of the Biology and Health Physics Division of Atomic Energy of Canada Limited, Chalk River, Ontario. For the past year he has been acting chief of the public relations office at the National Research Council of Canada.

C.S.I.R.O. National Standards Laboratory : Mr. N. A. Esserman

MR. NORMAN A. ESSERMAN, director of the National Standards Laboratory of the Commonwealth Scientific and Industrial Research Organization (Australia), has retired after twenty-three years service. Mr. Esserman graduated from the University of Sydney in 1916 with first-class honours in mathematics. He went to England in 1917 and worked in the Arsenal Branch of the

Munitions Office. On returning to Australia in 1920 he joined the Department of Defence, and afterwards became assistant superintendent of the Munitions Supply Laboratories (now the Defence Standards Laboratories) at Maribyrnong, Victoria. In 1938 he joined the Organization as officer-in-charge of the Metrology Section, one of the three units of the National Standards Laboratory. It was largely due to his experienced guidance that the Section was able to establish itself quickly and to make an outstanding contribution to Australia's war-time production. In 1958 he was appointed first director of the National Standards Laboratory. Mr. Esserman has been personally honoured by his election to the International Committee for Weights and Measures—a fitting climax to a career devoted to physical measurements and a tribute to the man who has had the unique distinction of playing the leading part in establishing the two main laboratories in Australia concerned with metrology.

C.S.I.R.O. Division of Mineral Chemistry : Mr. I. E. Newnham

Mr. Ivan E. Newnham has been appointed chief of the Division of Mineral Chemistry, Commonwealth Scientific and Industrial Research Organization (Australia). He is forty-one years old and graduated M.Sc. from the University of Melbourne in 1940. In 1942 he joined the Department of Aircraft Production and afterwards became supervisor of the Department's Testing Laboratories, where he was associated with the production of the first aircraft bearings made in Australia. During the later war years, while employed by the Newcastle firm of P. J. Taylor Pty., Ltd., he pioneered new techniques for the manufacture of automotive bearings. Since joining the Organization in 1947, Mr. Newnham's main interests have centred around the chemistry of the metals zirconium, hafnium and beryllium, all of which occur in Australian minerals. During 1950–55 he was engaged on research into methods for separating zirconium and hafnium, which occur together in the mineral zircon, in Australian beach sands. Pure zirconium is used as a container for uranium in the manufacture of atomic reactors, especially for use in nuclear submarines. Hafnium, which absorbs neutrons, is used for making control rods in reactors.

Physics at Sheffield: Prof. N. H. March

Dr. N. H. March has been appointed to the newly created second chair of physics in the University of Sheffield. He graduated at King's College, London, in 1948, and there began his research career under the direction of Prof. C. A. Coulson, then professor of theoretical physics at King's College. In 1950, March was appointed to an assistant lectureship in physics at the University of Sheffield, promoted successively to the grade of lecturer in 1953, and reader in theoretical physics only four years later. He has been an enthusiastic research worker from the beginning, and soon came to be regarded as one of the recognized authorities on the Thomas–Fermi statistical theory. In addition, he has maintained a lively interest in the electronic structure of molecules, in particular the electrical charge distribution. More recently, probably not uninfluenced by the activities of his experimental co-workers in Sheffield, he has turned to problems connected with the solid state. Here he has made important contributions to the distribution of charge around impurity centres. He now leads an active group of postgraduate research workers, and his school can confidently be expected to make substantial growth with the increased accommodation shortly to be available for the physics department in Sheffield.

Promotion of Science in Australian Schools

The fund which was established in England in November 1955, by a group of industrial organizations, to assist in increasing the number of scientists and technologists at the service of industry and of the country generally, has led to the establishment of a similar fund for a similar purpose in Australia. It was inaugurated at a meeting of industrial and commercial leaders, held in Sydney on March 20, 1958, under the chairmanship of Sir Edward Knox, and publicly announced on February 2, 1960. The Fund seeks to pursue its objects by stimulating and encouraging the teaching of science in suitable schools. Prior consideration is being given to independent boys' schools of standing, and the schools which are represented in the Headmasters' Conference of Australia are recognized as coming within this definition. It is intended to extend the range of consideration if sufficient funds become available. The Fund is now proceeding to assist chosen schools in the building of up-to-date laboratories and other rooms for the teaching of science. Attention is being given to the basic sciences, namely, physics and chemistry, and geology in suitable cases. Assistance to other sciences is not at present contemplated. In their discussion with schools, the Fund's representatives have endeavoured to encourage schools to plan with a long view so that it will be easy to add further accommodation later. The hope has been expressed that schools will endeavour to encourage a greater volume of science at the upper levels of secondary education and that they will seek to stimulate work of a more advanced standard. Contributions made or promised to the Fund to December 31, 1960, total £576,315. Although the amount is considerable, it is still far from sufficient to realize the Fund's full objectives, and more help is needed from others in the fields of industry and commerce. Further information about the Fund is described in a report which may be obtained from the Secretary, M.L.C. Building, Petrie Street, Canberra.

Shipbuilding Research in Britain

In the debate on shipping and shipbuilding in the House of Commons on July 13, the Minister of Transport, Mr. E. Marples, agreed that Parliament should attach the very greatest weight to research and development for both the shipping and shipbuilding industries. Better co-ordination is required in the future, he said, and shipbuilding research and shipping research should not be isolated departments but more and more together; this is now being discussed with the General Council of British Shipping. Referring to the loss of orders by the industry, to the charges of lack of modernization, inefficient management, demarcation and restrictive practices by the unions as well as to insufficient research and development, he said that a new and dynamic approach to production is required. The future of the industry is at stake, and for some time discussions have been proceeding between the two sides of industry about ways and means of improving its efficiency and removing obstacles to progress. If ths discussions do not achieve substantial progress in the near future, the Government intends to review the position with both sides of industry. Mr. Marples stressed the need for co-operation by both sides: without it we must fail. The question of research was particularly emphasized by Mr. A. Albu, and, in replying on the debate, the Parliamentary Secretary to the Ministry of Transport, Vice-Admiral J. H. Hallett, said that the British Shipbuilding Research Association is ably directed and undertakes research into production methods and techniques. It is hoped that the two industries will now jointly evolve some wider research organization which can handle problems of operation and of design and building as a whole, including possibly research on Diesel engines.

The Institution of Electrical Engineers

MUCH of the scientific work of the Institution of Electrical Engineers has been for many years conducted by four specialized sections (electronics and communications, measurement and control, supply and utilization). It has now been decided that it will be advantageous for the four sections to be replaced by three divisions, two of which will represent electronics and power interests, while the third, a general division, will cover activities of common interest to all electrical engineers, such as basic measurement, and technological education. Each of the divisions will comprise a number of technical groups designed to cover the specializations within its field, and the scheme provides for speedy re-adjustment and creation of those technical groups which will increase the flexibility which is now more than ever necessary to reflect the rapid developments of electrical science and engineering. In setting up a divisional structure, the Council has also recognized the increasing importance of electronics in electrical engineering, and the part being played by electronics engineers in the work of the Institution which has resulted in its present position as the professional home of electronics engineers.

Australian Journal of Experimental Agriculture and Animal Husbandry

THIS new journal is devoted to reports both on agronomy and animal husbandry. Judging from the contents of the first number, the journal is likely to cover a wide range of topics: it includes articles on the potassium content of some Western Australian soils, legumes in pastures, classification of Australian wheat varieties based on the granularity of their wholemeal and the relationships between digestibility of dry matter and digestibility energy content of ruminant diets. A pleasing feature is the inclusion of summaries of each paper in the list of contents—a departure which might be more widely followed. The journal is published quarterly by the Australian Institute of Agricultural Science for the Australian Agricultural Council and the subscription is £A.5 a year including postage.

Index Biologicus

UNDER the title *Index Biologicus* a periodical is being planned that will be a classified index of current papers from international literature in pure biology, giving titles, authors and journal references. It is intended that immediate notice will be given of all papers published in this already very extensive and rapidly expanding field, and that biologists will be provided with an information service comparable with those now available in other subjects, for example, *Index Medicus, Current Chemical Papers* and *Chemical Titles*. Scientific direction of the journal will be the responsibility of Biological and Medical Abstracts, Ltd., a non-profit company, limited by guarantee, that was formed by a number of learned societies when *British Abstracts* ceased publication so that the physiological and biochemical sections might be carried on. Like the present *International Abstracts of Biological Sciences*, which is the successor to Section *A* III of *British Abstracts*, the *Index Biologicus* will be published on a contractual basis for Biological and Medical Abstracts by the Pergamon Press. It is hoped that this publication will meet a real need and, moreover, it is expected that co-operation in the framing of scientific policy will be forthcoming from biological societies in Britain and overseas. The first issue of the journal is planned to appear in June 1962 or January 1963. Any comments or inquiries should be made to Dr. R. K. Callow, chairman, Biological and Medical Abstracts, Ltd., 4 Fitzroy Square, London, W.1.

Museum of English and Rural Life

THE annual report for 1960 of the Museum of English Rural Life at the University of Reading welcomes an acquisition from the British Council of some 300 examples of traditional design and fine workmanship (Pp. 19. 5 plates. Reading: The University. 1961. 1*s*.). The collection includes saddlery, wrought ironwork, small stools, turnery, basket work, pottery and textiles, and is of special value because the names and locations of the makers were known. More storage space has helped the re-arrangement of the reserve material, and increased workshop facilities have speeded the restoration of the larger farm implements and vehicles. The report of this organization is always noteworthy for the inclusion of notes on policy and other matters affecting the museum movement. On this occasion the curator, Mr. C. A. Jewell, contributes notes for collectors concerning the acquisition of folk-life material. It is rightly stated that traditional rural life in England was based on a relatively close-knit and self-sufficient community often with a distinct regional character. Although this has largely disappeared, scattered evidence still survives and practical and useful details are given on the collecting of objects and recording of information. The report is well illustrated.

Society for Folk-life Study

PLANS to form a Society for Folk-life Study will be discussed at a conference to be held in London during September 6–8, 1961. Membership will be open to all who are interested in the traditional patterns of life in both rural and urban communities, and especially in local dialects, crafts, folk-lore and customs. It is intended that the new Society will serve as a focus for the activities of all who take an interest in their cultural inheritance. Various well-known experts in this field of study have promised to speak at the conference. Further information can be obtained from Mr. J. G. Jenkins, St. Fagans, nr. Cardiff, who is the secretary of the steering committee.

The Nitrogen Problem

PROF. N. R. DHAR, in his presidential address to the forty-eighth Indian Science Congress held at Roorkee this year, reviewed the importance of nitrogen in crop production. He pointed out that synthetic nitrogen plants have been started in many industrially undeveloped countries, but they are handicapped by the heavy capital expenditure. The world demand for nitrogen fertilizers in 1960–61 is expected to be 8·1 million tons, of which Europe and the United States will together use 5·5 million tons. Prof. Dhar emphasized the desirability of building up phosphate reserves in the soil because natural fixation of atmospheric nitrogen in the soil is increased by adequate supplies of phosphate. Nitrogen is the key element in crop production; it is calculated that 100×10^6 tons of nitrogen are necessary for producing the world's food materials, excluding grass; but the chemical industry at present produces only 7×10^6 tons and legumes 5×10^6 tons. The nitrogen requirement of the world's grassland is estimated at 155×10^6 tons per annum, so that of the 250×10^6 tons of nitrogen necessary for the production of the major food crops of the world only 3 per cent comes from artificial fertilizers, 2 per cent from legumes, 2–3 per cent from precipitation and 2 per cent from organic manures. The rest is produced in the soil by microbiological activity on organic matter. Thus, industrial nitrogen supplies only a small part of the nitrogen requirement of crops, and a great deal can be done to increase land fertility by the better use of organic material supplemented by phosphate fertilizers to increase natural nitrogen fixation.

New Zealand Phytochemical Survey

During a survey of the New Zealand native flora for possible tumour inhibitory principles, extracts have been examined for the presence of alkaloids, leucoanthocyanins, saponins and triterpenes or steroids. In the latter work numerous fresh plants which are not extracted in bulk, and also herbarium specimens from the Auckland Institute and Museum, have been examined. The results of testing the New Zealand gymnosperms have now been reported (*N.Z. J. Sci.*, 4, No. 1; March 1961). The investigations show that alkaloids are not commonly found in species of the gymnosperms, notable exceptions being ephedrine from *Ephedra* species and taxine from *Taxus baccata*. No species of the New Zealand Cupressaceae or Podocarpaceae were found to contain alkaloids, but the heartwood of the sole representative of the Pinaceae, *Agathis austalis*, was found to give positive tests. The presence of an alkaloid has been afterwards confirmed when bulk samples of the heartwood were extracted and a water-insoluble alkaloidal fraction isolated in low yield. Attempts to isolate a pure compound have been unsuccessful. All the species gave positive tests for leucoanthocyanins, the barks of the plants being the richest source of these compounds. The isolation of the principle leucoanthocyanin from the bark of *Dacrydium cupressinum* has already been reported and has been shown to be a stereoisomer of leucocyanidin which is apparently not identical with any isomer previously isolated. That from the bark of *Dacrydium biforme* has also been isolated by one of the investigators but has not been obtained pure. It differs from that of *D. cupressinum*, giving delphinidin on hydrolysis.

Data on Research and Development

A preliminary report issued by the National Science Foundation (*Reviews of Data on Research and Development*, No. 28; June 1961. *NSF* 61–31) gives the capital expenditure of colleges and universities in the United States for research and development in the natural and social sciences in 1958 as 153·5 million dollars, of which 112 million dollars came from non-Federal sources. The remainder came from the Federal Government, being largely confined to the large research centres which colleges and universities administer for the Federal Government. Half this expenditure was in the biological sciences, 33 per cent in the physical sciences, 15 per cent in the engineering, and 3 per cent in the social sciences.

J.L.T. Industrial Research Fellowships

J. Langham Thompson, Ltd., are introducing a scheme of fellowships for young research workers, normally on a two-year tenure, with the intention that those appointed should carry out their research in the Company's laboratories in Watford, Hertfordshire. The fields of interest to the Company include: solid state, cybernetics, logical systems, electronics, circuit techniques, micro-waves, mechanics of solids, transducer techniques, optics. Candidates for these fellowships should have completed a higher degree, or have carried out original research of a high standard and have a clear appreciation of the research programme they wish to pursue. Further information can be obtained from J. Langham Thompson, Ltd., 176 High Road, Bushey Heath, Hertfordshire.

Sixth International Mineral Processing Congress

The sixth International Mineral Processing Congress will be held in Cannes during May 26–June 1, 1963. Organization is in the hands of la Société de l'Industrie Minérale and the following executive committee has been appointed: *President*, M. R. J. Testut; *General Secretary*, M. Pierre Gy; *Members*, MM. Astier, Emery, Ploix, Rey, Saint-Guilhem, Saverot, Seyer, Vielledent and Vuchot. As in the past, this Congress will be concerned with all aspects of mineral processing, and papers will be selected entirely on the basis of their scientific and technical value. However, the Committee wishes to place particular emphasis on the following subjects: (1) the concentration of iron minerals, including magnetic roasting but excluding agglomeration; (2) measurement, control and automation; (3) hydrometallurgy. Especial importance is also attached to the economic aspects of mineral processing and it is hoped that, whenever possible, authors will include data on installation and operating costs and comparative costs for alternative processes. The official languages of the Congress will be French, English, Russian and German, and provision will be made for simultaneous translation of discussions. An extensive series of visits to mineral processing plants and laboratories in Mediterranean countries will be arranged. The representative for the United Kingdom and the British Commonwealth is Prof. M. G. Fleming, Royal School of Mines, Prince Consort Road, London, S.W.7, to whom abstracts must be sent by October 15, 1961.

University News: Birmingham

Prof. William Henry Trethowan, professor of psychiatry in the University of Sydney, has been appointed to the chair of psychiatry; the date will be arranged.

Edinburgh

The following appointments have been announced: *Imperial Chemical Industries Research Fellows*, Juris Reinfelds (Adelaide) and Imre Laszlo (Debrecen), *Lectureships*, R. F. Thow (agriculture); Dr. L. M. Muggleton (electrical engineering); Benjamin Mitchell (veterinary surgery).

Leeds

New courses, leading to honours and ordinary B.Sc. degrees in food science, have been introduced, and the first students for them will enter the University in October 1962. The honours course extends over four years, and includes chemistry, physics, biology, statistics, bacteriology and biochemistry, taken in the appropriate pure science department in the first year. This is followed by courses in the chemistry, physics and microbiology of foodstuffs and food processing. The ordinary degree course is restricted to three years. Entry requirements include a high standard in chemistry and physics, with mathematics as the preferred third subject. The Procter Department of Food and Leather Science, under the Procter professor, A. G. Ward, has grown from the former Department of Leather Industries and will receive extended premises and additional staff for the new course. Postgraduate students will be able to take the postgraduate diploma in food science, involving study and research, or carry out research for the M.Sc. and Ph.D. degrees.

London

Mr. S. B. Watkins, head of the Department of Chemical Engineering at King's College, has been appointed to the University readership in chemical engineering tenable at that College. The title of reader in pure mathematics has been conferred on Dr. K. W. Gruenberg in respect of his post at Queen Mary College.

The title of professor emeritus has been conferred on the following: Prof. R. O. Buchanan, professor of geography in the London School of Economics and Political Science since 1949; Prof. L. P. Garrod, professor of bacteriology in St. Bartholomew's Hospital Medical College since 1937; Sir Austin Bradford Hill, professor of medical statistics at the London School of Hygiene and Tropical Medicine since 1945, and honorary director of the Medical Research Council's Statistical Research Unit; Sir Christopher Ingold, professor of chemistry in University College since 1930; Prof. W. H. Linnell, professor of pharmaceutical chemistry in the School of Pharmacy since 1944, and dean of the School; Prof. C. A. Mace, professor of psychology in Birkbeck College since 1944; Prof. D. M. Newitt, Courtauld professor of chemical engineering in the Imperial College of Science and Technology since 1945; Prof. F. R. Winton, professor of pharmacology in University College since 1938.

Announcements

The Earl of Halsbury, during 1949–59 managing director of the National Research Development Corporation, has been elected president of the Machine Tool Industry Research Association.

Dr. F. A. Robinson, at present director of research of Allen and Hanburys, Ltd., has been appointed chairman of the board of directors of the Crookes Laboratories in succession to Mr. N. B. Smiley, of Arthur Guinness, Son and Co., which acquired the Crookes Laboratories jointly with Philips Electrical Industries last November. Dr. Robinson is honorary treasurer of the Biochemical Society and the author of books on chromatography, antibiotics and the vitamin B complex.

Colonel J. F. Williams-Wynne has been appointed a member of the National Parks Commission. Colonel Williams-Wynne is a part-time member of the Merseyside and North Wales Electricity Board and a member of the Regional Advisory Committee of the North Wales Conservancy of the Forestry Commission and the Gwynedd River Board.

Lord Shawcross has been appointed a member of the Medical Research Council. He will serve as chairman of the Council in succession to Viscount Amory, who is resigning on his appointment as United Kingdom High Commissioner in Canada.

Mr. A. W. Jones (Fleming Radio (Developments), Ltd.) has been installed as president of the Scientific Instrument Manufacturers' Association of Great Britain for the year 1961–62 by Mr. G. C. Ottway (W. Ottway and Co., Ltd.), the retiring president, who now becomes a vice-president. The following honorary officers have been elected: *Vice-President and President-elect*, Mr. R. E. Burnett (Marconi Instruments, Ltd.); *Vice-President*, Mr. G. C. Ottway (W. Ottway and Co., Ltd.); *Secretary*, Major W. Logan (Avo, Ltd.); *Treasurer*, Mr. G. S. Sturrock (Kelvin and Hughes, Ltd.).

To celebrate the third centenary of the birth of Antonio Vallisneri, a past-president of the "Accademia Patavina di Scienze Lettere ed Arti", the University of Padua is organizing, during September 29–October 1, a symposium on "The experimental method in biology from the time of Antonio Vallisneri to the present". Further details can be obtained from the Organizing Committee, University, Via 8 Febbraio, Padova.

The Electronics Group of the Institute of Physics and the Physical Society announces that it is arranging a conference on "The Physics of Gas Discharge Devices", to be held at Leamington Spa during September 28–29. Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

New Laboratories for the Division of Food Preservation, Commonwealth Scientific and Industrial Research Organization, will be opened at North Ryde, New South Wales, on September 18. A Food Science Conference will follow, during September 19–22. Further information can be obtained from the Chief, Division of Food Preservation, Commonwealth Scientific and Industrial Research Organization, P.O. Box 43, Ryde, N.S.W.

The Autumn Meeting of the Institute of Metals will be held in Brussels during September 18–22. The programme includes the Autumn Lecture, to be delivered by Baron Snoy et d'Oppuers on September 18 (who will speak on "The Non-Ferrous Metals Industry in a Unified Europe"), and sessions on deformation and fracture, on surface friction and lubrication in metal working and on corrosion. Further information can be obtained from the Institute of Metals, 17 Belgrave Square, London, S.W.1.

Erratum. In the issue of July 29, p. 439, the date of birth of Sir Frederick Gowland Hopkins was given as June 30, 1861; this should read June 20, 1861.

LONG ASHTON RESEARCH STATION, BRISTOL

THE staff of the University of Bristol Agricultural and Horticultural Station at Long Ashton includes 47 graduate scientific officers and now totals 225, approximately double the pre-war figure. The basic work of the Station on all aspects of fruit culture and fruit utilization has similarly expanded, and there has been, in addition, a rapid growth in biochemical studies related to plant nutrition, a development stimulated by the formation in 1952 of the Agricultural Research Council Unit of Plant Nutrition (Micronutrients), the members of which were assimilated into the Long Ashton staff in 1959.

These increases in staff and research programmes have necessitated new laboratories and ancillary services. A comprehensive building scheme for the Station was drawn up in 1949, and, although the proposed plans have not all been implemented, a substantial instalment of the programme has now been completed. On July 6, Sir William Slater, formerly secretary of the Agricultural Research Council, declared open the buildings that have been constructed during the past five years. Of these, the three principal are : (1) a range of experimental glasshouses with attached laboratories and special-purpose rooms; (2) a refectory/common room adaptable for use as a hall for lectures and meetings; (3) the Wallace Laboratory, named after Prof. T. Wallace who retired from the directorship in 1957 after having served the Station for thirty-eight years.

The Glasshouse/Laboratory block, a building of 43,000 sq. ft., has six separate glasshouses, each 72 × 34 ft. with a ridge-height of 24 ft., constructed of a suspended 'Aluminex' framework with side walls infilled with 2-ft. brickwork. The houses, from west to east, are used respectively for propagation, plant nutrition (two houses), pomology, plant pathology and entomology. Each glasshouse communicates by a short corridor to a header house 220 × 18 ft. The southern half of the header house, adjacent to the glasshouses, forms an open gallery; the northern half is divided to form laboratories, stores, cold rooms and special-purpose rooms with controlled environment. A set of rooms for work on radioactive tracers is segregated at the east end of the header house; the west end houses the services for soil and plant preparation.

The main boiler house, attached at the west of the glasshouse block, contains three oil-fired automatic-

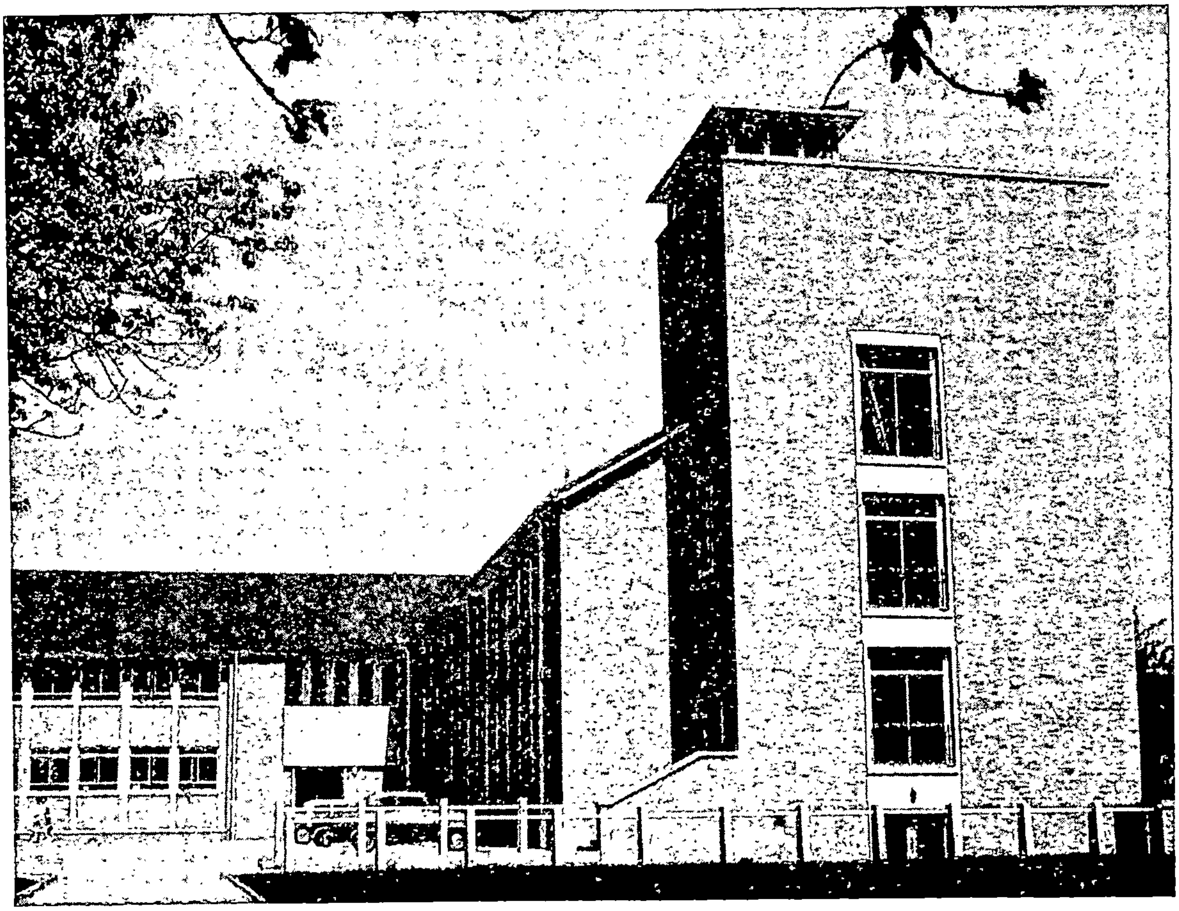


Fig. 1. The Wallace Laboratory of Long Ashton Research Station

ally controlled McNiel boilers, providing space heating and hot water for the glasshouses, the refectory and the Wallace Laboratory. Steam conveyed through underground conduits supplies heat exchangers for the production of hot water, which is passed through radiators, ceiling coils or hot-air circulators.

The Refectory/Common Room is a single-storyed building covering 5,300 sq. ft. It is faced with 'Reformite' reconstructed stone and has large french windows overlooking a pleasant expanse of the fruit plantations. The roof is of double-Roman tiles and the floor of polished Iroko wood blocks. The dining hall, 60 × 37 ft., connects through a folding partition to the common room 35 × 37 ft. With the partition open a hall 95 × 37 × 20 ft. high is provided. There is a well-appointed kitchen equipped with air extractors and refrigerated larder. The building is heated by a hot-air circulatory system linked to the main boiler house.

The Wallace Laboratory is a two-storyed, T-shaped building of 24,000 sq. ft. floor space, roofed and the walls faced in the same style as the refectory (Fig. 1). The entrance hall is panelled in apple-wood veneer cut from a tree of the cider variety 'Knotted Kernel' which formerly stood on the site. Some of its sister trees of the original cider orchard (planted in 1904) still flourish around the new laboratory. The western arm of the building houses the administration section on the ground-floor, and laboratories for analytical chemistry above. The southern limb of the ground-floor accommodates the pomology and plantations sections, with stores and organic chemistry on the first floor. The remainder of the ground-floor has laboratories for physical chemistry, statistics and willow research, with the section for nutrition of fruit plants above. There are extensive basement rooms for stores and heat-exchange equipment, with a tower at the southern end housing the water cisterns and capped by an observation platform for meteorological recording. The lofts house elaborate fume extractors and 'Plenum' heating equipment.

Among other buildings is one of 8,400 sq. ft. consisting of laboratories for biochemistry and plant physiology which was completed in 1956. During 1959–60, the director's room, the committee room and the library in the Barker Laboratory (the original laboratory building) were redecorated and re-furnished. Extensive improvements and additions have also been made to workshops, machinery sheds and roadways.

R. W. MARSH

THE BRITISH IRON AND STEEL RESEARCH ASSOCIATION

THE Sheffield Station of the British Iron and Steel Research Association, where open days were held on June 15 and 16, houses three of the Association's five main research divisions—the Steelmaking, Metallurgy (General), and Mechanical Working Divisions.

Steelmaking

The Steelmaking Division is responsible for research into all the modern processes involved in the conversion of iron into steel, and prominent among its present research projects are the control of electric-arc and open-hearth furnaces, continuous casting, and the use of oxygen in steelmaking operations.

The use of large electric-arc furnaces for the bulk production of steel is increasing and it is expected that, in 1965, these furnaces will account for one million tons more of Britain's total annual steel output than in 1960. Economy in the use of electrical power is becoming, therefore, a matter of increasing importance. The automatic power input controller (*Apic*), which has been developed recently by the Steelmaking Division for application to electric-arc furnaces, has already shown itself capable of achieving significant reductions in electricity consumption. Trials have been carried out satisfactorily on the Association's experimental furnace at Sheffield and on a 5-ton production furnace at Steel, Peech and Tozer, and further trials are now being carried out on another member's 30-ton furnace.

Continuous casting helps to achieve higher yields and greater uniformity of end product. A number of continuous casting plants has recently been installed or projected, including a twin-strand plant at the Abbey Works of the Steel Co. of Wales. This is based on the process developed by the Association in conjunction with the Continuous Casting Co. of Weybridge, and will be used for casting low-carbon slabs up to 48 in. × 8 in. in section. The Steelmaking Division's present investigations into this new process are directed mainly towards improved moulds and mould reciprocating cycles. Arising out of these investigations, a new design of mould has been evolved which enables three interconnected ingots to be cast simultaneously, these being afterwards separated and treated as individual castings.

During the past few years there has been a dramatic increase in the use of oxygen for steelmaking processes and the new vessels now being introduced into Britain, such as the *L.D.*, *Kaldo* and *Rotor*, will alone probably account for something like 14 per cent of the total annual steel production in 1965. In addition to pure refining duties, however, oxygen can be used for flame enrichment in open-hearth furnaces and also for pretreating hot metal. The Division is at present conducting trials of a new hot-metal pre-treating device which employs a simple reversal of the converter process principle. With this technique—termed spray refining—instead of passing oxidizing gases through molten metal, the metal is teemed through jets of oxidizing gas, thus breaking up the falling stream of metal and—by increasing the effective metal surface area—promoting rapid and efficient refining reactions.

The current research programme of the Steelmaking Division also includes investigations into control of open-hearth furnace combustion, the rapid desulphurization of electric steel and studies of subcutaneous segregates.

Metallurgy

The Metallurgy (General) Division is concerned with the physical, chemical and mechanical properties of iron and steel, and the effect on these properties of variables in the manufacturing processes. The Division's activities are divided into the broad categories of physical properties, mechanical properties, metal treatment and metallurgical analysis.

Research into physical properties is at present focused on the magnetic characteristics of silicon iron. During 1960, electrical power to the value of eleven and a half million pounds was wasted as a result of losses in silicon iron transformer cores. A reduction of as little as 5 per cent in these losses would result in an annual saving of more than half a million pounds. The Division's investigations into the factors contributing to core losses have already shown that reductions of up to 15 per cent could be obtained.

A better understanding of the factors affecting the fatigue limit of steel would greatly improve the possibilities of achieving better dynamic mechanical properties in high-tensile steels. Also in the context of mechanical strength, it has been established that the presence of certain elements in mild steels affects their resistance to brittle fracture at low temperatures. The Division's work in connexion with the mechanical properties of steel is mainly concentrated on these problems, although research is also being conducted on the mechanism of fatigue and more economical methods of fatigue testing.

Metal treatment research, the third of the Metallurgy Division's spheres of activity, consists primarily of investigations into methods of degassing molten steel. The presence of certain gases in steel often has a significant effect on the qualities that are expected of it. Excess hydrogen, as a specific example, may lead to cracking and other undesirable flaws in the finished product. The Division is at present engaged in investigating two methods of hydrogen removal by degasification techniques. In the first of these a continuous vacuum degassing unit is used, molten metal being poured into an evacuated chamber, degassed at a pressure of about 1/1,000th atm. and discharged into air. In the second method—termed 'jet degassing'—the hydrogen is removed by blowing a neutral gas such as argon, nitrogen or helium on to the surface of the molten metal by means of a lance. This second technique has achieved a reduction of eight parts per million to one part per million in the hydrogen content of an eight-pound melt, and the possibility of using this method on a larger scale is being examined.

The increased throughput rates which have been made possible by the use of oxygen in steelmaking practice have thrown greater emphasis on the need for faster metallurgical analysis. In addition to developing faster and more accurate physico-chemical analytical procedures, the Division is also therefore investigating the application to steel works requirements of the newer physical techniques of direct reading and X-ray fluorescent spectroscopy and electron-probe micro-analysis.

Supplementary to its main research programme, the Metallurgy (General) Division also provides services to other research divisions and departments of the Association, to industry, to research organizations and to universities.

Mechanical Working

The Association's research into all the processes by which ingots are formed into wrought metal products is carried out by the Mechanical Working Division. This Division's research programme is therefore spread over a wide range of manufacturing processes which may be broadly classified by end-product—sheet and strip, forgings, rounds and sections, and wire.

Because of the superior quality of product obtainable, the use of continuous annealing for blackplate has been increasing in the tinplate industry during the past few years. However, conventional continuous annealing plants are very large and costly, and inflexible in operation. Investigations by the Division on an experimental annealing line at the Swansea laboratories have resulted in the development of a process which, by greatly reducing the pass length, makes possible the use of a more compact plant, with a considerable saving in costs. Products of a commercial quality have been obtained and plans are now in hand for the installation of a pilot production plant at a member's works.

Other features of the Division's research into sheet and strip manufacture include a new process for aluminizing steel strip, the electrolytic smoothing of blackplate, investigations into the prevention of scale formation on coiled strip, and three new in-line inspection techniques. Some fundamental work is also being conducted by the Association's Physics Department on tandem-mill automatic gauge control with the aid of a new analogue computer which has been installed recently at the Battersea laboratories of the Association.

In the manufacture of forgings the Division's main research project is the automatic programme control of forging presses and manipulators. Simple programmed forging operations have already been carried out, both more rapidly and more accurately than in manual operation, and more complicated schedules are now being formulated. Forging research has also resulted in the development of a new procedure for closing ingot cavities by upsetting, and a heat reflector arrangement which, by conserving the heat in the workpiece, has proved capable of achieving a 30 per cent reduction in heating requirements. In addition, the Plant Engineering and Energy Division is at present conducting investigations of batch type forge furnaces on behalf of the Mechanical Working Division.

The Division is also now working on a number of problems associated with wire manufacture—such as wire cooling, continuous wire inspection and control, and the welding of high-carbon steel wire—and the manufacture of rounds and sections.

Development and Information Services

The Sheffield Station also houses the Steel User Section of the Association's Development and Information Services. This Section employs the Association's research resources to assist individual companies in their use of the industry's products. The services which it provides range from answering simple technical inquiries to more complicated investigations into metallurgical and other production problems.

The remaining research divisions and departments of the British Iron and Steel Research Association are located, as in the case of the Sheffield Group, in the area of the country most appropriate to the work for which they are responsible. The Ironmaking Division, for example, has a pilot-plant laboratory at Normanby, near Middlesbrough on the north-east coast, and a blast-furnace laboratory at the Imperial

College of Science and Technology in South Kensington. The Plant Engineering and Energy Division is based at the Association's Battersea Research Station, as are the Physics, Chemistry and Operational Research Departments, and there is also a laboratory at Swansea, in the South Wales tinplate area, where some of the Mechanical Working Division's research into sheet and strip manufacture is conducted. The Association's headquarters are at 11 Park Lane, London, W.1.

THE INTERNATIONAL COUNCIL FOR BIRD PRESERVATION

THE seventh Conference of the European Continental Section of the International Council for Bird Preservation was held in Stavanger, Norway, during June 19–24, and was attended by representatives of the National Sections of Austria, Belgium, Denmark, France, Germany, the Netherlands, Norway, Sweden, Switzerland and the United Kingdom, together with representatives of the International Wildfowl Research Bureau, Conseil International de la Chasse, International Union of Biological Sciences, and the International Union for the Conservation of Nature and Natural Resources.

The destruction of sea birds by waste oil has become a permanent item on the agenda of all conferences of the International Council, and it was noted with great satisfaction that Poland had ratified the International Convention for the Pollution of the Sea by Oil, and that the U.S. Senate had voted 92–0 to ratify this convention. The adhesion of Poland to the convention is of particular importance to Scandinavian countries for it now only requires the support of the U.S.S.R. to enable steps to be taken to declare the whole of the Baltic a prohibited zone for discharge of oil. The area in the Baltic in which oil discharge is now permitted has resulted in heavy pollution, particularly on the coasts of Sweden, where it has caused such great destruction of the long-tailed duck (*Clangula hyemalis*) that it is feared the status of this species is endangered. The representatives of the Netherlands, where systematic counts of birds killed by waste oil are carried out throughout the year, reported that there were distinctly less than before, and this statement was also supported from Belgium.

A resolution was adopted welcoming the decision of the Inter-Governmental Maritime Consultative Organization to convene, in the spring of 1962, a further conference on oil pollution of the sea, and urging that priority be given to the complete avoidance, at the earliest possible date, of discharge of persistent oils into the sea, and to the immediate provision of adequate facilities for the disposal of oily wastes at refineries, ports of loading and ports of discharge. The resolution further urged National Sections to press their Governments to instruct their delegates to the Inter-Governmental Maritime Consultative Organization conference in 1962 to support action on these lines.

The prejudice against any bird with a hooked beak dies hard, and though some countries reported an increase in certain species it was generally agreed that birds of prey were still being destroyed on a large scale and many species had critically decreased. The need for education on the valuable part these birds play in the balance of Nature was expressed in a resolution which also recommended that if an examination is required to secure a shooting licence a knowledge of identification of birds of prey should be required, in order that protected species should be distinguished from those allowed to be shot.

The destruction of bird life as a result of the use of pesticides has reached such proportions as to cause grave concern all over Europe, and a resolution was passed requesting Governments to adopt legislation by which all pesticides must be officially tested not only with regard to their effect on noxious animals and plants but also on wild life in general before they are permitted to be sold. Further, that Governments be urged to encourage research on the use of specific pesticides and other methods which limit the destruction of wild life, and to forbid or place drastic restrictions on the use of general pesticides that are dangerous to wild life, wherever effective and less dangerous materials can be made available.

The great increase in herring gulls (*Larus argentatus*) and the harm these cause to other species, particularly in Germany and the Netherlands, has been a problem for many years. In the Netherlands methods of control have been carried out with great success, the number of breeding pairs in 1959 and 1960 being reduced to almost half that of 1954. One of the factors in the great increase of this species is the easy availability of food in winter, through fish offal at ports, rubbish dumps, etc. In Denmark there is no problem as all fish offal is collected and used for food in mink farms or for fertilizers.

The present policy of land drainage in certain European countries, whether it be economic or not, presents a serious threat to many species of water and wading birds, and two areas in particular were the subjects of special resolutions. The first, the Marismas of the Guadalquivir in Spain, is the richest area in western Europe for marshland birds, particularly as regards migrating and wintering species. The disappearance of these marshes would mean not only the loss of the main European breeding ground of several rare species but would also have irreparable consequences to the status of migratory marsh birds in many European countries. On June 1 an international delegation of representatives of the International Wildfowl Research Bureau, the International Council for Bird Preservation, and the International Union for the Conservation of Nature and Natural Resources, with the Consejo Superior de Investigaciones Cientificas of Spain was received by the Minister of Education in Madrid to consider the possibility of acquiring an area of the Marismas as a reserve and to establish a biological research station there.

The second area, the Baie de L'Aiguillon (Vendée) on the Atlantic coast of France, with its large expanse of mud flats, provides an exceptionally favourable resting place for migratory birds which breed in northern Europe and rest there on their spring and autumn migration. The project to drain this area, for agricultural purposes of doubtful value, in a country where there is no lack of agricultural land is being strongly opposed by both French naturalists and sportsmen and their colleagues in other countries.

The proposals concerning the establishment of Council of Europe machinery for matters concerning nature conservancy and national parks were considered, and the prospect of a closer relationship between the Council of Europe and organizations for conservation of Nature was welcomed.

Among other subjects discussed were the traffic in European birds for cageing, bird protection in schools, the danger of the airgun and the increase of the starling (*Sturnus vulgaris*).

Prof. Sven Hörstadius (Sweden) was unanimously elected chairman of the European Continental Section in succession to Dr. Bøje Benzon (Denmark), and Dr. Gerth von Rokitansky (Austria), vice-chairman, in succession to Dr. G. A. Brouwer (Netherlands). Prof. S. O. Hörstadius, a former president of the International Union of Biological Sciences, was accorded an honorary degree by the University of Cambridge during the tercentenary celebrations of the Royal Society in 1960, and it may be apt to quote the Orator's citation on this occasion:

"We read in Lucretius these lines about the origin of animals: 'And many monsters also did the earth then try to create, springing up with strange face and limbs'. She did so to no purpose. But this man does the same to very good purpose. For beginning his studies from the egg in no mere proverbial sense, and using incredible penetration of mind and dexterity of hands alike, he has taken to pieces, down to their smallest components, the embryos of sea-urchins, worms and amphibia, and then re-assembled these into new animal organisms. Would anyone have believed before that two tissues cut from an embryonic newt combined could co-operate to form cartilage, and that, with the addition of a third, tooth would result ? What wonderful metamorphoses, and advantageous indeed to men ! For from this he has thrown light on the influence of part upon part and organ upon organ in the gradual construction of the adult animal, which could be of great value to the art of medicine. So we salute one who has at last set foot on the true way in an inquiry raised by Aristotle himself.

"But do not imagine he only examines and handles eggs. For he is an observer of the ways of birds, and in that most pleasant branch of study also has won world-wide fame.

"I present to you a professor of Uppsala, worthy heir of the studies of Linnæus of Uppsala—Sven Otto Hörstadius".

PHYLLIS BARCLAY-SMITH

IMMUNITY TO PROTOZOA

In the age of Metchnikow and Ehrlich, the subject of immunity had not become divided into the separate compartments of bacteria, fungi, protozoa, etc., as it has to-day, and a broader view was possible at that time. A recent symposium, organized by the British Society for Immunology at the Middlesex Hospital during June 1–2, represented an attempt by the Society to reverse the trend and to bring workers of one specialized field (protozoology) into the classical milieu. Various aspects of immunity to protozoa were thus presented, and, to some extent the idea of the Society was successful, many interesting papers were given, and these provoked some discussion.

The subject of the symposium, "Immunity to Protozoa", was introduced by P. C. C. Garnham, who gave a short historical survey, then a summary of the histological basis of immunity and finally a review of acquired and innate immunity in the parasitic protozoa. In his opinion, the realm of innate or natural immunity offered at present the most promising opportunities for research. All degrees of resistance of the host to the organism are found, which may be influenced by various internal factors like unsuitable blood components (later ably described by A. C. Allison in regard to the inhibitory action of sickle-cell hæmoglobin and glucose-6-dehydrogenase deficiency on *Plasmodium falciparum*), by external factors like removal of the spleen, and lastly by the site occupied by the parasite in relation to its host: immunity thus may be unable to touch the parasite when it is safely enclosed within a parenchyma cell of the liver, yet immunity will kill rapidly when it lies in an exposed position in the blood stream.

The symposium was honoured by the presence of Edmond Sergent, who came from Algiers to give a lucid account of premunition, a subject which has occupied him for the past 60 years and which he here crystallized into 26 precise definitions. Premunition is the basis of the subject, and yet has been much misunderstood; it was useful to have it described by the *Maître* himself. On the other hand, premunition is not the only form of immunity, and A. Corradetti described examples of true residual immunity in rats which had recovered from *Plasmodium berghei* and *Trypanosoma lewisi* infections respectively; in some cases, nothing could break down this resistance, and he clearly showed that the protozoa had been completely eliminated by the immunity response, while the rats proved to be immune for life.

Much of the original work on the cellular basis of immunity was carried out by W. H. Taliaferro, by his wife Lucy, and his collaborator, H. Mulligan. All these workers were present at the symposium, and the subject was introduced by W. H. Taliaferro, who described briefly the lymphoid-macrophage system and then went on to more recent work of a biochemical nature. This indicated that under the influence of the growth-inhibitory antibody, 'ablastin', nucleic acid synthesis in *T. lewisi* comes to a stop, although protein synthesis may continue at a low level. He also considered the phenomenon of 'avidity' or antigen-binding capacity. His explanation of the development of immunity in *T. lewisi* infections by the successive appearance of several different types of antibody was challenged by W. E. Ormerod, who suggested that the disappearance of young and dividing forms (the earliest of which inhabit the protected environment of the kidney capillaries) was not due to 'ablastin' but to an ordinary type of antibody which had a selective action on the young forms.

The subject of immunity cannot properly be considered apart from the nature of the damage inflicted by the parasite on the host, in other words, the mechanism of pathogenicity. B. G. Maegraith illustrated this by reference to non-invasive strains of parasites, which later change their habits and attack the tissue so that an increasing degree of virulence is attained. In *Entamoeba histolytica*, the special enzyme production of an amoeba determined its pathogenic potential. The actual cause of injury was thought, in the case of malaria, to be a soluble toxin which acted on the mitochondria of cells, destroyed their membranes, and brought respiration to an end. The chemical composition of this agent is still not precisely known, but it appears to be non-specific. This work seems to be on the brink of important new discoveries.

The symposium then continued by examining the phenomenon of immunity in: (*a*) blood infections; (*b*) tissue infections; (*c*) infections of the skin and mucous membranes, including the intestinal tract. The last-mentioned group stood out from the rest because some localization of immunity was often apparent.

Immunity in malaria was discussed by Avivah Zuckerman. *In vitro*, macrophages exposed to malarious chicken blood, in the presence of hyperimmune homologous serum, phagocytosed numerous normal as well as parasitized erythrocytes. Furthermore, in a series of simian, rodent and avian malarias, more anæmia occurred than could be attributed to destruction of red cells by emerging parasites. An autoantibody was postulated to explain these observations, and Coombs' tests were positive in two rodent malarias during the period of excessive anæmia. However, since positive antiglobulin tests were also observed in other anæmic states from which parasites were absent, the autoantibody hypothesis remains moot.

L. J. Bruce-Chwatt gave an interesting account of the variability of immunity in malaria inherited by an infant from its mother, and which might be derived either via the placenta (particularly in human beings) or via the milk (in rodents). There was no doubt of its existence in the hyperendemic regions of tropical Africa, and it formed a valuable barrier to infection in the earliest days of life, though the effect quickly wears off. Transplacental immunity is probably the explanation of the unexpectedly low rates of occurrence of parasites in infancy. The exact mechanism is still unknown, and it was suggested that the subject might be investigated further in monkeys, where the placental structure was not unlike that of the human.

Immunity in piroplasmosis was considered by R. Riek, and in a written communication by S. F. Barnett. The former described babesiosis in Australian cattle, demonstrating once more the peculiar tolerance of calves to *Babesia* infections in contrast to the susceptibility of the adults. He made some interesting references to the pathogenic effect of this organism, when present in a high density, on the invertebrate host, a subject which had been little discussed, perhaps because the arthropod is normally immune to any harmful effects of the protozoon which it carries. S. F. Barnett divided the *Theileria* species in cattle into three immunological groups: (1) *T. mutans* ; (2) *T. annulata*; (3) *T. parva* (which includes *lawrencei* and *bovis* in addition to *parva*). The last-mentioned group behaves differently because it produces a sterile residual immunity while the other two are followed by premunition. This worker stated that the endoglobular parasites and the intra-lymphocytic schizonts gave rise to distinct antibodies, and that after recovery from an endoglobular infection, the animal was still susceptible to the tissue forms, which were finally destroyed by lytic antibodies.

Immunity in trypanosomiasis was discussed in four papers. B. Weitz described his recent researches on exo-antigens in infections with the African trypanosomes. K. N. Brown analysed the antigens of *T. rhodesiense*, a highly labile organism which resorts to many changes in antigenicity, though reverting to the parent type after passage through *Glossina*. Two groups of antigens can be distinguished chemically by starch-gel electrophoresis and by ultracentrifugation ; one type is predominantly protein, the other is of unknown composition. This chemical approach has scarcely gone far enough yet for conclusions or principles to be drawn, but it offers promise for the future, especially when combined with the agar techniques using double diffusion of separating strains. A paper by M. A. Soltys and C. S. Folkers was presented by the latter and dealt with the effect of immunity on chemotherapy and chemoprophylaxis of the African trypanosomes of veterinary importance.

Two papers were given on toxoplasmosis. The first, by J. D. Fulton, gave a useful summary of the immunity reactions in this disease, and included details of the author's new agglutination test, using washed organisms from mouse peritoneal fluid. The results of this test corresponded closely with those of the Sabin–Feldman reaction, were constant, easy to read, and beset with none of the difficulties of the dye test. C. P. Beattie discussed the prevalence of the infection throughout the world in man, in other mammals and birds; but *Toxoplasma gondii* is a well-adapted parasite which rarely causes much pathogenicity to its host, either because most strains are of naturally low virulence or because of some special insusceptibility of the host. Youth, concomitant illness, and physical stress are factors which predispose to multiplication of the organism. Although several antibodies have been shown to exist in chronic infections, it is strange that none is particularly adequate in destroying the organism, perhaps because it eventually becomes enclosed in a cyst with an impermeable wall. But man has a natural resistance to the infection, which may be of the nature of premunition, the organism persisting in a latent form for years, and resembling in this and other aspects the tubercle bacillus.

Saul Adler described immunity in leishmaniasis, and discussed chiefly the reaction of man to the various cutaneous forms of *Leishmania*. He made a plea for the recognition and separation of these organisms into sub-species, of which there were two in the Old World and at least four in Latin America, causing the classical espundia in Brazil, uta in Peru, chiclero's disease in Central America and a curious form in Venezuela. These were distinct clinical entities, had special sandfly vectors, particular animal reservoirs, characteristic response to drugs and specific antibody reactions by which each form could be distinguished readily. He directed attention to the paradox of a protozoon making its home in cells of the lymphoid-macrophage system, the system which, *par excellence*, protects the host from protozoal infections. Nevertheless the host wins, and a persistent sterile immunity is the result.

Adler's paper formed a good introduction to the last part of the symposium, which concerned parasites localized in the skin or mucous membranes. The immunity reactions of this group have a peculiarly focal restriction, in contrast to the ordinary antibody response when the infection is generalized; but the difference disappears if these localized parasites are introduced artificially into the blood stream of the host, when a typical antibody reaction is the result. Muriel Robertson showed, however, that such antibodies, produced in response to *Trichomonas foetus*, were unable to reach the flagellates in their protected sites in the uterine wall; the production of antibody is a sequence of the presentation of antigen to the appropriate host cells; in trichomoniasis of cattle the antibody had a very limited value in destroying the infection.

C. Horton-Smith, P. L. Long, A. E. Pierce and M. E. Rose also examined the question of local immunity in coccidiosis and showed that a fowl which had recovered from an infection of *Eimeria tenella* in one cæcum was nevertheless immune in the other. These workers demonstrated that different stages of the life-cycle of coccidian parasites had their specific immunizing values. R. Augustin and A. P. Ridges. described similar work on *E. meleagrimitis* of turkeys.

In the early days, immunologists were much concerned in the practical aspects of cure or prophylaxis of disease: two papers in the present symposium show that this subject is still of interest to-day. S. Cohen and I. A. McGregor gave a most interesting account of the use of gamma-globulin in the cure of malaria. They collected sera from hyperimmune adults living in the Gambia and extracted the gamma-globulin component; this was then given in large doses to young children who were suffering from acute infections of the disease. Within 4 days, parasitæmia fell and by the ninth-day parasites were undetectable in 8 of the 12 cases studied. These investigations were accompanied by work on the turnover of gamma-globulin, on the lessening of hypergamma-globulinæmia as the result of specific therapy, etc., all of which observations tend to indicate that humoral immunity may be more important in malaria than was originally thought. The antibody appears to be contained in the 7-*S* fraction and not in the macroglobulin. P. E. C. Manson Bahr sent an important communication on a big field experiment he is carrying out in Kenya, in which he has immunized a population which is exposed to epidemic kala-azar with an avirulent ground squirrel strain of the organism. Preliminary work had shown that volunteers could be protected in this way.

The papers are being published in book form, and this should provide a modern review of many important aspects of the subject. P. C. C. Garnham

THE MUSEUMS ASSOCIATION

In the ideal environment of the City of Plymouth and its museums the sixty-seventh annual conference of the Museums Association was held during June 19–23, 1961.

The conference was officially welcomed by the Lord Mayor and this was followed by the presidential address by Dr. D. B. Harden (London Museum). In an interesting historical survey of the continual efforts of the Museums Association since 1945 to obtain Government grants for the provincial museums —and especially the smaller ones—he felt that there had been a certain amount of progress, for at last the Standing Commission for Museums and Art Galleries had been instructed to prepare a report. This was awaited with interest, though, meanwhile, the Association and museums throughout Britain were grateful to the Carnegie United Kingdom Trust for its continual help and advice. Reference was also made to the formation of the Area Councils and especially to the progress made by that in the south-west.

Sir Hugh Casson opened the discussion on display in museums, and in a provocative and entertaining address said that a lively imagination was necessary both in new buildings and in the adaptation of the older ones. He stressed that the building and display must be shared by the curator and the architect, and that it was essential to establish character and personality in a museum. A museum must be an active place—a lively cultural centre to arouse the sense of the curious.

Dr. D. A. Allan felt that the ideal display of natural history objects should include the clinical and the artistic. He also stressed the value of genuine objects, and said that it was important to achieve liveliness and realism. Large dioramas were not in the fashion at the present day. Mr. J. W. Y. Higgs (University of Oxford) dealt chiefly with folk material, and detailed the value of comparative displays—doing the same thing in different ways. He also referred to the need for more folk parks in Britain. Mr. Norman Cook (Guildhall Museum) stated that it was necessary to serve the public at different levels. He also felt that not all archæological exhibits should be treated as works of art, for it was not the curators' task to make everything look beautiful. As chairman of this session, Sir Frank Francis (British Museum) felt that there was no single solution to the problem of display for museums were both for the connoisseur and the ordinary visitor. The future may lie in some form of double exhibition.

A whole day was devoted to the many problems of conservation. Dr. P. Coremans (Brussels) opened the discussion, and said that great changes in the treatment of paintings was due to the scientific interest now taken in this subject. He described methods whereby it was possible to distinguish the layers of dirt, re-paint, varnish and the glazes which were an integral part of the picture itself. The extreme differences of opinion regarding restoration were also discussed. Mr. Norman Bronmelle (Victoria and Albert Museum) suggested a scheme for making modern scientific resources available to provincial museums, and Mr. H. Schubart (Bristol) spoke about the training of picture restorers. An art school basis was probably the best, but the creative impulse of the restorer must be directed entirely to conservation so that the welfare of the object became second nature.

An afternoon session was devoted to a symposium on modern developments of conservation in museums as opposed to art galleries. Dr. A. E. Werner

(Research Laboratory, British Museum) stressed the wider concept of conservation which involved successively preventive treatment, diagnosis of the causes of decay, research in the evaluation of new synthetic materials and scientific examination of the object before attempting treatment. Mr. H. W. M. Hodges (Institute of Archæology) said that at present there were only two academic training institutions in the world for conservators—in London and in New York. He detailed the training given in London and emphasized that conservation must be based on scientific knowledge. Mr. A. E. Rixon (British Museum (Natural History)) gave details of useful techniques for dealing with the preservation of fossils.

At the annual general meeting, Dr. Mary Woodall (Birmingham) was elected president for the ensuing year, and an invitation to hold the conference in Brussels in 1962 was accepted with gratitude.

Social events during the week included a civic reception and an annual dinner. At the end of the week organized tours to many of the museums and beauty spots in Devon and North Cornwall brought a memorable conference to a close.

F. S. WALLIS

DIGITAL COMPUTERS, THEIR USE AND CONTROL

A SYMPOSIUM on "Digital Computers, Their Use and Control" was organized by the Mathematics Department of Queen's College, University of St. Andrews, in collaboration with Standard Telephones and Cables, Ltd., during May 29–June 2. Standard Telephones and Cables, Ltd., provided the use of a *Stantec Zebra* computer for the whole course.

The first day was devoted to talks of a general nature to give a general appreciation of the use of computers. After an address of welcome by the Master of Queen's College, Prof. A. A. Matheson, Dr. R. J. Ord-Smith of Standard Telephones and Cables, Ltd., spoke on computer applications and this was followed by an address by Mr. W. A. Donaldson of Rolls Royce, Glasgow, entitled "Computers in Industry". The morning session was completed by a description of a transportation problem and its solution by Mr. W. A. E. Pillow of Standard Telephones and Cables, Ltd.

In the afternoon the symposium split into two sections. Those who wished to attend the whole course were given a demonstration of *Zebra* programmes and the remainder were given a brief introduction to programming.

The first day was attended by about eighty people of whom about thirty-five were from local industry and commerce and the remainder from the University. On subsequent days, about fifty people attended of whom ten were from outside the University.

The lectures on the remaining days were given largely by Dr. R. J. Ord-Smith and Mr. A. Y. Cooper of Standard Telephones and Cables, Ltd. They covered a comprehensive use of the *Zebra* simple code and an introduction to normal code. A lecture on "The Computer in the University" was given by Dr. A. J. Cole of Queen's College. By the end of the week, all delegates had written and run several programmes.

The members of Queen's College staff are grateful to Standard Telephones and Cables, Ltd., for providing such excellent facilities and in particular for the continued loan of a *Zebra* computer, which, in the week subsequent to the course had been in continual use for 8 hr. a day.

A. J. COLE

J. IBALL

A NEW APPROACH TO BIOLOGY TEACHING

A CONFERENCE, proposed and financed by the Gulbenkian Foundation, was held in the University of Birmingham during April 10–12, to discuss the biology syllabus for sixth forms, proposed by the Biology Panel of the Gulbenkian Enquiry 1959*. The conference was attended by seventy-five schoolteachers and several lecturers from teacher-training colleges. The majority of the members came from the West Midlands. Several topics were selected from the syllabus and a lecture was given on each.

During his introductory lecture Prof. O. E. Lowenstein pointed out that the University of Birmingham will be offering a new type of biological training in the near future. This will consist of an honours B.Sc. degree in biological sciences. Undergraduates will take a variety of biological courses which will cut across the traditional subject-boundaries and be in closer accord with the trends in modern biology in pure and applied research, in industry, in agriculture, etc. Various subject-combinations will be possible. It is anticipated that fewer students will complete courses of the traditional type leading to a degree in zoology or botany, although it will still be possible to specialize in selected branches of biology, for example, systematic botany, entomology, etc. It is difficult to predict whether other universities will adopt similar schemes; but the wind of change is blowing in this direction and during recent years in many university departments, even within the traditional courses of zoology and botany, the subject-matter has changed dramatically. Already in Birmingham the preliminary (= intermediate) course in biology has ceased to be virtually separate courses in botany and zoology and is now a closely integrated course following the lines recommended in the Gulbenkian Report.

It is against such a background of a rapidly evolving subject that this new approach to biology in sixth forms is presented.

During discussion there emerged considerable agreement on the need for a new approach, both to teaching and to examining, of the type put forward in the Gulbenkian syllabus, although it was recognized that there would be various difficulties in implementing the recommendations of the report. It was suggested that a first step in the right direction would be to persuade the Northern Universities Joint Matriculation Board to set up a pilot scheme to give the new syllabus a 'field trial'. The chairman was instructed to write to the Board and make this proposal. He was also asked to produce a memorandum listing a number of specific difficulties involved in teaching biology and to circulate the memorandum to the Institute of Biology, local education authorities and other bodies who might be interested.

---

* Copies can be obtained from the Registrar, The University, Birmingham 15.

The points which emerged during the discussion were as follows: (1) Lack of exclusive use of biology laboratories by many teachers. This makes adequate preparation for practical work very difficult or impossible. It also makes difficult the keeping of cultures, aquaria, etc. (2) Lack of time for collection of material and preparation for practical work. (3) Lack of technical assistance. (4) Lack of preparation rooms and storage facilities. (5) Lack of transport for collecting and field work. (6) Inadequate grants for the purchase of material, books and equipment, such as physiological apparatus, stereoscopic microscopes, phase-contrast microscopes, aquaria, cages, incubators, ovens, refrigerators, deep freezers, etc. These items include essential basic equipment for the proper study of biology. (7) It was recognized that staffing and salary problems might arise if zoology and botany were replaced by a single biology.

It is relevant to all these items that the conference was agreed that teachers of biology require more time for the collection of material and the preparation for practical work than teachers of most, if not all, other subjects. Many teachers at the present time have to 'make do' in the face of considerable difficulties. The adoption of a syllabus like that proposed would increase these difficulties. The problems involved in the day-to-day teaching of biology in schools require objective study. There is no logical foundation for the assumption that every science subject requires the same number of teaching-hours for its presentation. L. H. FINLAYSON

SCIENCE AND EDUCATION : THE TEACHERS' POINT OF VIEW

A POLICY Statement, "Science and Education"*, issued by the Science Masters' Association and the Association of Women Science Teachers, revises the preliminary statement published by the Science Masters' Association in 1957. The Statement is accompanied by separate syllabuses on chemistry, physics and biology for grammar schools compiled by panels of the Science and Education Committee for these subjects and amended in the light of comments received. The Policy Statement now issued concerns mainly grammar schools and represents the first part of the promised report. The second part will include a scheme for the general study of science in the sixth form, and two other panels are concerned with the training of teachers and with new experimental work necessary for teaching modern physical science.

The present 'scientific illiteracy' is attributed partly to a lack of factual knowledge but much more to a lack of understanding of the basic nature and aims of science. Science should be recognized and taught as a major human activity which explores the realm of human experience, maps it methodically but also imaginatively, and, by disciplined speculation, creates a coherent system of knowledge. As a human quest for truth, it is much concerned with basic values and is indeed an active humanity, and it follows that schools have the duty of presenting science as part of our cultural and humanistic heritage, to be taught in harmony with, not in opposition to, the various arts subjects which alone have hitherto been called humanities. The Committee recognizes that an adequate supply of scientists and technologists is needed and believes that its recommendations will help to meet that need, but it stresses the cultural aim because science has not yet been given its proper place in general education.

It may be apposite in these pages to comment how Sir Richard Gregory, with his passionate belief in the cultural value of science, would have welcomed such a statement from such a source. To ensure that science is a 'core' subject in grammar schools, as English and mathematics now are and as classics used to be, the Committee recommends that *all* pupils should follow a balanced course of science subjects up to the end of their fifth form year, and this course should lie within the range of a group of natural sciences, each of which is concerned with a limited aspect of human experience, though it is essential that this formal course, mainly involving the sciences of biology, chemistry and physics, with some astronomy and geology, if possible, should help pupils to appreciate the wider range of science as a whole. There should be no division into science specialists and arts specialists until beyond this level, though the Committee recommends that subject specialization should be retained in the sixth form but with a reduction of the detailed factual content of the Advanced Level syllabuses for the General Certificate of Education.

Such a reduction should release time for the broader course of science which the Committee recommends should be studied by all pupils in the sixth form, a course which would enable pupils to attain the scientific literacy described as 'numeracy' in the Crowther Report. The implications of these three general recommendations are outlined in the remainder of the Statement which describes, first, the introductory phase covering approximately the first two years of a grammar school course; secondly, an intermediate plan, usually three years up to about sixteen, when the Ordinary Level examination for the General Certificate of Education may be taken; and, thirdly, an advanced (sixth form) plan, offering a course indicated for all students and a course for the science specialists. These are further amplified in the three accompanying sections dealing, respectively, with chemistry, biology and physics, which set forth a syllabus which might appropriately meet the needs of all three phases of the proposed policy. The syllabuses seem well designed to meet some of the criticisms which have been made of the outlook of science graduates themselves, and the Statement and its accompanying detailed sections demonstrate that the two Associations are tackling the revision of syllabuses imaginatively as well as energetically. They are to be commended not simply to the attention of science teachers themselves but at least as much to scientists and the professional associations, as well as to the universities, for the recommendations can scarcely achieve their full effort without full co-operation by the universities and professional bodies. Moreover, considering the steps already taken by the National Science Foundation in the United States, and in view of the serious shortage of science teachers both the Minister of Education and the Minister for Science should be considering the wider implications, and the steps that will be required at a higher level if the recommendations are to be adopted not merely in grammar schools but also elsewhere.

---

* Science Masters' Association and the Association of Women Science Teachers. *Science and Education: A Policy Statement.* Pp. 12. 1s. net. *Chemistry for Grammar Schools.* Pp. 13. 2s. net. *Biology for Grammar Schools.* Pp. 24. 2s. net. *Physics for Grammar Schools.* Pp. 30. 2s. net. The Sections of Part 1 of the Science and Education Report to be read in conjunction with the Policy Statement. (London: John Murray (Publishers), Ltd., 1961.)

ATOMIC RADIATIONS AND LIVING ORGANISMS

SINCE 1955 various committees appointed by the U.S. National Academy of Sciences—National Research Council have been conducting a continuing appraisal of the effects of atomic radiations on living organisms. Various reports have come from sub-committees of the Committee on Pathologic Effects, and differ considerably in their approach and content[1].

The most generally valuable of the recent reports is that on *Inhaled Radioactive Particles* (ref. 1*a*). It contains, or provides references to, basic factual information on the nature of these particles, and the physiological and pathological mechanisms involved in their deposition in and translocation from the respiratory tract. The formidable complexity of the problems involved is clear.

Different processes are responsible for the retention of particles of different sizes and densities, and in practical situations the amount of radioactivity in a given particle is independent of the nature of the radioactivity and of the size of the particle. The rate of loss of radioactivity from particles deposited in the respiratory tract is not simply related to 'solubility' in body fluids. The problems of assessing radiation 'dose' to the tissue around a radioactive particle go to the root of what is meant by tissue dose, a concept which still awaits a fruitful synthesis of physical chemistry, cell biology and pathology, at least in relation to carcinogenesis. Although miners of radioactive ores have suffered a gross excess of cancer of the lung for several centuries, the relative importance of the different factors which may be concerned, including those non-radiative, is quite obscure.

Another report (ref. 1*b*) entitled *Long-Term Effects of Ionizing Radiations from External Sources*, is more discursive and covers the same ground as many fairly recent reviews: life-shortening, tumour production, cataract, fertility and ageing. Seven of the eleven sub-committee members come from the same university, five from the same department, and it is not unfair to say that this common background permeates the theoretical framework or point of view. The implication that ovarian damage in the adult human female is not irreversible (p. 27) would not be generally acceptable. The definition of threshold dose as a description of the possible response of a single animal (p. 2) seems likely to add further confusion to the existing conceptual muddle.

A short report (ref. 1c) on the *Effects of Ionizing Radiation on the Human Haematopoietic System* has also been issued, but much that is new is an expression of opinion, for example, on the proper clinical treatment for an individual exposed to a dose in the acutely lethal range. Routine blood counts at periodic intervals in occupationally exposed persons are "wasteful and unproductive in detecting low-dose radiation effects". When decisions are to be made involving exposure of populations, *society* must weigh the benefits against the potential health hazard (my italics).

R. H. Mole

---

[1] National Academy of Sciences—National Research Council. (*a*) Publication No. 848: *Effects of Inhaled Radioactive Particles: Report of the Subcommittee on Inhalation Hazards, Committee on Pathologic Effects of Atomic Radiation.* Pp. vi+78. (*b*) Publication No. 849: Report of the Subcommittee on *Long-Term Effects of Ionizing Radiations from External Sources*, of the Committee of Pathologic Effects of Atomic Radiation. Pp. v+82. (*c*) Publication No. 875: *Effects of Ionizing Radiation on the Human Hemapoietic System:* Report of the Subcommittee on Hematologic Effects, Committee on Pathological Effects of Atomic Radiation. Pp. v+14. (Washington, D.C.: Nationa Academy of Sciences—National Research Council, 1961.)

SLOW DECOMPOSITION OF EXPLOSIVE CRYSTALS AND THEIR DAMAGE BY FISSION FRAGMENTS

By Dr. F. P. BOWDEN, C.B.E., F.R.S., and H. M. MONTAGU-POLLOCK
Physics and Chemistry of Solids, Cavendish Laboratory, Cambridge

Thermal Decomposition

WITH many explosive solids the decomposition may begin slowly before it passes over into rapid explosion or detonation. The onset of explosions is usually governed by thermal considerations: if the rate of heat liberated by the slow chemical decomposition of the crystal is greater than that of heat lost by conduction, the reaction will accelerate to explosion[1]. The mechanism of slow decomposition is therefore of considerable importance in determining the explosive process. We have in this Laboratory been using electron microscopy and electron diffraction to study the early stages of the slow decomposition[2-5].

Similar methods have also been applied to non-explosive crystals[6,7]. In the earlier work on explosive crystals, replica techniques or the scanning electron microscope were used. More recently we have been using direct transmission electron microscopy—which reveals more detail—to study both thermal decomposition and the decomposition produced by fission fragments. Silver cyanamide (Ag_2CN_2) was the crystal chosen for this work. Although it is explosive it was found to be stable in electron beams of moderate intensity, and the crystals could be grown thin enough (about 100 molecules thick) to be observed in transmission. The main products of decomposition at low temperatures are silver, carbon and gaseous nitrogen. The formation, movement and aggregation of the liberated silver can be followed in some detail.

The crystals were heated in a furnace *in vacuo* under controlled conditions at temperatures ranging from 150° to 350° C. and then placed in the microscope for observation. They were supported on carbon films lying on the specimen grids. Diffraction patterns of selected areas were taken in order to identify undecomposed and decomposed material. As well as qualitative information on the decomposition process, rough measurements of rates of decomposition were obtained from estimates of the volume of silver liberated, with the help of metal shadowing.

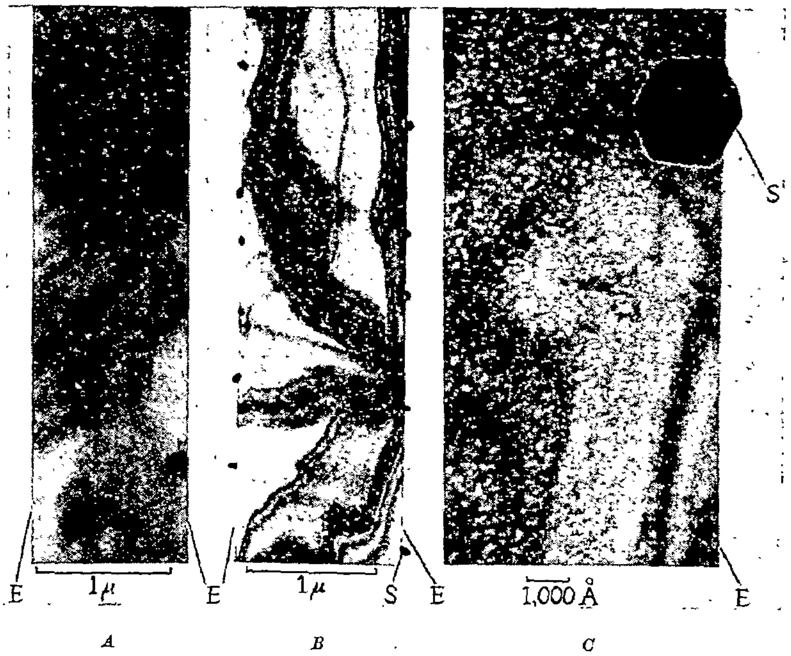


Fig. 1*A*. Symbols: *E*, true edge of crystal; *S*, silver aggregate. Undecomposed silver cyanamide crystal, about 200 Å. thick. The only visible details are the Bragg extinction contours, arising from slight elastic distortion of the crystal

Fig. 1*B*. Crystal in the first stage of thermal decomposition. A layer of the crystal extending from the surface to a depth of about 15 Å. has decomposed. The silver produced has diffused over the crystal surface and formed a few opaque specks at the the edges

Fig. 1*C*. Crystal in the second stage of thermal decomposition. Surface mobility of the silver is again apparent, with no evidence for nucleation of decomposition at the silver specks. Note the sharply bounded region of surface mottling

Fig. 1*A* shows an undecomposed crystal of silver cyanamide. Such crystals gave diffraction patterns consistent with the (100) orientation of the structure of silver cyanamide as determined by X-ray methods: this work will be described elsewhere and gives a monoclinic unit cell containing four molecules, with $a = 7{\cdot}26$, $b = 5{\cdot}92$ and $c = 6{\cdot}61$ Å., and with $\beta = 102°\ 20'$. The space group is $P2_1/c$.

Fig. 1*B* shows a crystal which has been heated for 2 min. at 200° C. A number of opaque specks can be seen at the edge of the crystal. Electron diffraction measurements show that these are single crystals of silver of random orientation. It would seem that the liberated silver is highly mobile and can migrate over the surface to sites of high surface energy on the crystal. These are frequently at the edge but may be elsewhere. These silver specks normally project from the crystal surface and, as they grow, develop more or less well-defined crystallographic faces (Fig. 1*C*); occasionally whiskers are seen. Nucleation, in the sense of decomposition spreading outwards from certain points, is never observed. Instead, the silver is liberated by an overall surface decomposition, before diffusing to the aggregation sites; this was deduced from plots of volume of silver per unit surface area of crystals against time at various temperatures, and confirmed by the observation that thermal decomposition was inhibited almost entirely if the crystals had previously been coated with a thin layer of carbon, evaporated on to the surface *in vacuo*. If the heating is continued for a sufficient length of time, the crystal is completely decomposed into crystalline silver and an amorphous layer which gives a diffraction pattern similar to that given by amorphous carbon (the possibility that some of this amorphous material may be formed by the polymerization of cyanogen[8] is not ruled out).

At temperatures below 230° C. the decomposition is found to take place in two stages. The first stage involves a progressive thinning of the crystal: the rate of this is of course dependent on temperature, and at 170° C., for example, it is about 0·7 Å./min. When the crystal has grown thinner by about 20 Å. (5 atomic layers), a second and much slower stage of the decomposition sets in. The thinning of the crystal continues at this stage until the crystal is completely decomposed, but the rate is about one-tenth of that observed in the first stage, for example, about 0·07 Å./min. at 170° C. It is possible that amorphous carbon formed during the first stage is responsible for the slowing down of the decomposition at the beginning of the second stage. As the decomposition proceeds, a mottling becomes visible, suggesting that the crystal surface is developing pits about 130 Å. in diameter, and consistent with the random surface decomposition process. The crystal in Fig. 1*C* is at this stage, and shows a characteristic often seen: the central part of the crystal is much more decomposed than the parts near the edges, where the mottling has not yet begun. The lines dividing these regions are perfectly straight, and there is evidence from other experiments that they may be associated with steps on the surface of the original undecomposed crystals.

If the crystals are heated in the presence of oxygen, an additional type of decomposition is observed:

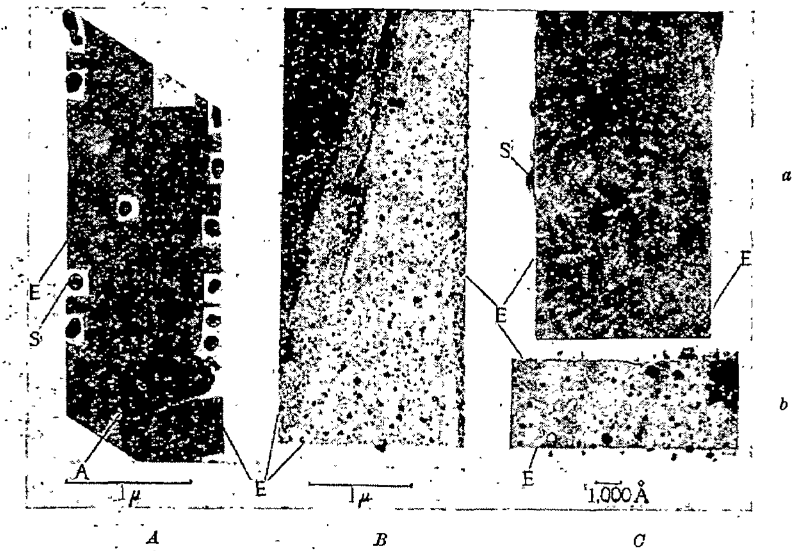


Fig. 2A. Crystal heated in the presence of oxygen. Additional decomposition takes the form of holes of crystallographic shape, starting around each silver speck and continuing to grow when isolated from it. Silver liberated by the growth of the holes has diffused over the crystal surface to aggregate at *A*

Fig. 2B. Crystal partially decomposed by the combined effects of heat and electron irradiation. A large number of specks of silver has appeared

Fig. 2C. (*a*) Crystal irradiated with uranium fission fragments (neutron flux density of $4\cdot3 \times 10^{15}$ n.v.t.). Each light area is a tunnel where material has been decomposed by a fragment: the dark areas are aggregated silver. (*b*) Crystal similarly irradiated (at a lower neutron flux density, $7\cdot2 \times 10^{13}$ n.v.t.)

the silver specks formed during the first and second stages cause the crystal to decompose through its whole thickness in their immediate neighbourhood, so that a hole appears around each speck (Fig. 2*A*). These holes rapidly take on crystallographic shapes and grow at a constant rate. The holes first become visible during the second stage, when the rate of growth of a silver speck is overtaken by the rate of growth of a hole. The liberated silver tends to diffuse over the crystal surface as before, rather than enlarge the new isolated silver specks. It aggregates at one or two specific sites.

If the electron beam intensity in the microscope is raised above a critical value, decomposition of the crystal occurs in the microscope. Under these conditions the liberated silver forms a large number of small specks distributed over the surface of the crystal (Fig. 2*B*). A similar distribution of the silver is obtained if the crystal is heated in a furnace to a higher temperature ($>$ 300° C.). If the beam intensity is raised still higher the crystal explodes giving silver and amorphous carbon. Heating to a high temperature will produce a similar explosion. If a cold stage is used in the microscope, the decomposition in the electron beam is inhibited. This behaviour is consistent with the view that the main effect of the intense electron beam is to heat the crystal to a high temperature.

Decomposition by Fission Fragments

Earlier experiments have shown that (with certain exceptions, such as nitrogen iodide) most explosive crystals do not explode when irradiated with a moderate intensity of neutrons or fission fragments[1]. Important changes are, however, produced and the explosive may be rendered more sensitive to subsequent thermal decomposition.

A study has been made in the microscope of the damage and of the tracks produced in silver cyanamide when it has been bombarded by uranium fission fragments. The damage of inert materials such as mica[9,10] and uranium oxide[11,12] has been described by others.

An experimental method developed by L. T. Chadderton of this Laboratory was used: silver cyanamide crystals in contact with thin foils of natural uranium were irradiated in the Harwell *Bepo* reactor for periods of 1 min.–10 hr. at a pile factor of 12. The grids were then examined by transmission electron microscopy as described above.

Fig. 2*C* shows a typical result of the irradiation. The light dashes and dots show that where each fragment has penetrated the crystal, material has evaporated or diffused away. The longest tracks represent paths of fragments travelling almost in the plane of the crystal, while dots indicate fragments that penetrated the crystal vertically. The mean track diameter is about 120 Å. The silver originally present in the volume occupied by a track has invariably diffused away from the track. On a few micrographs, an occasional fragment is seen to have caused greater damage than usual.

The greater part of the heat liberated by the passage of a fission fragment through a solid is due to ionization. Taking rough values of range and initial energy of a fragment in silver cyanamide to be 30μ and 80 MeV., the energy released is about 300 eV./Å. along a track. With the observed track radius of 60 Å., this gives a figure of about 650° C. (reached in about 5×10^{-11} sec.) for the maximum temperature of a molecule at the wall of a track. This implies that this is the maximum instantaneous temperature that a molecule can reach without decomposing: but the figure of 650° C. is necessarily very crude, because of the assumptions made in the calculation. However, the width of each light area is greater than in the inert materials, indicating that the damage is greater when additional heat is liberated by the decomposition of the material. This heat is of the same order as the heat supplied by the passage of the fragment, per unit length of track. It is not enough to produce explosion; this is consistent with estimates of limiting hot-spot sizes (10^3–10^5 Å.) for a number of explosives (see ref. 13 for further discussion).

The silver produced by the decomposition, in diffusing away from the sites where it is liberated, shows the same high mobility of the silver released from the crystals heated in the furnace.

Conclusion

The thermal decomposition studies have shown the importance of direct observation in investigating the physical processes involved. Nucleation at the interface between parent crystal and solid decomposition product has been observed only in the special case involving the initiation of holes of crystallographic shape. In general, the decomposition has been shown to take place everywhere on the crystal surface; and there is a suggestion that surface steps have some influence on the decomposition. The silver produced by the decomposition is very mobile on the crystal surface. The study of fission fragment tracks shows that this mobility applies also to silver liberated within the body of the crystal. It also confirms the earlier conclusion that the 'hot-spots' or 'hot cylinders' produced by fission fragments are too small to initiate explosion.

One of us (H. M. M.-P.) would like to thank the Master and Fellows of Trinity College for the award of a research scholarship, and the Department of Scientific and Industrial Research for a maintenance grant.

[1] Bowden, F. P., and Yoffe, A. D., *Fast Reactions in Solids* (Butterworths, 1958). See also *Initiation and Growth of Explosion in Liquids and Solids* (Cambridge University Press, 1952).

[2] Bowden, F. P., and McAuslan, J., *Nature*, **178**, 408 (1956).

[3] Bowden, F. P., and Camp, M., *Proc. Eighth Intern. Symp. on Combustion*, California, 1960 (to be published).

[4] Sawkill, J., *Proc. Roy. Soc.*, A, **229**, 135 (1955).

[5] Camp, M., *Proc. Fourth Intern. Cong. for Electron Microscopy*, 1958 (Springer-Verlag, Berlin, 1960).

[6] Goodman, J. F., *Proc. Roy. Soc.*, A, **247**, 346 (1958).

[7] Forty, A. J., *Phil. Mag.*, **5**, 787 (1960); **6**, 587 (1961).

[8] Brotherton, T. D., and Lynn, J. W., *Chem. Revs.*, 841 (Oct. 1959).

[9] Silk, E. C. H., and Barnes, R. S., *Phil. Mag.*, **4**, 970 (1959).

[10] Bonfiglioni, G., Ferro, A., and Mojoni, A., U.S. Air Force A.R.D.C. Contract No. *AF*.61 (514)–1333 (1960).

[11] Noggle, T. S., and Stiegler, J. O., *J. App. Phys.*, **31**, 2199 (1960).

[12] Bierlein, T. K., and Mastel, B., *J. App. Phys.*, **31**, 2314 (1960).

[13] Bowden, F. P., and Singh, K., *Proc. Roy. Soc.*, A, **227**, 22 (1954).

TERNARY COMPOUND FORMATION IN THE SYSTEM $CaO-Al_2O_3-MgO$

By J. H. WELCH

Building Research Station, Garston, Watford, Hertfordshire

THE quaternary system $CaO-Al_2O_3-SiO_2-MgO$ has been studied extensively at high temperatures because phase equilibria in this system provide basic knowledge of importance in understanding geological and industrial processes. Of the four ternary planes bounding the quaternary volume, the $CaO-Al_2O_3-MgO$ system is unique since its original and only investigators to date[1] found no evidence of ternary compound formation. Possibly, because of the apparently simple nature of the system and also its lack of geological significance, its long-established phase diagram has never been questioned. The system is, however, of considerable importance in understanding the potential compound formation in high-alumina cement, and work in this connexion has led to the discovery of two ternary compounds having the approximate compositions $7CaO.5Al_2O_3.MgO$ and $25CaO.17Al_2O_3.8MgO$.

The discovery of these compounds arose from the reported existence[2] of a quaternary compound, the composition of which was believed to be $6CaO.4Al_2O_3.MgO.SiO_2$. Attempts to confirm this reported formula were unsuccessful and led to an extensive investigation of adjoining compositions. In the course of this work, high-temperature microscope observations revealed that a homogeneous compound of composition $7CaO.5Al_2O_3.MgO$ could be prepared having optical and X-ray properties similar to those of the alleged quaternary compound. Since then, further work has disclosed that this ternary compound may be metastable at all temperatures, and is certainly so at temperatures above the solidus of the ternary system (1,344·5° C.) since it is observed to melt at 1,332° C. Its significance, however, resides in its solid-solution behaviour in the presence of silica. Preliminary observations suggest that a discontinuous series of solid solutions exist between $7CaO.5Al_2O_3.MgO$ and the compound gehlenite, $2CaO.Al_2O_3.SiO_2$. With increasing gehlenite content, these solid solutions first melt incongruently to yield a melt of $CaO.Al_2O_3$ and liquid, but congruent melting is established for compositions exceeding 40 per cent by weight gehlenite. It seems, therefore, that the quaternary compound reported might be accounted for as one of a series of solid solutions between two ternary compounds.

Following the initial discovery of the $7CaO.5Al_2O_3.MgO$ compound, a search was made in the system $CaO-Al_2O_3-MgO$ for a possible new primary phase field. Such a field was in fact located approximately by high-temperature microscopy and this was followed by a very precise quenching study of the indicated region. From a consideration of the course of crystallization of several of the compositions studied it became apparent that the very small primary phase field, which had been demarcated, could not be related to the $7CaO.5Al_2O_3.MgO$ compound.

It followed that a second ternary compound must exist in the system which bore a remarkable physical resemblance to the first. The two compounds in fact possess optical properties which are so similar that they cannot readily be distinguished by microscopic examination, although fortunately their X-ray diffraction patterns are quite distinct. The composition $3CaO.2Al_2O_3.MgO$ was at first assigned to the second of the two ternary compounds, but further examination of crystallization paths and homogeneity indicates that the almost contiguous and more complex composition $25CaO.17Al_2O_3.8MgO$ is more certainly correct. The similarity of optical properties suggested that there might be some solid-solution relationship between the two ternary compounds, but their separate co-existence has been checked by X-ray examination. As both compounds decompose on melting, their preparation in pure form presents considerable difficulty.

The 25 : 17 : 8 compound can be prepared in bulk by complete fusion at 1,650° C., followed by annealing of the rapidly chilled liquid at 1,200° C. Even with this treatment, several attempts may be necessary to suppress the formation of some magnesium oxide which, as the primary phase for this composition, tends to precipitate rather readily from the liquid. All similar attempts at bulk preparation of the 7: 5: 1 compound have failed; such heat-treatments

Table 1. PHASE DATA

$7CaO.5Al_2O_3.MgO$: Melts at 1,332° C.; may be metastable below this temperature with respect to $CaO.Al_2O_3$, $12CaO.7Al_2O_3$, and MgO.

$25CaO.17Al_2O_3.8MgO$: Melts incongruently at 1,350·5° C. with formation of $CaO.Al_2O_3$, MgO and liquid. Primary phase field terminates at:

(1) Reaction point; temperature 1,350·5° C. Equilibrium phases: $25CaO.17Al_2O_3.8MgO$, $CaO.Al_2O_3$, MgO and liquid. Composition (weight per cent): 42·6 CaO, 51·3 Al_2O_3, 6·1 MgO.

(2) Eutectic; temperature 1,346° C. Equilibrium phases: $25CaO.17Al_2O_3.8MgO$, $12CaO.7Al_2O_3$, MgO and liquid. Composition (weight per cent): 44·4 CaO, 49·9 Al_2O_3, 5·7 MgO.

(3) Eutectic; temperature 1,344·5° C. Equilibrium phases: $25CaO.17Al_2O_3.8MgO$, $12CaO.7Al_2O_3$, $CaO.Al_2O_3$ and liquid. Composition (weight per cent): 43·8 CaO, 50·9 Al_2O_3, 5·3 MgO.

Table 2. OPTICAL DATA

Composition	Refractive index (sodium light)	Birefringence	Optical character	Habit
$7CaO.5Al_2O_3.MgO$	$\alpha = 1·671$ $\gamma = 1·674$	Weak anomalous interference	Biaxial negative	Lamellar
$25CaO.17Al_2O_3.8MgO$	$\alpha = 1·675$ $\gamma = 1·678$			

have yielded a mixture of the 25 : 17 : 8 compound, $CaO.Al_2O_3$, and either $12CaO.7Al_2O_3$ or 'unstable $5CaO.3Al_2O_3$'. The latter compound has at various times been confused with the alleged quaternary compound $6CaO.4Al_2O_3.MgO.SiO_2$, but its separate existence has previously been established[3].

The 7 : 5 : 1 compound has only been isolated in minute quantity by carefully controlled supercooling of the melt conducted under continuous observation with the high-temperature microscope. Even by this technique it is only with the greatest difficulty that it has been found possible to grow single crystals of the compound, which exhibits a marked lamellar habit. The majority of compositions being studied at present in the hypothetical solid-solution series $7CaO.5Al_2O_3.MgO - 2CaO.Al_2O_3.SiO_2$ are, however, susceptible to bulk preparation by the method described.

Table 3. X-RAY POWDER DATA

Because of limitations on space, only the principal X-ray spacings for the two compounds are given here. Full powder-diffraction data are available on private application. The *d*-values here as *VS* (very strong), *S* (strong), *M* (medium) were obtained from measurements in a Guinier-type focusing camera, 114 mm. diameter, with copper *Kα* radiation

d (Å.)	*I*	*d*	*I*	*d*	*I*	*d*	*I*
			$7CaO.5Al_2O_3.MgO$				
3·72	*S*	2·776	*M*	2·145	*M*	1·802	*M*
3·36	*M*	2·724	*M*	2·086	*M*	1·784	*M*
3·09	*S*	2·473	*S*	2·032	*M*	1·764	*M*
2·93	*S*	2·390	*M*	1·966	*M*	1·524	*M*
2·89	*VS*	2·337	*S*	1·935	*M*	1·492	*M*
			$25CaO.17Al_2O_3.8MgO$				
4·21	*M*	2·682	*M*	2·029	*M*	1·681	*M*
4·07	*M*	2·602	*M*	2·008	*S*	1·669	*M*
3·91	*S*	2·493	*S*	1·955	*M*	1·646	*M*
3·79	*M*	2·478	*S*	1·942	*M*	1·629	*M*
3·72	*S*	2·460	*S*	1·937	*M*	1·626	*M*
3·63	*M*	2·404	*S*	1·927	*M*	1·619	*M*
3·58	*M*	2·378	*M*	1·913	*M*	1·574	*M*
3·10	*VS*	2·342	*M*	1·867	*M*	1·523	*VS*
2·89	*VS*	2·299	*M*	1·858	*M*	1·494	*M*
2·814	*M*	2·288	*M*	1·814	*M*	1·482	*M*
2·800	*M*	2·260	*M*	1·789	*VS*	1·464	*S*
2·784	*S*	2·211	*M*	1·765	*S*	1·454	*M*
2·772	*S*	2·153	*M*	1·734	*M*	1·442	*M*
2·723	*VS*	2·147	*M*	1·720	*M*	1·422	*S*
2·708	*M*	2·075	*M*	1·705	*S*		

Although I hope to publish a more complete description of this work eventually, it seems desirable to present a summary of the available information which is given in Tables 1, 2 and 3.

This information may be particularly useful if it is found that analogous ternary compounds exist in which FeO substitutes for MgO and Fe_2O_3 for Al_2O_3, as seems not unlikely.

This work is subject to Crown copyright.

---

[1] Rankin, G. A., and Merwin, H. E., *J. Amer. Chem. Soc.*, 38, 568 (1916).

[2] Parker, T. W., Proc. Third Internat. Symp. on the Chemistry of Cement, London (1952), 487 (Cement and Concrete Association, London, 1954).

[3] Aruja, E., *Acta Cryst.*, 10, 337 (1957).

FORMATION OF CHALCOPYRITE BY REACTION BETWEEN CHALCOCITE AND PYRRHOTITE IN COLD SOLUTION

By W. M. B. ROBERTS

Bureau of Mineral Resources, Geology and Geophysics, Canberra

THE simple metallic sulphides are readily precipitated from solution by the sulphide ion. These sulphides have also been precipitated from solutions by the action of sulphate-reducing bacteria[1].

Up to the present, however, the complex sulphides have been synthesized only at higher temperatures either by the reaction of elemental sulphur, copper and iron, or by solutions containing one or more of the constituents acting on the metals or their sulphides. Roy[2] showed that bornite situated along grain boundaries in pyrite changes to chalcopyrite when heated at 100–175° C. for 3–5 days. Park[3] carried out experiments on copper compounds—his work on sulphides was concerned with passing steam, steam and carbon dioxide, and steam and hydrogen sulphide over chalcopyrite and chalcocite. Schouten's[4] work involved the action of concentrated aqueous copper solutions on various sulphides, and showed that chalcopyrite can form in a few hours on marcasite, pyrite, and pyrrhotite at atmospheric pressure and at about 100° C.

Gill[5] quotes results using solid blocks of simple sulphides of iron and copper at temperatures ranging from 350° to 650° C. for periods of 3–48 hr. Bornite and chalcopyrite were formed.

The present attempts at the formation of the complex sulphide differ from the previous work in that they were carried out at room temperature, and that either distilled water or sea-water played a part in the reaction.

It was decided to attempt the formation of the complex sulphide in this manner when, during the

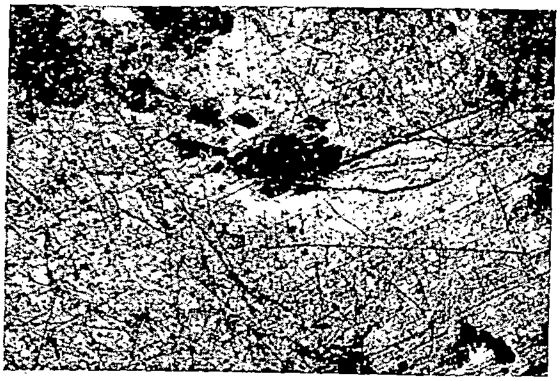


Fig. 1. Continuous coating of chalcopyrite on polished surface of chalcocite block (× 40)

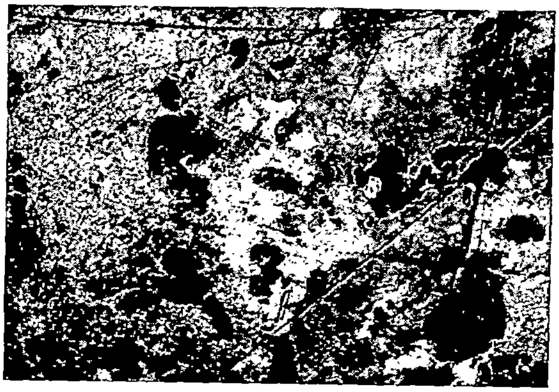


Fig. 2. Patchy coating of chalcopyrite on polished surface of chalcocite block. Chalcopyrite, dark grey; chalcocite, light grey (× 40)

course of preparation of blocks of sulphides for high-temperature synthesis, it was noted that, after about 5 min. contact between the wet ground faces of pyrrhotite (FeS) and chalcocite (Cu_2S), sufficient iron had been transferred to the chalcocite to give it a bronze colour on the ground surface. It was supposed that this iron had been removed from the surface of the FeS at an accelerated rate due to the presence of the water film at the interface, and that there was a possibility of a reaction with Cu_2S to form one of the copper iron sulphides.

Blocks about 1 mm. across were cut from naturally occurring chalcocite (Cu_2S), and pyrite (FeS_2), as well as from artificial pyrrhotite (FeS) made from iron filings and sulphur. A face was polished on these, and they were examined under an ore microscope to ensure that no other sulphides were present. Pairs of these minerals giving the combinations pyrrhotite–chalcocite and pyrite–chalcocite were held firmly together with their polished faces in contact, using clips made from heavy-gauge stainless steel wire.

A pyrrhotite–chalcocite and a pyrite–chalcocite pair were placed in a beaker of distilled water, and the same pairs placed in a beaker of artificial sea-water. Both beakers were allowed to stand at room temperature for five days. During this time it was observed that the pyrrhotite in both beakers formed a rim of hydrated iron oxide at the interface of the blocks within a few hours. After the first day a deposit of hydrated oxide was spread evenly over the bottom of both beakers. No apparent alteration took place on the exposed surface of the chalcocite blocks.

After five days the blocks were removed from their solutions and separated. The pyrite–chalcocite pairs in both sea-water and distilled water showed no reaction along the polished interface. The pyrrhotite–chalcocite pairs both had a brass-yellow deposit on the surface of the chalcocite. This deposit had covered the whole polished surface of the chalcocite from the distilled water (Fig. 1) but was patchy on the chalcocite from sea-water (Fig. 2).

In neither case was it found necessary to re-polish the chalcocite blocks for examination under the microscope; the new mineral had formed on the chalcocite, preserving the surface polish and scratches perfectly (Figs. 1 and 2). However, the surfaces of the pyrrhotite blocks had become etched, and had lost the original texture shown in the polished section (Figs. 3 and 4); but no new mineral had formed on their surfaces. The newly formed mineral was identified by X-ray diffraction as chalcopyrite ($CuFeS_2$). This mineral had not formed an intermediate layer between the two blocks—it had completely replaced part of the chalcocite; some of it had extended over the edges of the polished surfaces, along the sides of the chalcocite blocks. No other parts of the blocks exposed to the solutions showed any formation of chalcopyrite or any other change from the original material.

The depth of penetration of the chalcopyrite into the blocks of chalcocite was measured by cutting and polishing them in a plane normal to the polished surfaces. Microscopic examination at 1,100 magnifica-

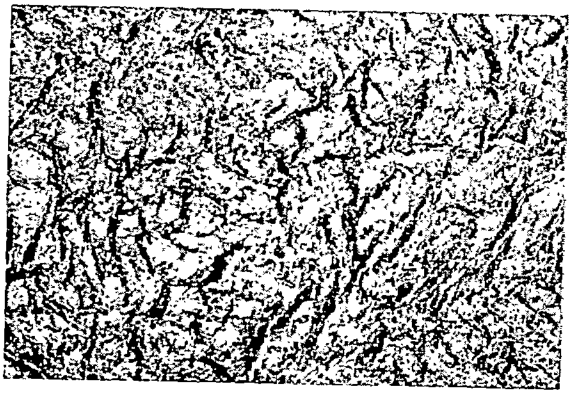


Fig. 3. Polished surface of pyrrhotite block before reaction (× 40)

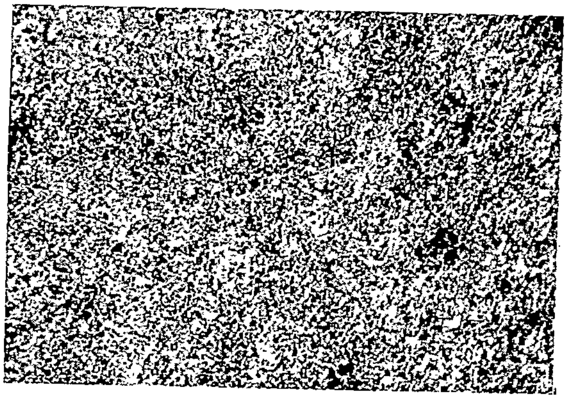


Fig. 4. Etched surface of pyrrhotite block after reaction (× 40)

tions showed that the penetration was highly irregular. It ranged from a sub-microscopic thickness to a depth of 0·11 mm. in the specimen placed in sea-water and to a depth of 0·13 mm. in that placed in distilled water. In one place chalcopyrite had formed to a depth of at least 1·5 mm. along a microscopic fracture.

It is of interest that, in these experiments, the iron moved into the copper sulphide, whereas Gill[5] found that the copper moved into the iron at a greater rate than the iron moved into the copper.

A block of chalcocite held in contact with a block of pyrrhotite for one month in an evacuated tube at room temperature showed a very faint brass-yellow tarnish on a small area of its surface, indicating that slight reaction had taken place even without the presence of a film of water between the blocks.

Some blocks of chalcocite were immersed in a cold solution containing ferrous ions, and another containing both ferrous and cupric ions, for ten days. No reaction was observed in any of the blocks. This seems to indicate that the reaction is not a result of general solution of iron from pyrrhotite and its subsequent reaction with chalcocite.

An electrolyte is evidently necessary for the reactions to proceed at a reasonable speed at room temperature. Both Cu_2S and FeS have very low solubilities:

Cu_2S, at 16° – 18° C.	2×10^{-47}
FeS, at 18° C.	$3{\cdot}7 \times 10^{-19}$

so that solution and recombination in the usual sense do not offer a reasonable explanation for the reaction because the quantity of $CuFeS_2$ is greater than could be expected to form from dissolved Cu_2S and FeS in the period involved in the experiment. Furthermore, a reaction was observed between Cu_2S and FeS even in the dry state. When the two sulphide blocks are brought into contact in water an electrolytic cell is formed, the e.m.f. of which, measured on a Cambridge *p*H meter, was found to be 0·51 V. This is responsible for the accelerated removal of Fe^{++} ions from the FeS and their transfer to the surface of the Cu_2S block to form the complex sulphide.

Both FeS and Cu_2S are capable of a high degree of non-stoichiometry. If non-stoichiometry is to persist in a crystal, electrical neutrality must be maintained—cation vacancies must be accompanied by a deficit of electrons to maintain neutrality—and therefore the degree of the non-stoichiometry is directly related to the diffusion properties as well as to the electronic properties of the crystals.

The behaviour of FeS in particular has been extensively studied, and it is known to be able to stand extreme departures from stoichiometry in the form of cation vacancies, without a change of phase. It would therefore be expected that where such great departures from stoichiometry are present they would be accompanied by a high diffusion constant.

Stone[6] has suggested a structure-sensitive diffusion by way of grain boundaries or Smekal cracks due to different mosaic structures in crystals. Barrer[7] gives some of the causes of this type of cracking in crystals along the surfaces of which diffusion can take place. As surface diffusion is much faster than volume diffusion it is possible that less activation energy is required for diffusion via cracks than via lattice vacancies.

Stone also suggests that the presence of impurities having different valencies from those of the normal crystal ions can have a marked effect in lowering the activation energy. If a divalent cation is substituted for a monovalent one in a crystal, electrical neutrality is maintained only by the formation of a cation vacancy. Cation vacancies of this type are present at all temperatures, and at the same concentration, and consequently their effect on diffusion will be much greater at lower temperatures at which there are fewer high-temperature vacancies.

The FeS used in this work is made up of randomly oriented small crystals the average size of which is 0·3 mm. The material has an irregular texture on the polished surface (Fig. 4), apparently the result of fractures developed during the cooling of an aggregate of anisotropic crystals. A semi-quantitative X-ray spectrographic analysis shows the impurities are: zinc, 0·5–1 per cent; copper, 0·1–0·5 per cent; lead, 0·1–0·3 per cent; arsenic, 0·05–0·1 per cent; manganese, 0·05–0·1 per cent; tin, trace; nickel, trace.

The Cu_2S used contains traces of nickel and iron as well as about 0·1–0·3 per cent bismuth. It is a polycrystalline aggregate having an average grain-size of about 0·6 mm. No cracks were observed on the polished surface at a magnification of 600, but the nature of the penetration of $CuFeS_2$ indicates that some diffusion has taken place along submicroscopic cracks as well as along grain boundaries.

The suggestions advanced by Stone suggest mechanisms by which low-temperature diffusion may take place. Both his conditions are fulfilled; these are the presence of impurities having a different valency and a large number of grain boundaries and fractures to act as channels for diffusion. In addition, the high degree of non-stoichiometry in these compounds enhances the possibility of diffusion at low temperature.

Once the iron has been removed from the FeS and brought to the surface of the Cu_2S, the complex sulphide will form because its lattice energy is greater than that of Cu_2S; the reaction goes in the direction where the free-energy change is negative.

Calculations of the unoccupied volume of the Cu_2S structure show that it has about 24 per cent unoccupied space compared with about 19 per cent for $CuFeS_2$. If the unoccupied volume bears an inverse relationship to the lattice energy, then it would be expected that $CuFeS_2$ would form at the expense of Cu_2S.

If this theory is valid, $CuFeS_2$ would form in preference to Cu_5FeS_4 (bornite), which has an unoccupied volume of about 23 per cent. Similarly its formation would be expected before the other copper–iron sulphides, cubanite ($CuFe_2S_3$) or valleriite ($Cu_3Fe_4S_9$), the unoccupied volumes of which are 22 and 41 per cent respectively.

I wish to thank Mr. L. L. Hawes of the Department of Chemistry, School of General Studies, Australian National University, for his helpful advice on some aspects of this work, and to acknowledge the help of G. J. G. Greaves, of the Bureau of Mineral Resources, in the study of the literature on sulphide synthesis. The photographs were taken by J. Zawartko, of the Bureau of Mineral Resources.

This work is published by permission of the Director, Bureau of Mineral Resources.

---

[1] Baas-Becking, L. G. M., *Econ. Geol.* (in the press).

[2] Roy, J. E., *Econ. Geol.*, **25**, 433 (1930).

[3] Park, C. F., *Econ. Geol.*, **26**, 857 (1931).

[4] Schouten, C., *Econ. Geol.*, **29**, 611 (1934).

[5] Gill, J. E., *Report Twenty-first Sess. Int. Geol. Cong., Copenhagen*, Part 16, 209 (1960).

[6] Stone, F. S., *Lattice Defects in Ionic Crystals—Chemistry of the Solid State*, 20 (Butterworth, 1955).

[7] Barrer, R. M., *Diffusion in and through Solids*, 313 (Cambridge, 1951).

CRYSTALLIZATION KINETICS IN POLYVINYLCHLORIDE AND THERMOELASTIC RELAXATION INDUCED BY ULTRASONICS

By Dr. V. NARDI

Istituto Nazionale di Fisica Nucleare, Istituto di Fisica dell Università, Padova

It is still an open problem to determine with certainty the molecular characteristics governing the crystallization kinetics in long-chain polymers. The importance of different elements has been recognized, such as length of repeating unit in the chain, kind and dimension of lateral substituent groups, geometrical irregularity in which they can be placed along the chains, etc.[1]. As Kitaigorodski's work[2] on monomeric organic crystals suggests, the molecular packing efficiency also has to be considered. It may be negligible for molecules with a very awkward shape, and crystallization will not occur. The packing efficiency depends also on the stereochemical regularity of the molecules. On the other hand, it has been observed[1] that this efficiency can be lower in the regular arrangements for any shape of molecule, so that dearth of packing efficiency remains a not very convincing cause of failure to crystallize. Furthermore, there are polymers in which the chain carbon atom carries two lateral groups of different size, for example, polyvinyl alcohol (—CH_2CHOH—$)_n$, and these reach crystalline form easier than other polymers with the same deficiency in regularity of position, along the chain, of substituent atoms having almost equal size. Besides the approximate criterion requiring, in connexion with substituent group sizes, some geometrical regularity in their position in space, it is not possible to determine whether a long-chain polymer will crystallize at all, and if so, how readily.

It seems reasonable, therefore, that for polymers regarded as non-crystalline, the crystallization is not really impossible, but only the right conditions to crystallize are not found during the preparation of the material. I have used polyvinylchloride (—CH_2—$CHCl$—$)_n$ in the form of 500 per cent stretched fibre with high homogeneity characteristics (molecular weight approximately 60,000) in order to see whether the amount of crystalline material, normally very low (about 10 per cent before stretching, about 40 per cent after stretching in hot water and suddenly quenched), can still be increased in drawn material and in order to get information on the rate of this transformation.

In these fibres, assuming that crystallization is favoured especially by the parallel orientation of molecules induced by the drawing process, there is no reason to assume that chain folding is the normal case[3], and I shall not consider the presence of morphological structures like spherulites, the crystallinity comprising crystallites (or fringed micelles) embedded in the amorphous material and oriented with the carbon chains along the fibre axis. The fibre has been radiated with 1 Mc./s. ultrasonics for different time-intervals t and different temperatures T_B of the water-bath in which the samples were placed during the radiation. For an equal radiation time t, the greatest increase in crystallinity has been found at $T_B \simeq 90°$ C., that is, the temperature where attenuation measurements show a maximum in ultrasonic absorption by the polymer[4]. (I have not carried out systematic measurements above this temperature.)

In Fig. 1 the observed increase is plotted in percentage weight of crystalline material versus the radiation time t ($T_B = 90°$ C.) as measured by X-ray analysis. Detailed information has already been given[5,6] on these measurements and on appreciable effects on the mechanical and thermoelastic properties of fibre due to this increase in crystallinity[7].

I shall discuss here briefly what kind of useful information about the crystallization kinetics one can get from a knowledge of relaxation phenomena inside the fibre and from the growing modality of crystallite under ultrasonic radiation. Information and references concerning these phenomena in polycrystalline solid material are given in ref. 8. I should like to mention the importance of the thermoelastic relaxation at the frequency used here. At this frequency the molecular motions are taking place between the isothermal limit and the adiabatic limit, and flow of heat between adjacent crystallites arises from internal friction. The ultrasonic radiation induces strains which are macroscopically homogeneous ($\lambda_{us.} \simeq 0{\cdot}2$ cm.). The elastic anisotropy of single crystallites embedded in the amorphous material, and their irregular and discontinuous arrangement, present also in this case of strong orientation along the fibre axis, renders it almost impossible for these

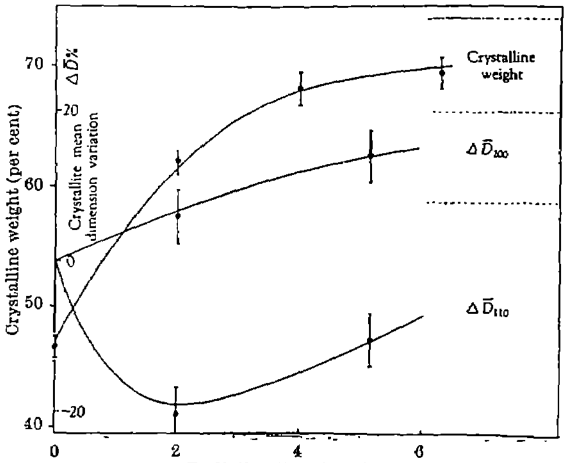


Fig. 1. Vertical axis left-hand scale gives crystalline weight per cent; the right-hand scale gives the change $\Delta\bar{D}$ per cent in mean crystallite dimensions $\bar{D}_{200}$ and $\bar{D}_{110}$, respectively, in the direction normal to the (200) and (110) planes, versus time of radiation. Dotted lines represent the limiting values for each quantity considered as obtained by fibre annealing and shrinkage at 150° C. in water

strains to be also microscopically homogeneous. Beside the flow of heat associated with these stress fluctuations, one has to consider also irreversible displacements in crystallite positions. These displacements can play a leading part in the crystallization process.

In order to account for the experimental observations on microscopic structure, one has to accept as true that inside the amorphous material many chain segments, all adjacent and already ordered parallel by stretching, have only a very low propensity to assume a packed configuration and consequently to crystallize. The reason for this, apart from molecular irregularity, is due I think to the fact that the movements of chain segments are hindered by the very low mobility of crystallites from which these segments ramify. The mutual distances between chain segments cannot be reduced by thermal oscillations, which do not affect the crystallite position sufficiently. From this description it seems clear why the 'effective' packing efficiency depends in a non-uniform way on regularity of chain structure, as the analysis of different polymers shows. In fact, some polymers may have, inside an amorphous region, almost parallel chain segments with quite regular structure and lateral groups of equal dimensions, but without the ability to move and, therefore, to form new nuclei, because of the very high friction associated with the smallest displacement of the binding crystallites. The chain segments will remain frozen in the positions assumed in the fibre production process, and crystallization will be arrested at low values for any interval of some limited time-scale of observation. In other polymers, also with different dimensions of lateral groups or some irregularity in their disposition along the chain, the friction for crystallite displacements may be very low, so that the relaxation processes bringing the chain segments to a close-packed configuration take place in a short time.

Accepting this picture, the ultrasonic action through change in local stress and temperature may be very relevant, as we have observed in polyvinylchloride. The rate of generation of new nuclei and their further growth strongly increase with the energy losses of the ultrasonic field. In polyvinylchloride the difference in substituent group dimensions is large (the Van der Waals's radii of hydrogen and chlorine atoms are respectively 1·10 Å. and 1·70 Å.). Atacticity, always present in a measure depending on the polymerization process used, may also prevent crystallization. On the other hand, the possibility of changing the degree of crystallinity to a very large extent, suggests that a more important element is the chain mobility and, therefore, the possibility for crystallites to realize irreversible displacements. This point of view is quite well supported by the experimental observation that, allowing the stretched polyvinylchloride fibres to recover their initial length in a thermal bath above the glassy transition temperature T_g, the amount of crystallinity may still increase up to 80 per cent in weight[6].

In Fig. 2 the general behaviour of the probability of nucleus generation, $P = \text{const.}\exp(-\Delta G/KT)$, is plotted versus temperature between the melting point T_m and the II-order temperature T_g. The width of the interval T_gS depends on chain mobility, strongly affecting the Gibbs free-energy variations ΔG. For chain segments inside the amorphous region, bound to not appreciably mobile crystallites, their mobility is very much reduced and the same applies to the probability of nucleus formation.

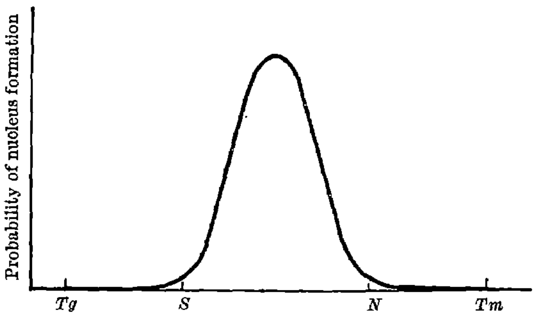


Fig. 2

Such a strong influence of very low crystallite mobility on that of the amorphous chain segments, which constitutes the essential point in the microscopic model assumed here, may also operate above T_g, where the polymer is in a rubber-like state, provided that the fibre has bounded ends, has been produced during ultrasonic radiation. In such a way fibre retraction is prevented; low mobility and strong orientation of chain segments in amorphous and crystalline regions are conserved. The thermoelastic relaxation induced by ultrasonics explains quite well the increase of crystallization with radiation time. If it is true that this relaxation governs the increase of crystallization with time, it becomes clear enough how the molecular characteristics can influence the crystallization process. They are essentially contributing to the same extent that the crystallite mobility and, consequently, that of the chains, is affected.

One has to observe that in the picture now presented, it is misleading to accept for the chain segments of the amorphous region, the possibility that all configurations consistent with the chain structure itself is approached. As a consequence, one has to change appreciably the evaluation of the configuration factor used in the Flory–Huggins theory and in the one-phase model of Frith and Tuckett[9], in order to determine the thermodynamic potentials.

In a general case, the generation of new nuclei may be described as taking place by two steps: in the first a parallel arrangement of chain segments is reached with high changes in entropy ΔS; in the second, the chains pack through small displacements, a monomer unit length at most, along their axis, in a configuration having a minimum internal energy U.

For stretched fibres, the parallel configuration is approached everywhere to a large extent, and the entropy contribution to the free-energy changes $\Delta F = \Delta U - T\Delta S$, originated by crystallization, is not appreciable ($\Delta S \simeq 0$). The increase in pressure and the relaxation phenomena induced by ultrasonics allow the energy barriers separating the amorphous from the crystalline configuration to be overcome. The possibility of new nuclei generated in already partially crystalline fibres must depend also on the extension of the amorphous regions, all interconnected, but approximately delimited by surrounding crystallites as set up in the stretching process. If the number of parallel chain segments in some of these regions is too low, no transformation of

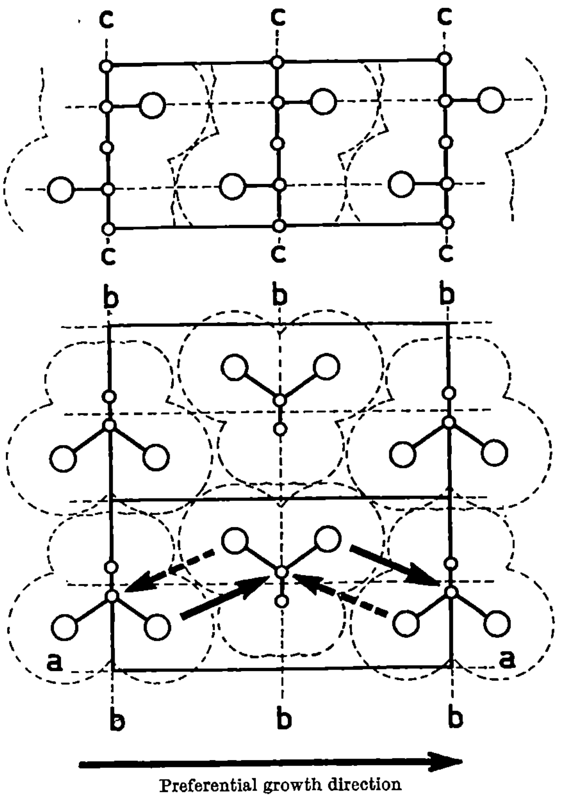


Fig. 3. Assumed crystalline structure with the axis $a = 10 \cdot 6 \pm 0 \cdot 1$, $b = 5 \cdot 4 \pm 0 \cdot 1$, $c = 5 \cdot 1 \pm 0 \cdot 1$ Å. A pair of strongly interacting dipoles is connected with → (in a different (00*k*) plane with respect to the other pair connected with - - -→)

embrions in new nuclei is possible and the crystallization will advance only by further growth of the confining crystallites. If the segment number is high enough, the variation in the Gibbs thermodynamic potential of n chain-segments changing to crystalline form, $\Delta G = Au^{2/3} + Bn$, may have the surface contribution $An^{2/3}$ smaller than Bn. In this case, a nucleus may be formed, and it also will grow after ultrasonic radiation stops and with quenching below T_g, when the relaxation in the neighbouring binding crystallites is extremely low.

Interesting information on this point has been obtained[4] by measurements based on X-ray analysis. I assume that polyvinyl chloride crystallizes in the orthorhombic system *Pacm* as has been shown by Natta and Corradini[10], according to the scheme shown in Fig. 3.

From the integral line-breadth of the observed reflexions, the mean width of the crystallite $\overline{D}_{200}$, in the direction normal to the (200) plane, increases with radiation time t. On the contrary, the mean width $\overline{D}_{110}$ decreases with radiation even if some recovery may be observed for high values of the radiation time. These results, with the increase in crystalline material, indicated in Fig. 1, suggest the conclusion that, under ultrasonic action, the polymer crystallizes both with formation of new crystallites and growth of those already present. The evidence that crystallization takes place preferentially in the direction normal to the (200) plane (along the a-axis), as I have often observed, is very important, in order to understand what kind of intermolecular force is the most influential in this process. One may explain the existence of this preferred direction by considering the strong forces between carbon–chlorine dipoles of neighbouring chains. In fact, the preferential growth of crystallites along the a-axis offers the possibility of packing the greatest number of dipoles with electric moment orientation of smallest potential energy, the opposite poles of two neighbouring chains being at the shortest distances in the assumed crystalline structure.

Considering that $\overline{D}_{110}$ is always smaller than $\overline{D}_{200}$ ($\sim$ 50 Å. and $\sim$ 60 Å. respectively) in the oriented or isotropic fibre states as obtained by stretching and shrinkage, this indicates clearly that in polyvinyl chloride crystallization the C—Cl dipole–dipole interaction is prevailing over the other Van der Waals's forces.

I thank Dr. M. Mammi, of Centro di Strutturistica Chimica del C.N.R., University of Padua, for essential help given in the analysis of the X-ray patterns carried out in that Institute and for many discussions. Thanks are due also to Dr. F. Smeraldi, technical director of Montecatini-Polymer, who has kindly furnished the polyvinyl chloride fibre prepared under known and reproducible conditions.

[1] Bunn, C. W., *J. App. Phys.*, **25**, 320 (1954).
[2] Kitaigorodski, A., *Acta Physicochimica, U.S.S.R.*, **22**, 309 (1947).
[3] Stuart, H. A., *Ann. New York Acad. Sci.*, **83**, 1 (1959).
[4] Maeda, Y., *J. Polymer Sci.*, **18**, 87 (1955).
[5] Nardi, V., and Pisent, G., *Proc. Third Intern. Cong. Acoustics* (Elsevier, Pub. Co., Amsterdam, 1959).
[6] Nardi, V., and Pisent, G. (in the press).
[7] Nardi, V., Italian Patent No. 20753 (2-12-1960).
[8] Kittel, C., *Reports on Progress ir Physics*, **11**, 205 (1947).
[9] Stuart, H. A., *Die Physik der Hochpolymeren*, **3**, B, 543 (1955).
[10] Natta, G., and Corradini, P., *J. Polymer Sci.*, **20**, 251 (1956).

INTERACTION OF LIPIDS AND TRYPSIN IN DIMETHYL SULPHOXIDE-WATER SYSTEMS

By Prof. A. LUKTON, H. DONOHUE and Prof. F. A. BETTELHEIM

Chemistry Department, Adelphi College, Garden City, New York

THE nature of the binding sites and binding forces between lipids and proteins is virtually unknown. The attempts that have been made to create model systems for the investigation of this binding have been hampered by solubility limitations. All the systems examined were heterogeneous and included: lipid colloidally dispersed in aqueous protein solution in the presence[1,2] or absence of dispersing agents[1,3], and lipid adsorbed on an inert solid surface and suspended in an aqueous protein solution[4]. The recent use of various non-aqueous lipid solvents in solvating proteins such as dimethyl sulphoxide

(DMS) and formamide[5], affords an opportunity of analysing lipid–protein complexing in a homogeneous system.

We have chosen trypsin for this work. It is particularly suitable because it has a relatively high solubility in water and in DMS and can be transferred from DMS to water with practically no loss in activity[5]. This transfer is necessary because the trypsin esterase activity can be determined only in aqueous medium. Preliminary efforts to demonstrate lipid–protein binding in DMS by observing changes in absorption spectra were unsuccessful. Other attempts to demonstrate strong lipid–protein interaction in DMS also failed. These included a continuous dialysis of lipid–protein DMS mixture against DMS and extraction of the mixture with petroleum ether. In each of these cases the lipid was removed easily. The only indication of an interaction between protein and lipid in DMS was the apparent decrease in enzyme activity.

Four different experiments were performed in observing the inhibition of enzyme activity by lipids:

(*a*) Enzyme and lipids were mixed simultaneously as follows: 3 mgm. of crystalline enzyme (Worthington) were dissolved overnight in 10 ml. of freshly distilled DMS. An 0·5 ml. aliquot of this solution was mixed with 0·5 ml. of a DMS solution of a lipid in concentrations varying from 0 to 4×10^{-3} *M* in DMS. The lipid–enzyme mixture was then added with stirring to 50 ml. of 0·02 *M tris* buffer at *p*H 7·90 which was 0·01 *M* in calcium chloride and 0·04 *M* in sodium chloride. Trypsin esterase activity was then determined by the usual procedure using benzoyl arginine ethyl ester as substrate.

The lipids used were stearic acid and a variety of carotenoids which were kindly supplied by Hoffman–La Roche, Nutley, New Jersey. The carotenoids were chosen to provide functional groups of differing polarity and because of their colour to make observations easier. Included were beta-apo-8′-carotenal *trans*-bixin, *cis-nor*bixin and vitamin *A*.

(*b*) A filtration experiment was performed as follows: Solutions were prepared and mixed as in (*a*). Before trypsin esterase activity was determined, however, the solution was filtered through a fine-pore (5μ) sintered-glass filter. All the colour was removed by this procedure.

(*c*) Mixing afterwards of lipid and enzyme: Solutions were prepared as in (*a*) except that the trypsin in DMS was added to the buffer solution followed by the addition of lipid in DMS.

(*d*) Later mixing of enzyme and colloidal carotenoid: Trypsin dissolved in DMS plus an equal volume of solvent were added to the buffer solution. This was followed by the addition of an aqueous colloidal solution of carotenoid which was prepared by introducing an acetone solution of the carotenoid into buffer and then evaporating the acetone *in vacuo*. The same final concentrations of carotenoid as in (*a*) and (*c*) were used.

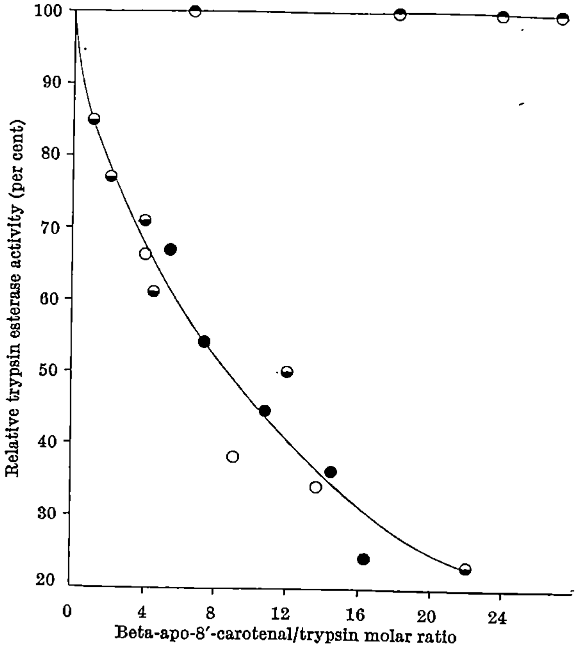


Fig. 1. Trypsin esterase activities at different beta-apo-8′-carotenal to trypsin molar ratios; ◓, subsequent addition of beta-apo-8′-carotenal in DMS to trypsin in DMS dispersed in buffer; ◒, simultaneous addition of beta-apo-8′-carotenal and trypsin in DMS to the buffer, first experiment; ○, same, second experiment; ●, simultaneous addition of beta-apo-8′-carotenal and trypsin in DMS to buffer and subsequent filtration

The results are summarized in Fig. 1 and in Table 1. Among the carotenoids examined the greatest inhibition was observed with beta-apo-8′-carotenal. In Fig. 1, one can see an increase in inhibition with increasing lipid to protein molar ratio which follows a logarithmic course. This occurs only when the lipid and enzyme are premixed in the solvent and, therefore, added together to the buffer. On the other hand, on additions afterwards of lipid in the solvent or the addition of lipid in colloidal aqueous suspension to the buffer into which trypsin in the solvent had been transferred before, there was no apparent reduction in activity. It is evident from the experiment in which the solutions were filtered after addition at the same time of lipid and enzyme to the buffer, that the filtrate showed approximately the same activity as that of the unfiltered material. Therefore, this indicates that the decrease in activity was the result of an aggregated lipid–protein complex of larger than colloidal size, making the enzyme unavailable for reaction. This was confirmed by experiments in which the precipitated complex was isolated in larger quantities. Only a small quantity (about 10 per cent) of the original activity was found in these complexes. At high lipid enzyme molar ratios, the activity determination became more erratic because of the fact that large amounts of enzyme were removed resulting in less accuracy. Other lipids inhibit trypsin esterase activity in a manner similar to that of beta-apo-8′-carotenal. In Table 1, the lipid to enzyme molar ratios required to reduce the enzyme activity to one-half of that in the absence of lipids are given. One can see a direct relationship between the extent of inhibition and the polarity of the lipid molecule[6].

Because of the relationship noted between the polarity of the lipid and extent of enzyme inhibition, activities were determined at various ionic strengths. Solutions of the buffer at *p*H 7·90 were prepared by varying the sodium chloride concentration to give values of ionic strength ranging from 0·065 to 0·50. Three different concentrations of beta-apo-8′-carotenal representing low, intermediate and high

Table 1. MOLAR RATIO OF LIPID TO PROTEIN AT 50 PER CENT RESIDUAL ESTERASE ACTIVITY

Lipid	Functional groups	Molar ratio
Beta-apo-8′carotenal	−CHO	8·3
Trans-bixin	−COOH, −COOCH,	11·8
Stearic acid	−COOH	15·0
*Cis-nor*bixin	−COOH, −COOH	20·0
Vitamin *A*	−OH	66·7

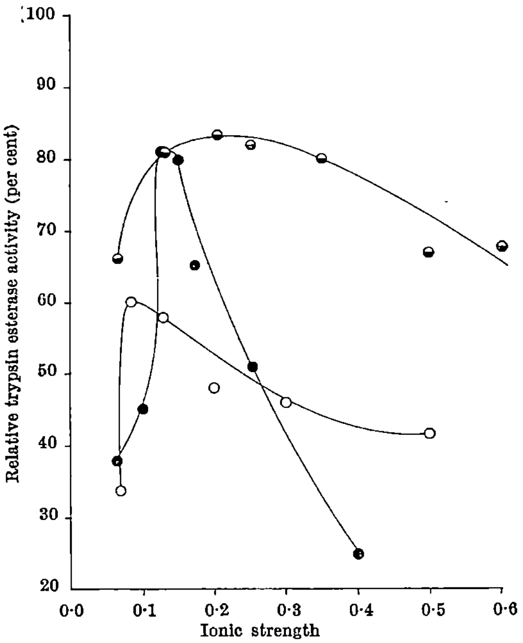


Fig. 2. Relative trypsin esterase activity at different ionic strengths and at different beta-apo-8′-carotenal to trypsin molar ratios; ◒, molar ratio 4 : 1; ●, molar ratio 9 : 1; ○, molar ratio 13·5 : 1

inhibition effects (beta-apo-8′-carotenal/trypsin molar ratio of 4 : 1, 9 : 1, 13·5 : 1) were examined in solutions of various ionic strengths. Trypsin esterase activity was determined as in 1(*a*).

The results are shown in Fig. 2. One can see two main features of the graphs: (*a*) the curves show maxima which appear at lower ionic strengths the higher the beta-apo-8′-carotenal/trypsin molar ratio; (*b*) the activities at an ionic strength of 0·065 followed the results of previous observations, that is, the activities decreased in a logarithmic fashion with the increasing beta-apo-8′-carotenal to trypsin molar ratio. A corollary to this is that the activity values at the maxima of the curves show the same trend.

This indicates that the interaction between protein and lipid is between the polar groups of the molecules or of the aggregate surfaces. At every molar ratio tried, a maximum appears in the ionic strengths of the medium versus activity curve. One may interpret this as two processes competing with each other. The first process which caused a rise in the relative activity with increasing ionic strength can be understood as the depression of interaction between the ionized surface of protein and the polar groups of lipid. One may interpret the second process, in which the activity decreased with increasing ionic strength, as the progressive aggregation of colloidal particles, that is, at high enough ionic strengths the depression of the charges on the protein surface not only decreased the interaction between the lipid and protein, but it also may have decreased the electrical double layer around the colloidal particle, thus enhancing aggregation.

The maxima in the activity curves, therefore, indicate the ionic strengths at which the second process starts to dominate the first. The fact that an inverse relation existed between the lipid to protein ratio and the ionic strength at which the maxima occurred supports this hypothesis.

It would appear that the decrease in enzyme activity was due chiefly to the aggregation of the lipid–protein complexes to larger than colloidal sizes.

We thank Dr. S. Vratsanos for many helpful discussions. This research was partly supported by a grant (*C*–3984 *BBC*) of the National Cancer Institute, U.S. Public Health Service.

[1] Karrer, P., and Strauss, W., *Helv. Chim. Acta*, 21, 1624 (1938).
[2] Sarda, L., Marchis-Mouren, G., and Desnuelle, P., *Biochem. Biophys. Acta*, 24, 425 (1957).
[3] Traitskiiand, G. V., and Tarasova, L., *Biokhimiya*, 20, 19 (1955); *Chem. Abst.*, 49, 9120 (1955).
[4] Avigan, J., *J. Biol. Chem.*, 234, 787 (1959).
[5] Rees, E., and Singer, S., *Arch. Biochem. Biophys.*, 63, 144 (1956); Vratsanos, S., *Arch. Biochem. Biophys.*, 90, 132 (1960).
[6] Smyth, C. P., in Weissberger, A., *Physical Methods of Organic Chemistry*, 1, Part 2, 1614 (Interscience Publ. Inc., New York, 1949).

RIGIDITY FACTOR OF GELATIN GELS

By Dr. AUDREY TODD
British Gelatine and Glue Research Association, 2a Dalmeny Avenue, London, N.7

PREVIOUS work[1-3] on gelatin fractions has disclosed an unidentified structural feature, termed the 'rigidity factor', which affects the rigidity of gelatin gels. Thus, it has been shown that variations between the rigidities of gels, set and matured either at 0° C. or 10° C., from different gelatin samples are related neither to differences in their molecular weights[2,3] (above a limiting viscosity number[4] $[\eta]$ in molar sodium chloride at *p*H 7 of about 40; that is, $\bar{M}_{\mu} \sim 115{,}000$ (ref. 5)) nor to possible differences in the average number of component chains per molecule[6]. Moreover, the considerable variation in gel rigidity (at high molecular weights) which can occur for gelatins derived by successive extraction from the same pretreated collagen is not accompanied by any apparent change in amino-acid composition[7]. The rigidity factor is reduced during thermal degradation in neutral or alkaline solutions[3]; but it is virtually unaffected by degradation in acid solution[3] or with enzymes or ultrasonics[8]. The present investigation was designed to discover whether the rigidity factor is related to the chain configuration, which would be reflected in the optical rotation of the gel.

Three gelatin samples of widely varying rigidity factor, but of the same high molecular weight and similar in chemical composition, were chosen for this investigation. Two of them (Nos. 76 and 127) were good-quality commercial lime-processed ox-hide gelatins; and the third (*AT*/1*a*), of very high rigidity, was prepared in the laboratory, as a neutral first extract from demineralized ossein (from the mixed bones of Indian cattle), by adaption of the accelerated alkaline pretreatment process of Ward[9]. The three gelatin samples would not differ significantly in amino-acid composition, since they were all derived from the same species of mammal[10]; and they would exhibit the low isoionic point $pI \sim 5$ typical of gelatins prepared by an alkaline process[11], corresponding to the conversion of most of the amide groups of the glutamine and asparagine residues of collagen to carboxyl groups[12].

Optical rotation and rigidity measurements were carried out on gels, from these three samples, which had been prepared under identical conditions and carried through exactly the same schedule of changes in temperature. Each gelatin sol, containing 5·5 gm. protein/100 ml. at pH 7, was filtered through a No. 3 sintered-glass funnel and used to fill either a 1-dm. jacketed polarimeter tube or a type A rigidity tube[13] at 40° C., and then quenched rapidly to 0° C. and left to mature overnight. Readings were taken at 10-min. intervals over a period of 23 hr. 15 min.–24 hr. 45 min. at 0° C., so that the mean of the 10 readings corresponded to 24 hr. maturing at 0° C. The temperature was then raised as quickly as possible (during 30 min.) in 10 deg. C. stages, taking care never to exceed the required temperature. Readings were taken at each temperature, particularly (10-min. intervals) over the 1½-hr. period corresponding to a mean of 3 hr. at 10, 20 and 30° C., and to 65 min. at 40° C. It was found in all cases that, over these 1½-hr. periods of measurement from 0 to 30° C., the optical rotation, as well as the rigidity[8], was changing very slowly and (within experimental error) linearly with time, so that the means of these observations were adequate for comparative purposes. At 40° C. there was no detectable change in optical rotation over the period of measurement. All temperatures were controlled to within ± 0·1 deg. C.

The optical rotation measurements were made for the sodium D line using a Bellingham and Stanley model A visual polarimeter with a screened light source in a dark-room at a half-shade angle of 8°. The glass scales were illuminated weakly by 2·5-V. torch bulbs set behind ¼-in. green 'Perspex', so that the dark adaptation of the eye was not destroyed between successive measurements. Zero readings were obtained by washing and refilling the polarimeter tube with distilled water (via the thermometer side-arm), without disturbing the end-plates, and repeating the measurements at each temperature. Following the suggestion of Bates *et al.*[14], condensation on the end-plates at temperatures below the dew-point was avoided by the addition of 'Perspex' chambers, containing silica gel and closed by a second pair of glass windows, to the ends of the polarimeter tube. To facilitate rigidity measurements on the weaker gels, carbon tetrachloride coloured with a small amount of a solid dye, 'Waxoline Red *O.S.*', was used instead of mercury[13] as the capillary fluid, and a dual system of manometers, one containing mercury and the other carbon tetrachloride, was employed to obtain and to record the required wide range of applied pressures. Protein concentrations at 40° C. were determined in duplicate by evaporating 20-ml. portions of the excess gelatin sol to constant weight in an oven at 105° C.; the residues were then corrected for the weights of inorganic material present by moistening them with concentrated sulphuric acid, charring them over a low flame, and igniting them in an oven at 550° C. for 2 hr.

The optical rotation measurements are shown in Table 1 and Fig. 1. From the results for duplicate runs on gelatin 127, the coefficient of variation of the specific rotation $[\alpha]_D$ is less than 1 per cent from 0 to 30° C. and only about 0·15 per cent at 40° C. Therefore, the differences between the specific rotations of the three gelatins are significant at each temperature. Sample 76/2/1 was a 50 per cent top fraction of an 87 per cent middle fraction obtained from gelatin 76 by coacervation with ethanol[15]. The specific rotation results for 76/2/1 are not significantly different, at any temperature, from those of the original gelatin 76 indicating that, at least for material of relatively high average molecular weight, the differences in molecular weight distribution are unimportant.

Table 1. SPECIFIC ROTATION OF GELATIN GELS AND SOLS

Gelatin No	76	76/2/1	127		*AT*/1*a*
$[\eta]$ in M NaCl, pH 7, 40° C.	61	63	64		62
Conc. gm. protein/100 ml.	5·591	5·452	5·461	5·504	5·365
pH	6·85	7·88	6·94	6·89	6·98
$-[\alpha]_D$ at 0° C.	301·9	306·5	329·3	326·9	334·1
10° C.	269·9	269·1	302·8	304·2	315·3
20° C.	208·7	212·0	257·3	255·9	264·8
30° C.	150·1	148·6	163·0	161·0	174·7
40° C.	141·3	141·4	144·2	143·9	144·6

The corresponding rigidity measurements for the three gelatins are given in Table 2. A slight increase of the apparent rigidity modulus G (that is, shear stress/shear strain) with strain was observed for some of the gels, and so measurements for all the gels were made at the same small maximum strain (at the tube wall) of 0·15 (or interpolated graphically to this value). Thus, although the values of G recorded in Table 2 are not true rigidity moduli, they should be close enough to the constant theoretical elastic moduli for comparative purposes[13].

When the specific rotations in Table 1 were plotted against the apparent rigidity moduli in Table 2, for the same gels after exactly the same treatment, the points lay on a single smooth curve, showing that $[\alpha]_D$ and G are closely related. In Fig. 2, a linear correlation is seen to exist between $-[\alpha]_D$ and $G^{\frac{1}{2}}$, where the values of G in Table 2 have been corrected

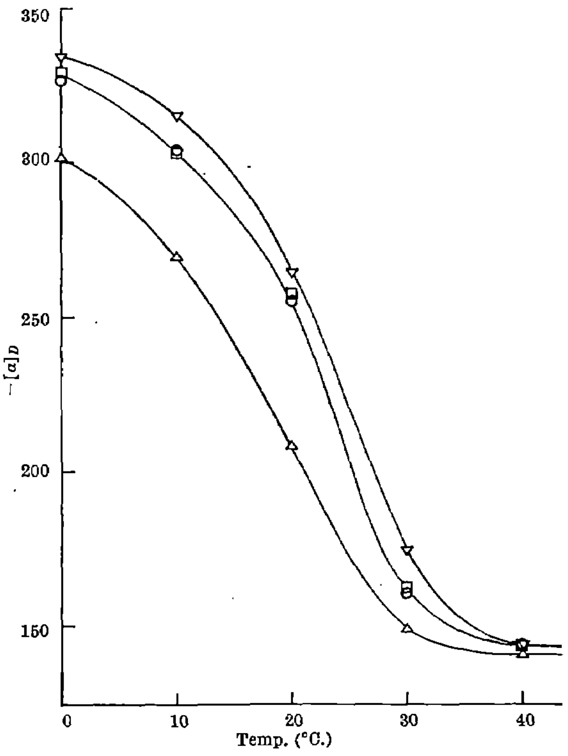


Fig. 1. Variation of specific rotation of three samples of gelatin: ▽, *AT*/1*a*; □, 127 run 1; ○, 127 run 2; △, 76

Table 2. Rigidity of Gelatin Gels

Gelatin No.	76	127		*AT*/1*a*
Conc. gm. protein/100 ml.	5·416	5·499	5·488	5·160
*p*H	6·95	6·96	6·97	6·98
G dynes/cm.^{2} at 0° C.	71,070	108,380	108,580	119,960
10° C.	45,910	72,230	72,180	80,370
20° C.	12,360	36,310	35,040	43,340
30° C.	~ 6	~ 19	~ 14	188

to a concentration C of 5·50 gm. protein/100 ml. (taking $G \propto C^2$, over at least a small range of C, from the work of Ferry[16] and Ferry and Eldridge[17]).

The conditions of these experiments (that is, gelatins of similar chemical composition at the same concentration and *p*H) were chosen so that the contributions to the optical rotation from the individual amino-acid residues and from environmental factors were as nearly constant as possible at each temperature. The fairly close values of the specific rotation for sols of the three gelatins at 40° C. (Fig. 1), where the molecules are present as random coils[18], is good evidence that this constancy was virtually attained. Hence, the increase in magnitude of the specific rotation for the gels must be, essentially, a measure of the amount of regular structure or chain folding in the gel phase (providing that only one type of folding is present, and that there is no appreciable variation in environmental contributions as the temperature falls). Thus, it follows from the correlation in Fig. 2, that the rigidity modulus of a gelatin gel, for a given concentration, is also determined by the amount of chain folding, and the 'rigidity factor' defines the capacity of a gelatin molecule to fold in a regular manner.

There is substantial evidence, from X-ray diffraction work[19] and from the similarity of the melting temperatures for mixtures of collagen or gelatin with ethylene glycol[20], that this regular folding in gelatin gels is the same type of triple helix present in native collagen[21]. The unusual negative chain-configurational contributions to the specific rotation given both by gelatin gels[22,23] and by soluble collagens[23] are also consistent with this view. An isotropic gelatin gel must, therefore, contain lengths of randomly orientated triple helix (formed from portions of three gelatin molecules), which bind the molecules into a network and confer stiffness on the structure. There appears, at present, to be no theoretical model for this type of structure. Qualitatively, however, its mechanical behaviour would be expected to resemble that of a frozen unstrained rubber inasmuch as the modulus of the unorientated crystallites (or helices in the gel) would be superimposed on that of the amorphous network[24]. It is not yet known whether a higher rigidity factor corresponds to more, or to longer, triple helices.

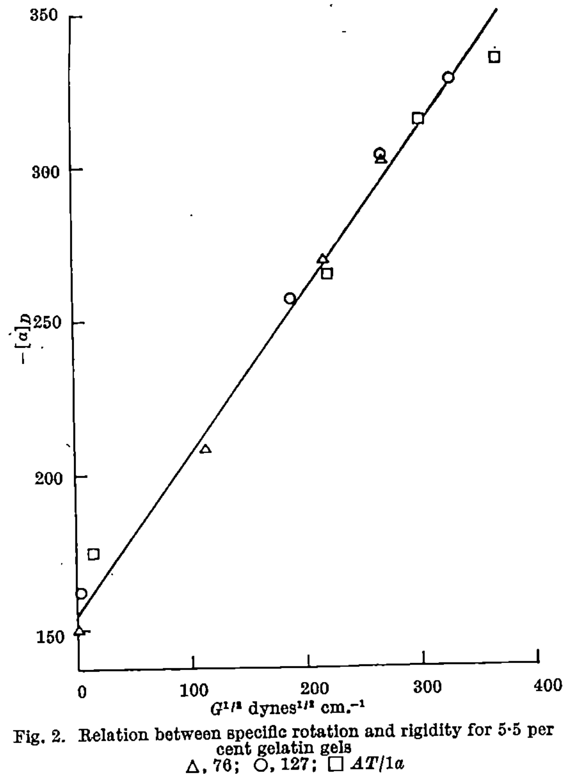


Fig. 2. Relation between specific rotation and rigidity for 5·5 per cent gelatin gels
△, 76; ○, 127; □ *AT*/1*a*

Although it has been established that the rigidity factor of a gelatin gel measures the facility of the molecules to take part in reforming the collagen triple helix, the precise nature of the structural feature which is responsible remains obscure. Further evidence that the molecular structure of the three gelatin samples is not completely identical is shown by the small but significant differences between the specific rotations of the sols at 40° C. in the absence of chain order. It is possible that this small variation in the contributions to the specific rotation from the individual residues is due to subtle differences in amino-acid composition which are not detectable by the present experimental techniques. A more likely explanation, however, is that differences in the mutual interactions of the individual residues are produced by changes of amino-acid sequence along the gelatin chains: the rigidity factor would then be determined by the proportion of residues in sequences capable of forming the collagen fold.

[1] Ward, A. G., *Nature*, **171**, 1099 (1953).
[2] Stainsby, G., Saunders, P. R., and Ward, A. G., *J. Polymer Sci.*, **12**, 325 (1954).
[3] Saunders, P. R., and Ward, A. G., *Nature*, **176**, 26 (1955).
[4] Stainsby, G., *Nature*, **169**, 662 (1952).
[5] Wootton, J. (to be published).
[6] Courts, A., *Biochem. J.*, **73**, 596 (1959).
[7] Eastoe, J. E., *Biochem. J.*, **61**, 589 (1955).
[8] Saunders, P. R., and Ward, A. G., *Recent Advances in Gelatin and Glue Research*, edit. Stainsby, G., 197 (Pergamon Press, London, 1957).
[9] Ward, A. G. (unpublished work).
[10] Eastoe, J. E., and Leach, A. A., *Recent Advances in Gelatin and Glue Research*, edit. Stainsby, G., 173 (Pergamon Press, London, 1957).
[11] Briefer, M., and Cohen, J. H., *Indust. Eng. Chem.*, **20**, 408 (1928). Briefer, M., *ibid.*, **21**, 266 (1929).
[12] Ames, W. M., *J. Soc. Chem. Indust.*, **63**, 277, 303 (1944). Bowes, J. H., *Research*, **4**, 155 (1951).
[13] Saunders, P. R., and Ward, A. G., *Proc. Second Int. Cong. Rheology*, 284 (Butterworths Scientific Publications, London, 1954).
[14] Bates, F. J., and associates, *Nat. Bur. Stand., Circular* C440, 103 (1942).
[15] Stainsby, G., *Faraday Soc. Discussion*, No. 18, 288 (1954).
[16] Ferry, J. D., *J. Amer. Chem. Soc.*, **70**, 2244 (1948).
[17] Ferry, J. D., and Eldridge, J. E., *J. Phys. Coll. Chem.*, **53**, 184 (1949).
[18] Boedtker, H., and Doty, P., *J. Phys. Chem.*, **58**, 968 (1954).
[19] Herrmann, K., Gerngross, O., and Abitz, W., *Z. physik. Chem.*, Abt. *B*, **10**, 371 (1930). Katz, J. R., Derksen, J. C., and Bon, W. F., *Rec. Trav. Chim. Pays-Bas*, **50**, 725 (1931).
[20] Flory, P. J., and Garrett, R. R., *J. Amer. Chem. Soc.*, **80**, 4836 (1958).
[21] Ramachandran, G. N., and Kartha, G., *Nature*, **176**, 593 (1955). See also review by Rich, A., and Crick, F. H. C., *Recent Advances in Gelatin and Glue Research*, edit. Stainsby, G. (Pergamon Press, London, 1957).
[22] Robinson, C., and Bott, M. J., *Nature*, **168**, 325 (1951). Robinson, C., *Nature and Structure of Collagen*, edit. Randall, J. T., and Jackson, S. F., 96 (Butterworths, London, 1953).
[23] Cohen, C., *J. Biophys. Biochem. Cytol.*, **1**, 203; *Nature*, **175**, 129 (1955).
[24] Leitner, M., *Trans. Farad. Soc.*, **51**, 1015 (1955). See also Treloar, L. R. G., *The Physics of Rubber Elasticity*, second ed. (Clarendon Press, Oxford, 1958).

TEICHOIC ACIDS AND THE STRUCTURE OF BACTERIAL WALLS

By Dr. A. R. ARCHIBALD, J. J. ARMSTRONG, Prof. J. BADDILEY, F.R.S., and J. B. HAY

Department of Chemistry, King's College (University of Durham), Newcastle upon Tyne

THE chemical composition of the walls of a number of bacteria has been examined by many workers[1,2] and much interest has been expressed in the possible function of the various components. Electron microscopy of stained sections of whole cells of *Bacillus subtilis* has shown that the wall of this organism appears to be composed of three layers (for example, ref. 3), although the interpretation of such micrographs may be ambiguous. We have now examined stained sections of the isolated walls of *Lactobacillus arabinosus*, *L. casei*, *L. fermentii*, *B. subtilis* and *B. cereus*, and observed three layers in each case.

Vigorous extraction of lyophilized walls of *L. arabinosus* completely removes the teichoic acid and a polysaccharide containing rhamnose, galactose and glucosamine[4]. After such extraction, the insoluble residue (50 per cent of the original weight of wall) consisted of the mucopeptide component, which is composed of alanine, glutamic acid, diaminopimelic acid, glucosamine and muramic acid; all the glucose, rhamnose, galactose and teichoic acid which were originally present in the wall had been removed. This mucopeptide material contained about 0·4 per cent of phosphorus, which was not present as teichoic acid[4]. The nature of the phosphorus is unknown, but it is of interest that after complete removal of teichoic acid from the walls of *Staphylococcus aureus* a similar quantity of phosphorus remained[5]. Furthermore, some twelve species of Gram-positive bacteria containing no teichoic acid in their walls nevertheless contained small amounts of phosphorus (0·2–0·6 per cent) chemically bound in the wall.

Electron microscopical examination of shadowed specimens of the mucopeptide from *L. arabinosus* showed that it retained the shape of the original cell wall, indicating that in these walls the mucopeptide is the component responsible for the structural integrity of the wall, the teichoic acid and polysaccharide being of minor importance in this respect. Similar results were obtained on extracted walls from *B. subtilis* which contained only traces of teichoic acid. This finding is similar to that of Park and Hancock[6], who showed that the residual material obtained after exhaustive extraction and enzymic digestion of whole cells of *Staph. aureus* had the composition of a mucopeptide and the appearance of cell walls. Moreover, we have observed that stained sections of extracted walls of *L. arabinosus* possess a three-layer structure similar to that of the original walls. This suggests that the mucopeptide is not homogeneous, a finding which is in accord with views expressed by others[7,8] on the basis of chemical investigations. The appearance of walls before and after removal of teichoic acid is shown in Fig. 1.

In view of modern opinion that walls are not solely rigid supports for the cell, it is interesting to consider possible functions of wall teichoic acids. As they do not contribute significantly to the rigidity of the wall, it is possible that they are concerned in cell metabolism. After isolation, they have charged phospho-mono- and di-ester groups and also free amino-groups[9,10]. Thus it might be expected that the provision of charged groups is one of the functions of teichoic acids. Consequently, it was necessary to investigate the manner in which teichoic acid is bound to the cell wall, and to determine whether the charged groups of teichoic acid exist as such in the wall structure.

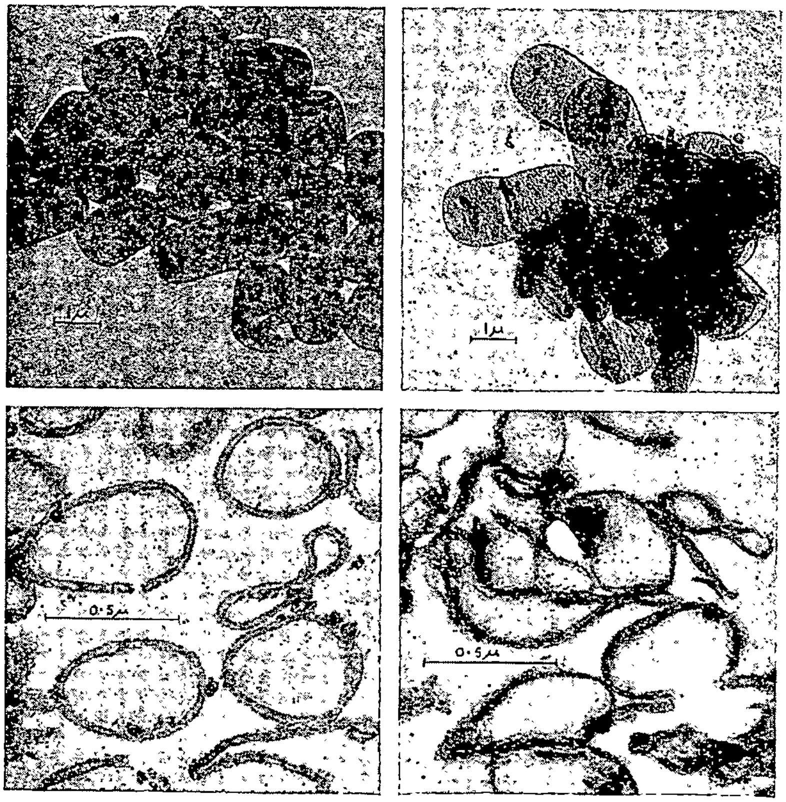


Fig. 1. Top left, walls of *L. arabinosus*; top right, walls of *L. arabinosus* after removal of teichoic acid and soluble polysaccharide; bottom left, section of walls of *L. arabinosus* stained with permanganate; bottom right, section of walls of *L. arabinosus* after removal of teichoic acid and soluble polysaccharide

It is unlikely that teichoic acids are bound to the walls by amide linkages involving the amino-groups of the D-alanine residues. These residues are joined to the teichoic acid molecule through exceptionally labile ester linkages which are nevertheless largely unaffected by the acid extraction procedures. It is improbable that amides would be hydrolysed under conditions which do not hydrolyse these ester linkages. Extraction of teichoic acid was effected more readily by trichloroacetic acid than by any of the other treatments described (Table 1). However, the amount of alanine released was greater in alkaline than in acidic or neutral solution, and the molar ratio of alanine liberated : teichoic acid phosphorus liberated was very much higher in alkaline than in acidic solution. For example, 2 *N* ammonia at room temperature released the alanine from walls, partly as alanine amide, without simultaneous release of comparable amounts of teichoic acid. Alanine and its amide were formed similarly from the walls of *Staph. aureus* (Oxford), *albus, citreus* and *Streptococcus faecalis*. It follows that teichoic acid is not bound to the wall by covalent linkages involving the amino-groups of the O-alanyl ester residues.

Table 1. EXTRACTION OF TEICHOIC ACID AND ALANINE FROM ISOLATED WALLS

Results are expressed as moles/mole of phosphorus originally present in the wall

	Reagent	Phosphorus in solution	Alanine in solution	Ratio alanine: phosphorus
L. arabinosus	5 per cent trichloroacetic acid, 24 hr., 28°	0·71	0·36	0·51
,,	0·1 *M* acetate, *p*H 6·5 24 hr., 28°	0·102	0·40	3·95
,,	0·1 *M* acetate, *p*H 9, 24 hr., 28°	0·15	0·61	4·0
,,	2 *M* ammonia, 3 hr., 18°	0·038	0·37	9·7
,, (pretreated with ammonia)	5 per cent trichloroacetic acid, 24 hr., 28°	0·44	0·02	0·05
B. subtilis	5 per cent trichloroacetic acid, 24 hr., 28°	0·88	0·36	0·41
,,	Water, 3 hr., 18°	0·023	0·02	0·91
,,	2 *M* ammonia, 3 hr., 18°	0·039	0·29	7·4
Staph. aureus (Duncan)	5 per cent trichloroacetic acid, 24 hr., 28°	0·67	0·55	0·82
,,	Water, 3 hr., 18°	0·059	0·055	0·93
,,	2 *M* ammonia, 3 hr., 18°	0·121	0·60	5·0

This conclusion has been confirmed for *L. arabinosus, B. subtilis* and *Staph. aureus* (Duncan) by treatment of their walls with fluorodinitrobenzene. The results are shown in Table 2. The reaction is heterogeneous in these experiments and thus is not necessarily complete, particularly as the walls studied may differ in their permeability towards the reagent. It is noteworthy that since reaction is usually effected at *p*H 7·8 or even *p*H 9 for several hours, a considerable proportion of alanyl ester groups would be expected to hydrolyse, liberating alanine or dinitrophenylalanine into solution. This was observed in the cases examined here. Thus, in the examination of terminal groups in bacterial cell walls which contain labile amino-acid residues, it is essential to examine the supernatant solution as well as the dinitrophenyl-walls, a procedure which, in the past, has not been followed[11].

Table 2. DINITROPHENYLALANINE FORMED ON TREATMENT OF WALLS WITH FLUORODINITROBENZENE

Conditions used were similar to those described by Salton (ref. 11). Results are expressed as moles dinitrophenylalanine/mole phosphorus originally present in walls

	Wall hydrolysate	Supernatant and water washings	Total
L. arabinosus	0·35	0·17	0·52
B. subtilis	0·17	0·11	0·28
Staph. aureus	0·32	0·18	0·50

The ribitol teichoic acids examined in detail (from *B. subtilis*[10] and *Staph. aureus*[5]) are polymers in which glycosylribitol units are joined together in a regular manner through phosphodiester groups at the 1- and 5-positions in the ribitol. Moreover, each polymer contains only one phosphomonoester group, which is situated exclusively at the 5- position of a terminal glycosylribitol unit. This has also been observed in the teichoic acid from *L. arabinosus* (unpublished observation). It follows that these compounds cannot be attached to the bacterial wall through phosphotriester linkages, as the fission of such linkages would result in the appearance of some 2,5-phosphodiester linkages or of some terminal phosphomonoester groups at positions 1 and 2 in the ribitol. Some polymer lacking a terminal phosphomonoester group would also be expected. These consequences are apparent from work in the nucleotide field[12]. Phosphotriester linkages of the type required would be extremely labile towards alkali, and this is inconsistent with the observed rate of liberation of teichoic acids from walls under alkaline conditions.

Thus, in the native walls the teichoic acid has free phosphate and amino-groups, and is presumably held in the wall through ionic linkages and hydrogen bonds. This would result in the walls bearing a charge, a conclusion supported by several observations. The ease of centrifugation of walls under various conditions can be explained on this basis, native walls being much more readily sedimented from dilute salt or trichloroacetic acid than from water. The ease of sedimentation of extracted walls in water is much greater than that of unextracted walls, and is not appreciably different from that of suspensions in dilute salt solutions. Moreover, in electron microscopical examination of natural and extracted walls we have observed repeatedly that, whereas in shadowed specimens and in stained sections native walls are well dispersed, extracted walls show a marked tendency to aggregate. These properties could arise through mutual electrostatic repulsion of the unextracted walls, this repulsive force being reduced after removal of the teichoic acid.

The ionic nature of teichoic acids in walls is consistent with the view that these compounds may regulate the passage of ions between the organism and its environment. It is perhaps significant that *Lactobacilli*, many of which contain teichoic acids in their walls, can grow in acid solutions and in the presence of fairly high (4 per cent) salt concentrations; similarly. *Staph. aureus, albus* and *citreus* grow in 10 per cent sodium chloride, *B. subtilis* in 7–12 per cent and *Strep. faecalis* in 6·5 per cent sodium chloride.

We acknowledge the award of a Frank Schon fellowship (to A. R. A.) and a Salters fellowship (to J. J. A.). We also thank Mr. A. L. Davison for the growth of bacteria, Mr. E. H. Boult and Miss B. Harris for electron microscopy, and the Medical Research Council and Nuffield Foundation for financial support. Walls of *Staph. aureus* (Duncan) were kindly provided by Dr. P. Mitchell.

[1] Salton, M. R. J., in *The Bacteria*, 1, edit. by Gunsalus, I. C., and Stanier, R. Y. (Academic Press, New York and London, 1960).

[2] Ikawa, M., *J. Biol. Chem.*, **236**, 1087 (1961).

[3] Tokuyasu, K., and Yamada, E., *J. Biophys. Biochem. Cytol.*, **5**, 123 (1959).

[4] Archibald, A. R., Baddiley, J., and Buchanan, J. G., *Biochem. J.* (in the press).

[5] RajBhandary, U. L. (unpublished observations).

[6] Park, J. T., and Hancock, R., *J. Gen. Microbiol.*, **22**, 249 (1960).

[7] Rogers, H. J., and Perkins, H. R., *Nature*, **184**, 520 (1959).

[8] Work, E. (personal communication).

[9] Armstrong, J. J., Baddiley, J., Buchanan, J. G., Carss, B., and Greenberg, G. R., *J. Chem. Soc.*, 4344 (1958).

[10] Armstrong, J. J., Baddiley, J., and Buchanan, J. G., *Biochem. J.*, **76**, 610 (1960).

[11] Salton, M. R. J., *Biochim. Biophys. Acta*, **24**, 9 (1957).

[12] Brown, D. M., Magrath, D. I., and Todd, A. R., *J. Chem. Soc.*, 4396 (1955).

MECHANISM OF ACTION OF ANTIGEN-ANTIBODY COMPLEXES: THEIR EFFECT ON MAST CELLS

By Dr. IVAN MOTA

Department of Histology, University of São Paulo

RECENTLY, Germuth and McKinnon[1] have made the important observation that soluble antigen-antibody complexes prepared in excess antigen produce anaphylactic shock when injected into unsensitized guinea pigs. A syndrome similar to anaphylaxis was also obtained by injection of soluble complexes in mice by Tokuda and Weiser[2] and in rats by Arocha and McIlreath[3]. The observation that in the above-mentioned animals the character of the shock induced by soluble antigen-antibody complexes was very similar to that seen in anaphylaxis has suggested that the sites of antigen-antibody action are the same regardless of whether anaphylaxis is induced by injection of antigen into an already-sensitized animal or by injection of soluble preformed antigen-antibody complexes. It is known that mast cell damage and histamine release occur in guinea pig active or passive anaphylaxis[4–6], as well as in rat and mouse active anaphylaxis[7–10], so I thought it would be interesting to find out whether soluble antigen-antibody complexes would duplicate damage to mast cells and release of histamine observed in anaphylaxis.

Crystalline bovine serum albumin was used as antigen. The antiserum was a pool of rabbit anti-bovine serum albumin containing 1·0 mgm. antibody nitrogen/ml.

To prepare the soluble complexes antigen was added to antiserum at equivalence, the mixture incubated at 37° C. for ½ hr. and kept for 24 hr. at 4° C. The precipitate was then washed three times with saline at 0–1° C., re-suspended in Tyrode solution containing a total amount of bovine serum albumin ranging from 5 to 8 times that used to precipitate antibody at equivalence, stored at 4° C. with occasional agitation for 48 hr. and cleared by centrifugation.

Twenty guinea pigs each weighing 250 gm. were injected intravenously with 2 ml. (2 mgm. antibody nitrogen) of the complexes preparation. Three of these guinea pigs died with emphysematous lungs, 10 presented severe shock and died overnight, 2 presented slight to moderate shock and 5 presented no effect. The emphysematous lungs were then fixed and stained for microscopical observation of the mast cells as described elsewhere[4]. However, a microscopical inspection of many fields did not reveal any mast cell degranulation. Nevertheless, it was considered that the amount of histamine released, although enough to induce emphysema, was insufficient to produce any visible mast cell damage and it was decided to perform *in vitro* experiments, in which the effect of the complexes on the mast cells could be detected more easily. Thus, 15 guinea pigs were killed by breaking the neck, their lungs, ileum and mesentery dissected out, and the activity of the complexes tried on these organs. The ileum was suspended in a Dale apparatus with 7 ml. capacity and the activity of preparations of the complexes tested by adding 0·4 ml. (400 μgm. antibody nitrogen) of them to the bath. The lung was cut in slices 0·8 mm. thick, washed in cold Tyrode solution, and 10–12 of these slices used as samples. They were then shaken in beakers closed with 'Parafilm' caps for 15 min. at 37° C. in 4 ml. (4 mgm. antibody nitrogen) of preparations of the complexes or in 4 ml. of Tyrode solution, after which the histamine content of the supernatants was assayed on the guinea pig ileum. The mesentery was cut into pieces which were incubated with the complexes under the conditions described for the lung, and then transferred to a 4 per cent solution of lead subacetate for fixation[4].

Six of the 15 ilea tested responded to the complexes with a definite contraction followed by desensitization as already reported[11]. However, no release of histamine or damage to mast cells could be detected when the lungs or mesentery of the same animals were incubated with the complexes. The same results were obtained when 1 per cent fresh guinea pig serum was added to the complexes. Twenty rats were injected intravenously with 2 ml. (2 mgm. antibody nitrogen) of the preparation of complexes. All the animals presented moderate to severe shock, but there were no fatalities. It was then observed that adrenalectomized rats were very susceptible to the effect of soluble antigen-antibody complexes and 15 rats adrenalectomized 48 hr. before were injected intravenously with 1 ml. (1 mgm. antibody nitrogen) of the complexes. All the animals showed severe shock and 12 died showing generalized intestinal congestion at autopsy. For microscopical observation of the mast cells, the skins of the snout and the tongue were fixed by injecting fixative through the carotid artery as described elsewhere[8]. However, no damage to the mast cells was observed, except for one rat in which there was generalized damage of these cells. This experiment was duplicated with a group of 12 adrenalectomized rats from which blood was collected 5 min. after injection of the complexes and the plasma histamine assayed on the ileum. Although all the animals showed severe shock no increase in plasma histamine was detected, except for one animal in which 0·5 μgm./ml. of histamine was found. Twenty mice were then injected with 0·5 ml. (0·5 mgm. antibody nitrogen) of the complexes. All the animals showed moderate to severe shock, and 9 of them died within 10 min. However, microscopical examination of their tissues did not reveal any damage to the mast cells. Pretreatment with *B. pertussis* aggravates the shock induced by soluble antigen-antibody complexes in mice[2] and 20 *pertussis*-treated mice were injected with 0·5 ml. of soluble complexes. Although 18 of these mice have presented severe shock and died within 20 min. no damage to the mast cells could be observed in their tissues.

These results showing that guinea pigs, rats and mice injected with antigen-antibody complexes in excess antigen present an anaphylactic picture similar to that observed in anaphylaxis confirm the results of several authors[1-3]. However, I have not been able to duplicate by means of soluble antigen-antibody complexes the damage to mast cells and release of histamine observed in anaphylaxis.

I consider these results show that neither release of histamine nor damage to mast cells occurs when guinea pig lung or mesentery is incubated with soluble antigen-antibody complexes, under conditions in which both damage to mast cells and release of histamine are promptly produced when antigen contacts sensitized tissues[5]. Furthermore, my results suggest that the complexes do not act as a histamine releaser or by sensitizing the tissues and reproducing passive anaphylaxis. Also the possibility that anaphylatoxin could have been formed by reaction between antigen-antibody complexes and serum proteins is ruled out as anaphylatoxin induces damage to mast cells similar to that produced by antigen-antibody reaction in anaphylaxis[12]. These results certainly do not exclude that soluble antigen-antibody complexes may be operative in anaphylaxis, but suggest that the complexes are not able, or at least not quite efficient, in duplicating the cytological aspects of anaphylaxis connected with liberation of histamine. However, that this may be possible under certain conditions is suggested by my observation that some of the adrenalectomized rats show damage to mast cells and release of histamine when injected with soluble complexes. This same fact, or a possible difference in the composition of the complexes used, may explain the results of Arocha and McIlreath[3], who have reported release of histamine from the isolated lung of the guinea pig or small intestine of the rat when perfused with soluble antigen-antibody complexes. However, the experiments reported here suggest strongly that soluble antigen-antibody complexes do not preferentially act by releasing histamine from the mast cells.

[1] Germuth, F. G., and McKinnon, G. E., *Bull. Johns Hopkins Hosp.*, **101**, 13 (157).
[2] Tokuda, S., and Weiser, R. S., *Science*, **127**, 1237 (1958).
[3] Arocha, H. G., and McIlreath, F. J., Twenty-first Intern. Cong. Physiol. Sc., Buenos Aires (1959).
[4] Mota, I., and Vugman, I., *Nature*, **177**, 427 (1956).
[5] Mota, I., *J. Physiol.*, **147**, 425 (1959).
[6] Humphrey, J. H., and Mota, I., *Immunol.*, **2**, 31 (1959).
[7] Mota, I., Thesis, Univ. of São Paulo (1953).
[8] Mota, I., *Brit. J. Pharmacol.*, **12**, 453 (1957).
[9] Mota, I., *Nature*, **182**, 1021 (1958).
[10] Carter, P. B., Higginbotham, R. D., and Dougherty, R. D., *J. Immunol.*, **79**, 259 (1957).
[11] Trapani, I. L., Gravey, J. S., and Campbell, D. H., *Science*, **127**, 700 (1958).
[12] Mota, I., *Immunol.*, **2**, 403 (1959).

CHROMATOGRAPHIC ANALYSIS OF INFECTIOUS RIBONUCLEIC ACID FROM POLIOVIRUS

By C. COCITO, A. PRINZIE and P. De SOMER

Rega Institute, Department of Virology, University of Louvain, Belgium

TWO metabolically distinct types of ribonucleic acids (RNA) have been recognized in mammalian and bacterial cells: a low molecular weight or soluble component (LMW-RNA) which is apparently free in the cytoplasm[1] and able to transfer the activated amino-acids to the ribosomes[2], and a highly polymerized form or high molecular weight component (HMW-RNA) linked to a protein moiety in a ribonucleoprotein structure (RNP, ribosomes) which is regarded as the major site of protein synthesis[3]. Another type of RNA is that of the mature particles of some plant and animal viruses (viral RNA), which seems able to direct the synthesis of the whole virus by a suitable host cell. Numerous publications have, in fact, reported the infectivity of naked RNA extracted with cold[4,5] or hot[6] phenol from different viral particles.

The system of cells infected with viral RNA from poliovirus presents a particular difficulty, because once extracted with phenol, most of the protein-free polio-RNA apparently remains no longer infectious[7]: a decrease of 3–5 log units is usually observed in different experiments, an upper limit of 1 per cent residual infectivity being only recently reported[8].

Some experiments of partition chromatography described in the present article were carried out to examine the relationships between cellular and viral RNA's, and to analyse the infectivity of protein-free RNA from poliovirus with a possible exclusion of cellular constituents. Our work is based on the use of a chromatographic column (protein-coated 'Supercel') which can separate the high molecular weight RNA (found by us only in the ribosomes) from the low molecular weight RNA (found mainly, but not only, in the supernatant on centrifugation of bacterial homogenates at 100,000*g*) and from the precursors contained in the phenol extracts of *E. coli*[9,10]. Such a column has also been used for research on deoxyribonucleic acids (DNA) from bacteriophages[9] as well as for the analysis of RNA metabolism in phage-infected bacteria[10] and of HeLa cells infected with different DNA- and RNA-viruses[11], because it allows separation of small amounts of nucleic acids without the necessity for previous isolation from large quantities of contaminating precursors. The column comprises analytical grade cellulose powder, protein-coated 'Supercel', 'Supercel' coated with albumin made basic by methylation and washed with 0·3 *M* buffered sodium chloride, 'Supercel'. Procedure of methylation and details for preparation of the column are to be found in refs. 9–14.

HeLa cells employed throughout this work were cultured in suspension in spinner flasks incubated at 37° C., using as growth medium Hank's solution lacking calcium and magnesium but supplemented with ten times as much phosphorus and with 5 per cent decomplemented calf serum and 0·5 per cent lactalbumin hydrolysate. Medium 199 added with carrier-free preparations of phosphorus-32 labelled orthophosphate to a specific activity of 1 or 2 µc. phosphorus-32/µgm. phosphorus-31 was used to yield radioactive cells. Virus stocks were prepared by infecting monolayers of either HeLa cells or monkey kidney primary cultures with multiplicities 1–5 of poliovirus type *I*, allowing an incubation of 20 hr. at 37° C. When labelled virus had to be obtained, after the contact with the virus, monolayers were washed with a phosphorus-free Hank's solution buffered at *p*H 7·6 with 0·01 *M tris* and then covered with medium 199 added with phosphorus-32 labelled inorganic phosphate. After lysis had occurred, cellular debris was spun down by low-speed centrifugation: spontaneous lysates thus obtained had the usual titre of 1 × 10^7–5 × 10^7 plaque-forming units (pfu)/ml. The virus was concentrated either by centrifugation at 100,000*g* for 2 hr., or by precipitation at *p*H 3·5 and 3° C. with hydrochloric acid and yeast-RNA as carrier: with the first method up to 10^5 pfu/ml. and with the second 10^3 pfu/ml. remained in the supernatant. Virus suspensions of high titre were also obtained by sonicating at 3° C. cells collected by centrifugation 10 hr. after infection with poliovirus, but these preparations were heavily contaminated by cellular RNA's and ribosomes. A further purification of the concentrated virus preparation could be achieved through a treatment with RNase at 37° C. followed by ultracentrifugation in the cold to remove the enzyme. Titrations of viable virus were usually performed on monolayers of trypsin-dispersed monkey kidney cells, following Dulbecco and Vogt's plaque-technique[15].

For the extraction of cellular RNA's, HeLa cells washed free from the radioactivity of the medium and suspended in 0·1 *M* sodium chloride buffered at *p*H 7·1 with 0·1 *M* sodium phosphates were extracted three times with water-saturated redistilled phenol at 60° C. and constant stirring: such procedure, however, proved to be unsuitable for the extraction of poliovirus, since a loss of the whole viral infectivity occurred when temperature exceeded 40° C. during the phenol treatment. For the preparation of viral RNA, concentrated suspensions of virus in the same buffered saline were extracted three times with phenol at 3° C.; residual phenol was removed by five extractions with peroxide-free ether and the latter eliminated by bubbling a stream of sterile nitrogen[10]. Preparations of cellular and viral RNA's were eluted from a protein-coated 'Supercel' column with a linear gradient of sodium chloride 0·3 → 1·5 *M* constantly buffered at *p*H 6·7 with 0·05 *M* phosphates[10]. The adsorption at 254 mµ and radioactivity of the eluate were continuously measured with ultra-violet adsorption and count-rate meters, and simultaneously recorded: measures with ultra-violet optics were omitted when infectivity of viral RNA had to be titrated on monkey kidney monolayers[15]. Molarity of a given fraction was obtained from the formula $C = C. + C_f(e^{x/v} - 1)e^{-x/v}$, where $C.$ = concentration of the mixer; C_f = concentration of the feeder; x = volume delivered less column displacement; v = vol. of the mixing vessel. A step-wise elution was also carried out with solutions 0·3 *M*, 0·5 *M*, 0·7 *M*, 1·0 *M* and 1·5 *M* sodium chloride buffered at *p*H 6·7, in which case the whole infectivity was eluted at 1·0 *M*. The optical density of the single fractions was measured in a Unicam spectrophotometer and the radioactivity with a gas-flow counter.

Fig. 1*b* shows the elution pattern of phosphorus-32 labelled compounds from HeLa cells: similarly to that observed with *E. coli*[10], peak *A* contains free bases, nucleotides and optically inactive phosphorus-32 labelled compounds (precursors), peak *B*, RNA of low molecular weight (LMW-RNA), and peak *D*,

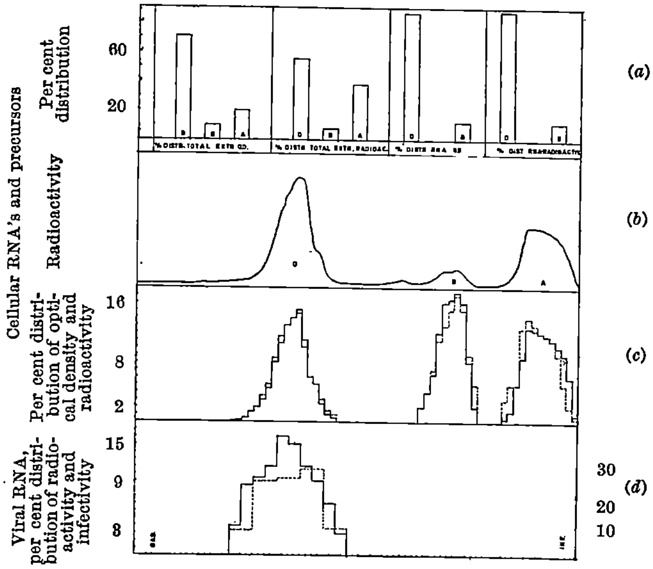


Fig. 1. Chromatographic patterns of cellular RNA's and precursors, and of infectious viral RNA from labelled poliovirus *a*, Per cent distribution of total optical density and radioactivity extracted from the cells among the three chromatographic fractions and similarly for the RNA-radioactivity and ultra-violet adsorption; *b*, elution pattern of phosphorus-32 labelled compounds extracted with phenol from HeLa cells grown for four generations in the presence of phosphorus-32 labelled orthophosphate. Peaks *B* and *D* represent cellular RNA's; precursors are contained in the fraction *A*; *c*, per cent distribution of optical density at 254 mµ (dashed lines) and radioactivity (continuous lines) among the three chromatographic peaks. Total ultra-violet adsorption and radioactivity within each peak considered equal to 100; *d*, per cent distribution of radioactivity (continuous lines) and infectivity (dashed lines) of viral RNA extracted with cold phenol from phosphorus-32 labelled poliovirus prepared on monkey kidney cell monolayers

of high molecular weight (HMW-RNA). The peak C of DNA, which is located between the two RNA-regions, does not appear because of the DNA-exclusion during extraction with hot phenol; it appears, however, when extraction is carried out at a lower temperature, that is, at 4°–40° C., and on addition of detergent, namely, sodium dodecyl sulphate 0·5–4 per cent, in which case the repartition of radioactivity among the three nucleic acid fractions is the following: $B : C : D = 8 : 32 : 60$. Figs. 1a and 1c show the distribution (per cent) of both ultra-violet adsorption and radioactivity among the three chromatographic fractions. Within the same area of (high molecular weight) RNA from the host cell, viral RNA is also eluted, as shown in Fig. 1d, in which the distribution of both radioactivity and infectivity for monkey kidney cell monolayers are reported (practically, 100 per cent of the viral RNA infectivity loaded on the column was recovered within this peak).

These experiments indicate that viral RNA from poliovirus behaves chromatographically in a similar way to cellular HMW-RNA, but quite differently from LMW-RNA. Differences between the two types of cellular RNA's in relation to the method of partition chromatography used have been discussed[9,10]. The recovery of infectious naked RNA from poliovirus by partition chromatography indicates that the protein-coated 'Supercel' column could be a suitable means for purification and isolation of viral RNA. In addition, our results may support further the view that isolated poliovirus-RNA is fully infectious, and exclude the participation of other viral or cellular components in such infectivity, a view that is in agreement with the theory of viral RNA as carrier of genetical information, since protein-free RNA's, but not nucleoproteins, seem to be eluted from our column. We have been unable to find any difference between inactive and infectious viral RNA from poliovirus extracted with phenol, and the reason for the partial inactivation at 0° C., when compared with the complete loss occurring at 60° C.

Note added in proof. While this work was in the press, Dr. L. Philipson (Rockefeller Institute, New York) informed us that with suitable gradients two fractions of high molecular weight RNA having sedimentation coefficients of 16 and 25–29 can be separated further on the type of column we have used, and that the DNA extracted with cold phenol from HeLa cells has an $S^0_{20,w}$ of 38, corresponding to a molecular weight of 32×10^6.

[1] Tissières, A., *J. Mol. Biol.*, **1**, 365 (1959).

[2] Zamecnik, P. C., Stephenson, M. L., and Scott, J. F., *Proc. U.S. Nat. Acad. Sci.*, **46**, 811 (1960).

[3] Hoagland, M. B., Zamecnik, P. C., and Stephenson, M. L., *Biochim. Biophys. Acta*, **24**, 215 (1957).

[4] Gierer, A., and Schramm, G., *Z. Naturforsch.*, **110**, 138 (1956).

[5] Fraenkel-Conrat, H., Singer, B., and Williams, R. C., *Biochim. Biophys. Acta*, **25**, 87 (1957).

[6] Wecker, E., *Virology*. **7**, 241 (1959).

[7] Prinzie, A., Schonne, E., and De Somer, P., *Arch. Virusforsch.*, **10**, 153 (1960).

[8] Koch, G., Koenig, S., and Alexander, H. E., *Virology*, **10**, 329 (1960).

[9] Mandell, J. D., and Hershey, A. D., *Anal. Biochem.* (in the press).

[10] Cocito, C., *Proc. Intern. Biophys. Cong.*, Stockholm (August 1961).

[11] Cocito, C., Prinzie, A., and De Somer, P., *Proc. Intern. Cong. Biochem.*, Moscow (August 1961). Cocito, C., and De Somer, P., *Proc. Intern. Biophys. Cong.*, Stockholm (August 1961). Cocito, C., Prinzie, A., and De Somer, P., *Biochim. Biophys. Acta* (in the press).

[12] Fraenkel-Conrat, H., and Olcott, H. S., *J. Biol. Chem.*, **161**, 259 (1945).

[13] Lerman, L. S., *Biochim. Biophys. Acta*, **18**, 132 (1955).

[14] Brown, G. L., and Watson, M., *Nature*, **172**, 339 (1953).

[15] Dulbecco, R., and Vogt, M., *J. Exp. Med.*, **99**, 167 (1954).

BLOOD-FEEDING, FECUNDITY AND AGEING IN *AEDES AEGYPTI* VAR. *QUEENSLANDENSIS*

By DR. M. M. J. LAVOIPIERRE

George Williams Hooper Foundation, University of California Medical Center, San Francisco

IN *Aedes aegypti*, as in many other culicines, a blood meal is essential for the maturation of the eggs, but it does not appear to be necessary for survival, since, under experimental conditions, this mosquito will live for many weeks on simple carbohydrates, notably certain hexoses and disaccharides[1]. This is not peculiar to mosquitoes, for in a wide range of biting and non-biting Diptera the normal life-span is attained on a carbohydrate diet[2]; the protein meal, apparently, is basically related to reproduction.

Adult female *A. aegypti* var. *queenslandensis* reared from well-nourished larvæ usually require only a single blood meal for the maturation of their oocytes[3], the number of eggs which develop depending on the host from which the meal is obtained[4]. After the first blood meal mated females may bite again, but their avidity for blood diminishes markedly as the eggs mature, biting being minimal just before oviposition[5]. After egg-laying, avidity once again becomes pronounced, and the cycle is repeated. On the other hand, in the case of the unmated insects, biting activity is high throughout the gonadotrophic cycle, although the number of eggs maturing and the speed at which they develop is no less than that of the mated flies. The unmated females retain most of the mature eggs during the remainder of their lives, deposition of the small number laid being extended over many weeks[6]; none the less, avidity for blood continues to be pronounced for several weeks, diminishing gradually as the population ages.

Since these results were published, I have shown that the blood-feeding drive is apparently closely related to oogenesis and to vitello-genesis. Supernumerary blood meals taken by unmated females were abnormally metabolized, producing, particularly in the ovary, aberrant changes which were especially noticeable in older flies. Moreover, the protein meal shortened the life-span of the unmated mosquitoes: a comparison (Figs. 1 and 2) of the survival-curves of mated and unmated insects, offered daily meals on human subjects, shows that the mated flies survived much longer than those which were unmated. The increase in death-rate

Table 1. 50 per cent mortality and life-expectancy of, and the number of eggs (calculated as a percentage) oviposited by, several populations of (*a*) mated and (*b*) unmated females of *Aedes aegypti* var. *queenslandensis* offered blood meals (for 10 min.), daily and at intervals, over a period of many weeks. In the mated group three cages were set up, each with a random number of mosquitoes—individuals which had not fed when the first blood meal was offered (the numbers are indicated in brackets) were removed and discarded; in the unmated series 140 previously fed mosquitoes were divided into two equal groups

Whether mated or unmated	Frequency of feeding	No. of mosquitoes	50 per cent mortality in days	Life-expectancy in days after 1st blood meal	Further life-expectancy in days at age 35 days	No. of eggs laid at 50 per cent mortality (calculated as a percentage)
Mated	Daily	104 (3)	69·0	65·1	35·5	44,166
	Every 8 days	85 (12)	81·0	77·0	46·5	33,761
	Every 16 days	62 (10)	91·0	80·9	53·0	26,613
Unmated	Daily	70	38·5	38·6	11·4	1,949
	Every 16 days	70	39·0	40·3	14·3	1,820

seems related to the metabolism of the blood meal, since virgin females of *A. aegypti* var. *queenslandensis* slightly outlive mated females when the populations are maintained on a sucrose diet alone[7]. In the case of certain other insects, however, for example, *Drosophila obscura* and *Periplaneta americana*, which discriminate much less than *A. aegypti* in their food-seeking, a diet which is responsible for high fecundity in mated females does not appear to reduce the life-span of the unmated females, which, indeed, live longer than their mated fellows[8].

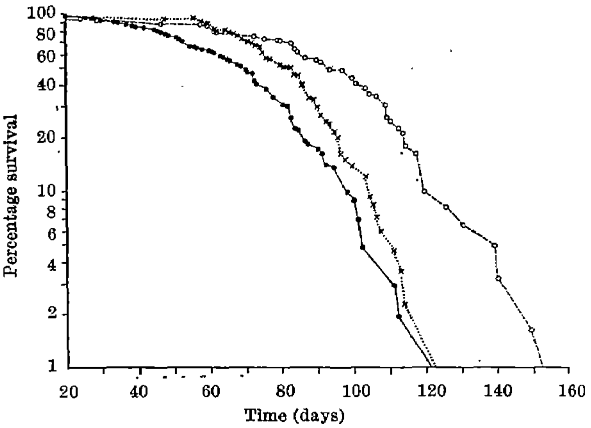


Fig. 1. Survival curves for comparable groups of mated *Aedes aegypti* var. *queenslandensis* offered blood meals daily ●, every 8 days ×, and every 16 days ○, plotted on semilogarithmic co-ordinates. The three groups were offered the initial meal on the same day

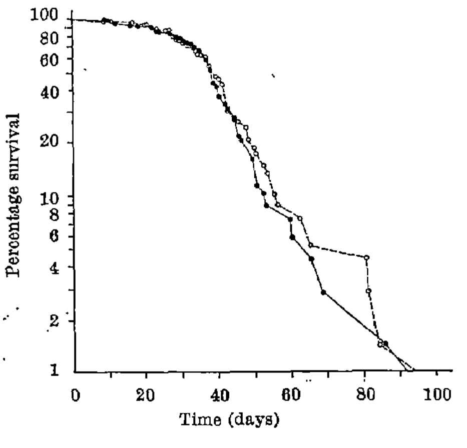


Fig. 2. Survival curves for comparable groups of unmated *Aedes aegypti* var. *queenslandensis* offered blood meals daily ●, and every 16 days ○, plotted on semilogarithmic co-ordinates. The two groups were offered the initial meal on the same day

Decreasing the frequency of feeding apparently scarcely influences the death-rate of the unmated females. The survival curves of two comparable populations, one fed daily and the other every sixteenth day, were found to approximate very closely (Fig. 2): calculations for life-expectancy differed little, a t-test giving $P > 0{\cdot}1$ and $< 0{\cdot}2$. In both populations the number of eggs deposited at 50 per cent mortality was small and almost the same (Table 1).

When, however, three comparable groups of mated mosquitoes were offered blood meals, daily, every eighth day, or every sixteenth day, the results were entirely different. The death-rate of the mated insects increased significantly with increased frequency of feeding, a t-test applied to the daily and 16-day groups giving $P = 0{\cdot}001$ (Table 1, Fig. 1). Associated with the more frequent opportunities to feed was a definite augmentation in the number of eggs laid, the daily-fed group laying 27 and 66 per cent more eggs than the eighth- and the sixteenth-day groups respectively at 50 per cent mortality.

These observations appear to indicate that while increased frequency of blood-feeding in mated females of *A. aegypti* var. *queenslandensis* allowed to lay eggs was accompanied by a marked increase in the death-rate of the mosquitoes, the effect seemed to be associated with augmentation in fecundity alone, for, in unmated females, which laid few eggs, increased feeding had no significant effect on the death-rate.

The results also suggest that the number of eggs laid by the mosquito is a parameter which may be of value when the probability of survival of mosquito populations both in the laboratory and in Nature is computed. The number of eggs laid depends on the number of ovipositions, and since the number of times a mosquito has oviposited can now be determined[9], the findings reported in this article may be of practical significance to investigators working on the dynamics of field populations of mosquito vectors of disease.

This work was supported in part by research grant *E*–3571 from the National Institutes of Health, U.S. Public Health Service. I also thank Dr. C. Keene, of the Kaiser Research Institute at Richmond, California, where part of this work was undertaken, and Rosalie Gill and Paul Budwiser for their assistance in carrying it out.

[1] Galun, R., and Fraenkel, G., *J. Cell. Comp. Physiol.*, **50**, 1 (1957).
[2] Downes, J. A., *Ann. Rev. Entom.*, **3**, 249 (1958).
[3] Macdonald, W. W., *Ann. Trop. Med. Parasit.*, **50**, 399 (1956).
[4] Pena, E., and Lavoipierre, M. M. J., *Rev. ibér. Parasit.*, **20**, 23 (1960).
[5] Lavoipierre, M. M. J., *Nature*, **181**, 1781 (1958).
[6] Pena, E., and Lavoipierre, M. M. J., *Rev. ibér. Parasit.*, **20**, 163 (1960).
[7] Pena, E., and Lavoipierre, M. M. J., *Rev. ibér. Parasit.*, **20**, 39 (1960).
[8] Maynard Smith, J., *J. Exp. Biol.*, **35**, 832 (1958).
[9] Macdonald, G., Detinova, T. S., and Bertram, D. S., *W.H.O. Mal.*, 238 (1959).

BRAIN WAVES AND 'BLOOD WAVES'

By HENRY R. PANTEK

Kresge Medical Research Institute, University of Michigan, Ann Arbor

IN search of an efficient method for localizing brain tumours, the temperature records in Fig. 1 were obtained from a normal female head with a modified Beckman d.c. breaker amplifier, a Leeds and Northrup paper recorder, and a copper-constantan thermocouple. The broken-line curve was taken with the thermocouple attached to the right temple and the solid line with it attached to the middle of the forehead about 4 cm. above the glabella. This latter record was obtained 24 hr. after the first. The subject sat comfortably in an arm-chair and was asked to relax with her eyes shut. At the first points *O*, about 5 min. later (the first 4 min. of the record is not shown in Fig. 1, the points *O* are shown at 0·6 min. later) the subject was asked to open her eyes and was immediately handed a multiplication problem. She finished multiplying a five-digit number by a four-digit number at the points *F* some 2 min. later. In this interval a large temperature change was noted. At points *C* she was asked to close her eyes and completely relax, and at the second points *O* was told to open them again. However, on the solid-line curve at point *O* she was given a second multiplication problem, whereas on the broken-line curve was engaged in a spirited conversation. From points *C* to *O* a temperature drop of about 3° occurred.

After examining the curves, it was decided that these variations in temperature or 'heat waves' were due to a change in blood-flow in these regions. Since it is known that problem solving is accompanied by fast electroencephalographic (EEG) waves[1] it was thought that these brain waves may be variations in potential between sites due to ions in motion in the blood vessels. That is, brain waves may actually be principally 'blood waves'. To test this electrohæmodynamic hypothesis a model was constructed as shown in Fig. 2, to simulate a simple blood vessel system. A 3-l. glass bottle filled with physiological saline was connected by 5 mm. inside diameter (all diameter dimensions given are inside diameter unless otherwise indicated) rubber tubing to a piece of polyethylene tubing 10 cm. long with a diameter of 2 cm. A hypodermic needle stylet *C* was forced half-way into this large tube in contact with the saline solution to serve as an EEG reference point or earth. The tube *B* was then connected to a glass *Y* connector *D*, and the two arms were each connected to No. 18 hypodermic needles. A duplicate of *D* connected to vessel *J* was coupled to it by two 20-cm. pieces of No. 18 (approximately 1 mm. diameter) polyethylene tubing via the No. 18 needles. Four size 35 suture wires (*E*, *F*, *G* and *H*) were forced into this small tubing 7·5 cm. apart to make contact with the saline solution. Wires *E* and *F* were connected to a flashlight battery to simulate d.c. potentials that may be present at a site. Wires *G* and *H* were connected to an EEG channel. The rubber tubing leading to the receptacle *J* had a diameter of 3 mm. The manometer *M* and the 3-mm. tubing *K* were connected by a glass *T* to bottle *A*. Whenever *K* was left open the solution in *A* was at zero pressure (or atmospheric pressure) and pressure and vacuum variations were applied by inhaling or exhaling into this opening. The records shown in Fig. 3 were obtained from this system.

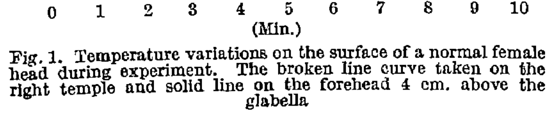


Fig. 1. Temperature variations on the surface of a normal female head during experiment. The broken line curve taken on the right temple and solid line on the forehead 4 cm. above the glabella

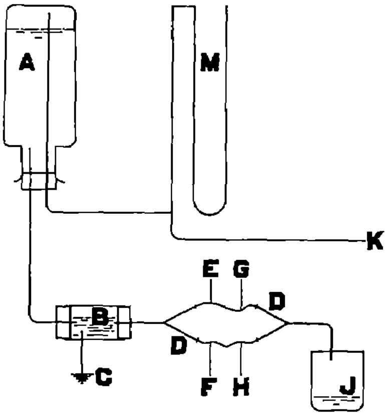


Fig. 2. Schematic representation of simulated simple blood vessel system

At atmospheric pressure the normal flow of the saline solution from bottle *A* through the system and finally into the container *J* was 60 c.c./min. The recording speed was 30 mm./sec. and each recording in Fig. 3 was obtained successively in a single experiment. Record *A* was obtained by creating a 20-mm. vacuum in bottle *A* and pinching off the opening at *K* with a clamp. This record occurred 144 sec. after the application of the vacuum. At this time the vacuum increased to 40 mm. due to the saline solution running out. It was observed that the waves at 3 per sec. are due to the formation and severing of drops at the end of the rubber tubing in container *J*. From an initial rate of about 6 drops/sec. and amplitude of 40 μV. the flow fell to 3 drops/sec. and the amplitude increased to 180 μV. This record might be compared with a left and right parietal petit mal record of a 9·5-year-old male child[3]. Record *B* is a

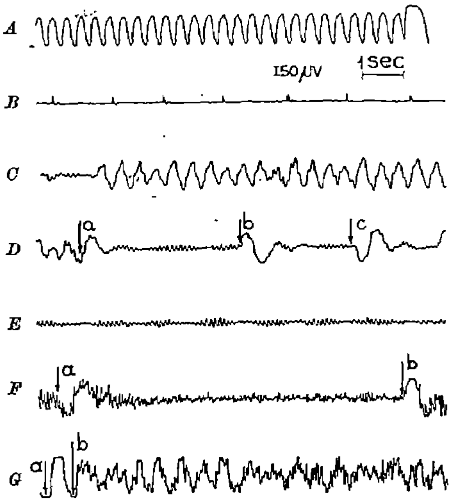


Fig. 3. Records obtained from simulated simple blood vessel system. Consecutive records from a single experiment

simulated heart beat which was obtained without a d.c. potential between E and F in Fig. 2. In this and later records the tube in container J was submerged below the level of the solution in J. The pressure at K was alternately built up to 30 mm. and suddenly released at intervals indicated by the distance between the spikes. When the pressure was alternately increased to 40 mm. no increase in amplitude of the spikes occurred, but remained at about 35 μV. Record C was obtained by squeezing the tube K about twice per sec. with the thumb and finger while it was clamped and the vacuum in A had slowly built up to 40 mm. Preceding this record there was a predominance of 10/sec. wave-forms with an amplitude of about 25 μV. while the vacuum was slowly building up. Occasionally big slow spikes and slow waves of 2–3 per sec. would appear. Amplitude modulated 9/sec. wave-forms such as in record E began appearing at about 33-mm. vacuum which occur in practice in the occipital region normally at any age. Record C can be compared with that of an 8-year-old boy in very deep sleep[2]. Record D occurred about 100 sec. before record E and the slow waves were produced by squeezing tube K while maintaining a 40-mm. vacuum. This record followed C by 20 sec., and the tube was squeezed with thumb and finger at point a, released at b, and again squeezed at c. Such slow waves apparently occur during light sleep of an adolescent. Record F was obtained by applying a pressure of 20 mm., which was released at point a and applied again at b. This recording is similar to an attention record where the eyes are closed and later opened. Recording G was made by first applying pressure rhythmically at about 1·5 times per sec. to point b (only one wave-form at this rate is included in the figure) and suddenly changing the rate at point b to twice per sec. These waves are similar to the paroxysmal waves in petit mal[3].

It is interesting that the heart-beat was reproduced without a d.c. potential and the other records with a d.c. potential applied. I think that these phenomena should be investigated further *in vitro* and *in vivo*, since it may explain the apparent discrepancies that occur when EEG patterns are compared with the location of certain brain lesions[4], their absence in hypothermia[5], and their presence over the area of a hemispherectomy, as I have observed.

---

[1] Adrian, E. D., and Matthews, B. H. C., *Brain*, 57, 355 (1934).

[2] Gibbs, F. A., and Gibbs, E. A., *Atlas of Electroencephalography*, 1 (Addison-Wesley Press, Inc., Cambridge, 1950).

[3] Gibbs, F. A., and Gibbs, E. A., *Atlas of Electroencephalography*, 2 (Addison-Wesley Press, Inc., Cambridge, 1952).

[4] Kahn, E. A., Bassett, R. C., Schneider, R. C., and Crosby, E. C., *Correlative Neurosurgery*, 41 (C. C. Thomas, Pub., Springfield, Illinois, 1955).

[5] Callaghan, J. C., McQueen, D. A., Scott, J. W., and Bigelow, W. G., *A.M.A. Arch. Surgery*, 68, 208 (1954).

FACTS AND THEORIES OF SHAPE DISCRIMINATION

In a recent article[1] Sutherland attempted to evaluate three theories of shape discrimination (actually, they are theories of the coding of information about shape in the brain, none is a complete theory as it stands.) The three theories are: Sutherland's own theory, which he labels theory A (ref. 2); a recent theory of Deutsch's, labelled theory B (ref. 3); a theory of mine, labelled theory C (ref. 4).

In his evaluation of these theories, Sutherland makes some incorrect statements about theories B and C, and does not mention some evidence of his own which is opposed to his own theory. The purpose of the present article is three-fold. First, to re-examine the evidence presented by Sutherland and to show that the conclusions he draws from it are unjustified; secondly, to point out several weaknesses in theory A which are purely logical and have nothing to do with the degree to which any one of the three theories has been verified or otherwise; and thirdly, to present some fresh observations which support theory C, but which do not support theory A. The discussion of the first topic will be confined mainly to the arguments put forward in Sutherland's article[1], and to theories A and C only, since Deutsch has already replied to Sutherland's criticisms of theory B (ref. 5).

Evaluation in Relation to the Various Theories

Sutherland[1] lists twelve groups of facts in his evaluation. Those facts which support both theory A and theory C will not be discussed; those which are claimed to give support to A but not to C will be discussed in the order and with the numbering given in Sutherland's article. His Fig. 1 is reproduced, together with the horizontal and vertical outputs of theory C, to clarify the ensuing arguments. A word about the outputs of theory C is in order. The two co-ordinates for an output are: on the ordinate, size of impulse; on the abscissa, time of occurrence. Thus each output is characterized by the sizes of the impulses generated and by the temporal pattern in which they occur, and either of these properties is sufficient to make an output distinctive and discriminable. Only in cases where there is little or no difference in *both* properties for both horizontal and vertical outputs respectively does the theory predict confusion of the associated shapes. The impulses are generated by a system which sums the extent of the contours of a shape on horizontal and vertical co-ordinates over a two-dimensional array[4].

(4) *Discrimination of broken vertical rectangle.* Sutherland points out that this shape (Fig. 1*a*) will be treated as a vertical rectangle in theory *A*, which is correct; but as a horizontal rectangle in theory *C*, which is incorrect, as can be seen by comparing the outputs for this shape with the outputs for vertical and horizontal rectangles shown in Fig. 2. This suggests that Sutherland has misunderstood theory *C*. The octopus and the rat both treat the broken vertical rectangle like a vertical rectangle, but Sutherland's assertion (p. 842): "... This result supports *A*, but is evidence against both *B* and *C*" is plainly incorrect.

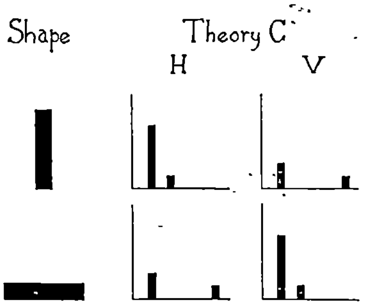


Fig. 2

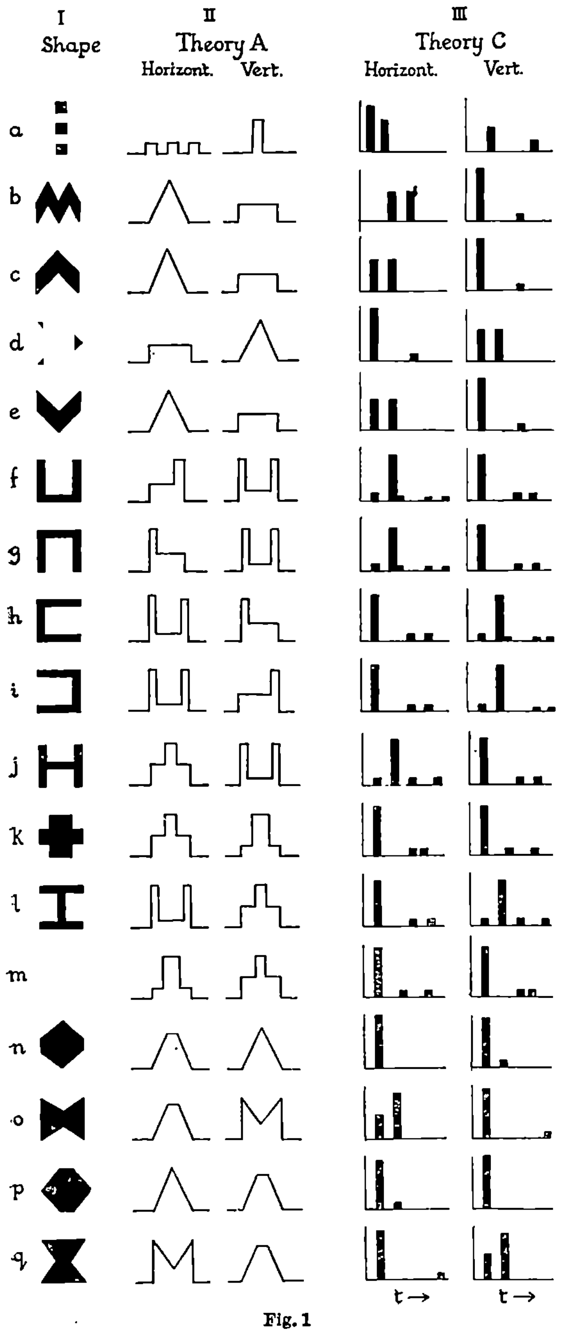


Fig. 1

(6) *Discrimination between diamond and oblique rectangles.* Sutherland is correct in stating that these figures should be indiscriminable on *C*, and since they can be discriminated by the octopus, he considers this a crucial test of theory *C*. However, inspection of his Fig. 2*a* shows that the diamond and rectangle used in his experiment are different in area (the dimensions reported elsewhere[6] are: square 5 cm. × 5 cm., rectangle 2 cm. × 10 cm.), and since he used 'filled in' shapes, or surfaces, rather than contours, it is obvious that the discrimination could be a simple brightness discrimination. Although some of the subsequent transfer tests suggest that discrimination was not based on brightness differences alone, no direct test of the effect of the brightness difference was made. As it stands, this finding can scarcely be considered a test of theory *C*, much less a crucial test.

(8) *Discrimination between shapes b, c, d and e* (*Fig. 1*). Theory *A* predicts that *b* and *c* should be indiscriminable, but they are discriminable. This is evidence against theory *A*, and to save it Sutherland has to introduce an additional assumption (ref. 1, p. 844 (1)). Inspection of the outputs for theory *C* in Fig. 1 shows that: (i) *b* and *c* should be discriminable, but with difficulty; (ii) *c* and *d* should be readily discriminated; and (iii) after training on *c* and *d*, *b* should be treated like *c*. Sutherland found all these three to be true, so his evidence supports *C* rather than *A*, since all three can be predicted from *C* without introducing additional assumptions. The lack of discrimination between *c* and *e* supports *A* and *C* equally.

(10) *Discrimination between up-down and right-left mirror images.* The fact that discrimination is possible appears to refute *C*, since inspection of the outputs for the pairs of shapes *f*, *g* and *h*, *i* (for example) shows that they are identical for each pair. However, Sutherland's use of filled-in shapes again makes his mirror-image experiments a poor test of *C*. While the overall brightness of shapes *f* and *g* is the same, for example, the distribution of brightness is different. Thus this pair could be discriminated on the basis of 'brighter above' (*g*) versus 'brighter below' (*f*). This possibility should at least be examined experimentally before adducing this evidence as a ground for refuting *C*. It may be noted that where there is

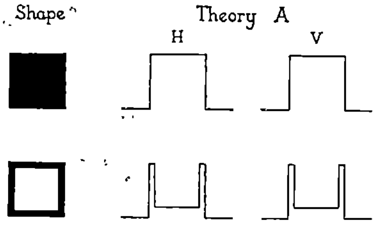


Fig. 3

not such an obvious difference in brightness distribution (as in shapes *c* and *e*), there is also no discrimination between the pair.

(12) *Discrimination of the pairs of shapes j, k; l, m; n, o; and p, q,* Inspection of the outputs for theory *C* shows that all four pairs should be easily discriminable, whereas theory *A* predicts that the pairs *j*, *k* and *n*, *o* should be less discriminable than the others, on the assumption of greater resolving power for the horizontal outputs (ref. 1, p. 843). Even if this assumption were incorporated (as it could be) in *C*, to account for the fact that horizontal rectangles of different extents are more readily discriminable by the octopus than vertical rectangles of different extents, this would still leave the pairs *j*, *k* and *l*, *m* equally discriminable, since both differ in both horizontal and vertical outputs in *C*. The same argument applies to the pairs *n*, *o* and *p*, *q*. So here are two cases where *C* yields a correct prediction, and *A* a false one, since all the pairs are discriminable. The remainder of the experimental findings are neutral, that is, equally explainable on either *A* or *C*.

Sutherland states (p. 843): "Theory *C* yields incorrect predictions throughout with the exception of some of the initial findings". In only two cases (diamond and oblique rectangle; mirror images) do Sutherland's experimental findings tend to refute *C*, and in both cases the evidence is vitiated by failure to implement a simple and obvious control condition. In five cases under (8) and (12) *C* yields a correct prediction, where *A* yields a false one; so Sutherland's evaluation of his theory as superior to *C* can scarcely be upheld on the evidence of his own experiments. Moreover, one piece of his own evidence not quoted by Sutherland is the fact that, apparently, the octopus transfers from discrimination of filled (surface) shapes to outlined (contour) shapes[6]. Theory *A* is a theory of surface discrimination, not one of contour discrimination. For theory *A* a contour shape shares nothing in common with a congruent surface shape except its outline, and the theory must deal with that outline as a 'surface'. The resulting outputs will be very different from the outputs for a surface shape. This is illustrated in Fig. 3, which shows the theory *A* outputs for a square, (*a*) as a surface shape, and (*b*) as a contour shape. Quite evidently the two are very different, and no transfer should occur from one to the other.

Logical Weaknesses in Theory *A*

Sutherland gets around this difficulty by assuming ". . . that some sort of filling-in mechanism occurs with outline shapes, although no attempt will be made to specify how this takes place"[6]. This is scarcely an acceptable avenue of escape from what looks like a crucial objection to theory *A*. In making this assumption, Sutherland specifically eschews any interpretation of theory *A* as a theory of contour discrimination. While this removes some ambiguity from his original statement of the theory[2], it leads one to question its plausibility. There are good reasons, both logical and factual, for supposing that contour properties are more important in shape discrimination than surface properties. Logically this is so, because the property of 'shape' in a stimulus display entails the property of 'contour' in that display, but does not entail the property of 'surface' as this has been used in the literature, to describe a difference in brightness, hue or saturation between the interior and exterior of a closed contour. A discriminating system based on contour properties is thus more general than a system based on surface properties. Practically, it seems unlikely that shape discrimination could be based only on properties of surface extent and not contours, since it has been shown for at least two types of visual system that surface and contour have different central representations[7,8]. (Young's demonstration of a marked layering of the plexiform zone in the octopus[9] suggests that the various layers might mediate different aspects of a stimulus display, such as surface and contour). Although there is no direct evidence to show what the relations are between these two types of representation, the preceding argument about the greater generality of contour discrimination suggests that, in the absence of physiological or behavioural evidence that 'filling in' occurs, the proper strategy to pursue is to assume that discrimination of shape is based on contour properties. Experimenting with outlined shapes should then yield results uncontaminated by the possibility of competing or confounding computations based on surface extent (this was pointed out some time ago in assessing earlier work on visual discrimination of shape[4]).

A further weakness of theory *A*, which has been pointed out previously[10], is that the manner in which row and column outputs are analysed is vague. The statement of theory *A* (ref. 2) suggests that this further analysis involves not only the magnitudes of the outputs but also their spatial distributions. For example, theory *A* outputs for shapes *f* and *g* in Fig. 1 are identical except for the fact that the peaks in the horizontal outputs occur in different positions relative to the total extent of the horizontal outputs: to discriminate between the pair, the analyser must register this difference. That is, since the recognition of shapes is supposed to occur independently of their position in the two-dimensional array, the analyser must be capable of registering differences in spatial patterns independently of their position ('peak to the right', or 'peak to the left' must be registered independently of whether these occur to the left, to the right, or in the centre of the horizontal output, for example). This problem, of recognizing a spatial pattern independently of its position, has always been a major difficulty for neural theories about the recognition of shapes (a difficulty which is overcome, incidentally, in theory *C*, by transformation of spatial into temporal patterns). An analyser which would perform this function would be of considerable theoretical importance, but Sutherland's statements on the topic have been too nebulous to make it possible to decide how the analyser is supposed to work, or whether it can really cope with the problem of registering spatial patterns in different positions. Further, it is difficult to see what the point is of postulating a two-dimensional array to separate horizontal and vertical components of a shape before

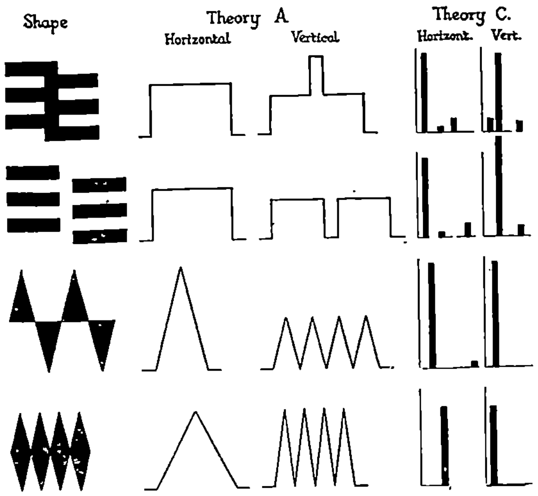


Fig. 4

input to the analyser. If the analyser has the properties required to deal with the analysis of the separate outputs of theory *A*, it should have the requisite properties for analysing shapes without prior separation into components, namely, the ability to register differences in spatial pattern independently of location.

Some New Evidence

In an attempt to gain some clear evidence in favour of either theory *A* or theory *C*, experiments were performed with rats and squirrels using the two pairs of shapes shown in Fig. 4. It can be seen by inspection of the associated outputs that theory *A* predicts good discriminability for the pair *a*, *b* and poor discriminability for the pair *c*, *d*: theory *C* predicts the reverse. These experiments will be reported in detail elsewhere; briefly, the results support theory *C* for both shapes in both rats and squirrels. In both cases there was a difference of discriminability in favour of the pair *c*, *d*. In the squirrel experiment the results are not quite conclusive, since only a small number of animals was available, and the experiment was in the nature of a pilot investigation. In the rat experiment, however, a *t*-test on the scores was significant ($P <$ 0·01), and in favour of theory *C*. It may be noted here that Sutherland has frequently reported significant differences for groups of animals in terms of a χ^2 test with one degree of freedom. Such a test is not appropriate as a test of differences between group means, since it takes no account of variability from one subject to the next. Thus, in addition to the difficulties already mentioned, a number of the results reported by Sutherland are based on inadequate statistical analysis; although the direction of the results is unambiguous, their conclusiveness is questionable.

The shapes used in the present experiment were surface shapes, rather than contour shapes. This may seem surprising, in view of the criticisms of the use of surface shapes made earlier. I used such shapes for the following reasons: (*a*) surface shapes should favour theory *A*, since that theory is a theory of surface discrimination, and it should involve complicating factors for a contour discrimination theory (namely, *C*); and (*b*) if (as seems scarcely possible) theory *A* could be interpreted as a contour discrimination theory, the use of surface shapes involves equal difficulties for both *A* and *C*. Thus, the use of surface shapes should either favour theory *A*, or be neutral between *A* and *C*: had contour shapes been used, the argument could have been put forward that the experiment was biased in favour of C.

Conclusion

To sum up: I believe that Sutherland's evaluation of theories *A* and *C* is faulty, and that the evidence he quotes supports theory *C* rather better than theory *A*. The faulty evaluation is in part due, apparently, to a misunderstanding of theory *C*. There are difficulties in his evidence for both theories, and either theory can only be saved by making additional assumptions.

Logical difficulties in theory *A* have been examined. Although the theory has not been stated clearly, it is evidently a theory which bases analysis on surface extent only, and not on the contours of figures. It seems unlikely that shape should be discriminated only in terms of surface and not in terms of contour, and physiological evidence to support this statement is available. This same evidence makes it perfectly plausible to suppose that contour can be 'abstracted' from surface shapes, and discrimination based on this abstracted information.

The analysis of horizontal and vertical outputs for theory *A* has been described quite as vaguely as the actual generation of those outputs, and to make the sort of analysis proposed comprehensible a far more thorough description is needed. Justification for separation into horizontal and vertical components is lacking, and this is a further weakness in the theory.

New evidence described here favours theory *C* over theory *A*, although the experimental conditions were biased in favour of *A*.

One may conclude by stating that neither on empirical nor on logical grounds is theory *A* superior to theory *C*. The logical weaknesses in theory *A* indicate that it should either be dropped or re-stated in a clear and unambiguous way.

P. C. DODWELL

Department of Psychology,
Queen's University,
Kingston, Ontario.

[1] Sutherland, N. S., *Nature*, **186**, 840 (1960).
[2] Sutherland, N. S., *Nature*, **179**, 11 (1957).
[3] Deutsch, J. A., *Nature*, **185**, 443 (1960).
[4] Dodwell, P. C., *Brit. J. Psychol.*, **48**, 221 (1957).
[5] Deutsch, J. A., *Nature*, **188**, 1090 (1960).
[6] Sutherland, N. S., *J. Comp. Physiol. Psychol.*, **53**, 95 (1960).
[7] Maturana, H. R., Lettvin, J. Y., McCulloch, W. S., and Pitts, W. H., *Science*, **130**, 1709 (1959).
[8] Gaze, R. H., *J. Physiol.*, **146**, 40 (1959).
[9] Young, J. Z., *Nature*, **186**, 836 (1960).
[10] Dodwell, P. C., *Nature*, **179**, 1088 (1957).

IN the preceding communication Dodwell takes me to task for the way in which I interpreted[1] my own experimental results on shape discrimination by *Octopus*. I agree with some of his general remarks. For example, he emphasizes at the outset that no complete theory of shape recognition in any species has yet been proposed. All he or I have suggested are ways in which information from patterned visual stimuli may be coded at some stage in the nervous system. These suggestions as to coding have been made to account for the relative difficulty animals have in discriminating between different pairs of shapes, the confusions they make between shapes, and the relative similarity of different shapes for a given species as revealed by transfer tests. A 'complete' theory would have to account for all the available evidence and would also be concerned not merely with how the stimuli are coded on the input side but exactly how the input as coded is further analysed and how the resulting neural output governs the responses made by the animal.

Unfortunately, I cannot agree with most of Dodwell's more detailed points. Many of them are based on assertion rather than argument. For example, he states that I use χ^2 to test for the difference between the performance of groups of animals, and rightly points out that this is the wrong statistic: in fact, in all papers written by me the significance of the difference between the performance of different groups of animals being trained to discriminate between different shapes has been evaluated by *t*-tests or analysis of variance and not by χ^2. Again, Dodwell suggests that the first stage of my own theoretical analysing mechanism (namely, breaking a shape up into its horizontal and vertical components) is unnecessary, since if the further analyser which analyses these projections "has the properties required to deal with the separate outputs of theory *A*, it should have the requisite properties for analysing shapes without prior separation into components": this is a complete *non-sequitur*. It is hard to see why Dodwell supposes that a mechanism which can analyse outputs on a one-dimensional neural array must be capable of analysing a two-dimensional array, and even if this were true there is no reason to suppose that the nervous system performs the complete analysis of shape in one stage.

Unfortunately, Dodwell does not state his own theory in the accompanying article. It may be helpful to the reader to have a brief statement of this theory[2]. He suggests that excitation representing the contours of shapes is projected on to a square array of cells in the brain. Impulses sweep across this square array in directions corresponding to the visual vertical and horizontal. Impulses travelling in either direction are said to be slowed down when they cross an area already excited by neural activity resulting from a contour present at the retina. The extent to which an impulse is slowed down is said to depend on the direction of the contour in relation to the direction in which the impulse is travelling. All impulses travelling in one direction are fed into a final common path: activity in this pathway represents the number of impulses arriving per unit of time. This theory is highly ingenious, but does not seem particularly plausible on neurological grounds, since it must involve some mechanism to synchronize the start of successive travelling waves of impulses. The pictures Dodwell presents in his Fig. 1 (column III) represent the outputs given by different shapes in the final common pathways: unfortunately, since he does not tell us how these outputs were derived, we have to take them on trust and many of them do not appear to conform to the rules he gives in his previous paper[2] (for example, those for shapes *b, c, j, l, n, o*). This doubt about how his outputs are derived makes it impossible to discuss some of the arguments based on these outputs. It may, however, be useful to discuss the crucial issues under each of Dodwell's main headings.

Evaluation in Relation to the Various Theories

Dodwell agrees that the ability of octopuses to discriminate between a diamond and an oblique rectangle constitutes a crucial test between his theory and my own. He is surely mistaken, however, when he says that the experiment I actually performed on this is inconclusive because I failed to equate the diamond and the rectangle for total brightness. He argues that since the area of the diamond used was 25 sq. cm. and the area of the rectangle was 20 sq. cm., it is possible that animals may have learned to discriminate between them in terms of the difference in the total brightness of each. This possibility is discussed in my paper reporting this experiment[3], and careful controls were run to test whether animals had been discriminating between the shapes in terms of their difference in brightness. Three findings reported in that paper rule out this interpretation: (1) If, after animals have learned the discrimination they are shown a rectangle with a *larger* area than the diamond, then, if they are responding in terms of brightness, they should respond to it in the way in which they had learned to respond to the diamond. When a large rectangle was shown to animals, they continued to respond to it in the way in which they had formerly responded to the original rectangle. (2) Similarly, when a diamond of *smaller* area than the original rectangle was shown, they continued to respond to it as they had done to the original diamond. (3) Animals were originally trained with white figures shown against a grey background: they continued to discriminate between a diamond and an oblique rectangle when they were presented with black shapes against the same background. The brightness of the black diamond was less than that of the original white rectangle. Despite these findings, Dodwell writes, "no direct test of the effect of the brightness difference was made".

Of the remaining points raised by Dodwell in this section, he is correct in saying that his theory (as well as mine) predicts transfer from a vertical rectangle to a broken vertical rectangle (*a*, in Fig. 1), and I was mistaken in supposing that it could not[1]. His other arguments, however, are vitiated by a failure to take into account the results of transfer tests. Thus, when octopuses are trained to discriminate between shapes which differ markedly in amount of contour (for example, shapes *j–q*), the ways in which they effect transfer show that something more is involved than an analysis along the horizontal and vertical axes. This means that no theory based merely on an analysis along these axes will account for all the results, and hence it is necessary to postulate some further analysing mechanism at work.

Logical Weakness in Theory *A*

Much of this section is taken up with a general accusation of vagueness in my own theory. As pointed out already, this charge is justified but applies to all theories of shape recognition hitherto proposed. However, it should be remembered that my own theory has been able to yield many detailed predictions which could be and have been tested, and that these predictions were rigorously derived from the theory Dodwell does not deny. As Dodwell rightly observes, the greatest area of vagueness lies in the fact that further mechanisms for analysing the output from rows and columns (that is, the shape of the 'projections' shown in Fig. 1 of Dodwell's article) have not been specified (for some suggestions see ref. 4). It would, however, be idle to speculate about this problem in default of evidence which would allow one to choose between alternative possibilities, and until the theory has received more thorough testing at its present level of complexity. Dodwell himself has not attempted to specify how the outputs yielded by his own mechanism are analysed.

He raises the important question of whether shapes are analysed in terms of their surface area or whether they are analysed in terms of their contours, and writes that my suggestion of a filling-in mechanism is vague. It is of course true that the contours of a shape are of importance in shape recognition, but what I am arguing is that it is the distances between contours that is important, not merely their orientation. Dodwell's mechanism operates primarily on the direction of contours in visual space: it does not analyse the distances between contours. It is for this reason that it predicts that animals should be unable to discriminate between a diamond and an oblique rectangle. All the contours of these figures run at 45° to the vertical and it is possible to draw the figures with the same total amount of contour as one another. It seems certain that, in discriminating between shapes, animals take account of the separation between contours and not merely of the directions in which they run, and for this reason Dodwell's theory is implausible. According to my theory, the octopus computes horizontal and vertical distances between contours: this would involve a filling-in mechanism for outline figures (incidentally, transfer to outline figures by octopuses is poor and sometimes non-existent[3,5]). It is important to be clear that a mechanism which operates *solely* on contour does not avoid the difficulties inherent in a filling-in process: thus if all contours present in a shape are analysed, animals that have learned to discriminate between unfilled outline figures should not effect transfer to the same figures with further contours inscribed within them. Evidence on this is lacking for the octopus, but Lashley[6] has found that rats show excellent transfer in just these circumstances. Thus a theory such as Dodwell's would need a 'blotting-out' mechanism to enable animals to disregard internal contours: this would be as complex as a 'filling-in' mechanism and indeed logically the two mechanisms reduce to the same thing.

Some New Evidence

In his final section Dodwell cites an interesting experiment he performed on rats and squirrels as providing evidence in favour of his own theory and against mine. He asserts that of the shapes shown in his Fig. 4, my theory (A) must predict that a and b would be more readily discriminable than c and d, whereas his own predicts greater discriminability of c and d than of a and b. These predictions are made on the basis of the outputs yielded according to the two theories and these outputs are shown in Fig. 4. The prediction Dodwell makes hinges on the difference between the outputs of a and b under my theory (A) being greater than the difference between the outputs of c and d, and vice versa under his theory (C). Examining the outputs in question as shown in Fig. 4, it is quite impossible to say under either theory whether the outputs of a and b are more or less different from one another than those of c and d: the reader can verify this for himself by inspection of this figure. Hence Dodwell's results, although of interest in their own right, are inconclusive with respect to the two theories under discussion. In order to say whether the outputs are more different for one pair of shapes than for another, it would be necessary to specify how the outputs themselves are analysed, which—as Dodwell himself points out—has not been done. Both theories can be made to make predictions, but only where the output for one pair of shapes is the same and the output for another different: Dodwell makes this point at the start of his section on evaluation, but he appears to have lost sight of it in designing a test between the two theories.

Conclusion

It is perhaps worth emphasizing why I have made no attempt in the above to deal individually with all the points raised by Dodwell: the details of this controversy are in fact in danger of obscuring the value of the experimental work on which the controversy is based. Dodwell and I are in agreement about the methods and aims of this type of work, and he clearly agrees that the evidence demonstrates that in the octopus an analysis of shape along horizontal and vertical co-ordinates is occurring. His own theory is valuable as a research tool, and this is all that I have claimed for my own. His theory prompted me to undertake the experiment on diamonds and oblique rectangles mentioned above, and this experiment yielded results of considerable interest irrespective of the rights and wrongs of any particular theory. The real importance of this type of experimental work lies in two aspects: (1) It demonstrates that it is possible to put forward hypothetical neurological mechanisms to explain behavioural findings, and that these mechanisms can be tested at a behavioural level. Disagreement arises at the present moment because none of the theories proposed has received sufficient systematic testing, and also none of the theories is complete. (2) Perhaps more important, the findings that have been obtained must eventually be linked with findings on the neuroanatomy and neurophysiology of the optic pathways. Only by having data on the functions mediated by these pathways will it be possible to interpret the meaning of the neuroanatomical structures and neurophysiological relationships which are at present being uncovered (cf. Young[7]). The final verification of any theory of shape discrimination must wait on further knowledge of this sort.

The work discussed in this article forms part of a project on "Stimulus Analysing Mechanisms", which is financed jointly by the American Office of Naval Research (Contract N 62558–2453) and the Nuffield Foundation. I am grateful to these bodies for their financial support.

N. S. SUTHERLAND

Institute of Experimental Psychology,
University of Oxford.

[1] Sutherland, N. S., *Nature*, **186**, 840 (1960).

[2] Dodwell, P. C., *Brit. J. Psychol.*, **48**, 221 (1957).

[3] Sutherland, N. S., *J. Comp. Physiol. Psychol.*, **53**, 95 (1960).

[4] Sutherland, N. S., *Nature*, **179**, 1310 (1957).

[5] Sutherland, N. S., *J. Comp. Physiol. Psychol.*, **53**, 104 (1960).

[6] Lashley, K. S., *J. Gen. Psychol.*, **18**, 123 (1930).

[7] Young, J. Z., *Nature*, **186**, 836 (1960).

LETTERS TO THE EDITORS

GEOPHYSICS

Connexion between Micropulsations and the Ionosphere

DUFFUS has recently pointed out a connexion between P_c occurrence and the F-region[1]. Other workers[2] have found correlation between P_t and f_0F_2 and it can reasonably be asserted that the ionosphere is a major factor in the transmission path by which these characteristic geomagnetic micropulsations are observed at ground-level. Recent work here has offered some further evidence on this subject.

For the purpose of this investigation P_c are defined as pulsations with a considerable element of continuity, lasting a number of hours, with periods between 10 and 60 sec., usually with a characteristic 'beating' appearance, and occurring predominantly in the day-time, although this last factor is not used for identification. P_t are short trains of oscillations with periods around 60 sec. which occur usually in otherwise quiet times at night. Fig. 1 shows typical examples of each class recorded on the Signals Research and Development Establishment's magnetometer. While it is generally accepted that these oscillations have their primary origin in solar emission, which by interaction with the Earth's atmosphere and magnetic field excites resonances in several possible ways[3-5], the exact manner of formation of each kind of pulsation is still unknown.

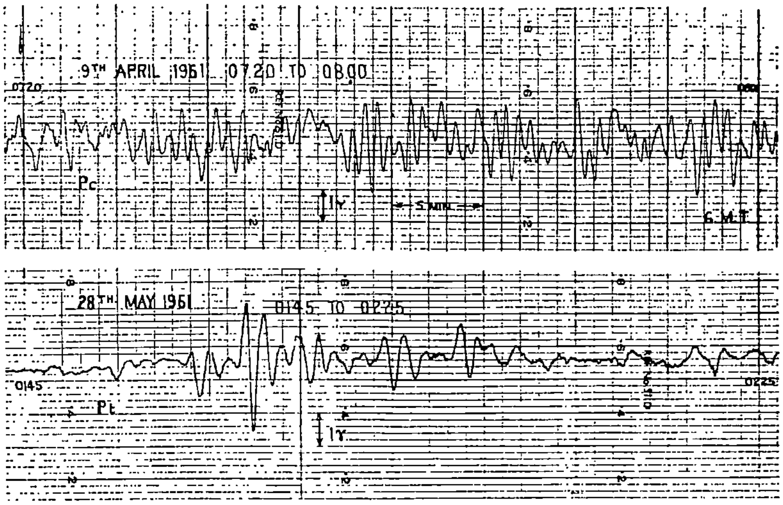


Fig. 1. Examples of geomagnetic micropulsations P_c and P_t observed at Christchurch, Hants

As part of a wider geomagnetic investigation, recording magnetometers have been set up at Christchurch (51° N., 2° W.) and Ascension Island (8° S., 14·5° W.), approximately 6,750 km. apart. Ascension is close to the geomagnetic equator so there can be no common lines of force with Christchurch. Inspection of simultaneous recordings from the two stations shows that P_t occur on both traces at the same time and with the same period, within the limits of discrimination of the system (Fig. 2). The amplitude at Christchurch is some 20–30 times that at Ascension. On the other hand, P_c, although occurring at about the same time and over the same frequency range, do not exhibit the same coherence (Fig. 3).

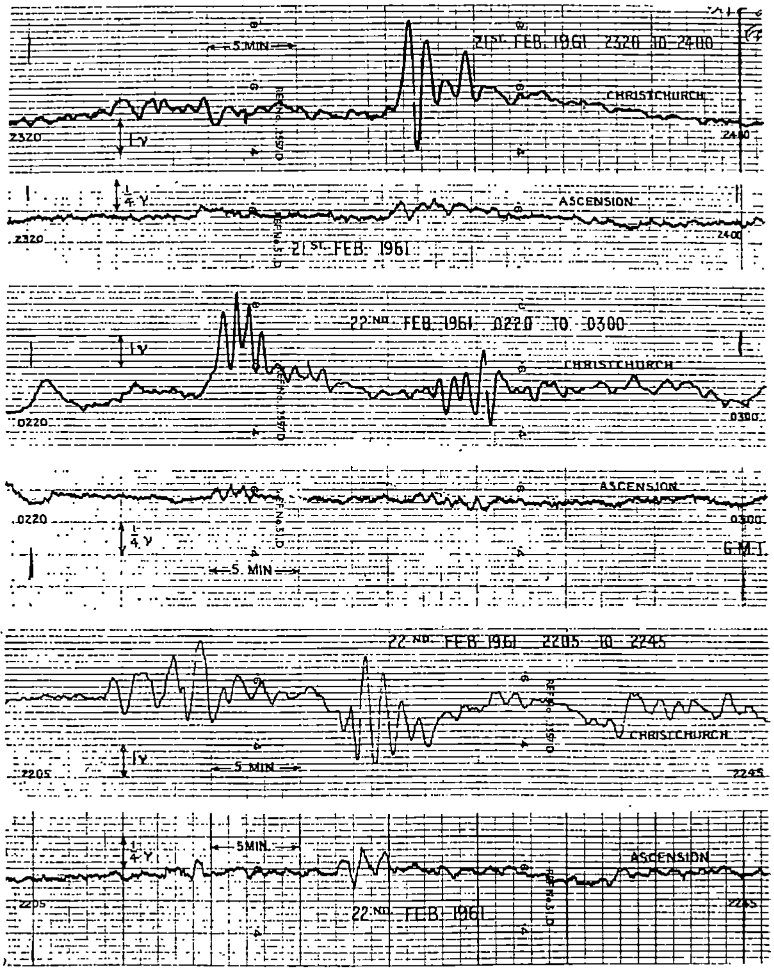


Fig. 2. Simultaneous recordings of micropulsations P_t at Christchurch, Hants, and Ascension Island

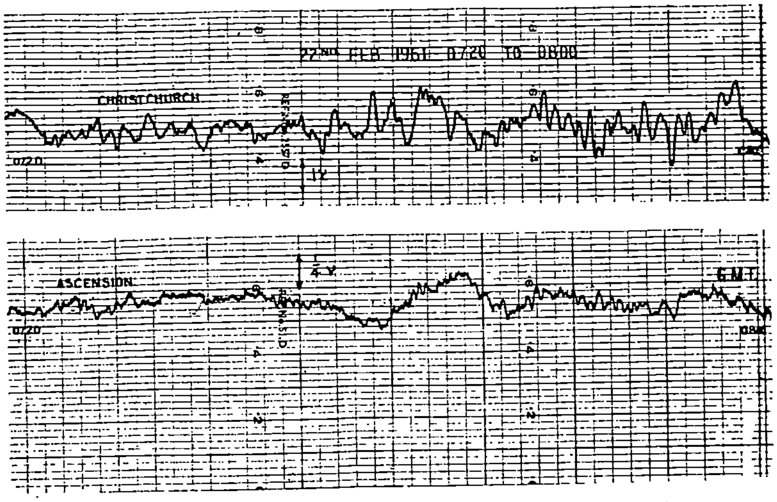


Fig. 3. Simultaneous recordings of micropulsations P_c at Christchurch, Hants, and Ascension Island

The seasonal variation of the start-up time (here defined as the time of the first fifth of the day's total active time) of P_c observed at Christchurch shows a definite correlation with sunrise (Fig. 4). This confirms the conclusion of Dr. Duffus that the low atmosphere has a modulating control over P_c and it also shows that this control varies markedly with latitude, in contrast to P_t.

The problem is to explain why P_t are night-time phenomena and are observed to be coherent over a great latitude-range, being of greater amplitude at high latitude, while P_c are recorded in the day-time and vary with latitude, both classes of pulsation being linked with the ionosphere. P_t may be oscillations of the outer atmosphere which are coupled into the ionospheric duct at the auroral belt, being propagated only in the low-attenuation night-time state of the ionosphere, while P_c may have a much more local and resonant transmission system, still originating in an outer atmosphere oscillatory mode. It is possible that part of their oscillatory content is inherent in the arriving corpuscular stream. Granulation waves of this frequency are known to exist in the photosphere[6-8].

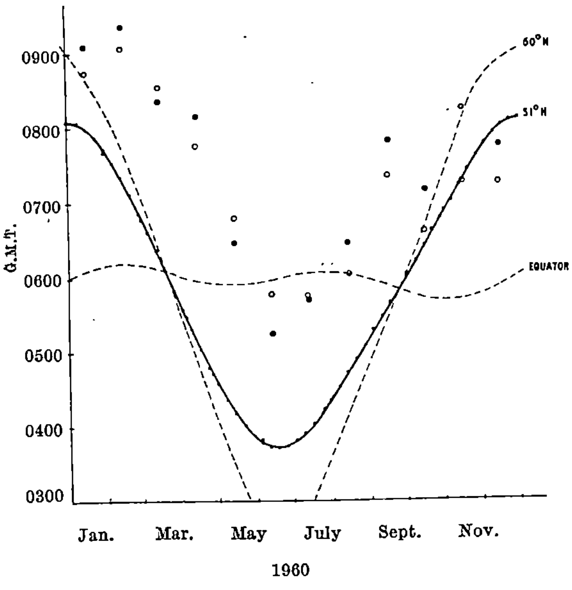


Fig. 4. Relation between mean monthly P_c start-up time and sunrise time at Christchurch, Hants. (Certain P_c are clear unambiguous displays. Weighed P_c have the hours of marginal P_c occurrence divided by 2 and added to the certain P_c.) Full line, sunrise 51° N.; ○, time of 1/5 days score weighed P_c; ●, time of 1/5 days score certain P_c; – – –, sunrise 60° N. and equator

Work at this Establishment is proceeding on the examination and classification of micropulsation activity together with a search for periodicities and cross-relationships. It is proposed to set up a magnetometer system with accurate timing between the stations and an increasing frequency coverage.

Acknowledgment is gratefully made to Mr. L. A. Brackstone of the Department of Scientific and Industrial Research at Ascension for his helpful co-operation.

C. M. Lock
P. J. Stevens

Signals Research and
Development Establishment,
Ministry of Aviation,
Somerford,
Christchurch, Hampshire.

[1] Duffus, H. J., *Nature*, **188**, 719 (1960).
[2] Afanasieva, *Proc. I.U.G.G. Meeting*, Utrecht (Sept. 1959).
[3] Dungey, J. W., *Ionospheric Res. Lab., Pennsylvania State Univ., Sci. Rep.*, No. 69 (Sept. 15, 1954).
[4] Kato, Y., and Akasofu, S., *Sci. Rep. Tohoku Univ.*, Ser. 5, *Geophys.*, 7, No. 3 (March 1956).
[5] Obayashi, T., and Jacobs, J. A., *Geophys. J. Roy. Astro. Soc.*, 1, No. 1 (1958).
[6] Alfven, *Cosmical Electrodynamics*, 147.
[7] Piddington, J. H., *Mon. Not. Roy. Astro. Soc.*, **114**, 638 (1954).
[8] Piddington, J. H., *Mon. Not. Roy. Astro. Soc.*, **116**, 314 (1956).

Stress Effects on Thermoremanent Magnetization

Hall and Neale[1] have reported accurate measurements of the directions of thermoremanent magnetization acquired by rock specimens which cooled under stress in a low magnetic field. They observed small, but consistent, differences between the directions of thermoremanent magnetization in rocks cooled under stress and without stress, and suggested that their observations conflicted with our results[2,3], which indicated the absence of measurable differences in isotropic rocks. However, we consider that the experiments of Hall and Neale are consistent both with our results and with our conclusions, which may be summarized as follows.

In an igneous rock which is cooled under stress in a small magnetic field and then released from the stress, the direction of thermoremanent magnetization differs from the field direction by an angle which is controlled both by the stress and by the intrinsic anisotropy of the rock, but if the intrinsic anisotropy is very small, then the angular difference is also very small, regardless of the stress which was applied[4] (at least up to 1,000 kgm./cm.[2]).

In our experiments, rocks which were shown by torque-meter experiments[5] to have anisotropies less than about 2 per cent were cooled under directed stresses up to 1,000 kgm./cm.^{2} in the Earth's field. Within our measurement error of 2°–3° we found that the thermoremanent magnetization after release of stress coincided with the field direction. Quite different results were obtained with anisotropic rocks; for example, in a rock with approximately 60 per cent anisotropy which was cooled under stress, thermoremanent magnetization was induced at angles between 5° and 60° away from the field direction. It is important to note, therefore, that although Hall and Neale have reported more accurate measurements of the directions of such magnetization in rock specimens, they were using specimens with appreciable intrinsic anisotropies, such that the magnetization acquired in the absence of stress differed from the field direction by as much as 4°. According to Stacey's[5] analysis, this implies intrinsic anisotropy of at least 13 per cent, which can scarcely be described as 'slight'; a limit of 10 per cent was suggested[5] as acceptable in rocks used for palæomagnetism.

Further experiments are needed on the interaction of stress and the intrinsic magnetic anisotropies of rocks. It may be necessary to impose a more stringent limit than hitherto on the anisotropy, which is acceptable in rocks used for palæomagnetism.

P. M. STOTT
F. D. STACEY

Department of Geophysics,
Institute of Advanced Studies,
Australian National University,
Canberra, Australia.

[1] Hall, J. M., and Neale, R. N., *Nature*, **188**, 805 (1960).
[2] Stott, P. M., and Stacey, F. D., *Nature*, **183**, 384 (1959).
[3] Stott, P. M., and Stacey, F. D., *J. Geophys. Res.*, **65**, 2419 (1960).
[4] Stacey, F. D., *Nature*, **188**, 134 (1960).
[5] Stacey, F. D., *J. Geophys. Res.*, **65**, 2429 (1960).

FROM the results of our pressure experiments[1] and more recent measurements of susceptibility anisotropy it appears that this parameter is of negligible importance in the production of thermoremanent pressure effects.

The scatter of thermoremanent magnetization directions mentioned in our previous communication is a maximum for the coarse-grained Ob Lusa dolerite rock type. A group of ten finer-grained contact specimens from the Ob Lusa dolerite dyke show a scatter of only ±1·5° about the mean direction (the ambient field direction), while showing a pressure-induced rotation of moment six times larger than that of the coarser specimens. It is these latter specimens that show a maximum scatter of ±4° about the ambient field direction.

Measurements of susceptibility anisotropy at low fields have been made at Cambridge on all the specimens used in the investigation. Anisotropies of 1 per cent or less between axial values of initial susceptibility were found for all the rock types employed with the exception of that having pyrrhotite as a magnetic component.

J. M. HALL

Applied Geophysics Department,
Imperial College of Science and Technology,
London, S.W.7.

[1] Hall, J. M., and Neale, R. N., *Nature*, **188**, 805 (1960).

ASTRONOMY

Holistic Approach to Selenology

THE proximity of exploration of the surface of the Moon demands a critical review of present selenological hypotheses. I wish to point out a defect inherent in modern selenological thought and to stress that there is a need for more information before developing contemporary theories more fully.

Theories of the formation of lunar craters may be divided into two classes: extrinsic (that is, impact—favoured by Baldwin and Urey) and intrinsic (that is, igneous—championed by Jeffreys and Spurr). Broadly speaking, the extrinsic hypotheses appeal to macroscopic features (for example, predominant circularity, the depth-diameter relationship, and the near-random distribution of craters) while the intrinsic theories appeal more to the finer features (including crater chains, summit craterlets, and crater polygonality).

The limitation to both approaches is that neither (in the first instance) distinguishes between primary and secondary features of the lunar surface.

Primary features are the large-scale structures as seen immediately after they were formed, and by secondary features I mean the alterations to the primary structures during their later life (this includes the effects of stress systems and erosion).

Consideration of the lunar grid system shows that large-scale stresses have been applied to the lunar surface during its history, and the effects of these stresses have been to obliterate information pertinent to the origin of the craters. In addition, completely new types of feature have arisen (for example, rill and valley systems) and some of these have been erroneously interpreted as primary features. Thus when one accepts the fact that the valleys radial to the Mare Imbrium are nothing more than a slightly more prominent element of the general grid system the collision hypothesis for the origin of this Mare loses considerable weight.

I would suggest, therefore, that the need at the present time is for a more expiscate approach. Many details of the lunar surface found recently do not fit in comfortably with any existing theories of crater formation, nor indeed with present ideas on secondary activity. We can only attempt to construct a complete theory when we have realized more of the properties of the features with which we are dealing.

BRIAN WARNER

University of London Observatory,
Mill Hill Park,
London, N.W.7.

Fielder, G., *Structure of the Moon's Surface* (Pergamon Press, London, 1961).
Warner, B., *J. Brit. Astron. Assoc.*, **71**, 116 (1961).
Warner, B., *J. Brit. Astron. Assoc.* (to be published).

PHYSICS

Use of a Different Type of Fluorescent Material as an Optical Maser

THE recent interest in the development of infra-red and optical maser systems has involved a number of different basic principles[1-3]. This communication is concerned with an alternative concept which utilizes the Stokes's shift associated with absorption and emission bands created by point defects in solids.

Preliminary calculations indicate that such a device can be made to operate.

We will be concerned with defect centres which possess non-overlapping absorption and emission bands. A specific example is the *F*-centre in potassium chloride, and this centre is selected as a representative example on which detailed calculations are performed since many of its properties are known. It is believed to consist of an electron trapped at a negative-ion vacancy. The broad absorption band associated with this defect centre shows a peak at 5600 Å. (2·3 eV.) while the emission band lies at 1·0μ. At temperatures below 80° K., the absorption-emission process is very nearly 100 per cent efficient so that for each photon absorbed one is emitted[4].

One can describe the principles involved in this suggested device by introducing a configuration co-ordinate curve (Fig. 1). The total energy is plotted along the ordinate, while the abscissa represents a general configuration co-ordinate. The frequency shift which occurs between the absorption and emission bands can be explained by referring to Fig. 1. The system in its ground-state at A undergoes the transition $A \rightarrow B$ when it is stimulated by the appropriate radiation. Then a radiationless transition, $B \rightarrow C$, follows, which lowers the vibrational level of the excited state. The system decays to a high vibrational level of the ground-state through the radiative transition, $C \rightarrow D$. Finally, the system relaxes back to A. Thus, the entire process may be visualized as a series of transitions between levels A, B, C and D. In view of the experimental data on emission, one may be certain that thermal equilibrium is established in the upper state before the emission of a photon. The non-radiative transitions from C to D are known to be small.

The relaxation times for the phonon-emitting transitions, $B \rightarrow C$ and $D \rightarrow A$, are approximately 10^{-12} sec., while the photon-emitting transition takes about 10^{-8} sec. Thus, it should be possible to make the ratio of populations in states C and D large by injecting radiation of the frequency $\nu_a = (E_b - E_a)/h$. On exposure to radiation of a second frequency, $\nu_e = (E_c - E_d)/h$, downward transitions are induced, resulting in the amplification of this second signal.

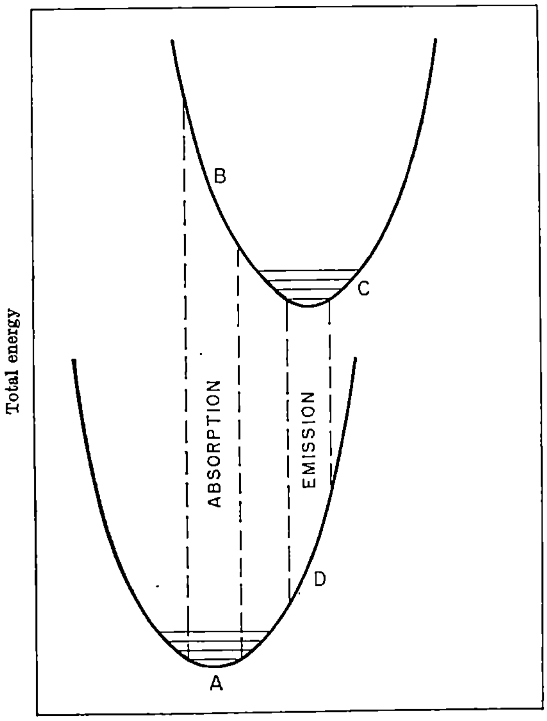


Fig. 1

The degree of amplification attained from such a system can be studied in terms of several parameters. The intensity of radiation emitted, I, is given by the relationship:

$$I = I_0 e^{\alpha' x} \tag{1}$$

where I_0 is the stimulating signal incident on the crystal, x is the distance the light travels and α' is a constant which corresponds to the absorption coefficient in the case of absorption. The absorption coefficient of a crystalline material containing defect centres is given by Smakula's equation as[5]:

$$\alpha = \frac{2}{9}\frac{e^2 f}{mc}\frac{(n^2 + 2)^2}{n^2}\cdot\frac{1}{\Delta\nu} N_a \tag{2}$$

where $\Delta\nu$ is the absorption band-width at half intensity; f is the oscillator strength associated with the defect centre; n_1 is the refractive index of the host crystal; N_a is the concentration of centres in the ground-state over that in the excited state; and e, m and c have their usual meanings.

If one now replaces $\Delta\nu$ with $\Delta\nu'$, the width at half height of the emission band, and N_a by N_c, one can adopt equation (2) to the case of emission since for any temperature of interest, $N_c \gg N_d$. In the case of the *F*-centre in potassium chloride:

$$\alpha' = 2{\cdot}7 \times 10^{-16} N_c \tag{3}$$

where f has been set equâl to 0·81. If $N_c = 10^{14}$, we find from equation (1) that x must be of the order of meters if one is to achieve a substantial gain; however, a measurable amplification could be attained if x were of the order of cm.

Consider next the power requirements to attain 10^{14} centres in state C. The life-time of this state in the absence of stimulating radiation is given by the einstein A coefficient as $5{\cdot}5 \times 10^7$ sec. when the effects of the dielectric are neglected. Hence, the life-time of the state C is $1{\cdot}8 \times 10^{-8}$ sec. and the required pumping power to maintain 10^{14} centres in state C is:

$$P = \frac{(h\nu)10^{14}}{1{\cdot}8 \times 10^{-8}} = 1{\cdot}27 \times 10^{22} \text{ eV./sec.} = 2{,}000 \text{ W.}$$

This is excessive if one hopes to maintain the required temperature; however, one may reduce this requirement by varying several parameters. The crystal dimensions could be reduced to 1 cm. × 1 cm. × 0·1 cm., thus reducing the power by a factor of 10. Furthermore, the device may be operated intermittently. Hence, the pumping power is reasonable for experimental purposes.

It should be pointed out that heavy *F*-centre concentrations will introduce non-linear effects owing to greater absorption at the face of the crystal than in its interior; however, these effects should not seriously affect the performance of such a device.

One mode of operating such a device would involve a thin crystal, two sides of which would be silvered and approximately parallel. The silvered sides would have two small apertures, thus permitting a long path for the radiation which is to be amplified due to multiple reflexions. The pumping radiation would fall on the face of the crystal at right angles to the stimulating radiation and would traverse the crystal in its shortest dimensions.

The noise inherent in such a system would be the result of spontaneous emission within the crystal; however, this spontaneously emitted radiation would be contained in a 4π steradian solid angle and very little of it would be travelling in such a direction as to emerge from the crystal through the small exit aperture. One may most probably measure the spontaneous emission emerging from a small exit aperture. However, we believe that the stimulated emission will be much greater under operating condition and the noise should not be a major problem. Thus, the spontaneously emitted radiation will lower the density of systems in the excited state, but should not be a serious limitation from the standpoint of noise.

JORDAN J. MARKHAM
DICKRON MERGERIAN

Physics Research Division,
Armour Research Foundation,
Chicago.

---

[1] Maiman, T. H., *Nature*, **187**, 493 (1960); *Brit. Comm. and Electron.*, **7**, 674 (1960).

[2] Sorokin, P. P., and Stevenson, M. J., *Phys. Rev. Letters*, **5** (7), 557 (1960).

[3] Javan, A., Bennett, jun., W. R., and Herriott, D. R., *Phys. Rev. Letters*, **6** (3), 106 (1961).

[4] van Doorn, C. Z., and Haven, Y., *Philips Res. Rep.*, **11**, 479 (1956). Becker, K., and Pick, H., *Nachr. Akad. Wiss. Gottingen*, Math.-physik. Kl., **2**, *a*, 167 (1956).

[5] Markham, J. J., *J. Phys. Chem.*, **57**, 762 (1953).

Dimensions of Electrical and Magnetic Quantities

FOR rather more than ten years the rationalized M.K.S. system of units has been generally accepted as the most satisfactory one to employ for practical calculations involving electrical and magnetic quantities[1,2]. This system side-steps the question of dimensions by introducing two unfortunate expressions—permittivity and permeability of free space—and assigning to them, respectively, the values $10^{-9}/36\pi$ farad/metre and $4\pi \times 10^{-7}$ henry/metre. Any dislike aroused by these terms may, however, be easily overcome by giving them new names: electric factor and magnetic factor, the question of units being left until later.

Unfortunately, the situation regarding dimensions is less satisfactory and still remains unsettled. L. H. A. Carr[3] has invoked the special theory of relativity to show that dimensionally Coulomb's law for the force F between charges q_1 and q_2 at a distance r apart in a uniform medium is $[F] = [q^2 L^{-2}]$, and from this relation all electrical and magnetic quantities can be expressed solely in the dimensions mass, length and time. Carr's proposal, however, does not seem to have been taken up and is not even mentioned in recent text-books[4,5].

An alternative suggestion is to regard q as a dimension in its own right[2]; but this settles nothing and leads to the singularly useless result that electromotive force $[E] = [ML^2 T^{-2} q^{-1}]$, implying that E is inversely proportional to q, whereas in fact the inverse is true. It is the purpose of this communication to advance evidence in support of Carr's proposal and to demonstrate that it leads to a very satisfactory direct physical relationship between electrical and magnetic quantities and their dimensions.

The gravitational force of attraction F between two masses is given by $[F] = [G M^2 L^{-2}]$, where G is the so-called gravitational constant and is necessary to make the equation balance dimensionally. However, gravitational attraction is independent of the medium and the introduction of G is quite arbitrary. Mass is only a convenient way of assessing the quantity of matter in a body, and it happens that in the gravitational equation its use leads to a rather awkward result dimensionally. An equally legitimate method for assessing a quantity of matter is to count protons or electrons, a neutron being supposed for this purpose to contribute one of each. It follows then that if two bodies are represented by $N_1 q_0$ and $N_2 q_0$, where q_0 is the elementary charge and N_1 and N_2 are pure numbers, $[F] = [q^2 L^{-2}]$ when $[M]$ in the gravitational equation is replaced by $[q]$ and $[G]$ is discarded as being unnecessary. From this it follows that $[q] = [M^{\frac{1}{2}} L^{\frac{3}{2}} T^{-1}]$, in agreement with Carr. It has been objected that dimensions with fractional exponents are meaningless, but this is merely a point of view, for if $[q]$ is taken as the fundamental quantity rather than $[M]$, then $[M] = [q^2 L^{-3} T^2]$ and the fractions disappear. Even though the relation $[q] = [M^{\frac{1}{2}} L^{\frac{3}{2}} T^{-1}]$ is not very attractive, the expression for the energy of a charged sphere, $\frac{1}{2}q^2/r$, is simple enough and the dimensions of q follow directly from this. Having established the dimensions of charge, it follows that $[E] = [M^{\frac{1}{2}} L^{\frac{1}{2}} T^{-1}]$. This relationship is equivalent to $[E] = [F]^{\frac{1}{2}}$ and is quite satisfactory since the force of attraction between two similar parallel plates, oppositely charged, can be directly measured and is found experimentally to be proportional to E^2, where E is the electromotive force between them.

The energy stored in a capacitor is $\frac{1}{2}CE^2$ and it follows therefore that $[C] = [L]$, and since E^2/R is the rate of doing work, $[R] = [L^{-1} T]$. Combining $[R]$ and $[C]$ we have $[R.C] = [T]$ and this result is independent of the nature of the medium. The identification dimensionally of RC with time is not difficult to accept when it is realized that the important criterion is that it shall be possible to measure time by means of a combination of resistance and capacitance. This is done by charging the capacitor to a convenient voltage E and connecting a resistor across its terminals. After time RC the voltage has dropped to $1/e$ of its original value, where $e = 2{\cdot}718$.

It is sometimes argued that capacitance cannot be physically equivalent to length, even though the capacitance of a sphere is proportional to its radius; but employing the criterion used for RC, namely that the dimension in question must be measurable by the quantity or quantities concerned, all that is necessary is to charge a parallel plate capacitor to a given voltage and then observe the rise in voltage as the plates are separated. The voltage is proportional to the distance apart of the plates and therefore the voltmeter used can be directly scaled to indicate distance.

Resistance also may be used to measure length in a direct manner since the resistance of a wire of constant cross-section is proportional to its length. However, resistance varies as length divided by area of cross-section, that is, $[L^{-1}]$, and in fact it is proportional to $[L^{-1} T]$, which is reasonable since for a fixed applied voltage resistance is inversely proportional to the mean velocity of electron flow. The impedance of free space (nearly 377 ohms) is obtained by multiplying the velocity of light in m./sec. by the electric factor $10^{-9}/36\pi$ and taking the reciprocal. Dimensionally this checks with $[R] = [L^{-1}\ T]$, for now, of course, the electric factor is dimensionless. The law of force between two charges in a uniform medium becomes $F = q_1\ q_2/\alpha\ r^2$, where α is proportional to the dielectric constant of the medium and is a pure numeric.

The dimensions of magnetic pole, m, are readily obtained from the relation $[m.q] = [M\ L^2\ T^{-1}]$ which is independent of the medium. From this equation, $[m] = [M^{\frac{1}{2}}\ L^{\frac{1}{2}}]$ and the law of force between two magnetic poles, m_1 and m_2, spaced a distance r apart in a uniform medium is given by $F = m_1\ m_2\ c^2/\beta r^2$, where c is the velocity of electromagnetic radiations *in vacuo* while β is proportional to the permeability of the medium and is a pure numeric. The relation $[m] = [M^{\frac{1}{2}} L^{\frac{1}{2}}]$ may not look promising but for a U-shaped magnet with poles at its extremities, the force between the poles produces a bending moment in the magnet and a very direct connexion between $[m^2]$ and $[ML]$ is apparent. The magnetic factor applicable to the rationalized M.K.S. system is proportional to β/c^2 and should therefore be given in units of sec.^{2}/m.^{2}.

The equation $[F] = [q^2\ L^{-2}]$ is the only one which leads to the plausible relationships discussed above. More general equations are $F = \dfrac{q_1\ q_2}{\alpha\ \varkappa_0\ r^2}$ and $F = \dfrac{m_1\ m_2}{\beta\ \mu_0\ r^2}$, where $\varkappa_0$ and μ_0 represent unknown properties of free space and $\varkappa_0\mu_0 = 1/c^2$. Let $\mu_0 = 1$ and $\varkappa_0 = 1/c^2$, from which we obtain $[E] = [M^{\frac{1}{2}}\ L^{\frac{3}{2}}\ T^{-2}]$, $[C] = [L^{-1}\ T^2]$ and $[R] = [LT^{-1}]$. These expressions cannot be simply related to the nature of the quantities concerned and, for example, capacitance becomes the inverse of acceleration, while resistance has the dimensions of velocity, the inverse of what might reasonably be expected.

A. E. MARTIN

Sir Howard Grubb, Parsons and Co., Ltd.,
Walkergate, Newcastle upon Tyne, 6.

---

[1] Schelkunoff, S. A., *Proc. Inst. Radio Eng.*, **36**, 827 (1948).

[2] Symposium, *Proc. Inst. Elec. Eng.*, Part 1, **97**, 235 (1950).

[3] Carr, L. H. A., *ibid.*

[4] Bleaney, B. I., and Bleaney, B., *Electricity and Magnetism* (Clarendon Press, 1957).

[5] Smith, C. J., *Electricity and Magnetism* (Edward Arnold (Publishers), Ltd., 1959).

CHEMICAL ENGINEERING

Inversion of a Laplace Transform arising from a Problem in Applied Chemical Kinetics

THE solution to a problem (Wood, T., unpublished work) arising from an analysis of a closed circuit consisting of a stirred-tank reactor and a tubular reactor in series, with first-order, isothermal, irreversible kinetics, involves finding the inverse of the Laplace transform :

$$\bar{c}(s) = \frac{1}{s + \beta_1 - \beta_2 . \exp(-\beta_3 s)}$$

where β_1, β_2, β_3 are known, positive, constants. The transform can be re-arranged:

$$\bar{c}(s) = \frac{\dfrac{1}{(s + \beta_1)}}{1 - \dfrac{\beta_2}{(s + \beta_1)} . \exp(-\beta_3 s)}$$

Since no tabulated transform could be found, the denominator was expanded as a power series, giving:

$$\bar{c}(s) = \sum_{n=0}^{\infty} \left[\frac{\beta_2{}^n}{(s + \beta_1)^{n+1}} . \exp(-n\beta_3 s)\right]$$

The inverse of the transform is now readily shown to be[1]:

$$c(t) = \sum_{n=0}^{\infty} \frac{\beta_2{}^n}{n!} . (t - n\beta_3)^n . \exp\{-\beta_1(t - n\beta_3)\} . H(t - n\beta_3)$$

where $H(t - n\beta_3) = 0$ when $t \leqslant n\beta_3$; $H(t - n\beta_3) = 1$ when $t > n\beta_3$.

The validity of the solution in this particular case was proved by its ability to satisfy the original problem and the given initial and boundary conditions. It would be of interest to know, in the general case, the conditions for which the approach adopted above is valid.

T. WOOD

Department of Chemical Engineering,
University of Sydney.

---

[1] Carslaw, H. S., and Jaeger, J. C., *Operational Methods in Applied Mathematics* (Oxford Univ. Press, 1953).

GEOLOGY

Continuation of the Great Glen Fault beyond the Moray Firth

THE Great Glen Fault was shown by W. Q. Kennedy[1] to be a sinistral tear fault on which a displacement of about 65 miles has occurred since Middle Old Red Sandstone time. E. M. Anderson[1] considered that "the curvature of the fault trace may indicate that we are dealing with the south-western sector of a major fault and that the main extension of the dislocation will probably lie to the north-east". This curvature is a gradual change of strike from about 040° in Loch Linnhe to about 035° at Inverness.

Examination of the submarine topography of the Moray Firth, Orkney and Shetland areas shows that a possible course for the Great Glen Fault lies through Shetland to the edge of the continental shelf north of Shetland[2]. The features which seem to indicate the continuation of the fault in this direction are shown in Fig. 1. Between Smiths Bank and the Caithness coast, a shallow submarine valley is especially well shown by the 30-fathom contour; the axis of this valley has a strike of 035° like the Great Glen Fault at Inverness. However, to pass along the axis of the valley, the strike of the fault north-east of Inverness has to change from 035° to 040° and then back again to 035° over a distance of about 40 miles.

To the north, the sea-floor east of Orkney is rather flat and featureless, and provides no evidence. If the fault crosses it with a gradual swing in strike towards the north, it runs into, and along, a trench to the south-west of Fair Isle shown up by the 50-fathom contour. The axis of this trench has a strike of about 025° to the south-west of Fair Isle and swings round to about 020° in its continuation up the west side of Shetland; it reaches its greatest depth of more than 70 fathoms at its northern end in a latitude of 60° N.

At the northern end of the trench, if the fault passes just east of the deepest part and to the west of The Deeps (a narrow submarine valley extending beyond the end of the trench and shown up by the 50-fathom contour) it runs ashore in Shetland at the head of Seli Voe and joins on to the Walls Boundary Fault[3]. The Walls Boundary Fault can be followed overland until it runs into the sea at Aith Voe. Still farther north it crosses the isthmus at Brae and can then be traced intermittently up the east coast of North Maven.

In the region between Seli Voe and Aith Voe, Old Red Sandstone intruded by granite lies to the west of the fault and gneisses and granite to the east. Finlay[3], and the Survey[4] report shattering, shearing and mylonitization on an extreme scale in the Old Red Sandstone and the granite, up to distances of more than a mile to the west of the fault. There is much shearing of the metamorphic rocks up to a mile to the east of the fault. At Brae, where the fault crosses the isthmus, it lies in a half-mile wide belt of intense shearing and shattering. On the east coast of North Maven, typical pegmatitic granite of the Graven Intrusive Complex of Delting[5] which is on the east side of the fault is in contact with low-grade schists and phyllites on the west side of the fault. About a mile farther west these metamorphic rocks are separated from a granite by a parallel fault. Intense shattering and shearing are associated with these faults.

The Walls Boundary Fault seems to be one of the most powerful dislocations in Shetland. No correlation across it seems possible; there is little similarity between either the metamorphic or the Old Red Sandstone rocks on either side of it. To the east, the Old Red Sandstone contains no contemporaneous or intrusive igneous material, while to the west it contains great thicknesses of volcanic ashes and lavas and is cut by a series of late granites. The Old Red Sandstones cut by the Walls Boundary Fault are considered by Finlay[3] to be of Lower Old Red Sandstone age. This indicates an age later than Lower Old Red Sandstone for a displacement of major proportions on the fault, and agrees with the findings of Kennedy for the Great Glen Fault.

North of Shetland the sea-floor is rather flat and featureless for some distance. However, the 100-fathom contour shows a small southward invagination on the strike of the projected fault if it continues its anticlockwise swing in strike (Fig. 2). To the north of the 100-fathom contour on the continental slope is a very large-scale feature of a type which would be expected if the continental slope itself had been intersected and offset by a sinistral tear fault with a displacement of about 50 miles.

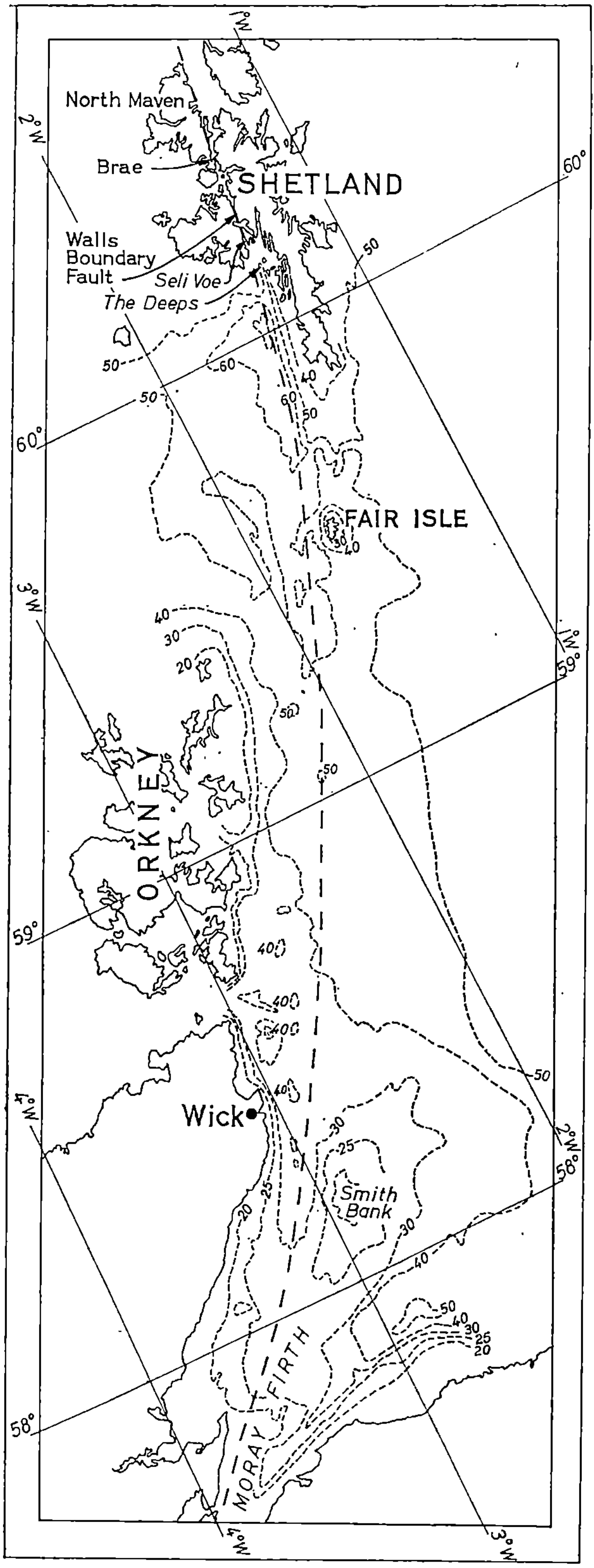


Fig. 1. Possible course for the Great Glen Fault through Shetland to the edge of the continental shelf north of Shetland

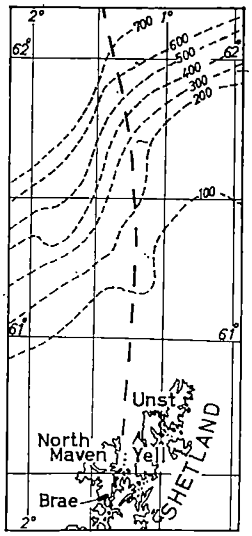


Fig. 2

The soundings on which Figs. 1 and 2 are based and several of the contours have been reproduced from British Admiralty Charts Nos. 115, 2182*c*, 2339, 3057, 1119 and 1118*b* with the permission of the Controller of H.M. Stationery Office and of the Hydrographer of the Navy, for the usual fee.

DEREK FLINN

Jane Herdman Laboratories of Geology,
University of Liverpool.

[1] Kennedy, W. Q., *Quart. J. Geol. Soc. Lond.*, **102**, 41 (1946).

[2] *British Admiralty Charts*, 1118*b*, 1119, 2182*c*, 2339, 3057, 115.

[3] Finlay, T. M., *Trans. Roy. Soc. Edin.*, **56**, 671 (1930).

[4] *Summaries Prog. Geol. Surv. Great Britain for 1932*, Pt. 1, 77 (1933).

[5] Flinn, D., *Quart. J. Geol. Soc. Lond.*, **110**, 177 (1954).

CHEMISTRY

Bond-Lengths in Solid Helium

RECENTLY, Vignos and Fairbank[1] reported the existence of a new solid phase of helium-4 which exists in a narrow range of pressure between 1·45° K. and 1·78° K. They have designated the new solid as the γ-phase. Its density is less than that of the α-phase (hexagonal closest packing) of helium with which it is contiguous. It is known[2,3] that in helium-3 at a temperature almost as low, there is a phase transition in the solid from hexagonal closest packing to a less-dense phase which is body-centred cubic. Reasoning by analogy, the authors believe the new phase will be found to have the body-centred cubic, *A*2, structure.

In 1947 Pauling[4] developed an equation which has been successful for treating covalent and metallic bonds and for estimating their change in length on a change in co-ordinating number. Thus, for a proposed change in structure, one could in many cases compute the molar volume of the new structure from the cell constants of the old one. The equation is given in the form:

$$R(1) - R_n = 0{\cdot}300 \log n$$

Here $R(1)$ is the single bond-radius of the element, R_n the radius along a bond direction in a structure, and n is the bond number. Furthermore, n equals v/N, where v is the valence and N is the number of bonds.

In the hexagonal closest-packed structure, each atom has twelve nearest neighbours and in the *A*2 structure eight. Because of the fewer neighbours in the cubic structure, the bond-radii will be shorter even though the molar volume may be greater. If bonds were formed with only the nearest neighbours, the decrease in bond-radius would be:

$$\Delta R = R_n(12) - R_n(8) = 0{\cdot}300 \log 12/8 = 0{\cdot}053\,\text{Å}.$$

However, in the *A*2 structure, there are six more neighbours at a distance only slightly longer than that of the nearest eight, and therefore some of the bond-forming power of the atom is used to make bonds with them. This results in the bond-number of the shorter bonds being less than $v/8$ and the correction in bond radius becoming less than 0·053 Å. Thus in the case cited by Pauling[4], of zirconium, which undergoes a phase transition of the kind discussed here, the bond-number of the stronger bonds is near $v/9{\cdot}24$ and ΔR is observed to be 0·037 Å.

Although the bonds in solid helium are mainly van der Waals, it is nevertheless instructive to apply Pauling's equation to the known phase change in helium-3. The molar volumes of helium-3 at 2·805° K. at the hexagonal-cubic transition line as deduced from the results of Grilly and Mills[2] are 19·088 c.c. and 19·206 c.c. respectively. From this one obtains $R_n(12) = 1{\cdot}776$ Å., $R_n(8) = 1{\cdot}730$ Å., and $\Delta R = 0{\cdot}046$ Å. The observed ΔR of 0·046 Å. corresponds to a bond-number of $v/8{\cdot}43$. This value of the bond-number is larger than that observed for zirconium partly because the unit cell of helium is larger and partly because the van der Waals forces have a short range. One would not expect these forces to form proportionally as strong a bond with the six farther neighbours as would be the case with structures having covalent or metallic bonding.

Returning to helium-4, if the new phase has the cubic body-centred structure as is supposed, one can compute the change in molar volume during the solid–solid transition. Referring again to the results of Grilly and Mills[2], the molar volume of solid helium-4 under a pressure of 29·88 kgm./cm.^{2} (near 1·723° K.) is 20·742 c.c. The method used in the experimental determination indicates that this is probably the volume of the hexagonal phase (Mills, R. L., and Grilly, E. R., personal communication). In accordance with this, the bond-radius, $R_n(12)$, of 1·826 Å. is obtained. If one uses as a first approximation the correction in bond-radius of 0·046 Å. that occurs in helium-3, then $R_n(8)$ will be 1·780 Å. The latter distance gives a molar volume for the cubic phase of 20·929 c.c. and a ΔV of transition to this form of 0·187 c.c./mol.

Swenson[5] reports the molar volume of solid helium-4 at 28·06 kgm./cm.^{2} pressure (1·6° K.) to be 20·95 c.c. If this solid is also in the hexagonal form, then one obtains 0·189 c.c./mole as the ΔV of transition. If, however, the solid has the *A*2 structure, then the ΔV of transition would be higher by only 0·014 c.c./mole.

Preparations are now under way at this Laboratory to determine the crystal structure of the new solid form.

This work was carried out under the auspices of the U.S. Atomic Energy Commission.

A. F. SCHUCH

Los Alamos Scientific Laboratory,
University of California,
Los Alamos, New Mexico.

[1] Vignos, J. H., and Fairbank, H. A., *Phys. Rev. Letters*, **6**, 265 (1961).

[2] Grilly, E. R., and Mills, R. L., *Ann. Phys.*, **8**, 1 (1959).

[3] Schuch, A. F., Grilly, E. R., and Mills, R. L., *Phys. Rev.*, **110**, 775 (1958).

[4] Pauling, L., *J. Amer. Chem. Soc.*, **69**, 542 (1947).

[5] Swenson, C. A., *Phys. Rev.*, **79**, 626 (1950).

Thermo-electric Phenomena in Electrodialysis Systems

ALTHOUGH several authors, notably Staverman[1] and Spiegler[2], have applied the methods of the thermodynamics of the steady-state to the treatment of electrodialysis processes, little or no attention has been given to the possible existence of heat fluxes in electrodialysis experiments. Of course, very useful relationships can be obtained between the flow of charge and the flow of matter through an appropriate treatment of an isothermal experiment, but we cannot say that we have described the system unless we consider the possibility of heat flows induced by these transport processes.

The argument in favour of this point of view may be roughly summarized as follows. We should like to find an expression for the rate of entropy generation, and if $\bar{S}$ is the entropy current in the steady system then:

$$\dot{S} = \nabla.\bar{S} = \frac{1}{T}\nabla.\bar{Q} + \bar{Q}.\nabla\left(\frac{1}{T}\right)$$

and by an argument based on the existence of local equilibrium within infinitesimal elements of the system:

$$\nabla.\bar{Q} = -\Sigma_i\nabla\mu_j.\bar{J}_j$$

where μ's are the total potentials (chemical, electrical, etc.) and the $\bar{J}$'s are currents of matter or electricity. A more complete discussion of this line of reasoning has been given by Callen[3]. In writing out a complete expression for the various currents that may exist in an electrodialysis system we must deal in the simplest case with at least four items: salt flow, solvent flow, electric current and a heat current. In an electrochemical system, it is convenient to formulate these flows in terms of the ions, the solvent and the heat. The electric current is, of course, the sum of individual ionic flows multiplied by the appropriate ionic charges.

Flow of positive ions $\Phi_1 = -L_{11}\frac{1}{T}\nabla\mu_1 - L_{12}\frac{1}{T}\nabla\mu_2 - L_{13}\frac{1}{T}\nabla\mu_3 + L_{14}\nabla\left(\frac{1}{T}\right)$

Flow of negative ions $\Phi_2 = -L_{21}\frac{1}{T}\nabla\mu_1 - \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad + L_{24}\nabla\left(\frac{1}{T}\right)$

Flow of solvent $\Phi_3 = -L_{31}\frac{1}{T}\nabla\mu_1 - \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad + L_{34}\nabla\left(\frac{1}{T}\right)$

Flow of heat $\Phi_4 = -L_{41}\frac{1}{T}\nabla\mu_1 - L_{42}\frac{1}{T}\nabla\mu_2 - L_{43}\frac{1}{T}\nabla\mu_3 + L_{44}\nabla\left(\frac{1}{T}\right)$

Elimination of all terms in ∇T simplifies the situation but clearly does not guarantee that $\Phi_4 = 0$.

I have begun a study of the coupling between the electrical currents and the possible heat flows in electrodialysis systems using the apparatus indicated schematically in Figs. 1 and 2. In the first experiments, made with the apparatus shown in Fig. 1, measurement of the voltage developed between the isothermal probes showed that an electrical current did indeed flow under the influence of a temperature gradient, and the sign of the potential indicated that, contrary to my initial expectation, the salt was becoming concentrated in the warmer part of the loop. Unfortunately, this particular apparatus is not suitable for obtaining quantitative relationships with thin-film ion exchange membranes.

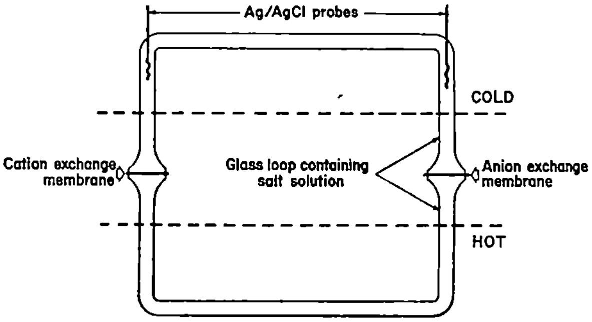


Fig. 1. Apparatus used to demonstrate coupling between heat flow and current flow in dialysis system. Salts tested, 1 *N* sodium chloride and 1 *N* lithium chloride. Left electrode is invariably negative when temperature is controlled as shown

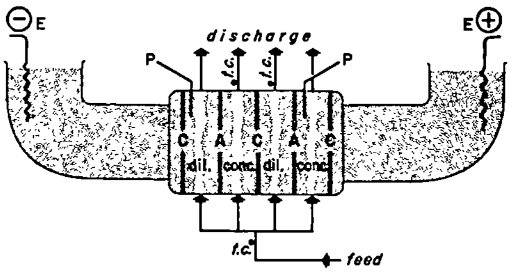


Fig. 2. Apparatus used to measure heat flow between diluting and concentrating compartments. *P*, Probes; *C*, cation exchange membrane; *A*, anion exchange membrane; *E*, power electrode (heavy silver/silver chloride mesh); *t.c.* ●, thermocouple

Some quantitative results have, however, now been obtained with the apparatus indicated schematically in Fig. 2. Electrodialysis experiments have been conducted in this system under adiabatic conditions. The liquid circulation-rates through the various compartments are made as nearly equal as possible; and, with the electric current and the circulation-rate adjusted so that there is little or no change in the concentration of the fluid as it passes through the alternating diluting and concentrating compartments, the changes in temperature in the streams indicated are measured. An estimate of the I^2R heating per cell is obtained from the current passed and the voltage developed at the probes. Under the conditions of these experiments, this loss is equally distributed between the cells; and, from this, the flow-rates, and the observed differences in the rise in temperature, we can calculate the amount of heat transferred into or out of a compartment due to transfer of salt across the membranes. The following preliminary results have been obtained:

Salt	Temperature rise Concentrated stream	 Diluted stream	Heat transferred per faraday
1 *M* Sodium chloride	0·275° C.	0·65° C.	3,030 cal.
1·27 *M* Lithium bromide	0·30° C.	0·50° C.	2,930 cal.
1·27 *M* Lithium bromide	0·10° C.	0·30° C.	3,240 cal.
8·4 *M* Lithium bromide	1·05° C.	1·70° C.	3,420 cal.
8·4 *M* Lithium bromide	1·38° C.	2·30° C.	3,510 cal.

These results are in rough agreement with those that can be calculated more directly from the fluid circulation-rates and the estimated heat capacities of the solutions used. In every case, flow of heat across the membrane is in a direction opposite to the flow of matter. I do not believe that these very substantial heat quantities are due to the existence of terms such as $-T\Delta S\nabla\left(\frac{1}{T}\right)$ (this term results from $\frac{1}{T}\frac{\partial\mu}{\partial T}\nabla T$ in the full expression for $\nabla\mu$) in the formulation that I have used to describe the currents of matter, electricity and heat, because, as has been shown by deBethune[4], the appropriate ΔS here is only of the order of a few cal./mole and in any event in the experiments I have performed $\nabla\left(\frac{1}{T}\right)$ is a very small quantity. The heat effect appearing here must be regarded as the electrochemical analogue of the Peltier effect.

Further work on a more refined apparatus in which we can further minimize thermal gradients is in progress, and a more complete report on the results is planned.

LLOYD H. SHAFFER

Research Department,
American Machine Foundry Co.,
Springdale, Connecticut.

---

[1] Staverman, *Trans. Farad. Soc.*, **48**, 176 (1952).
[2] Spiegler, *Trans. Farad. Soc.*, **54**, 1408 (1958).
[3] Callen, *Phys. Rev.*, **73**, 1349 (1948).
[4] deBethune, *J. Electrochem. Soc.*, **107**, 829 (1960).

Crystal and Molecular Structure of Diphenyl

X-RAY diffraction studies of crystalline diphenyl have been described by a number of authors[1-4] but the bond-distances and bond-angles have not been determined as accurately as in other aromatic compounds recently reported[5]. A detailed X-ray investigation of this structure has now been completed, and in view of the relevance of accurate structural information to modern valency theory, some results of this analysis are reported here.

Three-dimensional counter diffractometer data within the range $0 < \frac{\sin\theta}{\lambda} < 0.61$ have been refined by the method of least squares. The average estimated standard deviations:

$$\sigma_{xi} = [C_{ii}^{-1}\Sigma\omega\Delta^2/n - k]^{1/2}$$

(where C_{ii}^{-1} is the appropriate diagonal element of the inverse of the 9×9 matrix solved for each atom[6]) are 0·0016 Å. and 0·019 Å. for carbon and hydrogen co-ordinates respectively. The usual residual factor for 540 observed reflexions is 0·038; the space group is shown conclusively to be $P2_1/c$ and the molecule is therefore centrosymmetric and cannot be twisted as it is in the vapour phase[7].

The molecule is not strictly planar; the equation of the least-squares plane through the asymmetric unit[8] is:

$$-0·30584\,X + 0·55575\,Y + 0·77305\,Z' = 0·10114$$

while that of the mean molecular plane is:

$$-0·30891\,X + 0·55579\,Y + 0·77810\,Z' = 0$$

(These direction cosines refer to the orthogonal crystal axes a,b,c'.) The root mean square deviations from the ring plane are 0·0035 Å. for carbon atoms (maximum = 0·0049 Å.) and 0·012 Å. for hydrogen atoms (maximum = 0·017 Å.). The observed deviations of the carbons from this plane, while small, are statistically significant ($\chi^2 = 29·1$ with 3 degrees of freedom)[9] and are possibly due to intermolecular packing. For the molecular plane, the root mean square deviations are 0·0050 Å. and 0·019 Å. ($\chi^2 = 58·3$ with 4 degrees of freedom).

The bond-distances and -angles derived directly from the best least-squares co-ordinates are given with their estimated standard deviations in Fig. 1. These values require correction for the effects of molecular rigid body librations, and analysis[10] of the thermal vibration parameters in terms of such modes leads to the corrected distances, angles and estimated standard deviations given in Fig. 2. The effect of this correction is to increase all the carbon–carbon bond distances and to make all bond-angles more nearly equal to 120°. The deviation of the angles *BAF* (~ 17σ) and *ABC* (~ 10σ) from this value are, however, still highly significant. The difference (~ 8σ) between the distances *AB* and *CD* is also significant, and the differences *AB–BC* and *BC–CD* are just at the significance-level.

Since there are no significant differences between any two chemically identical but crystallographically non-equivalent distances or angles it seems unlikely that the estimated standard deviations are seriously under-estimated. In addition, since the least-squares results indicate no significant displacements of the ortho-hydrogens from a planar trigonal arrangement it appears that hydrogen–hydrogen repulsive interactions, to which the apparent non-planarity of the molecule in the vapour phase[7] has been attributed, cannot seriously affect bond-distances or bond-angles which involve only carbon atoms[14]. The observed deviations of the intra-ring carbon atoms from a regular hexagonal arrangement and the 0·20 Å. perpendicular separation between the planes of the two phenyl rings are probably due to intermolecular crystal forces. The effects of π electron delocalization across the linking bond should be small and, moreover, should increase the intra-ring bond-distances above the benzene value (1·397 Å.) whereas the observed values of *CD* and *BC* are shorter than this.

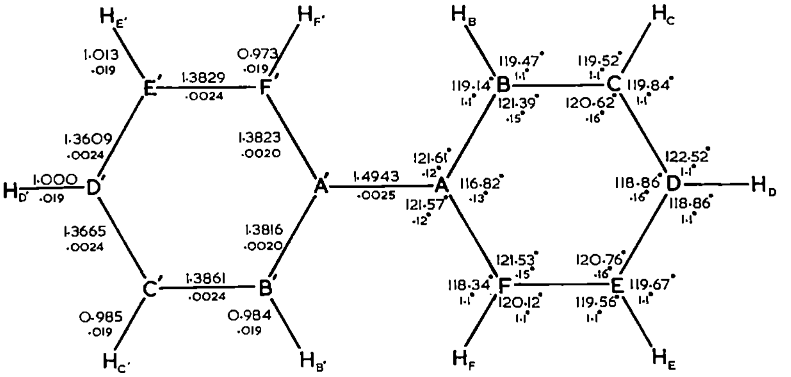


Fig. 1. Bond-distances and -angles uncorrected for libration

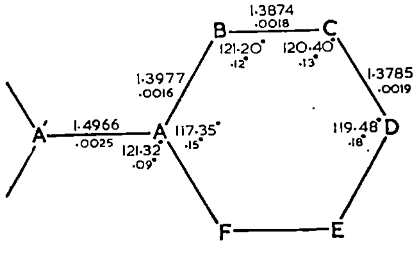


Fig. 2. Bond-distances and -angles corrected for libration

The intra-ring bond-distance is of considerable interest in relation to the recent discussion[11-15] on the effect of hybridization on carbon–carbon distances. The π bond-order derived from simple molecular orbital calculations is 0·37 (ref. 16), and more sophisticated self-consistent field calculations, such as have been reported for butadiene[12], indicate that the correct value is probably considerably less than this. The shortening due to double bonding must therefore be small and the observed distance of 1·497 Å. cannot differ greatly from that of a $C(sp^2) - C(sp^2)$ 'single' bond. This distance is significantly greater than 1·48 Å., which has been suggested[13] for the $C(sp^2) - C(sp^2)$ single bond distance. It is, however, consistent with the proposed distance of 1·51 Å. if, as has been suggested[17], the variation of bond-distance with bond-order is less rapid at low bond-orders than in other regions of the order-distance curve.

The calculations involved in this investigation were carried out on the University of London *Mercury* computer. I am indebted to Mr. O. S. Mills and Drs. J. S. Rollett and R. A. Sparks for making available their programmes for crystallographic calculations.

I am also indebted to Prof. C. J. B. Clews both for suggesting this problem and for his continual encouragement; to Prof. D. P. Craig and Drs. R. Mason and P. Pauling for much helpful discussion; and to the University of Western Australia for providing a research grant.

G. B. ROBERTSON*

Physics Department,
University of Western Australia,
Nedlands.

* Now at the William Ramsay and Ralph Forster Laboratories, University College, London.

[1] Dhar, J., *Indian J. Phys.*, **7**, 43 (1932).

[2] Dhar, J., *Proc. Nat. Inst. Sci. India*, **15**, 11 (1949).

[3] Kitiagorodsky, A. I., *Acta Physiochim. U.R.S.S.*, **21**, 575 (1946).

[4] Hargreaves, A., and Hasan Rizvi, S., *Proc. Chem. Soc.*, 122 (1961).

[5] For example, Cruickshank, D. W. J., *Acta Cryst.*, **9**, 915 (1956).

[6] *International Tables for X-Ray Crystallography*, **2**, 330.

[7] Allmeningen, A., and Bastiansen, O., *Det Kgl. Norske Videnskabers Selskabs Skrifter*, No. 4 (1958).

[8] Sparks, R. A., Ph.D. thesis, University of California at Los Angeles (1958).

[9] Cramer, H., *The Elements of Probability Theory* (John Wiley and Sons, Inc., New York, 1955).

[10] Cruickshank, D. W. J., *Acta Cryst.*, **9**, 754, 757 (1956).

[11] For example, Goodwin, T. H., *J. Chem. Soc.*, 4851 (1960).

[12] Mulliken, R. S., *Tetrahedron*, **6**, 68 (1959).

[13] Dewar, M. J. S., and Schmeising, H. N., *Tetrahedron*, **5**, 105 (1959).

[14] Cruickshank, D. W. J., and Sparks, R. A., *Proc. Roy. Soc.*, A, **258**, 270 (1960).

[15] Brown, M. G., *Trans. Farad. Soc.*, **55**, 694 (1959).

[16] Coulson, C. A., and Longuet-Higgins, H. C., *Proc. Roy. Soc.*, A, **195**, 188 (1948).

[17] Mason, R. (private communication).

Pyrene Dimers

THE striking change in the fluorescence spectrum from pyrene in benzene as a function of the pyrene concentration was studied by Förster and Kasper[1]. They concluded that the violet emission is produced by monomolecular pyrene while the blue emission is from transient dimers. Schmillen[2] has observed similar changes in the fluorescence spectrum from pyrene in solid hydrocarbons and has reported decay times of 8·9–9·9 nsec. for monomolecular pyrene and approximately 89 nsec. for pyrene complexes. The true value of the lifetime of pyrene dimers in liquid solutions has recently been the subject of discussion[3,4]. This communication reports the results of studies of changes in the fluorescence lifetime and in the emission spectrum of pyrene in paraxylene and in cyclohexane for various concentrations of pyrene.

Paraxylene and cyclohexane were purified with two vacuum distillations, but the pyrene (which, like the solvents, was obtained from the Eastman Kodak Co.) was used without further purification. The sample solutions were flushed with nitrogen before each measurement to obviate oxygen quenching and were handled with caution since pyrene is listed[5] as a carcinogen.

Decay times were measured using apparatus[6] which produced a pulsed beam of electrons of 75 keV. energy to excite the fluorescence, and a 1*P*28 photomultiplier and associated equipment for detecting, and graphically recording, the shape of the emitted light pulse. Each component in the fluorescence emission was studied by interposing either a 9863 Corning filter or a Wratten 2 series VI filter to pass the violet and blue components, respectively. This equipment can measure decay times conveniently over a range of 2–200 nsec. Longer components in the decay could be observed but not accurately determined.

Table 1. MEASURED DECAY TIMES

Solute	Solute concentration (gm./l.)	Filter type	Decay time (nsec.)
—	Pyrene crystals	Wratten 2 (blue)	91 ± 5
Xylene	0·1	Wratten 2 (blue)	91 ± 10
Xylene	0·5	Wratten 2 (blue)	91 ± 10
Cyclohexane	0·5	Wratten 2 (blue)	81 ± 10
Xylene	0·1	9863 (violet)	91 ± 5
Xylene	0·5	9863 (violet)	70 ± 4
Cyclohexane	0·5	9863 (violet)	65 ± 4

The measured decay times for several sample solutions are listed in Table 1. The two arbitrary values of the concentration of pyrene in xylene, 0·1 and 0·5 gm./litre, are chosen because with the former concentration the blue component in the emission spectrum is starting to assume its characteristic shape whereas, in the latter case, the blue component is quite prominent. No decay-time data are reported for a 0·1 gm./litre pyrene–cyclohexane solution as the fluorescence yield is too small to be measured accurately.

It is noted in Table 1 that the value for the violet component is, to a small extent, dependent on the solute concentration. The decrease in the decay time of the violet component with increasing solute concentrations coincides with the decrease in the intensity of the emission yield. For the same concentration (0·5 gm./l.) of pyrene, the value of the decay time and emission intensity of the violet component is less when the solvent is cyclohexane rather than xylene. These observations are consistent with one of two mechanisms which can explain the formation of the anomalous blue component at the expense of the violet component: (1), the existence of transient dimers or (2), energy transfer from monomeric pyrene to permanent aggregations.

Moreover, it was observed that the time taken for the blue component to rise is at least an order of magnitude longer than that of the violet component, and that it becomes shorter with increasing solute concentration. Since a long time in rising is indicative of a delay in the emission process, this observation supplies further evidence for either of the two postulates.

Fluorescence spectral measurements[6] were made to compute the energy-transfer parameter ε in an attempt to ascertain the existence and proliferation of permanent aggregations. A thin (0·05 mm.) sample of solution was excited with 265-mμ radiation from a Bausch and Lomb monochromator and the transmitted emission spectra from both solvent and solute were recorded by a Beckman *DK*-2 spectrophotometer. The exciting light is almost completely absorbed by the solvent. Some of this excitation energy is re-emitted and a portion is transferred to solute molecules. From the change in the intensity of the solvent yield the value of ε could be determined[6] and over the concentration range of 0·05–2 gm./l., ε, as a fraction of unity, could be expressed to a good approximation by the relationship $\varepsilon = 6{\cdot}8c/(1 + 6{\cdot}8c)$, where c is the solute concentration in gm./l. If, following Cohen and Weinreb[7], one interprets a linear dependence of c/ε versus c as an indication that only one level in the solvent molecule and only one species of acceptor molecule are active in the energy-transfer process, one must conclude that if there were permanent aggregations of pyrene they do not noticeably contribute to the deactivation of the excited solvent molecules.

Although these results provide supporting evidence for the assumption of transient dimers as postulated by Förster and Kasper[1], they are not sufficiently conclusive to eliminate other interpretations.

ISADORE B. BERLMAN

Radiological Physics Division,
Argonne National Laboratory,
Argonne, Illinois.

[1] Förster, Th., and Kasper, K., *Z. Elektrochem.*, 59, 977 (1955).
[2] Schmillen, A., *Naturforsch.*, 16, a, 5 (1961).
[3] Stevens, B., and Hutton, E., *Nature*, 186, 1045 (1960).
[4] Parker, C. A., and Hatchard, C. G., *Nature*, 190, 165 (1961).
[5] *Dorland's Illustrated Medical Dictionary* (W. B. Saunders Co., 1960).
[6] Berlman, I. B., *J. Chem. Phys.*, 33, 1124 (1960).
[7] Cohen, S. G., and Weinreb, A., *Proc. Phys. Soc.*, B69, 593 (1960).

2,6-Difluoropyridine

As part of a programme involving the investigation of general laboratory methods for the preparation of fluoropyridines[1], we wish to report the synthesis of 2,6-difluoropyridine. The only other recorded example of a pyridine containing more than one nuclear fluorine is pentafluoropyridine[2,3], which was prepared by methods best suited for the synthesis of perfluoro-aromatic compounds.

The present report describes the synthesis of 2,6-difluoropyridine from starting materials readily obtainable by standard laboratory procedures. Conversion of methyl 6-fluoropicolinate[4] (60·0 gm.) to 6-fluoropicolinic hydrazido (51·2 gm.), m.p. 120·5–121° (found: C, 46·7; H, 4·0; $C_6H_6FN_3O$ requires C, 46·5; H, 3·9 per cent), with hydrazine in methanol, followed by a modified Curtius-type degradation, gave 2-amino-6-fluoropyridine, m.p. 58–59° (found: C, 53·6; H, 4·3; N, 24·9; $C_5H_5FN_2$ requires C, 53·6; H, 4·5; N, 25·0). As it is known that fluorine atoms adjacent to aromatic ring nitrogens undergo facile hydrolysis in acid solution[5], it was found advisable to reverse the normal addition procedure of the Curtius degradation. Consequently, addition of *N* hydrochloric acid to a stirred slurry of sodium nitrite dissolved in water, 6-fluoropicolinic hydrazide (20·0 gm.), and ether gave 6-fluoropicolinic azide. This was decomposed in warm carbon tetrachloride to crude 6-fluoropicolinic *iso*cyanate, which was hydrolysed directly to 2-amino-6-fluoropyridine (7·2 gm.) with 50 per cent aqueous acetic acid. By use of Roe's modification of the Schiemann reaction[6], 2-amino-6-fluoropyridine (10·0 gm.) was converted to 2,6-difluoropyridine (2·8 gm.), b.p. 124·5° (743 mm.), n_D^{25} 1·4349 (found: C, 52·2; H, 2·6; N, 12·1; $C_5H_3F_2N$ requires C, 52·2; H, 2·6; N, 12·2).

The infra-red spectrum of 2,6-difluoropyridine in carbon tetrachloride, obtained with a Perkin–Elmer model 21 spectrophometer equipped with sodium chloride optics, showed absorptions at 1,607, 1,592 and 1,493 cm.$^{-1}$, providing evidence for a pyridine nucleus. Additional absorptions at 3,110 and 717 cm.$^{-1}$ contribute support for the proposed structure of this compound.

2,6-Difluoropyridine is exceptionally volatile and shows the same solubility characteristics as 2-fluoropyridine. In contrast to pentafluoropyridine, it does exhibit some basic properties in 50 per cent ethanol when titrated against 0·01 *N* hydrochloric acid. However, no hydrochloride was formed in ethereal hydrogen chloride and no picrate formed in ethanol.

An attempt to prepare 2,3-difluoropyridine from methyl 2-fluoronicotinate (10·0 gm.), b.p. 101° (10 mm.), n_D^{25} 1·4978 (found: C, 54·4; H, 3·9; N, 9·1; $C_7H_6FNO_2$ requires C, 54·2; H, 3·9; N, 9·0) by a similar route failed because hydrazine was found to react preferentially with the fluorine rather than the ester moiety giving methyl 2-hydrazinonicotinate (1·3 gm.), m.p. 106–106·5° (found: C, 50·4; H, 5·4; N, 25·4; $C_7H_9N_3O_2$ requires C, 50·3; H, 5·4; N, 25·1).

A more detailed report of this and related work will be published elsewhere.

G. C. FINGER
L. D. STARR

Section of Fluorine Chemistry,
Illinois State Geological Survey,
Urbana, Illinois.

[1] Finger, G. C., and Starr, L. D., *J. Amer. Chem. Soc.*, 81, 2674 (1959).
[2] Stacey, P. M., and Tatlow, J. C., *Nature*, 186, 231 (1960).
[3] Haszeldine, R. N., *et al.*, *Proc. Chem. Soc.*, 211 (1960).
[4] Roe, A., Cheek, P. H., and Hawkins, G. F., *J. Amer. Chem. Soc.*, 71, 4152 (1949).
[5] Miller, W. K., Knight, S. B., and Roe, A., *J. Amer. Chem. Soc.*, 72, 4765 (1950).
[6] Roe, A., and Hawkins, G. F., *J. Amer. Chem. Soc.*, 69, 2443 (1947).

RADIATION CHEMISTRY

Polarization induced by Radiation in 'Perspex'

IF, after irradiation of a thick piece of 'Perspex' with a sufficient number of electrons of a few MeV., the specimen is knocked with a sharp tool, a rather intense 'lightning flash' is seen and a tree-like crack pattern occurs. The foot of the stem is situated at the point which was struck. Sometimes the discharge occurs spontaneously towards any disturbance at the surface of the 'Perspex' block.

As the irradiated sample is thicker than the range of the electrons, it is clear that these electrons are stopped in the 'Perspex', mainly at depths corresponding to the more or less straight sloping part of the absorption curve. The 'Perspex' being a good insulator, negative space charge builds up in the sample, and when the dose of radiation is sufficiently high, a discharge can occur.

Gross[1] has already studied this phenomenon in some detail, but some of his statements do not agree with my observations. However, I wish only to report here on another property of the irradiated specimen.

I have bombarded several identical polished 'Perspex' blocks 13 mm. × 38 mm. × 47 mm. with $1{\cdot}2 \times 10^{14}$ electrons of 2·5 MeV. The current of the linear accelerator was held at 0·1 μamp. The beam entered the blocks in the middle of one of the largest faces. The blocks were examined with a polariscope with convergent light, the specimen being placed so that one of the largest faces was viewed. Before irradiation, no interference pattern was observed. Immediately after irradiation, an interference pattern could be seen that behaved as if the 'Perspex' block was a uniaxial double refractive crystal, the optical axis coinciding with the direction of the beam. One could observe a circular isochromate and the well-known dark cross, each line of which corresponds to the direction of the polarizer and analyser. This picture disappeared very quickly at first, but more slowly later on. After an hour, it was scarcely visible and after two hours it had disappeared completely, although it was still possible to create some cracking by knocking the sample. If the specimen was knocked when the interference pattern was still strong, the pattern disappeared nearly completely. From this we may conclude that in the circumstances considered the optical properties of the 'Perspex' bombarded with electrons is mainly due to an electrical effect, the remaining part being due probably to mechanical strain caused by the non-homogeneous increase of the temperature. It seems evident that something like the Kerr electro-optic effect is occurring. The charges in the 'Perspex' are more or less situated in planes perpendicular to the original direction of the beam, causing an electric field in that direction and giving the 'Perspex' the property of behaving as a uniaxial crystal with its axis along the electric field.

V. J. Vanhuyse
G. J. Vanpraet
J. F. Van Landuyt

Natuurkundig Laboratorium
der Rijksuniversiteit Gent,
Rozier 6,
Gent, Belgium.

---

[1] Gross, B., *Phys. Rev.*, **107**, 368 (1959); *J. Polymer Sci.*, **27**, 135 (1958).

BIOCHEMISTRY

Hexosamine and Sialic Acid Contents in Cells

Few data are available on the determination of glycoproteins containing sialic acid in tumour tissues, although the presence of an acid mucopolysaccharide in some kinds of tumour tissue has been reported. We determined the hexosamine and sialic acid contents in tumour cells, the HeLa cells and *C3H* ascitic hepatoma cells of different types, comparing them with those of non-malignant cells. The liver cells of *C3H* mice, and *FL* cells obtained by a tissue culture were used as non-malignant cells. The liver cell is generally considered to be a good example of the basic cell type. The types of the *C3H* ascitic hepatoma used were *MH* 134, *MH* 129 *P* and *MH* 129 *F*[1], which were inoculated into (*C3H*/*HeN*dd) F_1 hybrid mice, subcutaneously or intraperitoneally. The cells from solid tissue were prepared by use of a tissue presser. The cells were dried with acetone after washing with saline solution. The results of the determination are summarized in Table 1.

The sample used for the sialic acid determination was examined, and the absorption spectrum of the coloured solution which was produced by the resorcinol reagent was read; two peaks of absorption, with maxima at 450 and 580 mμ were obtained. This coincides with the results for sialic acid reported by Svennerholm[4]. From these results no significant difference in hexosamine and sialic acid contents was found between tumour cells and non-malignant cells. Prodi[5] reported the hexosamine contents of liver in rat, rabbit and guinea pig presenting values of 436, 408 and 405 γ/100 mgm. of dry weight. These results are only a little higher than the values obtained in *C3H* mouse liver. Hexosamine and sialic acid contents of the intact liver cells of the mice with intraperitoneally inoculated tumours were almost equal to the normal values, although the serum-level of sialic acid in mice with the same tumour was remarkably high[6]. Thus it was found that glycoprotein containing sialic acid is not stored in tumour cells as well as in the liver cells of tumour-bearing animals, even if the liver were a site of synthesis of these glycoproteins[7]. Furthermore, it may be concluded that tissue destruction is not a direct origin of the increase of serum glycoproteins in tumour-bearing subjects. The presence of a certain kind of substance which stimulates the production of sialic acid-containing substances in tumour-bearing animals is assumed. This assumption is partly based on the finding of the excretion of so-called 'κ-mucopolypeptide', which was stated to be a tumour-specific urinary mucopolypeptide[8].

Table 1. Sialic Acid and Hexosamine Contents of Cells

	Nitrogen	Hexosamine		Sialic acid	
	mgm./ 100 mgm. dry weight	mgm./ 100 mgm. dry weight	μgm./ mgm. nitrogen	mgm./ 100 mgm. dry weight	μgm./ mgm. nitrogen
FL cell	10·8	0·41	38	0·36	33
HeLa cell	12·0	0·37	31	0·46	38
Tumour cell					
MH 134 (i.p.)	12·9	0·48	37	0·26	20
MH 134 (s.c.)	14·4	0·42	29	0·36	25
MH 129*F* (i.p.)	14·1	0·40	28	0·36	26
MH 129*P* (i.p.)	14·2	0·36	25	0·32	23
Liver cell of *C3H* mice					
Normal	11·8	0·32	27	0·31	26
MH 129*F* bearing	12·6	0·33	26	0·33	26
MH 129*P* bearing	13·0	0·39	30	0·33	25

i.p., Intraperitoneally inoculated tumour; s.c., subcutaneously inoculated tumour.

Hexosamine was determined according to Boas (ref. 2), and sialic acid according to Svennerholm (ref. 3) using the orcinol reagent.

Atsushi Kimura
Yasushi Nagai
Koh-Iti Turumi

Department of Biochemistry,
Fukushima Medical College,
Fukushima.

Yoshiko Kawashima
Haruo Sato

Cancer Research Laboratory,
Research Institute for
Tuberculosis and Leprosy,
Tohoku University, Sendai.

---

[1] Sato, H., Kawashima, Y., Kanno, K., Munakata, H., and Saito, T., *Fukushima J. Med. Sci.*, **5**, 155 (1958).

[2] Boas, N. F., *J. Biol. Chem.*, **204**, 553 (1953).

[3] Svennerholm, L., *Acta Chem. Scand.*, **12**, 547 (1958).

[4] Svennerholm, L., *Biochim. Biophys. Acta*, **24**, 604 (1957).

[5] Prodi, G., *Proc. Soc. Exp. Biol. Med.*, **88**, 605 (1955).

[6] Turumi, K., Nagai, Y., Kimura, A., Kawashima, Y., and Sato, H., *Gann*, **50** (Supp. 76, 1959).

[7] Werner, I., *Acta Physiol. Scand.*, **19**, 27 (1950).

[8] Masamune, H., Hakomori, S., and Sugo, T., *Tohoku J. Exp. Med.*, **69**, 383 (1959).

Polarographic Examination of Sialic Acids

We have investigated systematically the polarographic wave of neuraminic acid derivatives with a dropping-mercury electrode ($m = 2 \cdot 86$ mgm./sec., $t = 2 \cdot 98$ sec.). The existence of a sialic acid wave was anticipated from the fact that structurally related substances such as pyruvic acid[1,2] and fructose[3,4] give well-defined polarographic waves. Three type of waves could be observed in N-acetylneuraminic acid solution (NANA): (*a*) a hydrogen wave in 0·1 *M* LiCl or in 0·01–0·1 *M* tetramethylammonium bromide (TMABr) under *p*H 7. The apparent half-wave potential is − 1·65 V. (s.c.e.) in 0·1 *M* TMABr and − 1·48 V. in 0·01 *M* TMABr. There is a linear relationship between the height of the wave and the square root of the height of the mercury column up to 45 cm.; and, under defined conditions,

Table 1. CHARACTERISTICS OF THE WAVES OBSERVED IN NANA SOLUTIONS AS COMPARED WITH THE WAVES OF PYRUVIC ACID AND OF FRUCTOSE

Substance	Condition or pre-treatment	30° C. Half-wave potential, V.	30° C. Height μamp./μmole	60° C. Height μamp./μmole	$\frac{h60°}{h30°}$	Per cent/ degree*
Pyruvic acid	TMAOH 0·01 *M*	−1·80	77·6	91·4	1·29	0·59
Fructose	LiCl 0·1 *M*	−1·90	21·0	61·5	2·93	6·4
Fructose	TMAOH 0·01 *M*	−1·95	25·0	66·5	2·65	5·5
NANA	TMAOH 0·01	−1·96	18·3	81·8	4·47	11·6
NANA	Heated 10 min. in TMAOH 0·01 *M*	−1·65				
		−1·95	53·8	74·3	1·38	1·27
NANA	Kept frozen freshly thawed	−1·87	33·0			
NANA	Same sample kept 1 hr. at room temp.	−1·82	26·0			
NANA	Same sample kept 2 hr. at room temp.	−1·86	10·0			

* Temperature coefficient of the wave in per cent change per degree, relative to the wave at 30° C.

a linear relationship between NANA concentration and the height of the wave. Under identical conditions, pyruvic acid ($4·05 \times 10^{-4}$ *M* in 0·01 *M* TMABr, under nitrogen) gives a hydrogen wave of the same height (36·7 μamp.) as NANA (39 μamp.). The slope of the straight line in which the height of the wave is plotted as the function of concentration of NANA is 0·3 μamp./μgm./ml. or 92·7 μamp./μmole/ml. (at 30° C. in 0·01 *M* TMABr). There is a marked sensitivity of the wave to ionic strength and also to the presence of oxygen.

(*b*) When a 0·4 μmole/ml. solution of NANA in 10 μmole/ml. of TMABr is neutralized by stepwise addition of 0·01 *M* TMA-phosphate buffer at *p*H 7, the hydrogen wave decreases and another wave appears on top of it and increases as the *p*H gets closer to neutrality. This second wave has an approximate half-wave potential of −1·75 V. This stepwise change of the two waves is shown by Fig. 1 (polarograms 7, 8, 9). This second wave, given apparently by the anion of NANA, was found in 0·01 *M* tetramethylammonium hydroxide (TMAOH), *p*H 10 (Fig. 1, polarogram 1). The apparent half-wave potential of this wave is independent of the mercury pressure and has a high-temperature coefficient (Table 1). It seems to be a wave controlled kinetically.

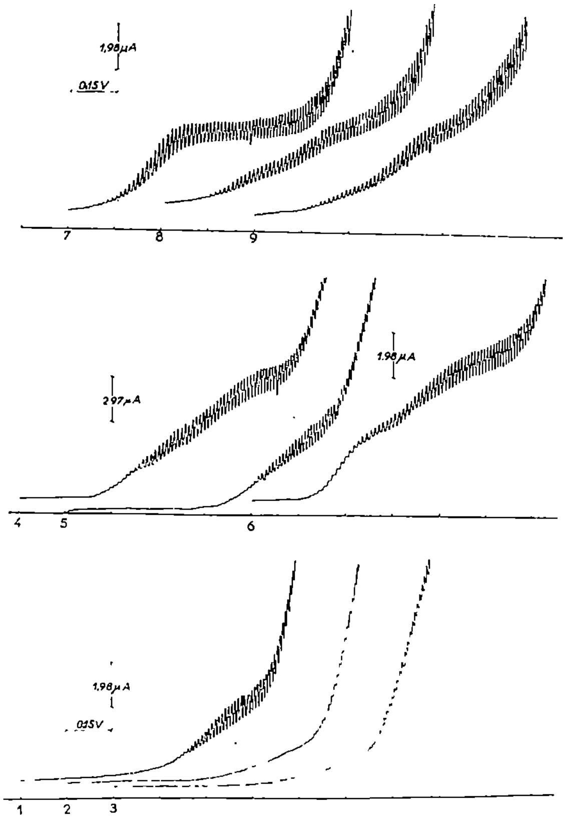


Fig. 1. Polarograms of NANA and related substances. (1) freshly thawed NANA, 1·62 μmole/ml. in TMAOH 0·01 *M* ; (2) same as (1) after 2 hr. at room temperature (24° C.) ; (3) same as (1), after 3·5 hr. at room temperature ; (4) pyruvic acid 1·60 μmole/ml. in TMAOH 0·01 *M* ; (5) fructose 1·60 μmole/ml. in TMAOH 0·01 *M* ; (6) NANA 1·60 μmole/ml. heated 10 min. in TMAOH 0·01 *M* recorded in the same solvent ; (7) NANA 0·4 μmole/ml. in TMABr 0·01 *M* (hydrogen wave) ; (8) Same as (7) after addition of 0·01 *M* $TMAPO_4$, *p*H 7, 0·6 μ*M* ; (9) same as (7) with 1·11 μ*M* $TMAPO_4$ buffer, all polarograms recorded at 30° C.

An interesting and as yet unexplained variation of the height of this wave was observed, which depended on the previous treatment of the NANA solution. Solutions adjusted to *p*H 4·5 (*p*H of maximum stability[6]) were kept frozen for several days. Freshly thawed solutions gave a higher wave than solutions kept at 4° C., or freshly thawed solutions allowed to remain for various periods of time at room temperature. The height of the wave decreased with time at room temperature. The phenomenon is apparently reversible within limits, since re-freezing and re-thawing of the material two or three times yielded in each case a wave of the same height as the originally frozen NANA solution. No carboxypyrol-derivatives could be detected by paper chromatography in frozen stored solutions, though small quantities were present in solutions kept at 4° C.

No definite statement can be made as to the nature of the reaction determining the wave-height. A ring-opening, as is the case with glucose or fructose[7], or a dehydration as shown for formaldehyde[8] may be the rate-controlling mechanism for this NANA wave.

(*c*) In heated NANA solutions (10 min. at 100° C. in 0·01 *M* TMAOH solution) a different type of wave was observed, which bears some relationship to the pyruvic acid wave (Fig. 1, polarogram 6). However, no pyruvic acid could be detected on chromatograms and the addition of lactic acid dehydrogenase and reduced diphosphopyridine nucleotide (0·005 *M*) to this solution produced a change in optical density at 340 mμ corresponding to only 10 per cent of the amount of pyruvic acid needed to account for the observed polarographic wave. No 2-carboxypyrol could be detected by paper chromatography in the heated solution. The height of the wave observed in heated NANA solutions is independent of the mercury pressure, but has a low-temperature coefficient (Table 1). Pyruvic acid in 0·01 *M* TMAOH gives a wave which decreases linearly with the square root of the mercury pressure.

More experiments are needed before any analytical application of this polarographic wave can be recommended.

The NANA was prepared from meconium according to the method of Zilliken *et al.*, and kindly provided by Dr. R. J. Winzler. Another sample re-crystallized three times was a gift from Dr. Saul Roseman, University of Michigan.

The work was supported by a grant from the National Institutes of Health (*E* 2183).

BARBARA ROBERT*
MAX E. RAFELSON
LADISLAS ROBERT*

Department of Biological Chemistry,
University of Illinois College of
Medicine,
Chicago.

* On leave from the Biochemical Department, Medical Faculty, Paris, and C.N.R.S.

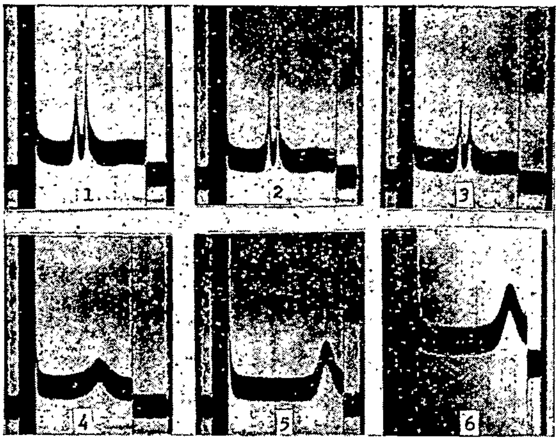
[1] Müller, O. H., and Baumberger, J. P., *Trans. Amer. Electrochem. Soc.*, **71**, 169 (1937).
[2] Brdicka, R., and Wiesner, K., *Coll. Czech. Chem. Comm.*, **12**, 138 (1947).
[3] Heyrovsky, J., and Smoler, I., *Coll. Czech. Chem. Comm.*, **4**, 521 (1932).
[4] Wiesner, K., *Coll. Czech. Chem. Comm.*, **12**, 64 (1947).
[5] Zilliken, F., Braun, G. A., and Gyorgy, P., *Arch. Biochem. Biophys.*, **63**, 394 (1956).
[6] Berggard, I., and Odin, L., *Arkiv för Kemi*, **12**, 581 (1958).
[7] Cantor, S. M., and Peniston, P., *J. Amer. Chem. Soc.*, **62**, 2113 (1940).
[8] Vesely, K., and Brdicka, R., *Coll.*, **12**, 313 (1947).

Sub-unit of Ribosomal Ribonucleic Acid from Yeast

RIBONUCLEIC acid (RNA) isolated by the phenol method from 80*s* ribosomes of yeast was demonstrated to contain two components of 19*s* and 28*s* [1]. In a previous paper[2], it was shown that by heat treatment the high molecular ribosomal RNA could easily be converted to a low molecular RNA with the sedimentation coefficient of about 4*s*. It is also the case for rat liver ribosomal RNA as demonstrated by Takanami[3]. The close agreement of the *s*-value of this heat-treated ribosomal RNA with that of soluble RNA led us to suppose that the 4*s* polynucleotide is the basic unit of all RNA's[2]. In the present work, it has been shown that trypsin, as in the case of heat treatment, can degrade ribosomal RNA into 4*s* sub-units.

Ribosomal RNA used was isolated by the phenol method from *Saccharomyces cerevisiae*, strain Kaneka. In the first series of experiments, the RNA was heated in 0·2 *M* sodium chloride for 10, 20, 40 and 60 min. at 90° C. The heated RNA solutions were observed in a Spinco *E* analytical ultracentrifuge with a schlieren optical technique at once or after dialysis against 0·1 *M* sodium chloride containing 0·01 *M* potassium phosphate buffer at *p*H 7·1. Heating for 10 min. caused a partial degradation of the RNA, giving rather continuously distributed products in the range 4*s*–13*s*, though a component of around 7*s* predominated (Fig. 4). The complete conversion to a 4*s* sub-unit ($S^0_{20} = 4 \cdot 3s$) was achieved by 40 min. heat treatment. Further heating up to 60 min. did not change the sedimentation pattern (Fig. 5).

Next, ribosomal RNA (0·5–1·0 per cent solution in 0·1 *M* sodium chloride–0·01 *M* potassium phosphate) was treated with three kinds of proteolytic enzymes respectively (all 0·15 mgm./ml.), that is, trypsin and chymotrypsin (Worthington Biochemical, United States, 2 × cryst.), chymotrypsin and pepsin (Nutritional Biochemical, United States, 2 × cryst.). The sedimentation analyses were directly made for the digest inclusive of added enzyme. The only effective enzyme in degrading the RNA was trypsin. Brief treatment of the RNA solution (20 min. at 25° C., *p*H 7·1) with this enzyme yielded polydisperse boundaries just as seen in the case of the short heat treatment. Treatment for 60–90 min., however, gave a single boundary of about 4*s* ($S^0_{20} = 4 \cdot 0s$) (Fig. 6), the sedimentation pattern being comparable with that of the 'stable sub-unit RNA' obtained by 60-min. heat treatment. Incubation for up to 90 min. at 25° C. at *p*H 7·1 without added enzyme (Fig. 1), or with chymotrypsin (Fig. 2), or incubation for 20 min. at 25° C. at *p*H 3·0 with pepsin (Fig. 3) did not affect the initial sedimentation pattern very much.



Figs. 1–6. Sedimentation patterns of yeast ribosomal RNA treated with heat or proteolytic enzymes (0·15 mgm./ml.). 1 per cent solutions in 0·1 *M* sodium chloride, 0·01 *M* potassium phosphate, *p*H 7·1. Sedimentation proceeds from right to left. Pictures taken about 30 min. after reaching 59,780 rev./min. (1) Untreated; (2) treated with chymotrypsin for 90 min.; (3) with pepsin for 20 min.; (4) heated for 10 min.; (5) heated for 60 min.; (6) treated with trypsin for 90 min.

A possibility that a trace of ribonuclease present in the trypsin preparation might be responsible for the observed effect of trypsin is probably excluded by the following tests using pancreatic ribonuclease (the action of ribonuclease having specificity different from pancreatic ribonuclease is still open to question, however). The RNA was incubated at 25° C. for 90 min. with a series of concentration of pancreatic ribonuclease (Worthington Biochemical, United States, cryst.) (0·001–1·0 μgm./ml.) and of trypsin (0·02–0·5 mgm./ml.), and the *s*-value of the main peak was recorded in relation to the concentration of the enzyme used (Table 1). In the case of ribonuclease, the higher the concentration of the added enzyme, the greater was the extent of the RNA degradation, no ultracentrifugally detectable 'end point' of

Table 1. EFFECTS OF PANCREATIC RIBONUCLEASE OR TRYPSIN ON YEAST RIBOSOMAL RIBONUCLEIC ACID

Ribonuclease		Trypsin	
Concentration of enzyme (μgm./ml.)	S_{20}*	Concentration of enzyme (mgm./ml.)	S_{20}*
0·000	19, 28†	0·000	19, 28†
0·001	10·5	0·02	9·50
0·005	7·65	0·10	5·33
0·010	5·11	0·15	3·99
0·025	4·69	0·20	3·93
0·100	4·19	0·40	3·75
0·150	3·10	0·50	3·86
1·000	2·00		

* S_{20} of the main peak, uncorrected.
† S_{20} at zero concentration.

Ribosomal RNA (1 per cent solution in 0·1 *M* sodium chloride, 0·01 *M* potassium phosphate, *p*H 7·1) was incubated with the enzymes for 90 min. at 25° C.

degradation being attained. On the other hand, degradation by trypsin clearly revealed a limitation; 0·15 mgm./ml. of trypsin was sufficient to degrade RNA into 4*s*, and no further change in *s*-value was observed with increasing concentration of trypsin.

An attractive hypothesis from the above results might be that the high molecular ribosomal RNA is composed of relatively stable 4*s* sub-units which are linked together through some peptide-like substance. Acid hydrolysates of the RNA preparation (6 *N* hydrochloric acid, 120° C., 8–24 hr.) were found to give ten amino-acids after two-dimensional paper chromatography or starch column chromatography which have been identified as glycine, glutamic acid, serine, alanine, aspartic acid, amino-butyric acid, lysine, threonine, leucine and valine (roughly decreasing in amount as found). Although the fact that the RNA contains a basic amino-acid, lysine, and no aromatic amino-acid is apparently compatible with the findings that the RNA is susceptible to trypsin, but neither to chymotrypsin nor to pepsin, the amount of lysine detected was too small to account for the number of lysine residue required for the sub-unit linkage. The effect of trypsin on the ribosomal RNA presented in this communication might be related to the degradative action of some non-enzymic proteins on the high molecular RNA from Ehrlich ascites tumour cells recently reported by Brown *et al.*[4].

EIKO OTAKA
YUKITO OOTA
SYOZO OSAWA

Biological Institute,
Faculty of Science,
Nagoya University.

[1] Maeda, A., *J. Biochem.* (Tokyo), **48**, 363 (1960).

[2] Osawa, S., *Biochim. Biophys. Acta*, **43**, 111 (1960).

[3] Takanami, M., *Biochim. Biophys. Acta*, **39**, 152 (1960).

[4] Brown, R. A., Ellem, K. A. O., and Colter, J. S., *Nature*, **187**, 509 (1960).

Quantitative Determination of Metadrenaline and Normetadrenaline in Normal Human Urine

SEVERAL authors have performed investigations on metadrenaline and normetadrenaline, metabolites of catecholamines, since their discovery by Axelrod[1]. Previous work, however, dealt mostly with the urine of rats[2], in which relatively large amounts of the metabolites are excreted, or the isotope tracer of authentic methoxycatecholamines[3] which can be detected easily by their radioactivity. Metadrenaline and normetadrenaline in normal human urine have never been determined quantitatively, because of their very low concentrations. This communication concerns the method of determination of methoxycatecholamines in urine of normal human beings.

An aliquot (5–10 ml.) of urine is adjusted to *p*H 1·0 with hydrochloric acid, and hydrolysed in a boiling water bath for 1 hr. After cooling in tap water, the urine is adjusted to *p*H 10·0 with sodium hydroxide, saturated with sodium chloride, and extracted with five volumes of ethyl acetate under vigorous shaking for 5 min. After centrifugation, the organic phase is separated by pipetting. The extraction is repeated three times. The extracts are combined, and evaporated under reduced pressure to dryness at a temperature below 60° C. The residue is dissolved in 2 ml. of methanol, and transferred to a small glass tube. This is repeated three times. The methanol extract is evaporated to dryness in the same way. The residue is dissolved in a small amount of methanol.

The methanol extract is placed on Toyo Roshi filter paper No. 51 (5 × 60 cm.), which has been pretreated with pyridine–acetic acid–water (1 : 10 : 89 v/v) just before use, at a distance of 8·5 cm. from the anode end. The paper is subjected to high-voltage paper electrophoresis for 2 hr., using *n*-hexane as refrigerating insulator. Operating voltage is 2,500–3,000 V., current 8–12 m.amp. Metadrenaline migrates about 40 cm. toward the cathode, and normetadrenaline about 42 cm., so that they are separated completely from other substances as well as from each other.

After the paper is dried at 60° C. for 20 min., diazotized *p*-nitroaniline is sprayed on both sides of the paper. Both metabolites react to give a violet colour. The paper is dried again, the bands of methoxycatecholamines are cut into pieces, and eluted with 4 ml. of alkaline methanol (methanol–2 per cent sodium carbonate, 2 : 1 v/v) for more than 1 hr. These eluates are assayed in a Beckman spectrophotometer model *DU* at the wave-length of 520 mμ. The uncoloured part of the paper is treated similarly and serves as a blank.

Table 1. AMOUNTS OF METADRENALINE AND NORMETADRENALINE IN 24-HR. URINE OF NORMAL SUBJECTS AND VARIOUS PATIENTS

Subject	Sex	Disease	Met-adrenaline (μgm./24 hr.)	Normet-adrenaline (μgm./24 hr.)
K. Y.	♀	Healthy	246	239
Y. H.	♂	,,	256	245
A. G.	♂	,,	254	186
K. Y.	♂	,,	172	135
T. K.	♀	,,	257	97
T. C.	♀	,,	125	147
H. S.	♂	,,	225	230
K. M.	♂	,,	104	127
K. O.	♂	Essential hypertension	153	156
J. K.	♂	,, ,,	211	143
S. O.	♂	,, ,,	255	143
S. S.	♂	,, ,,	199	96
S. S.	♀	,, ,,	120	119
M. T.	♂	,, ,,	105	160
S. S.	♂	Graves's disease	143	230
M. H.	♂	,, ,,	138	201
M. S.	♀	,, ,,	182	127
T. S.	♂	Parkinsonism	192	209
G. W.	♂	Primary aldosteronism	231	132
S. I.	♂	Hysteria	416	449
A. I.	♂	Phæochromocytoma	31,400	5,570
J. N.	♀	,,	7,700	8,270

Extraction (using *n*-butanol, *iso*butanol *iso*amylalcohol were examined) of the urine was found inadequate, for inorganic salts were extracted together, which interfered seriously with further procedures. The metabolites were not extracted into ethyl ether or ethylene dichloride as efficiently as into ethyl acetate. Purification of *O*-methylated catecholamines with ion-exchange resin was also unsuccessful.

High-voltage paper electrophoresis was found most suitable for the separation of the amines in the extract. Paper chromatography using several solvent systems did not result in good separation of them, as compared with the electrophoretic method.

When the urine was not hydrolysed, the estimated values were much lower than those after hydrolysis. Hydrolysis at approximately *p*H 0 (checked by test paper) resulted in lower yields as compared with that at *p*H 1·0. In accordance with Goodall *et al.*[4], incubation of urine with bacterial β-glucuronidase gave rise to no increase in metadrenaline and normetadrenaline values.

The minimal quantity detectable by the method was 0·2–0·3 μgm. for both amines. It was found after repeated estimations that errors of about ± 7 per cent are inevitable in the determination. The overall recovery of the amines added to urine was 80–90 per cent.

Twenty-two urine specimens from normal subjects and various patients were assayed (Table 1). Normal human beings excrete 100–300 μgm. of metadrenaline and almost the same amount of normetadrenaline daily in the urine. Patients with essential hypertension or hyperthyroidism excrete approximately the same quantities of metabolites as normal subjects. Two patients with phæochromocytoma excreted abnormally large quantities of metadrenaline and normetadrenaline.

Our method is relatively simple, reproducible and sensitive enough to estimate such low concentrations of metadrenaline and normetadrenaline as are excreted in the urine of normal human beings.

We are indebted to Dr. Julius Axelrod for his gift of metadrenaline and normetadrenaline.

Kaoru Yoshinaga
Chuichi Itoh
Nozomu Ishida
Tatsuo Sato
Yasuo Wada

Department of Internal Medicine,
Tohoku University School of Medicine,
Sendai, Japan.

[1] Goodall, M., *Pharmacol. Rev.*, **11** (Part 2), 416 (1959).

[2] Bernheimer, H., Ehringer, H., Heistracher, P., and Kraupp, O., *Biochem. Z.*, **332**, 416 (1960).

[3] Axelrod, J., *Science*, **126**, 400 (1957).

[4] Goodall, M., Kirshner, N., and Rosen, L., *J. Clin. Invest.*, **38**, 707 (1959).

Effect of Pituitary Growth Hormone on Cholesterogenesis

It has been reported earlier that injections of anterior pituitary growth hormone into adult female rats cause a decrease in the rate of synthesis of fatty acid and an increase in the rate of cholesterogenesis in the liver[1]. Inverse relationships of this type have also been described as occurring in diabetic rats[2] and in normal rats injected with thyroxine[3]. These results were obtained using labelled acetate as substrate. The availability of labelled mevalonic acid now permits some dissection of the pathway involved.

The rats used were adult females injected with 1 mgm. pituitary growth hormone 6 hr. before killing. After killing, the livers were rapidly removed and slices prepared with the aid of a Stadie–Riggs[4] microtome. 400 mgm. of slices were suspended in 5 ml. Krebs–Ringer bicarbonate buffer containing either 0·6 μc. sodium acetate-1-^{14}C or *dl*-sodium mevalonate-2-^{14}C with no carrier added. The flasks were gassed with an oxygen/carbon dioxide mixture (95 : 5) and were incubated for 3 hr. After this period the slices were collected by centrifugation and then suspended and homogenized in 4 ml. of 5 per cent trichloracetic acid. The precipitate obtained from this was washed with 5 per cent trichloracetic acid and then water, the supernatant being discarded in each case. The precipitate was then extracted with alcohol/ether (3 : 1) and, finally, ether. The combined organic solvent layers were evaporated and the residue re-extracted with petroleum ether (40°–60°). After separation of the phospholipids as the magnesium complexes, the supernatant was saponified and the free cholesterol extracted and precipitated as the digitonide. Fatty acids were then separated after acidifying the supernatant fraction. Samples from each of the separated fractions were counted as infinitely thin layers in a 'Tracerlab' *SC*–16 windowless counter.

The results showing the incorporation of the two radioactive substrates into fatty acids, phospholipids and cholesterol are given in Table 1.

It will be seen that the incorporation of both mevalonate and acetate carbon into phospholipids is reduced in animals treated with growth hormone although, as might be expected, the reduction is much greater in the case of acetate. The incorporation of acetate-carbon into the fatty acid fraction (derived mainly from glyceride and cholesterol esters with some free fatty acid) is also much reduced in the rats treated with growth hormone and to about the same degree as the reduction found in the fatty acids of the phospholipid fraction. However, the incorporation of carbons from mevalonic acid into the ester fatty acids appears to be slightly accelerated in the animals treated with hormone. This somewhat surprising result may mean that the fatty acids formed from mevalonate carbons differ from those derived from acetate.

Finally, it can be seen that while the incorporation of acetate carbon into cholesterol is speeded up by a factor of 4–6 times in rats treated with growth hormone, the rate of incorporation of mevalonate remains unchanged. Thus, the stimulating effect of the hormone on the rate of synthesis of cholesterol

Table 1. Effect of Pituitary Growth Hormone on the Incorporation of Mevalonate-2-^{14}C and Acetate-1-^{14}C into Cholesterol, Phospholipids and Fatty Acids of Liver Slices

Precursor	Condition of animal	Phospholipids		Fatty acids		Cholesterol	
		c./min./flask	Treated/control	c./min./flask	Treated/control	c./min./flask	Treated/control
Mevalonic	Control	215	0·68	1,411	1·27	26,700	0·9
	Hormone-treated	147		1,719		24,418	
Acetate	Control	8,356	0·21	16,619	0·24	1,860	6·1
	Hormone-treated	1,780		3,988		11,311	
Mevalonic	Control	221	0·42	855	1·26	21,120	1·0
	Hormone-treated	92		1,077		21,168	
Acetate	Control	6,782	0·20	14,827	0·20	2,117	4·1
	Hormone-treated	1,356		2,965		8,782	

appears to be located in that part of the pathway between acetyl coenzyme A and mevalonic acid. The earlier part of this pathway, leading to the formation of β-hydroxy-β-methyl-glutaryl co-enzyme A, is common to both cholesterol synthesis and acetoacetate formation. Since it has previously been shown that in rats treated 6 hr. previously with growth hormone the rate of hepatic acetoacetate output is much reduced[5], it would appear that the increased rate of formation of cholesterol is due to a diversion of the β-hydroxy-β-methyl-glutaryl co-enzyme A from acetoacetate formation, and an increased rate of its reduction to mevalonic acid by its reductase. The hormone appears to have no effect on the subsequent condensation of the mevalonate molecules to cholesterol. This finding is in keeping with the conclusion of Bucher, Overath and Lynen[6], who have suggested that the decreased rate of formation of cholesterol in starving animals is due to a reduction in the activity of the β-hydroxy-β-methyl-glutaryl co-enzyme A reductase.

A. L. GREENBAUM
R. S. LEAL*

Department of Biochemistry,
University College,
London, W.C.1.

* Present address: Laboratório de Radioquímica, C.E.E.N. Faculdade de Ciências, Lisbon.

[1] Greenbaum, A. L., and Glascock, R. F., *Biochem. J.*, **67**, 360 (1957).
[2] Hotta, S., and Chaikoff, I. L., *J. Biol. Chem.*, **198**, 895 (1952).
[3] Fletcher, K., and Myant, N. B., *J. Physiol.*, **144**, 361 (1958).
[4] Stadie, W. C., and Riggs, B. C., *J. Biol. Chem.*, **154**, 687 (1944).
[5] Greenbaum, A. L., and McLean, P., *Biochem. J.*, **54**, 413 (1953).
[6] Bucher, N. L. R., Overath, P., and Lynen, F., *Biochim. Biophys. Acta*, **40**, 491 (1960).

Blockage of Transformation of Tryptophan to Nicotinic Acid by Experimental Hepatitis in Mice

THE existence of biochemical lesions in the complicated path from amino-acid to vitamin in man, and in experimental animals suffering from various diseases, has been identified by a number of authors analysing urinary metabolites of tryptophan by means of paper and column chromatography[1-6]. These analyses have shown an accumulation in the urine of metabolites proximal to the lesion.

In the present work we have studied the chromatographic picture of urinary metabolites of the tryptophan–nicotinic acid cycle both in normal mice and in mice with experimental hepatitis induced by mouse hepatitis virus strain 3 (*MHV*-3) at different stages of multiplication, in an attempt to identify the level of a possible biochemical lesion. Since the spontaneous elimination of urinary metabolites of tryptophan is small, we have preferred to follow them after a dose of L-tryptophan.

Mice of Swiss stock, weighing about 15 gm. and fed on Voght and Moller's diet were used. The programme of one of our experiments was as follows: 100 mice were divided into 5 (*A*, *B*, *C*, *D*, *E*) groups of twenty animals. Ten mgm. of L-tryptophan were injected subcutaneously into each animal in order to maintain the normal excretory pattern. After five days, mice from groups *A*, *B*, *C* and *D* were inoculated by the intraperitoneal route with 100 LD_{50} virus *MHV*-3.

To study the excretory pattern of metabolites of the biological degradation of tryptophan in different phases of the multiplication of the virus, a second dose of tryptophan was given to groups *A*, *B*, *C* and *D* at 12, 24, 48 and 72 hr. respectively. The mice in group *E*, not inoculated with the virus, were also given a second dose of tryptophan simultaneously with group *A*.

In the 24 hr. following the injection of tryptophan, the urine from each group of mice was collected in opaque glass vessels containing 2 ml. of 50 per cent glacial acetic acid (1: 1), and was diluted with water to a volume of 5 ml. The urine was then centrifuged. Dalgliesh's[7] method of chromatographic analysis for urinary metabolites in the tryptophan–nicotinic acid cycle was used, but omitting the absorption on carbon and the subsequent treatment with phenol.

The analysis of the urine of the control mice (*E*) injected with L-tryptophan showed the appearance of conjugates of the kynurenines (R_F 0·1–0·15) of kynurenic and xanthurenic acids, of acetylkynurenine and sometimes of free anthranilic acid.

In contrast, the urine of the mice inoculated with the virus showed the presence of all these metabolites (except anthranilic acid) in greater and more constant quantities, and in addition, the presence of kynurenine and of 3-hydroxykynurenine in notable quantities. The chromatographic picture also showed the progressive growth of the metabolic lesion paralleling the aggravation of the viral hepatic lesion.

Other metabolites, such as 3-hydroxyanthranilic acid, have never been observed on normal mice or in mice inoculated with the virus.

From the results obtained, we conclude that in mice with experimental hepatitis caused by the *MHV*-3 virus a biochemical lesion occurs at the level of enzymic transformation of 3-hydroxykynurenine to 3-hydroxyanthranilic acid.

M. PIAZZA
F. TANCREDI
E. QUAGLIARIELLO

Department of Infectious Diseases
and
Institute of Biochemical Chemistry,
University of Naples.
Institute of General Physiology and Biochemistry
University of Camerino.

[1] Altman, K. I., and Miller, G., *Nature*, **172**, 868 (1953).
[2] Boyland, E., and Watson, G., *Nature*, **177**, 837 (1956).
[3] Musajo, L., Benassi, C. A., and Parpaiola, A., *Clin. Chim. Acta*, 1, 229 (1956).
[4] Quagliariello, E., Auricchio, S., Casale, M., and Tancredi, F., *Boll. Soc. Ital. Biol. Sper.*, **34**, 1918 (1958).
[5] Auricchio, S., Quagliariello, E., Rubino, A., and Vecchione, L., *Ann. Paediat.*, **194**, 129 (1960).
[6] Quagliariello, E., Auricchio, S., Saccone, C., and Sganga, W., *Boll. Soc. Ital. Biol. Sper.*, **35**, 343 (1960).
[7] Dalgliesh, C. E., *J. Clin. Path.*, **8**, 73 (1955).

Sucrases in *Phaseolus vulgaris*

DURING work on the oligosaccharides of *Phaseolus vulgaris*, L. (namely, verbascose, stachyose, raffinose and sucrose[1-3]—the raffinose family of oligosaccharides[1]), an examination was made of the enzymes catalysing the hydrolysis of these saccharides. α-Galactosidase and sucrase activities were found in the ungerminated and germinated seeds.

The α-galactosidase activity proved to be typical of that described by Courtois *et al.*[4-7] in extracts of coffee and haricot beans and by Pridham[8] in extracts of *Vicia faba*, since it removed galactose from verbascose, stachyose and raffinose *in vitro* to give sucrose. From the higher homologues, verbascose and stachyose, the galactose was removed stepwise (cf. Courtois *et al.*[4]), producing stachyose and raffinose respectively as intermediates. The α-galactosidase activity was found in extracts of both the embryos and cotyledons in the ungerminated and germinated seeds.

On the other hand, two types of sucrase activity were found. These differed in both properties and occurrence; one activity appeared to resemble the invertases previously demonstrated in extracts of other seeds[9–11] and in extracts of yeast[12], while the other activity ('alkaline sucrase') differed from these invertases in optimal *p*H of action and its apparent specificity.

The enzyme extracts were prepared by homogenizing fresh plant material with water or 0·01 *M* phosphate buffer, *p*H 7·2–7·5 (1/3–1/2 vol. for embryos and 3 vol. for cotyledons), centrifuging at 34,000*g* for 30 min. at 0° C. and dialysing the supernatant solution against 0·01 *M* phosphate buffer, *p*H 7·0. Usually for qualitative work an equal volume of enzyme extract was added to a volume of buffer 0·2 *M* containing 0·2 *M* substrate to give the required *p*H after mixing. The enzyme action was followed by conventional chromatographic techniques which would detect 1–2 per cent hydrolysis[13] of the substrates. For quantitative work the reducing sugars produced were estimated by the Nelson–Somogyi method[14] after removal of the protein from the extracts by precipitation with zinc sulphate–barium hydroxide[15].

The 'alkaline sucrase' activity could be found in extracts prepared from embryos and cotyledons of ungerminated and germinated seeds but could best be prepared from embryos of steeped seeds. On 0·1 *M* buffered sucrose solutions at 25° C., the activity was found to have a narrow optimal *p*H range with a maximum at *p*H 7·8. The activity was not lost on dialysis and phosphate ions were not necessary for its action since it functioned equally well on sucrose in borate buffer (0·1 *M*); however, it was inhibited by *tris*(hydroxymethyl)aminomethane (*tris*)-hydrochloric acid and *tris*-maleate buffers. An extract of 'alkaline sucrase' prepared as described from steeped embryos had an initial rate of inversion of sucrose of 3×10^{-4} moles per hr. on 0·1 *M* sucrose, *p*H 7·8 at 25° C., which fell off to a low rate after 24 hr. of reaction and 40–50 per cent inversion of the sucrose. The extract showed no action towards stachyose, raffinose, 1-kestose, and, moreover, showed no transferase activity[13] during its action on strong (20 per cent) sucrose solutions. A small amount of hydrolysis observed in the experiments with stachyose and raffinose was shown to be due to the action of the α-galactosidase. This produced some sucrose which was then hydrolysed by the 'alkaline sucrase'. No manninotriose or melibiose were found in these experiments. These oligosaccharides would be found if invertase were present (as in the experiments with germinated embryos). The extract had some action on maltose at *p*H 7·8 but had no action on melezitose. The rate of hydrolysis of the maltose was 0·41 the rate of hydrolysis of sucrose by this extract under similar conditions. However, after purification by an ammonium sulphate fractionation, the rate of maltose hydrolysis was 0·2 of the rate of hydrolysis of sucrose. This separation indicated that two different enzymes were present, namely, sucrase and maltase, since if only one enzyme were involved the ratio of rates of hydrolysis on the two substrates would remain the same after purification.

On the other hand, the sucrase similar to other invertases could only be found in extracts prepared from germinated embryos (cf. Pridham[8]). This activity had a broader optimal *p*H range with a maximum at *p*H 4·9–5·0. An extract, prepared as described from germinated embryos, had an initial rate of inversion of sucrose approximately the same as that of the 'alkaline sucrase' extract prepared from steeped embryos. However, the rate of action was linear with time up to 90 per cent inversion of 0·1 *M* sucrose and the sucrose was completely inverted after 12–14 hr. The extract hydrolysed stachyose and raffinose, producing manninotriose and fructose, and melibiose and fructose respectively; however, the α-galactosidase activity which was also present in the extract hydrolysed the oligosaccharides further to give galactose, glucose and fructose as the final hydrolysis products. The extract also hydrolysed 1-kestose, maltose and melezitose and showed characteristic transferase action on strong (20 per cent) sucrose solutions. The hydrolysis of raffinose and melezitose would indicate that both β-fructosido—and α-glucosido—invertases were present in the extract.

From these facts it seems, therefore, that the new enzyme 'alkaline sucrase' is not: (*a*) an α-glucosido- or a β-fructosido-invertase since it does not attack melezitose and raffinose, which are respective substrates for these enzymes; (*b*) an α-glucosidase proper since the enzyme does not attack melezitose and maltose; or (*c*) sucrose phosphorylase because the enzyme hydrolysed sucrose in the absence of phosphate ions. It is concluded that the observed activity must result from the presence of a new type of sucrase, apparently highly specific for sucrose. The metabolic significance of this enzyme is not yet known.

A sucrose-specific enzyme has been found in a haploid hybrid *Saccharomyces* strain by Hestrin and Lindegren[16], and it is also interesting to note that two phytases having different *p*H optima and two lipases having different *p*H optima have been reported to occur in germinating lettuce seeds[17].

We wish to express our thanks to Dr. J. B. Pridham for a sample of verbascose and to Dr. J. Edelman for a sample of 1-kestose.

R. A. COOPER
R. N. GREENSHIELDS

Department of Biochemistry,
University of Birmingham,
Edgbaston, Birmingham 15.

[1] French, D., *Adv. in Carbohyd. Chem.*, **9**, 149 (1954).
[2] Dupéron, R., *C.R. Acad. Sci., Paris*, **241**, 1817 (1955).
[3] Dupéron, R., *Rev. gén. Botan.*, **61**, 261 (1954).
[4] Courtois, J. E., Anagnostopoulos, C., and Petek, C., *C.R. Acad. Sci., Paris*, **238**, 2020 (1954).
[5] Courtois, J. E., and Petek, F., *Bull. Soc. Chim. Biol.*, **36**, 1115 (1954).
[6] Courtois, J. E., Anagnostopoulos, C., and Petek, F., *Enzymologia*, **17**, 69 (1954).
[7] Courtois, J. E., Anagnostopoulos, C., and Petek, F., *Congr. intern. biochim. Résumés communs. 2e Congr., Paris*, 240 (1952).
[8] Pridham, J. B., *Nature*, **182**, 1687 (1958).
[9] Brill, H. C., *Philippines J. Sci.*, **12**, A, 1 (1917).
[10] Courtois, J. E., Anagnostopoulos, C., and Petek, F., *Bull. Soc. Chim. Biol.*, **35**, 73 (1953).
[11] Hattori, S., and Shiroya, T., *Arch. Biochem. Biophys.*, **34**, 121 (1951).
[12] Sumner, J. B., and Myrback, H. (eds.), *The Enzymes* (Academic Press, Inc., N.Y., 1951).
[13] Bacon, J. S. D., and Edelman, J., *Biochem. J.*, **49**, 529 (1951).
[14] Nelson, N., *J. Biol. Chem.*, **153**, 375 (1944).
[15] Somogyi, M., *J. Biol. Chem.*, **160**, 69 (1945).
[16] Hestrin, S. S., and Lindegren, C. C., *Nature*, **165**, 158 (1950).
[17] Mayer, A. M., *Enzymologia*, **19**, 1 (1958).

PHYSIOLOGY

Active Cation Transport as a Pace-maker of Respiration

INHIBITION of metabolism leads to a gain of sodium and a loss of potassium by cells, and it is widely realized that the maintenance of concentration gradients of these ions between cells and plasma depends on a supply of energy from metabolism. This holds true for red cells that derive energy from glycolysis, for the majority of tissues that depend on respiration and for the few cells and tissues that actively transport cations with energy derived from either metabolic pathway. Cells appear to transport cations actively only when adenosine triphosphate (ATP) is being produced *in situ* or is supplied from an exogenous source, such as by injection into poisoned squid giant axons[1] or by introduction into red cells ghosts by the technique of 'reversible hæmolysis'[2]. ATP is necessary, therefore, and its rate of production either by substrate or oxidative phosphorylation is one of the factors that determines the rate at which active transport occurs.

The converse situation is that the rate of metabolism might be regulated by the rate of active transport. This is feasible in respiring cells because respiration is governed by the concentration of adenosine diphosphate (ADP) which is determined in turn by the activity of the various mechanisms causing the hydrolysis of ATP. Since one of these is the fission of ATP due to active transport, it is of interest to test experimentally if the cessation of active transport by specific inhibitors which do not directly inhibit respiration causes a fall in the rate of respiration.

Slices of brain and kidney cortex from rabbits are suitable experimental tissues, because they have a high consumption of oxygen, on which active cation transport depends[3], and also the rates of exchange of their sodium and potassium are high. The maintenance of the intra-cellular potassium concentrations has therefore been compared with the Qo_2 (µl./mgm. dry wt./hr.) after incubations of 1 hr. in a saline medium containing glucose (150 m*M* sodium chloride, 5 m*M* potassium chloride, 2 m*M* magnesium chloride, 10 m*M* sodium phosphate *p*H 7·4, 10 m*M* glucose). Two ways of abolishing active transport were tested at 37° C.: (1) exposure of the slices to the cardiac glycoside, ouabain; (2) replacement of sodium of the medium with choline. With brain slices, 5 µ*M* ouabain caused falls of 70 per cent in the potassium concentration (from 360 to 100 m. equiv./kgm. dry wt.) and of 50 per cent in the Qo_2 (from 16·7 to 8·0). Replacement of sodium of the medium with choline caused a fall in Qo_2 to 8·8 and a fall in potassium concentration to 110 m.equiv./kgm. dry wt.; no further fall occurred with 5 µ*M* ouabain. Similar results were obtained with kidney slices, in which 25 µ*M* ouabain caused a fall of 64 per cent in the potassium concentration (from 420 to 150 m.equiv./kgm. dry wt.) and of 45 per cent in the Qo_2 (from 13·2 to 7·2). In a choline (sodium-free) medium, the Qo_2 of 8·4 and the potassium concentration of 120 m.equiv./kgm. dry wt. were unchanged by ouabain. The fall in Qo_2 caused by ouabain thus occurred only in the presence of sodium in the medium and is of the same magnitude as the reduction caused by the removal of sodium.

To see if the component of the tissue which is sensitive to ouabain and sodium still controlled respiration after breaking up the cells, homogenates of brain cortex were made in four solutions so as to introduce (0·15 *M* sodium chloride) or to exclude (0·15 *M* potassium chloride, 0·15 *M* choline chloride and 0·25 *M* sucrose) sodium. Each solution also contained 2 m*M* magnesium chloride, 10 m*M* glucose and 2·5 m*M* dipotassium hydrogen phosphate. Incubations were then made with and without ouabain (5 µ*M*) both in the absence of sodium and after the addition of sodium to part of the sodium-free homogenates. Table 1 shows that, irrespective of the method of preparation, homogenates in a high-sodium medium were sensitive to ouabain, the Qo_2 being reduced by 32–48 per cent (line *c*). Omission of sodium from the medium caused falls in Qo_2 of 33–42 per cent (line *g*) and, in the absence of sodium, no further fall in Qo_2 was caused by ouabain (line *f*). The respiration of homogenates was thus equally reduced by ouabain and the deprivation of sodium in a way comparable to that of slices.

Since systems utilizing ATP are some of the most important regulators of cell respiration[4], it seems likely that the reduction in Qo_2 is due to the sensitivity to ouabain and sodium of one or more of the reactions leading to the formation of ADP from ATP. Recent work on adenosine triphosphatases in red cells[5], kidney[6], brain[7] and crab nerve[8] has indeed shown the existence of ATP-hydrolysing systems which require sodium for activation and which are inhibited by ouabain, and it has been suggested that they are intimately associated with active transport. The quantitative relation between the amount of ATP split and the amount of material actively transported is difficult to assess because of insufficient information of the intracellular concentrations of ATP, ADP and orthophosphate, and estimates based on the standard free energy of hydrolysis of ATP are dubious[9]. Nevertheless, the results with slices show that the maintenance of a high tissue-potassium concentration and the Qo_2 are reduced in a parallel manner by ouabain and the deprivation of sodium, suggesting a partial control of respiration by the transport mechanism. The stoppage of active transport reduces the demand for ATP and therefore explains the concomitant fall in Qo_2 of 40–50 per cent. Similar results with homogenates suggest the possibility either that other ATP-hydrolysing reactions are also sensitive to ouabain and sodium or that the transport mechanism continues to hydrolyse ATP in broken cells. The latter possibility would seem more likely in view of the correlation of the properties

Table 1. DECREASE IN Qo_2 OF BRAIN HOMOGENATE IN THE PRESENCE OF OUABAIN AND ON THE OMISSION OF SODIUM FROM THE MEDIUM. (*S.E.* of the means are given)

			Qo_2			
Main solute in the solution in which homogenate made			Sodium chloride	Potassium chloride	Choline chloride	Sucrose
No. of experiments			3	5	4	4
High sodium medium (112–150 m*M* sodium)	no ouabain	(*a*)	2·70	2·62 ± 0·23	2·33 ± 0·19	1·65 ± 0·11
	with ouabain	(*b*)	1·40	1·69 ± 0·07	1·59 ± 0·14	1·09 ± 0·06
	fall due to ouabain	(*c*) = (*a* − *b*)	1·30	0·93 ± 0·23	0·74 ± 0·18	0·56 ± 0·08
Sodium-free medium	no ouabain	(*d*)	—	1·57 ± 0·14	1·49 ± 0·10	1·11 ± 0·10
	with ouabain	(*e*)	—	1·51 ± 0·11	1·43 ± 0·07	1·00 ± 0·06
	fall due to ouabain	(*f*) = (*d* − *e*)	—	0·06 ± 0·10	0·06 ± 0·09	0·11 ± 0·05
Fall due to omission of sodium from medium	no ouabain	(*g*) = (*a* − *d*)	—	1·05 ± 0·32	0·84 ± 0·20	0·54 ± 0·08

of the active cation transport system in intact red cells with those of the ATP-hydrolysing system in fragment red-cell membranes[5].

The parallelism of the effects of ouabain and sodium on the maintenance of the tissue-potassium concentration and on the Q_{O_2} therefore suggests the tentative conclusion that an ATP-hydrolysing system associated with the active transport of cations may act as a pace-maker for about half the respiration of brain and kidney cortex.

R. WHITTAM

Department of Biochemistry,
University of Oxford.

[1] Caldwell, P. C., Hodgkin, A. L., Keynes, R. D., and Shaw, T. I.' *J. Physiol.*, **152**, 561 (1960).

[2] Gardos, G., *Acta Physiol. Acad. Sci. Hung.*, **6**, 191 (1954). Whittam, R., Ph.D. thesis, Univ. Cambridge (1957). Hoffman, J. F., *Fed. Proc.*, **19**, 127 (1960).

[3] Mudge, G. H., *Amer. J. Physiol.*, **165**, 113 (1951). Terner, C., Eggleston, L. V., and Krebs, H. A., *Biochem. J.*, **47**, 139 (1949). Pappius, H. A., and Elliott, K. A. C., *Canad. J. Biochem. Physiol.*, **34**, 1053 (1956).

[4] Krebs, H. A., and Kornberg, H. L., *Ergebn. Physiol.*, **49**, 212 (1957). Lardy, H. A., and Wellman, H., *J. Biol. Chem.*, **195**, 215 (1952). Siekevitz, P., and Potter, V. R., *J. Biol. Chem.*, **201**, 1 (1953). Chance, B., and Williams, G. R., *J. Biol. Chem.*, **217**, 383 (1955).

[5] Post, R. L., Merritt, C. R., Kinsolving, C. R., and Albright, C. D., *J. Biol. Chem.*, **235**, 1796 (1960). Dunham, E. T., and Glynn, I. M., *J. Physiol.*, **156**, 274 (1961).

[6] Wheeler, K. P., and Whittam, R. (unpublished results).

[7] Hess, H., and Pope, A., *Fed. Proc.*, **16**, 196 (1957). Jarnefelt, J., *Biochim. Biophys. Acta*, **48**, 104 (1961).

[8] Skou, J. C., *Biochim. Biophys. Acta*, **42**, 6 (1960).

[9] Whittam, R., and Davies, R. E., *Nature*, **173**, 494 (1954).

Effect of Thyroxine and 2,4-Dinitrophenol on the Rate of Utilization of Glucose

IT has long been recognized that 2,4-dinitrophenol increases oxidative metabolism. The increase of oxygen consumption after the addition of 2,4-dinitrophenol (DNP) is explained as being a result of dissociation between oxidative and phosphorylative processes. Similarly, thyroxine accelerates oxidation by uncoupling an oxidative phosphorylation. But DNP does not prevent the phenomena of hypothyroidism other than basal metabolism[1], and fails to change lipid metabolism[1,2] and nitrogen excretion, the effects of which are known to follow thyroxine administration[1]. Therefore, it was interesting to compare the effects of these agents on carbohydrate metabolism.

In the present experiments I studied the influences of DNP and thyroxine on the rate of utilization of glucose in normal and thyroidectomized rabbits (120 days after operation). The utilization of glucose was determined from the rapid intravenous glucose tolerance test (GTT) which was performed 4 hr. following the subcutaneous administration of DNP (7·5 mgm. rabbit) or thyroxine (0·25 mgm./kgm. body-weight). Immediately after a fasting blood sample of venous blood had been obtained from the vein of the ear, 0·5 gm./kgm. body-weight of glucose was injected intravenously during 10 ± 5 sec. The blood samples for estimation of blood glucose were drawn from the vein of the other ear at intervals of 10, 20, 30, 45, 60, 75, 90 min. after the end of the injection of sugar. From this intravenous GTT the rate of utilization of glucose may be expressed by determining the assimilation coefficient of glucose (K) as was described in previous papers[3,4].

The results of my experiments are summarized in Table 1. 4 hr. after the administration of thyroxine and DNP the fasting blood-sugar levels were slightly increased both in normal and thyroidectomized rabbits. In intravenous GTT it was observed that thyroxine accelerated the utilization of glucose in both groups of experimental animals as early as 4 hr. after administration. Similarly, DNP increases the rate of utilization of glucose in normal and thyroidectomized rabbits. The results of these observations suggest that the influence of thyroxine and DNP on carbohydrate metabolism is similar, in contrast to their effects on lipid or protein metabolism. Both agents accelerate the rate of utilization of glucose, but the precise mechanism of this action is not clear. The increase in the assimilation coefficient of glucose after the addition of DNP may be explained by insulin mobilization (probably due to its hyperglycæmic effect), as suggested by Hetényi *et al.*[5], or by the acceleration of the transfer of sugar across the muscle cell-membrane as observed by Randle *et al.*[6,7]. Further, it is interesting to note that thyroxine accelerates the utilization of glucose as early as 4 hr. following its injection, without any protracted latent period, which is in agreement with the experiments of Christophe and Mayer performed on rats[8].

L. MACHO

Endocrinological Institute,
Slovak Academy of Sciences,
Bratislava, Czechoslovakia.

[1] Sollmann, T. (edit. by), *A Manual of Pharmacology*, 7 (W. B. Saunders, New York, 1948).

[2] Castor, C. W., and Beinwaltes, W., *J. Clin. Endocrinol.*, **10**, 1026 (1950).

[3] Macho, L., *Acta Med. Scand.*, **160**, 477 (1958).

[4] Macho, L., *Arch. Internat. Physiol. Biochem.*, **66**, 260 (1958).

[5] Hetényi, jun., G., Issekutz, jun., B., and Winter, M., *Acta Physiol. Acad. Sci. (Hung.)*, **7**, 287 (1955).

[6] Randle, P. J., and Smith, G. H., *Biochem. J.*, **70**, 490 (1958).

[7] Morgan, H. E., Randle, P. J., and Regen, D. M., *Biochem. J.*, **73**, 573 (1959).

[8] Christophe, J., and Mayer, J., *Endocrinol.*, **65**, 475 (1959).

Table 1. VALUES OF FASTING BLOOD-SUGAR LEVELS (G) AND OF ASSIMILATION COEFFICIENT OF GLUCOSE (K) IN RABBITS BEFORE (G_1, K_1) AND AFTER THYROXINE (G_2, K_2) OR DNP (G_3, K_3) ADMINISTRATION

	No.	G_1	K_1	K_2	G_2	K_3	G_3
Normal rabbits	6	130 ± 3	1·08 ± 0·04	1·34 ± 0·07*	145 ± 10	1·52 ± 0·19*	151 ± 3†
Thyroidectomized rabbits	5	115 ± 7	0·86 ± 0·03	1·33 ± 0·08†	139 ± 16	1·63 ± 0·13†	126 ± 8

Statistical significance: * = $P < 0·05$; † = $P < 0·01$.

Determination of Spontaneous Plasma Fibrinolysis by a Practical Procedure

IN a recent review I analysed present aspects and principles of the various methods that are generally used for assessing the phenomenon of proteolysis in the blood. The extensive significance of *in vitro* determination of whole plasma spontaneous fibrinolysis, which expresses a more faithful and exact picture of the evolution of the fibrinolytic mechanism in the circulating blood, was emphasized[1].

As is known, the determination of the spontaneous fibrinolysis of the whole blood or of plasma in physiological conditions takes a long time[2,3]. Repeated attempts to shorten the time of lysis by a strong dilution of plasma[4] or the carrying out of the low-temperature technique[5] did not solve the problem sufficiently in so far as to establish an accepted practical method for the assessment and computation of the phenomenon of fibrinolysis. The only useful determination is obtained with the euglobulin method in the plasma, which according to different techniques that are described, offers an estimate of the phenomenon within 3–24 hr.[6-9]. This method is characterized by an expression for the spontaneous fibrinolysis of the plasma, but there are theoretical and practical considerations on the basis of which it becomes questionable if, and to what extent, the method is representative.

In this communication a practical procedure is described for the determination of spontaneous fibrinolysis in the whole plasma, based on the observation that the time of lysis of plasma under similar conditions of dilution, *p*H, recalcification and temperature is markedly shortened, if at first the received whole blood is diluted immediately in an appropriate buffer solution and, thereafter, the fibrin-clot is formed by recalcification of the supernatant fluid phase. Under these conditions the retraction of the clot is insignificant and thus the end-point of lysis can be observed.

Reagents :

(1) Buffer diethylbarbiturate (*p*H 7·40) :	
Sodium diethylbarbiturate	4·12 gm.
M/10 hydrochloric acid	144·00 ml.
Potassium oxalate	0·50 gm.
Sodium chloride	5·50 gm.
Water distilled at	1000·00 ml.
(2) *M*/600 calcium chloride	

With a 1·0 ml. calibrated precision-syringe, 0·5 ml. of venous blood is withdrawn and transferred immediately into a small tube containing 2 ml. of the buffer, which previously has been placed in a container of melting ice. The transfer is performed gently, while the mixing takes place automatically. The tube is centrifuged for 1 min. at 3,500 r.p.m. From the supercooled layer 1·0 ml. is taken at once and placed in a tube which is immersed in a 37° C. water-bath containing 1·0 ml. from the solution of *M*/600 calcium chloride. Clotting takes place in a few minutes. Time of lysis is determined by frequent checking.

The time of clot lysis varied between 6 and 18 hr. for almost the total series of cases, as this is calculated from the samples that were taken at the ninth hour, from 50 normal subjects of both sexes, 20–45 years of age (Fig. 1). In two cases a time less than 6 hr. (3 and 5 hr. respectively) was observed while for only 4 other cases the time of lysis was more than 18 hr. (21, 26, 27 and 32 hr.). The repeated trial of the method is satisfactory, because the different time of clot-lysis obtained in repeated determinations of the same plasma did not exceed 10 per cent.

The time-values obtained are much lower than with any hitherto known method of determination in whole plasma and comparable to the euglobulin method. The euglobulin method is used by many research workers as a method for the determination of the spontaneous fibrinolytic activity of the plasma, and this cannot be ascertained by virtue of the nature of the method, because lysis is only countable in the euglobulin fraction. These observations are of special import, particularly after recent work[6] showing that findings on the same samples of blood that are registered in the whole plasma and in the euglobulins fraction are not always in an analogous ratio. For example, examination of a hepatic patient, though it disclosed an increased fibrinolytic action in the whole plasma, did not record deviations worthwhile from the physiological point of view, by the method of the euglobulins.

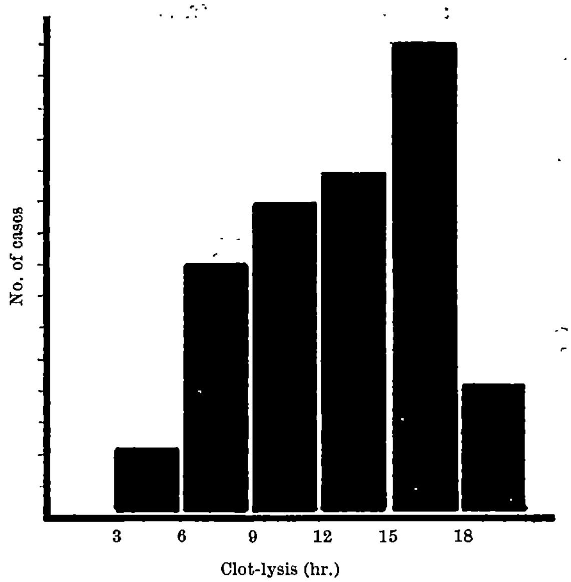


Fig. 1

The suggested procedure for measuring fibrinolytic activity of whole plasma represents a rapid technique and its simplicity is advantageous. This method requires 0·5 ml. of blood, the lysis of the fibrin clot is estimated accurately in a physiological *p*H and reproducibility is satisfactory.

H. YATZIDIS

Department of Internal Medicine,
Hippocratean University Hospital,
University of Athens
School of Medicine.

[1] Yatzidis, H., *Rev. Franc. Etudes Clin. et Biol.*, **4**, 383 (1959).

[2] Macfarlane, R. G., *Lancet*, i, 10 (1937).

[3] Ratnoff, O. D., *Bull. Johns Hopk. Hosp.*, **84**, 29 (1949).

[4] Biggs, R., Marfarlane, R. G., and Pilling, J., *Lancet*, i, 402 (1947).

[5] Fearnley, G. R., Balmforth, G., and Fearnley, E., *Clin. Sci.*, **16**, 645 (1957).

[6] Kowalski, E., Kopec, M., and Niewiarowski, S., *J. Clin. Path.*, **12**, 215 (1959).

[7] Milstone, H., *J. Immunology*, **42**, 100 (1941).

[8] Sherry, S., Lindemeyer, R. I., Fletcher, A. P., and Alkjaersic, N. *J. Clin. Inv.*, **38**, 810 (1959).

[9] Von Kaulla, K. N., *Circulation*, **17**, 187 (1958).

Effect of Bee Venom on Concentration of Blood Glucose and Liver Glycogen in Rabbits

COBRA venom and scorpion toxin are known to cause hyperglycæmia[1] whereas the *Walterinnesia* venum produces hypoglycæmia.

In the present experiments it was shown that bee venom, the effects of which resemble in many ways those of cobra venom, also causes hyperglycæmia and loss of liver glycogen. The blood glucose was determined by the Hagedorn and Jensen method[2] ; the liver glycogen after hydrolysis of the removed livers by the method of Good, Kramer and Somogyi[3].

Fig. 1 illustrates the hyperglycæmia produced by a sub-lethal dose of 5 mgm. bee venom injected subcutaneously into a 2-kgm. fasting rabbit. The effect did not occur when the injections were made into immunized rabbits.

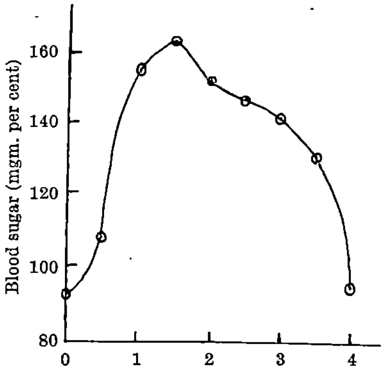


Fig. 1. Changes in blood-sugar level caused by injection of sublethal dose of bee venom

The fall in liver glycogen produced by 5 mgm. bee venom is illustrated for two groups of experiments. For each group five rabbits were used, one as a control, and the other four were killed 1, 2, 3 and 4 hr. after the injection and the liver removed.

Table 1. EFFECT OF SUBLETHAL DOSE

Time after injection (hr.)	Control	Liver glycogen (mgm./100 gm.) (mean value)	
		Experiment 1	Experiment 2
0	164·66	166·33	159·66
1	164	153·66	143·33
2	162	131	121·33
3	164	129·66	119
4	159	119·66	118

Since the maximal fall in liver glycogen occurred during the second hour, mobilization of liver glycogen may fully account for the hyperglycæmia, which was maximal 1½ hr. after the injection. The effect may be a direct action of bee venom on the liver, or brought about indirectly by the release of adrenaline from the suprarenals, since Feldberg[4] has shown that bee venom as well as cobra venom injected into the cœliac artery of the cat produced a sustained release of adrenaline from the suprarenals.

A. H. MOHAMED
K. ZAKI

Department of Physiology,
Faculty of Medicine,
Ain Shams University,
Cairo.

[1] Bertrand, M., and Vladesco, L. A., *C.R. Acad. Sci., Paris*, **209**, 585 (1939). Mohamed, A. H., *Nature*, **166**, 734 (1950).

[2] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, **135**, 46 (1923).

[3] Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, **100**, 485 (1933).

[4] Feldberg, W., and Kellawan, C. H., *J. Physiol.*, **90**, 257 (1937).

Action of the Hypothalamus

EXPERIMENTS with an analogue model of the heart, demonstrated by me[1], show that a pulse may arise, in a fluid-filled elastic system, in either of two ways: in the first, energy is cycled into the system from a mechanical source, corresponding to the physicochemical contraction of the ventricles ; in the second, pulsation can be derived from the static head of pressure with the aid of a length of elastic tubing arranged as a wave guide. The latter device, termed an 'oscillator', may be employed to time delivery of energy from the external source, in such a manner that the system is self-oscillating and that the two pulses, of differing origin, are synchronous.

In lesions of the conducting system of the heart, the coronary arterial system behaves, apparently, as an oscillator that utilizes the static head of pressure of the blood to develop a pulse. The same phenomenon is likely to occur at the circulus arteriosus, under normal conditions, however, due to the negative pressure in the internal jugular vein.

Examination of this possibility has led to the following explanation of the action of the hypothalamus :

Independently of the pulse from the heart, a vascular pulse originates in the perforating branches of the circulus arteriosus and is conveyed to the nerve cells in the nuclei of the hypothalamus. Provided the leading edge of the wave is sufficiently steep, the cells are excited to generate electrical impulses, which are utilized by the brain as timing signals.

There are at least two types of excitation. One, in the anterior hypothalamus, is associated with a positive temperature coefficient of frequency: a second, in the posterior hypothalamus, with a negative temperature coefficient of frequency. A third type of excitation probably occurs in the intermediate group of nuclei, with frequency unrelated to temperature. Similar physical relationships are found in connexion with the vibration of piezo-electric crystals, where the direction of the slice, with relation to the axes of the crystal, determines the sign of the temperature coefficient of frequency.

The importance of the structures around the circulus arteriosus suggests that the latter is the most important site in the body at which self-oscillation occurs. The major function of the sinus caroticus is, presumably, that of monitoring the diastolic blood pressure, to ensure stable operating conditions for the circulus arteriosus. Slight variations of frequency will result from changes of venous pressure in the thorax, due to respiration. This effect can be displayed on the model.

To prevent interference between the waves produced by the heart and those generated at the circulus arteriosus, it is essential that there be a co-ordinating mechanism. On one hand it is well known that signals—or nerve impulses—travel from the hypothalamus to the heart by way of the autonomic nervous system. On the other, it would appear that the signals from the heart to the hypothalamus are actually the heart sounds, conducted by the bones of the thorax and vertebral column to the region of the hypophyseal fossa, where they exert a 'pulsactor effect', of which the significance is explained elsewhere[2], on the surrounding vascular structures.

Examples of systems in which two synchronizing signals require co-ordination are to be found in radar. In one example, the transmitter takes signals from the master timing signal generator and, in addition, synchronizing signals from the indicator of the receiver : the more stable system determines the frequency. Applying the analogy to the human body, the hypothalamus is identified as the master timing signal generator.

JOHN ERSKINE MALCOLM

Postgraduate Medical School,
Ducane Road,
London, W.12.

[1] Malcolm, J. E., *Proc. Physiol. Soc.* (March 24, 1961).

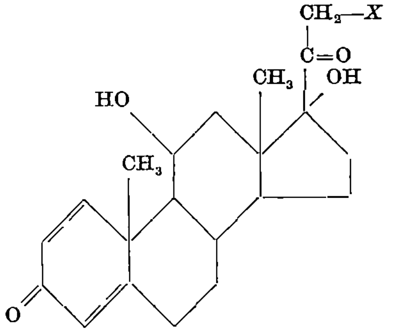
[2] Malcolm, J. E., *Nature*, **190**, 1114 (1961).

PHARMACOLOGY

Amino-derivatives of 11β,17α,21-Trihydroxy-3,20-Dioxo-1,4-Pregnadiene

The skeleton of corticosteroids was systematically modified, mainly in order to study the changes in the pharmacological effect of the compounds.

One of the objects of our experiments was the exchange of the C_{21} hydroxyl group of corticosteroids by amino- and substituted amino-groups, respectively.



(*a*) $X = -O-SO_2-C_6H_4-CH_3$; (*b*) $X = -I$; (*c*) $X = -1$ piperidyl; (*d*) X = 4-morpholyl; (*e*) $X = N(C_2H_5)_2$.

The 21-tosylate (*a*) of 11β,17α,21-trihydroxy-3,20-dioxo-1,4-pregnadiene and the -21-iodine compound (*b*)[1] derived from it served as initial substances in preparing the compounds mentioned.

One mole of the -21-tosylate of 11β,17α,21-trihydroxy-3,20-dioxo-1,4-pregnadiene (*a*) was treated in a tetrahydrofuran solution, in a nitrogen current, with two moles of piperidine at 55° C. for 5 hr. On dropping the reaction mixture into water, 11β,17α,dihydroxy-3-20-dioxo-21(1-piperidyl)-1,4-pregnadiene (*c*) was obtained in 93 per cent yield, in crystalline form; on recrystallizing from a mixture of tetrahydrofuran and petroleum ether, melting point 181° C. (decomposition) was found. Analysis: calculated, C 73·06; H 8·6 per cent; found, C 73·00; H 8·9 per cent.

Compound (*c*) has also been prepared by reacting one mole of the corresponding iodine compound (*b*) with two moles of piperidine under conditions similar to those mentioned. Melting point of product: 181° C. (decomposition). Melting point of the hydrochloride: 236–238° C. (decomposition). Analysis: calculated, C 67·31; H 8·1; Cl 7·6 per cent; found, C 67·07; H 8·1; Cl 7·8 per cent.

In a similar way, 11β,17α,-dihydroxy-3,20-dioxo-21-(4-morpholyl)-1,4-pregnadiene (*d*) was obtained from the tosylate (*a*) and iodine compound (*b*). Melting point 191–193° C. Analysis: calculated, C 69·91; H 8·1 per cent; found, C 69·62; H 8·2 per cent. Melting point of hydrochloride: 235–237° C. (decomposition). Analysis: calculated, C 64·65; H 7·7 per cent; found, C 64·38; H 7·6 per cent.

Similarly to the above-mentioned compounds, 11β,17α,-dihydroxy-3,20-dioxo-21-diethylamino-1,4-pregnadiene (*e*) has been prepared. Melting point of hydrochloride: 227° C. (decomposition). Analysis: calculated, C 66·44; H 8·41; Cl 7·86 per cent; found, C 66·20; H 8·28; Cl 7·61 per cent.

C_{21}-amino -derivatives similar to the corticosteroid derivatives mentioned have also been prepared in an essentially similar way by E. J. Agnello and G. D. Laubach[2].

The compounds prepared by the foregoing technique were subjected to pharmacological tests in the form of bases insoluble in water and of water-soluble hydrochlorides, respectively. Compounds of gluco-corticoid activity are known to possess the general feature of containing oxo-groups on their C_3 and C_{20} atoms, a hydroxyl or oxo-group on their C_{11} atom and a double bond between C_4 and C_5. Also, a common property of these compounds is the hydroxyl function on C_{21}. The α-positioned hydroxyl on C_{17} is absent in the case of corticosterone of similarly gluco-corticoid effect, although only moderately active. Thus, all known efficient substances actually bear an oxygen atom on C_{21}.

The compounds mentioned previously were examined by comparison with the effect of prednisolone. These investigations were carried out by two methods. The accumulation of liver glycogen was investigated on suprarenal gland ectomized rats, extracting the deposited glycogen with trichloracetic acid and determining glycogen by turbidimetry. The antiphlogistic action of the compounds was examined by the method of rat foot œdema determination evolved by us[3]. Male rats were used in these tests.

The experimental results obtained by both methods disclosed activities of about 80 per cent in respect to prednisolone in the case of the C_{21} piperidine derivative (*c*), of the C_{21} morpholyl derivative (*d*) and of the hydrochlorides of these compounds. Further, it was found that, while on treating rats *per os* with prednisolone the activity of this substance was about 50 per cent of that observed in the case of subcutaneous injections, the water-soluble derivatives were resorbed in 90 per cent.

On the basis of our experiments we can thus state that the C_{21} amino-derivatives of prednisolone possess a gluco-corticoid activity corresponding to that of prednisolone itself. Accordingly, the function of C_{21} does not seem to be an indispensable pre-requisite of gluco-corticoid activity. It appears that its function can be replaced by an amino-group. The water-soluble salts of the compounds prepared in this way are similarly efficient. From the gastro-intestinal tract these latter compounds are more readily resorbed than prednisolone—a substance insoluble in water.

Our experimental results will shortly be published in detail.

J. Tóth
Z. Tuba

Synthetic Laboratory,

L. Szporny

Pharmacological Laboratory,
Chemical Works of Gedeon Richter, Ltd.,
Budapest.

[1] Nussbaum, A. L. (Schering Corp.), U.S. Patent 2814632 (Nov. 20, 1957).

[2] Agnello, E. J., and Laubach, G. D., U.S. Patent 2920099.

[3] Szporny, L., and Fekete, Gy., *Arch. Exp. Path. Pharm.*, 238, 233 (1960).

Effects of Morphine-like Drugs in Chicks

Several workers[1–4] have used conscious chicks to study the actions of drugs. Chicks are particularly useful for drugs acting on the central nervous system, as their blood-brain barrier does not form until they are about 3 months old[5,6]. The effects of analgesic drugs on chicks were demonstrated at a meeting of the British Pharmacological Society[7] and are described here. Analgesic action was measured by the multiple toe-pinch method[8], which has been applied successfully to guinea pigs and rats. An untreated chick squeaks and shakes its foot when an artery clip is applied to one of its toes and these responses were used to test whether nociception was suppressed by drugs.

Morphine showed very low potency in suppressing these responses in chicks, in contrast with its potency in other species. While morphine (5–20 mgm./kgm.), subcutaneously or intraperitoneally, suppresses comparable responses in mice, rats and guinea pigs, 200 mgm./kgm. intravenously was required to produce similar analgesic action in chicks. This dose was very close to the *LD*50 and the therapeutic index was remarkably low. Table 1 shows the relatively low therapeutic indices (*LD*50/*ED*50) of the analgesic drugs tested and shows that morphine, methadone and 1-(β-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole (20684) have a far lower potency in chicks than in guinea pigs. Codeine, however, is about equipotent in the two species.

Table 1. The Analgesic Actions and Toxicities of Morphine-like Drugs in Chicks and a Comparison with their Analgesic Potencies in Guinea Pigs (ref. 8)

Compound	Chicks			Guinea pigs
	Analgesic *ED*50 (mgm./kgm.) intravenous	*LD*50 (mgm./kgm.) intravenous	$\frac{LD50}{FD50}$	Analgesic *ED*50 (mgm./kgm.) subcutaneous
Morphine	230	305	1·33	6·3
Methadone	>18	24	<1·33	3·0
Pethidine	>40	42	<1·07	20·4
Codeine	26	45	1·73	25·0
20684	3·2	>20	>6·25	0·0032

During this work, it was observed that the intravenous administration of morphine-like drugs quickly produced characteristic postural and behavioural effects in the chicks. These effects occurred at doses lower than those required to suppress responses to noxious stimuli. The chick exhibited marked ptosis, became restless and stiff-legged. Then it sat down with its feet spread apart and its back at 30–50° to the horizontal (Fig. 1). Some chicks whined occasionally and the intensity of their cries became stronger on provocation. These effects sometimes persisted for several hours but could be antagonized within a few minutes by nalorphine (50–100 mgm./kgm.), given subcutaneously or intravenously. Table 2 shows the approximate doses of analgesic drugs producing these characteristic and postural effects. Among many drugs of other types investigated, only atropine produced comparable postural effects.

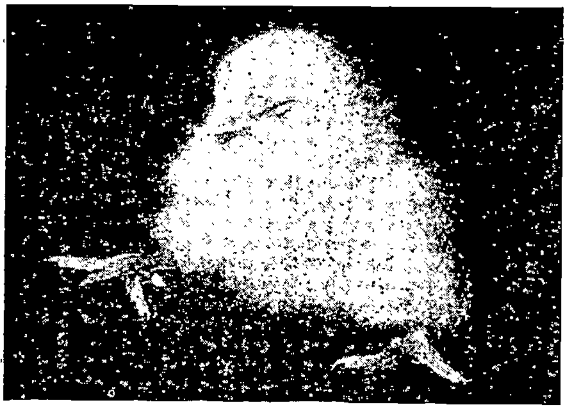


Fig. 1

Table 2. Approximate Minimal Doses of Analgesic Drugs producing Characteristic Postural Effects in Chicks

Compound	Dose (mgm./kgm.) intravenous
Morphine	20·0
Methadone	5·0–10·0
Pethidine	10·0
Codeine	15·0–20·0
20684	0·05

In conclusion, morphine and related drugs show only slight anti-nociceptive properties against acute mechanical stimuli in chicks, but cause postural and behavioural effects at low doses. Thus, in this species the side-effects of these drugs are more apparent than their anti-nociceptive action. The postural and other effects described appear to be characteristic of morphine and related drugs.

I thank Dr. H. O. J. Collier for his advice and encouragement, Dr. B. T. Warner for statistical advice and Ciba, Ltd., Basle, Switzerland, for a supply of 1-(β-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole.

C. Schneider

Department of Pharmacological Research,
Parke, Davis and Co.,
Hounslow, Middlesex.

[1] Selle, R., *Science*, **91**, 95 (1940).

[2] Clymer, N. V., and Seifter, J., *J. Pharmacol.*, **89**, 149 (1947).

[3] Buttle, G. A. H., and Zaimis, E. J., *J. Pharm. Pharmacol.*, **1**, 991 (1949).

[4] Zaimis, E. J., *J. Physiol.* (Lond.), **152**, 11 P (1960).

[5] Waelsch, H., *Biochemistry of the Developing Nervous System*, 196 (Academic Press, New York, 1955).

[6] Key, B. J., and Marley, E., *J. Physiol.* (Lond.), **155**, 39 P (1961).

[7] Schneider, C., *Brit. J. Pharmacol.*, **16**, 4 (1961).

[8] Collier, H. O. J., and Warner, B. T., *Brit. J. Pharmacol.* (in the press).

HÆMATOLOGY

Hæmoglobin *F* and the Genetic Control of Protein Structure

The publication of a hypothesis concerning hæmoglobin synthesis presented recently in *Nature* by Drs. Zuelzer and Robinson[1] prompts me to comment briefly. Their article concerns the genetic control of the synthesis of the peptide chains of the human hæmoglobins : A (adult) = $\alpha_2\beta_2$, F (fœtal) = $\alpha_2\gamma_2$, and A_2 (the minor adult hæmoglobin) = $\alpha_2\delta_2$. The symbols α, β, γ, δ are used to denote the peptide chains of which the hæmoglobin molecules are composed and also the genes controlling their structure. The authors propose that "the production of γ-chains is under the control of the same pair of genes which determines the formation of β-chains, once the maturation of the fœtus has reached the point where adult hæmoglobin can be made". Again, in cases of thalassæmia, for example, where there seemed to be a block in the synthesis of β-chains due to a substitution in the chain, "the simplest possible assumption seemed to be that the character or site of the substitution in the β-chain itself determined the nature of the by-product, γ or δ, accumulating in consequence of a block in the metabolic pathway of β-chain synthesis".

Zuelzer and Robinson's hypothesis suggests that the γ- and the δ-peptide chains represent alternative pathways in a synthetic mechanism which in the normal adult results in the production of β-peptide chains. The implication stated is that the alternative pathways to the γ- and δ-chains branch off from the main pathway at, respectively, earlier and later stages of assembly of the peptide chain. A peptide chain will be assembled under control of a β-gene to the stage where it contains n amino-acids, and then completed by the β-gene, if that is normal. However, if there is a mutational alteration in the β-gene (as in hæmoglobin *S*, refs. 2, 3, and 4), then a proportion of unfinished chains of length n will take the alternative pathway controlled by the γ-gene, provided the mutation in β occurs after the branch point.

The elegant work of Dintzis[5] has shown that the two types of peptide chain of rabbit hæmoglobin are made sequentially by stepwise addition beginning at the N-terminus of the chains. Bishop *et al.*[6] had previously indicated that growth of the chain begins at the N-terminus. The size of these growth steps is not clear, but it cannot be larger than a few amino-acids. The same situation presumably exists in man. In that case, and according to Zuelzer and Robinson[1], the peptide chain grows from the N-terminal valine residue until it has reached the nth amino-acid. Growth continues to completion and a normal β-chain results, if the β-gene is normal and the person is an adult. However, in the case of a sickle-cell homozygote, who is abnormal in both his β-genes, a large amount of fœtal hæmoglobin, that is, of γ-chain, is made. In this case[1], growth after the nth amino-acid has been added continues along the alternative pathway under γ-gene control to a finished γ-chain, which is a γ-chain normal in its amino-acid sequence. Here the hypothesis is in difficulties on two counts; first, since we know[4] that the sickle-cell abnormality of the β-gene alters amino-acid number 6 in the β-chain (glutamic acid to valine), the number n, which is supposedly the common sequence between β and γ, must be less than 6. This is a small proportion of the total of 146 amino-acids[7]; nearly 96 per cent of the β- and γ-genes must be separate and must be operating independently, according to Zuelzer and Robinson. Secondly, the N-termini of the β- and γ-peptide chains are reminiscent of one another in sequence, but by no means identical: β-chain = Val. His. Leu. Thr. Pro. Glu. Glu. Lys. Ser....; γ-chain = Gly. His. Phe. Thr. Glu. Glu. Asp. Lys. Ser. ... . Therefore, the number n, the common structural sequence, must be zero. In other words, the whole of the β- and the γ-genes are independent and separate, at least in so far as their function in producing separate and independent β- and γ-polypeptide chains is concerned, in contrast to Zuelzer and Robinson's hypothesis. The idea that the α-, β-, γ- and δ-chains are produced by four genes which are separate has been discussed by several investigators (summarized in ref. 8).

The δ-gene, according to Zuelzer and Robinson[1], takes over at a later stage in synthesis where m amino-acids are already in the chain ($m>n$). From our knowledge of the δ-peptide chain, m must be smaller than 9 amino-acid residues, since Stretton[9] has shown that the β- and δ-chains are probably similar in sequence up to position 9, where instead of a serine residue (β-chain) he finds a threonine. However, sickle-cell homozygotes produce only normal δ-chains and therefore m, the sequence of amino-acids common to β- and δ-chains, must be less than 6 residues. Therefore, at least 96 per cent of β- and δ-genes are separate genes, producing separately controlled peptide chains.

Although the basic concepts of Zuelzer and Robinson's hypothesis are untenable, at least in so far as they can be tested in the human hæmoglobins, it must be admitted that the presence of considerable quantities of fœtal hæmoglobin in their cases of thalassæmia minor is somewhat puzzling. Thalassæmia is a disease occurring in different forms, for which we have at least two hypotheses[10], but perhaps not yet the definitive explanations. However, it seems likely that most of the phenotypes found in the thalassæmias and other hæmoglobinopathies can be explained by the function and malfunctioning, and by the interaction, of the alleles of the α-, β-, γ- and δ-genes at present recognized. These genes are conceived as consisting of four separate genes controlling the synthesis of the four chemically recognizable hæmoglobin chains[8]. Where there is a drastic curtailment or block of the synthesis of one of the peptide chains, it seems to me more fruitful, and usually adequate, to consider consequent increases in the production of other peptide chains as a process of compensation; perhaps this is brought about by a change in the control mechanism which normally regulates the amounts of peptide chain made by each gene.

Note added in proof: The recent work of Schroeder, W. A., *et al.* (*Proc. U.S. Nat. Acad. Sci.*, 47, 811; 1961, and unpublished experiments) on the amino-acid sequences of the γ-peptide chain throws more light on the degree of similarity between the structure of the β- and γ-chains. The differences between them are fairly well spread along their lengths, and therefore regions of similarity are likely to be short and also scattered. In particular, at the C-termini of the β- and γ-chains only the last two amino-acids are the same, -Tyr.His, with dissimilar sequences before these. Therefore, any similarity between the β- and γ-chain genes at the ends corresponding to the C-termini of the peptide chains would have to be confined to a negligibly small proportion of the length of the genes.

V. M. INGRAM

Department of Biology,
Massachusetts Institute of
Technology,
Cambridge, 39.

[1] Zuelzer, W. W., and Robinson, A. R., *Nature*, **190**, 237 (1961).

[2] Neel, J. V., *Science*, **110**, 64 (1949).

[3] Pauling, L., Itano, H. A., Singer, S. J., and Wells, I. C., *Science*, **110**, 543 (1949).

[4] Hunt, J. A., and Ingram, V. M., *CIBA Symp. Biochem. Human Genetics*, 114 (1959).

[5] Dintzis, H. M., *Proc. Nat. Acad. Sci.*, **47**, 247 (1961).

[6] Bishop, J., Leaky, J., and Schweet, R., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1030 (1960).

[7] Braunitzer, G., Hilschmann, N., Hilse, K., Liebold, B., and Muller R., *Z. physiol. Chem.*, **322**, 96 (1960), and unpublished work.

[8] Ingram, V. M., in *Genetics*, Macy Found. Symp., edit. by Sutton, H. Eldon (1960).

[9] Ingram, V. M., and Stretton, A. O. W., *Nature*, **190**, 1079 (1961).

[10] Ingram, V. M., and Stretton, A. O. W., *Nature*, **184**, 1903 (1959).

THE basic assumption of our concept[1] of a common metabolic pathway with different end-products depending on the nature or location of substitutions within the hæmoglobin molecule is not the direction in which the synthesis of the polypeptide chain may proceed, but the very premise itself—that the build-up occurs in a stepwise fashion. This is the all-important conclusion which we reached on purely deductive grounds on the basis of the observation that mutations apparently involving β-chain synthesis, for example, the thalassæmias observed by us, could selectively determine whether an excess of γ- or δ-chains is formed.

At the time when we formulated this hypothesis, the concept of a stepwise build-up was a rather radical departure from the then prevalent ideas on the mode of operation of genetic templates. The almost immediate verification of this, our basic premise, by the work of Bishop[2], and that of Dintzis[3], which Ingram quotes, would seem to strengthen rather than weaken our hypothesis. Since no information was available at the time concerning the direction of the build-up, we refrained from making any explicit assumptions on this point. It is true that one can read the implication into our communication that we were suggesting the build-up of the molecule towards, rather than away from, the N-terminal. We were considering the alternative, but had no basis for a choice. We were interested rather in describing our concept in the simplest possible terms, and did not wish to confuse the reader by an alternative which was equally likely but would not materially affect the validity of the basic concept.

We are in complete agreement with Ingram's reasoning but not with his corollary. Now that it is known that the stepwise build-up in the formation of the molecule is at the N-terminal at least for rabbit, and very likely for human, hæmoglobin, it is clear that the correct model must involve convergence toward a common pathway a relatively short distance from the N-terminal, rather than divergence from the common pathway at that same point. It becomes simply a matter of reversing direction now that the necessary information is at hand. It is just as easily conceivable that, given a substitution near the N-terminal, the first few amino-acids are combined into an alternate pattern (for example, γ-chain type), from which the rest of the chain can arise in a normal manner along the common pathway, as it is to assume the opposite type of process. To that extent the hypothesis can now be made explicit. Instead of the 'branching-off point', we must speak of a 'point of convergence'.

Alternate explanations regarding the control of synthesis of hæmoglobin F are not particularly satisfying, as Ingram admits. It is, however, necessary for genetic theory to satisfy not only the structural relationships within the molecule, which no doubt will be clarified one way or the other in the near future, but also a large body of other pertinent observations. The theory of separate γ-genes runs into difficulties with respect to three sets of acts which can be reconciled with our present hypothesis.

One concerns the observations on thalassæmia minor with the stigmata of a β-gene mutation and divergent behaviour with respect to hæmoglobins F and A_2 which formed the basis of our theory[4]. The second is the increasingly well-documented allelism of the high F-gene with known β-genes such as S and C. Evidence from another laboratory will be forthcoming shortly to the effect that the γ-chains of high F carriers are identical in structure with those of normal fœtal hæmoglobin and that the high F in these individuals is evenly distributed throughout the red-cell population. This seems to us grounds for treating γ-chains as mutant β-chains under the control of the β-gene.

The third point is the uneven distribution of fœtal hæmoglobin in the red-cell population in various genetic and acquired disorders, for example, sickle cell anæmia, thalassæmia minor and leukæmia[5,6]. The concentration of hæmoglobin F in a few, rather than in all, cells suggests metabolic influences not under direct genetic control.

We are not attempting to defend at all costs a hypothesis which is, after all, a mere heuristic principle; but we think it is somewhat radical and premature to discard it on the basis of interpreting a relatively minor and peripheral implication as the substance of the argument.

Wolf W. Zuelzer
Abner R. Robinson

The Child Research Center of Michigan,
660 Frederick Street,
Detroit 2, Michigan.

[1] Zuelzer, W. W., and Robinson, A. R., *Nature*, **190**, 237 (1961).

[2] Bishop, J., Leaky, J., and Schweet, R., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1030 (1960).

[3] Dintzis, H. H., *Proc. U.S. Nat. Acad. Sci.*, **47**, 247 (1961).

[4] Zuelzer, W. W., Robinson, A. R., and Booker, C. R., *Blood*, **17**, 393 (1961).

[5] Betke, K., *Folia Haematol.*, **76**, 293 (1959).

[6] Coehn, F., and Zuelzer, W. W., *Thirty-first Ann. Meeting, Soc. Ped. Res.* (to be published).

PATHOLOGY

Immunological Study of experimentally induced Extrahepatic Cholestasis

Cohen *et al.*[1] have shown, by means of an immuno-cyto-chemical-fluorescence method, that gamma-globulin is present in the reticulo-endothelial cells of the sinusoids and fibrous tracts of human livers affected by hepatitis of various types and by post-necrotic cirrhosis. To test, in some measure, the unitary concept of liver disease put forward by Shorter *et al.*[2], it was decided to investigate whether similar changes occurred in livers damaged experimentally by the primarily extrinsic method of ligation of the common bile duct.

Rabbits of either sex and no particular breed but of 2 kgm. weight were used for the experiments. Under intravenous nembutal (Abbott) anæsthesia the common bile duct was exposed, ligated near its middle at two points separated by approximately 1 cm. and the intervening part excised. Penicillin powder was dusted over the operation site and the edges of the abdominal wound. Four groups of six animals were so treated and to each group was added a further control animal, which was subjected to exposure of the common bile duct but without ligation and excision of the latter. There were no operation deaths, and, apart from a small stitch abscess in the skin of one animal, no sepsis occurred.

The four groups, including their controls, were killed respectively at 3, 7, 14 and 28 days after operation and with each group a normal animal was also killed. The animals were killed by the intravenous injection of overdoses of nembutal. They were dissected immediately and portions of the right lobe of the liver and of the spleen were removed, frozen rapidly in a dry ice/alcohol mixture at −70° C. and stored at −30° C. while waiting to be sectioned.

Sections 6μ-thick were cut in a cryostat, exposed to a current of warm air (45° C.) for about 10 sec. and then washed for 5 min. in each of three changes of saline which was phosphate-buffered to pH 7·0. Each section was then treated for 30 min. with fluorescein-labelled anti-rabbit globulin of the sheep (Sylvana Chem. Co., New Jersey), washed as before in phosphate-buffered saline and mounted in glycerin. Immunological specificity was controlled by the treatment of two further batches of section either with non-conjugated sheep anti-rabbit globulin (Sylvana

Chem. Co.) or with normal sheep globulin (Wellcome Research Lab.) for 30 min., and, later, after washing in phosphate-buffered saline, with fluorescein-labelled sheep anti-rabbit globulin as before. These sections were also mounted in glycerin.

The sections prepared by both methods were examined immediately by fluorescent microscopy. The results were recorded by the method adopted by Cohen *et al.*, that is, the cells containing gamma-globulin were counted in each of at least three high-power (×40 objective; ×6 eyepiece) fields and the average for one field expressed thus: + = 2–3 cells; ++ = 4–6 cells; +++ = 7–9 cells; and ++++ = 10 or more cells.

In addition, sections from two experimental and both control animals in each group were prepared as above and then stained with hæmatoxylin and eosin; these sections were examined by bright-field microscopy.

Bright-field examination of the livers of the experimental animals in the first group, killed three days after operation, revealed marked accumulation of bile pigment in the hepatic parenchymal cells and bile ductules. Together with this cholestasis there were numerous foci of liver-cell necrosis, generalized enlargement of Kupffer cells, slight histiocytic and fibroblastic reactions in all the portal tracts and patchy proliferation of bile ductules. These changes were unaccompanied by any abnormality of the lobular architecture. The changes found in the second, third and fourth groups, examined 7, 14 and 28 days respectively after operation, were similar in nature and degree, with the exception of the fibroblastic reaction, to those present in the first group. In the later groups the fibroblastic reaction was greater and had also produced short collagenous projections from the portal tracts into the adjacent damaged parenchyma though without significant disturbance of the lobular pattern. In no case was there histological evidence of infection.

Gamma-globulin was found in cells lining the hepatic sinusoids of all the experimental animals of each group, but none was present in the portal tracts or elsewhere in the livers of these animals and there was none at any site in the livers of the control animals. The number of cells containing gamma-globulin increased with increase of the duration of cholestasis. The results are summarized in Table 1. Intracellular gamma-globulin was present in similar amounts in the splenic red pulp of the experimental and control animals of all the groups.

The sinusoidal cells in which the gamma-globulin was found appeared, on histological grounds, to be Kupffer cells. In no cases in the present experiments have these cells shown morphological changes indicating transition to plasma-cells. Although it is impossible to assess whether these cells elaborated or merely took up the gamma-globulin, the latter seems to be more likely in view of the enlargement of the cells, their known phagocytic powers and the lack of histological changes suggesting transition to plasma-cells.

The significance of these findings is obscure. It must be mentioned, however, that Havens[3] has conjectured that some forms of liver disease may be initiated or perpetuated by the formation of antibodies to the products of liver-cell necrosis, but whether this is the explanation of the liver damage which follows the experimental induction of extra-hepatic cholestasis must await the results of further investigations. On the whole, in agreement with Cohen *et al.*, it seems doubtful that the increase in hepatic gamma-globulin found in some diseases of the liver indicates the presence of any immunological process.

Table 1. CELLS CONTAINING GAMMA-GLOBULIN IN THE LIVER IN EXPERIMENTALLY PRODUCED EXTRA-HEPATIC CHOLESTASIS

Time interval in days	Groups	No. of animals	Liver	
			Cells containing gamma-globulin in portal tracts	Cells containing gamma-globulin lining sinusoids
3	Animals with ligated bile ducts	6	0	+
	Control animals	2	0	0
7	Animals with ligated bile ducts	6	0	+ to ++
	Control animals	2	0	0
14	Animals with ligated bile ducts	6	0	+ to +++
	Control animals	2	0	0
28	Animals with ligated bile ducts	6	0	+++ to +++
	Control animals	2	0	0

Finally, it seems that these results do not oppose a unitary concept of liver disease as a whole, since a change found in a wide variety of diseases of the liver, namely, the appearance of gamma-globulin in reticulo-endothelial cells lining sinusoids, has also occurred in uncomplicated experimentally produced extra-hepatic cholestasis.

ROY G. SHORTER
R. G. LACK

Department of Pathology,
St. Thomas's Hospital Medical School,
London, S.E.1.

[1] Cohen, S., Ohta, G., Singer, E. J., and Popper, H., *J. Exp. Med.*, 111, 285 (1960).

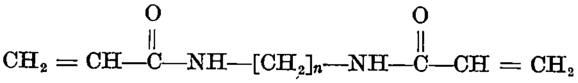
[2] Shorter, R. G., Paton, A., and Pinniger, J. L., *Quart. J. Med.*, 28, 43 (1959).

[3] Havens, W. P., *Internat. Arch. Allergy and App. Immunol.* (Basle), 14, 75 (1959).

N,N′-Alkylene-*bis*(Acrylamides), N-(Acrylamidomethyl)-3-Halopropionamides and Related Compounds: a New Series of Anti-tumour Agents

THE chemical, biological and clinical aspects of the 'alkylating agents' as anti-tumour agents have been reviewed thoroughly[1,2]. The available data indicate that at least two minimal requirements are necessary for possible alkylating agents[2]: (1) Such compounds must either have a certain chemical reactivity, or be converted *in vivo* to structures that do; (2) although polyfunctionality is not a rigid requirement for activity, the most active alkylating agents are polyfunctional. Functional groups with chemical reactivity are usually defined as those able to react with nucleophilic groups such as amino and mercapto, and the anions of carboxylic and phosphoric acids.

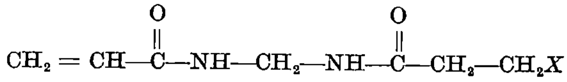
In the course of syntheses of compounds with potential anti-tumour activity, N,N′-methylene*bis*-acrylamide[3] [I, $n = 1$] was submitted for screening and found to inhibit markedly the growth of two transplanted mouse tumours (specific details later in this communication). Chemically, N,N′-methylene*bis*acrylamide fulfils the requirements for an active alkylating agent, since both α,β-unsaturated linkages can easily add mercaptans or amines[4]. Inasmuch as this structure represented a new type of anti-tumour agent, an effort was undertaken to define more precisely the structural characteristics necessary for anti-tumour activity. As a result, compounds of three related though distinct structure types were found to be active against at least one transplanted mouse tumour. The general structures are as follows:



$$CH_2=CH-\overset{\overset{O}{\|}}{C}-NH-[CH_2]_n-NH-\overset{\overset{O}{\|}}{C}-CH=CH_2$$

I

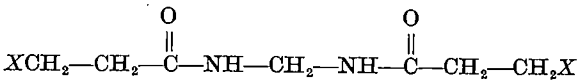
$n = 1,2,3,6,7$



$$CH_2=CH-\overset{\overset{O}{\|}}{C}-NH-CH_2-NH-\overset{\overset{O}{\|}}{C}-CH_2-CH_2X$$

II

$X = Cl,Br,I$



$$XCH_2-CH_2-\overset{\overset{O}{\|}}{C}-NH-CH_2-NH-\overset{\overset{O}{\|}}{C}-CH_2-CH_2X$$

III

$X = Br,I$

Except for the compound with $n = 1$, the compounds of type I were obtained by the action of acrylyl chloride or acrylic anhydride on the appropriate α,ω-alkylenediamine. The compound where $n = 1$ was obtained by the action of formaldehyde on two moles of acrylamide[3]. The compounds of type II were obtained by the action of N-(hydroxymethyl)acrylamide on the appropriate 3-halopropionamide in the presence of an acidic catalyst. The compounds of type III were obtained by the action of formaldehyde on two moles of the appropriate 3-halopropionamide.

Compounds of type I wherein one or both acrylamide residues were replaced by methacrylamide (one) or β,γ-buteneamide residues (one and two) were also found to possess anti-tumour activity, while crotonamide, cinnamamide and sorbamide residues gave inactive compounds. The full chemical details on all the above compounds, as well as on numerous other inactive compounds, will be published elsewhere.

In our Laboratories, compounds are screened for anti-tumour activity against a spectrum of three transplanted tumours in *C3H* mice: sarcoma 180, 6*C3HED* lymphosarcoma, and *C3H* mammary adenocarcinoma. The details of the screening procedure have been published[5]. All the active compounds described before inhibited the growth of the *C3H* mammary adenocarcinoma. In addition, N,N′-methylene*bis*acrylamide inhibited the sarcoma 180, and N,N′-ethylene*bis*acrylamide [I, $n = 2$] inhibited the 6*C3HED* lymphosarcoma. One compound, N-(acrylamidomethyl)-3-bromopropionamide [II, X = Br], inhibited all three screening tumours. Evaluation studies showed that this compound was effective against all three tumours by oral administration as well as by intraperitoneal injection. It prolonged significantly the survival-time of mice bearing the transplanted *C3H* mammary adenocarcinoma, but was ineffective against 'spontaneous' mammary adenocarcinomas. In common with the alkylating agents, N-(acrylamidomethyl)-3-bromopropionamide acts as a bone marrow depressant[6]. Detailed results of the screening and evaluation studies will be published elsewhere.

N-(Acrylamidomethyl)-3-bromopropionamide is now undergoing limited preliminary clinical trials.

A. S. TOMCUFCIK
S. D. WILLSON
Organic Chemical Research Section,

A. W. VOGEL
A. SLOBODA
Experimental Therapeutics Research Section,
Lederle Laboratories Division,
American Cyanamid Co.,
Pearl River, New York.

[1] Schmidt, L. H., *Ann. N.Y. Acad. Sci.*, **68**, 657 (1958).
[2] Montgomery, J. A., *Cancer Res.*, **19**, 447 (1959).
[3] Lundberg, L. A., *U.S. Pat.* 2,475,846; *Chem. Abstr.*, **44**, 873 (1950).
[4] Dalton, P. B., *U.S. Patents* 2,576,501–2; *Chem. Abstr.*, **46**, 1809 (1952).
[5] Dearborn, E. H., *Acta Unio Intern. Contra Cancrum*, **15**, No. 1, 76 (1959).
[6] Vogel, A. W. (to be published).

Two Phycomycetes isolated from Subcutaneous Phycomycosis in Uganda

In 1956, Lie Kian Joe *et al.*[1] reported two cases of a curious subcutaneous granuloma in children in Indonesia. The tissues showed a characteristic histological picture in the midst of which lay fungus filaments with occasional septa. From one case they isolated many colonies of a phycomycete, *Basidiobolus*, not hitherto known to infect mammals. Later this group of workers has reported three more cases[2]; *Basidiobolus* was isolated from two of them and an unidentified phycomycete from the third. A sixth case, probably infected in Indonesia, has been recognized by biopsy examination in England[3].

The general term 'phycomycosis' was suggested for diseases caused by members of the Phycomyces[4] to avoid the restricted connotation of mucormycosis and to cover cases in which a phycomycete was demonstrated by histology or culture, but closer identification was not possible.

A chronic progressive subcutaneous granuloma affecting only children and producing a characteristic picture on biopsy examination has been recognized in Uganda for many years[5]. It is locally known as 'creeping granuloma' and, though never proved, it has been regarded as most likely due to some helminthic infection largely because of the small 'tunnels' in the sections and the heavy infiltration of eosinophil cells around them. Jelliffe *et al.*[6], however, concluded that their patient with 'creeping granuloma' was suffering from a mycotic infection which they recognized as the subcutaneous phycomycosis of Lie Kian Joe.

About the same time, and unaware of the previous work, I also had come to the conclusion from a study of a series of biopsies that 'creeping granuloma' was a mycotic infection, and was afterwards able to isolate *Basidiobolus ranarum* from the next case presenting with this condition. Prof. Lie has seen the biopsy from this patient and agrees that the picture is identical with that of the Indonesian cases. The identity of the 'creeping granuloma' of Uganda and the subcutaneous phycomycosis of Indonesia therefore seems clear.

In connexion with the isolation of *Basidiobolus* in Uganda (the first from human tissue outside Indonesia), it is of great interest to note that the report referred to in ref. 5 is not accurate where it says a fungus had been sought in one case without result. A fungus was isolated from this case and it was identified by the Mycological Reference Laboratory at the London School of Hygiene as a member of the *Entomophthora* group, but as no connexion was then known between any human disease and such a phycomycete, it was considered of no significance. The records at that Laboratory have been consulted and confirm that the fungus was a phycomycete of the order Entomophthorales, probably an *Entomophthora* but certainly not a *Basidiobolus*. The evidence both from Indonesia and Uganda thus suggests that subcutaneous phycomycosis may be caused by more than one kind of phycomycete.

There is as yet no evidence either from Indonesia or Uganda of the way in which a fungus such as *Basidiobolus* is transferred from its normal habitat in the intestinal contents of frogs and beetles to the subcutaneous tissue of children, and this is now being investigated.

A. M. M. Wilson

Department of Pathology,
Makerere College Medical School,
Kampala, Uganda.

[1] Lie Kian Joe, Njo-Injo Tjoei Eng, Sutomo Tjokronegoro and Emmons, C. W., *Arch. Derm. Syph.*, **74**, 378 (1956).

[2] Lie Kian Joe and Njo-Injo Tjoei Eng, *Ann. N.Y. Acad. Sci.*, **89**, 4 (1960).

[3] Symmers, W. St. C., *Brit. Med. J.*, i, 1331 (1960).

[4] Lie Kian Joe, Njo-Injo Tjoei Eng, Sutomo Tjokronegoro, Schaafsma, S. J., and Emmons, C. W., *Amer. J. Clin. Path.*, **32**, 62 (1959).

[5] Proc. Mulago Staff Clin. Meeting, *E. Afr. Med. J.*, **33**, 365 (1956).

[6] Jelliffe, D. B., Burkitt, D. P., O'Connor, G. T., and Beaver, P. C., *J. Pediat.* (in the press).

BIOLOGY

Correlative Inhibition of Formation of Chlorophyll in the Leaves of Etiolated Pea Plants

Seventeen-day-old pea plants (*Pisum sativum* L., variety Přebohatý) grown in the dark were decapitated above the different nodes as illustrated by horizontal bars in Fig. 1. The decapitated plants and the isolated sections of the stems were placed in a window of the laboratory, left there overnight and on the next day exposed to daylight. After 24 hr. the chlorophyll formation in the individual organs was visually evaluated by means of a standard colour scale.

Fig. 1 shows that in intact control plants (*a*) the young leaves of the apex (*Aa*) and its region of elongation had become intensively green. The isolated stem tips (*Ab* ; *Af*) had formed chlorophyll to a lesser extent. In the decapitated plants chlorophyll formation took place mainly in the morphologically highest organ (*L2b* ; *L1c* ; *S2d*). Such organs and even the organs on isolated stem sections (*L2f*; *L1f*) usually formed more chlorophyll than the corresponding organs on intact plants (*L2a*; *L1a*) or on stem sections with the stem tip intact (*L2d*; *L1d*). The experiment clearly shows that in leaves chlorophyll formation is inhibited even in the absence of the apex if there is a morphologically higher, that is, younger node present.

In the presence of the basal parts, on the contrary, chlorophyll formation (compare *S2d* with *S2f* or *Ae* with *Af*) is stimulated. Individual organs differ in their capability to form chlorophyll according to their age if the inhibiting apical parts are removed as may be seen by comparing *L2b*, *L1c*, *S2d* and *S1e*. The fact that inhibition in the presence of apical parts is expressed not only in the control of the growth of lateral buds but also in the control of chlorophyll formation in the leaves and scales stresses the necessity to look on the plant as a well-co-ordinated whole[1,2,8]. The present concept of apical dominance[8] emphasizes the importance of the stem tip controlling directly or indirectly the growth of lateral buds. If the totality of the plant is to be taken into consideration it seems necessary to extend this concept to the active function of the transport system[3-5,7] in the theory of correlations until now somewhat underestimated.

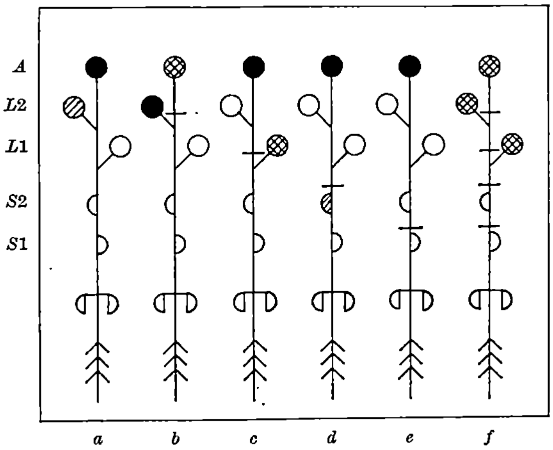


Fig. 1. Chlorophyll formation in the leaves and scales of etiolated plants and isolated stem sections of *Pisum sativum* after exposure to daylight for 1 day

In a normal etiolated pea plant it is the metabolic activity of the apex, driving the energy-requiring processes, that maintains the material, metabolic and electric gradients. These gradients in turn render possible a continuous flow of material to the apex, acting, with respect to this flow, as a metabolic sink or a centre of attraction. In such a process, positive[7] and negative[6] feedback mechanisms take part; auxins, gibberellins, kinetins and inhibitors together with their precursors represent probably important components of such systems.

If the transport, at least of some of the necessary material, by the conducting system is an active process, the 'pull of material'[5] by the conductive system continues even after the removal of the stem tip, so that one can speak about a 'memory' or 'inertia' of the system. This state probably has a more-or-less transient character; but it is supposed to play a decisive part in the first phases following the removal of the apex. Then, in analogy to a suddenly closed pipe line with an excessive pressure arising, the pulled material starts to flow to organs that were not reached before, partly due to resistances encountered.

The differences depending on age in chlorophyll formation by the leaves and scales are probably due to differences in such resistances of metabolic or mechanical character. It is assumed that similar relations participate in a variety of other phenomena studied by experimental morphology, such as the differences in mass production by the marginal buds of *Bryophyllum crenatum* leaves[2] or even the classical case of the cotyledonary bud inhibited by its cotyledon[2].

Apical dominance and similar phenomena are considered as special cases of metabolic correlations representing the basic principle of the totality of plant organism.

I wish to express my thanks to Prof. Rudolf Dostál for stimulating discussion.

Jiří Úlehla

Švancarova 35,
Brno.

[1] Čaljachjan, M. Ch., *Celostnost organizma v rastitelnom mire* (Jerevan, 1955).
[2] Dostál, R., *O celistvosti rostlin* (Prague, 1959).
[3] Fensom, D. S., *Canad. J. Bot.*, **37**, 1003 (1959).
[4] Kursanov, A. L., Brovchenko, M. I., and Pariskaya, A. N., *Fiziologia Rastenii*, **6**, 527 (1959).
[5] Nelsòn, C. D., and Gorham, P. R., *Canad. J. Bot.*, **37**, 439 (1959).
[6] Scott, B. I. H., *Austral. J. Biol. Sci.*, **10**, 164 (1957).
[7] Spanner, D. C., *J. Exp. Bot.*, **9**, 332 (1958).
[8] Thimann, K. V., *Rapports et Communications* 8e *Congr. Intern. Bot.*, Sect. 11, 114 (Paris, 1954).

Flower Initiation in the Carnation in Response to Photoperiod

In 1932, Laurie and Poesch[1] reported that for glasshouse carnation an increase in photoperiod during the winter months led to earlier production of flowers in the spring. There have been a number of similar reports since, but views have differed as to whether photoperiod affects the time of flower initiation or only the rate of subsequent development of the flower. Post[2] and more recently Rünger[3] have claimed that only the latter stage of development is affected. Blake[4], however, working in the laboratories here, demonstrated an effect on flower initiation such that plants grown in short days of 8 hr. had more leaves below the flower than plants grown in long days where an additional 9 hr. of illumination was given. The object of the present communication is to report results from three glasshouse experiments which show unequivocally that time of flower initiation in carnation is affected by photoperiod and that this effect can occur independently of the total radiation received by the plant.

In experiments 1 and 2, plants were grown from normal shoot cuttings. These were cut back about one month after planting and one axillary shoot was allowed to develop. In experiment 3, plants were grown from 'leaf-bud cuttings' comprising an axillary bud, its subtending leaf and a small portion of stem. The variety White Sim was used throughout. The basic treatment was 8 hr. of natural daylight (short day). In the other two treatments this was supplemented by 2 hr. of light from 'daylight' fluorescent tubes plus tungsten filament lamps, given either as 1 hr. at either end of the day (extended day) or as 2 hr. at the middle of the dark period (interrupted night). In each of the three experiments sampling of the plants and dissection of their apices showed that flowers had been initiated in the 'interrupted night' treatment, while plants in the other treatments were still vegetative (Table 1).

Table 1

Experiment No.	Date of planting	Date of sampling	No. of plants with flower primordia out of six sampled		
			Short day	Extended day	Interrupted night
1	28.1.60	26. 5.60	None	None	5
2	15.6.60	12.10.60	None	None	6
3	23.4.60	6.10.60	None	None	3

Further treatments in the experiments quoted, and further experiments now in progress here indicate that a number of factors can interact with photoperiod in effects on flower initiation. An understanding of these factors is desirable in considering any possible applications of photoperiodic treatments to the growing of carnations for cut flowers.

We wish to express our appreciation to the Agricultural Research Council, which has made a grant to support this work.

G. P. Harris
Juliet E. Griffin

University of Reading
Horticultural Research Laboratories,
Shinfield Grange,
Shinfield, Berks.

[1] Laurie, A., and Poesch, G. H., *Ohio Agric. Exp. Sta. Bull.*, 512 (1932).
[2] Post, K., *Cornell Univ. Agric. Exp. Sta. Bull.*, 787 (1942).
[3] Rünger, W., *Licht und Temperatur im Zierpflanzenbau* (Paul Parey, 1957).
[4] Blake, J., *Rep. Fourteenth Int. Hort. Congr. Netherlands*, 331 (1955).

Measurement of Leaf Colour

Leaf colour is a valuable index of nutrient status in flowering plants. Yellowing of the leaves is associated with deficiencies of nitrogen, magnesium, iron, manganese, zinc and molybdenum in many species.

The severity of leaf chlorosis has been recorded by subjective description, by colour photography and by estimation of the chlorophyll content. Chlorophyll estimations yield unambiguous results and have proved most satisfactory in experiments requiring a high degree of accuracy.

In many investigations it is desirable to measure the changes in colour which occur during maturation of the leaf. Chlorophyll estimations involve the destruction of the leaf, and, in consequence, large-scale experiments must be carried out in order to provide an adequate replication in each sample of leaves. As an alternative to this rather laborious procedure, a system of colour matching suitable for attached leaves has been adopted in recent experiments on lime-induced chlorosis, carried out at Sheffield.

A comparator has been made. The colours were prepared by mixing oil paints, which were applied to squares of absorbent paper (thick filter paper is suitable). The colours were arranged in series and matched against the Ridgway[1] colour standards (Table 1). Through the colour series there is a regular change in spectral composition. In order to match chlorotic leaves more easily (they are often bleached in addition to being yellow in colour) the colours at the yellow end of the comparator are of lower intensity.

Colour matching is carried out under standard conditions of white lighting provided by a Siemens-Ediswan Daylight Blue 60-W. lamp. Where leaves show an intervenal chlorosis, the colour matched is that of the intervenal areas.

The error involved in matching leaf colours varies according to the characteristics of the leaf. Species with waxy or glossy leaves are the most difficult subjects. In *Lathyrus montanus* and *Betonica officinalis*, the leaves of which have a matt surface, the error was found not to exceed 5 per cent in the 10–25 range of the colour series and 3 per cent in the 26–33 range.

Even in the standardized environment of a growth cabinet, the rates of leaf appearance in a population of seedlings germinated at the same time are not synchronous. For this reason, colour-values determined at fixed intervals of time after germination are not comparable. Trials have shown, however, that development of colour in successive leaves can be measured satisfactorily if the timing of the determinations is based on a biological unit of time. The unit is that occurring between the appearance of two successive leaves at the same growing point, that is, in each seedling, the colour of each foliage leaf is measured when its successor first appears and afterwards, at intervals determined by the appearance of successive leaves, at the same growing point. Provided that sufficient records are obtained and the behaviour of the seedlings is fairly uniform with respect to leaf colour, a series of curves or histograms may be constructed to represent the development of leaf colour during the seedling phase (Fig. 1). Where the primary shoot is non-persistent, being replaced by one or more secondary shoots soon after germination, the source of comparable data is limited. The system of colour-matching is unsuitable for grasses because growth is not monopodial and the leaf is not a unit of limited growth as it is in a dicotyledon.

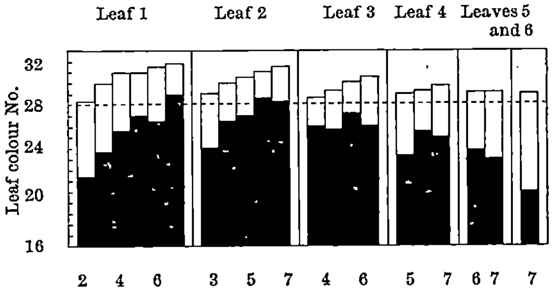


Fig. 1. Leaf colour measurements to show the onset of a permanent lime-induced chlorosis in seedlings of *Betonica officinalis*. Critical boundary line at colour number 28. □, On nutrient-sufficient brown earth; ■, on calcareous soil.

Table 1. Colour Series used in the Leaf Colour Comparator. Inset is the Range sufficient for most Species

Colour No.	Ridgway colour No.	Name of colour
1 2 3	23*B*	Pale lemon yellow
4 5 6	25*B*	Light greenish yellow
7 8	25*I*	Oil yellow
9	27*I*	Javel green
10		
11 12	29*I*	Cosse green
13 14 15	29*K*	Lettuce green
16 17 18	31*I*	Yellow green
19 20 21	31*K*	Parrot green
22 23	31*M*	Cedar green
24 25 26 27 28	33*I*	Scheele's green
29 30 31 32	34*K*	Grass green
33		
34 35	35*K*	Meadow green
36 37 38	35*M*	Antique green
39	37*M*	Diamine green
40 41	39*M*	Anthracine green
42	41*M*	Myrtle green

In comparative work on lime-induced chlorosis it has been found possible to construct, for each species, a critical boundary-line above which lie the colour development curves from control seedlings on a non-calcareous soil having sufficient nutrients. Values which fall below the boundary indicate some degree of chlorosis. The position of the boundary line varies according to the species, being at 25–26 in *Rumex acetosa*, 26–27 in *Betonica officinalis* and 30–31 in *Trifolium medium*.

Interspecific comparisons may be made in seedlings by examining the timing and magnitude of disparencies between the colour data for leaves of the experimental and control plants. A convenient method of summarizing this type of information is illustrated in Fig. 2.

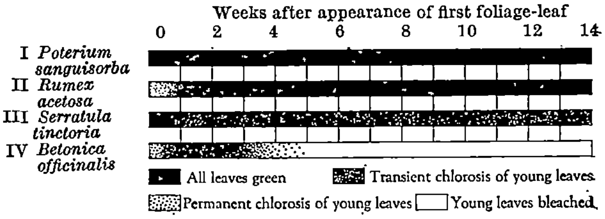


Fig. 2. The pattern of colour development in seedlings of four species growing on a calcareous soil. I, No chlorosis; II, transitory chlorosis; III, persistent, non-progressive chlorosis; IV, progressive chlorosis

The method is sensitive to the blue-leaf effects which are associated with an abundant supply of nitrogen. The comparator may not be used to follow the changes in colour in senescent leaves, as these are essentially changes in intensity and may be paralleled by the accumulation of masking piments.

J. Philip Grime

Department of Botany,
University of Sheffield.

---

[1] Ridgway, R., *Colour Standards and Nomenclature* (Washington, D.C., 1912).

Non-Lethal Pathological Infection of Roots

Rovira and Bowen[1] reported reduction in root elongation and in root-hair development following the addition of pasture soil suspension to seedlings of clover, tomato, *Phalaris tuberosa* and *Pinus radiata* raised in sterile agar or sand culture. They suggested the possibility of a complex interaction between plants and micro-organisms. Since there was no observable effect on the plant tops in their experiments, the reduced absorbing surface of the roots was apparently

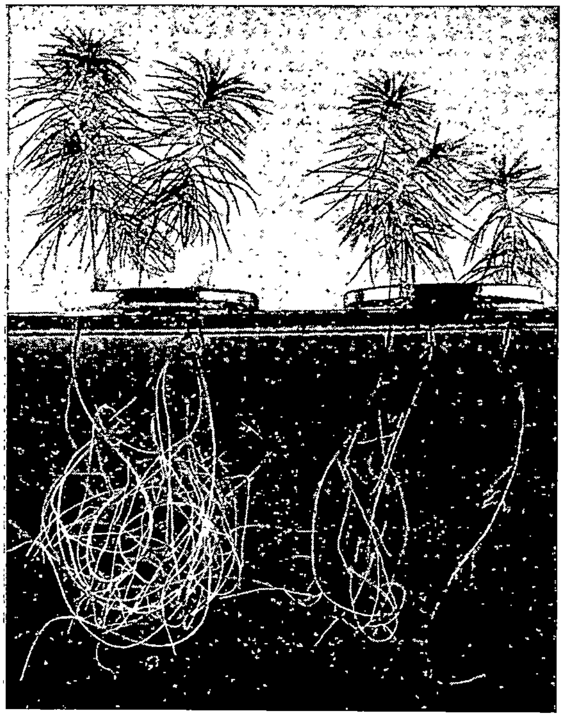


Fig. 1. Uninoculated control seedlings of *P. radiata* on left; plants on right are taken from liquid culture jar inoculated with *Phytophthora* sp. three months before

offset by the 'luxury' amounts of plant nutrients used.

While continuing investigations on a disease of *P. radiata* caused by *Phytophthora* spp. in New Zealand[2,3], some inoculation results were obtained in which many infected roots were drastically retarded without being killed. While the phenomenon recorded by Rovira and Bowen may have been similar to the stunting of roots and shoots without actual infection as reported by Kerr[4], it may have been due to the condition described here.

Newly germinated seedlings of *P. radiata* were grown in aerated culture solution for three months in a glasshouse. They were then inoculated by adding zoospores of a *Phytophthora* sp. which is widespread in pumice soil supporting a large 30–35-year-old plantation of *P. radiata*. Three weeks after inoculation, roots were found to be infected, being pale brown in colour and covered with mycelium and large numbers of sporangia. After a further three weeks many of the infected primary and secondary roots were dead, particularly in the distal portions of the root systems, but many others had resumed apical growth or were producing new lateral roots through infected cortical tissues. New root growth was very restricted by comparison with uninoculated check plants. After a further six weeks new root growth was still sparse and top growth of living plants was markedly depressed (Fig. 1). Shoot length was reduced by a third and the length of scale leaf by a fifth of that in the controls. The non-lethal infection and stunting of roots was obtained in aerated and non-aerated nutrient culture solutions or in tap water replacing the original culture solution. The pathogen was re-isolated from portions of retarded root.

Microtome sections of retarded roots showed that mycelium was confined to the cortex, being intracellular and reaching, but not penetrating, the endodermis. In dead roots mycelium had invaded all tissues, with occasional oospores occurring in the vascular cylinder.

Similar results were obtained in a further test with the same and other *Phytophthora* spp., including *P. cinnamomi* and *P. cactorum*. In some experiments infection resulted in death of complete root systems.

A significant feature of the results is that in the absence of control plants many of the diseased root systems would pass as healthy, the pale brown roots with infected cortical tissues being outwardly similar to roots undergoing normal suberization. In the field, where there are no controls to give a measure of root stunting, it is common to find rootlets with dark cortical tissues and white vascular cylinders which are still functional, judging by the possession of white elongating root tips.

Phytophthora spp. have been found to be widespread in New Zealand, in cultivated soil, exotic pine plantations and indigenous forests. If they and other soil fungi, in addition to being active pathogens on some plants, are affecting less-susceptible hosts in a manner similar to that described here, they must be regarded as constituting an important but not easily recognized biotic factor of the environment. In this role they could, for example, be of significance in some of the puzzling decline diseases in which there is an apparent lack of primary symptoms. They could also be predisposing agents in connexion with insect or fungus attack on upper parts of plants where low moisture content is a governing factor. By affecting host vigour they could influence ecological succession in natural plant communities.

F. J. Newhook

Plant Diseases Division,
Department of Scientific and Industrial Research,
Auckland,
New Zealand.

[1] Rovira, A. D., and Bowen, G. D., *Nature*, 185, 260 (1960).
[2] Newhook, F. J., *N.Z. J. Agric. Res.*, 2, 808 (1959).
[3] Sutherland, C. F., Newhook, F. J., and Levy, J., *N.Z. J. Agric. Res.*, 2, 844 (1959).
[4] Kerr, A., *Austral. J. Biol. Sci.*, 9, 45 (1956).

Wettability and the Capture, Carriage and Deposition of Particles by Raindrops

During rain the particulate matter of the atmosphere is washed to the ground, and it has been suggested that the wettability of the particles affects the efficiency with which they are picked up by the falling droplets. The rain-wash of fungal spores which are not easily wetted has been reported, however, by Gregory[1] and Hirst[2]. It was therefore decided to investigate the effect of surface wettability on the manner in which fungal spores are captured, carried and deposited by water droplets. The investigation was carried out with the simplest of apparatus and by direct observation under the microscope.

Capture and carriage. Water droplets approximately 2 mm. in diameter were caused to fall distances between 10 cm. and 25 cm. so that they then swept the conidiophores of *Verticillium alboatrum* or *Penicillium chrysogenum* before being caught in a mixture of oil and 'Vaseline'.

Droplets which swept *Verticillium* conidiophores invariably contained spores which exhibited Brownian motion within the droplet. No spores were ever observed within the droplets which had swept *Penicillium* conidiophores; but the spores were always observed on the surface of the droplet and most frequently at the boundary where the droplet rested on the oil and 'Vaseline' mixture. Evidently the readily wettable *Verticillium* spores enter the droplets with ease, whereas with the *Penicillium* spores, with their water-repellent surfaces and under the conditions of these experiments, impact velocities between spores and droplets were too low to overcome the interfacial energy at the surface of the droplets.

Since electrostatic charges have been shown to occur on raindrops by Chalmers[3] and on spores in air by Gregory[4], the possibility of an electrostatic attachment between water droplets and spores with water-repellent surfaces was investigated.

A mass of *Penicillium* spores held on the point of a steel needle was brought into contact with a water droplet produced at the tip of a hypodermic needle and subsequent events observed under the microscope. With both needles connected to earth, that with the spores was slowly moved away from the droplet. As this was done, it was observed that the part of the droplet in contact with the spores became drawn out, until finally, when contact ceased, the droplet assumed its original shape and a number of spores was left adhering to its surface.

Since both droplet and spores were earthed, it must be concluded that despite the water repellency of the spore surface, the spores were held to the droplet surface by molecular adhesion.

The manner in which fungal spores are captured and carried by rain or spray droplets is influenced by the wettability of the spore surface. The readily wettable spores of *Cephalosporium, Fusarium, Verticillium* and *Pullularia* enter droplets with ease and are carried within. At low impact velocities the 'non-wettable' spores of *Aspergillus*, *Cladosporium* and *Penicillium* are captured by adhering to the surface of the droplet.

Deposition of spores on water-repellent surfaces. Since most vegetation has water-repellent surfaces, it was decided to investigate whether surface wettability affected the deposition of spores by water droplets. In an initial experiment a microscope slide was covered on one surface with a mixture of 'Vaseline' and paraffin wax. A water droplet with its surface densely covered with 'non-wettable' *Penicillium* spores was placed at one end on the prepared surface, and the slide inclined and sharply tapped so that the droplet ran over it. As the droplet moved, most of the spores became attached to the hydrophobic surface and were visible to the unaided eye as a linear 'stain' along the course taken by the droplet. When the procedure was repeated with wettable *Verticillium* spores, and the slide examined under the microscope, no spores were seen in the track over which the droplet moved. When the experiment was repeated using plant leaves and the spores of other fungi, similar results were obtained.

It may be concluded, therefore, that when a droplet carrying spores falls on and moves over a hydrophobic plant surface, any 'non-wettable' spores brought into contact with the surface will tend to be deposited to the rear of the moving droplet, whereas wettable spores will either be washed away or deposited where the droplet comes to rest. During rain, the process is complicated by the splash-dispersal mechanism described by Gregory *et al.*[5] and the fact that through contact with water the wettability of a hydrophobic surface increases (Adam[6]).

Water droplets falling on hydrophobic leaf surfaces do not form a continuous film, but, to quote an expression of Adam, "pearl away in beads". It was observed that when a succession of droplets from a point source fell on a blade of grass, the beads did not run along the axis of the blade but 'pearled' off the edges, frequently alighting on an adjacent blade. This pearling away of water in beads from hydrophobic plant surfaces may in itself act as a spore dispersion mechanism distinct from the splash mechanism of Gregory *et al.*[5].

While it has not been investigated, in the removal of a radioactive dust cloud by rain, a 'non-wettable' fraction might tend to be deposited and retained on the surfaces of vegetation.

R. R. Davies*

Biological Department,
St. Thomas's Hospital Medical School,
London, S.E.1.

---

* Now at the Wright-Fleming Institute, St. Mary's Hospital Medical School, London, W.2.

[1] Gregory, P. H., *Trans. Brit. Mycol. Soc.*, **35**, 1 (1952).
[2] Hirst, J. M., *Trans. Brit. Mycol. Soc.*, **36**, 375 (1953).
[3] Chalmers, J. A., *Atmospheric Electricity* (Pergamon Press, 1957).
[4] Gregory, P. H., *Nature*, **180**, 330 (1957).
[5] Gregory, P. H., Guthrie, E. J., and Bunce, M. E., *J. Gen. Microbiol.*, **20**, 328 (1959).
[6] Adam, N. K., *Endeavour*, **17**, 37 (1958).

Taxonomy of *Xanthomonas* and *Pseudomonas*

Application of computers to problems of bacterial taxonomy has been suggested by Sneath[1], who described a method for handling large quantities of taxonomic data by an electronic computer. By this type of analysis, an outline of classification based on equally weighted features is obtained.

Hill[2] carried out an electro-taxonomic analysis of the *Staphylococci* and determined *Staphylococcus aureus*, *S. saprophyticus*, and *S. roseus* to be natural groups, whereas *S. lactis* and *S. afermentans* were not, since individual strains of these 'species' proved to be as different from each other as from the previous species. Other taxonomic studies involving the use of a computer[3] have yielded equally interesting results.

Work on the classification and taxonomy of the *Pseudomonas*, *Achromobacter*, *Flavobacterium* groups using the IBM 650 magnetic drum computer is being carried on in our laboratory[4,5]. The method clearly indicates generic and supra-generic groupings and there are indications, from our results and from others reported[6,7], that the genus-level may lie approximately at 60 per cent and the species-level at 75 per cent *S* ('similarity value'), meaning that organisms of the same genus will share about 60 per cent of their characters and strains will share 75 per cent or more of their characters.

Since *Xanthomonas* is distinguished from *Pseudomonas* by production of a yellow insoluble pigment and by plant pathogenicity, and since some *Pseudomonas* are plant pathogens, we became interested in applying the Adansonian method in an analysis of representatives of these groups to determine whether

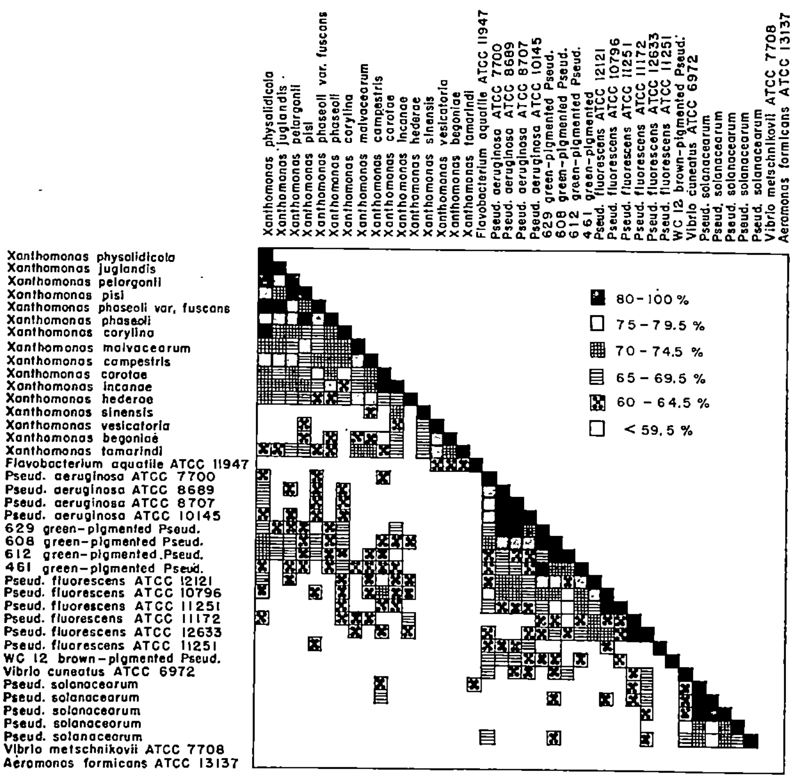


Fig. 1. Full table of S-values obtained from the analysis of forty representative strains, predominantly *Xanthomonas* and *Pseudomonas*

the separation of *Xanthomonas* from *Pseudomonas* is real or artificial (that is, taxonomic or determinative).

Some well-defined species of *Xanthomonas*, strains of *Pseudomonas solanacearum* which had been isolated from widely different geographical sources, and a number of named and freshly isolated *Pseudomonas* species were studied. All strains were tested, using standard bacteriological methods[8], in our laboratory and the 134 characteristics or 'states' obtained for each culture were punched on data cards. Calculations of S-values were performed by machine. The full S-table results are shown in Fig. 1.

It appears that three groupings were obtained: *Xanthomonas physalidicola* to *Flavobacterium aquatile*: *Pseudomonas aeruginosa* ATCC 7700 to *Vibrio cuneatus*; and the *Pseudomonas solanacearum* strains. *Vibrio metschnikovii* and *Aeromonas formicans* fall completely outside the *Pseudomonas–Xanthomonas* groups, in accord with results of other analyses[5].

The *Xanthomonas* species fall very clearly into two related groups which we have called *Xanthomonas* I and *Xanthomonas* II, for convenience. *Xanthomonas* II, the small group, contains only four species: *X. sinensis*; *X. vesicatoria*; *X. begoniae*; and *X. tamarindi*. All other species of *Xanthomonas* fall into Type I. However, within Type I, there is a small, tight clustering of the species *X. physalidicola* to *X. phaseoli*, which are so clearly related that they seem to form a 'taxonomic' species (intra-group $S = 78$ per cent). The five *Pseudomonas solanacearum* strains form a closely related group, quite distinct from *Xanthomonas* types, and probably constitute a true taxonomic species (intra-$S = 75$ per cent).

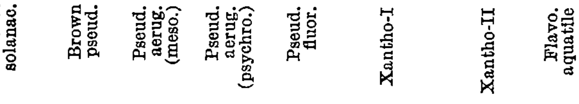


Fig. 2. Diagrammatic representation of inter- and intra-group S-values indicating group relationships

The fluorescent *Pseudomonas* form a large homogeneous group (intra-$S = 69$ per cent) within which three sub-groups can be delineated: a *Pseudomonas fluorescens* group and two *Pseudomonas aeruginosa*-like groups, one psychrophilic (461 to 629), consisting of organisms from marine sources and the other mesophilic, including all the ATCC *P. aeruginosa* cultures.

The two *P. aeruginosa* groups are so closely related (inter-$S = 74$ per cent) and individually so homogeneous (intra-$S = 75$ per cent and 85 per cent, respectively) that they could represent a single species with two varieties.

The maximum values for the inter-group S-values are shown in Fig. 2, the groups having been arranged according to the computed inter- and intra-S-values. It can be noted that *Xanthomonas* links with the *aeruginosa–fluorescens* block by a group relationship between the latter and *Xanthomonas* I, at a level close to the possible genus-level. *Xanthomonas* II is closely related to *Xanthomonas* I, but not to the principal *Pseudomonas* group. Indeed, it is interesting to note that *Xanthomonas* II has a nearly generic relationship with the ATCC *Flavobacterium aquatile*, which may not, however, be a *Flavobacterium*, since it has polar flagella.

As shown in Fig. 2, the *Pseudomonas solanacearum* species, which themselves produce a brown pigment, are linked to the *Pseudomonas* group through relationship with a sea-water-requiring brown-pigmented *Pseudomonas* species, but differ as much, or more, from the other *Pseudomonas* tested, as did the *Xanthomonas* species.

A more complete presentation of these results, with a discussion of the characteristics separating the groups, will be published in full elsewhere.

The *Xanthomonas* species were supplied by Dr. M. P. Starr, Department of Bacteriology, University of California, Davis, and the *Pseudomonas solanacearum* isolates by Dr. A. Kelman, Plant Pathology Department, North Carolina State College, Raleigh. The named strains of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Vibrio metschnikovii*, *Aeromonas formicans* and *Flavobacterium aquatile* were obtained from the American Type Culture Collection, Washington, D.C. Other pseudomonads were freshly isolated in our laboratory from marine sources.

ACADEMIC PRESS New York and London

Advances in ASTRONOMY and ASTROPHYSICS

A New Serial Publication

Edited by ZDENĚK KOPAL, *The University, Manchester*

VOLUME I *November 1961, about 325 pp.*

CONTENTS:

Distribution of Mass in Oblate Stellar Systems
By L. PEREK, *Astronomical Institute of the Czechoslovak Academy of Sciences, Prague*

The Shock-Wave Theory of Novae
By JOHN HAZLEHURST, *Yerkes Observatory, University of Chicago*

Polarization of Starlight
By K. SERKOWSKI, *Warsaw University Observatory*

Meteorites and Cosmic Dust
By D. W. PARKIN *and* W. HUNTER, *College of Technology, Liverpool*

The Properties and Problems of T Tauri Stars and Related Objects
By G. H. HERBIG, *Lick Observatory, University of California*

AUTHOR INDEX—SUBJECT INDEX.

Physics and Astronomy of the MOON

Edited by ZDENĚK KOPAL

September 1961, about 550 pp. approx. 107s.

CONTENTS:

The Motion of the Moon in Space
By DIRK BROUWER *and* GEN-ICHIRO HORI, *Yale University Observatory*

Libration of the Moon
By K. KOZIEL, *Cracow University*

Dynamics of the Earth–Moon System
By GORDON W. GROVES, *Universidad Nacional de México*

Photometry of the Moon
By V. G. FESSENKOV, *Academy of Sciences, Moscow*

The Polarization of Moonlight
By AUDOUIN DOLLFUS, *Observatoire de Paris, Meudon, France*

Lunar Eclipses
By F. LINK, *Astronomical Institute of the Czechoslovak Academy of Sciences, Prague*

Topography of the Moon
By ZDENĚK KOPAL, *The University, Manchester*

Interpretation of Lunar Craters
By EUGENE M. SHOEMAKER, *U.S. Geological Survey, Menlo Park, California*

Physical Observations of the Lunar Surface
By N. A. KOZYREV, *Poulkovo Observatory, Leningrad*

The Luminescence of the Lunar Surface
By J. F. GRAINGER *and* J. RING, *The University, Manchester*

Temperatures on the Lunar Surface
By WILLIAM M. SINTON, *Lowell Observatory, Flagstaff, Arizona*

Radio Echo Studies of the Moon
By J. V. EVANS, *Lincoln Laboratory, Massachusetts Institute of Technology, Lexington*

Origin and History of the Moon
By HAROLD C. UREY, *University of California, La Jolla*

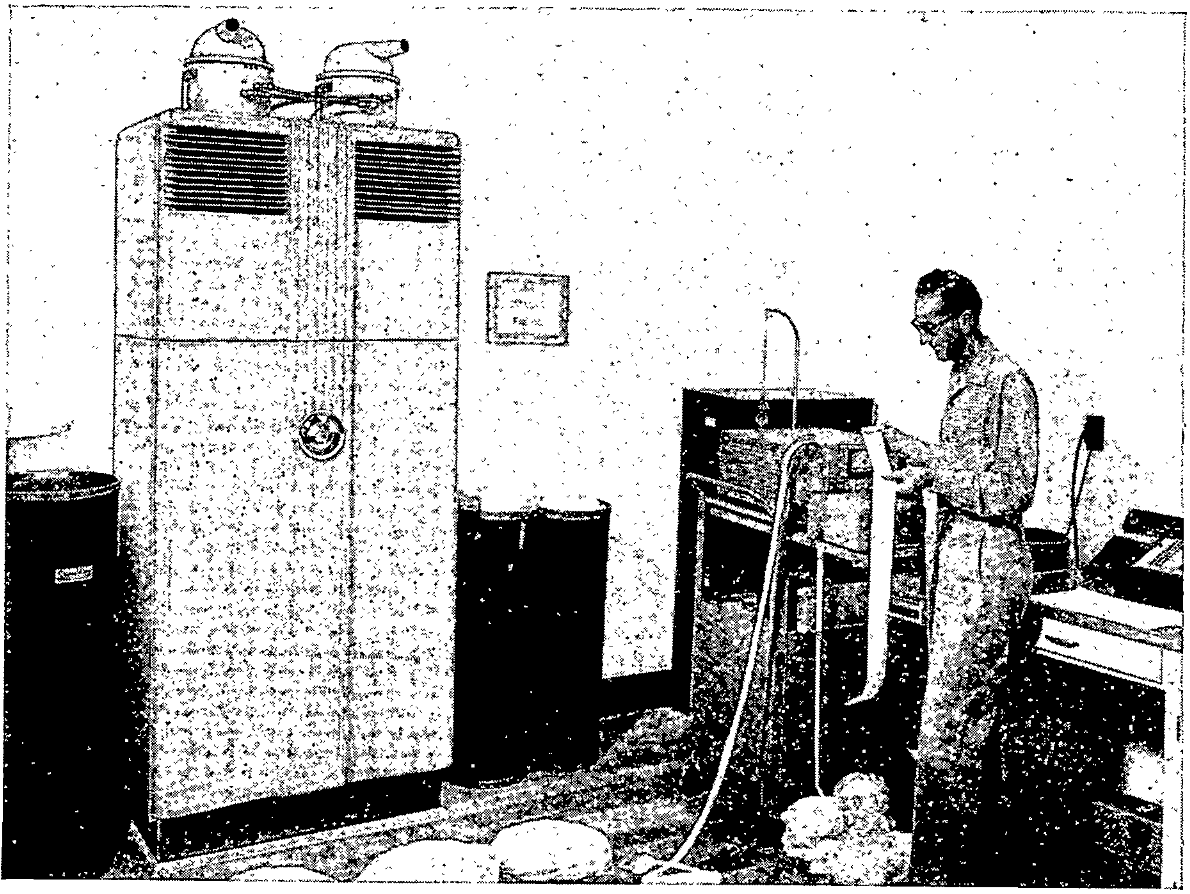
AUTHOR INDEX—SUBJECT INDEX.

Telephone : WHItehall 6631

111 FIFTH AVENUE, NEW YORK 3 17 OLD QUEEN STREET, LONDON, S.W. 1

CARLYLE

LABORATORY CONDITIONERS



give you 'PRECISION CONTROL' of temperature and humidity

No one knows better than you that varying air temperatures and humidity can affect the properties of materials, and the accuracy of instruments and experimental results. These problems can be overcome by the installation of a Carlyle Laboratory Air Conditioner. This completely self-contained unit—specially designed for laboratory work—will ensure year round control of temperature and humidity to within ±1°F and ±1½% R.H. The Carlyle Unit is the result of years of specialised experience in all phases of atmosphere control involving application of air conditioning and refrigeration to industrial plants and processes.

Capacities from 29,000 to 80,000 Btu/hr

Larger capacities are also available on special order. We shall be glad to co-operate with organisations in recommending the unit most suitable for their purpose.

CARLYLE

CARLYLE AIR CONDITIONING & REFRIGERATION LTD

1 King Street, St. James's, London S.W.1 Tel: WHItehall 5356

This investigation was supported by a grant (*E*-2417) from the National Institutes of Health, U.S. Public Health Service.

R. R. Colwell
J. Liston

College of Fisheries,
University of Washington,
Seattle, 5.

[1] Sneath, P. H. A., *J. Gen. Microbiol.*, **17**, 184, 201 (1957).

[2] Hill, L. R., *J. Gen. Microbiol.*, **20**, 277 (1959).

[3] Sneath, P. H. A., and Cowan, S. T., *J. Gen. Microbiol.*, **19**, 551 (1958).

[4] Colwell, R. R., and Liston, J., *App. Microbiol.*, **8**, 104 (1960).

[5] Liston, J., and Colwell, R. R., *Bacteriol. Proc.*, 78 (1960).

[6] Colwell, R. R., and Liston, J. (work to be published).

[7] Cheeseman, G. C., and Berridge, N. J., *J. App. Bacteriol.*, **22**, 307 (1959).

[8] Soc. Amer. Bacteriol., *Manual of Microbiological Methods* (McGraw-Hill Book Co., Inc., New York, 1957).

Effects of Kinetin and Indole-3-acetic Acid on Multiplication Rates of *Paramecium*

Kinetin (6-furfuryl aminopurine) is known to cause division in certain plant cells which could not otherwise divide[1]. But other cases are reported in which the rate of cell division is decreased after its application, for example, those of De Ropp[2], Kemp *et al.*[3], Pilet *et al.*[4], and Ham *et al.*[5]. How this substance affects the rate of division of animal cells is a question of interest. In a recent report[6], Guttman and Back re-analysed data and repeated experiments in which they had previously[7] found that kinetin causes an increase in the rate of division of *Paramecium caudatum* when added in low doses to the culture medium. Their later work[6] indicated that stimulation of cell division was present, but on a lower level than that previously reported. Coincident with the appearance of the second report of Guttman and Back, we reported elsewhere[8] the results of experiments in which kinetin, when used at the same concentrations and under similar conditions, was found to be inhibitory to division of *P. caudatum*.

Table 1 shows the results of a series of experiments in which comparisons were made between controls and treated *Paramecia* which were immediate descendants of one individual of a clone. In every series, there was inhibition of division at a significant level of confidence in kinetin-treated *Paramecia* as compared with untreated controls.

A smaller series of comparisons was made between rates of division in *Paramecia* treated with kinetin plus indole-3-acetic acid (IAA), and untreated controls. The results are given in Table 1. The results show that IAA appears to reverse the inhibition of division produced by 0·5 p.p.m. kinetin, and to counteract the severity of the inhibition produced by 1·0 and 1·5 p.p.m. kinetin. Antagonism between kinetin and IAA has been found in other experiments, for example, those of Wickson and Thimann[9] in lateral bud development.

Table 1. Mean Numbers of Divisions per 24-hr. Period of *Paramecia* treated with Kinetin (K) alone or in Combination with Indole-3-acetic Acid, as compared with Untreated Controls

No. of 24-hr. comparisons	Control	*S.E.*	Treatment solution (p.p.m.)			*t* value	Level of significance
			0·5 K.	1·0 K.	1·5 K.		
64	0·843	0·47	0·742			1·7	0·1
60	0·850	0·51		0·650		2·99	0·01
61	0·819	0·49			0·606	3·31	0·01
			0·5 K. +2 IAA	1·0 K. +2 IAA	1·5 K. +2 IAA		
41	0·607	0·48	0·607				
42	0·619	0·48		0·548		0·939	0·35
41	0·610	0·23			0·512	1·271	0·25

The method of treatment and handling of materials in our experiments were the same as those described by Guttman and Back[6], except that the nutrient medium we used was a snail-egg extract which had been filtered, sterilized, and adjusted to *p*H 6·8, rather than hay infusion. The ambient medium was changed every 24 hr., whether the *Paramecium* had divided or not. Cultures were maintained in depression slides at 25 ± 2° C. The kinetin used was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, and was freshly prepared.

Bacteria may have been transferred with the *Paramecium* from the stock culture; but the ambient medium as added daily for the test period was free from bacteria. In tests for a possible effect of kinetin on the bacterial population, Guttman and Back[7] found no change in its size or constitution. Lansford *et al.*[10] found no appreciable effect of kinetin alone on the growth of *Lactobacillus arabinosus*.

When Guttman and Back[6] re-analysed their results, recording division frequency for each day separately, their results showed a day-by-day pattern of divisions in kinetin-treated *Paramecia* which was approximately the same as the pattern for the controls, but at a higher level. Re-analysis of our Table 1 by this method, recording division frequency for each day separately, showed that the up-and-down pattern of the mean numbers of divisions for kinetin-treated *Paramecia* is the reverse of that for the control *Paramecia* in every case excepting for the third day in *Paramecia* treated with 0·5 and 1·0 p.p.m. kinetin. These show a decrease from day 2 to day 3, similar to the decreasing pattern for the controls. The re-analysis of the data on *Paramecia* treated with kinetin + IAA is shown in Fig. 1. The division pattern of the treated *Paramecia* is the reverse of the pattern for controls. Although the addition of IAA to the kinetin medium apparently reversed the inhibitory action of kinetin alone when the overall means for the three days are considered, day-by-day analysis shows that the reversal did not occur until day 2.

Woodruff[11], in numerous studies of division in *Paramecia*, found a regular cyclic rhythm of increase and decrease under normal conditions. The controls in our experiments and in those of Guttman and Back show day-to-day differences which might be part of such a cyclic rhythm, or which might be due to change in rate on transfer to a new medium.

In our experiments, the up-and-down rhythm pattern of division of *Paramecia* treated with kinetin was the reverse of the pattern for controls, that is, the number of divisions increased in treated *Paramecia* when it decreased in the controls, and vice versa. This also does not agree with the results of Guttman and Back, who found that the pattern of division for kinetin-treated *Paramecia* was similar to the pattern for the controls, with parallel increase and decrease in the number of divisions in all cases.

Guttman[12] in 1956 reported a stimulatory effect of kinetin on mitosis in cells of intact onion roots. Her data were obtained by counting mitotic figures in random fields of squash preparations. In similar work in which mitoses were counted in serial longitudinal sections of onion root tips, McManus[13] found an

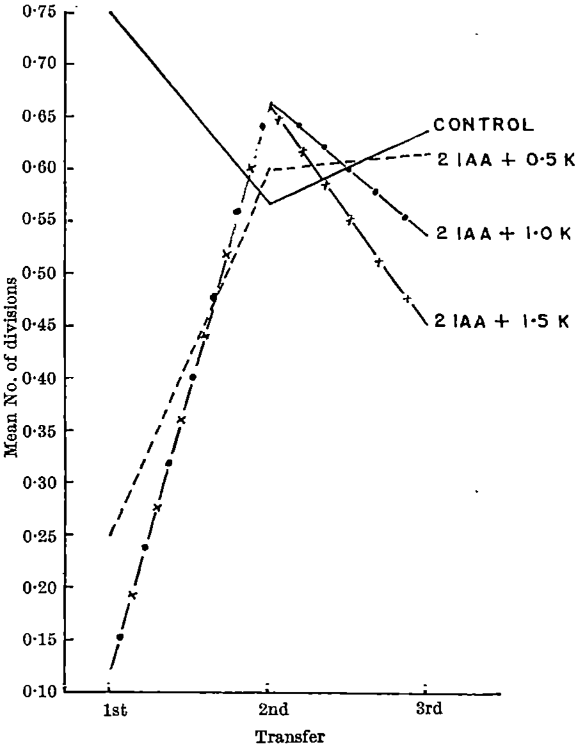


Fig. 1. Cell division in *Paramecium caudatum* treated with indole-3-acetic acid plus kinetin (parts per million). The influence of treatment on division frequencies after each of three daily transfers to new medium

inhibitory effect of kinetin, at comparable concentrations. Inhibition was also indicated by data obtained by Jensen and Pollock[14] in quantitative work on the deoxyribonucleic acid content of onion root tips treated with kinetin.

Whether or not kinetin occurs naturally in plant and animal tissue, clarification of the true nature of its effect on cell division under various conditions is of importance to our understanding of the growth process. Undoubtedly, interactions between kinetin and IAA and between them and other substances and events within the cell, which compel its division, must be very complex.

Mary Annunciata McManus
Kathleen Sullivan

Mount Mercy College,
Cedar Rapids, Iowa.

[1] Miller, C. O., Skoog, F., Okumura, F. S., Von Saltza, M. H., and Strong, F. M., *J. Amer. Chem. Soc.*, **77**, 2662 (1955).

[2] De Ropp, R. S., *Plant Physiol.*, 31, 253 (1956).

[3] Kemp, H. T., Fuller, R. G., and Davidson, R. S., *Science*, **126**, 1182 (1957).

[4] Pilet, F., Hildebrandt, A. C., Riker, A. J., and Skoog, F., *Amer. J. Bot.*, **47**, 186 (1960).

[5] Ham, R. G., Eakin, R. E., Skinner, C. G., and Shive, W., *J. Amer. Chem. Soc.*, **78**, 2648 (1956).

[6] Guttman, R., and Back, A., *Science*, **131**, 986 (1960).

[7] Guttman, R., and Back, A., *Nature*, **181**, 852 (1958).

[8] McManus, Sr. M. A., and Sullivan, K., *Proc. Iowa Acad. Sci.*, **67**, 501 (1960).

[9] Wickson, M., and Thimann, K. V., *Plant Physiol.* (Supp.), 31, 28 (1956).

[10] Lansford, jun., E. M., Skinner, C. G., and Shive, W., *Arch. Biochem. Biophys.*, **73**, 191 (1958).

[11] Woodruff, L. L., and Baitsell, G., *J. Exp. Zool.*, **11**, 339 (1911).

[12] Guttman, R., *Chromosoma*, **8**, 341 (1956).

[13] McManus, Sr. M. A., *Nature*, **185**, 44 (1960).

[14] Jensen, W. A., and Pollock, E. G., *Plant Physiol.* (Supp.), **33**, 15 (1958).

Cycle and Rhythm in the Ovulation of the Spider *Latrodectus geometricus* C. Koch

We define 'cycle' as that phenomenon represented by regular variations of internal origin. By 'rhythm', we understand a phenomenon represented by regular variations imposed by external conditions. 'Regularity' signifies a constancy of the interval separating two identical stages of the variation[1]. The ovulation of the cosmotropical spider *Latrodectus geometricus* C. Koch is at one time both cyclic and rhythmic.

Under laboratory conditions, with regular nutrition and prior fecundation, the adult *Latrodectus* lays an average of 22·56 cocoons at an interval of one every four days during the first part of its life. This interval becomes progressively longer until death intervenes. This metabolic ovarian cycle, established by observation of the laying of some thousands of cocoons, is illustrated in Fig. 1, which represents the percentage frequency (ordinate) of different time-intervals in days separating the laying of successive cocoons (abscissa)[2].

86·8 per cent of the cocoons are laid between 0230 and 0320 hr., with the relative maximum falling at 5.30. Under natural conditions this maximum is more sharply accentuated. Fig. 2 represents the observations made on several hundred cases, in which the numbers of cocoons produced (ordinate) are grouped against intervals of 1 hr. (abscissa).

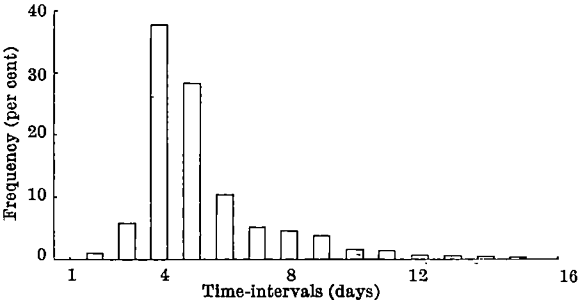


Fig. 1

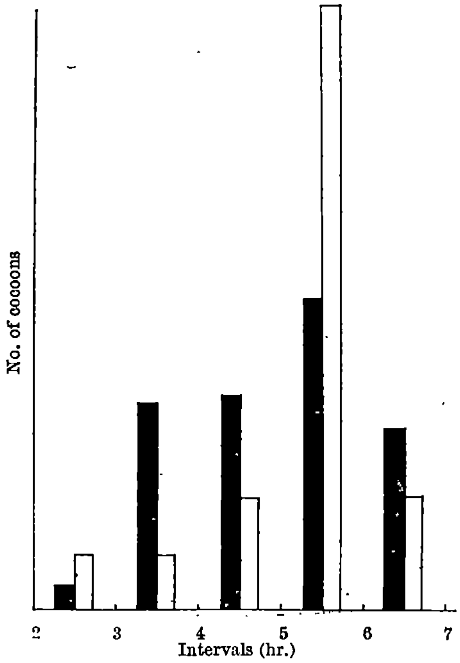


Fig. 2. ■, Laboratory; □, Nature

Observations on the production of 2,430 cocoons under varying controlled conditions (alternation of day and night with a displacement of 12-hr., period of daily illumination, temperature, humidity, time of feeding) have shown the only factor capable of influencing this rhythm, superimposed on the ovulation cycle, to be nycthemeral. By alternating the day and night cycle, the maximum intensity of ovulation can be shifted to fall between 1700 and 1800 hr., that is to say before an instantaneous change from obscurity to light, but the laying period is, however, more spread out than under normal conditions, 66 per cent of the cocoons being laid between 1400 and 2200 hr.[3]

A. Bouillon
R. Lekie

Department of Zoology,
University Lovanium,
Leopoldville, Congo.

[1] Reinberg, A., and Ghata, J., *Rythmes et Cycles biologiques* (Paris, 1957).

[2] Bouillon, A., *Studia Univ. Lovanium, Fac. Sci.*, 1, 10 (Leopoldville, 1957).

[3] Bouillon, A., and Lekie, R., *Studia Univ. Lovanium, Fac. Sci.*, 12. 5 (Leopoldville, 1960).

Significance of the Regeneration of Lateral Plates of the Stickleback (*Gasterosteus aculeatus* L.)

The taxonomy of the stickleback (*Gasterosteus aculeatus* L.) has excited the interest of zoologists for more than two centuries. Even now there are many unexplained questions concerning the differentiation within the species. The most difficult to explain is the variable number of lateral plates covering the body of this fish.

My earlier investigation, already described[1], of the process of regeneration of the lateral plates seems to question the popular conception that small losses in plates are caused by the mechanical tearing off of the plates in natural conditions. When the lateral plates of the stickleback were removed with the cutis, then the connective tissue, the subcutaneous tissue and a thin layer of muscles were regenerated by the organism by differentiation of the mesenchyme tissue. Plates removed from a considerable area of the body regenerated at the same time. This excludes the possibility of their being regenerated only in the proximity of the untouched plates. The discovery of the regeneration of the lateral plates which had been cut away completely is interesting, because so far all experiments carried out with dermal bones (which have the same structure and origin as lateral plates) have shown that regeneration takes place only when they were cut away only partially[2-5].

Continuing the investigation has shown recently that the regeneration of the amputated parts of the body of the stickleback sets in relatively quickly. The epidermis fills up the losses of the parts of the body removed within six days. In about fifteen days connective tissue penetrates in between the epidermis and the regenerating muscles. After 30 days the connective tissue differentiates into loose, elastic and subcutaneous kinds of tissue. A germ of a lateral plate appears on about the fiftieth day of the regenerating processes. Between the 100th and 150th day of regeneration, the lateral plate is usually restored, but sometimes its shape is irregular. In the case of a partial amputation of lateral plates, restoration of the loss takes place more quickly; this is natural, because the regenerated part profits by the cells of the old tissue.

It seems that this investigation throws some light on the complicated problem of the individual mutability of stickleback which is conditioned, among other factors, by a variable number of lateral plates.

Tadeusz Penczak

Department of Systematic Zoology,
University of Łódź,
68 Narutowicza str.,
Poland.

[1] Penczak, T., *Zeszyty Naukowe* U.L. (Łódź), Ser. II, *Nauki Mat. Przyrod.*, 7, 123 (1960).

[2] Beigel, C., *Bull. Int. Acad. Pol.*, Cl. Mat. Nat. (Cracovia), B, 655 (1910).

[3] Sauter, M., *Arch. Entw. Mech.*, 132, H. 1 (1934).

[4] Suworow, E., *Podstawy ichtiologii*, 304 (PWN, Warszawa, 1954).

[5] Wunder, W., *Umschau in Wissenschaft und Technik*, 3 (Leipzig, 1953).

Spiracle Control in Dragonflies

Hoyle[1] has shown that carbon dioxide has a direct action on the neuro-muscular junction of the closer muscle of the second spiracle of the locust, reducing the electrical responses and the tension developed in spite of maintained stimulation of the motor axons in the transverse nerve. However, this peripheral control mechanism does not seem to play an important part in the regulation of the intact spiracle which, except during flight, remains firmly synchronized with ventilation[2]. It might be expected to play a more important part in an insect the spiracles of which are not always synchronized with ventilation, such as the dragonfly. The adults of four species of Aeschnid dragonflies, occurring in Great Britain, have been examined and none shows ventilation synchronization in spiracles 1, 2 or 3. In the 15 species of Libellulidae from Great Britain and Uganda which have been examined, however, spiracle 1 opens with abdominal inspiration and closes completely or partially with expiration: at rest, spiracles 2 and 3 remain closed, but they may join spiracle 1 as inspiratory spiracles for a brief period after struggling or flight.

Spiracles 1, 2 and 3 are similar to the abdominal spiracles in possessing a closer muscle and in being opened by a cuticular elastic mechanism. The activity of the closer is controlled by the interaction of two mechanisms. In the first a central reflex, initiated by any movement—usually flight—inhibits the streams of motor impulses in the median nerves to spiracles 1, 2 and 3 so that they open instantaneously. They remain open throughout flight. The second mechanism depends on the peripheral action of carbon dioxide, and the site of action is most probably the neuro-muscular junction of the closer as in the locust[1]. Oscilloscope records show that the pattern of motor impulses in the transverse nerve to the closer muscle remains unaltered while the spiracle opens to locally administered 5 per cent carbon dioxide. In Aeschnids the spiracle opens with irregular fluttering movements, but in Libellulids it starts immediately to make movements synchronized with ventilation, although the other member of the pair remains closed. Each spiracle of the pair receives identical motor input, since the axons in the median nerve divide into right and left transverse nerves. In Libellulidae the motor input nearly always shows a rhythm, increasing in frequency with expiration and decreasing with inspiration. The rhythm is very marked when ventilation is strong, but may disappear during weak ventilation. In the absence of carbon dioxide, the lower (inspiratory) frequency is adequate to maintain a tetanus in the closer and the spiracle stays shut. Local administration of 5 per cent carbon dioxide to the spiracle makes the lower frequency ineffective so that the spiracle opens during inspiration and recloses with expiration. In 5–10 per cent carbon dioxide the higher frequency also becomes partially or completely ineffective so that the spiracle remains open. Carbon dioxide in the thoracic ganglia stimulates abdominal ventilation and this in turn gives a more strongly marked rhythm in the thoracic transverse nerves. It has been shown that, unlike the locust, there is no rhythmic ventilation centre in the thorax and, after removal of the abdomen, treatment of the thoracic ganglia with carbon dioxide does not alter the patterns of motor impulses in the transverse nerves. The reaction can take place only via the abdomen.

In this way the insect possesses a facultative synchronization mechanism which allows the spiracles to be coupled to the ventilatory rhythm in the presence of low concentrations of carbon dioxide, and it is clear that the peripheral control mechanism plays an important part. Reduced oxygen tensions have no comparable affect. Possibly similar facultative spiracle synchronization has been observed in the cockroach[3], and such mechanisms in which variation takes place peripherally, while the contribution from the central nervous system remains largely unaltered, may reflect the need for the arthropod nervous system to economize in central connexions[4].

The sensitivity of the response of the spiracle muscle to carbon dioxide has been shown to depend on the state of hydration of the insect. In partially desiccated dragonflies the spiracles do not open in less than about 5 per cent carbon dioxide in air, and after flight they close immediately. In well-hydrated insects they open in 1–2 per cent carbon dioxide, and they may remain open for as long as 2 min. after flight. Experimentally produced variations in the osmotic pressure of the hæmolymph have been shown to alter the spiracle muscle's sensitivity directly, both before and after denervation, so that internal osmometers relaying to the central nervous system need not be postulated, and again the reaction takes place entirely peripherally.

P. L. MILLER

Makerere College,
Kampala, Uganda.

---

[1] Hoyle, G., *J. Insect Physiol.*, 4, 63 (1960).
[2] Miller, P. L., *J. Exp. Biol.*, 37, 264 (1960).
[3] Hazelhoff, E. H., *Z. vergl. Physiol.*, 5, 179 (1927).
[4] Wiersma, C. A. G., *Cold Spring Harbor Symposia Quant. Biol.*, 17, 155 (1952).

MICROBIOLOGY

Occurrence of *Salmonellae* in Domestic Animals and Poultry in India

THIS communication presents the results of an investigation into the occurrence of *Salmonellae*, usually in the symptomless carrier-state, in domestic animals and poultry in the State of Uttar Pradesh in India.

Bacteriological examination was mainly confined to fæcal samples; however, small pieces of liver, spleen, gall-bladder, lungs and intestine with its contents were also collected from domestic animals and poultry for examination.

The methods of isolation of *Salmonellae* used were on the lines adopted by Smith and Buxton[1] and Edwards *et al.*[2] with minor modifications. *Salmonella* genus-specific 0–1 bacteriophage was also used as advocated by Cherry *et al.*[3]. The serological typing was done by one of us at the Armed Forces Medical College, Poona. The strains were also sent to Dr. P. R. Edwards, Chamblee Georgia, U.S.A., and to Dr. Joan Taylor, Salmonella Reference Laboratory, London, for serological typing.

A total number of 2,970 domestic animals including poultry (1,332 fowls; 206 dead in shell embryos; 68 pigeons; 110 ducks; 455 cattle; 290 buffaloes; 163 sheep; 91 goats; 247 pigs and 18 rats) were examined during the course of the investigation. The occurrence of *Salmonella* species was demonstrated in 24 fowls, 7 dead in shell embryos, 1 duck, 8 cattle, 9 buffaloes, 4 goats, 3 pigs and 1 rat, making a total of 57 isolations.

Seventeen species of *Salmonellae*, including a new sero-type encountered during this investigation, are as follows (the figures in brackets indicate the number of isolations):

Fowls: *anatum* (2); *champaign* (2); *chester* (1); *hvittingfoss* (2), *matopeni* (1); *pomona* (1); *richmond* (3); *san-diego* (1); *stanley* (1); *weltevreden* (10).
Dead in shell-embryos: *richmond* (7).
Duck: *san-diego* (1).
Cattle: *aba* (1); *charity* (1); *magwa* (2); *matopeni* (1); *pomona* (2); *mathura* (1).
Buffaloes: *anatum* (1); *enteritidis* var. *jena* (1); *magwa* (1); *pomona* (1); *typhimurium* (1); *weltevreden* (4).
Goats: *magwa* (1); *matopeni* (1); *weltevreden* (2).
Pigs: *anatum* (1); *newport* (1); *Stanley* (1).
Rats: *anatum* (1).

A new species of *Salmonella*, which has been named *Salmonella mathura* with antigenic formula (9), 46 : i : e, n, z 15, has been isolated from cattle. The biochemical behaviour of this species, as studied by Dr. F. Kauffmann, International Salmonella and Escherichia Centre, Copenhagen, is as follows: adonitol, rhamnose, salicin, inositol, lactose and sucrose: negative after 30 days.

Positive reaction after 1 day in: dulcitol, sorbitol, arabinose, xylose, maltose, mannitol, glucose and trehalose. Gas production in mannitol and glucose. Stern's glycerolfuchsin broth negative after 8 days.

Hydrogen sulphide in ferrous chloride gelatin, negative. Gelatin, negative.

Ammonium glucose and ammonium citrate, positive. Potassium nitrate, positive; Voges-Proskauer, negative; methyl-red, positive; urea, negative.

d-tartrate, +1; *l*-tartrate, delayed and irregular, *i*-tartrate, +2; sodium citrate, +2; mucate, +1.

It is interesting to note that several of the species which probably have been isolated so far only from human beings are being encountered in animals. The importance of such occurrence from the epidemiological and public health point of view is obvious and further work is in progress. Investigation is also being conducted for the further characterization of the new species, *S. mathura*.

We are indebted to Major L. D. Sachdeva, of the Armed Forces Medical College, Poona, for the help and guidance in sero-typing the cultures, and Dr. Joan Taylor, of the Salmonella Reference Laboratory, Colindale, London, and Dr. P. R. Edwards, Chamblee Georgia, U.S.A., for sero-typing of the strains. We gratefully acknowledge the kind help of Dr. William B. Cherry, Chamblee Georgia, U.S.A., for

supplying 0–1 phage, and of Dr. J. B. Shrivastava, National Salmonella Centre, Kasauli, Punjab, for his help, and Dr. F. Kauffmann, International Salmonella and Escherichia Centre, Copenhagen, for final confirmation of the new species, and to Sri C. V. G. Choudary, principal of the Veterinary College, Mathura, for providing the necessary facilities.

V. K. Sharma
C. M. Singh

Department of Pathology and
Bacteriology,
U.P. College of Veterinary Science
and Animal Husbandry,
Mathura, India.

---

[1] Smith, H. W., and Buxton, A., *Brit. Med. J.*, i, 1478 (1951).

[2] Edwards, P. R., Bruner, D. W., and Moran, A. B., *J. Infect. Dis.*, 83, 220 (1948).

[3] Cherry, W. B., Davis, B. R., Edwards, P. R., and Hogan, R. B., *J. Lab. Clin. Med.*, 44, 51 (1954).

Transfer of Virulence in *Rhizobium trifolii*

So far, very few reports concerning transformations in *Rhizobium* have appeared. Krasilnikov[1] reported as early as 1941 that non-infective strains turned infective when they were grown for several months in a filtrate of a highly infective strain. By ultrasonic treatment and with preparations of deoxyribonucleic acid (DNA) Balassa[2,3] has obtained transformations concerning several properties, for example, infective ability under formation of new host specificity, increase or decrease in nitrogen-fixing ability, variation in colony types, and changes in antigenic structure.

I happened to meet with a case of transformation in *Rhizobium trifolii*. As part of a study of the infection process a polysaccharide was prepared from an infective strain[4]. As it had been observed (unpublished results) that inoculated plants formed nodules 1–2 days earlier when this preparation was added, the polysaccharide was added to plants inoculated with a non-infective strain to see whether it might help the avirulent bacteria to overcome the infection barrier.

The clover strain *A* used in the experiment described here has been studied thoroughly by Nutman[5,6]. The original strain lost its infection ability in 1940 (called Bart *A*), but a virulent line (*A* 1) was contained in an isolate from a nodule produced by the original strain. *A* 11 is an avirulent and *A* 121111 a virulent line, isolated from *A* 1. 226, the other clover strain, is a Swedish strain isolated by Bjälfve and so far as known not related to the *A* strain.

A polysaccharide from strain 226 was prepared[7] from a 2-wk.-old culture (Dorn's A_5-medium[8]) by precipitating the whole culture in alcohol, dissolving the precipitate in water and separating the cells by centrifugation. The clear solution was deproteinized according to Sevag[9], precipitated again, and dialysed against distilled water. After another precipitation, the preparation was dried *in vacuo* and a white water-soluble material was obtained.

Seeds of *Trifolium repens* (var. Morsö, Svalöf, Sweden) were germinated aseptically in tubes containing slopes of Jensen's seedling agar. After 2 days, they were inoculated with a few drops of a suspension of *Rhizobium* (Table 1, series 1–8). Immediately after inoculation, 2 mgm. of the 226 polysaccharide in water solution was added. The appearance of the first nodule in days after inoculation and the number of nodules after 2, 3 and 4 wk. are recorded in Table 1.

Table 1. PLANTS OF *Trifolium repens* INOCULATED WITH AVIRULENT (BART *A* AND *A* 11) AND VIRULENT (*A* 121111 AND 226) CLOVER BACTERIA WITHOUT (–) AND WITH (+) POLYSACCHARIDE PREPARATION FROM STRAIN 226 (2 MGM./TUBE). EACH TUBE CONTAINED 10 ML. OF JENSEN'S AGAR. THE NUMBER OF NODULES ARE MEAN VALUES OF FIVE REPLICANTS

Series No.	Bacterial strain	Poly-saccharide addition	Appearance of the first nodule in days after inoculation	Mean number of nodules per tube after 2 wk.	3 wk.	4 wk.
1	Bart *A*	–	–	–	–	–
2	*A* 11	–	–	–	–	–
3	*A* 121111	–	10	3·3	8·5	12·5
4	226	–	9	2·7	7·0	10·0
5	Bart *A*	+	9	2·5	7·3	9·3
6	*A* 11	+	9	3·5	9·0	12·0
7	*A* 121111	+	7	6·7	12·0	15·0
8	226	+	7	5·8	8·5	10·3
9	Bart *A*	–	–	–		
10	Bart *A*·2	–	8	4·2		
11	Bart *A*	+	8	6·4		
12	Bart *A*·2	+	6	7·4		

Bacteria from the nodules produced by the formerly avirulent Bart *A* in the presence of the polysaccharide were isolated (Bart *A*·2). The colonies had the smooth appearance of 226, not rough like Bart *A*.

New seedlings were inoculated with Bart *A* and Bart *A*·2 with and without polysaccharide 226 (Table 1, series 9–12). From this experiment it is evident that Bart *A* has become infective. A serological test showed that Bart *A*·2 did not react with antiserum prepared from the *A* strain (*A* 121111), indicating that the new strain has not recovered the antigenic properties lost by the *A* strain, but has gained something new, most likely from strain 226. Investigations into the nutritional requirements of the involved strains showed that strain 226 and Bart *A*·2 are greatly stimulated by pantothenic acid, but that Bart *A* is not.

The most likely explanation of the above results is that a transformation induced by the polysaccharide preparation (which might contain DNA) has taken place. This evidence and the earlier report[4] point to the surface material of the *Rhizobium* cells as playing an important part in their ability to infect the root hairs of the appropriate host.

Detailed work on the importance of the capsular material for the infection process is in progress and will be published elsewhere.

I wish to express my indebtedness to Dr. P. S. Nutman for the opportunity to work at his laboratory. My stay at Rothamsted was financed by a grant from Magnus Bergvalls Stiftelse.

Hans Ljunggren

Soil Microbiology Department,
Rothamsted Experimental Station,
Harpenden, Herts.
and
Institute of Microbiology,
Royal Agricultural College,
Uppsala 7.

---

[1] Krasilnikov, N. A., *C.R. Acad. Sci., U.R.S.S.*, 31, 75 (1941).

[2] Balassa, R., *Acta Mikrobiologica* (Hungary), 2, 51 (1954).

[3] Balassa, R., *Nature*, 188, 246 (1960).

[4] Ljunggren, H., and Fåhraeus, G., *Nature*, 184, 1578 (1959).

[5] Nutman, P. S., *J. Bact.*, 51, 411 (1946).

[6] Nutman, P. S., *Heredity*, 8, 35 (1954).

[7] Wiley, B. B., and Scherp, H. W., *Canad. J. Microbiol.*, 4, 505 (1958).

[8] Dorn, M., *Zbl. Bact.*, II Abt. 109, 120 (1956).

[9] Sevag, M. G., *Biochem. Z.*, 273, 419 (1934).

VIROLOGY

Infectious Ribonucleic Acids derived from Mouse Brains infected with Two Kinds of Arbor Virus Group *B*

INFECTIOUS ribonucleic acid (RNA) has been extracted by phenol treatment[1] from cells infected with animal viruses. In Group *B* of the arbor viruses, it has been recently reported that the RNA extracted from mouse brain infected with Murray Valley encephalitis virus[2] or Japanese *B* virus[3] and from Ehrlich ascites tumour cells infected with West Nile encephalitis[4] are infectious. The purpose of this present communication is to add a datum concerning the extraction of infectious RNA from mouse brains infected with dengue fever virus to the reports of viral RNA already published, and to compare the effects of cold- and hot-phenol extractions on the yields of infectious RNA from the cells infected with Japanese *B* encephalitis virus and dengue fever virus, respectively.

Five-day-old mouse brains infected intracerebrally with the Hawaiian strain of dengue fever virus (*M*-184) and three-week-old mouse brains infected with the Nakayama strain of Japanese *B* encephalitis virus (*M*-55) were employed as the sources of the materials for extraction of RNA. The brains were removed from exsanguinated mice shortly after the appearance of signs of involvement of the central nervous system, ground in a chilled mortar, and a 10 per cent suspension was made with sterile distilled water. Coarse particles were removed by low centrifugation, and the supernatant fluid was used for the experiment. The supernatant fluids were extracted three times with an equal volume of cold 80 per cent phenol at 4° C. for 3 min. following the procedure of Gierer and Schramm[1], or with an equal volume of molten phenol (50° C.) and shaken at 50° C. for 3 min. by Wecker's method[5]. After three such extractions the aqueous phase was shaken five times with peroxide-free ether and the excess ether was removed by bubbling with nitrogen. The aqueous phase obtained by each extraction was reconstituted to the original volume with sterile distilled water. Infectivity titres of the samples, expressed as LD_{50}, were determined by the intracerebral inoculation of serial 10-fold dilutions in mice, 0·02 ml. for three- to five-day-old mice and 0·03 ml. for three-week-old mice. Clarified 10 per cent infected brain suspension and the RNA preparation were regarded as 10^{-1} dilution. The results obtained are shown in Table 1.

Both infectious RNA of Japanese *B* encephalitis virus and dengue fever virus could be easily extracted by phenol treatment, and the yields of infectious RNA of these viruses were the same as that of poliovirus, that is, they amounted usually to about 0·1–0·01 per cent of the crude virus preparation. There were no differences between the effects of cold- and hot-phenol extraction on the yields of RNA derived from the two viruses.

The ultra-violet absorption spectra of these preparations of RNA were typical for nucleic acid. If such an extracted preparation was treated with 50 μgm./ml. of ribonuclease for 10 min. at room temperature prior to inoculation, its infectivity was completely abolished, whereas identical treatment of the virus suspension had no such effect. The preparations of RNA of these viruses had no hæmagglutinating activity under the conditions which were used for these viruses.

Table 1. INFECTIVITY OF TWO KINDS OF ARBOR VIRUS GROUP *B* AND RIBONUCLEIC ACID PREPARATIONS

Material	Animal for titration (−log LD_{50}/ml.)	
	Suckling mouse	Adult mouse
Japanese *B* encephalitis virus:		
Crude virus	8·9	8·5
RNA extracted by cold phenol	4·7	4·2
RNA extracted by hot phenol	5·5	3·8
Virus + RNase 50 μgm./ml., 22° C.; 10 min.	9·2	7·5
RNA* + RNase 50 μgm./ml., 22° C.; 10 min.	—†	—
Dengue fever virus:		
Crude virus	8·1	6·0
RNA extracted by cold phenol	4·7	4·5
RNA extracted by hot phenol	4·9	5·0
Virus + RNase 50 μgm./ml., 14° C.; 10 min.	8·0	*N.T.*
RNA* + RNase 50 μgm./ml., 14° C.,; 10 min.	—	*N.T.*

* RNA was extracted by hot-phenol treatment.
† No mice died when given undiluted inoculum.
N.T. = Not tested.

It might be noted, as indicated in Table 1, that, in the case of dengue fever virus, there were no differences between the infectivity titres of RNA for suckling mice and that for adult mice; whereas if adult mice were used for titration, the titres of the crude virus were approximately 100 times lower than that in suckling mice. Therefore, the ratio of virus to RNA in adult mice was much nearer unity than that in suckling mice.

Experiments are now being carried out in the extraction of RNA from adult mouse brains infected with dengue fever virus and suckling mouse brains infected with adult mouse-adapted dengue fever virus.

MASAHIRO NAKAMURA
Department of Microbiology,
Kurume University School of Medicine,
Kurume, Japan.

[1] Gierer, A., and Schramm, G., *Z. Naturforsch.*, **11**, *b*, 138 (1956).
[2] Ada, G. L., and Anderson, S. G., *Nature*, **183**, 799 (1959).
[3] Nojima, T., *Report from Virus Lab., Kyoto Univ.*, **1**, 101 (1958).
[4] Colter, J. S., Bird, H. H., Moyer, A. W., and Brown, R. A., *Virology*, **4**, 522 (1957).
[5] Wecker, E., *Virology*, **7**, 241 (1959).

Relationship of Tobacco Mosaic Virus (TMV) Lesion Number and Concentration to the Rate of Lesion Production on Pinto Bean

IN experiments which required the daily counting of TMV lesions on pinto bean leaves (*Phaseolus vulgaris* L.) for the purpose of determining specific rates of lesion-appearance, it was noted that those leaves which had the greatest final number of lesions produced a greater percentage of their lesions earlier. Since comparisons of rates of lesion development could be seriously affected by differences arising from this, an attempt was made to determine its cause and extent.

Bald[1] had observed the same phenomenon when potato virus *X* was inoculated into *Nicotiana tabacum* L. (var. White Burley), but could not obtain these results when this virus or TMV was inoculated into *N. glutinosa* L. He suggested that either adjacent incipient lesions enhanced production of early lesions or else the greater the number of infective particles entering an infection site, the more rapid the rate of virus multiplication and hence lesion appearance. The first suggestion is not compatible with our results and the second seems unlikely, since it is generally held that only one virus particle can infect one site[2,3].

According to Lauffer and Price[3] the rate of development of symptoms should be a function of concentration of virus, since Bryan and Beard[4] had observed this to be true for rabbit papilloma virus. They postulated that high virus concentrations should be associated with a high probability that every susceptible spot in a scarified area would become infected. Our results with TMV tend to confirm this hypothesis.

In order to investigate this problem more thoroughly a series of concentrations of purified TMV in distilled water, 7, 129, 430, 1,290 and 12,900 μgm./ml., was inoculated to primary pinto bean leaves selected randomly from 10-day-old plants. Inoculation was accomplished by rubbing several drops of virus solution gently with a glass rod over the 600 grit 'Carborundum' sprinkled leaf surface. 1 hr. after inoculation, leaves were washed in running tap water and incubated in a constant light and temperature chamber at 30° C. Lesions were counted at intervals, until new lesions failed to appear. The percentage of the final total of new lesions appearing at each count was plotted against time after inoculation (Fig. 1).

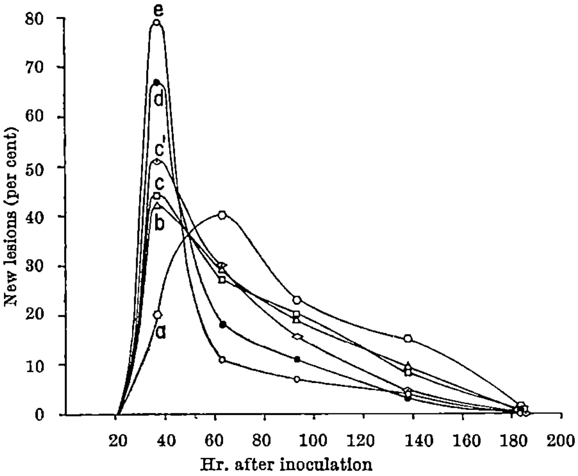


Fig. 1. The effect of tobacco mosaic virus concentration on the rate of appearance of lesions on primary leaves of pinto bean. Virus concentration, number of half-leaves and mean number of lesions per half-leaf for each curve: (*a*) 7 μgm./ml., 6, 9; (*b*) 129 μgm./ml., 8, 48; (*c*) 430 μgm./ml., 8, 61; (*c'*) 430 μgm./ml., 4 of the 8 half-leaves of (*c*) with the greatest number of lesions, 82; (*d*) 1,290 μgm./ml., 6, 60; (*e*) 12,900 μgm./ml., 6, 78. Note that for each time the number of new lesions appearing in the graph is expressed as the percentage of the final total

Examination of the count 37 hr. after inoculation (Fig. 1) shows conclusively that the percentage of new lesions is a function of the virus concentration. It is clear that higher concentrations bring about a higher percentage of lesions appearing early and a lower percentage of those appearing late than do lower virus concentrations. It appears, too, that the difference in the initial rate of appearance of lesions becomes less pronounced as the concentration increases.

Acceleration in appearance of lesions by higher concentrations of TMV was also obtained in two other experiments; one, comparing 0·5 μgm./ml. with 50 μgm./ml. TMV on 18 pinto bean leaves where the average number of lesions per half-leaf for the low concentration was 10 and for the high concentration 62, and the other comparing TMV at 5 μgm./ml. with 50 μgm./ml. on 6 pinto bean leaves where the mean number of lesions per half-leaf for the low concentration was 38 and for the high concentration 93.

An additional observation is that, even though the average number of lesions per half-leaf produced by the 430 μgm./ml. and 1,290 μgm./ml. inocula is about the same, a much greater percentage of the lesions appeared earlier in the case of the more highly concentrated inoculum, as can be seen by comparing the 37 hr. counts (Fig. 1, *d*, *c*). On the other hand, *c'* in Fig. 1, which represents the four half-leaves of the 430 μgm./ml. inoculated group with the most lesions, shows an increased rate of development of lesions as compared with this group as a whole (Fig. 1, *c*, *c'*). Additional comparisons of leaves with high and low lesions counts in four separate experiments averaging fourteen leaves each have confirmed this finding. From calculations[3] based on the number of mixed infections obtained from TMV and aucuba mosaic virus on *N. langsdorffii* Weinm., it seems reasonable to assume that a number of sites within the many-celled area that comprises a lesion by the time it is seen are often exposed and infected in the process of mechanical inoculation. It would follow also that the probability of virus particles initiating an infection at these sites increases with the concentration of the inoculum. Such composite lesions would be likely to appear sooner. The virus concentration is, however, only one variable.

Noting the range of time over which new lesions form for the relatively dilute 7 μgm./ml. inoculum (Fig. 1, *a*), where the percentage of composite lesions should be at a minimum, it appears that sites must vary considerably in the time required for them to produce virus and finally a visible lesion. If such a variability in sites exists, it is possible that at higher concentrations, when more than one site within a lesion area is infected, the site which produces virus most rapidly will determine the time of appearance of the lesion. The time of appearance, then, would depend on the probability of an early site being infected and would be a function of the total number of interactions of virus sites. It is clear that this latter condition depends not only on virus concentration but also on variability in host susceptibility and inoculation technique as well.

This leads us to our finding that when a variety of lesion counts is obtained from a given inoculum, the leaves having the greatest final number of lesions have also the highest percentage of early appearing lesions. Here again, differences in inoculation and host susceptibility have increased the number of interactions of virus sites, resulting in both a higher number of lesions and a higher number of susceptible sites occupied within a lesion area. This results in more and earlier lesions.

It seems apparent, then, that the number of lesions is not what determines the rate of appearance of lesions, but is only a reflexion of the infectivity of the inoculum, inoculating technique and host susceptibility, all of which actually affect the rate. Number of lesions can, however, be used as an index of the number of virus-site interactions.

This work was aided by an American Cancer Society Institutional grant.

JOHN PIACITELLI
VINCENT SANTILLI

Biology Department, University of Buffalo,
Buffalo 14, New York.

[1] Bald, J. G., *Ann. App. Biol.*, 24, 33 (1937).

[2] Wildman, S. G., *The Viruses*, edit. by Burnet, F. M., and Stanley, W. H., 2, 1 (Academic Press, New York, 1959).

[3] Lauffer, M. A., and Price, W. C., *Arch. Biochem.*, 8, 449 (1945).

[4] Bryan, W. R., and Beard, J. W., *J. Infectious Diseases*, 66, 245 (1940).

Chromatography of Tobacco Mosaic Virus (TMV) on Chitin Columns

While performing experiments on infected tobacco plant fibre to determine the virus-releasing enzymic activity of crude mushroom extracts (Townsley, P. M., unpublished results), it was noted that added chitin strongly adsorbed tobacco mosaic virus. At least a portion of the adsorbed virus could be released with concentrated phosphate buffers. This investigation describes the chromatographic properties of chitin for TMV.

90 gm. of chitin (Eastman Organic Chemicals) was reduced to a fine powder in a porcelain–'Carborundum' ball mill, in about 18 hr. The powder was suspended in 1 litre of *N* sodium hydroxide, stirred for about 0·5 hr. and collected in a Buchner funnel. The chitin pad was washed thoroughly with distilled water and then resuspended in 1 litre of 2 *N* hydrochloric acid. After settling for at least 0·5 hr. the top milky layer was decanted and discarded. The sediment was collected on a Buchner funnel, washed first with water, then with 95 per cent alcohol, and finally with ether.

10 gm. of prepared chitin was suspended in 250 ml. of water, and allowed to settle for 20 min. The top layer was discarded. The sediment slurry, now containing approximately 5 gm. of air-dried chitin, was poured into a chromatographic column, to form a column 17 × 1·3 cm. The column was washed with 25 ml. of 1 *N* hydrochloric acid, followed by 50 ml. of water, then 150 ml. of 0·1 *M tris*–hydrochloric acid, *p*H 6·8, and finally equilibrated with approximately 75 ml. of 0·01 *M tris*–hydrochloric acid, *p*H 6·8 (similar equilibration of the column with phosphate buffer, *p*H 6·8, prevented the column from adsorbing all the added TMV). The eluate from the column was approximately *p*H 6·8.

0·5 ml. of tobacco mosaic virus solution containing 0·3–2 mgm. of virus in 0·01 *M* sodium phosphate buffer, *p*H 6·8, prepared by two alternate cycles at 6,000*g* and 78,000*g* (ref. 1), was added to the column and the virus washed into the column with three successive 1-ml. aliquots of 0·01 *M tris*–hydrochloric acid, *p*H 6·8. 3 ml. of the latter buffer was then placed on the column and the column connected for linear gradient elution. The linear gradient progressed from water to 0·5 *M* K_2HPO_4. The eluate was collected in 4·3-ml. aliquots at 10-min. intervals in a fraction collector equipped for continuous optical density recording at 254 mμ.

As measured by optical density the virus was almost wholly accounted for in the two eluted peaks (Fig. 1). The results were reproducible, but the amount of virus in each peak varied. No explanation is offered for the appearance of two major peaks rather than one as might have been expected from the virus purified by ultra-centrifugation.

Material from both the peaks was birefringent and infective. Preliminary, comparative infectivity tests directly on elution, using six half-leaves of the local lesion host, *Nicotiana glutinosa* L., for each of the peaks, indicated that the material eluted at *p*H 7·2 was from four to ten times as infective as that eluted at *p*H 7·7. Spectrophotometric measurements gave a 260/280 mμ ratio of 1·23 and 1·29 respectively, for the peaks in the eluting solvent.

As a chromatographic agent for TMV chitin would appear to be very useful in view of the mild treatment required for adsorption and elution of the virus.

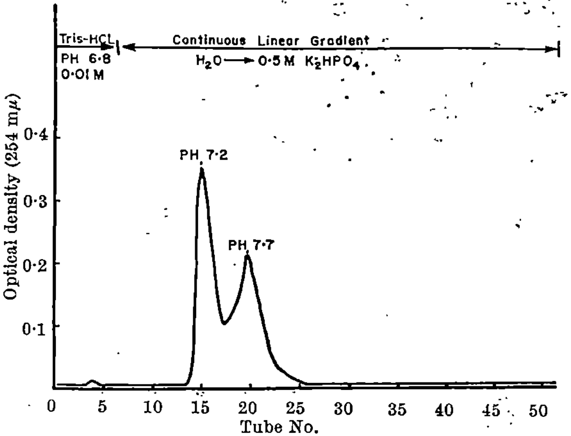


Fig. 1. An elution curve of tobacco mosaic virus from a chitin chromatographic column

Preliminary experiments have also shown that chitin may be used with similar ease for nucleic acid chromatography.

P. M. Townsley

Research Station,
Canada Department of Agriculture,
6660 N.W. Marine Drive,
Vancouver 8, B.C.

[1] Townsley, P. M., *Canad. J. Biochem. Physiol.*, 37, 1025 (1959).

CYTOLOGY

Peripheral Position of Sex Chromatin

In examining buccal smears for sex chromatin, one finds a surprisingly high incidence of sex chromatin bodies not merely at the nuclear membrane but also more precisely at the periphery of what appears to be a disk-shaped nucleus. The following discussion will provide evidence that, as in tissue cultures[1], the sex chromatin is characteristically at the edge of the nuclear disk in squamous cells *in vivo*. In establishing this let us first consider that squamous nuclei *in vivo* are flattened. This may be seen in mucosal smears when a mucosal fragment is folded over (Fig. 1). It is evident, too, that the cells in fragments in the smear maintain the same relative position to one another that they had *in vivo*, and that one is seeing the cells as one would have seen them in looking directly down on to the mucosal surface. Further, in most nuclei which can be reliably scored,

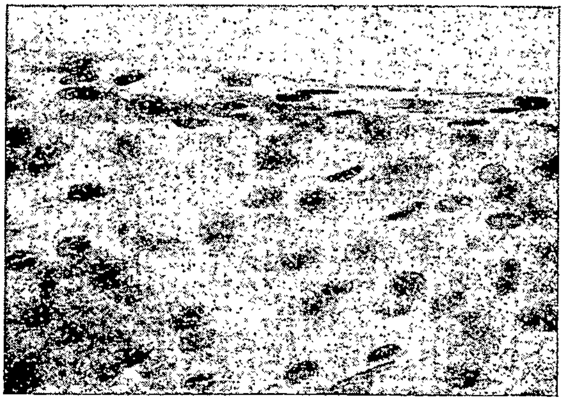


Fig. 1

one can visualize all, or almost all, the nucleoplasm by focusing up and down. Thus one can visualize the sex chromatin regardless of its position in the nucleus.

What is the probability that the sex chromatin body would, by chance alone, appear at the periphery of nuclei as they lie in the optical plane ? Let us assume that the nucleus as observed is an approximate ellipse. If the sex chromatin body lies within a zone bounded by the edge of the nucleus and a locus of points no greater than the thickness of the sex chromocentre measured from the outer to the inner edge (that is, this distance is the altitude when the sex chromatin body is triangular, the radius if the chromatin is plano-convex, and so on), then it will appear to be at the edge of the nucleus. The probability that the sex chromatin will be at the periphery is found by dividing the area of the peripheral zone by the total area of the nuclear ellipse. (Calculations based on volumes are more complicated but give results of the same order of magnitude.)

Ten positive nuclei were selected at random, photographed, and the appropriate dimensions determined. The average thickness of the sex chromatin was slightly less than $0{\cdot}6\mu$, while the nuclei averaged about $7{\cdot}2 \times 9{\cdot}4\mu$. $0{\cdot}6\mu$ is smaller than the average figure reported by Prince, Graham and Barr[2] for the short axis of the sex chromatin body, hence in order to weigh the assumptions against the inference being favoured here, the larger figure will be used. The small area in question, that is, the numerator of the probability fraction, is found to a satisfactory approximation by multiplying 0·8 by the circumference of the ellipse. If the width of nucleus is $2a$, the length is $2b$, then the numerator is $0{\cdot}8 \times 2\pi\sqrt{\{(a^2 + b^2)/2\}} = 21\mu^2$; the denominator equals the area of the nuclear ellipse, $\pi ab = 54\mu^2$. Hence the probability that the sex chromatin will lie at the periphery of the nuclear disk equals 21/54 or, approximately, $P = 0{\cdot}39$; that is, about 39 per cent of the positive nuclei might be expected to show sex chromatin at the periphery by chance alone.

Good thionin-stained[3] buccal smears from ten subjects were used in determining the actual incidence of peripheral sex chromatin. One hundred nuclei were counted as being negative, unscorable, or as having peripheral or non-peripheral sex chromatin bodies. Nuclei were listed as unscorable if the observer could not decide whether they were positive or negative because the nuclei were pyknotic, folded over, disrupted or the like. They were scored as having non-peripheral sex chromatin if they had a non-peripheral chromocentre comparable in size (but not necessarily in morphology) to the sex chromatin. Counts were made by me (observer A), and by another microscopist (observer B). The latter had no knowledge of the purpose of the counts. On the average for both observers, about 86 per cent of the positive nuclei showed the sex chromatin at the periphery. Generally higher counts of observer A could properly be attributed to greater experience with sex chromatin assay, but unconscious bias cannot be ruled out.

Since observer B found a somewhat smaller percentage of peripheral chromocentres than did observer A, observer B's data are used in a test of significance. Observer B counted 252 positive nuclei of which 206 had peripheral chromatin bodies, while in 46 there were non-peripheral bodies. Using the theoretical expectation of 39 per cent peripheral chromocentres gives corresponding figures of 98 peripheral and 154 non-peripheral. $\chi^2 = 199{\cdot}5$, $P < 0{\cdot}005$. This evidence favours the view that the sex chromatin is characteristically at the periphery of the nuclear disk in smears, and since the relative orientation and shape of the nuclei are similar, the same observation applies to cells *in vivo*.

In addition to conditions in tissue cultures[1] mentioned here, preferential localization of sex chromatin has been described also for the cigar-shaped nuclei of smooth muscle cells[4], where the chromatin tended to be at one tip or the other. The mechanisms responsible for these localizations remain to be elucidated.

This work was supported in part by a U.S. Public Health Service research grant C–4866 (C1) Genetics, National Cancer Institute.

I thank Dr. Robert Day and his staff at the Sonoma State Hospital, California, for providing the buccal smears; and Mr. Steven Storey, Stanford University School of Medicine, who acted as observer B.

CHARLES P. MILES

Department of Pathology,
Stanford University,
School of Medicine,
Stanford, California.

[1] Miles, C. P., *Cancer*, 12, 299 (1959).

[2] Prince, R. H., Graham, M. A., and Barr, M. L., *Anat. Rec.*, 122, 153 (1955).

[3] Klinger, H. P., and Ludwig, K. S., *Stain Technol.*, 32, 235 (1957).

[4] Castro, N. M. de, and Sasso, W. da S., *An. Pac. farmacia Odont. S. Paulo*, 11, 249 (1953). Moore, K. L., and Barr, M. L., *Acta Anat., Basel*, 21, 197 (1954). Hungerford, D. A., Donnelly, A. J., Nowell, P. C., and Beck, S., *Amer. J. Human Genet.*, 11, 215 (1959).

Chromosomal Elimination induced by 6-Aza-Uracil

DURING preliminary work in this Laboratory dealing with the effects of some antimetabolites on cell division in *Allium cepa*, it was noted that 6-aza-uracil brings about chromosomal elimination. This elimination occurred at metaphase and anaphase. Root tips from bulbs after reaching a length of 2–3 cm. were treated for 6 hr. with a 1 per cent aqueous solution. The solution was prepared by first grinding the powder and dissolving it in warm tap water. In order to clarify the location of the spindle attachment region of the chromosomes the root tips were treated with a 1 per cent aqueous solution of colchicine for a half-hour prior to fixation. On each preparation the number of normal metaphases, anaphases and telophases and the number of these stages showing elimination were counted. The eliminated chromosomes were studied in detail to determine whether or not the same chromosomes were always eliminated. Aceto-orcein smears and sections stained in iron alum hæmatoxylin were used.

Following 6-hr. treatment with the chemical 13 per cent of the division figures showed eliminated chromosomes. Further examination of mitotic figures indicated that anaphase figures showed this phenomenon more frequently than any other stage with an average of 31·7 per cent of the figures showing elimination. In different roots anaphase elimination ranged from 16 to 54 per cent.

Of 456 metaphases analysed, 6·4 per cent showed eliminated chromosomes. Of these, 66 per cent eliminated one chromosome (Fig. 1A), 24 per cent eliminated two chromosomes, 7 per cent eliminated three chromosomes and 3 per cent eliminated six chromosomes. In most of these cells the metaphase chromosomes were clumped and the eliminated

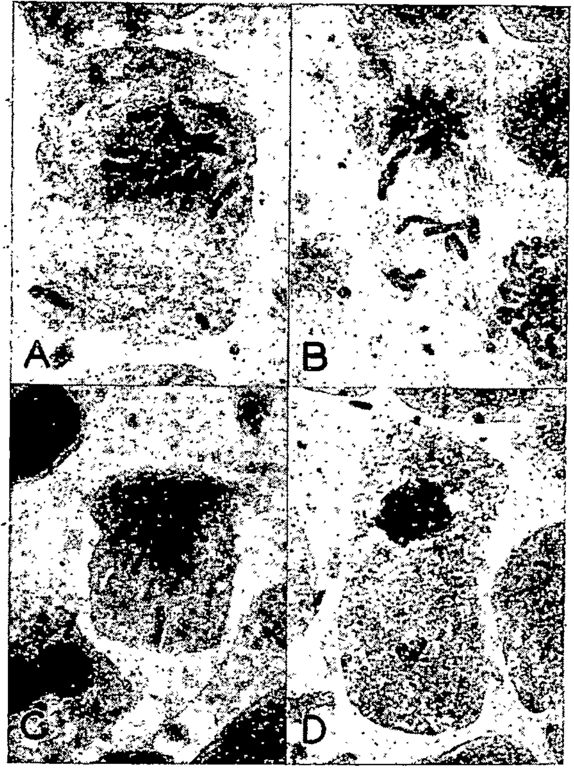


Fig. 1. Photomicrographs of aceto-orcein smear preparations of cells from the root tip of *Allium cepa* showing chromosomal elimination. *A*, metaphase; *B*, anaphase; *C*, anaphase; *D*, telophase. (× 750)

chromosomes were some distance from this mass and near the cell membrane. Sectioned material showed that these divisions with eliminated chromosomes occur in both the plerome and the periblem of the meristematic region. The eliminated chromosome was not always the same. Chromosomes with median, sub-median and sub-terminal centromeres were eliminated. These nuclei were apparently in prometaphase when affected by the chemical.

The anaphase picture is even more aberrant. In some cases two anaphase groups were noted with lagging chromosomes. It is assumed that such cells were in anaphase when influenced by the chemical. In other cases one large anaphase group was present with a number of chromosomes scattered throughout the cell (Fig. 1*B*). A similar effect was noted by Deysson[1] in *Allium sativum* following treatment with sodium oxalate and sodium citrate. In still other cases one large anaphase group was present with a single eliminated chromosome near the periphery of the cell (Fig. 1*C*). In other cases there appears to be no orientation at all. Two hundred and fifty-two anaphase figures were analysed. Of these 31·7 per cent showed eliminated chromosomes. Of these cells showing elimination 35 per cent eliminated one chromosome, 15 per cent eliminated two chromosomes, 16·3 per cent eliminated three chromosomes, 10 per cent eliminated four chromosomes, 7·5 per cent eliminated five or six chromosomes, 3·7 per cent eliminated eight chromosomes and 2·5 per cent eliminated nine or ten chromosomes. The types of chromosomes were eliminated at random.

At telophase in most cases there was a tightly clumped mass of chromosomes in various stages of reversion with one, two, three or four chromosomes near the periphery of the cell forming micronuclei (Fig. 1*D*). In other cells there were two telophase groups of chromosomes, but with apparently varying chromosome numbers. Even following normal anaphases cell-plate formation is apparently interfered with and binucleate cells frequently result. It is possible that these cells were affected in late anaphase, since anaphase separation itself was not interfered with. In those cells showing chromosomal elimination, the resulting nuclei are frequently of unequal size. Telophase figures were difficult to analyse. Of 210 cells studied, 11 cells or 5·2 per cent showed eliminated chromosomes. Of the cells 54·5 per cent eliminated one chromosome and 18·2 per cent eliminated two chromosomes.

Several investigations have been undertaken to ascertain the exact mechanism responsible for chromosomal elimination. Geyer-Duszynska[2] proposed that certain cytoplasmic factors inhibit chromosomal elimination, but she did not suggest any mechanism for the initiation of elimination of chromosomes. However, Avanzi[3] threw some light on the subject by showing that thallium acetate inhibits the functioning of the centromere in some chromosomes which afterwards lag at anaphase. Brachet[4] and Janaki-Ammal[5] also suggested a defective centromere as an explanation for chromosomal elimination. Nicklas[6,7] stated that elimination of chromosomes was due to failure of normal chromosomal fibre action. Vaarama[8] presented evidence that split spindles and timing disturbances resulted in the uneven distribution of chromosomes at the end of metaphase and his diagrams show the failure of some chromosomes to be included in the spindle structure.

Elimination of chromosomes by treatment with 6-aza-uracil may find a possible explanation in the fact that this substance is an analogue of uracil which is a normal constituent of ribonucleic acid. Davies[9] showed that in situations where the normal nucleic acid metabolism of the cell has been upset, the centromeres behave abnormally. He stated that a close interrelationship exists between the centromeric activity, nucleic acid metabolism and chromosomal behaviour. Evidence that ribonucleic acid is needed for the functioning of the spindle was reported by Kaufmann and Das[10], who showed that after treatment with ribonuclease, mitotic abnormalities were found, such as contraction, stickiness, adhesion and clumping of chromosomes, production of aneuploid and polyploid chromosome complexes, tripolar and multipolar spindles, binucleate cells and lagging of chromosomes at anaphase.

This investigation was aided by a grant (*G* 7091) from the National Science Foundation.

M. COLETTE MAHONEY*
E. R. WITKUS
C. A. BERGER

Biological Laboratory,
Fordham University,
New York 58.

---

* Predoctoral Research Fellow, American Association of University Women.

[1] Deysson, G., *Pathologie et Biologie*, Tenth Congr. Intern. Biol. Cell., 9, 500 (1961).
[2] Geyer-Duszynska, I., *Bull. Acad. Polon. Sci.*, **6**, 371 (1958).
[3] Avanzi, S., *Caryologia*, **9**, 131 (1956).
[4] Brachet, J., *Arch. Biol.*, **65**, 1 (1954).
[5] Janaki-Ammal, E. K., *Nature*, **146**, 839 (1940).
[6] Nicklas, D. B., *Chromosoma*, **10**, 301 (1959).
[7] Nicklas, R. B., *Chromosoma*, **11**, 402 (1960).
[8] Vaarama, A., *Hereditas*, **35**, 136 (1949).
[9] Davies, D. R., *Chromosoma*, **8**, 221 (1956).
[10] Kaufmann, B. P., and Das, N. K., *Chromosoma*, **7**, 19 (1955).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Tutorial Student (graduate or about to graduate) in Geography to read for a higher degree and give a limited amount of assistance by demonstrating—The Registrar, King's College (University of London), Strand, London, W.C.2 (August 9).

Lecturer (with a good honours degree, and teaching and/or research experience) to teach Physics to General Degree and G.C.E. Advanced Level; and Grade B Assistants (2) (preferably with teaching and/or research experience) to teach (*a*) Physics and (*b*) Mathematics—The Principal, Norwood Technical College, Knight's Hill, London, S.E.27, quoting Ref. FE.3a/N/1926/7 (August 12).

Research Assistant (with a first- or second-class honours degree in physics, and prepared to register for a higher degree) in the Department of Physics for work in the field of electron spin resonance—The Registrar, The University, Nottingham (August 12).

Lecturer or Assistant Lecturer (with a good honours degree in electrical engineering or physics, with special qualifications in one of the following branches: electrical machines, plasmas, materials, high vacuum or solid state electronics) in the Department of Electrical Engineering—The Secretary, The University, Edinburgh (August 14).

Lecturer (with a specialist interest in and experience of climatology and meteorology) in Geography—The Registrar, University College, Singleton Park, Swansea (August 14).

Assistant (with a special interest in pig husbandry) in Animal Husbandry—The Secretary, The University, Aberdeen (August 19).

Demonstrators in the Department of Geology—The Registrar, University College of North Staffordshire, Keele, Staffs (August 19).

Junior Research Fellow in Pure Mathematics—The Registrar, The University, Sheffield (August 19).

Research Assistant (honours graduate in biochemistry or microbiology, or closely related fields with subsidiary biochemistry) in Microbiological Biochemistry to carry out research on problems of the regulation of metabolism in bacteria—The Secretary of University Court, The University, Glasgow (August 19).

Senior Lecturer (with outstanding qualifications and achievements in a relevant field of electrical engineering or physics) in Electromagnetism—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15 (August 19).

Electronics Designer (with a degree in physics or electrical engineering, or a comparable qualification) in the Department of Chemistry to collaborate with members of the teaching staff in designing and constructing electronic equipment to be used in research work in the Department—The Registrar, The University, Manchester 13 (August 21).

Principal of Thanet Technical College—The Clerk to the Governors, Education Office, Denemount, The Vale, Broadstairs (August 22).

Lecturer in African Anthropology—The Secretary, School of Oriental and African Studies, University of London, London, W.C.1 (August 31).

Research Assistant in the Department of Civil Engineering, to work with Dr. J. M. Zarek on problems of flow measurements under pulsating conditions—The Registrar, King's College, Strand, London, W.C.2 (August 31).

Senior Lecturer in the Department of Biochemistry (Sub-Department of Malting and Brewing)—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15 (August 31).

University Librarian; and Chairs of Agriculture, Archæology, Education, Zoology, Botany, Chemistry, Physics, Biochemistry, Mathematics, Geography, Pharmacy, Civil Engineering and Sociology in the University of Ife, Nigeria—The Registrar, University of Ife (Ibadan Branch), Ibadan, Nigeria (August 31).

Director of Research—Prof. A. C. Frazer, British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey (September 1).

Assistant in Zoology in the Department of Natural History—The Secretary, The University, Aberdeen (September 2).

Research Assistant (preferably male, with an honours degree in social anthropology or psychology) in the Department of Social Anthropology, to conduct an industrial study in the Midlands—The Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh (September 4).

Senior Lecturer (with a higher degree or equivalent qualifications and interested in promoting and leading research) in Mathematics in the Broken Hill Division of the University of New South Wales—The Agent General for New South Wales, 56–57 Strand, London, W.C.2, and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (September 8).

Research Associate (graduate in psychology) in the Department of Social and Administrative Studies, University of Oxford, for a project, sponsored by the National Science Foundation, Washington, concerned with perception—The Secretary, Barnett House, 40 Wellington Square, Oxford (September 10).

Lecturer or Assistant Lecturer in Agricultural Zoology at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 15).

Senior Lecturer or Lecturer in Physics at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

Teaching Fellow (with an honours degree or equivalent qualifications) in the School of Mathematics, University of New South Wales, Australia—The Agent General for New South Wales, 56–57 Strand, London, W.C.2, and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (September 19).

Chair of Veterinary Physiology—The Secretary to the University, The University, Edinburgh (October 1).

Analyst (preferably B.Sc. (Chemistry), and G.R.I.C., or equivalent qualifications) in the Department of Organic Chemistry, for duties mainly concerned with the operation and care of optical equipment including infra-red and nuclear magnetic resonance spectrometers—Mr. W. A. Wightman, School of Chemistry, The University, Leeds 2.

Assistant Experimental Officer (with a pass degree in biology (including zoology), F.I.M.L.T. or equivalent qualifications, and preferably some experience of histological techniques) in the Physiology Department to assist with histological investigations related to the endocrine research programme of the Department—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 61/22.

Forestry Officer (with an honours degree in forestry and a sound practical approach and ability to train and guide supervisory staff, preferably with experience in tropical forestry), to deal with all aspects of forestry work in Hong Kong, particularly with the preparation of management plans and in-service training—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/51/07/T.

Geologist (with at least a first- or upper second-class honours degree in geology, and preferably specialized in petrology) with the Falkland Islands Dependencies Survey for service in the Antarctic for two years—The Crown Agents, 4 Millbank, London, S.W.1, quoting Ref. M3C/53437/NF.

Graduate to participate in biochemical research on coagulation in normal human and human cancer tissue—Dr. W. A. Boggust, Department of Biochemistry, Trinity College, Dublin.

Graduate (with a degree in botany or zoology, and preferably previous experience in cytological work) for work in a section investigating cytogenetic effects of radiation—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berks.

Lecturer or Assistant Lecturer in Physics at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

Lecturer or Assistant Lecturer (particularly interested in petrology and mineralogy) in Geology—Prof. D. Williams, Geology Department, Imperial College of Science and Technology, London, S.W.7.

Lecturer (with special qualifications in pure mathematics) in Mathematics (Grade I or II)—The Registrar (Room 22, O.R.B.), The University, Reading.

Librarian to take charge of a technical library—The Director of Research, The Natural Rubber Producers' Research Association, 48–56 Tewin Road, Welwyn Garden City, Herts.

Microbiologist or Biochemist (recent graduate) for one year's sponsored research on antibiotics—Dr. J. G. David, 9 Gerrard Street, London, W.1.

Research Assistant (preferably with experience in cytology), to take part in a programme designed to study human chromosome abnormalities and associated disorders—The Secretary, Genetics Department, The University, Sheffield 10.

Research Assistant to take part in chromosome studies on human leukæmias and other forms of malignant disease using tissue culture techniques—The Administrative Officer, Mount Vernon Hospital, Northwood, Middlesex.

Research Fellow (with Ph.D. degree or equivalent research experience) in the Chemistry Department, to work in collaboration with Dr. J. J. Kipling on the carbonization and graphitization of high polymers—The Professor of Chemistry, The University, Hull.

Senior Research Associates (2) (preferably experienced electrical engineers or physicists with an electrical engineering background) in the Department of Physics for the development of the proton synchrotron—The Assistant Registrar (Science), The University, Edgbaston, Birmingham, 15.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

The National Foundation for Educational Research in England and Wales. *Technical Education Abstracts from British Sources*, Vol. 1, No. 1 (September–December, 1960.) Pp. 57+xxvii. Subscription rate: 42*s*. per volume of four quarterly issues. (London: National Foundation for Educational Research in England and Wales, 1961.) [305

British Museum (Natural History). The Culicine Mosquitoes of the Indomalayan Area. Part 5: Genus *Aëdes* Meigen, subgenera *Mucidus* Theobald, *Ochlerotatus* Lynch Arribalzaga and *Neomelaniconion* Newstead. By P. F. Mattingley. Pp. 62. (London: British Museum (Natural History), 1961.) 15*s*. [305

University of Reading: National Institute for Research in Dairying. Report, 1960. Pp. 164. (Shinfield: University of Reading: National Institute for Research in Dairying, 1961.) [305

Chelsea College of Science and Technology. Prospectus, Session 1961–62. Pp. 35. (London: Chelsea College of Science and Technology, 1961.) [305

Ministry of Housing and Local Government. Pollution of water by Tipped Refuse: Report of the Technical Committee on the Experimental Disposal of House Refuse in Wet and Dry Pits. Pp. 141+7 plates. (London: H.M. Stationery Office, 1961.) 7*s*. net. [315

Machine Tool Industry Research Association. News Letter, No. 1 (May, 1961). Pp. 19. (Manchester: Machine Tool Industry Research Association, 1961.) [315

Production Engineering Research Association of Great Britain. PERA Report No. 50: Drilling Carbon Steels. Pp. 21. (Melton Mowbray: Production Engineering Research Association of Great Britain, 1961.) [315

University of Reading: Museum of English Rural Life. Report, 1960. Pp. 19 (5 plates). (Reading: The University, 1961.) 1*s*. [16

National Society for Clean Air. Clean Air Year Book, 1961. Pp. 80. (London: National Society for Clean Air, 1961.) 2*s*. 6*d*. net. [16

C.I.B.A. (A.R.L.), Limited. Technical Notes, No. 221 (May, 1961): Aerolite in Scotland. Pp. 12. (Duxford: C.I.B.A. (A.R.L.), Ltd., 1961.) [16

Bulletin of the British Museum (Natural History). Zoology. Vol. 7, No. 7: The Dealfishes (Trachipteridae) of the Mediterranean and North-East Atlantic. By G. Palmer. Pp. 335–351+plate 62. (London: British Museum (Natural History), 1961.) 8*s*. [26

Committee of the Privy Council for Agricultural Research. Report of the Agricultural Research Council for the year 1959–60. Pp. ix+209. (Cmnd. 1362). (London: H.M. Stationery Office, 1961.) 9*s*. net. [26

The University of Leeds. Publications and Titles of Theses, 1958–9. Pp. 79. (Leeds: The University, 1961.) 2*s*. 6*d*. [26

Oundle School Natural History Society. Report for 1960. Pp. ii+45. (Oundle, Peterborough: Oundle School Natural History Society, 1961.) [26

A Study Outline. Prepared by Brian Groombridge. Pp. 57. (London: National Union of Teachers, 1961.) 1*s*. 6*d*. [66

The Imperial Forestry Institute, University of Oxford. Thirty-sixth Annual Report, 1959–60. Pp. 32. (Oxford: Imperial Forestry Institute, The University, 1961.) [66

Department of Scientific and Industrial Research: Forest Products Research. Special Report No. 15: Working Stresses for Structural Laminated Timber. By W. T. Curry. Pp. v+50+4 plates. (London: H.M. Stationery Office, 1961.) 4*s*. net. [66

National Union of Teachers. Some Thoughts on Higher Education: a speech delivered by Sir Wilfred Martineau at the Annual Conference, Brighton, 1961. Pp. 12. Sixth Form Studies: an address by H. D. P. Lee to the Grammar Schools Meeting at the Annual Conference, Brighton, 1961. Pp. 16. Looking Ahead: an address by Sir Ronald Gould, General Secretary of the N.U.T. to the Union's Annual Conference at Brighton, 1961. Pp. 16. (London: National Union of Teachers, 1961.) [66

Department of Scientific and Industrial Research: Road Research Laboratory. Road Research Technical Paper No. 52: The Use and Control of Vegetation on Roads and Airfields Overseas. By K. E. Clare. Pp. vi+42+8 plates. (London: H.M. Stationery Office, 1961.) 3*s*. 6*d*. net. [66

Public Investment and Export. By Sir Walter Benton Jones, Bart. Pp. 7. (Grantham: Sir Walter Benton Jones, Irnham Hall, 1961.) [66

International Nickel Company (Mond), Ltd. Nickel in *S.S. Canberra*. Pp. ii+22. (London: International Nickel Company (Mond), Ltd., 1961.) [66

Department of Scientific and Industrial Research. Hydraulics Research 1960: The Report of the Hydraulics Research Board with the Report of the Director of Hydraulics Research. Pp. iv+79+8 plates. (London: H.M. Stationery Office, 1961.) 5*s*. 6*d*. net. [76

Building Research Station Digest (Second Series), No. 11: Heating in Local-Authority Flats. Pp. 4. (London: H.M. Stationery Office, 1961.) 4*d*. [76

Other Countries

Bulletins de la Société Royal de Zoologie d'Anvers. No. 15: Les Kiwis (Apterygiformes dans les Systemes de Classification. Par Prof. R. Vorheyen. Pp. 11. No. 16: Liste des Mammiferes ayant figuré ou figurant dans les collections du Jardin Zoologique de la Société Royale de Zoologie d'Anvers, depuis sa fondation en 1843 jusju au mois de Juin 1960 (inclus), avec én regard l'année la plus reculée au cours de laquelle leur présence fut étable. Données réunies par Dr. Agatha Gijzen. Pp. 18. No. 17: Contribution à l'Osteologie et à la Systematique des Ratitae. Par Dr. R. Verheyen. Pp. 16. No. 18: l'*Anomalurus jacksoni* de Winton: Répartition, Biologie et Observations en Captivité. Par U. Rahm. Pp. 16 (5 planches). No. 19: Les Poissons d'Aquarium du Bassin du Congo. Par J. G. Lambert. Pp. 50 (27 planches). No. 20: Observations sur la Natation chez les Reptiles. Par R. R. Tercafs. Pp. 19 (6 planches). (Anvers: Société Royale de Zoologie d'Anvers, 1960 et 1961.) [305

Académie Royal de Belgique. Annuaire pour 1961. Pp. 320+7 planches. (Bruxelles: Académie Royale de Belgique, 1961.) [305

Kirkia, Vol. 1, 1960–1961. Edited by H. Wild. (Journal of the Federal Herbarium, Salisbury, Rhodesia and Nyasaland.) Pp. 164+20 plates. (Salisbury: Government Printer, 1961.) 30*s*. [305

Uganda Electricity Board. Thirteenth Annual Report and Accounts for the year ended 31st December, 1960. Pp. 48. (Kampala: Uganda Electricity Board, 1961.) 5*s*. [305

Food and Agriculture Organization of the United Nations. Animal Health Branch Monograph No. 3: A Laboratory Handbook of Veterinary Diagnostic Procedures. By A. J. Stevens. Pp. ii+70. (Rome: Food and Agriculture Organization of the United Nations, 1961.) [305

Annals of the New York Academy of Sciences, Vol. 92, Article 1: Vitamin C. By J. J. Burns, C. G. King and 53 other authors. Pp. 1–332. (New York: New York Academy of Sciences, 1961.) [305

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 42 (April, 1961): Rapid Growth in Populous Areas. Death Claim Payments Exceed $568,000,000 in 1960. Accident Fatalities in Canada. Tuberculosis is Being Wiped Out. Pp. 12. (New York: Metropolitan Life Insurance Company, 1961.) [315

United States Department of Agriculture: Agricultural Research Service. Technical Bulletin No. 1227: A Revision of the Species of *Monopsyllus* Kolenati in North America (Siphonaptera, Ceratophyllidae. By Phyllis T. Johnson. Pp. ii+69 (22 plates). (Washington, D.C.: Government Printing Office, 1961.) 35 cents. [315

United States Department of the Interior: Geological Survey. Water-Supply Paper 1535–C: Calculation and Use of Ion Activity. By John D. Hem. Pp. iii+17+1 plate. (Washington, D.C.: Government Printing Office, 1961.) [315

International Centre for the Study of the Preservation and the Restoration of Cultural Property, Rome. Works and Publications, III: Climatology and Conservations in Museums. Pp. 201–289. (Reprinted from *Museum*, Vol. XIII/No. 4, 1960.) (Rome: International Centre for the Study of the Preservation and the Restoration of Cultural Property, Via Cavour 256, 1960.) [315

Bulletin of the Bingham Oceanographic Collection, Peabody Museum of Natural History, Yale University. Vol. 17, Article 4: Studies on Spawning Behaviour, Egg Masses, and Larval Development in the Gastropod Genus *Conus*. 2: Observations in the Indian Ocean during the Yale Seychelles Expedition. By Alan J. Kohn. Pp. 51. (New Haven, Conn.: Peabody Museum of Natural History, Yale University, 1961.) 2 dollars. [16

Institut Royal Meteorologique de Belgique. Observations Ionospheriques—Station de Dourbes. Février, 1960. Pp. 26. Mars, 1960. Pp. 26. Publications, Série A, No. 16: La Station d'Aerologie de l'Institut Royal Meteorologique de Belgique. Par P. Defrise, A. Maenhout et J. Van Mieghem. Pp. 24. (Bruxelles: Institut Royal Meteorologique de Belgique, 1961.) [16

Fact Sheets on U.S. Nuclear Power Projects. Third edition. Pp. iii+51. (New York: Bozell and Jacobs, Inc., 230 Park Avenue, 1961.) [16

Food and Agriculture Organization of the United Nations. Report of the FAO/WHO Seminar on Problems of Food and Nutrition in Africa South of the Sahara, Lwiro, Bukavu (Congo), 18–29 May, 1959. (FAO Nutrition Meetings Report Series, No. 25.) Pp. ix+93. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1961.) 5*s*.; 1 dollar. [26

National Academy of Sciences—National Research Council. Nuclear Science Series. NAS–NS 3022: The Radiochemistry of Vanadium. By J. L. Brownlee, Jr. Pp. v+76. 75 cents. NAS–NS 3029: The Radiochemistry of Ruthenium. By E. I. Wyatt and R. R. Rickard. Pp. vii+78. 1 dollar. (Washington, D.C.: Office of Technical Services, Department of Commerce, 1960 and 1961.) [26

United States Department of Commerce: Coast and Geodetic Survey. Publication 65–1, Part 19: Plane Coordinate Intersection Tables (2½ minute), Massachusetts. Pp. iii+27. 25 cents. Technical Bulletin No. 16 (January, 1961): Erosion and Sedimentation, Eastern Chesapeake Bay at the Choptank River. By G. F. Jordan. Pp. ii+8+1 map. 40 cents. (Washington, D.C.: Government Printing Office, 1961.) [26

Legislative Council of Fiji. Council Paper No. 5 of 1961: Geological Survey Department—Annual Report for the year 1960. Pp. ii+10. (Suva: Geological Survey Department, 1961.) 1*s*. 6*d*. [26

Chicago Natural History Museum. Fieldiana: Geology. Vol. 14, No. 5 (April 26, 1961): Eurypterids of the Devonian Holland Quarry Shale of Ohio. By Erik N. Kjellesvig-Waering. Pp. 79–98. 85 cents. Fieldiana: Zoology. Vol. 41, No. 3, (April 2, 1961): New Caledonian Land and Fresh-Water Snails: an Annotated Check List. By Alan Solem. Pp. 413–501. 2.50 dollars. Vol. 42, No. 9 (April 7, 1961): Philippine Zoological Expedition, 1946–1947. A New Genus and Species of Cantacaderina Lace-Bug from the Philippines. (Hemiptera: Tingidae). By Carl J. Drake. Pp. 115–118. 20 cents. (Chicago: Chicago Natural History Museum, 1961.) [66

United States Department of the Interior: Geological Survey. Professional Paper 303–D: Geology of the Shaviovik and Sagavanirktok Rivers Region, Alaska: Exploration of Naval Petroleum Reserve No. 4 and Adjacent Areas, Northern Alaska. Part 3: Areal Geology. By A. Samuel Keller, Robert H. Morris and Robert L. Detterman. Pp. iii+169–222+plates 21–26. (Washington, D.C.: Government Printing Office, 1961.) [66

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 60–31: Belleville and Wellington Map-Areas. Ontario, (30 N/14 and part of 30 N/15 W½; 31 C/3 and part of 31 C/2 W½). By B. A. Liberty. Pp. ii+9+2 maps. 50 cents. Geological Maps—Preliminary Series. Map 41–1960: Big Bald Mountain, Northumberland County, New Brunswick. Map 43–1960: Mingo Lake, Baffin Island, District of Franklin, Northwest Territories. Map 48–1960: St. Mary Bay, Digby, Yarmouth and Charlotte Counties, Nova Scotia and New Brunswick. Map 50–1960: North Spirit Lake, Kenora District, Ontario. Map 52–1960: Whiskey Jack Lake, Manitoba. Map 1–1961: Hopewell, (West Half), Nova Scotia. (Ottawa: Queen's Printer, 1960 and 1961.) [66

Annals of the South African Museum. Vol. 46, Part 1: Cercopithecoid Fossils, including a New Species, from Taung, Cape Province, South Africa. By Leonard Freedman. Pp. 1–14+plates 1–10. 36c. Vol. 46, Part 2: Some New Caddis Flies (Trichoptera) from the Western Cape Province, III. By Dr. K. M. F. Scott. Pp. 15–34. 33c. Vol. 46, Part 3: Some South African *Cladocera* collected by Dr. A. D. Harrison. Pp. 35–46. 21c. Vol. 46, Part 4: Pogonophora from South Africa. By Eve C. Southward. Pp. 47–53. 13c. (Cape Town: South African Museum, 1961.) [66

Companhia de Diamantes de Angola (DIAMANG). Servicos Culturais. Dundo—Lunda—Angola. Museu do Dundo. Publicacoes Culturais No. 52: Subsidios para o Estudo da Biologia na Lunda—Estudos Diversos (XX). Pp. 271. (Lisboa: Companhia de Diamantes de Angola, 1961.) [66

United States of America. Scientific Progress, The Universities, and the Federal Government: Statement by the President's Science Advisory Committee. Pp. v+33. (Washington, D.C.: Government Printing Office, 1960.) 15 cents. [96

Indian Forest Records (New Series.) Statistical—1957. Vol. 1, No. 1: A Statistical Study of the Variability of Physical and Mechanical Properties of *Tectona grandis* (Teak) Grown at Different Localities of India and Burma and the Effects of the Variability on the Choice of the Sampling Plan. By K. R. Nair and H. K. Mukerji. Pp. i+49. Rs. 8.00.; 13*s*. Silviculture. Vol. 10, No. 9: Standard Volume Tables for *Dipterocarpus macrocarpus* Vesque. By S. K. Seth and S. N. Dabral. Pp. 239–246. Rs. 1.55.; 2*s*. 6*d*. (Delhi: Manager of Publications, 1960.) [126

Editorial and Publishing Offices of "NATURE"

MACMILLAN & CO., LTD.

ST. MARTIN'S STREET, LONDON, W.C.2

Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to

T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.

Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office

FURTHER ADVANCEMENTS IN SPACE RESEARCH

COMMENTING on the latest Soviet achievement in space science, last year's president of the British Association for the Advancement of Science, Sir George Thomson, said: "If you have done a thing once it is not difficult to do it several times. It seems to me a cheerful Bank Holiday event, and we should thank the Russians for arranging it". After pondering these remarks they would appear less waggish and more profound than at first. The first remark is, however, rather naïve, for there can be no doubt that this second successful attempt to put man into orbit embraced more scientific 'things', with consequent results from which much can be gleaned, than were inherent in the first successful attempt, with Major Yuri Gagarin in the space-vehicle. The second remark, however, needed making. The news would be cheerful whether the feat was achieved by Soviet scientists, American scientists or those of any other nationality: for here, once again, is the manifestation of great triumphs for scientists, coupled with the heroism of another—this time Major G. S. Titov, of the Soviet Air Force.

The spaceship-satellite *Vostok 2*, 1961τ, launched from the U.S.S.R. at 06.00 hr. U.T. on August 6, carried the second man to travel in orbit around the Earth. The satellite, which weighed 4,730 kgm. excluding the final-stage rocket, was projected into an orbit inclined at 64° 56′ to the equator and having an orbital period of 88·6 min. Its height above the Earth varied between 178 km. at perigee and 257 km. at apogee. Its radio transmitters operated on frequencies of 15·765, 19·995, 20·006 and 143·625 Mc./s. *Vostok 1*, which carried Major Gagarin in the first manned orbital flight on April 12 (see *Nature*, **190**, 307; 1961), made one revolution about the Earth. *Vostok 2* made 17 revolutions, and, since the orbital period was such that the geographical track on the seventeenth revolution was almost identical with that on the first, Major Titov made his descent in the same region as Major Gagarin. Major Titov landed safely near Saratov, some 500 miles south-east of Moscow, after a flight lasting 25 hr. 18 min. The main object of this second orbital flight was to test the effects on a human being of a longer journey in orbit. Major Titov reported that his condition was excellent throughout his epoch-making flight, and the prolonged period of weightlessness appeared to have no ill-effects. He slept for 8 hr. and ate several meals, taking the food from tubes, as well as operating various manual controls in the satellite, observing the Earth from one of the three portholes, and sending messages to the peoples of the countries he passed over.

On this second occasion more details of experimental conditions were revealed, and even some preliminary results announced—an excellent and cheerful omen for scientific advancement on an international scale. Hitherto, far too much lip-service has been paid by Soviet and American political leaders to the ideal of sharing their scientific results; but little has so far accrued.

There can be no gainsaying the fact that the Soviet scientists are in the vanguard of space travel; but it does not follow that they are ahead of others in space research *in toto*. There are many who believe that the U.S.S.R., through their awe-inspiring achievements, are deliberately playing to the political gallery. It is obvious that their successes are enhancing the political prestige of the Soviets throughout the world, especially in scientifically and culturally backward and emergent countries. But it would be wiser for world leaders in policy to look on these successes in space-travel as the result of hard and inspired scientific endeavour made by scientists who are highly respected and encouraged by their political leaders.

Even in their own country, certainly outside it, Gagarin and Titov are presented to the public as great heroes, which they surely are. But it is essential to recognize that the real prime-movers in these fantastic feats are the scientists and technologists who, through their ingenuity and devoted effort, have made such feats possible. The lay public, both Soviet and millions of others, will acclaim the heroism of men such as Gagarin and Titov; but world leaders would be better advised to take heed of the words of Prof. Nikolay Barabashov, who said: "The general triumph of Soviet *scientists and engineers* consists in that they have created a spaceship controlled by man".

There are still too many otherwise intelligent people who grudgingly now admit that we have had science thrust upon us. They will continue that attitude at their peril, for this is the Age of Science, and, like it or not, we must do all we can to give moral, and especially practical, support to our scientists (*in all* branches). This is best done by learning and teaching more about science, for then shall we treat scientists for what they really are—world leaders in their own sphere, not 'backroom boys'.

Walter Bagehot said that: "Governments are only strong when public opinion is definable and decided". The strength of democratic Governments must now be enhanced by the support of a public opinion inspired by knowledge of, and greater respect for, science and scientists.

NATIONAL ORGANIZATION OF SCIENTIFIC RESEARCH

THE last report of the Council for Scientific and Industrial Research stressed the growing importance of the Council's influence on university research. This necessarily arises out of the Council's concern with the balance of research within and between the scientific disciplines, and its effort, at the request of the Advisory Council on Scientific Policy, to bridge any important research gaps which come to light and thus promote a healthy balance of research effort. It also arises as a counterpart of the Department's expanding programme of research grants and training awards, which are bound to affect university developments quite apart from the attempts of the Council to encourage co-operative effort, as, for example, in grants for the provision of nuclear reactors for training and research or by specia encouragement for research in certain fringe subjects, such as biophysics, geophysics, meteorology and oceanography, where the Council found insufficient research effort in the country.

Generally, the Council regards the balance of research effort as reasonable in the main streams of development within the chief scientific disciplines, though, to keep this effort adequate and to develop it flexibly, substantial and increasing resources must be given to research through existing channels. The Council is already prepared to make grants for longer periods than hitherto and help initiate projects which for one reason or another seem to require its specially extended support; but nevertheless, when university researches are well established, the Council considers that they are best supported from normal university funds, and the Council welcomes the action of the University Grants Committee in making arrangements for the universities to seek funds for the new quinquennium 1962–67, to continue researches which have been established with the aid of grants from the Department of Scientific and Industrial Research. The Council has already invited the universities concerned to consider whether they should seek funds from the University Grants Committee to carry on most of the research programmes receiving current grants and is discussing these arrangements with the universities.

In this situation, a statement on "Scientific Progress, the Universities and the Federal Government", issued last November in the United States by the President's Science Advisory Committee, to which Dr. Glenn T. Seaborg, chairman of the United States Atomic Energy Commission, made some reference in addressing the American Physical Society on April 26, on atomic energy research, is of particular interest. Dr. Seaborg did not discuss the Commission's programme of applied research; his address was rather on basic research in physical science, and before discussing the Commission's activities in basic research he quoted freely from the Science Advisory Committee's statement to illustrate his own fundamental philosophy of basic science.

The statement, which deals essentially with the problems arising in the advancement of science by basic research and the graduate education of scientists, stresses first the urgent need for scientific progress and for investment in science to that end, without, however, either overlooking the returns such investment also yields in the quality and humanity of our investigation or suggesting that scientific research and scientific education are the only kinds of learning that matter. It affirms unequivocably that basic research and graduate education go best together at every possible level, and equally it insists on the disappearance of the barriers between 'pure' and applied science. Basic research depends in the end on the imaginative powers and scientific skill of its practitioners, and partly because of this the apprentice scientist, even if his subsequent career were not attained in basic research, learns best in an atmosphere of creative research with the depth of understanding of a subject that only comes with such experience.

The President's Science Advisory Committee is under no illusions that this always obtains in the universities or in the Federal agencies, but it suggests that if a research field is to attract able young scientists, it generally needs roots in the universities. A scientific field which has no research life in the universities is at a grave disadvantage in recruiting new talent, nor can research be sustained without teaching, and it is always important for research institutions to maintain effective connexions with students. Not all basic research should or could be pursued in universities, but where it is done elsewhere the Committee believes that special efforts should be made to take advantage of its educational value.

The Committee thus came to consider the role of the Federal Government, which by 1957–58 was providing 70 per cent of the funds for research in American universities, three-quarters of which came from either health or defence agencies. The use of research contracts and research grants is then appraised, and, in spite of the high quality of much research, particularly in nuclear physics, carried out under Government contracts, the Committee regards the research grant, on balance and in the long run, as the better instrument for basic research. It points out, however, that the support of research projects does not, by itself, fully meet the needs of the Federal Government, and that no narrow or single instrument or method of action will serve the Government's purpose.

This appears particularly in considering the fields of science which are comparatively neglected and for which it is difficult for the universities to find funds or facilities. Like the Advisory Council for Scientific Policy and the Council for Scientific and Industrial Research in Britain, it suggests that such areas of scientific promise call for action by the central Government in initiative and support, and one such area instanced is oceanography. Nevertheless, the statement suggests that the greatest interest of the central Government is to see that university science is as strong as possible, and whether basic research and graduate education in the United States are adequate in quantity and quality depends primarily on the Government: either the Federal Government finds the policies and resources which permit the universities to flourish and discharge their duties adequately, or no one will.

The Committee recognizes that the American universities have themselves much to do and that they have been slow in finding effective ways of encouraging scientific research and training at all the new levels and in all the new ways that scientific advance makes possible. Graduate education is not so good as it should be, and new fields of study are ignored because they inconveniently cross departmental barriers. The Committee does not, however, share the view that Government support is necessarily subversive of academic freedom, though it recognizes its dangers; but it suggests that the most important single task of the universities is to maintain their own standards of freedom and excellence in this period of growing dependence on the State. In the United States, as in Britain, the main trouble in the universities is neither failure of understanding nor communication, but simply lack of means, and it is from this similar context that the recommendations of the statement derive their special interest in Britain at this juncture.

Some specific conclusions should, however, be noted; first, not only on account of their relevance to the general organization of scientific effort, but also equally for their bearing on the expansion of the universities and the colleges of technology. Stress is laid first on the support of excellence. It is of the first importance that national support for both research and teaching should aim at sustaining and reinforcing outstanding work wherever it may be found. Examination of the implications of this conclusion could go far to resolve some recent controversies, and it should be noted that the President's Science Advisory Committee also considers that additional centres of excellence are urgently needed, that graduate education should be expanded and include a genuine experience of research, and that it is important to attract a larger number of talented students to science as a career. Graduate education in science itself needs constant modernizing, and its financing should be continuously and flexibly reinforced, particularly by fellowships.

From what has already been noted it might be expected that the Committee would conclude that new fields of research and education need particular encourgement and that separate research institutions should be avoided wherever possible It is emphatic that in the absence of special considerations the university is the best place for basic research and concludes that new laboratories for special fields should be attached to the universities whenever it is practicable, and that universities should make full educational use of such facilities. It is interesting to note the Committee's opinion that scientists outside the universities can play a fruitful part in graduate education, and it is not only in the United States that there are opportunities demanding exploration and exploitation by the Government, by industry, by universities, and by colleges of technology.

Of the Committee's recommendations, some six were selected by Dr. Seaborg to express his own philosophy on basic research. First, there is the general responsibility. "In view of the growing importance of scientific research to National security and welfare, all parts of the National community should assume a greater responsibility for supporting, strengthening and expanding basic research in graduate education." Secondly, turning to Government responsibility, "Federal support for basic research and graduate education in the sciences should be continued and flexibly increased so as to support excellence where it already exists and to encourage new centres of outstanding work". Thirdly, "The Federal Government should continue and enlarge the practice now followed with great success in a few agencies of providing research support over long terms and for broad objectives". Fourthly, "The Government should strengthen its ability to establish general policies governing its support of basic research and graduate education at the universities". Fifthly, as regards the universities, "Universities should accept primary responsibility for ensuring that their growing partnership with the Government reinforces their freedom and excellence", and sixthly, and generally, "It should be a general basis of policy and action that basic research and the education of scientists go best together, that they are inseparable functions of the Universities".

These recommendations are cited by Dr. Seaborg to illustrate his own approach to basic research, an approach which has not been modified by his experience as chairman of the Atomic Energy Commission. He does not suggest that these are the only important recommendations of the report, but it is interesting to note how largely the Advisory Council of Scientific Policy in Britain appears to be guided by similar views. Nevertheless, the connexion between research and teaching is emphasized in a much more forthright manner than has been apparent in recent discussions in the United Kingdom, and the stress laid on excellence gives no support for the mediocrity and triviality which Sir Eric Ashby and others have criticized.

If this report, therefore, contains much that is of interest to the University Grants Committee and the Robbins Committee also, as well as to the Ministry for Science, Dr. Seaborg's examination of the policies and programmes of the Atomic Energy Commission in the light of these findings is of particular interest in the context of adjustment of Britain's scientific effort with which the Advisory Council on Scientific

Policy is concerned. Dr. Seaborg points out that the budget of the National Science Foundation has already been increased by some sixty-five million dollars to implement some of these recommendations, and two-thirds of this is for support of basic research in the sciences and to improve research facilities. Further, he stresses the close co-operation and exchange of information that already exist between the Commission and the other four Federal agencies that disburse about 90 per cent of Federal funds for basic research. There is some reason for concern as to whether that liaison is sufficiently effective in Britain, and this may be one reason for the misgivings that have been openly expressed as to the deployment of our scientific man-power. At the Baird Memorial Lecture in Glasgow, in May, Dr. S. C. Curran suggested that scientific advance in some fields was hamstrung by the excessive effort being made in such fields as nuclear energy, for example, and on the other hand, the existing relations between the Atomic Energy Authority and the Central Electricity Generating Board appear to offer at least some possibility of duplication of effort. It is by no means clear that the Board's new nuclear laboratory on which £1 million is to be spent, with a staff of 250–500, can do anything which could not be done with the Authority's existing plant and research teams. While there are advantages in the decentralization of nuclear research and engineering, they will not be realized without a careful review of organization and some paring of the effort for which the Authority has hitherto been receiving a consulting fee of £300,000 from the Board.

Dr. Seaborg's remarks about the Atomic Energy Commission's relations with the National Science Foundation and with the universities are of special interest at the present time. Under an agreement with the Foundation, the Foundation takes care of the Commission's projects which involve support of university research and training reactors, but while the universities are part of the foundation on which the United States nuclear energy programme is built, the pattern of arrangements varies widely. At Brookhaven National Laboratory, for example, which is managed for the Commission by Associated Universities, Inc., some 170 scientists are engaged in research and about 300 more from university faculties spend considerable time each year at the Laboratory on research projects. The Argonne National Laboratory is operated under a contract with the University of Chicago, but the thirty-one constituent schools of Associated Mid-West Universities play a vital part in the Laboratory's programme with more than 100 postgraduate research workers, while the Laboratory also operates the International Institute for Nuclear Science and Engineering. Again, at the Lawrence Radiation Laboratory in the University of California, Berkeley, where about 250 graduate workers are employed, basic research is undertaken by faculty members on a part-time basis and by full-time non-academic personnel. Again, at the Oak Ridge National Laboratory, the Union Carbide Nuclear Co. co-operates in making the facilities available to universities, for example, through the Oak Ridge Institution of Nuclear Studies, comprising 39 universities in the south.

Dr. Seaborg gives some figures to indicate the extent of the Commission's research effort. At the end of the past fiscal year, the Commission had more than 900 contracts totalling more than thirty-six million dollars for unclassified basic research in the physical and biological sciences with some 162 educational institutions. Some 330 schools have been assisted with grants totalling 18·5 million dollars, and loans of nuclear materials valued at eleven million dollars and about 1,000 fellowships have been awarded. Basic research supported by the Commission in the physical sciences is mainly in physics and mathematics (including high- and low-energy nuclear physics, computor research and mathematics), metallurgy and related sciences; chemistry and controlled thermonuclear research.

Dr. Seaborg referred to the way in which the Commission is seeking to strengthen research in the metallurgy and material sciences throughout the country, and also to the existence of areas where imaginative contributions of the highest creative type were needed to advance the country's welfare not simply in the nuclear energy programme but in such fields as the long-range transmission of electric power, long-range prediction of weather, the desalting of sea-water, and the application of biophysics and biochemistry to the whole problem of living matter. He was prepared to consider allowing the Commission's National Laboratories to contribute to the solution of such problems even if this went beyond their present functions.

The choice of method or organization is bound to be influenced by the objective or subject of a research project and that alone is sufficient reason for requiring variety and flexibility in the organization of research, whether fundamental or applied. While the points made by the President's Science Advisory Committee and by Dr. Seaborg are not new, they direct attention to first principles to which full regard should be paid alike by the University Grants Committee in considering the expansion of university education and by the Advisory Council on Scientific Policy and the Council for Scientific and Industrial Research in guiding the deployment of Britain's research effort. Furthermore, the extent to which that expansion and research effort are interlocked points to the importance of some co-ordinating authority at a higher level, if frustrations or waste are not to occur. Both through its research grants and its post-graduate training awards the Department of Scientific and Industrial Research cannot now help strongly influencing university development or even policy, however carefully it may seek to avoid infringing academic influences. For example, after the Department's last annual report was issued a grant of £750 was announced towards a project to which the University Grants Committee and the National Institute for Research in Nuclear Science was also contributing.

It is important that Britain's organization for the support of research should be flexibly and also effectively co-ordinated; the choice of project poses difficult problems also. With whatever justice it may be argued that our present expenditure on nuclear research, whether through the Atomic Energy Authority or more generally, is excessive, those who so argue are usually concerned at the neglect of a particular field. Dr. S. C. Curran, for example, was concerned at our neglect of space research and lack of a satellite programme. Here again we are concerned with a field calling for particularly costly equipment, but Sir Harrie Massey, in a recent statement referring to the growing interest in space research in British universities, suggested that such research could be considerably extended without distorting our scientific effort. This, he thought, was not a narrowly specialized field of work, but required more facilities, and collaboration in a European organization should not preclude a separate programme of research in Britain.

At the anniversary dinner of the Biochemical Society on March 29, Lord Hailsham said that it was important that not merely the structure but also the philosophy of our present arrangements for the organization and encouragement of research should be brought home to scientists. That is true enough, but it is no less important that the magnitude of Britain's effort should be seen to be in proportion to national needs, and it is from misgivings as to the scale of our effort that much of the present malaise arises. That is, of course, a main theme in the pamphlet, *Science and the Future of Britain*, issued in March by the Labour Party, but it is not simply a party political issue; it is fostered also by procrastinations and petty-mindedness that, as in such matters as scientific libraries, have already squandered resources in both finance and man-power.

Lord Hailsham quite rightly insists that the scientific planning of our research effort must be done by scientists themselves. Ultimately they must decide on the project and the means by which it is to be secured. That, however, ultimately turns on the availability of resources, and while, as Lord Hailsham said, the function of the Government is to see that the necessary research was proceeding and that its results were being applied, the fact that for the most part Government acts here indirectly through encouraging science in the universities, stimulating industrial research and organizing research institutes in fields where necessary, does not affect the fundamental issue of the availability of adequate resources.

The Minister for Science appears to be satisfied that the Research Councils, the Atomic Energy Authority, and the Advisory Council on Scientific Policy provide him with an adequate advisory structure, though some questions of ministerial relations could well be asked. He referred on this occasion particularly to the relations between the Department of Scientific and Industrial Research and the Medical Research Council and the University Grants Committee; the value of independent sources of finance for research is indisputable. Nevertheless, we come back once more to the question of the scale of the research effort in Britain. If the existing structure has proved its ability to prevent dissipation of our research effort on projects which are not easily justified in relation to viable national needs, it is the more important that its scale should be commensurate with real needs rather than political convenience. It is at the magnitude of our effort as well as at its deployment that Lord Hailsham should be looking, and making sure that departmentalism is no more an obstacle at ministerial level than in universities or elsewhere to the full exploration of new opportunities or a cover for waste and conservatism. Further, the implications of the relation between teaching and research also deserve examination, and if the President's Science Advisory Committee is right in its conclusion, some recent developments such as that at the Radar Research Establishment call for examination by the Minister for Science from that point of view if precious resources of man-power and equipment are to be used to the fullest advantage.

A REFERENCE BOOK FOR BIOCHEMISTS

Biochemists' Handbook

Edited by Dr. Cyril Long. Consultant Editors: Prof. Earl J. King and Prof. Warren M. Sperry. Pp. xxii + 1192. (London: E. and F. N. Spon, Ltd., 1961.) 168*s*. net.

THIS handbook caters for fairly big-handed biochemists: it consists of almost twelve hundred pages of techniques, tables, facts, figures and references on a very wide range of biochemical topics, and includes the contributions of 171 authors.

The book is divided into six main sections. Section 1, called "Chemical Data", deals in the main with commonly used biochemical techniques like spectrophotometry, chromatography and manometry, but contains, in addition, information about isotopes, an excellent account of volumetric analysis (with many tables of buffer solutions and a very full list of acid dissociation constants), a brisk contribution on organic chemical nomenclature, and some useful data on the composition, structure and analysis of polysaccharides.

Section 2, "Data on Individual Enzymes", deals with nearly three hundred enzymes, which are arranged in groups according to the type of reaction they catalyse—hydrolysis, transfer, dehydrogenation, etc. Then follow a hundred pages on "Metabolic Pathways", giving a condensed and up-to-date account of important reaction schemes. This chapter is intended primarily for biochemistry teachers.

Section 4 (pp. 637–936) is called "Chemical Composition of Animal Tissues and Related Data". Presented mainly in the form of tables, this section takes us tissue by tissue through one, and sometimes several different species of animal to give an extraordinary wealth of data (46 pages are given to the composition of blood alone). Section 5 concerns itself with the chemical composition of plant tissues, and is considerably shorter than the previous chapter despite having "been given somewhat greater prominence than is customary". After a brief introduction on plant nomenclature and classification (a subtle 'dig' at biochemists who are all-'chemist' and no 'bio-' ?), subdivisions are made between one type of chemical constituent and another. Individual presentations vary, but in general the compounds, with their chemical characteristics and natural sources, are listed with appropriate references, in tabular form. The book ends with a short section on "Physiological and Nutritional Data", which is rather on the fringe of biochemistry and has a fairly strong medical bias.

If, as the authors state in the preface, this book is meant to serve as a source of material for research biochemists, criticism could be levelled at some sections. The chapter on enzymes is so condensed and indigestible that probably both specialist and non-specialist alike will prefer to reach for 'Dixon and Webb'. One or two accounts of enzymes do not cover the subjects fully, and tend to be a bit unsatisfying because the selection of data has been too stringent. (The account of β, 2–1 fructanases, for example, does not even mention the work of Bacon and Edelman.) Also, in Section 1, perhaps it might have been a better idea in a book of this kind to have concentrated more on drawing up fuller lists of data (R_F values of more compounds in the chapter on paper chromatography, for example) rather than to have written so much on theory and technique, which are covered extensively in more specialized text-books. When the book concentrates on condensed information in the form of tables, figures and references (which is most of the time), it is excellent.

There are, however, one or two misprints; on pp. 953–4, '*Hordenum*' appears four times: it should be *Hordeum*. '*Liriodendro*' is printed instead of *Liriodendron* on p. 951. On p. 114 ribonucleose is written for ribonuclease, and on p. 515, the word 'succharides' might cause some hilarity.

The main strength of the book lies in its references, and in this respect alone it more than justifies the authors' claims that it should be regarded primarily as a source of biochemical material. If information is sought, one is almost certain to find it in the *Biochemists' Handbook*—and even if it is not there the references will indicate the source.

D. RECALDIN

REVIEWS OF GENETICS

Principles of Genetics

By Prof. Eldon J. Gardner. Pp. vii+366. (New York and London: John Wiley and Sons, Inc., 1960.) 60*s*.

Principles of Plant Breeding

By Prof. R. W. Allard. Pp. xi+485. (New York and London: John Wiley and Sons, Inc., 1960.) 9 dollars; 72*s*.

Genetic Research

A Survey of Methods and Main Results. By Prof. Arne Müntzing. Pp. 345. (Stockholm: LTs Forlag, 1961.) n.p.

THESE three books are written for different readers. The first is intended "primarily for the college student taking his first course in genetics" and presents the fundamentals of genetics accurately in a straightforward and readable manner. The first eight chapters are concerned mainly with Mendel's experiments, the mechanics of the transmission of hereditary units, their interaction and the ways in which they can be located on the chromosomes by studies of linkage and recombination. Then, among other topics, the author deals with structural alterations of chromosomes, sex determination, mutation and alleles and compound loci. He indicates some present trends in chapters on physiological genetics and population genetics. One of the pleasing features of the book is the skilful way in which experimental results from both higher and lower organisms are woven into the text; and with equal facility, the student is given a hint of the usefulness of statistics and chemistry in modern genetics. It is a pity that the book is so expensive.

Prof. Allard's book is not greatly different in scope from some of the others on this subject. The reader is assumed to have a knowledge of cytogenetics, and emphasis is placed on principles of plant breeding rather than on breeding procedures for particular crops. After a brief discussion of patterns of evolution in cultivated plants and a general account of their reproductive systems, the author separates self-pollinated and cross-pollinated crops and, in each, in turn, deals with the genetic basis of breeding and with breeding methods. In my own opinion, Prof. Allard loses some possible clarity by not considering the roles of genotype and environment in continuous variation apart from mating systems. One of the most admirable sections of the book is that on breeding for resistance to disease, in which the interactions of host and parasite are very fully discussed; unfortunately, resistance to virus diseases is completely overlooked. The author writes in a vigorous and lucid style, but at times the subject-matter is condensed to the point of oversimplification; contrary to the author's statements, cultivated potatoes are, regrettably, classified into several species and many diploid edible bananas are sterile. Several Latin names are wrongly quoted and there are other printing errors that could well be corrected in subsequent printings.

Prof. Müntzing attempts in his book to cover the whole field of pure and applied genetics for the benefit of "everybody working with living material or teaching biology". Indeed, in slightly more than 300 pages, he deals with more topics than are included in the two other books under review. The reader will find chapters on all the traditional aspects of genetics —Mendelism and the developments therefrom, the structure of chromosomes, speciation, plant and animal breeding and eugenics. The facts are described accurately and clearly, but in order to cover so much ground many behaviours have to be treated in a somewhat cursory way; for example, the topic of self-sterility in plants is dismissed with a brief account of gametophytic incompatibility; a short description of the biochemical genetics of eye colours in *Drosophila* serves as a picture of the gene-controlled formation of pigments in plants and animals. Nevertheless, the account of genetical investigations in bacteria and viruses and the discussion of the nature of the gene are masterly condensations of available information. I was left with the impression that the book is too advanced for the amateur geneticist but, without a more extensive bibliography, falls short of being a satisfactory text-book for the intending professional. It is an unfortunate compromise. The book is well reproduced with excellent illustrations and diagrams. That the text is a translation is still evident; apart from clumsy sentences, words are sometimes wrongly used: "bacteriophage . . . creeps . . .", "electron microscopes . . . large power of dissolution".

KENNETH S. DODDS

PROGRESS IN CHEMISTRY

Modern Aspects of Inorganic Chemistry

By Prof. H. J. Emeléus and Dr. J. S. Anderson. Third edition, revised and reset. Pp. xi+611. (London: Routledge and Kegan Paul, Ltd., 1960.) 35*s*. net.

Radioactive Tracers in Chemistry and Industry

By Pascaline Daudel. Text augmented and revised by the author. Translated by Dr. Ulli Eisner. Pp. xi+210. (London: Charles Griffin and Co., Ltd., 1960.) 36*s*. net.

Annual Reports on the Progress of Chemistry for 1959

Vol. 61. Edited by Dr. R. S. Cahn. Pp. 476. (London: The Chemical Society, 1960.) 40*s*.

THE book by Emeléus and Anderson is now so well known that any detailed description of its contents is superfluous. The new edition has been carefully revised, new sections have been added, and some of the text in earlier editions which is now less important has been omitted, so that the size of the book has not been substantially increased. This is important for two reasons. First, the very reasonable price of a book of this character has been maintained. Secondly, the amount of material which can be dealt with in lecture courses and read by students is limited, and while a book of this size provides something which an average student may be expected to master, a larger book would be less acceptable.

Among the many new topics dealt with, mention may be made of a short but instructive account of ligand field theory, the chemistry of ferrocene and related compounds, sulphur nitride and its derivatives, and much new material in the chapters on co-ordination compounds. The last subject, with the main emphasis on chemistry, has always been a notable part of the book, and I know of no other account which can match it for clarity and completeness. In most of the other sections which were dealt with in previous editions, such as the boron and sulphur hydrides, significant new material has been added. The whole book is worthy of the highest praise from the point of view of the selection of material and also for its treatment.

One or two minor matters which were noticed are mentioned here not for criticism but in the hope that the authors may see their way to consider them in a future edition. I think the rather short section on molecular orbitals, which is quite largely drawn on in later parts of the book, will prove difficult to many students. The symbols for hybrid states on pages 61 and 69 are not clearly defined. If another four or so pages were added to this section it could be made clearer. The short table of dissociation energies on page 101 seems to be based on a quoted source of 1947, and it might have been pointed out that some of the values are still doubtful. I did not notice any reference to 5-valent manganese, and the section on metal hydrides on page 416 should be brought up to date by inclusion of the new preparation of cuprous hydride; the rather sceptical treatment seems now scarcely justified. The higher oxide of sulphur described on page 473 has been shown not to exist. In some cases the statement that "the structure is unknown" is misleading; there is little doubt, for example, that iodine dioxide is I_2O_4, since it is diamagnetic. The new formula for graphitic oxide does not seem to be mentioned. On page 52 bivalent americium is mentioned but on page 597 it is said not to exist. It now seems that N_2O_3 is $ON{\cdot}NO_2$ rather than a compound with an oxygen linkage as shown on page 466, and it is scarcely correct to say on page 468 of hyponitrous acid that "the structure has not been determined", since the dipole moment of its esters clearly indicates a trans-configuration. These are all small matters which little affect the accuracy of the book as a whole and the obvious care with which the information has been sifted.

The subject of radioactive tracers is now so important both in chemical laboratories and in industry that the short account of it by Daudel is welcome. The biological applications are not included. The text is clear and easy to read and outlines of laboratory methods are given which could enable a reader to judge whether a method is likely to be of interest in his field or not. This applies particularly to the industrial applications, since there is obviously a large field here which may not be very well known to those engaged in technical processes. The sections are followed by detailed references to the literature which seem to be brought up to the year 1955. The series of reports now being issued by the National Academy of Sciences, Washington, under the auspices of the National Research Council, will provide further information. It seems to me that this modest book should find a place in every works laboratory.

The *Annual Reports* issued by the Chemical Society continue to provide, in a commendably small volume, a survey of progress in chemistry which for compactness and completeness is unrivalled. All chemists have cause to be grateful to those who participate in the difficult task of surveying the fields of knowledge which they are so well fitted to traverse. The more recent policy of attempting to cover, however briefly, all the important work of the year, rather than to provide reviews of selected parts of it, which is possible in the case of inorganic and organic chemistry, seems on the whole to be a good one. In physical chemistry it is scarcely possible, and selected topics are treated from year to year, the material which has accumulated in the interval being brought together. The book is one which every chemist and every advanced student should have, and its relatively low price puts it well within his reach.

J. R. PARTINGTON

MEGALITHIC TOMBS OF FRANCE

The Prehistoric Chamber Tombs of France

A Geographical, Morphological and Chronological Survey. By Glyn Daniel. Pp. xx+282+32 plates. (London: Thames and Hudson, Ltd., 1960.) 70*s*. net.

TO win appreciation for some phase of antiquity on the strength of modern excavations is far easier than what has been undertaken here—where the antiquities concerned have been known for centuries, until only yesterday often excavated badly, and celebrated in a literature in which perverse and obsolete terminologies have run riot. Dr. Daniel, supported by his wife, and with backing from many quarters as well as friendly French co-operation, has for long been working towards a systematic account of the megalithic and related stone tombs of France, their form and contents, and their placing in the frame of European prehistory in the third and second millennia B.C., to which in general they belong. This book, by no means his first study of megalithic structures, is his best so far. It is not too hard to read. Though he as well as others will certainly follow it with further work, yet he has broken the back of the subject's principal difficulties. These, above all in classification and terminology, were formidable in a manner made peculiarly acute by the history of this study among the French, which he traces in a sympathetic chapter. The core of the work then comes in the 150 pages describing the tombs themselves, using, from the multitude of some 6,000 recorded, the numbers intelligible to-day in each of seven main regions: the North, Brittany, the Centre-West, the South-West, the South, the Centre, and the East. To visit these or their remains, perhaps 1,500 of them, the Daniels have travelled, tramped, recorded and tramped again, cheerfully making friends by friendliness and amassing information; the mass is here given to us critically sifted and classified, and steadfast readers will find that it makes sense. The steadfastness is of course put to some strains, though the worst is not the terminology, because Dr. Daniel has worked so hard to straighten the tangle of both French and English theoretical and descriptive terms. The most unnecessary is his assumption that readers can do without any sketch of the background in Mediterranean and Iberian lands whence French tomb-building was initially inspired. For of his two main categories, passage-graves come all from Iberia, whether Atlantic-wise to Brittany or Mediterranean-wise to the South, while gallery-graves may now be Iberian too, now adapted in the South from rock-tombs in Sardinia. One is supposed to have looked up classes of pottery, too, such as Bell-beaker or Polada, elsewhere beforehand.

The same applies to the chronology. For nearly all the dates used are borrowed, without reason for them given, from other lands. In the second millennium, most are slightly too low; in the third, the whole scheme may need drastic raising to meet carbon-14 determinations, which began to invade the megalithic field just when the book was finished. Yet this again means that it is timely: it tells us *what* the pattern is that carbon-14 may alter; and without that, the new method would have looked like making confusion worse confounded. The book deserves a serious welcome.

C. F. C. HAWKES

MATHEMATICAL DELIGHT

The Language of Mathematics

By Dr. Frank Land. Pp. vii+264. (London: John Murray (Publishers), Ltd., 1960.) 21*s*. net.

IN an essay which formed part of the symposium "The Quality of Education", Dr. J. Bronowski put forward in 1947 the thesis that mathematics was a language. For many people this statement would seem to be an exaggeration if their remembrance of the subject was little more than a series of tricks designed to get the 'right answer' by the application of routine processes. Yet a little reflexion will show clearly that some, at least, of the essential constituents of a language are certainly present. There is a vocabulary (which like most may be merely remembered); there is certainly a grammar, the rules of which must be rigidly followed. It is a necessary means of communication about the real world, and here the limits need not be set within national boundaries. What has been so long neglected is the æsthetic appeal of this discipline, rigid in its logic but thrilling in the power of its 'literature' and its 'poetry'. The past thirty years has been a time of amazing development at the frontiers of knowledge in this field, but there has also been astonishing progress in the methods of teaching at all stages from the primary school onwards.

Dr. F. W. Land, the professor-elect in education at the University of Hull, carried out an investigation into the "attitude and attainments of 2,000 grammar school leavers", and the study revealed the need to present the fundamental principles of mathematics in a new light for the mature reader. The Shell International Petroleum Co. made it possible for Dr. Land to embark on this project, and the present volume is his very successful attempt to satisfy this need. The book is published in two bindings, and there is now available a set of exercises which will add to the profit of those who approach it with serious intentions.

It is in every respect a delightful book; merely to turn its pages is an attractive occupation and the reader is drawn again and again to browse, to ponder and then to read with understanding. Little in the way of prior knowledge or acquired ability in mathematics is necessary from those who begin. If they are prepared to think as they read, the author's enthusiasm and clarity of expression, supported by Margaret Clark's beautiful illustrations, will carry them along. I read it at a single sitting and have no hesitation in saying that it should form part of the general studies course in every sixth form, as well as being a source of pleasure and profit to every intelligent adult.

The first six chapters are devoted to arithmetic, but they range far outside the limits of commercial calculations, which is all that so many students know about the concept of number. There is an attractive treatment of the problems of the calendar, and a careful discussion of the British system of units. Then come four chapters concerned with the generalizations which we know as algebra, though here again the range is wide and the treatment exciting; the use of graphs leads to such unexpected considerations as the whirls on the shell of *Nautilus* and the florets in the heads of Compositae. The second fundamental concept of mathematical thought, the concept of space, includes within its two chapters much that is traditional in geometrical teaching, but map projections and the four-colour problem, some elementary topology and the applications of Euclid's golden section, show how Nature has her laws which man may understand and use though he cannot control them. The final chapter, on statistics, is perhaps the only part of the book in which considerable demands are made on the reader, for though the treatment of normal distribution is easy and interesting, the development of correlation and the use of scatter diagrams may cause some readers to put aside the last few pages for 'another day'.

A tribute is due to Will Carter, who designed the volume; his collaboration with the printers has produced a book of lasting value and immediate appeal. The proof reading was carefully done for there are few misprints. Such blemishes as the statement (p. 171) that the three angles of a triangle "add up to a straight line", and the drawings (p. 156), which are neither isometric nor perspective, can easily be removed with a second edition, and this should soon be needed. The book ought to be in every public library in the country—or rather its place on the shelves should permanently be empty.

W. J. LANGFORD

FUTURE OF NUCLEAR PHYSICS IN AUSTRALIA*

By Prof. E. W. TITTERTON, C.M.G.

Research School of Physical Sciences, Australian National University, Canberra

RESEARCH into nuclear physics nowadays falls into two main categories—what I shall call low-energy and high-energy nuclear physics. In general terms I would define low-energy nuclear physics as embracing the energy-range for bombarding particles between zero and some 50 MeV. Under these conditions the incoming bombarding particle is of sufficiently long wave-length to see the target nucleus as a whole; that is, we are able to study the properties of all nuclei available to us. At higher energies, up to the maximum presently available from accelerating machines, which is 25,000 MeV. or 25 GeV., the wave-length of the incoming projectile is small compared with the nuclear size and the interaction is no longer with the nucleus as a whole. To the bombarding particle, all nuclei look the same—just an assembly of neutrons and protons. The interactions are nucleon–nucleon interactions, and tell us little about nuclear structure; among other things, they lead to the production of mesons and strange particles which are associated with nuclear force fields and which, therefore, are of great importance.

To see what the Australian role in these fields is likely to be in the future, it is perhaps easiest to survey, first of all, the present position. Early in March this year, a major conference on nuclear physics was held in the Australian National University, Canberra. In many ways it marked a turning-point for Australia—partly because it was held to coincide with the final stages of erection of a new accelerating machine at the Australian National University, and partly because, for the first time, outstanding overseas research workers felt it to be worth while to travel to Canberra especially to take part in the meetings and learn what was going on in the field in Australia. In the four days of the meeting, virtually all current work in Australia was reviewed.

Sixty-two papers were read, of which twenty-eight came from the Australian National University, eleven from the University of Melbourne, six from the University of Sydney, four from the University of Adelaide and one each from the Universities of Tasmania and Western Australia; the remainder were papers given by overseas visitors. The vigorous growth of the work in the country can be judged from the fact that at a similar conference organized by the Australian National University in 1958, the total number of papers presented from all sources was twenty-eight; the output of work increased considerably in the three-year interval.

In Table 1 a summary is given of work going on in the Australian universities. It will be seen that of the ten universities, seven have some work in the field, if one includes work in cosmic-ray physics. In recent years, as is shown, there has been a very strong development of theoretical nuclear physics in the country. This came about partly because of the intrinsic importance of such work, but more importantly because big capital investment is not required and, apart from computer time, expenditure for theoretical work is limited to salaries and the provision of office accommodation. The situation is quite different in the case of experimental nuclear physics, especially if one leaves aside the rather specialized work in cosmic rays. Worth-while work in nuclear physics necessitates big capital expenditure for laboratories, accelerating machines, detecting equipment and data processing, while considerable staffs of technical assistants are required to maintain the very complicated equipment and aid the experimental work. Because of this heavy demand on money and on staff, such work is pursued only in the University of Melbourne and at the Australian National University. The former has four accelerating machines—one variable-energy cyclotron capable of yielding protons up to a maximum of 12 MeV., two electrostatic generators capable of voltages up to 800 KeV., and an electron synchrotron yielding a peak energy of 18 MeV. Of these, the cyclotron is a major experimental tool, and has, for some time, been turning out important experimental results.

Table 1. Summary of Nuclear Research Work now in Progress in Australian Universities

University	Scientist in charge	Type of work
Australian National University	Sir Mark Oliphant	Particle (high-energy) physics including the design and construction of a 10,000-MeV. proton accelerator.
	Prof. K. J. LeCouteur	Theoretical work in nuclear physics.
	Prof. E. W. Titterton	Experimental work in nuclear physics.
University of Melbourne	Prof. C. B. O. Mohr	Theoretical work in nuclear physics.
	Prof. D. Caro	Experimental work nuclear physics.
	Dr. V. D. Hopper	Experimental work in cosmic-ray physics.
University of Sydney	Prof. H. Messel	Theoretical work in cosmic rays.
	Prof. S. T. Butler	Theoretical work in nuclear and particle physics
	Prof. C. B. McCusker	Experimental work in cosmic-ray physics.
University of Adelaide	Prof. H. S. Green	Theoretical work in nuclear physics and high-energy physics
University of New South Wales	Prof. J. M. Blatt	Theoretical work in nuclear physics.
University of Tasmania	Dr. A. G. Fenton	Experimental work in cosmic-ray physics
University of Western Australia	Dr. H. Thies	Certain photonuclear experiments.

---

* Substance of the presidential address delivered to Section A of the Australian and New Zealand Association for the Advancement of Science meeting in Brisbane, May 30, 1961.

At the National University there are four accelerating machines, including a 1,200-KeV. Cockcroft–Walton accelerator, an 8-MeV. cyclotron, a 33-MeV. electron synchrotron and a 12-MeV. tandem electrostatic accelerator completed in May of this year. Nearing completion is a 2-MeV. Van de Graaff generator which will serve in the dual role of an accelerator in its own right and also as an injector to allow helium ions to be accelerated in the tandem. Under construction there is a 10-GeV. (10,000 MeV.) proton synchrotron which will be powered by a giant homopolar generator capable of currents in excess of a million amperes.

This last machine, which is being constructed by a group working under Sir Mark Oliphant, incorporates novel features, many of which were essential to allow the machine to be built with the funds and staff available. Thus an orbit of about 30 ft. diameter was chosen, and this requires restraining magnetic fields at the orbit of 80,000 gauss or so. Such fields cannot be achieved with an iron magnet, and an air-cored system was therefore chosen. This necessitates currents of peak value near to 1½ million amp., requiring a homopolar generator with liquid metal brushes as power source. The penalty which one pays for this 'poor man's' high-energy accelerating machine is a low pulse-rate—approximately 1 pulse/10 min. In competition with overseas machines in the same energy-range, where pulse-rates are one every 1 or 2 sec., this means that there is a severe intensity disadvantage. The field of experimental work in high-energy physics with this machine when it is completed will therefore be limited.

The homopolar generator, however, will be a most useful power source for other purposes—for example, certain investigations in the plasma physics field.

This accelerator of Oliphant's is likely to be the only one built in the high-energy field in Australia. The reason is that investments now being made for high-energy accelerators of 10 GeV. and upwards overseas are in the range of £A.10 million–£A.100 million with similar sums of money per installation required for ancillary experimental equipment. Moreover, the teams of people required to build, operate and use such equipment approach, for one installation, the entire physics staffs of the combined Australian universities. It is therefore clear that in the future, Australia will be unable to compete with the big nations in the field of high-energy experimental particle physics. Some work can go on profitably in cosmic-ray physics, but this is likely to be on the more geophysical aspects of the subject rather than in the nucleon–nucleon interaction field where, again, the intensity advantage and flexibility of experimental arrangement make an accelerating machine the highly preferable tool. There is no reason, however, why effective theoretical work in the high-energy field should not be carried out in Australia, and it is encouraging to note that small groups are already in being in Adelaide, Canberra and Sydney.

To return to the field of low-energy nuclear physics, I should remark that, in addition to the work going on in the universities, there is a group at the Australian Atomic Energy Establishment, Lucas Heights, Sydney, whose task it is to make certain nuclear measurements of importance to the atomic energy programme. It is called the Reactor Physics Division, and was responsible for the nuclear measurements made in Australia's first reactor *Hifar*, and it has now acquired a smaller reactor named *Moata* for the needs of its own work. Most of its work is of an applied nature, but some of its studies in neutron physics, for example, neutron diffraction, will be of wider interest.

A joint Universities–Atomic Energy Commission organization called the Institute for Nuclear Science and Engineering is now beginning to operate effectively. It has offices and laboratories at Lucas Heights, and members of staff of any Australian university desiring to use the unique facilities at Lucas Heights are encouraged to go and work there. Although the Institute will be able to support some nuclear physics work, this is likely to be a small part of its overall programme since the nuclear problems which can be tackled using a fission spectrum in a reactor are limited in variety. It is to be expected that most experimental effort will go into chemistry, metallurgy, solid-state physics, biology and medicine, and perhaps geophysics.

It will be apparent, then, from this brief survey that the future of nuclear physics in Australia will reside, on the experimental side, in the fields of low-energy nuclear physics with some peripheral activities in cosmic rays and plasma or thermonuclear physics. But there is no reason why, on the theoretical side, work should not range over the whole field providing always that our theoretical colleagues give adequate backing to the low-energy experimental programmes going on in the country.

It was for reasons of the type outlined above that in considering the future programme at the Australian National University we chose to make a major investment in the field of low-energy nuclear physics by building a tandem electrostatic generator which is the most flexible and sophisticated tool available to the experimenter at the present time.

But, first, I want to offer reasons why I believe it is essential to have at least two flourishing schools of experimental nuclear physics research in the country.

The training received by a graduate in going to a Ph.D. degree in nuclear physics is about the broadest I know. The student must learn to handle a wide variety of equipment—electronics, both valve and transistor, vacuum physics, thin film preparation, accelerating machines, a wide variety of light and ionization detectors, multichannel memory storage devices, data processing systems and so on—merely as tools for doing the job of an experiment in nuclear physics. Then he must have an adequate understanding of the basic theory to be able to interpret the results properly. I am inclined to agree with Dr. Louis Rosen, a recent visitor to our Laboratory from the U.S. Atomic Energy Commission, who said: "We find that training in nuclear physics is the best a physicist can get. Such men have great breadth of outlook and a remarkable diversity of knowledge. They can turn their hands at any time to solve difficult problems of technology which continually arise in the country".

This point was demonstrated most effectively during the War, when it was found that the nuclear physicists recruited from the universities, both in the United Kingdom and the United States, rapidly took over the leadership in the development of radar and operational research methods before going on to develop the nuclear reactor—the power source of the future—and the uranium isotope separation methods required to obtain the highly fissile materials, plutonium and uranium-235, necessary to manufacture the nuclear bombs which finished the War.

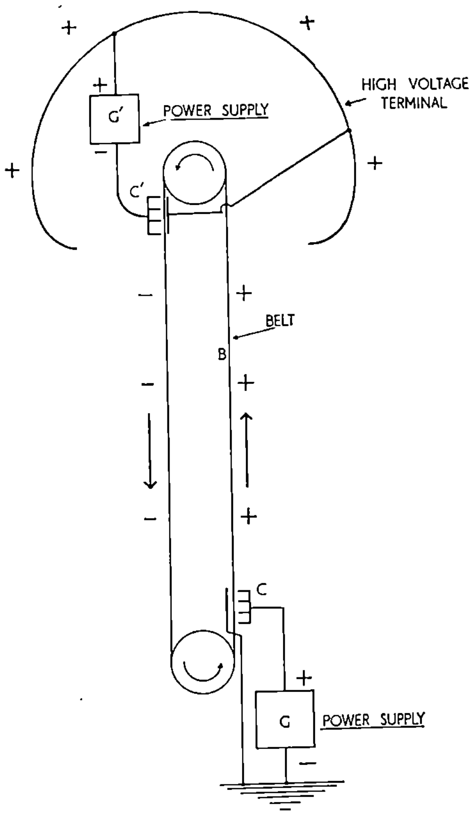


Fig. 1. Schematic diagram of the original (single ended) Van de Graaff accelerator. Charge from a power supply *G* is sprayed into a moving belt *B* and carried to a hollow spherical terminal *T* where it is removed. Charge of the opposite sign can be sprayed into the belt from the power supply *G′* and carried from the terminal if desired. The practical voltage limit to such a machine is 6–7 million volts

Out of these nuclear developments has grown the biggest industry in the world—the nuclear or, as it is popularly called, the atomic energy industry. This will provide an ever-increasing proportion of the world's power resources as fossil fuels become depleted. and it will become essential in the long-term development of Australia. The most promising energy source after fission resources become exhausted—and they will have a life-time of some 50,000 years—is again a nuclear one. It arises through the synthesis of heavier elements from lighter ones through fusion or thermonuclear reactions similar to those we now know provide the major source of stellar energy.

Fission and fusion then are two compelling practical reasons why any forward-looking country must study low-energy nuclear physics.

My final reason is probably the most important. Although we have an ever-increasing knowledge of the properties of nuclei, we are still far from understanding the detailed structure of nuclei, and the seat of the very strong nuclear forces which bind the nucleons together in a nucleus. It is a major aim of nuclear physics research to try to provide such understanding.

To illustrate the scale on which one needs to operate in low-energy nuclear physics these days, I can do no better than to describe our new installation in Canberra. The ingredients for nuclear experimentation are: (1) appropriately prepared targets; (2) an accelerating machine with which to produce the fast particles with which to bombard the target; (3) detecting and recording equipment to observe the particles or radiation leaving the target as a result of nuclear reactions.

Target preparation is an art in itself, and will not be discussed here, but I should say something about the other two since this is where the big expenditure and effort lie.

To-day, for precision work in nuclear physics, the choice of the experimentalist is for an electrostatic accelerator of the type first built by R. J. Van de Graaff in the United States. Such machines are exceptionally useful for investigating properties of nuclei because they produce highly collimated beams of bombarding particles the energy of which can be varied continuously and smoothly, and they yield particles which are nearly monochromatic in energy.

The operating principle of a conventional electrostatic generator is illustrated in Fig. 1. An endless belt, *B*, of rubberized fabric, runs over two pulleys placed one above the other. Electric charge from a power supply, *G*, is sprayed on to the lower end of the rising half of the belt by means of corona discharge from points on a metal comb, *C*. The charge is carried up by the belt and at the top enters a spherical hollow metal terminal, *T*, to which the charge is transferred from the belt by means of another comb, *C′*. Alternatively, charge of the opposite sign can be sprayed on to the belt from another power supply, *G′*, inside the terminal. In this way, the positive charge on the terminal is continually built up, the potential obtained being limited only by the rate at which it leaks off, or by electrical breakdown to the surroundings.

An evacuated acceleration tube, consisting of alternate sections of glass and metal, connects between the terminal and earth potential. An ion source mounted at the upper end of this tube supplies positively charged particles such as protons, deuterons or tritons, and these are accelerated through the potential difference between the terminal and the ground. The higher the maximum voltage to which the terminal is required to be raised, the longer the accelerator tube needs to be and the larger the diameter (and therefore the electrical capacity) of the terminal must be to withstand the voltage stress.

In actual fact it turns out that 6–7 million volts is the practical limit, partly because, beyond this figure, the total stored energy in the terminal ($\frac{1}{2}$ CV^2) is so great that any odd arc discharge from it to the surroundings causes great damage to the system. Unfortunately, 6 million volts are insufficient for many of the problems we want to attack, and it became necessary to improve on this. The machine just completed at the Australian National University will allow particles to be accelerated to more than 12 MeV. and is a tandem accelerator. It employs ion exchange techniques for increasing beam energy which were suggested many years ago by W. H. Bennett, but which at the time were not practical. Renewed interest in the idea occurred when physicists learned how to produce negatively charged hydrogen ions, that is, protons to which two electrons have been attached. Such ions can be produced with an intensity of about 2 parts in 100 when a beam of protons, accelerated through a potential difference of about 20 kV., is allowed to pass through hydrogen gas. Under such conditions the velocity of the protons corresponds approximately to the velocities of the outer orbital electrons in the gas atoms and leads to the formation of the negative ions. This negative ion beam is then analysed magnetically so that unwanted particles, such as neutral particles and electrons, are removed from the ion beam. The clean ion beam is then injected into the tandem accelerator, as indicated in Fig. 2. The negative ions are accelerated by the positive voltage on the central high-voltage electrode of the machine. When they reach this terminal the negative ions are moving with a centre of mass velocity which is large compared with that of the outer electrons about a nucleus. In these circumstances, by allowing the ion beam to pass through a tube containing gas (called the 'stripper' tube), electrons are stripped off and the emergent beam consists of positively charged particles. The gas pressure in the stripping canal can be maintained at a low enough value so that the energy lost in passing through the gas is negligible (less than 0·5 keV.). The positive ions so formed are then pushed away from the positively charged central terminal and are accelerated in the second half of the stack. The energy of the emergent beam is therefore twice that of the terminal voltage and, with 6 MV. on the terminal, the emergent beam has an energy of 12 MeV.

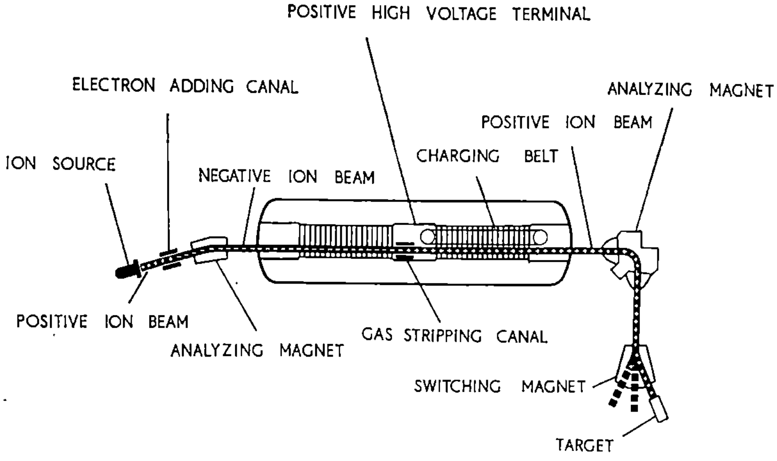


Fig. 2. Functional diagram of the operating principles of a tandem electrostatic accelerator

The tandem generator has many possibilities for experimental work. It is the most flexible accelerating machine yet built, for the reason that the ion source and the target assembly are both at earth potential and readily accessible to the experimenter (the ion source in the conventional Van de Graaff generator, Fig. 1, is in the high-voltage terminal within the pressure shell of the machine and is inaccessible).

Proton and deuteron beams with energies of about 12 million volts and oxygen ions with energies of 30–40 MeV. can be readily obtained. In the more distant future, ion beams of lithium, fluorine, and any other element capable of forming negative ions (a criterion which includes about half the Periodic Table) may be produced.

Unfortunately two very important projectiles, helium-4 and helium-3, do not form negative ions long-lived enough to be accelerated with intensity comparable with protons, deuterons or tritons. In the near future, therefore, we are planning to install an injector which will make such acceleration possible. This will use a conventional 2 million volt electrostatic machine in which He$^+$ ions are accelerated to about 700 keV. (which is an optimum figure for the purpose), neutralized in a charge-exchange canal, injected into the tandem machine and allowed to coast, without gaining energy, to the high-voltage terminal. There, as before, they are stripped of two electrons, becoming He^{++}, and in falling through the potential difference of 6 MV. in the second accelerator tube gain 12 MeV. of energy to emerge with a total of 12·75 MeV. The injection machine will be mounted on a turn-table and railway arranged so that, when not in use for helium-ion injection to the tandem, it can be run back into an adjacent laboratory, rotated through 140° and used as a 2-MeV. accelerator in its own right in conjunction with an appropriate analysing magnet which can switch the beam into either of two directions for experimental work.

This example of the helium-ion injector illustrates one of the great advantages of the tandem accelerator. Because the ion source is at ground potential and readily accessible, there are virtually no limits to the power consumption, size and complexity of the injector system. For example, mμsec. time of flight techniques (which are important in many experiments) can be applied very readily with the tandem generator, since radio-frequency deflexion and bunching can be carried out on the low-energy beam before injection. Further, it may be possible in the future to polarize the nuclei of negative ions and eventually produce beams of polarized ions, which would open up entirely new fields of experimentation.

Preliminary experiments with the tandem accelerator show that the peak-to-peak ripple at 5·1 MV. on the high-voltage terminal under beam conditions is 1·6 kV. with a slow drift of 0·5 kV. The voltage stability of the beam is therefore better than one part in a thousand, and to make use of this it is necessary that precision detecting equipment be available in the target area.

Two major units will be available for precision experiments—a 24-in. double focusing 180° spectrometer and a 65-cm. Buechner particle spectrograph, both of which can analyse the charged particles leaving a target under bombardment. The former incorporates a 20-ton magnet having a specially shaped magnetic field of value quadratically dependent on the radius of the particle trajectory in it. In cylindrical polar co-ordinates there arises a radial component of the field in addition to the z-component, enabling focusing in the z-direction to be effected as well as radial focusing. By shaping the field so that the coefficient, α, of the first-order term of $H_z(r)$ is exactly one half, the radial and z-focuses coincide for all values of object distance, and in our spectrometer object distances 9–26 in. from the field boundary can be chosen. The size of the image given by such a magnet depends on the object size and aberrations. Aberrations can be reduced considerably by choosing the coefficient, β, of the quadratic term in $H_z(r)$ correctly. The pole-face profile is therefore complicated, but successful achievement leads to a greater solid angle of particle acceptance. In the present instrument, for equal object and image distances the solid angle of acceptance is 0·01 steradian at 0·1 per cent energy resolution for fields up to

THE COMMONWEALTH LIBRARY
OF SCIENCE, TECHNOLOGY AND ENGINEERING

Chairman of the Honorary Editorial Advisory Board:
Sir Robert Robinson O.M., F.R.S.

The first volume of a 1,000 *volume original paperback series in aid of education*

ON TEACHING MATHEMATICS

A report on some present-day problems in the teaching of mathematics, being the outcome of discussions and lectures at the **Southampton Mathematical Conference, April 1961,** *under the Chairmanship of*

Bryan Thwaites

Can Britain survive in a Technological Age ?

Ultimately it all depends on education, and upon school mathematics in particular. Today British schools are desperately short of mathematics teachers, of whom British universities are not producing enough. The weakening foundations of our educational structure are swaying beneath the ever-increasing load of science and technology. Even the best and fastest rescue operation will take a decade to right it all. Questions in the House of Lords, in the Commons, and letters in *The Times* have foretokened the anxious years ahead.

What are our chances of remaining a first-class industrial power ? How bad are things ? What must be done ? ON TEACHING MATHEMATICS contains the findings and recommendations produced in answer to these questions by professional mathematicians drawn from schools, universities and industry at the 10-day Southampton Conference.

It is most appropriate that the first volume to be issued in this Library should be this important book on the teaching of mathematics, which will undoubtedly have a profound effect on and be a great help to everyone who teaches mathematics or is interested in the wider field of education.

The Pergamon Press leads amongst the international scientific publishing houses, and is proud to play its part in current affairs of great moment.

This valuable paperback is available from all booksellers, or in case of difficulty direct from the Publisher, price 7/6 net ($1.25).

PERGAMON PRESS
OXFORD LONDON NEW YORK PARIS

Headington Hill Hall, Oxford. 4 & 5 *Fitzroy Square, London, W.*1. 122 *East* 55*th Street, New York* 22, *N.Y. Gauthier-Villars,* 55 *Quai des Grands-Augustins, Paris* 6

GD604

What's he up to?

He's weighing to an accuracy of 1 MICROGRAM!

He's doing it on the filing cabinet just to show you—he could do it on anything else just as easily. He's not worried about levelling, draughts, temperature or vibration.

And he's not worried about his results; he knows that with the CAHN ELECTROBALANCE he has an accuracy of 0.05% and a reproducibility of 0.02% on each of the instrument's six scale ranges—0–1mg to 0–100mg—and with no 'ifs' or 'buts.'

This weighing will take him less than one minute. Then he'll hand the balance back to the colleague he borrowed it from—with one hand: it weighs only 12 pounds. And he knows it will give just the same first-class performance next time he borrows it—because he can calibrate it in a matter of a few seconds every time he sets up to weigh.

He's a happy man. And so is his Director. Because this *one* versatile, portable CAHN ELECTROBALANCE replaced *four* conventional micro-balances—and does the same work, MORE ACCURATELY, MORE EASILY and FASTER.

Two other versions of the Cahn Electrobalance are available. One has the capacity to weigh small samples up to 1gm much more easily, quickly and accurately. The other is an automatic version of the balance featured above.

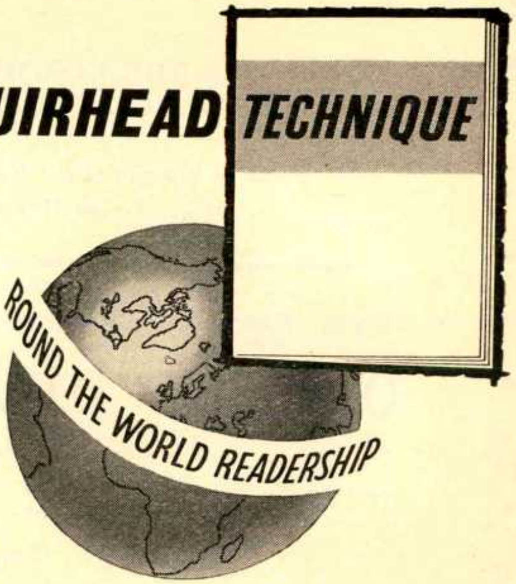
THE CAHN ELECTROBALANCE

is a revolution in micro-weighing
Write to-day for further details

SHANDON

SHANDON SCIENTIFIC COMPANY LIMITED
6 CROMWELL PLACE, LONDON SW7 KNIGHTSBRIDGE 1131

MUIRHEAD TECHNIQUE



This quarterly journal of instrument engineering contains information of latest developments. It is available post free on request—ask to be put on the mailing list.

MUIRHEAD & CO. LIMITED, Beckenham, Kent, England. *Telephone: Beckenham 4888*
MUIRHEAD INSTRUMENTS LTD., Stratford, Ontario, Canada. *Telephone: 271-3880*
MUIRHEAD INSTRUMENTS INC., 441 Lexington Ave., New York 17, N.Y., U.S.A. *Telephone: Murray Hill 2-8131*
513

BECK
LOW POWER BINOCULAR MICROSCOPES

The many uses in industry and science for which this type of microscope is suited are fully explained in booklet, post free on application.

•

R. & J. BECK Ltd.
69/71 MORTIMER STREET
LONDON W.1

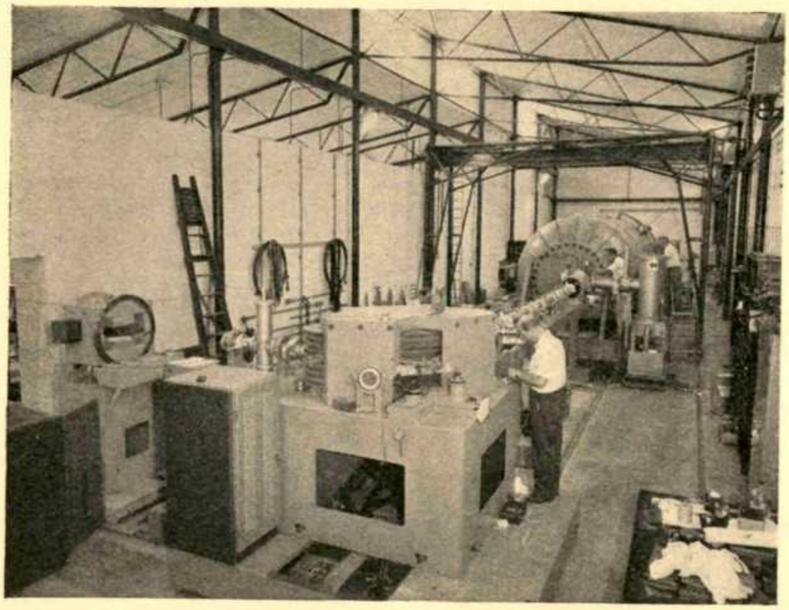


Fig. 3. The tandem accelerator at the Australian National University viewed from the high-energy end. In the foreground is the 90° analysing magnet. On the left (not yet connected to the machine) is the switching magnet which allows three beams, + 25°, 0 and − 25°, to be obtained. The accelerator pressure vessel is in the background

10,000 gauss—sufficient to analyse protons of energy up to some 25 MeV. In use it is mounted on a trolley and is fed with current through flexible cables so that it can be rotated around a target from + 10 to − 155° to analyse reaction products as a function of angle.

The second major piece of equipment—the Buechner spectrograph—will be installed early in 1962. It differs from the spectrometer in that it will accept and analyse particles having a wide range of energies simultaneously; in fact, at one field setting particles in the energy-range from E to $2{\cdot}4\,E$ are recorded. In this case the magnet employs a uniform field having a circular boundary which focuses particles from the target located outside the field along a hyperbolic focal surface also outside the field after a mean deflexion of 90°. Again an energy resolution better than 1 part in 1,000 can be achieved and recording is by nuclear emulsion, the plate being bent to take up the shape of the focal surface. With this device the solid angle of acceptance is much smaller—about $1{\cdot}4 \times 10^{-3}$ steradian—but the photographic emulsion detector can integrate the particle spectrum over a lengthy run and over the wide range of energies. At a field of 12,000 gauss the spectrograph will record protons and α-particles up to 35 MeV. and deuterons up to 17-MeV. energy. The magnet weighs 16 tons and can be rotated about the incident beam from −10° to +130° to enable angular distributions to be measured.

In addition to the spectrometer and spectrographic methods of analysing reaction products, the more conventional electronic—gas counter, scintillation counter—and nuclear emulsion techniques will be employed.

The more recently developed silicon surface barrier detectors are likely to have very wide application; they are compact, with a 100 per cent detection efficiency for charged particles, a linear energy-dependence over a wide range and extremely short pulse rise time. In some of those we have made in the laboratory, energy resolutions of 1 per cent have been achieved for α-particles and this necessitates the use of low-noise amplifiers. Unfortunately, at present, the layers are thin for protons and detect linearly only up to some 3 or 4 MeV. These counters, however, are ideal for many purposes—particularly the simultaneous study of particles emitted at a number of angles from a target.

Most detectors present a problem of pulse-height analysis—determination of the spectrum of pulses arising from them and multichannel kicksorters capable of accepting the data, sorting it into 400 or 512 channels, storing it for the duration of a counting period and then printing it at the end of the run are necessary. For reliability these devices are largely transistor operated; we have two in operation and will install another two in the course of the next year.

The foregoing review will indicate that in the immediate future experimental nuclear physics is well cared for in the Melbourne and Canberra laboratories, while theoretical nuclear physics is flourishing both in these laboratories and in other universities too (Table 1). The question may be asked whether there should be more work in the general field. This must be answered in relation to the problem of a balanced development of physics in the country. To set up a new and effective Department of Experimental Nuclear Physics would require a capital investment of between half a million and one million pounds, with running expenses of perhaps £150,000 per annum. However enthusiastic one may be about the discipline, it must be admitted that in the present stage of national development, such funds put into other, possibly less-glamorous and less-spectacular but no less-important, parts of the physical sciences, would ensure a better balance in the overall development of physics in Australia. But we, like other Western countries, will have to build further universities in the next two decades and it may be that expansion of nuclear activities could take place then.

Along with some others of my colleagues, I have become concerned at the way research in physics is being represented to the public. In the public mind there is a tendency to believe that the building of nuclear reactors, the placing of satellites into orbit, the production of nuclear bombs and the plans for men to visit the Moon are science. Such projects do, of course, use the knowledge gained in past research, but at best they are applied science, and more generally steps in a development programme; as such, of themselves, they add little to our store of fundamental knowledge.

Pure research in physics cannot be carried out in the full glare of publicity of Press, radio and television. There is nothing certain about it; it flourishes quietly and methodically in the laboratory. Its results go to acknowledged responsible scientific journals both in Australia and overseas, and its merit is judged by colleagues in the international community of scientists.

THE ROAD RESEARCH LABORATORY

OPEN days were held by the Road Research Laboratory (Department of Scientific and Industrial Research) on Wednesday and Thursday, May 3–4, 1961.

The work of the Traffic and Safety Division was shown at the new Road Research Track at Crowthorne, Berkshire. The track is three miles long and is in the form of a figure eight with a large paved area 900 ft. in diameter in the centre. A detailed description of the track has been given in *Nature* (188, 894; 1960).

The display at Crowthorne comprised static exhibits in marquees and in an underground laboratory, and several demonstrations of the work on vehicles, skidding, road user behaviour, and traffic.

In one marquee, equipment used in traffic studies was on view and included traffic counters, a recording speedmeter, data recorders and multi-channel telemetry equipment for traffic studies over wide areas.

Traffic and Safety

Demonstrations were given of some of the work undertaken in the application of electronic guidance to motor-vehicles.

A car was driven along a bend on part of the track in which detector loops were buried in the road. The passage of the car caused lights to be switched on, and remain on until the car was three loops away. In fog such a device could reveal to a driver in a car how far ahead the road was clear. Similar detectors could be used to check the speed of passing vehicles and warn drivers when they were exceeding the speed limit.

In another demonstration of the use of electronic control, a car with its windscreen blacked out was driven along a straight section of the track. The driver was guided by his instruments, which were actuated by two coils at the front of the vehicle to pick up impulses from a guide wire on the ground. An audible signal was transmitted to the car, and for the purpose of demonstration was amplified over a loudspeaker system. When the driver was on course the signal was heard loud and clear, when the driver moved away from his predetermined course the signal was weak. The transmission is actually made from the roadside via the guide wire beneath the centre of the track. On a road equipped with a guide wire this system could be used by traffic police to communicate with a motorist who had a suitably modified radio installed in his car.

A possible development from these investigations, which are at present in their early stages, would be to link the signals direct to the controls of the car and thus drive the car automatically. In fact, in some runs the car steered itself entirely by electronics and was kept within 6 in. of the guiding cable.

The greater resistance to skidding, and the shorter braking distances, that can be achieved with tyres having tread rubber with a high hysteresis loss was demonstrated by two identical cars, one following the other and about 30 ft. apart, at a speed of about 40 miles per hour on a wet, slippery section of the track. At a given signal, each driver applied his brakes as in an emergency stop; the car equipped with conventional tyres travelled farther in coming to rest than the one fitted with tyres with tread rubber with a high hysteresis loss. Experiments have shown that by this means braking distance can be reduced by about 14 per cent.

Another series of demonstrations showed the advantage of fitting devices which prevent wheels from locking when brakes are applied. A car was driven at 45 miles per hour on a wet surface, and the driver applied his brakes as hard as possible to lock the wheels. When using Maxaret units to prevent wheel locking the driver did not lose directional stability and was able to retain steering control and bring the car to a stop in a much shorter distance.

To help analyse the cause of accidents the Laboratory is using a camera suspended on a 40-ft. collapsible pole which protrudes from the roof of a van. The camera photographs, from above, the exact position of vehicles that have been involved in an accident. By this means it is hoped to obtain information on deficiencies in vehicle design and road layout.

The Laboratory has been co-operating with the glass industry in attempts to improve vision through toughened glass windscreens when they shatter. By reducing the fragmentation of the glass to between five and ten particles per inch in the part of the windscreen immediately in front of the driver he is given reasonable vision when the glass is shattered. The effectiveness of the new glass was demonstrated by driving a car with a shattered windscreen and manœuvring it around obstacles at about 20 miles per hour.

The problem of getting information across to road users in the most efficient way was shown by a demonstration in the central area. Signs were mounted on a car which was driven towards the spectators, who were then invited to record by pressing push-buttons when they could read the signs; the distances at which they could do this were clearly indicated. Signs with different types of lettering were used.

About a hundred people are killed each year on the roads in Britain as a result of cars striking lighting columns. The Laboratory is examining the design and positioning of columns with the view of minimizing injuries to road users. A film showed the effect of an 8 h.p. car hitting four types of lighting column—concrete, tubular and thin sheet steel, and aluminium.

The vehicle detectors now in use on the road can register moving vehicles only; they cannot therefore distinguish between dense stationary traffic and the complete absence of vehicles. Two devices that can detect the presence of moving and stationary vehicles are now under development; both depend on the change in the magnetic field produced by a nearby metal object.

The Laboratory has been examining the possible applications of vehicle-presence detectors and a demonstration was given of the detection of congestion in a roundabout. The equipment was also used to indicate imminent 'locking up' at a signal-controlled roundabout.

A prototype device was shown that is being used in experiments with vehicle-actuated traffic signs on high-speed roads. As shown in a series of demonstrations, it ensures that the traffic signals will not change from green to amber when a driver approaches an intersection at such a speed that he can neither stop safely nor clear the intersection before the 'red' appears. A transistorized form of the device is now under development by manufacturers.

Materials and Methods of Construction

The work of the Materials and Construction Division was shown at Harmondsworth, Middlesex, where research is undertaken on pavement design, soil moisture and surface drainage, bituminous materials, concrete and special problems such as those arising from snow and ice and the measurement of surface irregularity. The Laboratory's Tropical Section is also at Harmondsworth.

A number of exhibits dealt with the problem of designing road pavements in relation to foundation conditions and the traffic to be carried. The Laboratory has many full-scale road experiments in progress in which the performance of short sections laid on heavily trafficked routes is being studied. The main variables are the thickness and type of both surfacing and base in the case of flexible roads and the thickness of slab and weight of reinforcement for concrete roads. Results from experiments which have been in progress for periods up to twelve years were shown.

Severe frost, which occurs on average every 10 years, can cause damage to road foundations. In a refrigerated road full-scale freezing tests on a typical road section were in progress. Apparatus was also on view for studying the moisture retention and swelling of soils, and photographs showed the relation of this work to practical problems associated with pavement failures.

The function of a road pavement is to spread the wheel-loads so that the stresses in the soil and other layers of the road structure do not cause unacceptable permanent deformation. This load-spreading depends on the elastic properties and thickness of layers in the road. The stresses in the structure and in the soil can be determined theoretically and they are also being measured by equipment developed at the Laboratory. Exhibits were concerned with dynamic stresses measured under different forms of flexible construction and with the effect of repeated applications of these stresses.

In one building were assembled rainfall recorders and flow-measuring apparatus devised at the Laboratory for use in research on surface-water drainage. The main objective of this work is the design of drainage systems both in relation to their efficiency and their cost. Apparatus is being devised for printing hydrological data directly on to tape for analysis by an electronic digital computer. A working model was shown of a novel automatic recording salt-velocity apparatus devised jointly by the Road Research Laboratory and the Hydraulics Research Station. The apparatus comprises a device for injecting charges of salt solution into the sewage (each charge is triggered at a pre-set depth of flow by a water-level recorder), two pairs of electrodes in the sewer and apparatus for recording the change in conductivity as the salt charges pass the electrode. This enables the velocity of flow between the electrodes to be measured for a range of depths; a relation between the rate of flow and the depth of flow is then deduced. Also on display was an analysis of records of rainfall and run-off for twelve catchment areas, employing the rational (Lloyd-Davies) method, the unit-hydrograph method and the Road Research Laboratory hydrograph method. Two methods of calculating sewer sizes for design purposes were on show; both methods were devised at the Laboratory from the results of research into rainfall and run-off. In one method use is made of an electronic computer.

Bituminous binders are viscous liquids adhering to the surface of roadstone. Viscosity and adhesion are therefore two primary properties, and an exhibit described problems connected with the measurement of viscosity, the effects of water on adhesion, and the use, in wet weather, of adhesion agents to prevent damage to a surfacing. The resistance of the binder to hardening under the action of weather plays a major part in road performance; research on hardening was described.

There is at present considerable interest in the possibility of using bituminous materials in the construction of road bases. These materials combine the advantages of a bound material with a large measure of flexibility. Investigations are in progress in the Laboratory on the mixtures to be used, and experimental sections have been included in the full-scale experiments already discussed. The specifications at present recommended were shown.

On rolled asphalt wearing courses in which the stone content is 45 per cent or less, it is normal practice to lay a final application of coated chippings. To spread the chippings uniformly by hand requires skilled labour and slows up the work even where there is no shortage of skilled manual workers. A working model was shown of a machine now under development that spreads coated chippings at a predetermined rate. The machine consists of a hopper and rotating metering device carried on rollers which travel on the surface of the newly laid rolled asphalt. In the construction of new roads the spreader would be carried on rubber-tyred wheels on the concrete edge strip or on the base-course outside the mat being laid.

A group of exhibits illustrated the factors that affect the riding quality of a concrete road and the procedures needed to obtain good riding quality. Experiments undertaken by the Laboratory have shown that, when efficient spreading and compacting machines are used, the riding quality obtained is of a very high standard, the average irregularity index being 32 in. per mile with only 25 per cent of the values greater than 40 in. per mile. No significant differences in riding quality were obtained when different mixes were used, and different mix proportions did not appear to be important when modern power-propelled plant was used.

One of the problems investigated by the Laboratory is the possibility of using prestressed concrete for roads. Tests have been undertaken to determine the performance of thin sections of prestressed concrete. An exhibit showed the comparative costs of reinforced and prestressed concrete roads, work done in collaboration with the Belgian Centre de Recherches Routières, loading tests and measurement of subgrade restraint, and the development of a jack for prestressing slabs by cables with minimum interference with construction. In co-operation with the Belgian research organization a prestressed concrete road was built at Zwartburg, Belgium, in 1960. The experimental road is more than two miles long and includes prestressed, plain and reinforced concrete slabs. The performance of the road under traffic is being observed, and the possibility of carrying out loading tests will be considered at some future stage.

In recent years sections of road have been constructed with heating wires placed in the upper layer of the road structure to prevent the formation of ice and frost, and to melt snow. A control device automatically switches on the heat when the road surface is cold and wet. The control device was shown in operation. The heating system has been installed in the underpass at Hook on the Kingston by-pass road (*A*.3) and in the one nearing completion at Hangar Lane, Ealing (*A*.40), on a bridge at Basildon, Essex, and on roads in Edinburgh, Slough and Harmondsworth. The Laboratory has also assisted with the design of the heating systems for many sections of road, including the Hammersmith fly-over, the Hyde Park Corner under-pass in London and for the approach ramps to the new Clyde tunnel in Glasgow.

Tropical Section

One of the main aims of the Tropical Section is to systematize knowledge of the engineering properties and occurrence of the soils and roadmaking materials in different territories. Exhibits illustrated: the nature and engineering properties of soils in individual territories and areas, including the use of aerial photography in terrain evaluation; work on tropical gravels, their use in road building and their suitability for stabilization with Portland cement and hydrated lime; stabilization of sands—full-scale experiment in Nigeria employing bitumen and Portland cement; tests on tropical aggregates; the relation between climate and moisture conditions under roads and runways; pavement design in the tropics.

The rapid growth of road traffic in developing countries is both a factor in the development and a symptom of it. Examples were shown of investigations in Uganda and North Borneo to determine the effects of providing roads on the rate and character of agricultural development. In the expanding towns and cities overseas there are already severe traffic problems and there is at the same time opportunity to plan the future growth of the towns so that the facilities required by future traffic are integrated with the needs of the community as a whole. Examples were shown of investigations undertaken in Hong Kong, Kingston (Jamaica) and Lagos to ease present traffic problems and to plan future needs.

Scottish Branch

The Scottish Branch of the Laboratory held open days during June 7–8 at Thorntonhall, Glasgow. The Branch was established in 1949 with the view of investigating problems of particular interest to Scotland, making known to all concerned with roads and traffic in Scotland the results of work undertaken at the parent Laboratory in England, and encouraging the application of the results of research.

While many of the exhibits displayed at Thorntonhall were those originally on view at Crowthorne and Harmondsworth, there were a number that illustrated the work undertaken in Scotland.

An investigation is being carried out on the effect of the new Forth road-bridge on the traffic patterns in the region. An exhibit described with photographs and maps an origin-and-destination survey carried out during the whole of one week in August 1960. Vehicles were stopped at three survey stations and more than 8,200 drivers, or about 43 per cent of the total, were questioned to obtain information on the traffic in the region before the opening of the new bridge. Analysis of the information obtained has not been completed, but preliminary results show that 22 per cent of the journeys start and terminate in Glasgow or Edinburgh, and about 34 per cent in five major cities.

The effect of new towns on the generation of traffic and on traffic patterns is being studied using East Kilbride as a 'guinea pig'. Such studies should assist planners in predicting facilities required for new towns, for example, the number of lock-up garages that should be provided. In East Kilbride there is one car for every three families.

There is a shortage of crushed stone in certain parts of Scotland, and the Laboratory has been examining the possibility of using morainic (glacial) gravels for the construction of road bases and surfacings. For the base, glacial gravels are mixed with cement to produce a 'lean concrete', for surfacings they are mixed with bitumen to provide a reasonably cheap and durable wearing course. Since 1956 many short sections of road containing glacial gravels—each with a different composition—have been laid in various parts of Scotland. The results so far are very promising.

Peat occurs in many regions in Scotland. This soft, water-filled, organic material is compressed when a road is constructed on it and this often results in an uneven road surface with a poor riding quality. A group of exhibits showed how the Laboratory is tackling the problem of building roads on peat.

OBITUARIES

Prof. A. W. P. Wolters

PROF. A. W. P. WOLTERS, who died recently, was the first holder of the chair of psychology in the University of Reading and played an outstanding part in dissolving academic distrust of his subject and achieving its recognition. His patience in handling the problems of its acceptance in the outside world was effective as well as exemplary. As deputy president, during 1936–38, and as president, during 1939–41, of the British Psychological Society, he took a leading part in the difficult transformation of a somewhat ungainly association into an incorporated body with specific professional, as well as scientific, aims. Wolters, too, was among the first to urge the opportunity and the need to employ people with a psychological training in the event of war. Unusual hardihood and a complete lack of aggressiveness enabled him to survive the official flood of waste-paper and committee proceedings which resulted. The idea that objective study of the human organism is not irrelevant to human welfare and efficiency thus gained some degree of official recognition in war and survived to spread effectively in peace.

Wolters learnt his psychology, both academic and practical, the hard way. As a fourteen-year-old pupil-teacher, he had charge of the mental defectives "who littered the enormous classes" of the Board School where he taught. Perhaps this experience stood him in good stead later on. His catholic intelligence responded to such education as came the way of a pupil-teacher, and in due course he entered University College, Reading, where he was to live and work for almost the rest of his life. W. L. de Burgh, the professor of philosophy, gave him such scope as was available to introduce psychology, and the good judgment with which Wolters handled this admirable but hazardous opportunity ensured a much earlier start for an independent department and honours school at Reading than was possible in many other places. His quiet advocacy, too, prevailed among manifold claims and restricted resources and somehow saw to it that a specially designed laboratory was built. Here, in the years which followed the War, he built up a school of psychology modest in size, but the quality of which aroused the envy and admiration of his colleagues elsewhere. At the same time his self-effacing talents were devoted to his University—he became deputy vice-chancellor in 1947—then preparing for complete removal to Whiteknight's Park, and beset by the difficulties of post-War expansion. His moderation and his constructiveness in meeting these problems were indispensable.

Wolters's modesty and simplicity were inherent and free of contrivance, his perceptiveness unforced. His qualities stirred young people much, but himself only a little, to intellectual achievement. His main explicit contribution to psychology—an extension of Head's concept of *Schema* into the field of conceptual thinking—is an original contribution the full implications of which probably remain to be appreciated. But the inspiration of his teaching and the gentleness with which he deflated the pretentious will long be remembered and admired. R. C. OLDFIELD

Prof. A. F. Kapustinsky

ANATOLY FEDOROVICH KAPUSTINSKY was born in Zhitomir on December 19, 1906, and died in Moscow on August 26, 1960. He studied chemistry in the University of Moscow, from which he graduated in 1929. His main field of research was in physical chemistry and thermodynamics. He was particularly interested in work on the energetics of chemical reactions, in the energy of the crystal lattice, in crystallochemistry and its application to geology. In 1933 he proposed the second principle of crystallo chemistry, namely, that the energy of the crystal with its attendant properties is determined by the number of ions, their radii, their valencies, and their degree of polarization. At the same time he proposed a simplified equation for the energy of the crystal lattice in ionic crystals, and in 1943 he produced a revised equation, which enabled him to calculate the energy of the crystal lattice of compounds with complex ions. Still later, he produced a number of equations applicable to various crystallochemical problems.

Besides these problems, Kapustinsky tried to solve many others, especially in thermodynamics. In collaboration with B. A. Shmelev, he devised a new method of physico-chemical analysis which enabled them to study complex equilibrium systems. He also published numerous papers dealing with thermochemical methods, with ionic compounds, electrolytes and chemical technology. By introducing the zero period into the Periodic Table he developed the idea of secondary periodicity which he linked with the periodic structure of atomic nuclei. This led him to discuss matter subjected to high pressure and then to apply these ideas to his new "Theory of the Earth" (see *Nature*, 180, 1245; 1957).

During his busy life, Kapustinsky occupied the following posts: professor of physical chemistry at the University of Gorky (1933–37); the same post at the Moscow Steel Institute (1937–41) and also at the University of Kazan (1941–43); professor of general and inorganic chemistry at the Chemical-Technological Institute in Moscow (1943–60). In 1939 he was elected corresponding member of the Academy of Sciences of the U.S.S.R.

Kapustinsky's publications are varied and numerous. Besides pure research, he published some thirty papers dealing with topics related to the history of chemistry. He also edited many books translated into Russian.

In private life Kapustinsky was a most jovial fellow and a very versatile conversationalist, and his death at the early age of fifty-four was deeply felt by his friends, of whom he had many.

S. I. TOMKEIEFF

NEWS and VIEWS

Applied Mathematics at Manchester : Dr. F. Ursell

THE Beyer professorship of applied mathematics at the University of Manchester is to be filled by Dr. F. Ursell from October 1, following the departure of Prof. M. J. Lighthill for the Royal Aircraft Establishment in 1959. Dr. Ursell is at present a lecturer in the Department of Applied Mathematics and Theoretical Physics, University of Cambridge. He took the Mathematics Tripos at Cambridge during the early years of the War (in company with his predecessor in the Beyer professorship), and spent the remainder of the War at the Admiralty Research Laboratory, Teddington. The introduction which he gained there to problems associated with water waves evidently made a deep impression, for he has pursued such problems steadily and successfully since that time, and has done much to revive interest in a 'classical' subject which—like so many others—was thought to be well understood and well tilled until war needs revealed deficiencies. In 1947, he was elected to a research fellowship at Trinity College, Cambridge, for a dissertation embodying his research on water waves. A short time later, he proceeded to the University of Manchester, where he held an I.C.I. Fellowship, returning to Cambridge to take up a lectureship in 1951. He was eventually elected to a research fellowship at King's College, and has received other tributes to his research, which is characterized by great analytical power. He is interested in a wide variety of mathematical techniques, and can be expected to maintain the high reputation of Manchester as a centre of teaching in applied mathematics.

Electrical Engineering at Birmingham : Prof. J. T. Allanson

MR. J. T. ALLANSON has been appointed to the recently established second chair in the Electrical Engineering Department in the University of Birmingham. In this post he will be responsible primarily for the undergraduate work of the Department. Prof. Allanson entered the Department of Electrotechnics in the University of Manchester in 1938, taking his B.Sc. in 1942 and his M.Sc. in 1944. Although he had worked at the University on waveguides, he took for a year a post with Callenders Cables, Ltd., concerned with power cables and the cross-channel petrol pipe-line, and then went to Ultra Electric, Ltd., for two years to work on general electronics, including radio receivers. In 1947 he was appointed head of the Electrical Laboratory at the Telegraph Construction and Maintenance Co., Ltd., and was concerned once more with cables. In 1949 he was appointed lecturer in electrical engineering in the University of Birmingham, becoming senior lecturer in 1957. He is a man of very wide interests, embracing philosophy and biology as well as engineering; and, indeed, his strongest research interests lie in the field of nervous systems and particularly the mechanisms of hearing, and he has made some original contributions to this in co-operation with colleagues in the Department of Physiology. He has a strong natural interest in electrical net-work theory, and has published a number of papers on this topic, being particularly concerned with net-work synthesis. But his strongest enthusiasm is probably for teaching and students, and he has experimented in a number of ways to find better methods of teaching and to improve staff–student understanding and co-operation. He is also very interested in university administration. His appointment will give much satisfaction to his colleagues.

The Royal Scottish Museum, Edinburgh :
Dr. Douglas A. Allan, C.B.E.

On September 15, Dr. Douglas A. Allan retires from the directorship of the Royal Scottish Museum, Edinburgh, the destinies of which he has controlled for nearly seventeen years. After graduating at Edinburgh he became assistant to the professor of geology in Edinburgh and afterwards was appointed lecturer in geology in Armstrong College (now King's College, University of Durham), Newcastle upon Tyne. Work on Lower Carboniferous geology in Fife earned him a Ph.D., and later on Lower Old Red Sandstone tectonics along the Highland Border a D.Sc. In 1929 he was appointed director of Liverpool City Museums. In 1945 he became director of the Royal Scottish Museum, and returned to his native Edinburgh to complete his active professional career. Under him, the Museum has played a much more prominent part both nationally and internationally, for he has served on the Joint Committee of the Carnegie United Kingdom Trust for twenty-five years, advising on the allocation and utilization of development grants, was chairman of the British National Committee of the International Council of Museums (Unesco) for seven years, and this year is president of the editorial board of *Museum* (Unesco). In 1952 he was director of the first Unesco seminar on "Museums and Education", held in New York. Under his directorship, the Royal Scottish Museum has been modernized in display techniques, has rendered wide service to research in many fields, and has embarked on schemes of periodical exhibitions of educational and cultural value. His early exploring adventures are reflected in his four years tenure of the presidency of the Royal Scottish Geographical Society and his official museum tours in North America, Asia and Central Africa.

Mr. Ian Robertson Finlay

Mr. Ian Finlay, who succeeds Dr. D. A. Allan, was born in New Zealand but educated in Edinburgh, graduating M.A. with honours in the history of art. He joined the staff of the Museum in 1932 and was successively assistant keeper and, since 1955, keeper of the Department of Art and Ethnography. He is particularly interested in display methods in all types of museums and will bring his undoubted talents to the front at an important time in the history of the Museum, which has practically completed its major overhaul since the Second World War. Mr. Finlay is secretary of the Royal Fine Arts Commission for Scotland, a Freeman of the City of London and a Member of Livery of the Worshipful Company of Goldsmiths, London. He has a strong interest in contemporary Scottish crafts and is a leading authority on Scottish gold and silver, having written several books and broadcast on numerous occasions. Last year he was awarded a Leadership grant by the United States Government to enable him to visit museums and galleries in North America.

Mr. Cyril Aldred

Mr. Cyril Aldred succeeds Mr. Finlay as keeper of the Department of Art and Ethnography of the Royal Scottish Museum. Born in London, he was educated at Sloan's School and entered later on a course of study in the History of Art at King's College, London, and the Courtauld Institute, graduating as a B.A. in 1936. He was appointed assistant keeper in the Department of Art and Ethnography in the Royal Scottish Museum in June 1937, and has served that Department with distinction ever since, except for one lengthy break with the Royal Air Force during the Second World War, and two years as associate curator in the Egyptian Department in the Metropolitan Museum of Art, New York. From an early stage he has taken a very strong interest in Egyptology and the life and arts of ancient Egypt, a field in which he now has an established reputation, and in which he has published a considerable amount of work, both on the monograph and the popular level. A competent practitioner himself in gold and silver craftwork, he is particularly well qualified to judge standards and methods in the works in his care, and brings a new attitude to the study of the ancient Egyptians to that more commonly brought by the philologists. He has already made a notable impact on museum techniques by his staging of exhibitions, both temporary and permanent, and in the latter, in Edinburgh, introduced the novel idea of employing contemporary writings to explain and illuminate his ancient Egyptian exhibits—rather than the 'hindsight' view of a twentieth-century savant.

Veterinary Anatomy at Liverpool :
Prof. A. S. King

Dr. A. S. King, senior lecturer in veterinary anatomy in the University of Bristol, has been appointed professor of veterinary anatomy in the University of Liverpool. He is thirty-nine years of age, and was educated at Felsted School, Essex, and the Royal Veterinary College, London, where he obtained the degree of B.Sc.(Vet.Sci.) in 1946 and the degree of Ph.D. in 1956. During July 1945–December 1950 he held teaching appointments at the Royal Veterinary College, London. In January 1951 he was appointed lecturer in veterinary anatomy in the University of Bristol, and senior lecturer in veterinary anatomy in 1960. During 1960 he held the post of visiting associate professor in anatomy in the School of Veterinary Medicine, Philadelphia. Dr. King has been a member of the consultant editorial board of *The Journal of Small Animal Practice* since its inception.

National Reference Library of Science and Invention

Aslib Proceedings for July (13, No. 7) includes a report of the discussion meeting on the National Reference Library of Science and Invention arranged in London on May 9, at which Prof. R. S. Hutton had urged the paramount importance of the potential users' requirements in planning the Library, and expressed his doubts as to the adequacy of present plans and intentions. Sir Frank Francis said that the British Museum would have preferred to have the new Library entirely on the Bloomsbury site, but use of the South Bank site would permit full operation by 1965, whereas development of the Bloomsbury site would not be completed until 1985. The National Library of Science and Invention would be one of a number of departmental libraries of the British Museum, the work of which would be thereby co-ordinated. There would be a separate science library in Bloomsbury devoted to the historical material; current material would be held on the South Bank. The new Library would be limited to 500,000 volumes because the appropriate committee of the Advisory Council on Scientific Policy thought this was the largest number compatible with open access. All material discarded from the National Reference Library would go to the British Museum collections. Research was needed on documentation services for scientists and provision had been made for this in the plans. It had been hoped to include rooms for lectures and exhibitions, but, in view of the limited space on the South Bank, these facilities and accommodation for investigations into the use of scientific literature would be provided in Bloomsbury. Investigations were being made to determine the reference services that were needed and the results would be made public. A general reference service would be provided at Bloomsbury. In the discussion the need for a first-class reference and information service was stressed, and also the importance of a rapid shuttle service between Bloomsbury and the South Bank, making full use of the latest possible techniques. Prof. Hutton said that his fears had only been partly dispelled: the fundamental importance of the Library was not grasped by policy makers in or near the Government.

The Role of University Extra-Mural Libraries in Great Britain

In *Library Association Pamphlet* No. 21, E. P. Pritchard reviews the aims, organization and methods of university extra-mural libraries in Great Britain and the results so far achieved (Pp. 32. London: The Library Association, 1961. 5*s*., and 3*s*. 6*d*. to Library Association members). This is a comparatively recent development, and in some areas library services for extra-mural courses have an air of improvization, although in others present needs are well on the way to being met. Mr. Pritchard visualizes, however, a considerable growth during the next fifteen years in this side of university work, largely through an increase in the number of extra-mural students who are graduates or have been in a sixth form. He believes the co-operation of extra-mural libraries, public libraries and the National Central Library, which already exists in several areas, could meet almost any foreseeable needs, provided: (1) that library services and the resources devoted to them receive the same emphasis in extra-mural work as in university internal teaching; (2) that each type of library makes the kind of contribution it is best fitted to make; and (3) that tutors never forget the importance of reading in adult education and lose no opportunity of applying this principle. Mr. Pritchard gives an admirably concise survey of various problems and difficulties encountered in practice and the methods of overcoming them.

Zoological Society of London: Annual Awards

The Zoological Society of London will, from 1962 onwards, make the following annual awards for contributions to zoology. These will be in addition to the Society's Gold, Silver and Bronze Medals which have been in existence since 1847.

The Scientific Medal of the Society, which was instituted in 1938, will be presented to a zoologist, less than forty-five years of age, for distinguished work in zoology. The Prince Philip Prize will be awarded to a pupil of zoology, on the basis of an account of some practical work in the field of animal ecology. This will be open for competition to all pupils of zoology in schools in Great Britain, Northern Ireland, the Channel Isles and the Isle of Man, who are studying for the General Certificate of Education A-level examination or an examination of equivalent standard. The award will be a sculpture in bronze, "Animal Head", by Mr. Henry Moore. The entries will be in the form of an essay of not more than 3,000 words, the first award being made in April 1962. Full details will be sent to schools in September of this year, but the regulations can, in the meantime, be obtained from the Zoological Society (Education Department), Regent's Park, London, N.W.1.

Two further awards for contributions to zoology, also sculptures by Henry Moore, have been made possible through the generosity of Granada Television Network. The Stamford Raffles Award, commemorating the founder of the Zoological Society, will be awarded to an amateur zoologist for contributions to zoology, or to a professional zoologist for contributions which extend beyond his field of specialization. This sculpture, "Animal Form", will first be awarded in April 1962. The Thomas Henry Huxley Award will be presented annually to a postgraduate research student in a university in Great Britain and Northern Ireland, for outstanding original work in zoology submitted as a doctor's thesis. This award will also be a sculpture, "Bird", the first award being made in April 1962. Mr. Henry Moore has generously agreed that a limited edition of each of the sculptures may be made, thus establishing the awards for some years. Thereafter the form of award will be reviewed.

Employment of Graduates: a Durham University Survey

The fifteenth annual report of the Durham University Appointments Board, and the third to be published, expresses the view that the demand for graduates of all kinds, from many different types of employers, is still rising, and, generally speaking, more Durham graduates and postgraduate students were seeking first appointments in 1960 than ever before (Fifteenth Annual Réport. Pp. 9. Durham:

Durham University Appointments Board, 1961). Fewer men seemed to be applying to the Atomic Energy Authority, but the Board did not notice the diminished interest in the heavy electrical plant industry found elsewhere. Of male graduates in science taking up paid employment, 13 per cent went straight into school teaching, compared with 12 per cent in 1959; for women, the corresponding figures were 67 per cent and 58 per cent; 50 per cent of the men graduating in arts and 70 per cent of the women went into teaching, and of these, 75 per cent attended teacher training courses. Comment is made on the very low present-day demand for geologists, the acute shortage of mathematicians (the proportion entering teaching has in consequence declined), the increased demand for arts men from industry and the increased recruitment to the public service, though public service overseas attracted only one recruit. The serious lack of information bearing on the proper employment of the woman arts graduate is stressed.

Vision Research

THE appearance of yet another scientific journal is not necessarily a cause for rejoicing, but Pergamon Press is certainly to be congratulated on its initiative in founding *Vision Research*, an international journal the first number of which appeared in June (1, Nos. 1/2; June 1961. Pp. iv + 201. Annual subscription rates: (*A*) for libraries, government establishments, research laboratories, etc., £10 (30 dollars); (*B*) for individuals who place their orders directly with the publisher and certify that the journal is for their personal use, 70*s*. (10 dollars). London and New York: Pergamon Press, 1961). Several different disciplines have an interest in the subject of vision—psychology, physiology, ophthalmology, physics, photo-chemistry, genetics—and the subject has applications in very many different fields. It is too much to hope that all papers on vision can be channelled into a single journal, but if the best of them find their way into *Vision Research* this will be an immense gain to visual scientists, who at present have to search through a great variety of journals. It will also enhance the prestige of the science itself and stimulate more rapid progress in the subject. The first number has been dedicated very fittingly to Commander Dean Farnsworth, who was the guiding spirit when the project was first under discussion, but who died while the plans were still in the formative stage. A board of editors, under the chairmanship of Dr. T. Shipley, of Miami, is supported by a large honorary editorial advisory board drawn from some twenty different countries and representing the leading workers in the field. Success in a venture of this kind depends most of all, perhaps, on maintaining a high standard in the quality of the papers that are accepted for publication and on the quality of the publication itself. The first number has got away to a good start on both counts.

National Museums of Southern Rhodesia

IN the annual report of the National Museums of Southern Rhodesia for 1960 reference is made to the planning that has been undertaken for the provision of three new museums buildings at Salisbury, Bulawayo and Umtali (Pp. 22. Bulawayo, Salisbury, and Umtali: National Museums of Southern Rhodesia, 1961). To further these important projects the Administrative Section of the organization was moved to Salisbury as being better placed strategically to cover the three units. The move also relieved the Administrative Section, at a time of intensive planning, of the detailed work of any one unit. There have been several changes in the staff, and Colonel H. L. Boultbee, curator of the Queen Victoria Museum, has completed his contract with the Trustees. Much research has been undertaken and members of the staff contribute to an impressive list of publications.

Maccabaean Prize and Medal

ENTRIES are now invited for the 1962 Maccabaean Prize of twenty-five guineas and a Bronze Medal, which is awarded annually by the Faculty of the History of Medicine and Pharmacy of the Worshipful Society of Apothecaries of London. Competitors, who must be less than thirty years of age on March 15, 1962, will be required to submit before that date an essay of not more than 6,000 words on any subject connected with the history of medicine or pharmacy. Further details can be obtained from the honorary secretary of the Faculty, Dr. F. N. L. Poynter, The Wellcome Historical Medical Library, The Wellcome Building, Euston Road, London, N.W.1.

Plastics Institute Lectures on "Plastics"

THE Plastics Institute is again arranging a series of lectures on "Plastics", to be held at the City of London College, Moorgate, in the autumn. The lectures are of an introductory nature and are intended for non-technical staff. Applications for registration forms should be sent to the City of London College as soon as possible. The programme is as follows: "Types of Plastics", by Dr. C. A. Redfarn (October 2); "Plastics and the Chemical Industry", by Dr. E. S. Narracott (October 9); "The Structure of the Plastics Industry", by C. J. G. Stanley (October 16); "Plastics as Replacement Materials", by K. B. Bartlett (October 23); "Creation of New Markets by Plastics Materials", by C. W. Welch (October 30); "Plastics and Packaging", by Dr. G. Swift (November 6); "The Plastics Industry and the Rubber Industry", by J. A. Rhys (November 13); "The Future", by Dr. V. E. Yarsley (November 20).

University News: Leeds

THE following appointments have been announced: Dr. P. Grosberg, to a research chair of textile engineering in the Department of Textile Industries; Dr. P. R. Brook and Dr. G. A. Morrison, to be lecturers in the Department of Organic Chemistry; Mr. R. S. Davidson, formerly demonstrator in the Department, to be Brotherton Research Fellow in the Department of Organic Chemistry; Dr. I. G. Gass, to be lecturer in the Department of Geology; Mr. W. Houghton-Evans, to be lecturer in architectural engineering in the Department of Civil Engineering; Mr. M. Kirk, at present lecturer in the Department of Geography, to be lecturer in the Department of Social Studies; Mr. P. Levi, to be Bernard Hickson Fellow in the Department of Botany; Dr. G. A. Salmon, to be J. W. Wootton Research Fellow in the Department of Chemistry; Dr. Jean E. Wilkinson, to be Medical Research Council Research Fellow in the Department of Medicine.

The following grants and donations, among others, have been received: £1,000 from the Yorkshire Council of the British Empire Cancer Campaign, for the Department of Physical Chemistry; £15,000 a year for seven years from the Anglo-American Corporation of South Africa, Ltd., to continue the work of the Research Institute of African Geology, and

£13,362 during two years from the Department of Scientific and Industrial Research for an investigation into African rocks, for the Department of Geology; £1,000 from Courtaulds, Ltd., for the Department of Textile Industries; £1,000 during two years from Messrs. W. L. Ingle, Ltd., for the Procter Department of Food and Leather Science; £2,470 from the Ministry of Aviation for a research project in the Department of Metallurgy, Houldsworth School of Applied Science; £2,897 during two years from the Department of Scientific and Industrial Research for a research project in the Department of Biochemistry.

London

The following appointments have been announced: Prof. W. D. Gill, professor of geology and mineralogy in Trinity College, Dublin (see *Nature*, 172, 1082; 1953), to the chair of oil technology tenable at the Imperial College of Science and Technology; Mr. S. B. Watkins, head of the Department of Chemical Engineering at King's College, to the University readership in chemical engineering tenable at that College; Dr. Brian Abel-Smith, lecturer in social science at the London School of Economics and Political Science, to the University readership in social administration tenable at that School; Mr. L. P. Foldes, lecturer at the London School of Economics and Political Science, to the University readership in economics tenable at that School; Mr. Harry Townsend, lecturer at the London School of Economics and Political Science, has been appointed to the University readership in economics tenable at that School.

The title of professor of civil engineering has been conferred on Mr. J. K. T. L. Nash, in respect of his post at King's College; that of professor of powder technology has been conferred on Dr. H. E. Rose, in respect of his post at King's College; that of reader in economics on Mr. J. Wiseman, in respect of his post at the London School of Economics and Political Science; that of reader in engineering science on Dr. J. M. Zarek, in respect of his post at King's College; that of reader in pure mathematics on Dr. K. W. Gruenberg, in respect of his post at Queen Mary College.

Sheffield

Contributions to the University's Development Fund, the appeal for which was launched early in 1960, now total £480,975. While the major portion of the cost of the University's expansion is being borne by the Government, the University is required to make a contribution from its own funds towards the total allocation. It is to this end that the Fund will be used.

Announcements

H.M. The Queen has been pleased to approve the grant of a Charter to the British Institution of Radio Engineers. The grant of the Charter is recognition of the vital part played by radio and electronic engineering in modern times.

Mr. Ronald Beresford Dew has been appointed as visiting professor of industrial administration in the Faculty of Technology at the Manchester College of Science and Technology.

Dr. R. M. Laws, a principal scientific officer at the National Institute of Oceanography, Wormley, Surrey, has been appointed senior investigator in the Nuffield Unit of Tropical Animal Ecology, and will take up his duties at the Unit's field station at Mweya, Queen Elizabeth National Park, Uganda, in the autumn.

A summer school on corrosion has been arranged by the Metallurgy Department, Battersea College of Technology, and will be held during September 25–28. The topic will be "Corrosion Problems in the Electrical Power Industry". The course has been arranged in conjunction with the Corrosion Group of the Society of Chemical Industry, and will be given by independent lecturers. Enrolment forms can be obtained from the Secretary (Summer School), Battersea College of Technology, London, S.W.11.

The second conference on "Nuclear Reactor Chemistry" and the fifth conference on "Analytical Chemistry in Nuclear Reactor Technology" will be held in Gatlinburg, Tennessee, during October 10–12, under the sponsorship of Oak Ridge National Laboratory. Further information can be obtained from W. R. Grimes, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee.

A special meeting of the Iron and Steel Institute of Great Britain and the Metallurgical Society of the American Institute of Mining, Metallurgical, and Petroleum Engineers is to be held in the United States and in Canada, and at the Metallurgical Society's annual congress in Detroit, during October 17–November 8. Further information can be obtained from K. Headlam-Morley, the Iron and Steel Institute, 4 Grosvenor Gardens, London, S.W.1.

A symposium on "Corrosion in the Nuclear Energy Industry" is being organized by the Société de Chimie Industrielle, 28 rue Saint-Dominique, Paris (7e), and will be held during October 19–20. The symposium will be concerned specifically with corrosion in sheath materials.

A symposium on the freeze-drying of foodstuffs will be held at the Department of Chemistry and Food Technology, Borough Polytechnic, London, during October 19–20. The symposium will deal with the development of freeze-drying techniques, a survey of the commercial equipment at present available and a discussion of the potentialities of the technique in various branches of the food industry. Nutritional aspects will also be included. Pilot plant and model freeze-drying equipment, samples of freeze-dried products and packaging materials will be on display. Further details can be obtained from the Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

An interscience conference on "Antimicrobial Agents and Chemotherapy", sponsored by the American Society for Microbiology, is being held at the Hotel Commodore, New York City, during October 31–November 2. The first day will be devoted exclusively to infectious diseases, and the second and third to various aspects of antimicrobial agents, including their chemical evaluation. Further information can be obtained from the American Society for Microbiology, 19875 Mack Avenue, Detroit 36, Michigan.

The Research Fund of the Chemical Society provides grants for the assistance of research in all branches of chemistry. Applications for grants should be sent to the General Secretary, Chemical Society, Burlington House, Piccadilly, London, W.1, not later than November 15.

THE BRITISH HYDROMECHANICS RESEARCH ASSOCIATION

THE Laboratory of the British Hydromechanics Research Association at Harlow was open to visitors during June 13–15, when several hundred representatives from industry and the academic world saw details of the research carried out.

The Association specializes in the industrial application of fluid flow and serves a very wide range of industries. Some recent problems investigated are: the ventilation of mines; the design of centrifugal pumps; water hammer in pipe lines; rotary and reciprocating seals; cavitation; the hydraulic transport of solids; friction losses in pipe fittings; and hydraulic design of civil engineering structures. These problems are typical of the extensive interests of the Association. The numerous and apparently unrelated difficulties which arise in such industrial applications of fluid flow often have a fundamental similarity, and for this reason can sometimes be solved through the experience of specialists in fluid mechanics. The Association has been building up such a team in the twelve years since its formation, and can now offer members a pool of such specialized knowledge. Despite the name 'Hydro' in the title, the Association is interested in all fluids, both gaseous and liquid, and its membership includes such diverse industries as hydraulic machinery, instruments, paper, chemicals, aircraft, oil, brewing and the nationalized industries of coal, electricity and transport.

In its research on centrifugal pumps, the Association aims at establishing the sources of loss within a pump, which should lead to a better understanding of the way in which the efficiency of the pump can be improved. This work involves investigations of the Reynolds number effects on pump performance, the relation between impeller and volute characteristics and the overall machine characteristic, and the measurement of disk friction loss. The exhibits demonstrated the refined instruments necessary to study the distribution of velocity and pressure within a rotating impeller. This approach to the problem of centrifugal pump research will incidentally assist in the establishment of acceptable laws for the scaling up of model-pump tests, and for the use of air tests for the prediction of water-pump performance.

Of additional interest to those involved in the testing of hydraulic machinery was an exhibit summarizing the variety of items which have been studied relating to the design of pump test-circuits. These have included the use of guide vanes to prevent swirl and the development of a cascade design which will be more rigid than conventional cascades, and less sensitive to upstream-flow conditions. A 'pepper-pot' valve has also been developed which has the dual advantages of good control characteristics and low loss when fully open. For any circumstances where it is required to produce a good velocity distribution upstream of a test pump or flow-measuring device, a 'flow straightener' has been designed. This combines the features of a graded resistance and a honeycomb, and will reduce considerably the extent of straight pipe required upstream of a pump or flow-meter. A new understanding of the conditions of flow within a shielded pitot tube has enabled a simple shield configuration to be devised, which renders the pitot tube insensitive to the direction of flow over an angle of about 40°.

The Association has been able to assist in the solution of several mixing problems, and an exhibit devoted to this subject illustrated several different aspects of mixing. One of these is a study of the mixing of a jet of water discharged into a shallow lake. The purpose of the research was to check that adequate mixing took place when power-station cooling water was discharged into a lake, so as to avoid the risk of warm water re-circulating into the intakes, and to reduce the danger to the fish population. Another application of techniques of mixing is the development of an in-line blending unit which successfully mixes, in given proportions, fluids of widely differing viscosities. This uses an orifice designed to have a discharge coefficient which does not vary with Reynolds number.

Two recent items of research were on show, which were of particular interest to makers and users of control valves. The incidence of cavitation in such valves introduces risk of material damage to the valve seat, and, by increasing the loss through the valve, interferes with its effective operation as a control unit. The exhibit reminded makers and users of the importance of checking for cavitation, and showed the importance of a dimensionless 'cavitation number' in defining the incidence of cavitation. Research is also being undertaken on the oscillation of relief valves, a frequent source of trouble in hydraulic engineering. The model valve on show demonstrated that unstable oscillations could occur at several frequencies, depending on the damping.

The water-driven vacuum pump is a device familiar to laboratory workers, but it may not be appreciated that the same technique is becoming more widely used as an economic means of maintaining vacuum in power stations and chemical plant. The essential design features of these pumps were demonstrated with the aid of a transparent model, which showed the importance of ensuring good mixing between the driving fluid and the entrained gas.

When air is released under water from a line source a rising vertical current is induced which, when it reaches the surface, divides into two surface water currents. The windward side current is capable of breaking approaching waves, so that relatively calm water is found in the lee side of such a 'pneumatic breakwater'.

At the request of a Government research establishment, the British Hydromechanics Research Association has studied the relationship between air flow and jet velocity, and has found means of accounting for the effects of slip of the air bubbles.

The exhibits relating to models of civil-engineering structures and pump sumps demonstrated the increasing use of models to ensure the achievement of design requirements, and to devise modifications which could lead to economies in head loss.

For spillways, the function of the model is primarily to study the most suitable design of stilling basin to dissipate the energy of the water at the bottom of the structure. This aspect was illustrated by a model, at present being tested, of the spillway for the Sasuma Dam in Nairobi.

Models of the cooling-water intakes for the Sizewell nuclear power station were also on show. One, an air model, was being used to determine the head losses at the junctions between the intake shafts and tunnels. As a result of the tests made on this model, modifications to the hydraulic design which naturally had to be compatible with the structural engineering requirements have reduced the head losses due to the junctions by 40 per cent.

The diversity of interests of the Association was of advantage in this case, as the modifications were based on experience gained previously in the study of ventilation schemes of mines. These intakes will be located in a tidal estuary, and a water model has been constructed to examine the effects of tidal flow and tide-level on the flow and head loss in the intakes, and the possibilities of the formation of air entraining vortices.

The Association is also active in the rapidly expanding field of 'industrial hydraulics'. There are many problems involved in the design and utilization of high-pressure oil hydraulic machinery, and a particular example is the use of slipper pads to transmit the thrust from a swash plate to the pistons in an axial-piston pump. An analysis of the conditions for minimum power consumption of these slipper bearings has shown that the minimum occurs when the viscous drag loss is three times as large as the flow loss, although this ideal cannot always be attained as the resulting film gap would be too small. A new apparatus has been built to test slipper bearings under dynamic conditions.

Examples of machines embodying design features of this nature were seen in the form of two experimental power transmissions. One is for applications which require a rapidly variable speed, and is a 2 horse-power ball-piston pump/motor machine. The extremely low inertia of this unit is demonstrated by the fact that full reversal of rotation at 3,000 r.p.m. takes about 0·01 sec. The second exhibit was designed for a motor-vehicle drive where a prime requirement is high efficiency. This unit employs a differentially coupled pump and motor and has an efficiency exceeding 90 per cent, over a wide range of speed.

The Association has been active in work to ensure compatibility between a hydraulic system, and the hydraulic fluid. A rig was demonstrated for the evaluation of hydraulic brake fluids to study, over a wide range of temperatures, such factors as brake response, leakage, seal life and fluid deterioration. A committee has recently been formed to draw up a specification which will define adequately the properties required of a fluid to be used in particular applications.

Recently, the efforts of the Association in research on fluid sealing have been increased considerably by special contributions from members. This research is directed towards studying the physical processes involved in sealing in mechanical seals, stuffing boxes, and reciprocating shaft seals. A new test rig has been built to study the behaviour of mechanical seals at pressures up to 3,000 p.s.i. In particular, the rig will be used to investigate the possibility that the present limits on the pressure rating of this type of seal are due to mechanical distortion.

In reciprocating-shaft seals it has been found that the leakage is proportional to the square of the shaft speed, and independent of pressures above 1,000 p.s.i. The leakage increases and the friction decreases as the fluid viscosity is increased.

Although the exhibition was primarily of the present research work, an equally important function of the Association is to assist members in the solution of day-to-day problems involving fluid flow, and in issuing an *Abstract Bulletin* and technical reports which keep the membership in touch with the work carried out in other laboratories.

A problem common to all research associations is that of ensuring that the designers and development engineers, who will benefit most from the experience available at the laboratory, are kept informed of the service which is available. To this end, a mobile unit has been fitted out with exhibits demonstrating all the aspects of the work of the Association. The visits made so far by the unit, to member firms, have resulted in a fruitful interchange of ideas between the research staff of the Association and their colleagues in industry. S. T. Bonnington

BLOOD GROUPS OF ANIMALS OTHER THAN MAN

A CONFERENCE on "Blood Groups in Infra-human Species", organized by the New York Academy of Sciences, was held in New York during May 15–16. The programme of papers arranged by Drs. D. B. Amos and C. Choen very fully covered much present-day research on the identification of blood groups in animals other than man, and their use in a variety of pure and applied sciences. On the first day several papers described work with animals useful in medical research. Drs. R. D. Owen (California Institute of Technology) and D. R. Anderson (School of Aviation Medicine, Brooks Air Force Base, Texas) had collaborated on a study of blood groups in the rhesus monkey, with the object of providing markers for transplanted erythropoietic tissue in irradiated animals. Blood group systems in the dog had been thoroughly investigated by Drs. S. N. Swisher, L. E. Young and N. Trabold (Rochester School of Medicine, N.Y.), and used in experimentation of clinical interest. The rabbit is valuable in comparative research on hæmolytic disease of the new-born, and for this a knowledge of its blood groups was an essential pre-requisite. Dr. C. Cohen (Battelle Memorial Institute, Columbus, Ohio) pointed this out in his review paper, while making an eloquent plea for research workers to co-operate more closely in establishing nomenclature of blood group systems. Dr. Cohen also provided an illustration of the wider interest of the conference with his discussion of genetical aspects and natural selection. The usefulness of rats as laboratory animals was exemplified by the inclusion of three papers about them. Dr. R. D. Owen reported on genetic systems of antigens which had proved abundantly useful as tools in a variety of experimental studies, in particular of red cell chimærism following either parabiosis, embryonic injection or bone marrow injection after X-irradiation. The interest of Drs. A. E. Bogden and P. M. Aptekman (Biochemical Research Foundation, Newark, Delaware) was in inherited hæmagglutinogens of rats and their relationship to histocompatability factors. The presence or absence of one hæmagglutinogen determined growth or rejection of a tumour, and this system showed a number of other interesting parallels with the H-2 factors of mice. In a careful review paper, Dr. J. Palm (Wistar Institute, Philadelphia), described her own work with blood groups and histocompatibility factors of rats and related it to that of other investigators.

The first of three papers on mice was a review by Dr. D. B. Amos (Roswell Park Memorial Institute, Buffalo) in which most emphasis was naturally enough placed on the classical H-2 system of tissue and red cell antigens. A clear account of the serological and genetical complexity of this system was given. Drs. R. Basch and C. A. Stetson (Bellevue Medical Center, New York) followed with a description of results obtained using an assay method for those antigens of mice which elicit cytotoxin and hæmagglutinin production (antigens which they believe to be absolutely identical). A wide range of isoantigen activity—as judged by ability to absorb or neutralize cytotoxins—was found among various mouse tissues. Further detailed chemical results included the important finding that in liver homogenates antigenic activity was concentrated in the lysosome-rich fraction. Finally with mice, Dr. J. W. Goodman (Oak Ridge National Laboratory) described experiments in which blood or bone marrow, or both, were injected into irradiated mice, and the fate or establishment of the donor-type cells followed by serological means, either hæmolytic, for red cells, or cytotoxic, for lymphoid cells.

The remainder of the papers on the first day were of more general zoological interest, rather than specifically medical. Dr. J. Buettner-Janusch (Department of Anthropology, Yale) had studied the reactions of the red cells of various primates with various antisera prepared to agglutinate human A or B red cells. This work was intended primarily as an aid to anthropological investigations. In connexion with an entirely different sort of population study Dr. G. F. Ridgway (U.S. Bureau of Fisheries, Seattle) gave a fascinating account of the extremely difficult work involved in analysing blood groups of salmon and trout, primarily using isoimmunizations. Dr. W. H. Hildemann (University of California at Los Angeles) interpreted the term 'blood groups' in its very widest sense in considering the literature and his own work on genetic specificities of amphibians and reptiles. Individual differences in red cell antigens have been found in only five species; no intraspecific differences in serum proteins—as determined electrophoretically—have yet been found, although their existence as raw material for speciation must be postulated, in view of the known ordinal, familial or interspecific differences. In contrast to the above sparse evidence of diversity, tissue transplantation studies yielded evidence of a high degree of intraspecific antigenic diversity. The role of individual red cell antigens in speciation has of course been the main theme of the classical researches of Dr. M. R. Irwin *et al.* on pigeons and doves, and an authoritative account of this work was presented by Dr. D. H. Shaw (University of Wisconsin, Madison). Other aspects mentioned included the study of hybrid substances, and the use of blood-group factors as markers in somatic mutation work and in radiation chimæra research (the latter being Dr. Shaw's own particular contribution). The final paper on the first day was introduced humorously by the chairman as being about blood groups of bacteria. Dr. G. F. Springer (University of Pennsylvania, Philadelphia) had continued his previous work on the immune origin of so-called natural anti-human B antibodies —in which 'germ-free' chicks were inoculated with $E. coli\ O_{86}$—by studying the chemistry of the bacterial substances responsible for the cross-reactivity. Among various findings were striking resemblances to human blood group substances, as shown by the composition of the carbohydrates produced by hydrolysis.

The second day's programme was devoted almost entirely to blood-groups of animals of agricultural interest. The first of four papers on chickens was a general survey by Dr. D. G. Gilmour (School of Agriculture, Cambridge, England). One point especially emphasized was that the chicken has particular advantages as an experimental animal for the study of selective factors influencing the polymorphism of blood groups. Considerable evidence on this aspect was already available. Dr. W. E. Briles (De Kalb Agricultural Association, De Kalb, Illinois) followed with a meticulous account of experimental techniques leading to the discovery of further blood group systems in chickens, of which six were described, five probably new. Evidence on the relationship between B locus blood group genotypes of female parents, and the economic performance of their 'hybrid' offspring (four-way crosses of inbred lines), was presented by C. P. Allen (Hy-Line Poultry Farms, Johnston, Iowa). This paper was especially interesting, being indicative of the possibility of the application of blood groups in some systems of commercial poultry breeding. Work on a red cell agglutinogen of chickens identified by certain plant seed extracts was described by Drs. S. L. Scheinberg and R. P. Reckel (U.S. Department of Agriculture, Beltsville). The occurrence of this factor is both inherited and influenced by hormonal state, occurring in genetically competent females only on the onset of egg-laying. Pigs were the subject of two papers, the first being a very clear account of the state of knowledge of their blood groups by Dr. E. Andresen (Iowa State University, Ames), much of whose own work had been done at the Royal Veterinary and Agricultural College, Copenhagen. His interest was primarily in genetic studies, and to a lesser degree in hæmolytic disease of the new-born. This condition was the prime interest in the second paper on pigs by Drs. R. Saison and D. G. Ingram (Ontario Agricultural College, Guelph), who reported on some systems previously described, and other new ones. They also briefly mentioned studies of blood groups of mink.

The report on horse blood groups by Dr. D. Franks (Department of Pathology, Cambridge, England) was largely concerned with hæmolytic disease of the new-born foal, and included much important material of veterinary and comparative medical interest. Dr. Franks's suggested interpretation of some of his serological findings—variation in amount of antigens in different genotypes—as being due to a *cis-trans* position effect provoked one of the most lively discussions of the meeting.

The last afternoon session was opened by Dr. C. Stormont (University of California at Davis), who presented a highly competent and scholarly review of blood groups in cattle. In one cattle system, *J*, the antigenic substance is found in soluble form in various body fluids and tissues, and is absorbed on the red cells. This was the subject of a contribution by Dr. W. H. Stone (University of Wisconsin, Madison), who discussed physiological, genetical and chemical aspects. Dr. J. Gasparski (Polish Academy of Science) was unfortunately unable to attend the meeting, and his paper—on red cell antigens of the European bison identified by cattle blood typing reagents—was read for him. A definitive account of blood-group systems of sheep was given by Dr. B. A. Rasmusen (University of Illinois, Urbana), and the meeting concluded with a fascinating account, presented by Dr. A. Eyquem (Pasteur Institute, Paris), of blood group investigations by himself and Drs. L. Podliachouk and P. Millot. Species involved were chimpanzees, horses, mules, donkeys, sheep, pigs and cattle.

The papers described above are to be published as soon as possible in the *Annals of the New York Academy of Sciences*, and should remain definitive for some time to come. In addition, the meeting provided an excellent opportunity for exchange of opinions between workers in widely diverse fields, and there can have been few attending who did not acquire ideas for new approaches. Discussions were especially enlivened by the presence of some guests noted particularly for their research on human blood groups, among whom may be especially mentioned Drs. W. C. Boyd, S. D. Lawler, P. A. Levine and A. S. Wiener. Other activities associated with the meeting included an evening discussion meeting, which resolved to form an Immunogenetics Society of America, another evening discussion on blood-group nomenclature, and a most enjoyable dinner in honour of Dr. M. R. Irwin, arranged by some of his past students.

D. G. GILMOUR

DESIGN AND USE OF RESPIRATORS

A JOINT meeting of the Ergonomics Research Society and the British Occupational Hygiene Society at Porton on July 5 and 6 was devoted to the study of respirators. The Societies were welcomed by Dr. E. A. Perren, director of the Chemical Defence Experimental Establishment (C.D.E.E.). The first paper, by C. G. Trotman (C.D.E.E.), dealt with the history of the subject. Dust masks were much the oldest form of respirator but were not really significant until a performance standard for industrial dusts was established in 1933. Canister respirators for protection against industrial gases grew from the chlorine box respirator of the First World War. The Chemical Works Regulations of 1922 enforced the provision of respirators of an adequate standard; since then, systems of coding and testing respirators for practically all known industrial and agricultural hazards have been laid down.

R. G. Dorman (C.D.E.E.) discussed fibrous filters for protection against aerosol particles. Sizes near $0\cdot3\mu$ diameter are the most difficult to filter; collisions with filter fibres are less probable for particles having low inertia and small Brownian motion so that they tend to penetrate the filter relatively easily. Experimental demonstration of a minimum of filtration efficiency at a certain particle size proved the general validity of the theory of filter action. The resin–wool filter was described, which combined extremely low air-flow resistance with high efficiency, due to electrostatic action. Methylene blue and sodium aerosol testing, with flame photometer detection, were also discussed, and desirable standards of protection indicated.

F. A. P. Maggs (C.D.E.E.) gave an account of the removal of gases by activated charcoal. Adsorption is a matter of the surface area of the charcoal and the number of molecules of gas, so that a high molecular-weight gas is retained better than one having small molecules. The life-time of a respirator against volatile, low-molecular-weight vapours may be quite short and there is some difficulty about giving proper warning of incipient failure to the user.

J. E. Cotes (Medical Research Council Pneumoconiosis Research Unit) dealt with physiological aspects of respirator design, including breathing apparatus supplied with oxygen. Factors of importance included dead space, flow-resistance, vision, fit of face-piece, and leakage. In addition to protecting the wearer, breathing sets with closed circuit made it possible to help him in situations involving physiological stress by humidifying, cooling and adding oxygen to the air which he breathed.

The ergonomics of the respirator was dealt with by R. J. Shephard (C.D.E.E.), who described methods of assessing the loss of performance resulting from wearing respirators. Deterioration of specific functions was slight, and the expenditure of energy occasioned by dead space and resistance only amounted to a few per cent of maximum effort under average conditions. General discomfort, sweating and unusual activity, however, reduced tolerance unless the subject were adequately motivated.

The work of ventilation was analysed by E. A. Cooper (Department of Anæsthesia, Newcastle upon Tyne), who directed attention to the contrasting resistance laws which were relevant to laminar flow in filter beds and turbulent flow in pipes and valves; the latter rises much more rapidly with an increase in the velocity of breathing. By considering the mechanism of respiration and cardiorespiratory function it was possible to lay down allowable standards of resistance.

Respirators, besides being protective devices, have been used in physiological research since the days of Lavoisier. H. S. Wolff (Medical Research Council, Hampstead) dealt with such applications. Mouthpieces were often used in place of masks, for brief experiments, but were uncomfortable and promoted salivation. Masks were important for measuring expenditure of energy from consumption of oxygen; types produced for anæsthetics, for airmen and for gas-masks were commonly adapted for physiological research, and various measuring devices could be attached to them.

Communication between persons wearing breathing equipment was the subject of a paper by J. Ernsting and J. E. Gabb (Royal Air Force Institute of Aviation Medicine). The situation is complicated in aircraft by the effects of reduced pressure on auditory acuity. Speech gives rise to respiratory flow patterns which impose a severe strain on the flow capacity of respiratory equipment.

J. R. Carr (Royal Navy) spoke of the design of underwater breathing apparatus. The lungs of the user must be pressurized to the equivalent of the surrounding water, which results in a rapid increase with depth of the mass of gas consumed. Long endurance therefore necessitates either re-breathing or a surface supply of gas. Gases under pressure may affect man adversely, and psychological and physiological hazards may be introduced by limitations of the breathing set.

F. Lavenne and P. Leyh (Institut d'Hygiene des Mines, Hasselt, Belgium) compared the performance of two closed-circuit breathing sets, one working with compressed oxygen and the other with liquid air, and an open-circuit apparatus supplied with compressed air. Exercises performed at different temperatures showed that the inspired air temperature depended mainly on release of heat by absorption of carbon dioxide and was lowest in the open circuit apparatus. Light weight was an important point.

Industrial gas and dust masks were analysed by H. J. R. Letts (C.D.E.E.), who emphasized the influence on design of legislation requiring approved types. Dangers due to limitations of the absorbing medium are not serious, but the risk from leakage at the face-piece is significant and difficult to measure; a 10 per cent leakage due to physical activity was common, and wearability of a mask was an important attribute because of the user's tendency to avoid discomfort by discarding it. Leakage through the face-piece seems to be a fundamental limitation of the container respirator and may have to be avoided by electrically driven individual fan units.

The iron and steel industry uses respirators to a large extent, as was explained by J. Graham Jones (Richard Thomas and Baldwin, Ltd.). Major problems were carbon monoxide, from blast furnaces, and dust. For the former, only air-line masks had been successful, and compressed-air points were provided on furnace platforms. In the Fire Service, F. C. S. Shirling (Home Office) stated that respirators were useless, because of oxygen deficiency, and self-contained breathing sets were used which had a useful life up to 1 hr. An overriding factor in design was the necessity for instant availability.

The final session was conducted with Mr. A. C. Peacock (formerly of C.D.E.E.) in the chair, who also read a paper on the manufacture and testing of respirators. He suggested that it would be advantageous if users were to collaborate in producing a limited number of designs, which should be manufactured by a single organization.

S. T. Hermiston, R. F. Hounam and R. P. Rowlands (United Kingdom Atomic Energy Authority) listed the various types of apparatus for protecting people who handled radioactive substances. The problems encountered in other applications are here augmented by the necessity of decontaminating the appliances after use. For this reason, a worker is issued with a respirator from a pool instead of its being fitted to him individually, a practice which drew some criticisms.

The final application of respiratory devices discussed was the important one of mine rescue, by C. R. Senneck (Safety in Mines Research Establishment). A new closed-circuit liquid-oxygen set has outstanding performance; it weighs 33 lb. fully charged and gives cool dry air for more than 2 hr. During heavy work at 90° F., respiratory cooling of more than 1 kcal./min. is available and the resistance is only 1 cm. water at 300 l./min.; operation is automatic.

C. N. DAVIES

FOUR DECADES OF STATE FORESTRY IN GREAT BRITAIN

THE publication of the forty-first annual report of the Forestry Commission in Great Britain for the year ended September 30, 1960,* marks the first year of State forestry activity in its fifth decade of existence. In temperate countries it takes more than four decades to create new forests, a process which does not end with the planting of so many trees on so many acres. In the year 1959/60 the State planted 61,700 acres and private enterprise 36,900 acres, making a total of 98,600 acres. If this rate continues and if we assume that the average length of rotation, making allowance for the slow growth to be expected on much of the land being planted, is 80 years, then it would seem that the final wooded area will, in that time, amount to 8,000,000 acres plus 2,000,000 acres already existing, or 10,000,000 acres in all. If this is the avowed objective, the position at the moment is reasonably good. The competition for land in Great Britain has not prevented the Forestry Commission from adding during the year some 60,000 acres of plantable land to bring its total holding thereof to 1,618,400 acres. In describing the year's work it is interesting to find, however, that private forestry is placed before State forestry. This may be merely politeness, but things have certainly moved a long way since the last chairman expressed the view that private enterprise in forestry was doomed. It has greatly strengthened its position in recent years and the policy of paying grants in aid to the tune of £1,150,000 a year has given it a remarkable boost. The dedication of woodlands to productive forestry has advanced to the extent that the area of private woodlands eligible, or about to become eligible, for State aid is now 837,476 acres. There is thus quite a healthy balance between the managed woodlands of the State and those of the private owner. It is surely time to abolish, if possible, the needless complication of having two bases of dedication, for only two of the 562 schemes in operation are on basis II.

The report introduces to the public the concept of the 'working-plan', which is, in fact, a plan of management for the forest. It is defined as being "in effect, a blueprint for a forest or group of forests" and "a direction to succeeding generations of foresters ensuring that in all stages of development the basic objectives are kept clearly in mind". One might ask what is a blueprint ? Have British foresters become that mechanically minded ? In 1960 a new working-plan code came into operation. The prescribed format contains no fewer than 43 chapters, and, if the code is to be rigorously applied, it would appear that the technical staff will have even less time to undertake essential technical operations and may even be helping to dig its own grave.

---

* Forestry Commission. Forty-first Annual Report of the Forestry Commissioners for the Year ended 30th September, 1960. Pp. 69 + 9 plates. (London: H.M. Stationery Office, 1961.) 5s. net.

So far, the Forestry Commission has been able to sell all its felled material, which amounted in 1959–60 to 20·1 million hoppus feet from a total area of forest of 1,283,256 acres. This presumably included the 500,000 hoppus feet blown down, equivalent roughly to the growth on 10,000 acres. While the problem of marketing is easy in those forests near industrial centres it will become acute in the next decade or two in the more remote forests, the location of which can readily be seen from an examination of the maps provided with the report. Improved methods of exploitation and transport are receiving attention and may be the solution, involving greater use of machines. Too much machinery used in forestry operations will kill employment in the country and increase it in the towns, however, which runs counter to a policy of re-population of the former. The wide scatter of the State forestry operations can be seen from the maps mentioned. This adds greatly to the risk of fire. The cost of fire protection is a heavy item in the creation from scratch of pure coniferous forests and £273,000 was spent thereon in 1960. Out of a total area of 1,618,400 acres of forest, only 1,596 acres, or 0·1 per cent, were lost by fire, the greater part through an excess of zeal.

A paragraph describes what has been done to preserve the remnants of the natural pine forests which are owned by the Forestry Commission. It mentions "a broad belt of the eastern Highlands" where the native Scots pine is a dominant species. The meaning of this is obscure. It is claimed that investigations began in the early days of the Commission into methods of treatment of the natural stands it had acquired. Details are not given, but the investigations were of the most meagre description and the main method of treatment has unfortunately been to plant up the greater part of these areas with introduced exotic conifers. The attention being devoted to other important indigenous species, especially oak and birch, is woefully inadequate, even in the research programme.

Forest research is the subject of a separate annual report. A relatively large staff is engaged in this work but its organization is far from satisfactory. It has engaged in numerous projects, many of which seem to be of minor significance. The main aim of research should undoubtedly be the improvement of sylviculture. This remark owes its origin to a statement in the report that fertilizers have been applied from the air to checked spruce stands in Cornwall, with impressive results. For how long will these results last and what was the cost ? Much research work owes its origin to faulty sylviculture, and in the improvement in their sylviculture British foresters have a great deal to learn. Forestry in Britain has suffered from a lack of trained personnel of the higher ranks and still has a long and arduous task before it is to realize its main objectives.

Regarding the financial cost to Great Britain of the establishment and working of the Forestry Commission, this is an appropriate year in which to consider this, because, when the Commission was set up in 1919, it was claimed that it would be self-supporting in forty years' time. The total net sum spent since the start, after allowing for income other than Treasury grants, is £118,219,420, on which presumably some rate of interest is charged. Far from being now self-supporting, in 1960 payments were £13,930,162, of which only £3,130,639 was met by operational income from sales, etc. A Parliamentary vote of an additional £10,702,000 was still required. There is a somewhat naïve explanation of why forestry is not turning out to be the "counter to depopulation in the uplands" that it has for long been claimed to be. Almost any other industry could justify its existence by claiming that it gives indirect employment in other industries.

The manner of presentation of the Forestry Commission report is odd. The author of it is presumably the secretary of that body, but the report is signed by ten other persons and is presented to two Ministers in conformity with legislative requirements. To what extent each of the eleven persons in question takes responsibility for the contents remains uncertain. There is no minority report. Whether the two Ministers who receive it sit down and discuss it in detail, or whether each deals with it in turn, is doubtful. It should be mentioned, however, that the Forestry Commissioners can rely on advice from national committees, regional advisory committees, a Home-grown Timber Advisory Committee, a Committee on the Utilization of Home-grown Timber, a Mechanical Development Committee and an Advisory Committee on Forest Research. In all, between 150 and 200 persons are named in the report as having some say in the running of British forestry. This restricts the number of competent independent reviewers of their activities to a very few and makes their task invidious

M. L. ANDERSON

POTATO VIRUS DISEASES

ALTHOUGH conferences, especially those restricted to a particular subject (therefore small) can be very useful for providing opportunities for exchanging views, their proceedings are often superfluous. Most of the interesting information they contain has already appeared in scientific journals or in reviews as well. Recently, it has become customary to include at the end of each paper the discussion which it has stimulated, but most of it is trifling or irrelevant to the paper.

This criticism applies to the proceedings of the fourth conference on potato virus diseases, held at Braunschweig, during September 12–17, 1960*. As it is only three years since the last conference, it is not surprising that the twenty-three papers (175 pages) it contains have very few advances to report.

Recently, Dr. Stegwee discovered that potato leaf roll virus multiplies in the vector *Myzus persicae* and has now reported more evidence for this. Of special interest is the finding of P. Ehrhardt that infected aphids show significant decrease in oxygen consumption. V. Moericke discusses some electron micrographs of the salivary glands of *M. persicae*. (Unfortunately the micrographs are not reproduced.) Those from infected aphids contain rod-shaped particles (50 × 200 mμ) which are suspected to have some connexion with leaf roll virus if they are not the virus particles themselves.

---

* Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig. Proceedings of the Fourth Conference on Potato Virus Diseases, Braunschweig, 12–17 September, 1960. Edited by J. Brandes, R. Bartels, V. Völk and C. Wetter. Pp. 175. (Wageningen: H. Veenman and Zonen, N.V., 1961.)

The papers by C. Wetter and J. Brandes report some recent work at the Biologische Bundesanstalt, Braunschweig, Germany, which increases our scope for grouping viruses on serological relationships. Using Freund's adjuvant, antisera to viruses, having rod-shaped particles, were produced with precipitation titres of up to 1 : 500,000 (unusually high titres for viruses). With such antisera it has been possible to demonstrate serological relationships between viruses previously considered unrelated. Even more unexpected is the relationship found between viruses differing in particle-length such as between potato virus X (520 mμ) and white clover mosaic virus (480 mμ).

There are five papers on the resistance of potato varieties to virus infection, the inheritance of resistance and some practical aspects in testing for resistance. However, there is only one paper on field experiments. This may be the fault of the selection committee and not that this important kind of work is lacking.

In recent years soil-borne viruses are in the mode. In this respect the *Proceedings* are up to date, but of the several viruses discussed only potato rattle virus is appropriate to the conference. From the three papers attempting to review "some aspects" of virus research none contains anything new which has not been previously discussed in other review articles.

B. KASSANIS

CHROMOSOMES OF *NEUROSPORA*

RESEARCH workers at the University of Texas are investigating the genetic and nutritional simplicity of the red bread mould, *Neurospora*, to map the locations, on particular chromosomes, of the determinants of biochemical reactions by which particular substrates are metabolized for utilization in life processes. The work is expected to lead to a clearer definition of the gene.

The red bread mould *Neurospora* has long been regarded as one of the most useful of genetical experimental organisms. Pure strains can be multiplied a million-fold in a few days without genetic change. Each cell nucleus contains only a single set of genes, so recessive characters are not hidden by dominants. *Neurospora* can be grown in pure culture on a chemically known medium containing only sugar and a vitamin B (biotin) plus a few inorganic substances. In the natural state, the mould possesses the biochemical apparatus to make, from these simple materials, all the other constituents of its cells. Thus when a genetic element is disrupted experimentally by irradiation, the mutation may be expressed and mapped in terms of the measurable changes which occur in the simple nutritional requirements of the organism.

A large group of such nutritional mutations of *Neurospora* have been described by R. P. Wagner, C. E. Somers and A. Bergquist at the University of Texas in Austin. Plotting the distribution of the mutations on a map of *Neurospora* chromosomes, Wagner's group traced them all to a limited area of the fifth chromosome. Wagner's report is preliminary, but appears to record the first demonstration, in any organism with a single or double chromosome complement, of a direct relationship between one small segment of a single chromosome and a particular set of biochemical reactions. (Damage in the segment of chromosome affected directly the transformation of precursors of two important amino-acids—isoleucine and valine.)

The results also indicate a linear arrangement of the chromosomal material, with its active elements arranged to correspond to the order of sequence of the biochemical reactions it controls.

Wagner's work is supported by the Division of General Medical Sciences and the National Cancer Institute of the U.S. Department of Health, Education and Welfare. The publication *Highlights of Research Progress in General Medical Sciences*, 1960, presents a summary of Wagner's work and that of other investigators who are sponsored by the Division*.

---

* United States Department of Health, Education and Welfare. Public Health Service Publication No. 815: *Highlights of Research Progress in General Medical Sciences*, 1960. Items of Interest on Research Studies and Research Training Programs supported by the Division of General Medical Sciences, National Institutes of Health, Bethesda 14, Md. Pp. v+45. (Washington, D.C.: Government Printing Office, 1961.) 25 cents.

A CONCEPT OF ATOMIC EXISTENCE

By PROF. W. H. WATSON
Department of Physics, University of Toronto

IN classical terms we have a manageable concept of physical existence. We represent things in a space-time map. Usually the processes by which recognizing, naming and surveying are accomplished are not shown in this map. Nevertheless, they could be, and whenever there is doubt or unclarity in their application, they are so represented for the purpose of clarifying representation. So long as the precision of representation does not approach the limits set by the Uncertainty Principle, all the apparatus and procedure just referred to can be used. We have confidence in ordinary language and rely on the training of physicists needed for applying it successfully in this context. The force of this consideration is illustrated in the skilful elaboration of classical explanation by Thirring in the first chapter of his *Principles of Quantum Electrodynamics*.

At the microphysical level, of course, there are physical impediments to applying ideas that we take for granted in the unformalized improvisation necessary in using classical mechanics. The purpose of this brief article is to expose one aspect of our thinking that can be liberated from the pre-suppositions inherent in our forms of speaking about physical representation.

The classical picture of radiation misleads us with a picture of 'something' coming out of the source like a bullet from a gun. But to particularize the thing a label cannot be used; to name it one must point to the events connected by it.

For example, we know that the creation of a β-particle by a nucleus is associated with a detectable event—the recoil of the nucleus; and we know also that the β-particle can be detected in a scintillation counter. In the absence of a physical interaction by which the particle could be 'observed' on the way, the emitting nucleus is connected with the detector in one jump. Observing it in between creates a different phenomenon.

Motion in quantum physics is a transformation from one event to a later one. With the events, somehow or other an existence is connected. Classically, we want to put a thing at each event. The phenomenon of interference shows that we have to associate the existence with both events. The connexion between the events is an atomic existence: it cannot be cut.

The events that are connected are, in fact, not point-instants, although we may for mathematical convenience represent them as such, subject, of course, to the limited precision allowed by the Uncertainty Principle in applying the representation. So long as the events are well separated in space and time, this limitation may be left out of account in discussing the existence of atomic entities. Our idea must depend on essentially classical supports, namely, the events observed and recorded by means of devices we can see and handle, and the substantiality of the objects on which we depend to observe.

So far as physical observation is concerned where interference is involved, there is no existence between the source event and the detection event. So the kinematic order of ordinary physical space-time does not correspond to spatial possibility for the individual atomic existence. Yet the existences connect pairs of events in ordinary physical space-time. When under the stimulus of the bullet analogy for radiation we think of things launched into space, we fail to appreciate that we are ordering independent possible atomic existences into a series with a common initiating event (or one fictitiously arranged to be so) and a variable terminating one. But to get to a particular termination, one does not go through intervening members of the series, unless there are physical effects actually occurring by which to label the track. This then bears a resemblance to classical observation of a moving object.

A particle of a radiation field connects an emission event with a detection event (and it may also involve other events). The existence at the emission event is the recoil, or some such change in the emitter that can be observed. A corresponding existence is established at the detector. These two events, as it were, bound the existence of a particle which is said to leave the source and enter the detector, and is thought of as moving in the intervening space. Provided that the representation is crude enough, this view is practically effective. If sharper representation is attempted, the classical view of motion and the attendant possibilities of interpolating observations that do not disturb the physical process of the motion being observed are no longer allowed. Only if we accept the transition between the two events at the source and detector as the existence of the particle concerned are we on safe ground in relation to the interference of probability. In so far as we do not need to bring into account the physical effect of distant objects, we have the entity localized in an extended region that includes both events—but then we cannot imagine it moving within the region in a classically acceptable sense. Only if we can interpose events at which physical interactions take place that can be observed is the picture of classical motion applicable. Thus motion in quantum mechanics with non-classical statistics is represented by a set of separate existences which overlap on our map, whereas what corresponds to the classical case is a series of consecutive jumps in a continuing existence. The distinction is shown in Fig. 1.

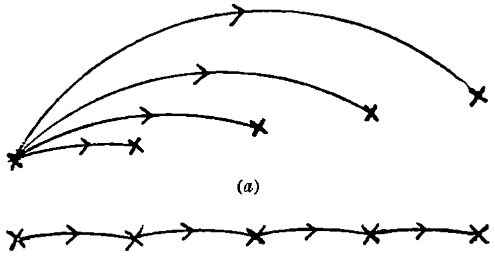


Fig. 1. The events being connected are marked ×. (a) Quantum connexions and overlapping existences; (b) classical series of consecutive jumps in a continuing existence

If we wish to represent the variable termination as a moving point governed by a continuous law of motion, we cannot have the point move in ordinary physical space because we must eliminate the intervening possibilities of ordinary physical space between source and detector.

We may exhibit in an elementary way how to achieve this. Consider the intersection of a rectangular axis triad ($OABC$) with a sphere of unit radius centred at O. Let the points of intersection be a, a', b, b', and c, c'. The directions Oa, Oa', Ob, Ob', Oc, Oc' stand for six distinct logical possibilities with which we are all familiar in ordinary language, namely, up-down, right-left, fore and aft. Vectors joining O to points (x) of the sphere other than a, a', b, b', c, c', do not stand for logical possibilities in this system. The vector Ox is a linear combination of the basis vectors Oa, Ob, Oc. (Here $Oa + Oa' = 0$, where + stands for the logical 'or'. So $Oa' = -Oa$. This is a necessary part of the logic of the system, since Oa and Oa' are mutually exclusive.) Ox does not represent one of the six logical possibilities, but it could represent a statistical combination of these possibilities, provided that we have regard to the laws of probability in establishing the representation. The mathematical elaboration of this is obvious, since the sum of the squares of the components of Ox is unity. The important point for my purpose here is that some points x are traversed by the representative point on the sphere in continuous transitions between pairs of the six logical elements of our system. A jump in the discrete system is then regarded as a continuous change according to some law which we have not yet specified. Once the continuous law is given, however, a track is specified on the sphere, and in going from Oa to Ob, for example, would pass continuously through points between Oa and Ob on the track.

This scheme of representation may be extended to a system of n possibilities and a representation in n-dimensional linear vector space. Imagine possibilities for existence on a lattice (h,k,l) where h, k and l are integers. We shall denote a variable point of the lattice by the integer triad (ξ, η, ζ). The mathematical identity:

$$u(\xi,\eta,\zeta) = \sum_{h,k,l} u(h,k,l)\ \delta_{\xi h}\delta_{\eta k}\delta_{\zeta l}$$

$0 \leqslant h,k,l \leqslant N$, where δ_{mn} is the Kronecker delta symbol, can be read as a vector equation in the space in which each

$$e_{hkl} = \delta_{\xi h}\delta_{\eta k}\delta_{\zeta l}$$

is a basis unit vector with $e_{hkl}\, e_{h'k'l'} = 0$ or 1 according as the corresponding primed and unprimed subscripts differ or agree. $u(h,k,l)$ is the hkl-component of the vector $u(\xi,\eta,\zeta)$; it may be a continuously variable number of absolute magnitude $\leqslant 1$, and indeed, corresponding to our previous spherical representation:

$$|u(\xi,\eta,\zeta)|^2 = \sum_{h,k,l} |u(h,k,l)|^2 = 1$$

Thus we have a N^3-dimensional linear vector space in which $u(\xi,\eta,\zeta)$ is represented as a vector.

Now consider displacement from $(0,0,0)$ to (a,b,c) on the lattice, that is, through points (ξ,η,ζ) for which the succession of co-ordinates is governed by the following rule (A); in each step only one of the integers, ξ,η,ζ, changes by unity while the other two remain unchanged. On the lattice, this resembles continuous motion in the continuum.

The corresponding transformation from $u(0,0,0)$ to $u(a,b,c)$ in the vector space proceeds via rotation from one co-ordinate axis to the next. But clearly, if u_{hkl} and $u_{h+1,k\,l}$ are positive:

$$u(\xi,\eta,\zeta) = u(h,k,l)\delta_{\xi h}\delta_{\eta k}\delta_{\zeta l} + u(h+1,k,l)\delta_{\xi h+1}\delta_{\eta h}\delta_{\zeta l}$$

represents a vector between e_{hkl} and $e_{h+1,kl}$. That is, whereas on the lattice we are concerned with integral steps between points of the lattice, in the vector space we can prescribe a continuous transition through the corresponding basis vectors in turn. Further, if we wish to allow only the integral connexion $(0,0,0) \rightarrow (a,b,c)$ on the lattice, we may prescribe a continuous one-parameter family of vectors bounded by $u(0,0,0)$ and $u(a,b,c)$, which includes none of the basis vectors except e_{000} and e_{abc}.

Thus this representation makes possible continuous transition (or motion) in the vector space corresponding to the connexion between two widely separated points of the lattice without requiring that intervening points of the lattice be traversed in accordance with the rule A.

Let us now pass to the representation of possibilities for existence in the continuum of physical space by letting N tend to infinity. The basis vectors now become:

$$\delta(\xi - x)\delta(\eta - y)\delta(\zeta - z)$$

corresponding to the spatial positions (x, y, z) and the sum over h, k, l is replaced by an integral over physical space. The integral connexion between two points of physical space, one corresponding to the source and the other to the detector of a physical particle, is represented in the vector space (which is now ∞-dimensional) by a continuous transition which need not require the vector to pass through any of the basis vectors.

In quantum mechanics the domain of numbers in which the components of the vectors vary is not the continuum of real numbers hitherto assumed above, but that of complex numbers (the grounds for this are given by H. Weyl in *Gruppentheorie und Quantenmechanik*, §4).

As an example of the law of continuous (unitary) transformation in the Hilbert space we may show the equation:

$$\frac{\hbar}{i}\frac{d\mathbf{x}}{dt} + H\mathbf{x} = 0$$

where $\mathbf{x}$ is the unitary vector and H is the Hermitian matrix for the energy (Weyl, *op. cit.*, §15).

In representing the emission of an atomic particle from a source, we should normally choose as the initial vector, consistent with our knowledge of the emitter, one of the characteristic outgoing spherical waves (an eigen function of the angular momentum). The location of the source is then indefinite to the extent determined by a momentum uncertainty equal to twice the momentum of the emitted particle. But when the source is observed to recoil, we have to choose a different vector to represent the outgoing particle: because of our enforced ignorance in the circumstances we have imagined, it cannot be an eigen vector of position or momentum, and it cannot be one of angular momentum. The particular vector judged appropriate for this representation carries the information needed with the dynamical law to determine the probability of the free particle being detected by a suitable detector placed in space to receive it. Thus the unitary vector associates a probability of detection with each point of physical space representing the position of a possible detector, and consequently with each member of the family of atomic existences that can be associated with the event in which the source recoils in a particular direction and 'a particle is emitted'.

If some diffracting or other physical system is interposed between source and detector, the 'state vector' for the particle is transformed by dynamical interaction and the new probability for detection at a given place is determined by the modulus squared of the component of the transformed vector in the functional direction, $\delta(x - x_D)\delta(y - y_D)\delta(z - z_D)$, (x_D,y_D,z_D) being the position of the detector.

In the functional space each possibility of existence is represented by a ray of 'unit length' from the origin. Only the particular axes that are eigen functions of 'the positional co-ordinates of the particle' correspond to the existences in physical space. Any other ray does not correspond to a point of physical space at all, because it involves in a sense every point of physical space. Thus in the functional space we have possibilities for existence that are not possibilities for existence in ordinary space. They can be interpreted statistically, however, and this is what quantum mechanics does.

In classical representation the physical thing is located in the event. This act in the representing process may stand for a quite different and more elaborate pattern of behaviour than merely marking the map, for it involves actual things and other processes. Whatever is done must be acceptable as a support for attaching to the event a name by which an existence inheres in the event. According to the conception presented here for atomic existence, we should attach the name to the atomic transition between source event and detection event. In a crude representation in which these events are not distinguishable, the name is in effect attached to a single event, as required by the classical representation with which we use the word 'existence' in ordinary language.

As to the existence of electrons in an atom or nucleons in a nucleus, physicists seem to be in no difficulty at the level of ordinary language, because the physical forces localize the particles sufficiently well to permit its unrefined use.

TIME-INTEGRATED MEASUREMENTS OF SUBMARINE IRRADIANCE

By RONALD CURRIE and LAURENCE DRAPER

National Institute of Oceanography, Wormley, Godalming, Surrey

IN the course of trials of a new type of self-recording integrating irradiance meter for use under water, some measurements of ecological interest have been made in two Scottish lochs.

The irradiance meters are designed to be suspended at a number of depths from a free-floating buoy and, in the course of a day, to integrate the total irradiance at these points. The final instruments will be designed to record the scalar irradiance in conjunction with measurements of the photosynthesis of the phytoplankton[1], but at present they have been used with flat-plate (cosine) collectors primarily to test the recording mechanism. No allowance has been made for this in presenting the results, and the integrated measurements do not take into consideration the alteration in angular distribution of the radiation as the day proceeds. Nevertheless, since the measurements were made under water, discrepancies from the true picture of downward irradiance are likely to be minimized through the effects of refraction at the surface and scattering by suspended particles in the water which tend to orientate the under-water radiation towards the vertical[2].

Through the courtesy and help of the director and staff of the Scottish Marine Biological Association Laboratory at Millport, we have been able to use these meters at two stations, one in Loch Striven and one in Loch Fyne. On both occasions the instruments were suspended at depths of 3, 5, 8·5 and 11 metres from sunrise to sunset in an unshaded position.

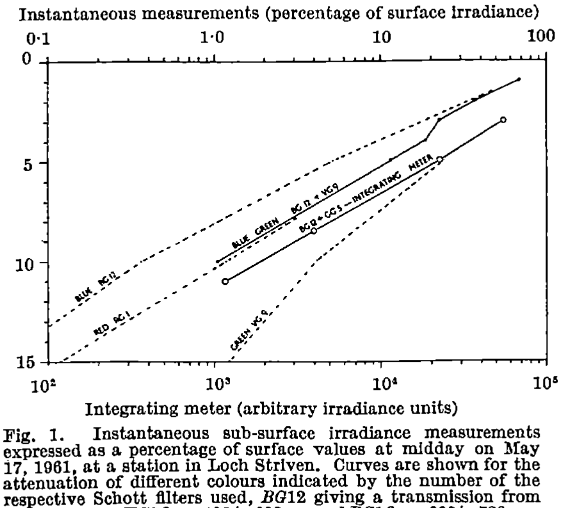


Fig. 1. Instantaneous sub-surface irradiance measurements expressed as a percentage of surface values at midday on May 17, 1961, at a station in Loch Striven. Curves are shown for the attenuation of different colours indicated by the number of the respective Schott filters used, *BG*12 giving a transmission from 860 to 480 mμ, *VG*9 from 480 to 600 mμ and *RG*1 from 600 to 720 mμ. The instantaneous measurements in the blue-green (*BG*12 + *VG*9) are compared with the integrated measurements (*BG*12 + *GG*5), the latter being expressed in arbitrary units

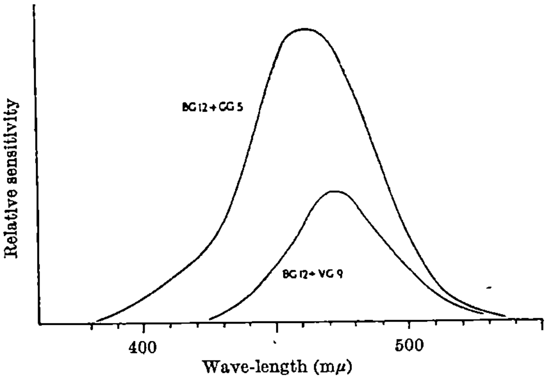


Fig. 2. The relative response of the cells and filter combinations used for blue-green instantaneous measurements (*BG*12 + *VG*9) and integrated measurements (*BG*12 + *GG*5)

Typical integrated measurements are shown in Fig. 1, where they are compared with instantaneous measurements which were made at midday on both occasions by the method recommended by Atkins *et al.*[3].

The integrating meters are fitted with a filter combination consisting of a 1-mm. thickness each of Schott *BG*12 and *GG*5 glass. The spectral sensitivity of these filters in conjunction with the photocell (Fig. 2) covers a 50-mμ band centred on the wave-length of maximum transmission in oceanic waters. This sensitivity lies close to that of the meters used for instantaneous attenuation measurements in the blue-green (Fig. 2) which were fitted with a combination of 2 mm. thicknesses of Schott *VG*9 and *BG*12 glasses.

Comparing the measurements in this part of the spectrum, it is clear that the decrease with depth of the irradiance integrated over a whole day follows a pattern very close to that of the attenuation measured instantaneously at midday. The corresponding attenuation coefficients 2·30 ($\log I_{\lambda,z} - \log I_{\lambda,(z+1)}$) (ref. 4) are shown in Table 1.

The close similarity between these measurements is very interesting, for it lends support to a method

Table 1. ATTENUATION COEFFICIENTS FOR INSTANTANEOUS MEASUREMENTS AND FOR THE IRRADIANCE OVER THE DURATION OF A DAYLIGHT DAY, IN THE 5–10 M. LAYER IN TWO SCOTTISH LOCHS

	Date	Instantaneous measurement Filters *BG*12 + *VG*9	Irradiance integrated over one day Filters *BG*12 + *GG*5
Loch Striven	May 17, 1961	0·482	0·493
Loch Fyne	May 18, 1961	0·532	0·559

commonly used in marine ecology in which the total radiant energy at different depths in the sea is computed by applying instantaneous attenuation measurements to an integrated record of the sea surface irradiance[5,6].

To the best of our knowledge these are the first direct measurements of sub-surface irradiance measured over a protracted period. While the agreement between these and the instantaneous measurements is good, it should be remembered that only the downward sub-surface irradiance has been considered, and that the measurements were made under ideal conditions with only slight sea-surface disturbance and a relatively high noon solar altitude (53°). We hope to extend these measurements to a consideration of scalar irradiation under water in diverse weather conditions.

Finally, an interesting feature of the optical characteristics of the water in these two lochs is demonstrated by the very high rate of attenuation of blue light (Fig. 1) measured with a Schott *BG*12 filter with its maximum transmission at about 430 mμ. This strongly selective absorption must only be ascribed to a strong yellowish coloration of the water, probably from dissolved organic substances like the "gelbstoff" of Kalle[7] and perhaps for the greater part originating from the humic acids in the fresh water inflow into these lochs.

A description of the integrating irradiance meters will be published shortly[8].

[1] Currie, R. I., *U.G.G.I. Symp. Radiant Energy in the Sea*, Helsinki, 1960 (in the press).

[2] Tyler, J. E., *Bull. Scripps Inst. Oceanogr.*, **7**, No. 5, 363 (1960).

[3] Atkins, Clarke, Petterson, Poole, Utterback and Ångström, *J. du Conseil*, **13**, 1, 37 (1938).

[4] Sverdrup, H. U., Johnston, M. W., and Fleming, R. H., *The Oceans* (Prentice Hall, Inc., New York, 1946).

[5] Jenkin, P. M., *J. Mar. Biol. Assoc. U.K.*, **22**, 301 (1937).

[6] Talling, J. F., *New Phyt.*, **56**, 29 (1957).

[7] Kalle, K., *Conseil. Perm. Int. Explor. Mer, Rapp. et Proc-Verb.*, **109**, Pt. 3 (1939).

[8] Draper, L., *J. Sci. Instrum.* (in the press).

FIBROUS SILICA

Dr. WOLFGANG HALLER

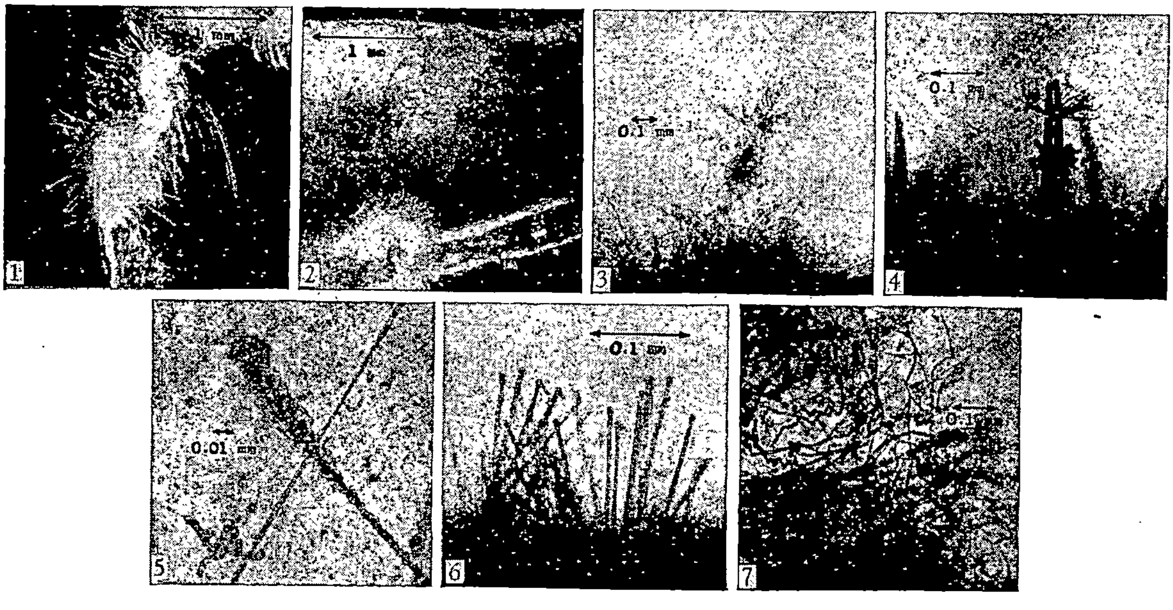
National Bureau of Standards, Washington 25, D.C.

FIBRES of silica were grown on electrically heated platinum surfaces by exposing the platinum target to nitrogen-diluted mixtures of silicon tetrafluoride and water vapour. Before striking the target, the mixture was passed through an electrically heated platinum tube maintained above the temperature of the target. The growth of the fibres on the target could be observed microscopically.

The fibres so obtained have circular cross-sections without any visible indication of faces. Diameters of fibres range from less than 1μ to 50μ. Their lengths range from 10μ to millimetres, and are limited mainly by the availability of growth space which has a homogeneous temperature field.

The refractive index of the fibres approximates the known refractive indices of vitreous silica (1·46) and is lower than the indices of any known forms of crystalline silica. Rotation of a fibre around its longitudinal axis reveals no optical anisotropy. Birefringency can be observed by rotating the fibres around axes perpendicular to their longitudinal axis. Because of the curvature of the fibres and the rather slight birefringency, it is difficult to distinguish if the birefringence extends uniformly over the full length or is segmentally modulated.

Figs. 1–7 illustrate typical forms of fibres. All fibres were grown at target temperatures of approximately 1,100° C. Lowering the target temperature



Figs. 1–7

produces colloidal silicious deposits, rather than fibres. Fibres start to grow straight or slightly curved, mostly originating from limited areas of the target. Clusters of this kind are shown in Figs. 1 and 2. The cluster in Fig. 1 originates from a corner of the platinum target. The cluster in Fig. 2 grew in a fan-shaped arrangement around the circumference of a thermocouple wire.

Frequently, secondary branching can be observed, as shown in Figs. 3 and 4. In these pictures the parental fibres are still visible. Another secondary growth occurs if fibres grow out of the optimal-temperatures field or if temperature fluctuations occur. In this case, the fibres terminate their growth with heads of opaque finely dispersed silica (Fig. 1) or the surface of the fibre becomes coated with colloidal silica (plume-shaped growth at the centre of Fig. 5).

Observation of fibre growth under the microscope conveys the impression that the fibres are in a plastic state. A fibre, having terminated its growth, may become shorter and thicker (Fig. 4) or may develop a spherical head at the point (Fig. 6). These spherical heads are as transparent as the rest of the fibre, while the colloidal deposits are opaque to light. The thickened fibre in Fig. 4 added later a mustachioed growth to its obese shape. Occasionally fibres suddenly change their direction of growth; the helical and zigzag shaped fibres shown in Fig. 7 owe their form to spontaneous angular bending of already grown, originally straight fibre segments. In addition to this drastic bending at distinct regions of the fibres, which is not reversible and which appears to be the product of flow or slip, the fibres constantly perform slight twisting and bending motions of synchronous character. Microscopical observation at growth temperature gives the impression of looking on a colony of water plants, drifting in a slow current. These motions are cyclic and reversible and may well be due to strains produced by fluctuations in the temperature field.

Spectrochemical analysis of the fibres produced only the emission lines of silicon. Water and fluorine contents were not determined, but are expected to be low since these compounds and their possible reaction products with silica have high vapour pressure at growth temperatures.

X-ray diffraction investigations of a multitude of fibres by the powder method showed amorphous bands plus faint but distinct diffraction lines, indicating a small degree of crystallinity. Selected fibres were mounted and investigated by single-crystal X-ray diffraction methods, but were only found to be completely amorphous.

A more detailed study of the fibre-forming conditions and more structural studies are being pursued. The most likely cause for the deposition of the silica into oblong, statistically and energetically unfavourable forms, is the action of some directing lattice force, although other directing mechanisms such as electrostatic agglomeration and thermal effects may be possible.

PYROLYSIS AND GAS-LIQUID CHROMATOGRAPHY ON THE MICROGRAM SCALE

By C. E. ROLAND JONES and A. F. MOYLES,

Research Department, Vinyl Products, Ltd., Butter Hill, Carshalton, Surrey

WE are now able to describe an improved pyrolyser unit for use in conjunction with a gas-liquid chromatograph.

Whereas the previous model[1], designed for use with a chromatograph equipped with a gas density balance detector[2], required a sample size of 2–3 mgm., the new unit, coupled with a Pye argon chromatograph, needs only 20–30 μgm. In this way the essential requirements for pyrolysis are better observed, in particular the criterion of a very thin sample film.

The improved pyrolyser (Fig. 1) consists of a 22-cm. length of 1 × 0·0015 single core 'Pyrotenax' so fashioned at the lower end that the sheath is partially cut away for 5 cm. and the insulating medium removed; the central conductor is shortened by 4 cm. and the end turned over to form a hook. A brass bush, 6 mm. long and 2·5 mm. in diameter, drilled to 20 wire gauge along its major axis and drilled and tapped to accept a 12 *BA* grub screw as illustrated, is brazed on to the inside of the cut-away at the bottom of the sheath. The filament is a 10 cm. length of 34 s.w.g. nichrome wire coiled to a diameter of 1·5 mm. (about 10 turns) one end of which is hooked over the end of the central conductor while the other is held captive in the brass bush after the length has been adjusted to give a resistance of 2 ohms. The upper end of the 'Pyrotenax' has the sheath removed for about 1·5 cm. to expose the central conductor and connexions are made in a conventional manner. The glass *T*-piece consists of a *B*.14 cone and a side entry for the carrier gas stream;

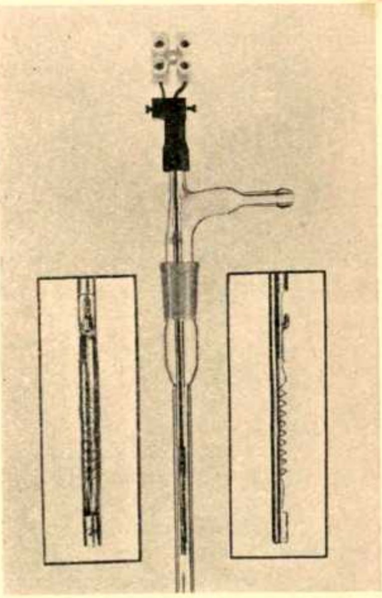


Fig. 1. General view of improved pyrolyser with details showing the filament mounting

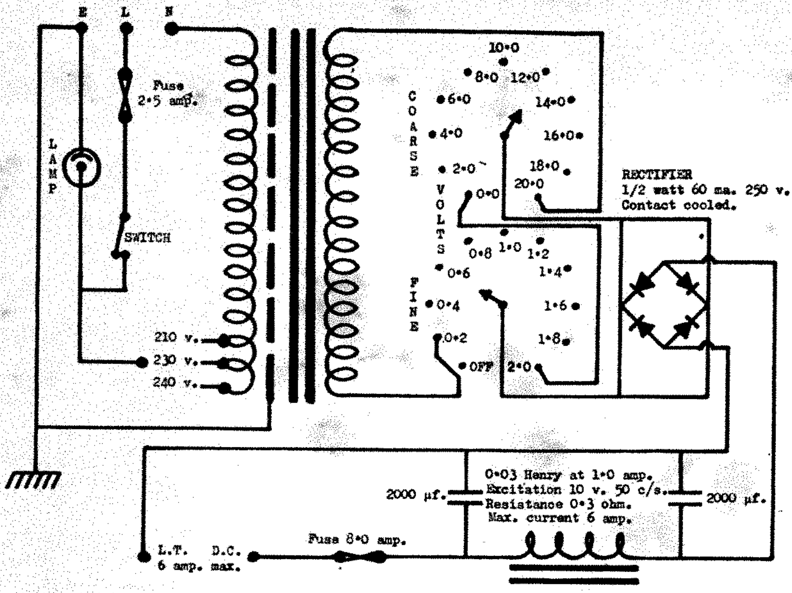


Fig. 2. Firing circuit for improved pyrolyser

the 'Pyrotenax' is held in position by means of a short length of thick-walled rubber tubing. The firing circuit (Fig. 2) is the low-tension section of a Radford 'Labpack'[3], which gives 0–22 V. by 0·2 V. steps up to a load of 6 amp.

The filament is calibrated by plotting applied potential versus temperature (measured under actual operating conditions with a Cambridge disappearing filament pyrometer).

The sample, in the form of a dilute solution or dispersion (about 1 per cent w/w concentration), is applied with a fine camel-hair brush to one or two coils toward the centre of the filament and dried under an infra-red lamp.

Comparison of the chromatograms obtained by pyrolysing a styrene homopolymer on both the milligram and the microgram scales (Fig. 3) is of interest, for the monomer yield rises from about 90 per cent on the milligram scale to near quantitative on the microgram scale. This, and the analogous behaviour of a methyl methacrylate homopolymer, are evidence for the occurrence of secondary reactions in the thicker film, probably due to the diffusion of fragments through the degrading polymer despite a reasonably short time of residence in the vicinity of the filament[4]. It is clearly advantageous, therefore, to work at the microgram-level.

The catalytic effect of the filament surface can be demonstrated at the milligram-level by comparing the pyrolyses of styrene and methyl methacrylate homopolymers using nichrome and gold-plated nichrome wires; the latter surface gives slightly simpler chromatograms. However, at the microgram-level no such differences are apparent when the pyrolyses are conducted on nichrome, gold-plated platinum and platinum filaments.

The versatility of the technique is illustrated by the following examples:

(i) Although many synthetic polymers are readily soluble in a wide range of organic solvents, it is often desirable to sample an aqueous polymer emulsion or a modified aqueous polymer emulsion direct without

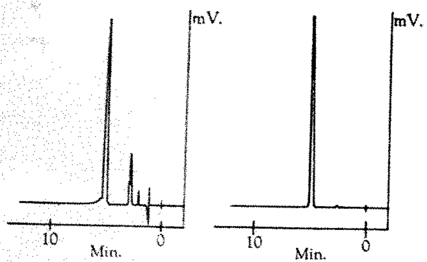
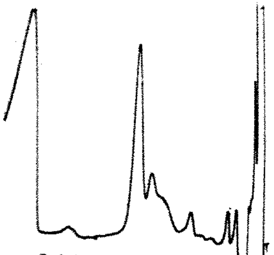
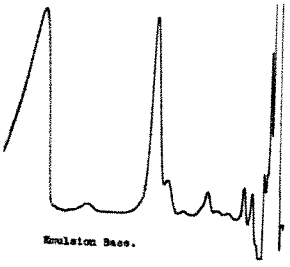


Fig. 3. Comparison of the pyrolysis chromatograms of a styrene homopolymer on the milligram scale (left) and the microgram scale (right) under otherwise similar conditions



Emulsion Paint



Emulsion Base.

Fig. 4. Pyrolysis chromatograms of an emulsion paint and the emulsion base obtained by pyrolysing at 700° C. and chromatographing at 82° C. on a 4-ft. column prepared with 12½ per cent w/w poly-(ethylene glycol succinate) on 100–120 mesh acid-washed 'Celite' 545. Detector setting, 1,250 V. at maximum sensitivity. Argon carrier gas at 24 ml./min. Chart speed, 6 in./hr. Time of each analysis, 40 min.

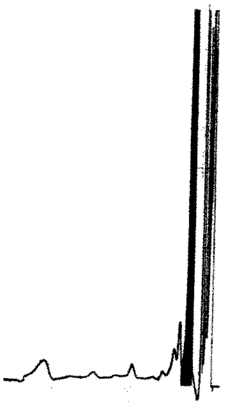


Fig. 5. Pyrolysis chromatogram of a sample of a motor-cycle fairing. The shaded peak is due to styrene. Conditions as for Fig. 4. Analysis time, 60 min.

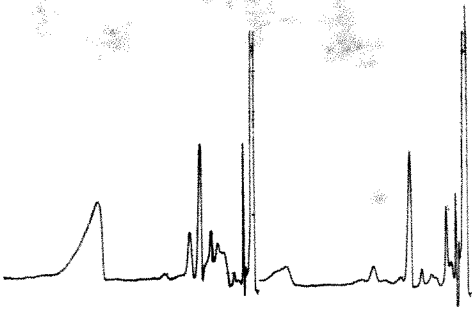


Fig. 6. Pyrolysis chromatograms of two samples of a silicone rubber. Sample 1 (left), sample 2 (right). Conditions as for Fig. 4. Time of each analysis, 50 min.

any preliminaries. This is exemplified in Fig. 4, which shows the characteristic identity of the polymer without separation from the inert constitutents.

(ii) Fig. 5 shows application to a fabricated polymer, a glass-fibre reinforced polyester, sampled by filing with a smooth Swiss file; the resultant powder mixed with 1 per cent w/w of gum tragacanth is slurried with water and then applied to the filament. Obviously, many materials may be treated in this way if they cannot be taken up into solution; a sample such as coal, for example, may be ground to an impalpable powder. It is desirable that filed or powdered samples should pass a 200 B.S.S. sieve.

(iii) Fig. 6 illustrates the pyrolyses of two specimens of a high-temperature silicone rubber, sampled by filing at – 70° C. (grinding at a somewhat lower temperature would be equally effective). Silicone rubber No. 1 retains its properties after prolonged use at 300° C., while silicone rubber No. 2 breaks down in use at 200° C. Quite clearly, there are chemical differences between the specimens.

We wish to thank the Directors of Vinyl Products, Ltd., for permission to publish this article, and we are indebted to Mr. G. E. J. Reynolds for helpful advice, and to Mr. M. Harrison for assistance with the practical work. Thanks are also due to Mr. F. Jackson, of the Woodall-Duckham Construction Co., Ltd., for the loan of a disappearing filament pyrometer.

---

[1] Jones, C. E. R., and Moyles, A. F., *Nature*, **189**, 222 (1961).

[2] Martin, A. J. P., and James, A. T., *Biochem. J.*, **63**, 138 (1956).

[3] Radford Electronics, Ltd., Ashton Vale Trading Estate, Bristol 3, England.

[4] Grassie N., and Melville, H. W., *Proc. Roy. Soc.*, A, **191**, 1 (1949).

EFFECT OF POLYVINYL ALCOHOL ON THE DISTRIBUTION OF IODINE-131 IN THE INTERNAL ORGANS, FLUIDS AND EXCRETA OF RABBITS

By H. F. NOWAK and J. KUCHARSKI

Department of Pathological Anatomy and Department of Medical Physics, Bialystok Medical Academy, Poland

ON the basis of experiments on mice given aqueous solutions of polyvinyl alcohol to drink in concentrations of 0·1 per cent–10 per cent for 210 days, it was found that this polymer is a non-toxic compound and causes a slight increase in the glycogen in the liver[1]. Experiments carried out with polyvinyl alcohol on rabbits showed that an 8 per cent solution at *p*H 7·3 and viscosity 1·7 possesses properties similar to plasma. This solution, when injected intravenously, brought about an excessive deposition of glycogen in the liver cells and Browicz-Kupfer cells during the first 4 weeks after injection, and, 3 months after injection, the liver glycogen content was similar to that of the controls. An increase in the number of normoblastic cells was noted in the marrow[2]. In experiments on *Tendipes* f. l. *plumosus* in aqueous solutions of polyvinyl alcohol, a 50 per cent increase in hæm was discovered[3]. Polyvinyl alcohol, administered intravenously to rabbits together with a shock dose of trypan blue, was effective in preventing the appearance of shock symptoms, and when administered to rabbits during the initial phase of shock, diminished the shock symptoms and thus prevented the death of the animals[4]. This polymer can, in addition to the above-mentioned properties, bind dyes and toxins and increase the kidney excretion coefficient of these substances.

In view of these properties, revealed in our previous experiments, our aim was to examine the effect of polyvinyl alcohol on the distribution of iodine-131 in animal organs.

The experiments were carried out on 20 rabbits of both sexes, weighing between 2·5 and 3 kgm. The rabbits were divided into two series, and each series was divided into two groups, (*a*) and (*b*). Rabbits 1–5, group (*a*), series I, were given 10-μc. iodine-131 intravenously in 10 ml. of physiological salt solution. Rabbits 6–10, group (*b*), series I, received 10 μc. iodine-131 in 10 ml. of polyvinyl alcohol, intravenously. All the rabbits of series I were killed 4 hr. after administration of iodine-131 with physiological salt solution and polyvinyl alcohol.

Rabbits 11–15, group (*a*), series II, received intravenous injections of 10 μc. iodine-131 in 10 ml. of physiological salt solution. Rabbits 16–20, group (*b*), series II, were given 10 μc. iodine-131 in polyvinyl alcohol, intravenously. All the rabbits of series II were killed 17 hr. after injection. Before killing the animals of both series, 3 ml. of blood were taken for testing. After centrifuging, the serum was separated from the morphological elements. When the rabbits had been killed, they were dissected. In order to detect radioactivity, the urine, fæces, bile, parenchymal organs, endocrinal glands, lymphatic nodes and marrow were taken for testing. All the tissues were dried at 80° C., after which they were triturated in a mortar, and the resulting powder put into specially prepared bags of polyvinyl chloride (as a protection against radiation). After weighing, the powder was placed under the mica end-window of the chamber in the Geiger–Müller, BAT-type counter and the number of impulses in a certain period of time was recorded.

At the same time, sections of organs were taken from each rabbit for histological examination. They were fixed in 6 per cent formalin solution and then,

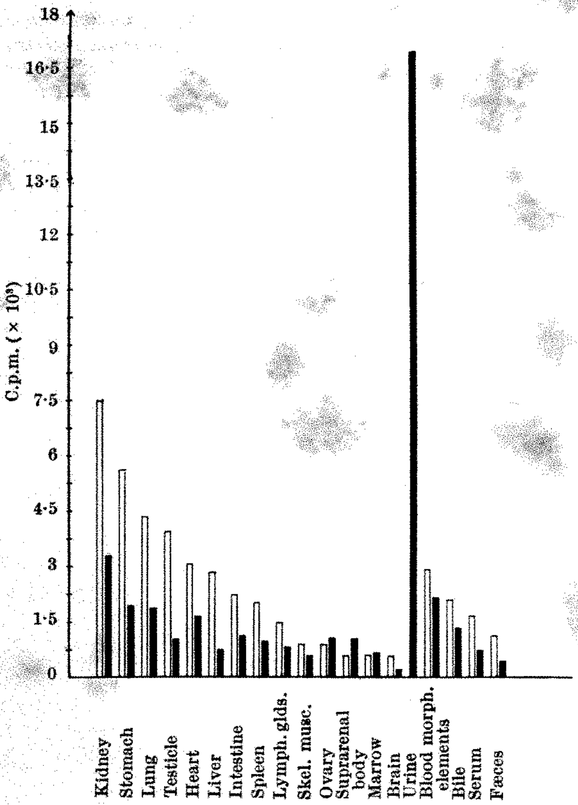


Fig. 1. Average findings from 5 rabbits injected intravenously with iodine-131 in physiological salt solution and from 5 rabbits given labelled iodine in 8 per cent aqueous solution of polyvinyl alcohol. The rabbits were killed 4 hr. after injection. The various columns represent activity of 1 gm. of dry mass of the organs of fluids, and of excreta, expressed in impulses per min. after intravenous injection of: white, iodine-131 in physiological salt; black, iodine-131 in polyvinyl alcohol

after dehydration, embedded in paraffin. The sections were stained with hæmatoxylin and eosin. The results are given in Figs. 1–3. The various columns represent 1 gm. of dry organic mass, fluids and excreta in counts per min. after intravenous injection of iodine-131 in physiological salt or polyvinyl alcohol. In calculating the radioactivity, self-absorption of radiation in the various samples was taken into consideration.

In the microscopical picture of the lungs of series I, group (*a*) rabbits, the walls of the alveoli were thickened. The epithelial cells of the alveoli were œdematous, with strongly acidophilic protoplasm. The mucous membrane of the bronchus showed hypertrophic and hyperplasic traits. The same changes were evident in the capillary vessels of the endothelium and the parabronchiole branch of the pulmonary artery. In the walls of the lung alveoli and in the blood vessel lumen, a great number of acidophilic leucocytes were seen. In the parabronchiole lymphatic nodules, hypertrophic and hyperplastic characteristics were observed.

The cardiac muscle displayed basophilic degeneration as well as single acidophilic leucocytes. The liver cells were enlarged, their trabecular outline obliterated and the nuclei of the Browicz–Kupfer cells clearly hyperchromatic. In the portal spaces, single acidophilic leucocytes were evident.

The bile duct epithelium showed hypertrophic characteristics. The epithelial cells of the straight kidney caniculi had a light-coloured protoplasm and gave the impression of being swollen. Among them, single acidophilic leucocytes were observed.

The suprarenal body displayed hypertrophy of the cortical layer with focal hyperplasia of the glomular layer. Single acidophilic leucocytes were seen in them. The stomach mucous membrane contained, in the superficial layer, single acidophilic cells. The lymphatic nodules of the appendix possessed hypertrophic characteristics and a considerable admixture of acidophilic cells. The glandular epithelium of the mucous membrane was grossly swollen and in places mucously degenerated.

In the rabbits of series II (*a*), killed 17 hr. after injection, there were considerably less acidophilic cells; on the other hand, mononuclear cells, similar in appearance to plasmatic cells, were increased in number.

In the sections of rabbit organs from series I and II (*b*) with polyvinyl alcohol, the acidophilic cells were less numerous than in group (*a*), with the physiological salt solution. The changes in the cells were absent, nor was there evidence of hyperplasia.

It was experimentally demonstrated that the rabbits which were given iodine-131 with polyvinyl alcohol were found to have a considerably different content of iodine-131 (in counts per min.) in their various organs, fluids and excreta, as compared with the rabbits given iodine-131 in physiological salt solution. In the thyroid gland, 4 hr. after administration of iodine-131 with polyvinyl alcohol, the radioactive iodine which accumulated was six times greater than in the thyroid of rabbits given iodine-131

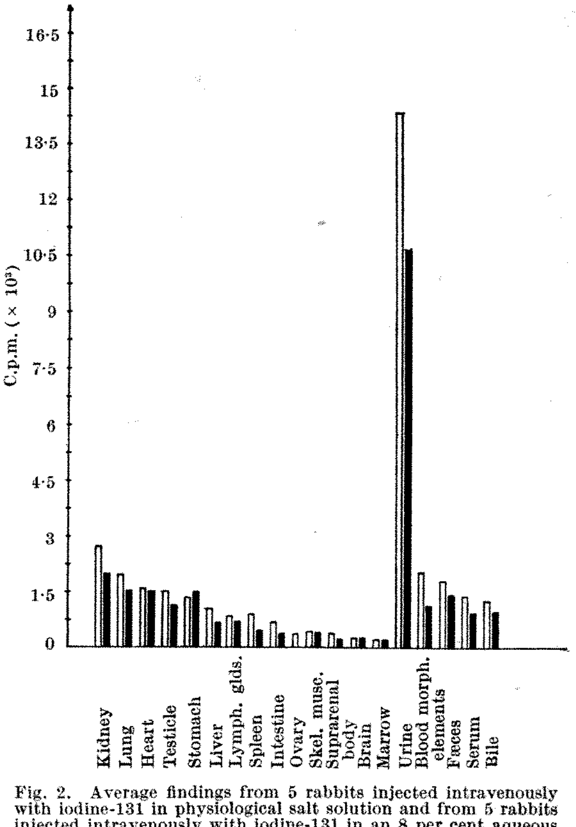


Fig. 2. Average findings from 5 rabbits injected intravenously with iodine-131 in physiological salt solution and from 5 rabbits injected intravenously with iodine-131 in an 8 per cent aqueous solution of polyvinyl alcohol. The rabbits were killed 17 hr. after injection. The various columns represent the activity of 1 gm. of dry mass of the organs, fluids or excreta expressed in impulses per min. after intravenous injection of: white, iodine-131 in physiological salt; black, iodine-131 in polyvinyl alcohol

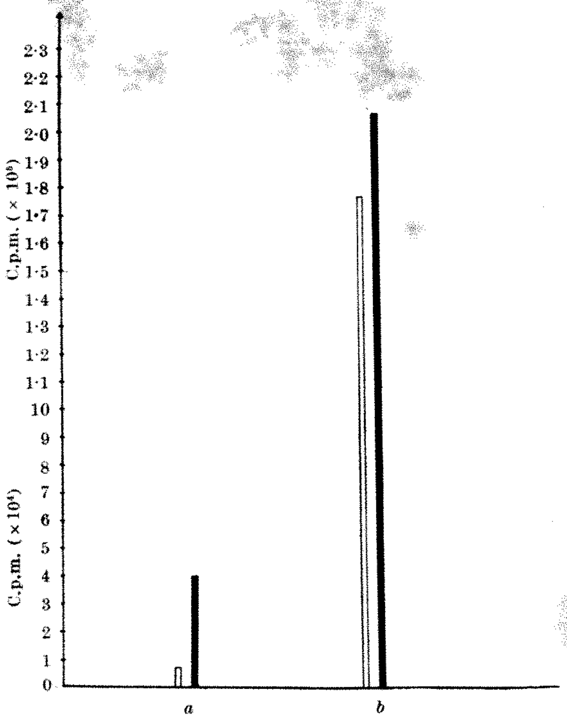


Fig. 3. Average findings from 5 rabbits which were given intravenous injections of iodine-131 in physiological salt solution and from 5 rabbits injected with iodine-131 in 8 per cent aqueous solution of polyvinyl alcohol. The rabbits were killed (*a*) 4 hr. after, (*b*) 17 hr. after injection. The various columns represent the activity of 1 gm. dry mass of degenerated thyroid, expressed in counts per min. after intravenous administration of: white, iodine-131 in physiological salt; black, iodine-131 in polyvinyl alcohol

with physiological salt solution. However, in the parenchymal organs of these rabbits, the amount of iodine-131 was less (by practically half) than that of the iodine-131 in the organs of the rabbits to which it was administered in physiological salt solution. After the injection of polyvinyl alcohol, the rabbits excreted urine in large amounts, especially during the first 4 hr. We recorded 1,650 counts per min. in the urine. Anuria was observed to occur during the first 4 hr. in the rabbits given iodine-131 with physiological salt solution. 17 hr. after injection of iodine-131 with polyvinyl alcohol, there was still more radioactivity to be found in the thyroid of these rabbits than in those which had received iodine-131 with physiological salt solution. Radioactive iodine accumulating in the parenchymal organs had a harmful effect on these organs, as was seen from the microscope pictures. Administration of iodine-131 with polyvinyl alcohol caused it to collect more rapidly in the parenchymal organs and thus the possibility of injury to these organs is considerably reduced.

In our experiments on rabbits, we also showed that the radioactive iodine given with polyvinyl alcohol and collected in the thyroid gland was six times greater than when given in physiological salt solution. Therefore, it can be concluded that doses of iodine may be reduced to a sixth, and thus the pathological changes in the parenchymal organs, occurring on administration of radioactive iodine, are even further diminished.

---

[1] Nowak, H. Fr., and Rejniak, L., *Patologia Polska*, IX, **4**, 381 (1958).

[2] Nowak, H. Fr., *Patol. Pol.* (in the press).

[3] Nowak, H. Fr., Czeczuga, B., and Rejniak, L., *Nature*, **188**, 119 (1960).

[4] Nowak, H. Fr., *Roczniki Akademii Medycznej w Bialymstoku*, **5**, (1959).

CONSTITUTION OF CELLULOSE MICROFIBRILS

By D. T. DENNIS and PROF. R. D. PRESTON, F.R.S.
Botany Department, University of Leeds

THE difficulty in isolating a native cellulose yielding glucose only on hydrolysis has long been recognized. Until comparatively recently the non-glucose sugars included in the hydrolysates of cellulose have been regarded as derived from residual unextracted hemicelluloses. The identification of cellulose with the microfibrils visible in the electron microscope, and the evidence that apparently pure microfibrillar material yield other sugars as well as glucose sometimes in amounts up to 50 per cent of the total sugar[1], show that the microfibrils are more complex than at first sight appeared. Since the microfibrils of, for example, *Rhodymenia* cellulose yield approximately equal amounts of glucose and xylose then they must at least contain molecular chains both of xylose and glucose. It is equally possible that the molecular chains may be mixed.

On various grounds it appears reasonable to suppose that the chains running down the centre of a microfibril form a core which is highly crystalline and that this core is clothed in a 'cortex' in which the chains, while still parallel, are not regularly spaced[2]. It is clearly possible to suggest further that this difference in the degree of crystallinity could be due to the presence in the cortex only of, for example, xylose residues, whether in the form of xylan chains or of mixed glucan-xylan. This possibility can to some extent be tested.

According to Ränby[3] and, later, Mukherjee and Woods[4] and others, cellulose microfibrils can under certain conditions be broken down to short rodlets which Ränby identifies with the crystallites—micelles—first suggested by Nägeli as the crystalline units in cellulose and later found necessary to explain some features of the X-ray diagram. If it were possible even approximately to associate these rodlets with short lengths of the crystalline core of the microfibril then analysis of these rodlets would serve to decide whether or not this central core is homogeneous. This article concerns the relevant observations.

Holocelluloses varying widely in hemicellulose content were prepared by the method of Jermyn and Isherwood[5]. With the exception of *Porphyra* these were treated with 4 per cent potassium hydroxide at 20° C. for 4 hr., a process known to remove most of the alkali-soluble hemicellulose with no damage to the microfibrils. Part of the residue was hydrolysed and the sugars identified as described by Cronshaw *et al.*[1]. The rest was treated with 2·5 *N* sulphuric acid at 100° C. for 24 hr. and washed until it formed a colloidal solution[3]. The dispersed phase

Table 1

Source of cellulose	Holocellulose *	Holocellulose after 4 per cent KOH *	α-Cellulose †	Colloidal cellulose *	Acid from colloidal cellulose *
Elm wood	glucose *S* xylose *M*	glucose *S* xylose *W*		glucose *S*	glucose *S* xylose *S*
Pine wood	glucose *S* mannose *M* xylose *M*	glucose *S* mannose *M* xylose *W*		glucose *S* mannose (trace)	mannose *S* xylose *S* glucose *S* galactose *W*
Rhodymenia	glucose *S* xylose *M*	glucose *S* xylose *S*	glucose *S* xylose *S*	glucose *S* xylose (trace)	glucose *W* xylose *S*
Ulva	glucose S xylose *W* rhamnose (trace)	glucose *S* xylose *W* rhamnose (trace)	glucose S xylose *S*	glucose *S*	glucose *S* xylose *M* rhamnose *W*
Laminaria	glucose *S* uronic acid *W*	glucose *S* uronic acid *W*	glucose *S* uronic acid *M*	glucose *S*	glucose *S*

* *S*, strong; *M*, medium; *W*, weak, spot on chromatogram.
† From Cronshaw *et al.* (ref. 1).

was precipitated by the addition of a few drops of 0·1 *N* hydrochloric acid and the precipitate was washed by centrifugation into acetone and dried *in vacuo*. The resulting powder was hydrolysed and the constituent sugars determined both in this hydrolysate and in the acid extract. At each stage the material was examined by electron microscopy and by X-ray diffraction analysis.

The analysis of the celluloses after the various treatments is given in Table 1.

The colloidal celluloses contain at most only traces of non-glucose sugars even though these are abundant in the parent microfibrillar material.

Breakdown of microfibrils to rodlets therefore involves the removal of the bulk at least of non-glucose sugars. Since, as shown for phloem fibres by Ränby[3] and by Mukherjee and Woods[4] and for our material during the present investigation, the rodlets still yield the X-ray diagram of cellulose 1 the molecular chains cannot have been widely separated at any stage of extraction, and it seems likely that they represent in only slightly modified form the original central core of the microfibrils. The evidence suggests therefore that this crystalline core is cellulose in the 'true' sense (the 'eucellulose' of Myers and Preston[6]) and that the non-glucose sugars of the microfibrils are confined to the outer layers. This is of considerable importance both in terms of the mechanism whereby the microfibrils may be synthesized and because it means that even in celluloses containing a low percentage of non-glucose sugar residues the exclusion of these from the central core increases the proportion of them lying on the surface of the microfibrils to a level greater than might otherwise be expected. Except when so rigorously extracted that a cellulose preparation falls to a colloidal solution, its reactions with non-swelling material may be those of xylan and/or mannan and not of a glucan.

[1] Cronshaw, J., Myers, A., and Preston, R. D., *Biochim. Biophys. Acta*, **27**, 89 (1958).

[2] Frey-Wyssling, A., *Science*, **119**, 80 (1954). Cronshaw, J., and Preston, R. D., *Nature*, **181**, 248 (1958). Preston, R. D., *Intern. Rev. Cytol.*, **8**, 33 (1959).

[3] Ränby, B. G., *Acta Chem. Scand.*, **3**, 649 (1949). Ränby, B. G., and Ribi, E., *Experientia*, **6/1**, 12 (1950).

[4] Mukherjee, S. M., Sikorski, J., and Woods, H. J., *J. Text. Inst.*, **43**, 1196 (1952). Mukherjee, S. M., and Woods, H. J., *Biochim. Biophys. Acta*, **10**, 499 (1953).

[5] Jermyn, M. A., and Isherwood, F. A., *Biochem. J.*, **64**, 123 (1956).

[6] Myers, A., and Preston, R. D., *Proc. Roy. Soc.*, B, **150**, 447 (1959).

BREAKDOWN OF [2-^{14}C]-GLYCINE DURING PAPER CHROMATOGRAPHY

By A. K. HUGGINS and V. MOSES

Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, S.E.5

IN the course of work on the metabolism of [2-^{14}C]-glycine by animal tissues, it has been observed that up to 40 per cent of the glycine may decompose with the formation of a number of radioactive substances when paper chromatograms are developed with phenol/water followed by *n*-butanol/propionic acid/water. It seems probable on the basis of chromatographic properties that the major breakdown product is neither glyoxylic nor glycollic acid. Unless quantitative measurements were made, the decomposition of glycine would not normally be detected, since the breakdown products did not give a positive ninhydrin reaction. Fowden[1] has reported losses of up to 30 per cent with glycine when chromatograms developed with phenol/water were dried with hot air. The influence on the rate of breakdown of glycine by various solvents, and of pre-treatment with oxalic acid of the paper used for chromatography, has been investigated.

[2-^{14}C]-glycine (0·1 μc.; 0·026 μmole) (Radiochemical Centre, Amersham, Bucks) was spotted on Whatman No. 4 filter paper, either untreated or previously immersed for 2 hr. in 1 per cent (w/v) oxalic acid, and then rinsed ten times during 48 hr. with distilled water. Chromatograms were run using the following solvent systems: (i) phenol (72 per cent w/w)/water (British Drug Houses phenol detached crystals B.P.C.); (ii) phenol (72 per cent w/w)/water, after distillation of the phenol over 0·1 per cent (w/w) aluminium turnings and 0·05 per cent (w/w) sodium bicarbonate[2]; (iii) *n*-butanol/propionic acid/water (45·9, 23·4, 30·6 per cent v/v)[3]; (iv) pyridine (80 per cent v/v)/water. One-dimensional chromatograms were run using each solvent on both oxalic acid-treated and untreated paper. Two-dimensional chromatograms were run on both types of paper using phenol/water or redistilled phenol/water followed by *n*-butanol/propionic acid/water, and again with the

Table 1. EXTENT OF GLYCINE BREAKDOWN DURING CHROMATOGRAPHY WITH VARIOUS SOLVENTS, AND THE EFFECT OF PRETREATING THE PAPER WITH OXALIC ACID

Pretreatment of paper	Dimensions	First solvent	Second solvent	Carbon-14 (per cent) not in glycine after chromatography	Carbon-14 (per cent) not glycine appearing during drying of phenol*	Protective effect of using the acid solvent in the first dimension*
None	1	Ph.	—	13·5	—	—
"	1	Redist.-Ph.	—	4·4	—	—
"	1	But.-Prop.	—	2·1	—	—
"	1	Pyr.	—	4·7	—	—
"	2	Ph.	But.-Prop.	37·6	22·0	—
"	2	Redist.-Ph.	But.-Prop.	21·8	15·3	—
"	2	But.-Prop.	Ph.	12·5	—	− 3·1
"	2	But.-Prop.	Redist.-Ph.	2·0	—	− 4·5
Oxalic acid	1	Ph.	—	12·8	—	—
" "	1	Redist.-Ph.	—	1·1	—	—
" "	1	But.-Prop.	—	0·2	—	—
" "	1	Pyr.	—	2·4	—	—
" "	2	Ph.	But.-Prop.	22·6	9·6	—
" "	2	Redist.-Ph.	But.-Prop.	4·1	2·8	—
" "	2	But.-Prop.	Ph.	8·6	—	− 4·4
" "	2	But.-Prop.	Redist.-Ph.	0·9	—	− 0·4

* Calculated as: sum of breakdown in both solvents − (breakdown in phenol/water + breakdown in *n*-butanol/propionic acid/water).

Abbreviations: Ph., phenol/water; Redist.-Ph., redistilled phenol/water; But.-Prop., *n*-butanol/propionic acid/water; Pyr., pyridine-water.

solvents used in the reverse order. All chromatograms were dried for 24 hr. in a current of air at room temperature. Radioactive substances on the chromatograms were located by radioautography using 'Kodirex' No-Screen X-ray film (Kodak, Ltd.), and the radiocarbon in each compound was counted directly on the paper with an end-window Geiger–Müller tube[4] connected to a scaler.

The results are presented in Table 1, which reports the percentage of the carbon-14 on each developed chromatogram present in substances other than glycine. With phenol as the first solvent, there was streaking of radioactive material ahead of the glycine spot, suggesting breakdown of the glycine during chromatography. When this was followed by development in the second dimension with *n*-butanol/propionic acid/water, the streaked material was well separated from glycine (Fig. 1). In the acid solvent, the most radioactive area of the streaked material lay directly ahead of glycine, and it is probable that this represented glycine which decomposed while the chromatogram was being dried following development with phenol. Development in the acid solvent then separated this material from glycine. From the sum of the extent of glycine breakdown during development in phenol/water and in *n*-butanol/propionic acid/water, estimates were made of the amount of decomposition of glycine while phenol was being removed from the paper by aeration: these values are included in Table 1.

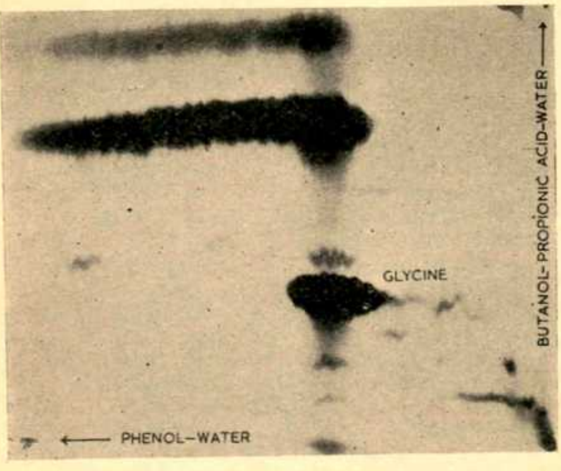


Fig. 1. Radioautogram of chromatogram of [2-^{14}C]glycine. Paper: Whatman No. 4 pretreated with oxalic acid. Solvents: phenol/water (first dimension); *n*-butanol/propionic acid/water (second dimension)

The breakdown of glycine during chromatography in *n*-butanol/propionic acid/water and in pyridine/water was low with untreated paper, and was reduced by treating the paper with oxalic acid. Breakdown in phenol/water was largely eliminated by distillation of the phenol, and there was a further improvement on treatment of the paper with oxalic acid, particularly with respect to the amount of breakdown of glycine during drying of the chromatogram. Development of the chromatogram with *n*-butanol/propionic acid/water prior to phenol/water exerted a protective effect on the decomposition of glycine in phenol, since the total breakdown after consecutive development in these two solvents was less than the sum of the observed breakdowns in each solvent separately.

Dr. J. R. Catch, Radiochemical Centre, Amersham, Bucks, has suggested that the extent of breakdown of glycine might be related to the very small quantities (2 µgm.) of the amino-acid which were applied to the paper. Three chromatograms were run using untreated paper, with phenol/water as the first solvent, and *n*-butanol/propionic acid/water as the second. [2-^{14}C]Glycine (0·1 µc.; 2 µgm.) was applied to the paper, either with no carrier or in the presence of 20 µgm. or 200 µgm. of unlabelled glycine. The percentages of the total carbon-14 not in glycine after development of the chromatograms were as follows: no carrier, 46·3; 20 µgm. of carrier, 39·4; 200 µgm. of carrier, 30·5. Thus, the breakdown of glycine was influenced by the total quantity of the amino-acid present on the chromatogram, but even large amounts of carrier provided partial protection only.

Fleming and Crosbie[5] have reported recently a non-enzymic transamination between glycine and glyoxylate which is catalysed by hydroxyl ions and inhibited at *p*H values less than 6. At *p*H 7 the reaction was catalysed by copper ions and inhibited by ethylenediamine tetraacetic acid. The *p*H of our paper, with and without treatment with oxalic acid, and before and after running in various solvents, is given in Table 2. Semi-quantitative determinations of the copper content of the solvents were performed by the phosphomolybdic acid method described by Feigl[6], and these values are reported in Table 3. It appears

Table 2. COMPARISON OF *p*H OF OXALIC ACID-TREATED AND UNTREATED PAPER BEFORE AND AFTER DEVELOPMENT IN EACH SOLVENT (Abbreviations as for Table 1)

Pretreatment of paper	*p*H of paper before development	Solvent	*p*H of paper after development
None	6·7	Ph.	5·5
,,	6·7	Redist.-Ph.	5·5
,,	6·7	But.-Prop.	4·8
,,	6·7	Pyr.	6·8
Oxalic acid	4·9	Ph.	4·8
,, ,,	4·9	Redist.-Ph.	4·8
,, ,,	4·9	But.-Prop.	3·3
,, ,,	4·9	Pyr.	5·0

Table 3. COPPER CONTENT AND *p*H OF SOLVENTS (Abbreviations as in Table 1)

Solvent	*p*H	Approx. copper content (μgm./ml.)
Ph.	4·8	20
Redist.-Ph.	4·8	20
But.-Prop.	2·5	10
Pyr.	9·2	0

from these results that the copper content of the solvents is not an important factor affecting glycine breakdown during chromatography, since there was no detectable difference in the amounts of copper present in the phenol before and after distillation; yet the extent of glycine breakdown was greatly reduced by distillation. The *p*H of the paper and of the solvents would seem to be of greater significance, as the degree of glycine breakdown on oxalic acid-treated paper (*p*H 4·9) was always less than with untreated paper (*p*H 6·7), even when pyridine/water (*p*H 9·2) was used as the solvent. The protective effect on glycine of developing the chromatogram first in the acid solvent is probably due to the incomplete removal of the acid during drying in air, resulting in a low *p*H of the paper after development in this solvent.

We have some evidence that similar chromatographic considerations may also operate in the case of other amino-acids, particularly L-[^{14}C]glutamic acid, and it would therefore appear advisable for the chromatography of amino-acids involving phenol as a solvent constituent to use an acid paper and to purify the phenol by distillation. Pretreatment of the paper with oxalic acid may also be beneficial as a result of binding any copper ions which may be present.

We wish to thank the Medical Research Council and the Empire Rheumatism Council for grants toward the cost of this work.

---

[1] Fowden, L., *Biochem. J.*, **48**, 327 (1951).

[2] Draper, O. J., and Pollard, A. L., *Science*, **109**, 448 (1949).

[3] Benson, A. A., Bassham, J. A., Calvin, M., Goodale, T. C., Haas, V. A., and Stepka, W., *J. Amer. Chem. Soc.*, **72**, 1710 (1950).

[4] Fuller, R. C., *Science*, **124**, 1253 (1956).

[5] Fleming, L. W., and Crosbie, G. W., *Biochim. Biophys. Acta*, **43**, 139 (1960).

[6] Feigl, F., *Qualitative Analysis by Spot Tests*, second English edition, 42 (Nordemann, Pub. Co., Inc., New York, 1939).

PHARMACOLOGICAL CHARACTERIZATION OF THE OXYTOCIC PEPTIDES IN THE PITUITARY OF A MARINE TELEOST FISH (*POLLACHIUS VIRENS*)

By PROF. H. HELLER and B. T. PICKERING
Department of Pharmacology, The University, Bristol
AND
J. MAETZ and F. MOREL
Departement de Biologie, Commissariat a l'énergie atomique, Saclay, Gif-sur-Yvette, Seine et Oise, France

PITUITARY extracts from teleost fishes have been shown to contain at least two oxytocic substances[1,2]. In the pollock or pollack (*Pollachius virens*) one of these substances has been identified as arginine^{8}–oxytocin (arginine vasotocin) by amino-acid analysis[2,3]. Arginine^{8}–oxytocin has also been chemically characterized in neurohypophysial extracts from frog[4] and chicken[5]. Chromatographic[2] and pharmacological[2,6] evidence has been advanced for the presence of arginine^{8}–oxytocin in the neurohypophysis of reptiles as well as in that of other amphibians and birds.

The second oxytocic principle found in pollock pituitary extracts was found to have many of the properties of oxytocin. Relative to its oxytocic potency, it showed very weak antidiuretic activity and, like oxytocin, had a much lower frog water balance activity than vasotocin[1]. When chromatographed on paper in butanol / acetic acid / water (4 : 1 : 5) (refs. 1 and 2) or eluted from a column of 'Amberlite *CG*-50' (ref. 2) it was found in the same position as oxytocin.

The 'second' oxytocic peptide in pollock pituitary extracts has now been compared with oxytocin ('Syntocinon', Sandoz) by further pharmacological tests. The sample of peptide used was prepared by paper chromatography of the material obtained from the region of oxytocin when pollock pituitary extract was eluted from a column of 'Amberlite *CG*-50' as in ref. 2. The active eluate from the paper chromatogram was freeze-dried and the resulting solid dissolved in 0·25 per cent acetic acid containing 0·5 per cent chlorbutol as preservative. This solution was used in all the biological assays described here.

The following tests were performed (the same batch of 'Syntocinon' = synthetic oxytocin, was used as standard throughout): (*a*) *Isolated rat uterus assay with and without magnesium:* Munsick[7] has shown that the action of many synthetic analogues of oxytocin on the isolated rat uterus is potentiated by the presence of magnesium in the suspension fluid. He showed also that the degree of potentiation varied from one peptide to the other. Accordingly, the unidentified pollock peptide was assayed on rat uteri suspended in either Munsick's[7] modified van Dyke–Hastings solution[8] without magnesium ions or in the same solution containing 0·5 m*M* Mg^{++}/l. The solution of the pollock peptide had a potency of 7 *U*/ml. in the absence of magnesium, but in the presence of this ion its potency was increased to

28 *U*/ml. Since 'Syntocinon' was the reference standard in both sets of assays, the increase in potency of the pollock peptide clearly differentiates it from oxytocin. (*b*) *Avian depressor assay*[9] *:* Estimations of the potency of the pollock peptide by this method gave a mean of 16·3 *U*/ml. compared with 7 *U*/ml. in assays on the isolated rat uterus without magnesium. (*c*) *Effect on sodium transport through the isolated frog skin* (natriferic activity[10]): unlike oxytocin, the unknown pollock peptide almost completely lacks the power to stimulate the sodium current produced by *Rana esculenta* skin *in vitro*. The mean natriferic potency was found to be 350 m*U*/ml. or 1/20 of the oxytocic activity. (*d*) *Effect on water movement through isolated frog bladder* (gravimetric assay[11,12]): as compared with oxytocin, the second pollock principle exhibited only a slight enhancing effect on the osmotic permeability of the *Rana esculenta* bladder. The mean activity was 750 m*U*/ml. or 1/9 of the oxytocic activity. (*e*) *Effect on water movement through the isolated frog skin* (volumetric assay[13]): The potency of the unidentified pollock peptide to enhance the net water flux through the isolated skin of *Rana esculenta* was very low, that is, about 350 m*U*/ml. or 1/20 of the oxytocic activity. (*f*) *Effect on water uptake by intact frogs:* in one *in vivo* experiment (21 animals) on *Rana esculenta*, the water-balance activity of the solution containing the unidentified pollock peptide was found to be less than 1 *U*/ml., that is, its potency in this test was less than 1/7 of that in assays on the rat uterus without magnesium ions. Low sensitivity of 'winter frogs' prevented further experiments.

The low potency of the unknown 'second' pollock oxytocic principle in all the amphibian tests is in striking contrast to the high potency shown by the 'first' oxytocic principle which has been chemically characterized as arginine^{8}–oxytocin[2,3]. Compared with oxytocin, for example, the latter was found in a few preliminary experiments to be about 20 times more potent on the sodium transport across frog skin and 200 times more active on the water movement through the isolated frog bladder. It was already known to be about fifty times more active in the frog water balance test *in vivo*[2].

Fig. 1 shows the difference in the potencies of the two oxytocic principles found in pollock pituitary extracts with reference to their action on sodium transport across frog skin.

The effect of oxytocin on the renal water excretion of rats varies according to the conditions of the experiment[14,15], but it has been shown that the hormone has a weak antidiuretic action in intact rats with a high water load. Assayed against 'Pitressin', 'Syntocinon' was found to have an antidiuretic activity corresponding to 1·1 per cent of its oxytocic potency[15]. When the unidentified pollock peptide solution was compared with 'Pitressin', its antidiuretic action was equal to a mean of 13 m*U*/ml. or to 0·2 per cent of its oxytocic activity.

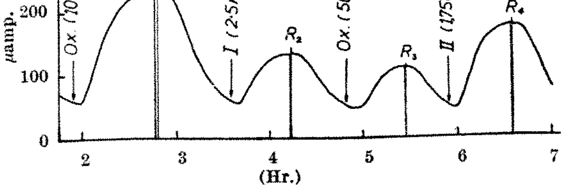


Fig. 1. Comparison of the effects of the two oxytocic pollock peptides and of oxytocin on the 'sodium current' (ref. 10) produced by frog skin. Ordinate: 'short circuited current' (in μamp.); abscissa: time, hr. Peptides added at arrows (in brackets: oxytocic = rat uterus activity). *Ox* = synthetic oxytocin; *R* = rinsing; I = the first oxytocic principle characterized as arginine^{8}–oxytocin. Note that it was about 20 times *more* potent than oxytocin. II = The second oxytocic principle. Note that it was about 20 times *less* potent than oxytocin

In a few preliminary antidiuretic assays[16] on *Rana esculenta* the antidiuretic activity of the unknown pollock peptide was likewise found to be very weak, while the 'first' oxytocic peptide showed a high antidiuretic activity similar to that of arginine^{8}– or lysine^{8}–oxytocin.

Table 1 shows the 'pharmacological spectrum' of the solution containing the unidentified oxytocic peptide in pollock pituitary extracts. The standard in each of the assays shown was synthetic oxytocin ('Syntocinon'). If, therefore, the pollock peptide had been identical with oxytocin, the activity ratio (last column of the table) would have been unity in each type of assay. The fact that all the assays on amphibians gave activity ratios very much less than unity indicates that oxytocin, if present at all, could only have been present in very small amounts.

Table 1. POTENCY OF THE UNIDENTIFIED ('SECOND') OXYTOCIC POLLOCK PEPTIDE ASSAYED AGAINST OXYTOCIN ('SYNTOCINON', SANDOZ) (Note that, by definition, the activity ratios would be 1, had the unknown substance been oxytocin)

Assay method	Potency (*U*/ml.)	Ratio of activity to rat uterus ($-Mg^{++}$) potency
Rat uterus without Mg^{++}	7	1
Rat uterus with 0·5 m*M* Mg^{++}/l.	28	4
Chicken blood pressure	16·3	2·3
Natriferic activity (isolated frog skin)	0·35	0·05
Water-balance activity (isolated frog skin)	~0·35	~0·05
Water-balance activity (isolated frog bladder)	0·75	0·11
Water-balance activity (intact frog)	<1	<0·15

Until the unknown peptide is completely purified, it cannot be excluded that the preparation used in the present investigation contained other very similar peptides. However, the results presented are sufficient to demonstrate that teleost pituitary glands may contain not only arginine^{8}–oxytocin (arginine vasotocin) but also another oxytocic peptide not found in the neurohypophysis of mammals. It would thus appear that the diversity of neurohypophysial active peptides in the vertebrate phylum is greater than hitherto assumed.

[1] Pickering, B. T., and Heller, H., *Nature*, **184**, 1463 (1959).
[2] Heller, H., and Pickering, B. T., *J. Physiol.*, **155**, 98 (1961).
[3] Heller, H., and Pickering, B. T., *J. Physiol.*, **152**, 56P (1960).
[4] Acher, R., Chauvet, J., Lenci, M.-T., Morel, F., and Maetz, J., *Biochim. Biophys. Acta*, **42**, 379 (1960).
[5] Chauvet, J., Lenci, M.-T., and Acher, R., *Biochim. Biophys. Acta*, **38**, 571 (1960).
[6] Sawyer, W. H., Munsick, R. A., and van Dyke, H. B., *Nature*, **184**, 1484 (1959).
[7] Munsick, R. A., *Endocrinology*, **66**, 451 (1960).
[8] van Dyke, H. B., and Hastings, A. B., *Amer. J. Physiol.*, **83**, 563 (1928).
[9] *British Pharmacopoeia* (The Pharmaceutical Press, London, 1958).
[10] Maetz, J., Morel, F., and Race, B., *Biochim. Biophys. Acta*, **36**, 317 (1959).
[11] Bentley, P. J., *J. Endocrin.*, **17**, 201 (1958).
[12] Sawyer, W. H., *Endocrinol.*, **66**, 112 (1960).
[13] Morel, F., Maetz, J., and Lucarain, Cl., *Biochim. Biophys. Acta*, **28**, 619 (1958).
[14] Brunner, H., Kuschinsky, G., and Peters, G., *Arch. Exp. Path. Pharmakol.*, **228**, 457 (1959).
[15] Berde, B., and Cerletti, A., *Helv. Physiol. Acta*, **14**, 129 (1956).
[16] Uranga, J., and Sawyer, W. H., *Amer. J. Physiol.*, **198**, 1287 (1960).

EVIDENCE OF A 'HUNGER MOTIVATIONAL' SYSTEM IN THE LATERAL HYPOTHALAMUS OF THE RAT

By Dr. P. J. MORGANE
Department of Physiology, University of Tennessee Medical School, Memphis 3

MUCH interest has recently been focused on the higher nervous control of food and water intake, particularly in the hypothalamic 'centres' concerned with the mechanisms of 'feeding' and 'satiety'. Several investigations[1-5] have tended to stress the importance of longitudinal hypothalamic fibre trajectories, especially the medial forebrain bundle, as being intimately involved in the motivation of feeding and drinking behaviour. Thus, Olds[4] classifies the lateral hypothalamic area along the medial forebrain bundle as a part of his 'reward' or 'pleasure' system, stimulation of which is 'preferred' by a hungry animal to actual feeding. Miller[6] has consistently found that stimulation of the lateral hypothalamic area in the perifornical area elicits feeding and drinking responses in satiated rats. His work indicates that the feeding and drinking areas are largely co-extensive with drinking responses slightly more prominent from the dorsal perifornical area. Miller has also shown that this stimulation evokes several properties of actual 'hunger' in that it would motivate learned food-seeking responses. Grossman[7], using the implanted cannula technique, has shown that injection of crystalline adrenergic substances into the peri- and supra-fornical areas elicits feeding in satiated animals and that acetylcholine injection caused water-sated animals to consume significantly more water. Furthermore, hungry rats would actually leave food, and drink water following acetylcholine injections.

In earlier experiments[8,9] I have shown that the medial forebrain bundle is not a crucial system in the effectuation of spontaneous feeding and drinking behaviour, since lesions in this bundle, singly and in combinations, anterior and/or posterior to the level of the 'feeding centre' did not chronically affect the intake of food or water. Only with lesions at the level of the ventromedial nuclei was the lateral hypothalamic syndrome of adipsia and aphagia produced, and these effects were radically different in nature depending on whether the lesions were in the mid-lateral or far-lateral hypothalamus. Mid-laterally lesioned animals showed a 'reversible' type of aphagia and adipsia, while far-laterally lesioned animals showed a more drastic 'failure' with no reversal tendencies even when carried 45 days on tube feedings. It was postulated that crossing fibre systems, mostly pallidofugal, were the critical elements in the organization of the feeding and drinking 'centres' since these were interrupted after far-lateral lesions but not after mid-lateral or perifornical lesions. The more drastic downhill course in the animals lesioned in the far-lateral hypothalamic area correlated well with degeneration in the pallidofugal fibre systems. Furthermore, it was shown afterwards[10] that lesions directed to the origins of these systems in the internal segments of the globi pallidi reproduced faithfully the far-lateral hypothalamic syndrome.

I have also shown[11] that the lateral hypothalamic area seems to be organized in a complex manner into a more medial 'hunger motivational' system and a far-lateral 'metabolic' system[11]. Animals lesioned medially and perifornically showed predominantly a 'motivational' failure toward food and water since this adipsic–aphagic state was easily reversible after forced feedings depending on the extent of the lesion. Some of these medially lesioned animals showed adipsic and aphagic reversal even when given special positively motivating foods such as chocolates, malts, etc., and it is now thought that all these animals are potentially reversible with proper post-operative support. On the other hand, far-laterally lesioned animals with pallidofugal fibre degeneration or animals with lesions in the pallida themselves are not so easily reversed. In my experience with 15 far-laterally lesioned rats supported by tube feeding, 5 died in spite of this treatment, 9 died of aphagia and adipsia after the tube feedings were discontinued (between 12 and 45 days post-operatively in the various animals), and only 1 showed reversal (resumption of spontaneous feeding and drinking behaviour) after 43 days of tube support. Of 9 animals lesioned in the pallida, 5 died during tube support while the remaining 4 reverted to adipsia and aphagia after tube removals (20–48 days) and died shortly thereafter. Five other animals lesioned in the pallida and not given tube support all died of aphagia and adipsia with food and water freely available. Thus, the far-laterally lesioned animals were not merely showing 'motivational inertia' to water and food but some additional defect not compensated for by actually delivering food and water to the gastro-intestinal tract. I have termed this a 'metabolic' failure as opposed to the purely 'motivational' failures of the mid-lateral and perifornically lesioned animals[10].

In the present experiments bipolar stainless-steel electrodes 0·20 mm. in diameter were implanted stereotaxically in the lateral and dorsal perifornical areas as well as in the far-lateral hypothalamic area. The stereotaxic co-ordinates used were: anterior planes 5·8–4·9 of DeGroot[12], parasagittal planes 1·0–1·7 for the perifornical implantations and 1·7–2·5 for the far-lateral implantations, and horizontal planes −1·5 to −3·0. This range of loci corresponded to several areas immediately around the fornix columns and in the areas of dispersion of pallidofugal fibre systems. Rectangular pulses of 0·2 msec. duration, 100 pulses per sec., were obtained from a 'Tektronix 162' waveform generator and a 161 pulse generator which drove a cathode follower output stage. The output from the cathode follower was fed to the electrodes through a General Radio isolation transformer. Current was monitored on a 'Tektronix 502' oscilloscope for each pulse series. The current generally used was 2 m.amp.

As a result of these stimulations additional support was added to the theory of the dual nature of the

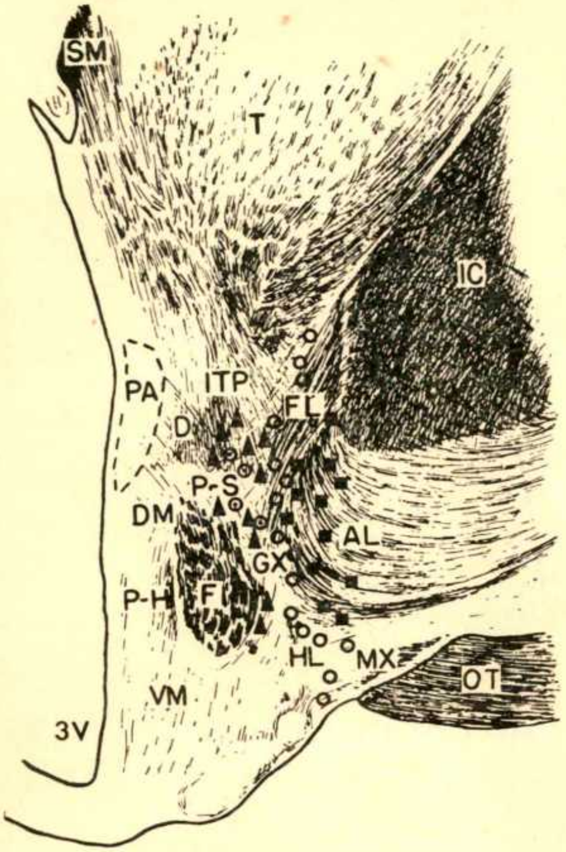


Fig. 1. Frontal section through the brain of the albino rat at anterior plane 5·8 of DeGroot with fibre systems indicated. Feeding responses to electrical stimulation and drug injection at points indicated by ▲; drinking responses to electrical stimulation and drug injection at points indicated by ⊙; no effects to either electrical stimulation or drug injection at points indicated by ○; feeding responses evoked by electrical and adrenergic stimulation at points indicated by ■. *SM*, stria medullaris; *T*, thalamus; *IC*, internal capsule; *PA*, paraventricular nucleus; *ITP*, inferior thalamic peduncle; *D*, dorsal hypothalamic area; *FL*, fasciculus lenticularis of Forel; *P-S*, paraventriculo-supraoptic fibres; *DM*, dorsomedial hypothalamic nucleus; *P-H*, pallido-hypothalamic fibres; *F*, fornix; *GX*, dorsal supraoptic commissure, pars dorsalis (Ganser); *AL*, ansa lenticularis; *HL*, lateral hypothalamic area; *VM*, ventromedial hypothalamic nucleus; *OT*, optic tract; *MX*, dorsal supraoptic commissure, pars ventralis (Meynert); *3V*, third ventricle

organization of the 'feeding centre' and in the first part of the present work confirmation of Miller's[6] work has been obtained. In 8 animals electrical stimulation of the dorsal and lateral perifornical areas has resulted in selective feeding or drinking responses in satiated states. The areas stimulated to produce feeding and drinking behaviour are shown in Figs. 1 and 2, which represent the two sterotaxic frontal planes utilized. Both these figures show that feeding and drinking responses were obtained mostly from the dorsal and lateral perifornical areas and that these response areas were distinctly separated from other response areas in the far-lateral hypothalamic area by regions of no response. However, after lesioning the medial forebrain bundle anterior and posterior to the level of the 'feeding centre' and repeating these perifornical stimulations (same animals with electrodes in place) no feeding or drinking responses were obtained in any of the animals. On the other hand, it was previously shown[11] that sated animals stimulated in the far-lateral hypothalamic area would feed even with the medial forebrain bundle lesioned. These far-lateral stimulated animals would not, however, run an electrical barrier to press a lever for food. Before lesioning the medial forebrain bundle the animals stimulated in the far-lateral hypothalamic areas would both feed in the sated state and run the electrical barrier to press a lever for food. This evidence certainly indicates essential differences in behaviour produced by the mid-lateral (or perifornical) stimulation as opposed to far-lateral stimulation. Figs. 1 and 2 also indicate the areas in the far-lateral hypothalamus stimulated in the present experiments. Stimulations in the areas shown provoked feeding in sated animals regardless of whether or not the medial forebrain bundle was intact.

Also in the present experiments we compared Grossman's studies as to the effects of injecting adrenergic and cholinergic drugs into the dorsal and lateral perifornical areas with the effects seen after these same substances were injected into parts of the far-lateral hypothalamus. Selective feeding and drinking responses were obtained from the perifornical areas, the adrenergic substances producing feeding in the sated state and the cholinergic substances 'pure' drinking responses. In these animals (with injection needles in place), after section of the medial forebrain bundle anterior and posterior to the level of the 'feeding centre', no feeding or drinking responses could be evoked by injection of these drugs into the perifornical areas. In another group of animals injection of adrenergic and cholinergic drugs into the far-lateral hypothalamus produced somewhat different results. Adrenergic drugs did

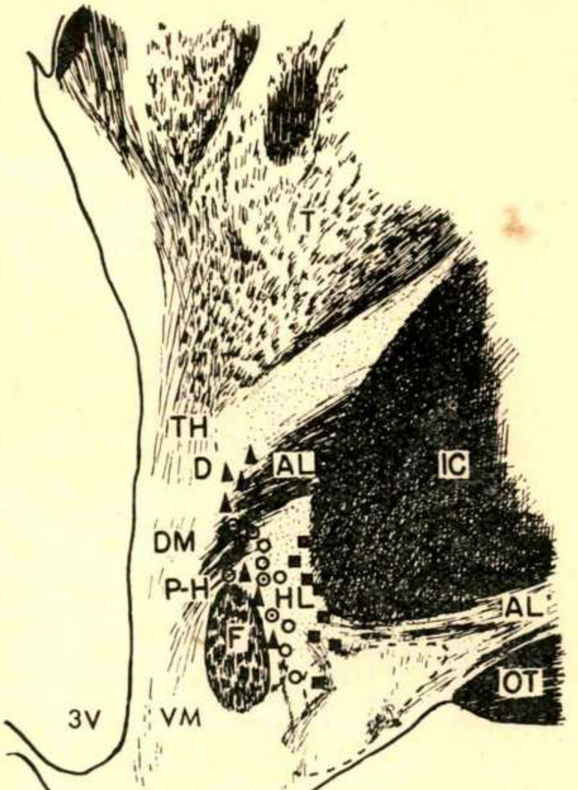


Fig. 2. Frontal section through the brain of the albino rat at anterior plane 4·9 with fibre systems indicated. Feeding and drinking responses designated as in Fig. 1. Notations for fibre systems and nuclei as in Fig. 1. *TH*, thalamo-hypothalamic fibres

provoke feeding in the sated state, but no 'pure' drinking responses could be obtained with cholinergic substances. After section of the medial forebrain bundle in this far-lateral group, however, feeding responses in the sated state were still easily obtainable following injection of adrenergic materials. These feeding responses were obtained from the same areas of the far-lateral hypothalamus as those that responded to electrical stimulation (Figs. 1 and 2). It is clearly seen from these figures that the critical areas are directly in the dispersion fields of the pallidofugal fibre systems (ansa lenticularis and lenticular fasciculus of Forel).

It is concluded that the mid-lateral and perifornical portions of the lateral hypothalamus contain 'hunger motivational' systems important in food drives. Intactness of the medial forebrain bundle seems necessary for this system to function properly since this system can only be activated (by stimulation or drug injection) in sated animals to produce further eating (or drinking) if the medial forebrain bundle is intact. Oddly enough, stimulation farther laterally in the far-lateral hypothalamus will cause sated animals to feed even when the medial forebrain bundle is lesioned; on the other hand, certain learned food-seeking responses are not provoked by far-lateral stimulation when the medial forebrain bundle is disjoined. It thus seems that the far-lateral mechanism is the more 'basic' feeding system and is mostly dependent on activation of pallidofugal fibre trajectories having primary influences perhaps on neuro-metabolic regulations. The medical and perifornical systems seem to be purely 'motivational' in nature, that is, are concerned only with the 'urge' of the animal to approach food and water.

This work was supported by grants *B*–2601 and *B*–2797, U.S. Public Health Service, National Institutes of Health.

[1] Delgado, J. M. R., and Anand, B. K., *Amer. J. Physiol.*, **172**, 162 (1953).

[2] Olds, J., *J. Comp. Physiol. Psychol.*, **51**, 675 (1958).

[3] Bower, G. H., and Miller, N. E., *J. Comp. Physiol. Psychol.*, **51**, 669 (1958).

[4] Olds, J., *Science*, **127**, 315 (1958).

[5] Olds, J., Travis, R. P., and Schwing, R. C., *J. Comp. Physiol. Psychol.*, **53**, 23 (1960).

[6] Miller, N. E., *Fed. Proc.*, **19**, 846 (1960).

[7] Grossman, S. P., *Science*, **132**, 301 (1960).

[8] Morgane, P. J., *Fed. Proc.*, **19**, 292 (1960).

[9] Morgane, P. J., *J. Comp. Neurol.* (in the press).

[10] Morgane, P. J., *Amer. J. Physiol.* (in the press).

[11] Morgane, P. J., *Science*, **133**, 887 (1961).

[12] DeGroot, J., *Verhandel. Konikl. Ned. Akad. Wetenschap. Afdel. Natuurk.*, Sect. 2, No. 4 (1959).

EVIDENCE FOR A NEW ENZYME CONNECTED WITH PROTEIN SYNTHESIS

By HANS G. BOMAN and INGVAR SVENSSON

Institute of Biochemistry, University of Uppsala

THE enzymic reaction which incorporates amino-acids into soluble RNA has been so extensively studied that only review references can be given here[1-4]. The first step involves the formation of an enzyme-bound amino-acid adenylate compound. The second step is the transfer of the activated amino-acid to the 2′- or 3′-hydroxyl of the terminal adenosine residue in the soluble RNA. The overall reaction is summarized by the following formula:

$$\text{amino-acid}_1 + \text{adenosine triphosphate} + \text{soluble}$$
$$\text{RNA}_1 \overset{\text{enzyme}_1}{\rightleftarrows} (\text{amino-acid}_1)\text{-RNA}_1 + \text{adenosine}$$
$$\text{monophosphate} + \text{inorganic pyrophosphate} \quad (1)$$

Most probably there is at least one specific acceptor RNA molecule and one specific enzyme for each amino-acid, and the same enzyme ('the activating enzyme') should be able to carry out the activation and the transfer to the soluble RNA. Purifications of more than a dozen activating enzymes from various sources have been reported[1-4]; but so far most of these preparations have not been obtained in a pure and homogeneous form. It may also be significant that these enzymes show very low figures for turnover number when such calculations are possible for the overall reaction (1).

In this article we are recording a few experiments which may contribute additional information about reaction (1). We have prepared two partially purified enzymes: the arginine activating enzyme from *Escherichia coli*[5] and the methionine activating enzyme from yeast[6]. The methionine incorporating activity of these two enzymes in combination has been found to be up to twelve times as large as the sum of their separate activities. This finding, together with kinetic observations and metal ion effects, is taken as evidence for the existence of a new enzyme connected with reaction (1).

The RNA was prepared from bacteria by extraction of the whole cells with 90 per cent phenol followed by alcohol precipitation of the total RNA from the water phase. The soluble RNA was extracted from the precipitate by cold *M* sodium chloride[7] and further purified by precipitation with lanthanum nitrate[8]. The lanthanum salt of soluble RNA was dissolved in 0·5 *M* ethylenediamine tetraacetic acid of *p*H 4·7 and finally dialysed against distilled water for 15 hr. The activity of the activating enzymes was measured as the ability to incorporate C-14 amino-acids into soluble RNA. The assay volume was either 0·5 or 0·2 ml. and the reaction mixture contained varying amounts of soluble RNA and activating enzyme, 0·11 m*M* C-14 L-amino-acid, 2 m*M* adenosine triphosphate, 2 m*M* glutathione, 4 m*M* magnesium chloride and 0·1 *M* *tris*-maleate buffer, all adjusted to *p*H 7·0. The reaction was terminated by precipitation with 0·01 *M* lanthanum nitrate in 0·5 *M* perchloric acid. Carrier RNA was added, the precipitate was washed and the radioactivity determined with a windowless gas-flow counter as described in detail elsewhere[5].

During the study of the partially purified arginine activating enzyme, it was found to be gradually inactivated at 0° and *p*H 4·1. The time-dependence

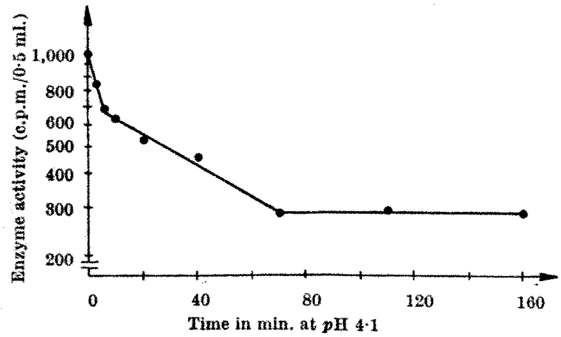


Fig. 1. Denaturation of arginine activating enzyme at 0° and *p*H 4·1. Enzyme activity measured as C-14 arginine–RNA formed in 15 min. at 37°. Assay volume 0·5 ml. with 4·3 μgm./ml. of enzyme and 4·0 mgm./ml. of soluble RNA. The activity is plotted on a logarithmic scale and is given in c.p.m. corrected for background and the mean blank, but not for self absorption

for this denaturation is shown in Fig. 1. The assay was performed with an enzyme concentration of 4·3 μgm./ml. and a soluble RNA content of 4 mgm./ml., conditions which make the enzyme rate-limiting for more than 15 min. Three blanks were used: one with enzyme heated to 70° for 10 min. gave 160 c.p.m., one with 0·02 *M* ethylenediamine tetraacetic acid *p*H 6·8 gave 150 c.p.m., and one with 0·002 *M* *p*-chloromercuribenzoate gave 165 c.p.m. The mean value of these blanks has been subtracted from the data given. Fig. 1 shows that about 25 per cent of the enzyme activity is stable at *p*H 4·1 and that the denaturation cannot be a single first-order reaction, but might be the sum of two such reactions. It has been checked that a partially denatured sample cannot be reactivated by standing at *p*H 7·0.

Berg and his associates[3] have compared the incorporation of methionine into *E. coli* soluble RNA when using methionine activating enzymes from the same source and from yeast. They found the bacterial enzyme to incorporate more than twice as much as the yeast enzyme. This result has been interpreted as a heterogeneity in the methionine accepting RNA[3,4]. We have used the working hypothesis that Berg's observation could be related to the results given in Fig. 1 and that such systems might permit the assay of different enzymes involved in the transferring of the same amino-acid to the soluble RNA. An attempt was thus made to purify the arginine activating enzyme from yeast (*Saccharomyces cerevisiae C*836) and it was found that the preparation *AS*-1 of the methionine activating enzyme described by Berg[6] was a potent source of enzyme for the incorporation of both arginine and methionine as seen from Table 1. It can also be seen that the two enzyme preparations have almost the same specific activity for the methionine incorporation, but that in combination they have about 13 times higher specific activity.

Table 1. RATE CONSTANTS AND SPECIFIC ACTIVITIES FOR THE ENZYME PREPARATIONS

Incorporation in soluble RNA with	C-14 L-arginine		C-14 L-methionine	
Enzyme preparation	Rate in c.p.m./min.	Spec. act.	Rate in c.p.m./min.	Spec. act.
Arginine activating enzyme from *E. coli*	72·2	20·2	1·0	0·014
Methionine activating enzyme from yeast	7·5	0·014	12·8	0·016
Methionine activating enzyme + arginine activating enzyme	94·4	—	167	0·210
Methionine activating enzyme + *p*H 4 treated arginine activating enzyme	—	—	25·6	0·027

The experiments with both enzymes in combination were performed with the same amount of the same enzyme preparations which were tested alone. The experiment in the last line was carried out with another preparation of methionine activating enzyme with a specific activity of 0·028. The rate constants are derived from the initial slopes of time-curves using 0·2 ml. assay volume. The specific activities are given in mμmoles of amino-acid incorporated at 27° (in an excess of soluble RNA) per min. and per mgm. of protein.

The effect of the combined enzyme preparations has been further examined in kinetic experiments of various types. Fig. 2 shows two parallel experiments when arginine activating enzyme was added to a reaction with methionine activating enzyme. The result is a significant increase in the formation of methionine–RNA, while the control experiment with arginine activating enzyme shows only a minor incorporation. The addition of more methionine activating enzyme after 60 min. does also give an increased formation of methionine–RNA indicating that soluble RNA does not become rate-limiting during the experiment. One of the experiments in Fig. 2 was carried out with C-14-methyl labelled L-methionine (represented by circles) the other with C-14-(C2) labelled DL-methionine (represented by squares); both obtained from California Corporation for Biochemical

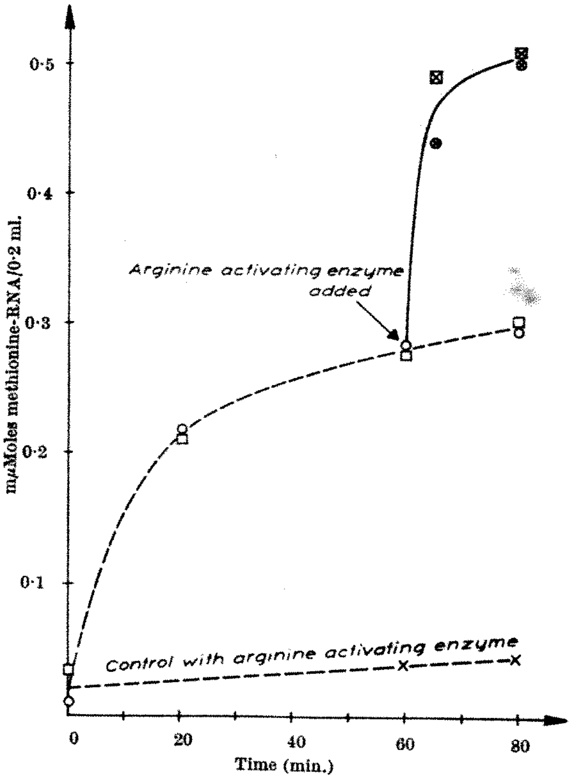


Fig. 2. Two parallel kinetic experiments at 27° with 1·24 mgm./ml. of methionine activating enzyme and 3·1 mgm./ml. of soluble RNA. Arginine activating enzyme (final conc. 0·17 mgm./ml.) was added to the reaction mixture after 60 min. Aliquots of 0·2 ml. were withdrawn at various times and assayed for C-14 methionine-RNA. Circles represent L-methionine with methyl-C-14, squares DL-methionine with (C2)-C-14. The crosses represent an experiment with 0·098 mgm./ml. of arginine activating enzyme, 2·6 mgm./ml. of soluble RNA and L-methionine with methyl-C-14

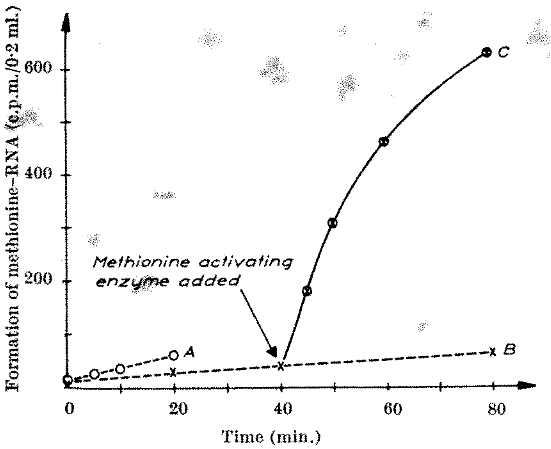


Fig. 3. Kinetic experiment at 27° showing the formation of methionine–RNA using 0·75 mgm./ml. of methionine activating enzyme (curve *A*) and using 68 μgm./ml. of arginine activating enzyme (curve *B*). After 40 min. was added to the latter reaction mixture methionine activating enzyme to a final concentration of 0·75 mgm./ml. (curve *C*). The concentration of soluble RNA was 4·5 mgm./ml. for curves *A* and *B*, 4·1 mgm./ml. for curve *C*. Assay volume 0·2 ml. The activity is given in c.p.m. and corrected only for background

Research, Los Angeles, 63. The agreement between the two experiments rules out a transmethylation reaction.

Both the enzyme preparations were stored at – 18° C. with 50 per cent ethylene glycol as antifreeze. In these conditions, the arginine activating enzyme has been stable for 7 months, while the methionine activating enzyme gradually has become less active. Fig. 3 shows the time-curves for the formation of methionine–RNA using a somewhat inactivated preparation of methionine activating enzyme (curve *A*) and a normal preparation of arginine activating enzyme (curve *B*). After 40 min. the same amount of the same preparation of methionine activating enzyme was added to the latter reaction mixture as was used for curve *A*. The suddenly increased formation of methionine–RNA obtained by the combined enzymes (curve *C*) corresponds to about a nine-fold increase in specific activity.

Most enzymic reactions are characterized by the fact that the enzyme concentration is rate-limiting in the beginning, while the substrate concentration becomes limiting towards the end of the reaction. Curves corresponding to this type of kinetics are also found for the formation of arginine–RNA using the arginine activating enzyme and for the formation of methionine–RNA using both the arginine and the methionine activating enzymes together. However, the curve obtained with the methionine activating enzyme alone is approximately logarithmic with time (as in Fig. 2) and similar curves are obtained also with arginine activating enzyme which has been kept at 0° and *p*H 4·1 for 80 min. Such logarithmic curves may be expected when a rate-limiting component is disappearing in a first-order reaction.

Some factors which could be expected to influence the rate or the equilibrium position of reaction (1) have been tested for their possible effects. Both the enzyme fractions have been pre-incubated at 27° for 80 min. without any detectable change of activity. The addition of crystalline pyrophosphatase[10] and purified venom 5′-nucleotidase[11] had no influence on the course of the reaction. An instability of one of the enzyme fractions or a trapping of the reaction products can thus be eliminated as interpretations.

However, it was found that in the presence of magnesium, the addition of calcium, cobalt or manganese ions has a pronounced stimulating effect on the methionine activating enzyme preparation, but a slight inhibitory action on the preparation of arginine activating enzyme. On the other hand, the addition of ashed preparations of the respective enzymes had scarcely any effect on the reaction. Thus the sudden increase in the formation of methionine–RNA observed with both enzymes together (Figs. 2 and 3) cannot be a metal-ion effect.

All the results reported here could be explained by the following hypothesis, which is based on two assumptions. (*a*) The activating enzyme is consumed during the course of the reaction (1). It could be that the reaction produces a chemical change in the enzyme or that 'the enzyme-substrate compound' formed is not split into 'product and enzyme'. The methods used do not distinguish between these alternatives, but both may be expected to give logarithmic time-dependences. (*b*) A second enzyme exists which is capable of regenerating the methionine, as well as the arginine activating enzyme. This 'regenerating enzyme' is present in an excess in the arginine activating enzyme preparation, which cannot be regarded as highly purified despite its high specific activity (see Table 1). The methionine activating enzyme was purified about fifty-fold[6], but has probably been freed of the 'regenerating enzyme', which explains both its relatively low specific activity and the stimulation by the arginine activating enzyme (Table 1).

The 'regenerating enzyme' may have its maximum activity with magnesium ions, which explains the general agreement[1–4] about the requirement for this metal ion. We have determined that the arginine activating enzyme has its maximum activity with 0·01 *M* magnesium but that about 50 per cent activity is obtained with manganese. The alanine activating enzyme from pig liver cytoplasm requires cobalt[12], and the tyrosine activating enzyme from rat liver has recently been shown to require potassium[13]. Other activating enzymes may show optimum activity with other metal ions such as calcium, iron and manganese, all of which are known to be present in RNA preparations[14], but which are removed by our treatment with ethylenediamine tetraacetic acid. Preliminary experiments have indicated that besides methionine this would be the case with the enzyme for phenylalanine.

The difference between the methionine activating enzymes from yeast and from bacteria observed by Berg *et al.*[9] can thus be explained by assuming that their preparation of the bacterial enzyme contains the 'regenerating enzyme' and that this is absent in the preparation of the yeast enzyme. Zillig *et al.*[15] have reported that the amount of leucyl–RNA which can be formed by their '*p*H 4 enzyme' is directly proportional to the amount of enzyme used, and suggest that the normal reaction product is a complex of amino acyl soluble RNA and enzyme. Our interpretation would be that the 'regenerating enzyme' is denatured by the *p*H 4·5 precipitation used by these authors, as well as in our *p*H 4·1 experiments with the arginine activating enzyme (Fig. 1 and Table 1). Such a denaturation would explain why enzyme preparations subject to the acid treatment show the anomalous dependence on enzyme observed.

In conclusion, we feel that our results are in agreement with the previous findings of Berg's and Zillig's groups, and that together with our own data they support the hypothesis of the existence of a 'regenerating enzyme' coupled to the activating enzymes which catalyse reaction (1). Such a coupling could be a powerful control mechanism, but speculations about its possible function *in vivo* may await further experimental data. It is a consequence of our hypothesis that in the presence of soluble RNA reliable measurements with activating enzymes cannot be performed without an excess of 'regenerating enzyme'.

This work has been supported by Grant No. *RG*–7576 from the U.S. Public Health Service.

Note added in proof. Dr. Paul Berg has kindly informed us that soluble RNA from bacteria can be obtained in a form in which a fraction of the chains lacks the adenosine end-group. Consequently the effect of the arginine activating enzyme preparation could be due to incorporation of adenosine monophosphate, which would increase the number of acceptor sites for methionine. However, preincubation of our soluble RNA with the arginine-activating enzyme preparation and adenosine triphosphate has failed to demonstrate any increase in the number of acceptor sites for methionine.

[1] *Symp. Amino Acid Activation. Proc. U.S. Nat. Acad. Sci.*, **44**, 67 (1958).

[2] Lipmann, F., Hülsmann, W. C., Hartmann, G., Boman, H. G., and Acs, G., *J. Cell. Comp. Physiol.*, **54**, Supp. 1, 75 (1959).

[3] Novelli, G. D., *Ann. Rev. Microbiol.*, **14**, 65 (1960).

[4] Hoagland, M. B., in *The Nucleic Acids*, edit. by Chargaff, E., and Davidson, J. N., **3**, 349 (Academic Press, New York, 1960).

[5] Boman, H. G., Boman, I. A., and Maas, W. K., in *Biological Structure and Function* (I.U.B./I.U.B.S. Symp., Sept. 1960) (Academic Press, New York, 1961).

[6] Berg, P., *J. Biol. Chem.*, **222**, 1025 (1956).

[7] Method developed in the laboratory of Dr. F. Lipmann.

[8] Hammarsten, E., Hammarsten, G., and Teorell, T., *Acta Med. Scand.*, **68**, 219 (1928).

[9] Bergmann, F. H., Berg, P., Preis, J., Ofengand, E. J., and Dieckmann, M., *Fed. Proc.*, **18**, No. 751 (1959).

[10] Kindly obtained from Dr. M. Kunitz and prepared according to *J. Gen. Physiol.*, **35**, 423 (1952).

[11] Björk, W., and Boman, H. G., *Biochim. Biophys. Acta*, **34**, 503 (1959).

[12] Webster, G. C., *Biochem. Biophys. Res. Com.*, **2**, 56 (1960).

[13] Holley, R. W., Brunngraber, E. F., Saad, F., and Williams, H. H., *J. Biol. Chem.*, **236**, 197 (1961).

[14] Wacker, W. E. C., and Vallee, B. L., *J. Biol. Chem.*, **234**, 3257 (1959).

[15] Zillig, W., Schachtschabel, D., and Krone, W., *Z. physiol. Chem.*, **318**, 100 (1960).

PHOTOPHOSPHORYLATION AND THE HILL REACTION

THE rate of the Hill reaction has been reported to be stimulated strongly by concomitant photophosphorylation, that is, by synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and orthophosphate[1–4]. Warburg *et al.*[5] have pointed out that this stimulation of the rate of photoreduction is an effect of *p*H, but the warning appears to have gone unheeded. Most recently, for example, Good[6] has used such a stimulation to distinguish between phosphorylating and non-phosphorylating electron transport; he suggests that the latter should be subtracted from the former to obtain the true ratio of micromoles ATP formed to electron pairs transferred (P/2*e* ratio), and concludes that this value is 2. Good cites a similar value of 2 reported by Krogmann and Vennesland[7] for the stoichiometry of oxidative photophosphorylation. We must point out, however, that the assumptions underlying the latter calculation are quite different from those made by Good, and may or may not be correct. At the same time, our own work on the dependence on *p*H of the Hill reaction leads us to the conclusion that Warburg is correct in attributing rate stimulation by associated phosphorylation to effects of *p*H.

Punnett[8] has noted that freshly prepared chloroplasts photoreduce ferricyanide most rapidly at a rather alkaline *p*H. With spinach chloroplasts freshly prepared in 0·35 *M* sodium chloride, the *p*H optimum we observe is generally greater than *p*H 8. The rates at the maximum, in saturating light, range up to 600 μmoles per mgm. chlorophyll per hr. at 15° C., and may be as much as four times faster than the rates measured at *p*H 7 with the same chloroplast preparations. Fig. 1 shows the dependence on *p*H of photoreduction and photophosphorylation in 0·05 *M* *tris*(hydroxymethyl)aminomethane buffer, where the abscissa represents the initial *p*H of the reagents employed. Reactions were carried out in 25-ml. conical flasks incubated at 15° C. with air as the gas phase. Each vessel contained in micromoles: *tris*(hydroxymethyl)aminomethane, 100; potassium ferricyanide, 10; magnesium chloride, 10; and, where indicated, potassium phosphate and adenosine diphosphate, 3·4 in a final volume of 2·0 ml. *Tris*, phosphate, and adenosine diphosphate solutions were adjusted to the indicated *p*H with the use of hydrochloric acid and sodium hydroxide and a Leeds and Northrup *p*H meter. Chloroplasts, freshly prepared in 0·35 *M* sodium chloride and used without prior washing, containing 113 μgm. of chlorophyll were added to the vessels at zero time. The reactions were initiated and terminated by turning the light on and off; illumination was from below for 4 min., with shaking, and with white light of about 5,000 ft.-candles. Reaction mixtures were deproteinized by adding 0·50 ml. of 20 per cent (w/v) trichloracetic acid and centrifuging. Residual ferricyanide was determined on an aliquot of the supernatant by direct reading at 400 mμ in the Beckman *B* spectrophotometer. Residual orthophosphate was determined on a separate aliquot by the method of Fiske and Subbarow[9]; the reduced phosphomolybdate complex was read in a Lumetron colorimeter with a 650-mμ filter, after centrifuging to remove the precipitate of ferrocyanide–molybdate. Chlorophyll was determined by Arnon's method[10].

The upper curves represent ferricyanide photoreduction in the presence of ADP and orthophosphate (solid circles) and in the absence of both ADP and orthophosphate (open circles). The largest effect of these reagents (20 per cent stimulation) is seen at the lowest *p*H. This effect diminishes as the initial *p*H approaches the *pK* of the buffer (8·2), and it could be enhanced by the use of lower buffer concentrations. This behaviour can be accounted for on the basis of the formation of hydrogen ions during ferricyanide photoreduction (equation 1) and the consumption of hydrogen ions during the synthesis of ATP (equation 2):

$$2\mathrm{Fe(CN)}_6^{3-} + \mathrm{H_2O} \rightarrow 2\mathrm{H}^+ + 2\mathrm{Fe(CN)}_6^{4-} + \tfrac{1}{2}\mathrm{O}_2 \qquad (1)$$

$$\mathrm{ADP}^{3-} + \mathrm{HPO}_4^{2-} + \mathrm{H}^+ \rightarrow \mathrm{ATP}^{4-} + \mathrm{H_2O} \qquad (2)$$

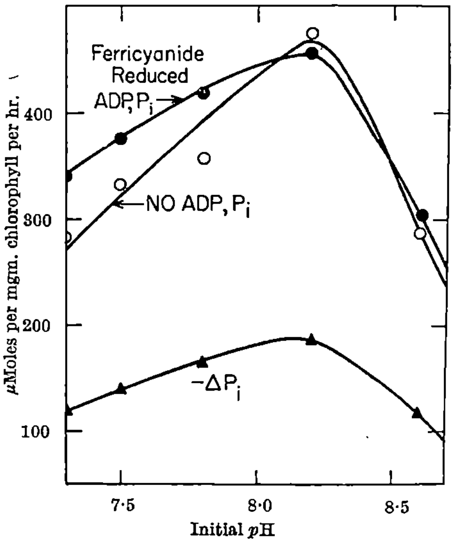


Fig. 1. Dependence on *p*H of ferricyanide photoreduction and phosphate uptake by spinach chloroplasts in the presence and absence of a phosphorylation system

The lower curve of Fig. 1 shows the rate of phosphate consumption accompanying ferricyanide photoreduction in the presence of ADP and phosphate. It should be noted that the rate of phosphorylation here is proportional to the rate of ferricyanide reduction and that the P/2*e* ratio is about 0·8 throughout the *p*H range. If we were to apply the correction suggested by Good[6] for non-phosphorylating electron transport in the calculation of the P/2*e* ratio, values for the latter in this experiment would range from four to infinity.

The reaction conditions detailed in Fig. 1 have been employed extensively in our laboratory for the study of the stoichiometry of the Hill reaction and associated photophosphorylation. An examination of published results from other laboratories[4] shows that very large stimulations of ferricyanide photoreduction by ADP have been obtained with rather different reaction mixtures which contain ATP, higher concentrations of phosphate and lower concentrations of chlorophyll and ferricyanide, assayed with shorter illumination periods. Table 1 shows that we can also obtain a stimulation of the Hill reaction-rate under these conditions, even in well-buffered systems. The correct interpretation of the nucleotide effects shown in Table 1 is not entirely clear, and would seem to require further investigation. But from numerous experiments such as that illustrated in Fig. 1, it is clear that the addition of ADP and orthophosphate to adequately buffered chloroplast suspensions can elicit a good rate of photophosphorylation without appreciable stimulation of the rate of ferricyanide photoreduction. Thus we are in agreement with Warburg's conclusion, namely, that photophosphorylation can occur as a result of the Hill reaction, but that the rate of the latter is independent of the ATP synthesis which may or may not accompany it.

Table 1. EFFECT OF ADP AND ATP ON FERRICYANIDE PHOTOREDUCTION BY SPINACH CHLOROPLASTS

The reaction mixture contained in μmoles: *tris*(hydroxymethyl)-aminomethane, 82; potassium phosphate, 68; potassium ferricyanide, 2; magnesium chloride, 10; sodium chloride, 70; in a final volume of 3·0 ml., at *p*H 7·8, and chloroplasts containing 74 μgm. chlorophyll. Illumination was for 2 min.; other conditions and assay as described for the experiment in Fig. 1

Additions	Ferricyanide photoreduction (μmoles per mgm. chlorophyll per hr.)
None	282
1 μmole ADP	425
0·5 μmole ATP	172
1 μmole ADP + 0·5 μmole ATP	403

This work was supported by a research grant from the U.S. National Science Foundation.

MARY STILLER
BIRGIT VENNESLAND

Department of Biochemistry,
University of Chicago,
Chicago, Illinois.

[1] Arnon, D. I., Whatley, F. R., and Allen, M. B., *Science*, **127**, 1026 (1958).
[2] Avron, M., Krogmann, D. W., and Jagendorf, A. T., *Biochim. Biophys. Acta*, **30**, 144 (1958).
[3] Avron, M., and Jagendorf, A. T., *J. Biol. Chem.*, **234**, 967 (1959).
[4] Krogmann, D. W., Jagendorf, A. T., and Avron, M., *Plant Physiol.*, **34**, 272 (1959).
[5] Warburg, O., Krippahl, G., Gewitz, H. G., and Volker, W., *Z. Naturforsch.*, **14b**, 712 (1959).
[6] Good, N. E., *Nature*, **188**, 661 (1960).
[7] Krogmann, D. W., and Vennesland, B., *J. Biol. Chem.*, **234**, 2205 (1959).
[8] Punnett, T., *Plant Physiol.*, **34**, 283 (1959).
[9] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, **66**, 375 (1925).
[10] Arnon, D. I., *Plant Physiol.*, **24**, 1 (1949).

THE following considerations compel me to reject the *p*H theory:

(1) There is always much less formation of hydrogen ion associated with the reduction of flavins than with the reduction of ferricyanide. When flavins function catalytically in the reduction of oxygen, as in my usual test system, there is no hydrogen ion formation at all. Nevertheless, I find that the reduction of flavin mononucleotide is stimulated to the same extent as the reduction of ferricyanide by the presence of the phosphorylating system.

(2) The acid consumed in the phosphorylation of adenosine diphosphate reappears when the resulting adenosine triphosphate is used in the synthesis of glucose-6-phosphate. Yet the stimulation of ferricyanide and flavin reduction which is associated with the phosphorylation is independent of the presence or absence of hexokinase and glucose.

(3) Chloroplasts stored below *p*H 7·0 rapidly lose all ability to respond to the components of the phosphorylating system. These aged chloroplasts continue for a long time to reduce ferricyanide in the absence of the phosphorylating system at the same rate as freshly prepared chloroplasts. Consequently, the phosphorylating and non-phosphorylating electron transport systems must differ in at least one labile reaction step. This fact precludes the possibility that the rate-stimulation observed during phosphorylation represents the effect of improved conditions, *p*H or other, on a single process.

(4) The evidence presented by Stiller and Vennesland in support of Warburg's *p*H hypothesis contradicts the experience of most workers. I have carefully considered buffering problems and have established satisfactory buffer-levels by exhaustive experimentation without encountering phenomena of the kind described by the Chicago group. I believe that the high rates they attribute to "adequate buffering" are, in fact, an expression of the hitherto unrecognized uncoupling effect of high concentrations of salts. This effect of salts on chloroplasts will be documented in detail elsewhere. Chloroplasts I have

used exhibit the familiar pattern of behaviour regardless of the alkalinity and the concentration of the buffer when they are suspended in media low in ions; the complete phosphorylating system always causes large increases in the rate of ferricyanide reduction and there is always a quantitative relationship between the increase of rate and the amount of adenosine triphosphate formed. On the other hand, these same chloroplasts in 0·35 *M* sodium chloride are similar to, but not identical with, the Stiller–Vennesland chloroplasts. The condition described by Stiller and Vennesland, phosphorylation without stimulation, would be kinetically reasonable if their chloroplasts were both partially uncoupled and partially inhibited (as mine seem to be in 0·35 *M* sodium chloride). Thus the uncoupling might be sufficient to allow rates to rise to the maximum of a partially inhibited process which would then become rate-limiting. Yet this amount of uncoupling might still permit considerable phosphorylation; hence there is phosphorylation but no increase in the rate of electron transfer. However, the effects of salts on chloroplasts are very complex, with many as yet unexplored ionic interactions. Consequently there is little point in attempting a detailed interpretation of the Stiller–Vennesland observations at present.

N. E. Good

Pesticide Research Institute,
University sub-Post Office,
London, Ontario.

Stiller and Vennesland (first communication) present a timely warning with respect to the interpretation of experiments measuring the rate of the Hill reaction when ferricyanide or other redox dyes are reduced. I agree that the *p*H of the reaction mixture can fall during the course of the reaction if insufficient buffer is present; and further that a concomitant phosphorylation of ADP will prevent this fall. My measurements also show a fairly sharp optimum at about *p*H 8; thus a downward drift in *p*H would cause a lower rate of dye reduction or of oxygen evolution.

It is another question, however, whether or not this sort of artefact is actually responsible for previously reported phenomena[1-3]; and whether in the complete absence of a drift of *p*H the rate of the Hill reaction is stimulated due to an accompanying phosphorylation reaction.

In our first account of stimulation of rate by adding ADP, magnesium and orthophosphate[1], which confirmed the earlier observation of Arnon *et al.*[4]; and also in our published work since then[2,3], the reactions were run at room temperature, in 3 ml. total volume containing either 0·030 or 0·040 mgm. of chlorophyll, 40 μmoles of *tris* buffer at *p*H 8, 70 μmoles of sodium chloride and 1·5 μmoles of ferricyanide. (Unfortunately the original tables (ref. 1) omitted mention of the *tris*; its presence was indicated only indirectly by reference to earlier papers.) The illumination period was 2 min., so that initial rates only have been measured. The control reactions, to which no other reagents were added, reduced at most one-third of the ferricyanide present, or 0·5 μmole. It seems quite unlikely that the equivalent amount of hydrogen ions generated could have lowered the *p*H significantly in the presence of 40 μmoles of *tris* at *p*H 8. A re-check of the same reaction conditions shows at most a *p*H fall of 0·1 unit in the control reactions. This could not be enough to account for more than a minor share of the difference in rate between the phosphorylating and non-phosphorylating reactions.

I have re-examined the rate of ferricyanide reduction as a function of *p*H, using 150 μmoles of *tris* buffer in 3 ml. volume as suggested by Stiller and Vennesland, in order to eliminate any possible drift of *p*H. The *p*H's of the reaction mixtures were all measured after illumination ended as a further precaution against undetected drifting. The results shown in Fig. 1 are from a typical experiment out of five repetitions. All points represent the average of duplicate samples, containing, in 3 ml. total volume, 0·040 mgm. of chlorophyll, 70 μmoles of sodium chloride, 150 μmoles of *tris* at an appropriate *p*H and 1·5 μmoles of potassium ferricyanide. Where indicated to be present, there were 30 μmoles of phosphate adjusted to the same *p*H as that of the *tris*, 10 μmoles of magnesium chloride, 0·2 μmole of ATP or 1·5 μmoles of ADP. The reaction mixtures were illuminated in small test-tubes for 2 min. at room temperature, at 5,000 ft.-candles (the source was an incandescent lamp with 5 cm. of water filter between the lamp and the reactions). The *p*H's shown were those of the reaction mixtures, determined on each sample after the reaction was ended. Following this, the mixtures were killed by adding 0·3 ml. of 20 per cent trichloracetic acid, the proteins removed by centrifugation, and optical density of the supernatant solution measured at 420 mμ in a Beckman spectrophotometer. The ferricyanide reduced was measured as the change in optical density from that of zero-time control, and no appreciable change could be seen without illumination. The chloroplasts were prepared by grinding spinach leaves in a buffer containing 0·40 *M* sucrose, 0·02 *M* *tris* buffer and 0·01 *M* sodium chloride at *p*H 8·0. They were centrifuged once, resuspended in 0·40 *M* sucrose/0·03 *M* sodium chloride, and used without washing. The entire *p*H series was accomplished within an hour and a half after grinding the leaves, the higher *p*H's being measured first.

The results show that reactions with and without a simultaneous phosphorylation have a similar response to *p*H; but at any one *p*H the rate is usually doubled owing to addition of ADP. As before[1],

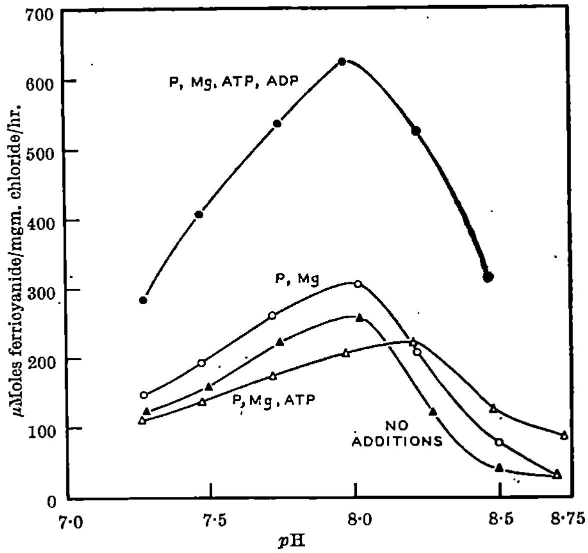


Fig. 1. Ferricyanide reduction by the Hill reaction as a function of *p*H of the reaction mixture

ATP at low concentrations has an inhibitory effect, so that if ATP, magnesium ions and orthophosphate serve as a control, the rate may be tripled owing to addition of ADP. These results correspond to those of Table 1 in the communication by Stiller and Vennesland. Although I agree that the interpretation of these results is not clear if they stand alone, we have previously[1] presented results from various other types of experiments, all of which supported the concept that the actual process of simultaneous phosphorylation was responsible for the observed increase of rate.

It was recently discovered that the rate of reduction of triphosphopyridine nucleotide (TPN) is stimulated by adding all three reagents required for phosphorylation (ref. 5, and San Pietro and Keister, in preparation). Although the extent of such stimulation may be four-fold, or even more, a *p*H drift of no more than 0·03 unit can be seen under the reaction conditions used (San Pietro and Keister, personal communication). Furthermore, the *p*H response for reduction of TPN appears to be considerably less steep than that for ferricyanide reduction.

In view of all these results, I must take exception to the generalization implicit in the statement of Stiller and Vennesland attributing "... rate stimulation by associated phosphorylation to effects of *p*H". The statement obviously cannot apply to the results obtained under the conditions of my Fig. 1, or of Table 1 of Stiller and Vennesland. This interpretation of stimulation of rate has a chance of being significant only when the Hill reaction is allowed to proceed for longer periods of time, generating major amounts of hydrogen ions (as is usual when manometric procedures are used, for example). It should be noticed that in Fig. 1 of Stiller and Vennesland the 4-min. reaction period with a fairly high chlorophyll-level leads to the generation of approximately 4 μmoles of hydrogen ions, rather than the 0·5 μmole or less under my conditions.

It is always possible that under differing conditions a particular phenomenon may not appear. Using the reaction conditions as described by Stiller and Vennesland for their Fig. 1, I have confirmed their observation that the Hill reaction-rate is unchanged (or shows no more than a 5–10 per cent stimulation) due to a simultaneous phosphorylation. I have found that one condition contributing significantly to the smaller effect is the low concentration of phosphate employed ($1{\cdot}67 \times 10^{-3}$ *M*) compared with that needed for a maximal rate of phosphorylation[1,6]. It seems to me reasonable that in a complex system with a very significant basal level at all times, close to optimal conditions will be required for the induced reactions to become apparent. The possible role of other factors in diminishing the rate-stimulation by ADP, magnesium ions and orthophosphate is at present under investigation.

As Stiller and Vennesland point out, the variations possible in relative rates between phosphorylating and non-phosphorylating reactions make the calculation of the P/2*e* ratio after subtracting the basal rate an extremely questionable operation. It seems to me that this same demonstrable variation also invalidates the generalization implicit in the statement of Stiller and Vennesland, "... that photophosphorylation can occur as a result of the Hill reaction, but that the rate of the latter is independent of ATP synthesis which may or may not accompany it".

Work reported here was supported by grant *RG* 3923 from the National Institutes of Health.

André T. Jagendorf

McCollum-Pratt Institute,
The Johns Hopkins University,
Baltimore 18, Maryland.

---

[1] Avron, M., Krogmann, D. W., and Jagendorf, A. T., *Biochim. Biophys. Acta*, **30**, 144 (1958).

[2] Krogmann, D. W., Jagendorf, A. T., and Avron, M., *Plant Physiol.*, **34**, 272 (1959).

[3] Avron, M., and Jagendorf, A. T., *J. Biol. Chem.*, **234**, 967 (1959).

[4] Arnon, D. I., Whatley, F. R., and Allen, M. B., *Science*, **127**, 1026 (1958).

[5] Davenport, H. E., *Biochem. J.*, **77**, 471 (1960).

[6] Jagendorf, A. T., and Avron, M., *J. Biol. Chem.*, **231**, 277 (1958).

BIOLOGY OF REPRODUCTION IN *RUBUS*

By Dr. G. J. DOWRICK

Department of Biological Sciences, Wye College, nr. Ashford, Kent

APOMIXIS has, in the past, been frequently defined to include diverse types of vegetative reproduction such as bulbil formation and vegetative proliferation[1]. In this account the use of the term is restricted to embryo formation from a cell which has not undergone meiosis and has not been fertilized. It is usually assumed that many polyploid species of *Rubus* reproduce either by facultative or obligate apomixis. Thus *R. laciniatus* ($2n = 4x = 28$) is believed to be an example of a facultative apomict while *R. caesius* ($2n = 28$) is reported as being entirely apomictic. In *Rubus*, unreduced egg cells have been considered to be aposporic in origin, and it was assumed that embryos develop without fertilization. Development of embryos is, however, dependent on pollination which, presumably, is necessary for fertilization of the endosperm (unreduced pseudogamy). The results presented here show that the breeding behaviour of tetraploid *Rubus* species does not follow this accepted pattern, but that embryos develop from haploid embryo-sacs arising by a normal meiotic sequence from megaspore mother cells.

Three tetraploid species, *R. caesius*, *calvatus* and *laciniatus*, have been investigated and in all three species meiosis was normal and embryo-sac formation followed the *Polygonum* type of development. Development has been studied in particular detail in *R. caesius* since this species has been stated to be an obligate apomict[2]. All stages of meiosis have been found in the megaspore mother cell and at MI there were 14 bivalents. Subsequent chromosome separation was normal and a young tetrad was seen regularly. All stages in the development of the embryo-sac have been observed with the exception of the third embryo-sac division. There was no evidence of

Table 1. CHROMOSOME NUMBERS IN PROGENIES OF SEEDLINGS FROM *R. laciniatus*

	No. of seedlings								
	$2n=$ 14	$2n=$ 21	$2n=$ 25	$2n=$ 27	$2n=$ 28	$2n=$ 29	$2n=$ 30	$2n=$ 35	$2n=$ 42
R. laciniatus ♀ ×									
$2x$* ♂		10	1		6		1	3	
$2x$† ♂		6			9			6	
$4x$ ♂	1				13				1
Self	1			1	21	1			1
$5x$ ♂					4				
$6x$ ♂					2				
$7x$ ♂					2				
	2	16	1	1	57	1	1	9	2

* × *R. ulmifolius*; † × *R. tomentosus*.

restitution and embryo-sacs, where counts could be made, had a haploid chromosome number. Thus there was no evidence of apospory.

Chromosome numbers of progeny from crosses between these three tetraploid species and other species of different ploidy have been determined. As an example of the variation found in chromosome numbers counts from crosses involving *R. laciniatus* are shown in Table 1. It will be seen that 62·3 per cent of the seedlings had the same chromosome number as the maternal species. The phenotypic appearance of these plants made it clear that they were identical to *R. laciniatus*. However, from the embryological evidence it is now known that they cannot be apomictic, since no unreduced embryo-sacs were formed.

In addition to these maternal-like seedlings there were both reduced and unreduced sexual seedlings, as shown by the plants having 21 and 35 chromosomes in families derived from diploid male parents. The former are considered to have been derived from fertilized reduced egg cells; the latter from reduced egg cells which, before fertilization, have become diploidized in the way described here. Plants with 21 chromosomes could be distinguished readily from those with 35 on phenotypic characters. The *laciniatus* leaf character is phenotypically recessive in the triploid and relatively more dominant in the pentaploid, where the proportion of *laciniatus* chromosomes is higher. The high frequency of plants with 35 chromosomes was unexpected and this class has not been reported previously. Relatively few seedlings with chromosome numbers different from *R. laciniatus* were formed in families derived from parents of higher ploidy. The two haploid seedlings with 14 chromosomes form a fourth class of progeny. These were derived directly from reduced egg cells. In addition, there were four aneuploids in the 91 seedlings examined.

The origins of two classes of progeny, the triploid sexuals and haploids, present no difficulty since the former are simply the products of reduced, fertilized egg cells while the latter have been developed from reduced unfertilized egg cells. Both classes have been reported previously and will not be considered further.

Since all megaspore mother cells in this material were seen to undergo meiosis, the production of tetraploid maternal-like and of pentaploid sexual progeny requires more careful consideration.

The former could arise in one of two ways: either restitution at the first division of the egg cell or 'fusion' of the nuclei within the embryo-sac, that is, automixis, must occur. Evidence of diploid parthenogenesis is found in *Petunia nyctaginiflora*[3]. This species, when pollinated with either *Salpiglossis variabilis* or *Nicotiana tabacum*, produces only homozygous *Petunia nyctaginiflora* seedlings. These are formed as a result of diploidization by restitution in the egg cell. Previous workers have suggested that automixis was a reproductive mechanism found in *Rubus*, although it has usually been considered to be of little general significance[4,5]. In the present material it was impossible to establish the method whereby doubling of haploid nuclei in the embryo-sac occurred. Since the genetic consequences of the two possible methods suggested above are the same, the critical establishment of the method of doubling is of minor significance.

Pentaploid sexual progeny must arise from egg cells which have undergone meiosis, become diploidized and later fertilized.

A further class of progeny which has been termed *sub-sexual* has been described by previous workers[6,7]. Phenotypically these are plants similar to the maternal species, have the same chromosome number, but are not genetically identical to it. Sub-sexual progeny are assumed to arise from diplosporic egg cells, which are themselves derived from megaspore mother cells in which a normal meiotic prophase is followed by restitution. Such egg cells have undergone genetic crossing over, but retain the somatic chromosome number. There is no cytological evidence for the occurrence of such a process in the tetraploid *Rubus* species studied.

One of the genetical implications of the reproductive mechanism now put forward is that the tetraploid species concerned are homozygous. However, since they are almost certainly amphi-diploids, as shown by their cytological behaviour during meiosis, there will be homozygosity only between the chromosomes of each of the two complexes which together make up the tetraploid. They will be heterozygous as a result of differences between the two complexes. Crossing-over, though it occurs, will not result in segregation, and diploidization either in the egg cell or as a result of automixis will immediately restore the full chromosome complement of the female parents. The sub-sexual plants might then arise from occasional allosyndetic pairing and crossing-over between the chromosomes of the two different complexes.

It has been shown before that the proportion of sexual progeny produced by a supposed apomict may differ with different pollinating parents. Further, it has been shown that species which in particular circumstances are sexual are, in different circumstances, also apomictic[8]. Thus *R. vitifolius*, when pollinated with diploid *R. idaeus*, gives only sexual progeny, whereas the same species when pollinated with the tetraploid form of *R. idaeus* gives both sexual and 'apomictic' seedlings. The breeding of this species is most easily explained on the basis that only one type of embryo-sac is formed, that is, sexual, and the subsequent development varies according to the pollinating parent used. If both sexual and apomictic seedlings were produced, it is difficult to understand why, in the cross involving diploid *R. idaeus*, apomictic progeny are not produced. These results also suggest that diploidization occurs at the first division of the egg, rather than as a result of automixis since, if the latter mechanism occurred, maternal-like seedlings would be expected from the cross using diploid *R. idaeus*.

Much of the evidence for apomixis in *Rubus* has been derived from chromosome counts[9]. The chromosome counts of apomictic seedlings will be identical to those of the maternal species from which they arose. Many of the counts cited involve self and open pollinations. Since sexual seedlings derived from such crosses may also retain the maternal chromosome number, they are, however, inadmissible. Nevertheless, pollinations between parents of widely different ploidy do frequently produce a high proportion of maternal-like seedlings. In crosses between tetraploid female parents and various male parents, Einset found that the number of reduced parthenogenetic seedlings (43) was almost as great as the total number of sexual seedlings (46) and higher than might be expected when compared with the number of supposed apomictic tetraploids (592). These relative frequencies suggest that reduced embryo-sacs are of much greater significance than hitherto realized.

Two other recent accounts of reproductive mechanisms in *Rubus* are relevant to the present work. Christen found evidence in *R. caesius* that only sexual embryo-sacs are formed[10]. Similarly, Markarian and Olmo suggested that there was no evidence for the formation of aposporic embryo-sacs in another 'apomict', *R. procurus*[11]. It was assumed by these workers that the form of the species studied must have been atypical of the species generally, or that the aposporic stages of embryo-sac formation had been missed. Neither of these possibilities is true of the present work. Clones of *R. caesius* from England, Finland and Poland have now been examined and in all only reduced sexual egg cells were formed. Also, the frequency with which the sexual stages of development were seen makes it impossible to consider that the development of aposporic embryo-sacs has been overlooked.

The high proportion of seedlings with 35 chromosomes, which, as shown here, were derived from pollination of *R. laciniatus* with diploid male species gives still further evidence against an apomictic mechanism. Fertilization of aposporic egg cells would be unlikely to occur if apomixis, as is generally supposed, enables a species, which would become extinct under a sexual system, to reproduce itself.

The evidence not only from this study but also from previous work suggests, therefore, that apomixis in its commonly accepted sense, is not an important reproductive mechanism in tetraploid forms of *Rubus*. The production of maternal-like progeny, though superficially simulating apomixis, depends solely on the formation of reduced egg cells.

Much of this work was carried out at the John Innes Institute, and I am particularly grateful for the stimulating guidance of Mr. Watkin Williams and for the technical assistance of Miss P. Dow.

[1] Stebbins, G. L., *Botanical Rev.*, **10**, 507 (1941).
[2] Lidforss, B., *Z. indukt. Abstamm.-u VererbLehre*, **12**, 1 (1914).
[3] Redinger, K., *Biol. Zentralbl.*, **58**, 142 (1938).
[4] Thomas, P. T., *J. Genet.*, **40**, 119 (1940).
[5] Gustafsson, A., *Acta Univ. Lund*, **42**, 1 (1946).
[6] Darlington, C. D., *Recent Advances in Cytology*, second edit. (Churchill, 1937).
[7] Haskell, G., *Heredity*, **7**, 409 (1953).
[8] Crane, M. B., and Thomas, P. T., *Nature*, **143**, 684 (1939).
[9] Einset, J., *Amer. J. Bot.*, **38**, 768 (1951).
[10] Christen, H. R., *Ber. schweiz. Bot. Ges.*, **60**, 153 (1950).
[11] Markarian, D., and Olmo, H. P., *J. Heredity*, **50**, 131 (1959).

SOME CHARACTERISTICS OF MITOCHONDRIA EXTRACTED FROM HIGHER PLANTS

By Dr. WALTER D. BONNER, Jun., and DINOR O. VOSS

Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia

With the exception of one brief note[1] there are no instances in the literature showing control of respiratory activity in mitochondria isolated from higher plants. The purpose of this article is to show some of the characteristics of higher plant mitochondria that do possess such respiratory control.

There are instances on record (cf. ref. 2) which show a modest increase in plant mitochondrial oxidative activity (up to twice) in the presence of adenosine diphosphate. It is possible to obtain mitochondria which show no utilization of oxygen following addition of substrate (succinate) but which are maximally active following addition of a minute amount of adenosine diphosphate. The increase in rate of utilization of oxygen following addition of adenosine diphosphate is usually about ten times. In such instances (Fig. 1) the utilization of oxygen does not decrease on exhaustion of adenosine diphosphate, and there is a curious induction period following its addition before steady-state conditions are achieved. The induction period may well be related to the requirement for adenosine triphosphate for succinate oxidation (see discussion in ref. 3). The dependence of this type of plant mitochondria on adenosine diphosphate and the lack of control is not due to activity of mitochondrial adenosine triphosphatase but rather to several factors which sediment with starch and the mitochondria. The preparation of plant mitochondria that show oxidations controlled

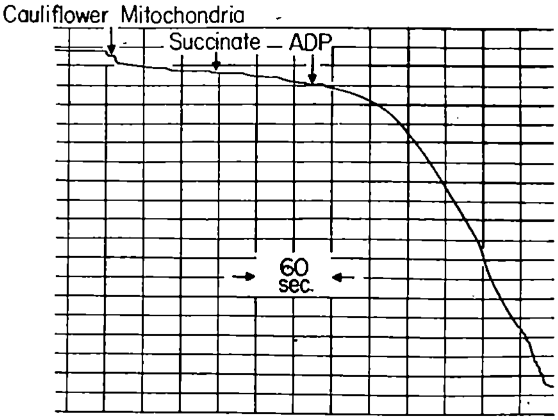


Fig. 1. Polarograph trace of the oxygen utilization by cauliflower mitochondria. Mitochondria suspended in a phosphorylating medium which consisted of: mannitol, 0·3 *M*; potassium chloride, 0·01 *M*; magnesium chloride, 0·005 *M*; potassium dihydrogen phosphate, 0·01 *M*; versene, 10^{-4} *M*; *p*H 7·4. Succinate = 0·005 *M*; the concentration of adenosine diphosphate resulting from each addition was 125 *μM*

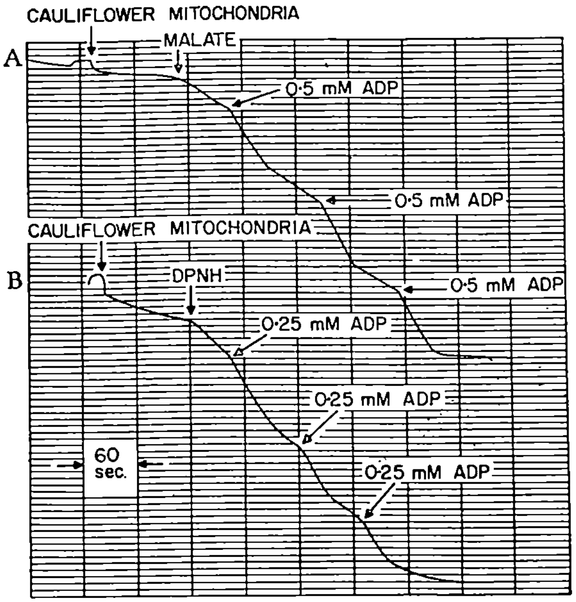


Fig. 2. Polarograph traces of oxygen utilization by cauliflower mitochondria in the presence of malate, trace *A*, and reduced diphosphopyridine nucleotide, trace *B*. Malate = 0·005 *M*, 2·5 mgm. reduced diphosphopyridine nucleotide added, conditions as in Fig. 1

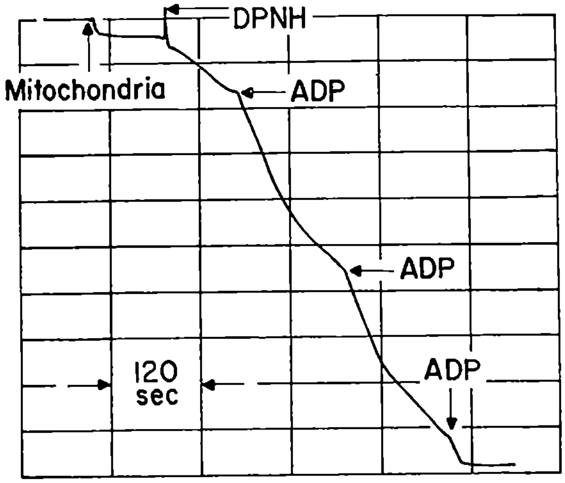


Fig. 3. Polarograph trace of the oxygen utilization by sweet potato mitochondria in the presence of reduced diphosphopyridine nucleotide and adenosine diphosphate. 2·5 mgm. reduced diphosphopyridine nucleotide added, conditions as in Fig. 1

by adenosine diphosphate depends on several factors, one of which is the careful separation of the mitochondria from the starch fraction.

Cauliflower (*Brassica oleracea*) and sweet potato (*Ipomea batatas*) have been shown to be excellent chlorophyll-free sources for mitochondrial investigations[1,2,4] and are easily obtained from local markets in some places.

Mitochondria were isolated in the following manner. 150 gm. of cold, diced tissue were ground in a Waring blendor for 5 sec. in 300 ml. of grinding medium. The grinding medium consisted of 0·6 *M* mannitol, 0·6 *M* sucrose (75 per cent mannitol, 25 per cent sucrose), 0·5 per cent cysteine (free base), and 10^{-3} *M* versene. The *p*H of the medium was 7·2. The partially disintegrated tissue resulting from the treatment in the Waring blendor was ground in small aliquots in an unglazed porcelain mortar, the grinding being continued until a smooth paste was obtained. The disintegrated tissue was squeezed through muslin and the resulting suspension was centrifuged at 1,000*g* for 5 min. The mitochondria were then sedimented by centrifuging 7 min. at 10,000*g*. The mitochondria were washed twice in the mannitol–sucrose mixture but without the addition of cysteine or versene. After each washing the sedimented mitochondria must be separated carefully from the starch fraction. The *p*H of all suspensions is one very critical factor in the preparation of mitochondria from cauliflower. With cauliflower, the *p*H must be kept between 7·2 and 7·5; with sweet potato, the *p*H is not such a critical factor.

Sweet potato and cauliflower mitochondria actively oxidize a few substrates. Fig. 2*A* shows the time-course of malate oxidation by cauliflower mitochondria, and it may be seen that the presence or absence of adenosine diphosphate considerably alters the rate of oxidation. In marked contrast to intact mitochondria from animal tissues, the plant mitochondria not only rapidly oxidize malate but also reduce diphosphopyridine nucleotide. The rate of oxidation of reduced diphosphopyridine nucleotide is also controlled by the presence or absence of adenosine diphosphate (Figs. 2*B* and 3). This control has been obtained in mitochondria derived from sweet

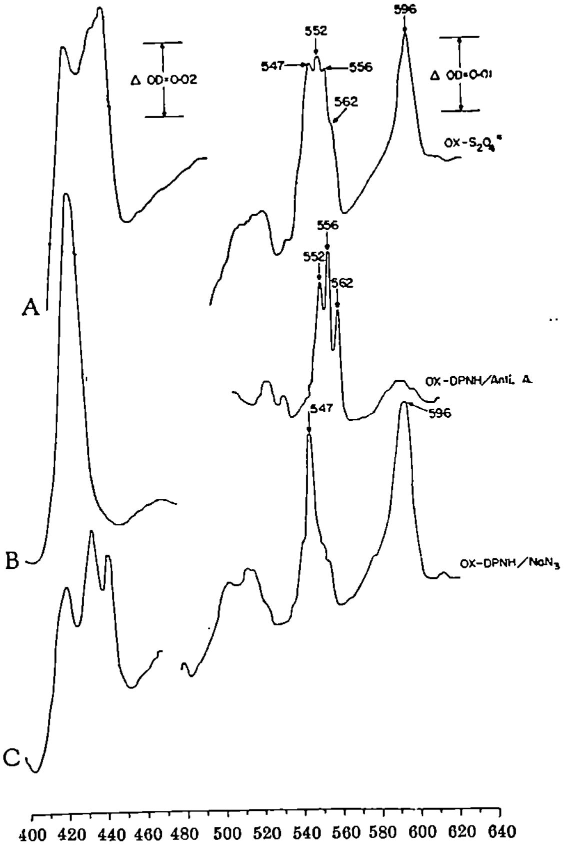


Fig. 4. Low-temperature (–190° C.) difference spectra of sweet potato mitochondria. Curve *A*, the difference in absorption between oxidized and hydrosulphite-reduced mitochrondria. Curve *B*, the difference in absorption between oxidized mitochondria and mitochondria reduced with reduced diphosphopyridine nucleotide in the presence of antimycin *A*. Curve *C*, the difference in absorption between oxidized mitochondria and mitochondria reduced with reduced diphosphopyridine nucleotide in the presence of azide

potato, potato (*Solanum tuberosum*), cauliflower, broccoli, beetroot and cucumber. It would seem that the oxidation of 'external' reduced diphosphopyridine nucleotide is a phenomenon common to plant tissues, and represents one of the marked differences between plant and animal mitochondria.

The cytochrome components of sweet potato mitochondria have been delineated through the use of a sensitive rapid scanning split-beam spectrophotometer[5] adapted for use at low temperature[6]. The spectra were obtained in the absence of glycerol as described by Bonner[7]. Low-temperature spectrophotometry allows increased absorption and increased delineation, afforded by the sharpening of absorption bands at such temperatures[8]. A low-temperature difference spectrum between aerobic and hydrosulphite-reduced sweet potato mitochondrial suspensions shows maxima at 596, 562, 556, 552 and 547 mμ (Fig. 4*A*). A similar spectrum of the difference in absorption between aerobic mitochondria and mitochondria reduced with reduced diphosphopyridine nucleotide in the presence of antimycin *A* shows maxima at 562, 556 and 552 mμ (Fig. 4*B*). Finally, a difference spectrum between aerobic mitochondria and mitochondria reduced with reduced diphosphopyridine nucleotide in the presence of azide shows two main peaks at 596 and 547 mμ (Fig. 4*C*). These difference spectra show maximum absorption at 596 and 547 mμ, corresponding to the maxima of cytochromes $a + a_3$ and c respectively. Since the maxima at 562, 556 and 552 appear in the spectrum obtained in the presence of antimycin *A*, they are interpreted to represent the absorption maxima of three different b cytochromes.

The cytochromes of sweet potato mitochondria appear to be identical to those which have been described in mitochondria derived from a variety of plant tissues[7]. The cytochromes present in higher plant mitochondria are remarkably similar among plant species, but show marked differences from animal mitochondria, especially in the nature of the b components. It is our hope that the preparation of reasonably intact mitochondria, as described here, will provide the much-needed material for the characterization of the respiratory chain in plants.

[1] Bonner, W. D., and Ito, T., *Fed. Proc.*, **19**, 35 (1960).

[2] Hackett, D. P., Rice, B., and Schmid, C., *J. Biol. Chem.*, **235**, 2140 (1960).

[3] *IUB–IUBS Symp. on Biol. Structure and Function*, Stockholm (Academic Press, New York, in the press).

[4] Laties, G. G., *Plant Physiol.*, **28**, 557 (1953).

[5] Chance, B., in *Methods in Enzymology*, edit. by Colowick, S. P., and Kaplan, N. O., **4**, 273 (Academic Press, Inc., New York, 1957).

[6] Estabrook, R. W., *J. Biol. Chem.*, **223**, 781 (1956).

[7] Bonner, W. D., in *Proc. Haematin Enzyme Symp.*, Canberra, edit. by Falk, J. E., Lemberg, M. R., and Morton, R. K. (Pergamon Press, London, in the press).

[8] Keilin, D., and Hartree, E. F., *Nature*, **164**, 254 (1949).

GIBBERELLIN-LIKE SUBSTANCES IN PLANTS

By MARGARET RADLEY

Imperial Chemical Industries, Ltd., Pharmaceuticals Division, Akers Research Laboratories, Welwyn, Herts

Leguminous Root Nodules

IT has been shown[1] that in clover excision of the first-formed nodules, and also of the root-tips, stimulates further nodulation. Plants grown in association[2] or in succession[3] on a limited volume of medium can inhibit nodulation of an inoculated leguminous plant. The donor of the inhibiting effect need not be a leguminous plant. When charcoal[4] is added to the rooting medium, nodulation-rate is stimulated. It has also been shown that root-nodule formation on leguminous plants is reduced in *Phaseolus*[5,6] and in some species of *Trifolium*[7] by treating the foliage with gibberellin.

The content of endogenous gibberellin-like substances in nodules and roots has now been examined by the bioassay of extracts of these tissues. The extractions were carried out with ethyl alcohol, and the solutions were purified as previously described[8]. The extracts were then applied in alcohol to the leaves of dwarf pea seedings (var. Meteor). The growth of these plants was compared with others treated with known amounts of gibberellic acid.

Table 1 gives the results of three experiments using French beans and garden peas. The first experiment was carried out with young bean seedlings, one month old. In the second experiment old plants, from which pods had been gathered, were used. In neither case was any gibberellin-like activity detected in the roots, whereas a high concentration was found in the nodules. Dwarf and tall varieties of pea were compared. In both cases a low concentration of active substances was detected in the roots and a high concentration in the nodules. The nodules of the dwarf variety appeared to be particularly rich in these substances.

In addition, two small-scale experiments were carried out with French bean plants grown in water culture with and without inoculation with *Rhizobium*. In one experiment the nodulated roots and the culture solution in which they were grown both contained material with gibberellin-like activity, whereas none was detected in the uninoculated sample. In the other experiment the same amount of activity was found in the shoots of both groups of plants and some activity in the nodules of the inoculated plants, but none was found in the roots or the culture solutions of either group.

No activity was detected in a 2-gm. sample of root tips from 3-week-old French beans grown in soil;

Table 1. GIBBERELLIN-LIKE ACTIVITY IN ROOTS AND NODULES

Species	Tissue extracted (gm.)	μgm. gibberellic acid equivalent per kgm. tissue
I. *Phaseolus vulgaris* var. Masterpiece	Nodules 8·2	9·8
	Roots 8·2	0
	,, 24·9	0
II. *Phaseolus vulgaris* var. Masterpiece	Nodules 8·2	17·1
	,, 16·8	26·2
	Roots 8·2	0
	,, 108·5	0
III. *Pisum sativum* var. Meteor	Nodules 0·3	240
	Roots 0·3	0
	,, 69·3	0·2
var. Improved Pilot	Nodules 0·26	35·4
	Roots 0·26	0
	,, 59·7	0·93

but French bean seedlings grown in water culture secreted material with gibberellin-like activity into the culture solution for at least two weeks after germination. At least 0·01 μgm. gibberellic acid equivalent per plant was detected. Runner beans, peas, wheat and radish secreted smaller quantities into the culture medium.

These results suggest that the nodule-inhibiting factor produced by nodules and root tips is probably a gibberellin, and this may diffuse out into the surrounding medium in some circumstances.

The plants inoculated with *Rhizobium* in water culture were provided by Mr. W. K. Smith.

[1] Nutman, P. S., *Ann. Bot.*, N.S., **16**, 79 (1951).
[2] Nutman, P. S., *Ann. Bot.*, N.S., **17**, 95 (1953).
[3] Nutman, P. S., *Ann. Bot.*, N.S., **21**, 321 (1957).
[4] Turner, E. R., *Ann. Bot.*, N.S., **19**, 149 (1955).
[5] Galston, A. W., *Nature*, **183**, 545 (1959).
[6] Thurber, G. A., Douglas, J. R., and Galston, A. W., *Nature*, **181**, 1082 (1958).
[7] Fletcher, W. W., Alcorn, J. W. S., and Raymond, J. C., *Nature*, **184**, 1576 (1959).
[8] Radley, M., *Chem. and Indust.*, 877 (1959).

Seaweed (*Fucus vesiculosus*)

ALTHOUGH gibberellin-like substances have been found in many species of flowering plants, none has yet been reported from lower plants, apart from the fungus *Gibberella fujikuroi* and the yeast *Candida pulcherrima*[1]. Nevertheless, there are reports of a response to applied gibberellin in ferns[2,3], a moss[4], a liverwort[5] and green algae[6,7], suggesting that gibberellins may play some part in regulation of growth. A substance with gibberellin-like activity has now been shown to occur in a seaweed.

4,000 gm. of *Fucus vesiculosus* was collected and stored at − 18° C. For the first experiment 500 gm. was extracted with ethyl alcohol for 18 hr. at room temperature. The alcohol was removed by filtration and pressing, and the tissues were re-extracted with fresh 70 per cent aqueous alcohol for 4 hr. The alcoholic solutions were combined and were then treated as previously described[8]. The purified extracts were applied in alcohol to the leaves of dwarf pea seedlings of the variety Meteor. Three doses of the extract were compared with known amounts of gibberellic acid. This test was carried out in duplicate, one batch of plants being grown in a greenhouse in the natural lighting conditions of July, and the other batch grown under continuous artificial light only at a temperature of 15° C. The results are given in Table 1.

Table 1. GIBBERELLIC ACID EQUIVALENT (μGM./KGM. FRESH WEIGHT)

Fresh weight equiv. of extract (gm.) per plant	Bioassay conditions Greenhouse	Artificial light
50	7·2	0·76
10	12·0	2·1
2·5	6·8	5·2

Thus all extracts promoted growth considerably; this is evidence for the presence of gibberellin-like substances. The relative decrease in activity in the higher doses tested, when the plants were receiving artificial illumination, suggested that inhibitors might be interfering with the response.

The remaining 3,500 gm. of material was then extracted twice with alcohol, filtering but not pressing the tissue each time, and the alcoholic solution was concentrated in a climbing film evaporator. The acidic fraction of this was adsorbed on to a column of charcoal/'Celite' (5 : 1), and was eluted with water containing increasing amounts of acetone. Six fractions were recovered from the aqueous acetone and were dissolved in ethyl alcohol.

In a preliminary series of bioassays aliquots of all eluates were concentrated and applied to dwarf pea plants. Gibberellin-like activity was found only in the 50 per cent acetone eluate. In a similar experiment carried out under artificial light in which 1·0 μgm. gibberellic acid was added to each eluate it was found that the 90 per cent acetone eluate (an aliquot equivalent to 100 gm. of tissue) reduced the response to gibberellic acid to about half that expected; there was no evidence for the presence of an inhibitor in the 50 per cent acetone eluate.

The 50 per cent acetone eluate was then bioassayed at three dosage-levels (Table 2). The lower level of activity found, compared with that recorded in Table 1, may have been due to loss in storage or to less-efficient extraction. Part of this eluate was chromatographed on paper in *n*-butanol/1·5 N ammonia (3 : 1). The paper was divided into 10 parts, eluted with alcohol and bioassayed on dwarf peas. All the activity was found between R_F 0·3 and 0·4. Under these conditions the R_F of gibberellic acid was 0·38 and that of gibberellin A_5 was 0·59. The other known gibberellins with a similar R_F to gibberellic acid are gibberellins A_1 and A_6 (ref. 9); thus the active material present in *Fucus vesiculosus* may be one of these.

Table 2. GIBBERELLIC ACID EQUIVALENT (μGM./KGM. FRESH WEIGHT)

Fresh weight equiv. of extract (gm.) per plant	Bioassay conditions Greenhouse	Artificial light
125	0·45	0·36
25	0·52	0·40
4	trace	trace

Chromatograms of the 90 per cent acetone eluate were run in the same solvent system. An inhibitory substance present at R_F 0·6–0·7 was demonstrated by a lettuce germination test and by the lettuce seedling assay[10] in which the response to gibberellic acid was reduced. On the other hand, there was no significant reduction of the response of dwarf peas to gibberellic acid, and it is not clear whether this is in fact the substance causing the reduction in gibberellin response in the earlier experiments. Inhibitor β, which is found in many higher-plant tissues, has a similar R_F in this solvent system[11,12]. An inhibitor of gibberellin-induced growth found in seed extracts has been described by Corcoran and Phinney[13]. Its mobility in this solvent system is not known.

[1] Krassilnikov, N. A., *et al.*, *Doklady Akad. Nauk SSSR*, **123**, 1124 (1959).
[2] Knobloch, I. W., *Amer. Fern J.*, **47**, 134 (1957).
[3] Wardlaw, C. W., and Mitra, G. C., *Nature*, **181**, 400 (1958).
[4] Mitra, G. C., and Allsopp, A., *Nature*, **183**, 974 (1959).
[5] Asprey, G. F., Benson-Evans, K., and Lyon, A. G., *Nature*, **181**, 1351 (1958).
[6] Conrad, H., Saltman, P., and Eppley, R., *Nature*, **184**, 556 (1959).
[7] Provasoli, L., *Biol. Bull.*, **113**, 321 (1957).
[8] Radley, M., *Chem. and Indust.*, 877 (1959).
[9] MacMillan, J., Seaton, J. C., and Suter, P. J., 138th Meeting of the Amer. Chem. Soc. (1960), *Advances in Chemistry*, **28**, 18 (1961).
[10] Frankland, B., and Wareing, P. F., *Nature*, **185**, 255 (1960).
[11] Luckwill, L. C., *Nature*, **169**, 375 (1952).
[12] Kefford, N. P., *J. Exp. Bot.*, **6**, 129 (1955).
[13] Corcoran, M. R., and Phinney, B. O., *Plant Physiol.*, **33**, Supp. xl (1958).

AN ENDOGENOUS RHYTHM IN THE RATE OF DARK-FIXATION OF CARBON DIOXIDE IN LEAVES OF *BRYOPHYLLUM FEDTSCHENKOI*

By DIANA M. WARREN and MALCOLM B. WILKINS

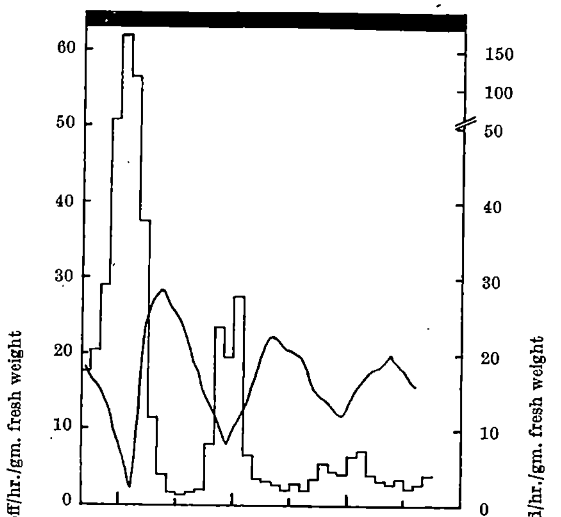
Department of Botany, University of London, King's College, 68 Half Moon Lane, London, S.E.24

LEAVES excised from the succulent plant *Bryophyllum fedtschenkoi* and maintained in darkness, at a constant temperature of 26° C., and in an air-stream initially free of carbon dioxide, exhibit an endogenous circadian rhythm in their rate of carbon dioxide emission[1,2]. The rhythm is inhibited by light but recommences when darkness is restored, the phase being determined by the time at which the light is extinguished. Furthermore, exposing leaves maintained in darkness to a light perturbation of 5 hr. duration at the crest of a peak has no effect on the phase, whereas a similar perturbation offered between the peaks induces a phase shift in which the first post-treatment peak occurs 18–19 hr. after the recommencement of darkness. The rhythm of emission of carbon dioxide is present in leaves excised from plants grown during long days and short days[1].

It has been suggested[1] that this rhythm may be due to the periodic activity of a dark, carbon dioxide fixation mechanism acting on an aperiodic rate of production of respiratory carbon dioxide by the tissue and the present work was designed to test this hypothesis.

The experimental plants were grown in cabinets in which illumination was provided by a Philips high-pressure mercury vapour lamp and four 150-W. tungsten lamps. Long-day illumination extended from 2400 hr. until 1600 hr. and short-day illumination from 0800 hr. until 1600 hr. The technique adopted in these experiments was as follows: three leaves were placed in each of a number of specially constructed glass tubes through which an air stream, initially free of carbon dioxide, passed at a rate of 1·5 l. per hr. The tubes were immersed in a water bath at 26° C. and illuminated at an intensity of 3,000 lux until darkness commenced. At 2-hr. intervals following the onset of continuous darkness, the flow of air free of carbon dioxide to one of the tubes was interrupted and $^{14}CO_2$ liberated in the closed tube. The concentration of carbon dioxide in the tube at the commencement of the 2-hr. feeding period was 0·66 per cent and the specific activity 88·4 µc./m.mole. At the end of the feeding period the tube was cleared of labelled carbon dioxide and the leaves were killed in boiling 80 per cent alcohol. They were then macerated and extracted successively with 80 per cent alcohol, 50 per cent alcohol and water, when no activity remained in the solid residue. The extract was reduced to a known small volume and the total carbon dioxide fixed by the leaves during the feeding period determined by estimating the radioactivity present in a sample of the extract. The uptake of carbon dioxide is expressed as µgm. carbon dioxide per hr. per gm. fresh weight of tissue. Simultaneous control experiments were conducted in which the rate of carbon dioxide output of two samples of leaves was monitored with an infra-red gas-analyser incorporated in an apparatus similar to that used in previous studies[1-3].

In the first two experiments leaves were excised from the plants between 1400 hr. and 1500 hr. and transferred to continuous darkness at 1600 hr., that



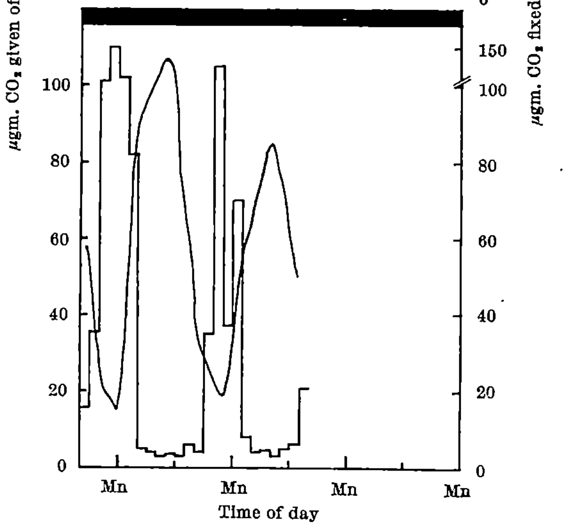
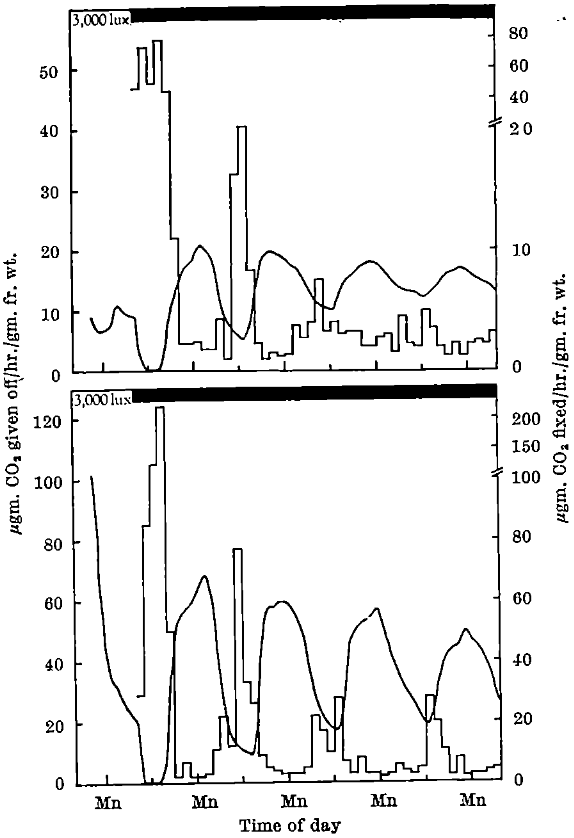


Fig. 1 (top). Rhythms in the rate of fixation (histogram) and output (continuous curve) of carbon dioxide in excised short-day leaves of *B. fedtschenkoi* maintained at 26° C. and in continuous darkness which commenced at 1600 hr. The black area of the bar above each figure indicates darkness. Ordinates, µgm. CO_2 fixed or given off/hr/gm. fresh weight of tissue; abscissa, time of day, Mn (midnight)

Fig. 2 (bottom). Rhythms of fixation (histogram) and output (continuous curve) of carbon dioxide in leaves taken from a plant grown in long days and transferred to darkness at 1600 hr.

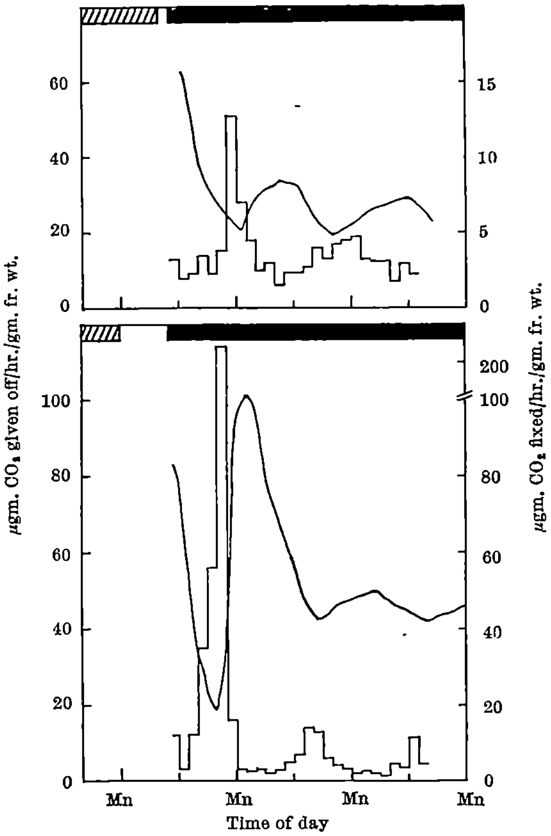


Figs. 3 and 4. Changes induced in the phase of the rhythms of fixation (histogram) and output (continuous curve) of carbon dioxide in short-day (Fig. 3, above) and long-day (Fig. 4, below) leaves by postponing the onset of continuous darkness from 1600 hr. to 0800 hr. the following morning

is, at the same time that darkness commences in the growth cabinets. The subsequent rate of dark fixation of carbon dioxide by short-day leaves showed a distinct rhythm in which the first peak occurred at 2400–0200 hr., 8–10 hr. after the onset of continuous darkness, and further peaks at intervals of approximately 23–24 hr. (Fig. 1). A similar result was obtained when this experiment was repeated with leaves taken from a plant grown in long days (Fig. 2). Continuous darkness again commenced at 1600 hr. and the first peak of the rhythm of fixation occurred approximately 8 hr. later at 2400 hr., with subsequent ones at about 23-hr. intervals. In each experiment the peaks of the rhythm of fixation occurred at times closely similar to the troughs in the rhythm of net output of carbon dioxide and vice versa. The slightly earlier occurrence of the first peak of the fixation rhythm in long-day leaves is consistent with a previous finding[1,4] that the transient in the output rhythm decreases as the duration of the previous light treatment is increased.

The phase of the rhythm of fixation of carbon dioxide can be changed if the onset of darkness, which normally occurs at 1600 hr., is postponed for several hours. Leaves were excised and placed in the tubes in the usual manner between 1400 hr. and 1500 hr. and then illuminated at an intensity of 3,000 lux until 0800 hr. the following morning. The rate of carbon dioxide fixation by both short-day (Fig. 3) and long-day (Fig. 4) leaves in the subsequent continuous darkness showed a distinct 23–24-hr. rhythm in which the first peak occurred at approximately 1400 hr., that is, about 6 hr. after the light was extinguished. Figs. 3 and 4 show that the rhythm of carbon dioxide fixation persists for at least 4 days in both short-day and long-day leaves held in darkness and at 26° C. In each figure the peaks of the fixation rhythm again occur at similar times to the troughs in the output rhythm.

In two further experiments the leaves were excised between 0800 hr. and 0900 hr. and transferred to darkness at 1000 hr.; both the short-day and the long-day leaves therefore experienced darkness 6 hr. earlier than if they had remained in the growth cabinets. It is clear from Fig. 5 that when short-day leaves are darkened at 1000 hr. the first peak of the rhythm of carbon dioxide fixation occurs at 2400–0100 hr. and the next peak 22–23 hr. later. The peaks occur, in fact, at the same times of day as those in the experiment shown in Fig. 1, where the leaves were darkened at 1600 hr. On the other hand, if long-day leaves are darkened at 1000 hr. the first peak of the fixation rhythm occurs at approximately 1900 hr., 9 hr. after the onset of darkness (Fig. 6), and subsequent ones at intervals of approximately 23 hr. It is clear, therefore, that while the early onset of darkness does not modify the phase of the rhythms of fixation or output of carbon dioxide in short-day leaves, in long-day leaves



Figs. 5 and 6. Rhythms of fixation (histogram) and output (continuous curve) of carbon dioxide in short-day (Fig. 5, above) and long-day (Fig. 6, below) leaves transferred to continuous darkness early in the day at 1000 hr. The hatched area of the illumination programme bar represents the darkness experienced by the leaves in the growth cabinets prior to the onset of the day (unshaded area) in which they were excised and darkened early. For explanation see text a phase shift is induced in which the first peaks of the fixation and output rhythms occur approximately 9 and 19 hr. respectively after the recommencement of darkness.

The invariable coincidence between the times of occurrence of the peaks of the fixation rhythm and the troughs of the output rhythm and vice versa in Figs. 1–6, irrespective of the time of day at which darkness commences, indicates that the rhythm of emission of carbon dioxide in the leaves is, in fact, a manifestation of the fixation rhythm re-fixing respiratory carbon dioxide at certain times in the cycle and not at others.

The induction of a phase-shift in the rhythms of both fixation and output of carbon dioxide by exposing long-day, but not short-day, leaves to darkness at 1000 hr. was to be expected on the basis of results already published[2,3]. If the onset of the dark period immediately prior to the day during which the leaves were transferred to darkness at 1000 hr. is considered as the time when continuous darkness commenced, then the curtailed day which the leaves received afterwards can be regarded as a light perturbation. In the case of long-day leaves this perturbation commenced at 2400 hr., that is, between the peaks of the rhythm of carbon dioxide output, and this would induce a phase-shift in which the first post-treatment peak would occur 18–20 hr. after the end of the treatment. In the short-day leaves the perturbation did not commence until 0800 hr., that is, until the crest of the peak of the output rhythm had been reached, and the phase of the rhythm is unaffected by a perturbation offered at this position in the cycle. Furthermore, the duration of the perturbation experienced by the long-day leaves was 10 hr., whereas that experienced by short-day leaves was only 2 hr.

Except when the leaves are, in effect, merely exposed to a light perturbation at the crest of a peak of the output rhythm, it is clear that the phase of the rhythms of output and fixation of carbon dioxide is determined by the time at which darkness commences and may be set accordingly at any desired time of day. Moreover, the results presented in Figs. 5 and 6, and those already published[2,3], show that the phase of the rhythms in leaves maintained in darkness is reset by a light perturbation only if it is administered in that part of the cycle where carbon dioxide fixation is occurring.

In attempting to locate the basic, oscillating chemical system in the leaf tissue it is important to determine whether the rhythm of carbon dioxide fixation is the only component of the output rhythm or whether there is also a rhythm in the rate of production of respiratory carbon dioxide. Preliminary experiments using the Warburg technique have not hitherto yielded evidence of a rhythm in the rate of respiration (oxygen uptake) of either whole or dissected leaves maintained in darkness and at 26° C. If the rate of production of respiratory carbon dioxide is aperiodic and corresponds to the rate of carbon dioxide emission of the leaves at the crests of the peaks of the output rhythm, then there should be a quantitative agreement between the maximum rates of fixation of carbon dioxide and the maximum decreases in the rates of carbon dioxide emission by the leaves in the corresponding troughs of the output rhythm. However, it is not possible to make a close quantitative comparison between the maximum rates of carbon dioxide fixation and the maximum decreases observed in the rate of carbon dioxide output of the leaves because, first, the rate of fixation of carbon dioxide by the leaves is a function of the ambient concentration of the gas, and secondly, the rates of fixation of carbon dioxide recorded in this work refer to the total fixation of the carbon dioxide liberated into the tubes and do not include the refixation of respiratory carbon dioxide which must occur during the 2-hr. feeding period when the leaves are incorporating the labelled gas.

The endogenous rhythm in the rate of dark fixation of carbon dioxide is undoubtedly the major component of the rhythm of carbon dioxide output in the leaves. Whether or not it is the only component is now the subject of a detailed investigation.

We thank Prof. T. A. Bennet-Clark for providing laboratory facilities for this work, and the Agricultural Research Council for financially supporting one of us (D. M. W.).

[1] Wilkins, M. B., *J. Exp. Bot.*, **10**, 337 (1959).
[2] Wilkins, M. B., *J. Exp. Bot.*, **11**, 269 (1960).
[3] Wilkins, M. B., *Cold Spring Harbor Symp.*, **15**, 115 (1960).
[4] Wilkins, M. B., *Proc. Roy. Soc.* (in the press).

CALCULATION OF THE AREAS OF ISOLATED OR OVERLAPPING NORMAL PROBABILITY CURVES

By DR. D. MORISON SMITH and JOHN C. BARTLET
Food and Drug Directorate, Tunney's Pasture, Ottawa, Canada

A MATHEMATICAL method has been developed for the calculation of the areas of normal probability curves, even when they overlap one another[1]. The method was used in that paper for the analysis of gas chromatograms obtained with a differential detector system. It should be possible to apply this method to any display of overlapping curves which exhibit probability characteristics. There are many procedures in various fields which apparently give such displays besides gas chromatography; for example, there are the curves obtained on plotting a given chemical property versus emergence time or fraction number in column partition chromatography; the optical density curves obtained by photoelectric scanning either through an angle in X-ray spectrometry or over a wave-length in emission spectra or across the spots in the direction of their motion in paper chromatography or electrophoresis; the sedimentation diagrams obtained on the ultracentrifuge with materials of high molecular weight; and some mass spectrometry curves, particularly those obtained on machines using the time of flight principle. The optical density curves obtained from photographic plates of emission spectra or Debye–Scherrer X-ray diffractions may also be probabilistic in nature, but calculations for such curves will depend on variations in the photographic response with wave-length and intensity. There is also the possibility that this method might apply to some very simple, uncomplicated infra-red or ultra-violet spectra.

The first step in the application of this method is to determine whether isolated peaks fit the requirements for normal probability curves. From Sheppard's tables of the normal probability integral[2], it is found that the width of the normal probability curve equals one standard deviation, σ (sigma), at 0·882 of the peak height, h, equals 2σ at 0·607 h and 3σ at 0·324 h. If the curve conforms to a normal probability curve, the values of σ derived from measurements of peak-width will be constant. The area may then be calculated from the equation: area, $A = h\sigma\sqrt{(2\pi)}$.

For the determination of the areas of overlapping normal probability peaks, there must be some means of predicting the σ values these peaks would have if they were isolated. In the case of chromatographic processes, the distribution of a given compound is controlled by random mechanisms such as diffusion within a phase or transfer between phases. The repeated application of these random mechanisms leads to broadening the peaks (increased σ) as the process continues. In gas chromatography, when σ is plotted versus the retention times of the peaks, a straight line is obtained[1]. The work of Craig[3] indicates that the same increase in σ occurs in countercurrent distribution and in column chromatography. For other processes, σ may be constant, or depend on certain experimental variables, for example, width of slit in an optical system or angle from the incident beam, in a diffraction system. All the above variations of σ with time, fraction number, angle, slit width, etc., are experimental variables which can be correlated to values of σ in a calibration curve. Sigma can then be read from this curve and the σ values of overlapping peaks can be interpolated.

When two additive normal probability curves overlap, the contribution of one curve to the apparent height of the other can be calculated. The information necessary for this correction was obtained from Sheppard's tables[2]. For convenience, it is presented graphically on probability paper in Fig. 1 with the ordinate of the normal probability curve, converted to percentage of peak height, plotted against the distance between the peaks measured in units of σ.

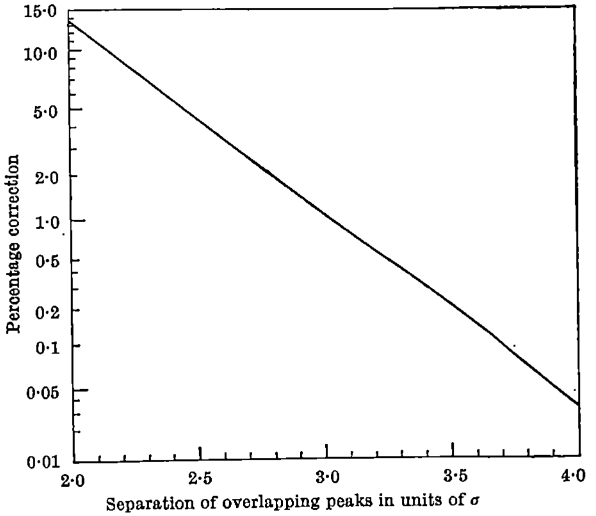


Fig. 1. Corrections of peak height for given separations of peaks measured in units of σ of the interfering peak

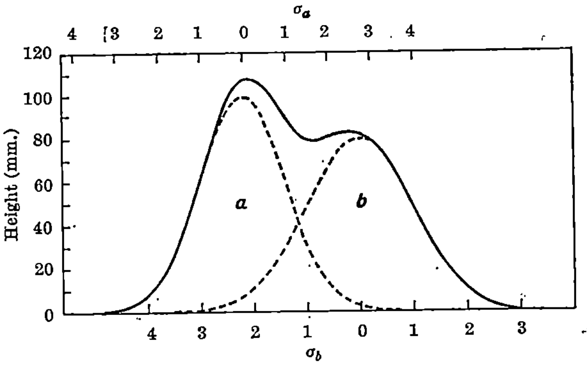


Fig. 2. Overlapping normal probability curves

A sample calculation of the mutual correction of two peaks is as follows. Consider the two peaks in the construction in Fig. 2. From previous experiments carried out under the same experimental conditions, either on the two isolated components for most accurate work, or on various mixtures, the values of σ and positions of the two peaks are interpolated. In the example under consideration, the values of σ obtained from a calibration graph are, for the first peak, (a) 20 mm. and for the second peak, (b) 25 mm.; the peaks are 55 mm. apart before photographic reduction for publication. The contribution of peak b to the height of peak a at the mean of peak a is dependent on the distance a is from b in terms of σ_b, which is 55/25 or 2·2 σ_b. This corresponds to a percentage contribution of 8·8 per cent of 82 mm., the observed height at b, that is, 7·2 mm. The observed height at a, 107 mm., is then corrected to 99·8 mm. by the deduction of the contribution of peak b. This figure is then used in the calculation of the true area of peak a. Similarly, the contribution of peak a to the height of peak b at its mean depends on the distance apart of a and b in terms of σ_a, which is 55/20 or 2·75 σ_a, corresponding to a contribution of 2·2 per cent of the 99·8 mm. of peak a or 2·2 mm. to be deducted from the height of peak b, giving 79·8 mm. for the true height of peak b. The construction actually used a height of 79·8 mm. for a and 99·7 mm. for b, giving an accuracy for the method within the limits of linear measurement on such a construction.

This accuracy was obtained with what is only the the first of a series of iterative corrections. It has also been observed that when the peaks are 4σ or more apart, no corrections need be made in the measured peak height.

It can be seen from Fig. 1 that there is a slight horizontal displacement of the observed peaks when they approach as closely as 2σ. The effect of this is insignificant; in fact, in many cases, if the peaks are separated enough to show as defined shoulders, direct measurement of height will give values by calculation for the areas which are within the inherent precision of the method itself.

This method may be extended to more than two peaks with the appropriate interrelated series of multiple corrections. Even with skewed probability curves, a similar treatment can be used to find the area of overlapping peaks[1].

[1] Bartlet, J. C., and Smith, D. M., *Canad. J. Chem.*, 38, 2057 (1960).

[2] *Biometrika Tables for Statisticians*, 1, 104 (Camb. Univ. Press, London, 1954).

[3] Craig, L. C., *Anal. Chem.*, 22, 1346 (1950).

LETTERS TO THE EDITORS

PHYSICS

Photography of Čerenkov Light from Extensive Air Showers in the Atmosphere

SEVERAL authors[1-4] have shown that Čerenkov light from extensive air showers in the atmosphere can be detected above the background light of the night sky, and that a single photomultiplier can be used for detecting showers of about 10^{15}-eV. primary energy at distances up to 600 m. from the shower axis.

We have used an image intensifier system, triggered by amplified pulses from a 5-in. photomultiplier, in an attempt to photograph the showers. The system consists of a Schmidt mirror 30 cm. in diameter, and nominal aperture of *f*0·5, but with usable area only 300 cm.². It has an acceptance cone of half-angle 17° about the zenith and is focused for infinity on the 5-in. diameter cathode of an image intensifier (Westinghouse *WX*4171). This is run continuously and integrates night-sky light over the decay time of the *P*15 phosphor, nominally 1 μsec. It is coupled optically to a three-stage cascaded intensifier (R.C.A. *C*73491) which is normally gated off, and which is followed by an intensifier orthicon, with kinescope display. The intensifier system has previously been used with a scintillation chamber and is described elsewhere[5]. Under present conditions we are limited by night-sky background, and operate the system well below full gain.

Light pulses are accepted by the photomultiplier over a cone of half-angle 50°, so that selection is isotropic over the field of view of the Schmidt system, but not all selected showers will be visible to it. Operation has been possible for 8 hr. under clear sky conditions and 32 shower pulses have been observed. A random pulse is applied a few seconds after each Čerenkov pulse, to obtain a comparison picture. Ten of the Čerenkov photographs have large spots, 3–5° in angular diameter. A typical event is shown in Fig. 1. No spots of comparable size or brightness have been observed in any of the 32 random pictures, though stars appear in both Čerenkov and random pictures. On covering the system, the field becomes dark. The evidence indicates that we are photographing the Čerenkov radiation from extensive air showers.

In the case illustrated, the first-magnitude star α Lyræ (Vega) also appears in both exposures, and this can be used to set a lower limit for the brightness of the shower pulse. It is known from previous work with the system that the effective integration time of the first phosphor is approximately 10 μsec., about ten times the nominal decay-time of the fast component. The total visible flux from the star during this time is roughly 10 photons/cm.². From the film density we estimate that the intensity of the shower pulse is of the order of 50 photons/cm.², consistent with a shower size of 10^5 particles. Accurate estimates are difficult because of non-linearities in the kinescope display and film response.

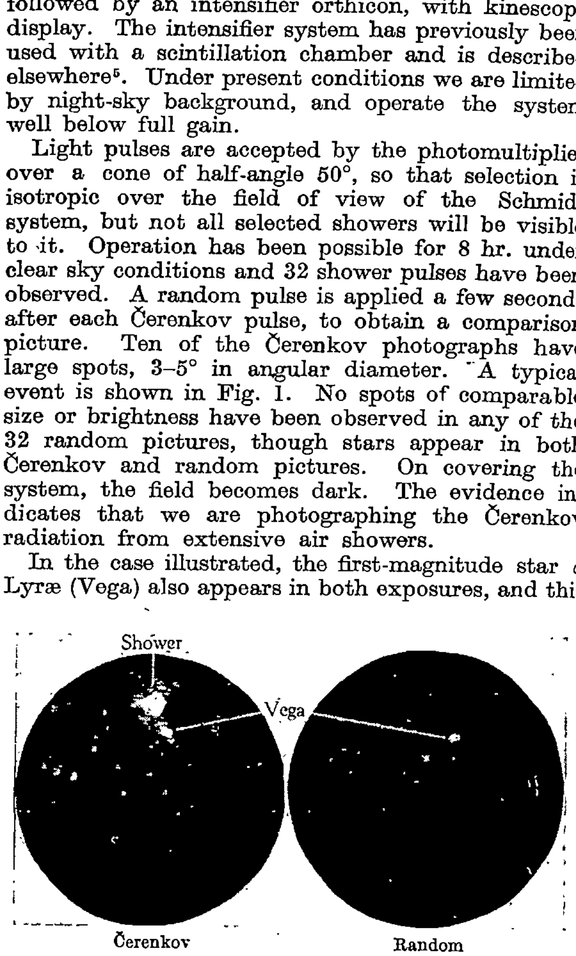


Fig. 1

The centres of the shower spots, which correspond presumably to the directions of the showers, can be determined with accuracy better than 0·5°. This combination of high directional precision with relatively high detection-rate suggests that the technique may be of value in the search for showers initiated by primary γ-rays.

The work was carried out at the Agassiz Observatory of Harvard University (100 m. above sea-level) and we would like to thank the Committee of the Observatory for allowing us the use of an astronomical building as well as Mr. K. Richards for his help in constructing and operating the system. One of us (N. A. P.) thanks the O.E.E.C. for the award of a senior visiting fellowship at the Massachusetts Institute of Technology. The experiment was supported in part through A.E.C. Contract *AT*(30-1)—2098, by funds provided by the U.S. Atomic Energy Commission, the Office of Naval Research, and the Air Force Office of Scientific Research.

D. A. HILL

Massachusetts Institute of Technology,
Cambridge, Mass.

N. A. PORTER

University College,
Dublin.

[1] Jelley, J. V., *Čerenkov Radiation*, 212 (Pergamon Press, London, 1958).

[2] Cudakov, A. E., *Rep. Moscow Conf. Cosmic Rad.*, **2**, 50 (1960).

[3] Brennan, M. H., Malos, J., Millar, D. D., and Wallace, C. S., *Nature*, **182**, 973 (1958).

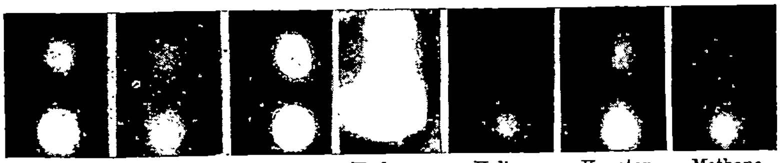
[4] White, J., Porter, N. A., and Long, C. D., *J. Atmos. Terr. Phys.*, **20**, 40 (1961).

[5] Caldwell, D. O., *Rep. Belgrade Conf. Nuclear Instruments* (in preparation

An Autoradiographic Effect

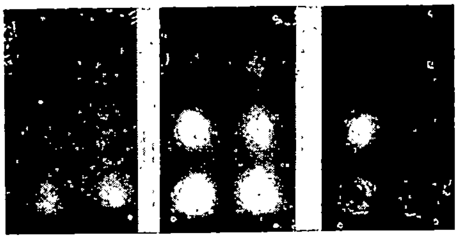
WHILE using X-ray films in another investigation, autoradiographic effects were obtained from many ordinary substances or mixtures of them. Materials such as zinc metal powder alone or mixtures of metal powders in a medium such as dried 'Glyptal' varnish were used. Such mixtures were put in holes in wooden or plastic blocks and the films attached to the blocks with drawing-pins or tape. A cardboard separator was used around the edges of the blocks to keep the films out of contact with the materials, leaving an air-gap of about 1 mm. Exposures of 48 hr. were generally used.

The combination of materials used in most of these experiments was a mixture of aluminium filings in 'Glyptal' varnish with addition of small amounts of castor oil sometimes. The following experimental



Room air · Vacuum (continuously pumped) · Argon · Hydrogen · Helium · Krypton · Methane (natural gas)

Fig. 1



30° F. · 65° F. · 90° F

Average temperature

Fig. 2

results are for such mixtures, but similar results were obtained from mixtures of other materials and also from metal filings or powders alone, notably zinc. Metal powders are not necessary for the effects, for they were also obtained from mixtures of organic materials.

The following series of experiments were performed to find out their effect on the autoradiations that were obtained:

Effect of ambient atmosphere on the radiation: Blocks were made up and exposures made in different atmospheres. The block, in each case with film attached, was put in a box impervious to light and placed in a bell jar in which the atmosphere was changed for each exposure. Results are shown in Fig. 1. All exposures were from the same block and were for 48 hr. The pressure in each case was a full atmosphere, except for vacuum, which was about 25μ or what is obtained from a rotary oil vacuum pump. There was no 'out-gassing' of the materials. Gases used such as argon, helium and krypton were taken from supplies used for lamp making and were dry. Hydrogen was dried with a small tube containing phosphorus pentoxide.

It may be noted that the kind of atmosphere affects greatly the kind of radiation results obtained from the same materials. The intensity is changed, and in some cases the wood of the block is made to radiate as shown in the case for vacuum. The highest intensity was obtained for hydrogen. The effect of additions is shown here, because the lower spot was from the mixture with a few per cent castor oil, while the upper one had no castor oil.

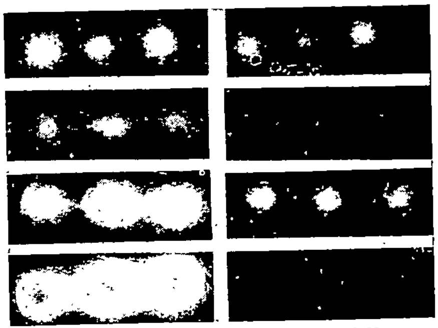
Effect of ambient temperature: The effect of ambient temperature is shown in Fig. 2. The same block was used for all cases. It may be noted that ambient temperature changes the results as to intensity of radiation and also the areas of the block which produce the radiation. At the first two temperatures the spot radiates as a whole, but at the third the radiation comes mostly from the edges of the spot.

Transfer of radiation: This is an effect which was found to occur if exposed, but not developed, films were stored next to each other for as much as 24 hr. This effect was studied by itself, and the results are shown in Fig. 3. On the left side are original radiations from the blocks, but on the right side are results from films which were not exposed to blocks, but were left in contact with the films on the left before they were developed. The time of such contact was 24 hr. or more. As shown, definite transfers were made and it should be noted that the transfers are not in proportion to the intensity of the original films. This would indicate that the agency causing the transfer is not the same as that which affects the X-ray film. Such transfers were obtained from materials other than films exposed to the radiating blocks, such as sheets of gelatine and metal foil.

Attempts were made to produce such transfers from films exposed to known radiations such as ordinary light, X-rays (about 1·5 Å.), β-rays from tungsten-185, γ- and β-rays from iron-59 and infra-red light. In all cases the original films (except the infra-red) had images comparable with those obtained from the blocks, but no transfers were obtained. The film is not sensitive to infra-red.

Experiments were performed to find out how long the originally exposed film would produce such transfers. A film was exposed to a radiating block for 48 hr. It was then left in contact with various other films which were changed at 24-hr. intervals. The results are shown in Fig. 4. The original film is on the left and the others are the films that were in contact with the original for the first 24 hr., second 24 hr., third 24 hr. respectively. The third film did not reproduce, but the images were discernible in the negative. Thus the activity of the original film persisted for 72 hr. or more.

It should also be stated that, for the transfer, the two films have to be as close as 1 or 2 mm. between them.



Original films · Transferred films

Fig. 3

Original film · First 24 hr. · Second 24 hr. · Third 24 hr.

Transfer film

Fig. 4

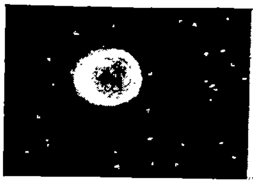


Fig. 1

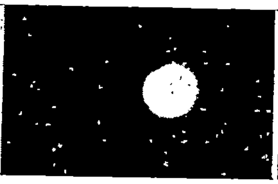


Fig. 2*a*

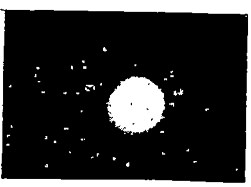


Fig. 2*b*

Fig. 1. Film exposed to hydrogen peroxide vapour for 1 hr.
Fig. 2*a*. As in Fig. 1
Fig. 2*b*. 'Transfer' from 2*a*. Time of transfer, 22 hr.

Range of radiation: This, of course, depends on the materials involved. Exposures at a distance of 3 in. produced the effect on the X-ray film. Using the usual extrapolation techniques, the indication is that the radiation may extend to as much as 6 in. Also at 3 in., the same results were obtained with the radiating materials above, below or to one side of the films. It is considered that this would not occur if the effect were due to a gas or vapour.

Also, the effect on the X-ray film has been obtained through thin sheets of aluminium foil, mica and up to 0·5 mil. 'Mylar' sheet. Whether the actual original radiation goes through these materials is open to question, for it may be another case of transfer as described here, the intervening materials re-radiating to the film on the other side.

Life of the radiating bodies: Experimental results indicate that, so long as the materials remain in the same condition, they will continue to radiate in the same way. Since, however, some oxidize, others such as plastic materials polymerize and set, etc., their radiating characteristics change.

All films referred to as X-ray films were Eastman 'No screen' X-ray films. A few attempts were made to use other films such as 'Tri*X*' and 'Panatomic', but without success.

The literature as listed here[1-3] mentions the 'Russell effect', which ascribes the effect of various materials on photographic films to the production of hydrogen peroxide, which reacts chemically with the films to produce the effect. In those cases ordinary films were used, and there is no doubt that hydrogen peroxide affects the films. In the above work, the effects were obtained only on X-ray film and were obtained under conditions such as vacuum, inert atmosphere and time where no such peroxide would be formed. This would indicate the presence of a radiation of a type for which these X-ray films are designed. It is therefore suggested that these radiations are a soft electron or β-type radiation with a range of as much as 6 in. from the source. The agency producing such radiations is somewhat obscure. It would have to be something which depends on the kinetic condition of the source, since production of the radiations is varied by conditions such as atmospheric content and pressure, vacuum, temperature, etc. More experimental work is needed to determine the exact nature of the agency causing these radiations.

Stuart McLean

Lamp Research and Development
Operation,
General Electric Co.,
Nela Park, Cleveland 12, Ohio.

[1] Russell, W. J., *Proc. Roy. Soc.*, **61**, 424 (1897).
[2] Grunberg, L., and Wright, K. H. R., *Nature*, **170**, 456 (1952).
[3] Meleka, A. H., and Barr, W., *Nature*, **187**, 232 (1960).

We wish to report the following experiments which were designed as a consequence of McLean's observations reported here.

In our experiments we also used X-ray film, Kodak 'Crystallex', which is a standard material for X-ray diffraction work. Two series of experiments were carried out. In the first, the film was exposed for various times to hydrogen peroxide vapour by placing it above a dish containing the liquid. The film was then processed in the normal way. An example is shown in Fig. 1. (Compare with Fig. 1 of McLean.) In the second series of experiments a film which was exposed to hydrogen peroxide vapour, but not developed, was placed in contact with a fresh film. The two films were then developed and a transfer effect was observed as can be seen in Fig. 2*a* and 2*b*. (Compare with Fig. 3 of McLean.) We believe that the vapour particles attached to the original film were transferred to the second, resulting in the same effect as in the first series of experiments.

We made no attempt to reproduce McLean's 'transmission' effect using hydrogen peroxide; little information is given in the original work. It would be interesting, for example, to have such data as the porosity of the aluminium foil used, also the relative intensities obtained.

A. H. Meleka
W. Barr

Metal Physics Section,
British Iron and Steel Research
Association,
140 Battersea Park Road,
London, S.W.11.

Initial Vibrations of a Supported Beam

The theory of steady flexural vibrations of a supported beam is well known, but that concerning the initial vibrations produced by impact seems to have received little attention. We have therefore carried out experiments on the subject.

Flexural vibrations of metal strips were initiated by striking the strips normally to the greatest area with pendulum-type hammers. The vibrations were examined by using a variable-reluctance transducer coupled through an amplifier to an oscilloscope. The movable component of the transducer was fixed to the beam or was formed by the beam itself when steel ones were used. The conversion characteristics of the transducer were found to be nearly constant with frequency for the small amplitudes of vibrations obtained in the experiments. The metal beams were all 2·54 cm. × 0·318 cm. × 40 cm., and were supported on knife-edges placed as closely as possible to their ends. Hammers made of various materials were used and were shaped to give approximately point contacts on the beams. Soft hammers, such as those made from cork or rubber, were found to result in a single impact, as shown by George[1] and Cliffe[2] for soft hammers striking pianoforte strings.

Figs. 1 and 2 show the oscilloscope trace obtained when a mild-steel beam was struck by a soft hammer at its centre, where the transducer was also located on the reverse side of the beam. In Fig. 1 the small timing pulses are spaced at intervals of 20 msec.

while in Fig. 2 the pulse spacing is 2 msec. In these figures the first apparent half-cycle of the fundamental mode of vibration is clearly seen to be of smaller amplitude and of shorter duration than the subsequent half-cycles. The same features were found in many of the photographs obtained by altering the positions of the transducer and the impact and by using beams of different metals.

The impulsive force acting at the hammer is not known, and attempts will be made to elucidate the force–time relationship which exists during impact, but the anomalous first half-cycle can be explained by assuming a form for the impulsive force and then following the method of Carslaw and Jaeger[3]. The force P_t acting at the centre of the beam due to the hammer is assumed to be given by $P_t = P_0(\exp(-at) - \exp(-bt))$, where P_0, a and b are all large positive constants and P_t is the force at time t. Fig. 3 shows graphs of $\exp(-at)$ and $\exp(-bt)$ from which a graph giving $(\exp(-at) - \exp(-bt))$, and hence the form of the impulsive force, can be constructed. Such a form agrees with that suggested by Goland *et al.*[4], although the ways in which impacts were obtained in this earlier work are different from those in the present experiments.

The displacement y_t of the centre of the beam at time t is then given by:

$$y_t = A_1\cos(kt) + B_1\sin(kt) + A_3\cos(9kt) + B_3\sin(9kt) + A_5\cos(25kt) + B_5\sin(25kt) + \ldots + A_n\cos(n^2kt) + B_n\sin(n^2kt) + \ldots - C\exp(-at) + D\exp(-bt) \quad (n \text{ odd})$$

where k, A_n, B_n, C and D are positive constants. k depends on the elasticity and dimensions of the beam. If $y_t = 0$ at $t = 0$, A_n, C and D are related by:

$$A_1 + A_3 + \ldots + A_n + \ldots - C + D = 0$$

Other considerations suggest that the amplitudes A_n and B_n are inversely proportional to the square of n. Thus, a simplified synthesis of y_t using only the terms having coefficients A_1, B_1, C and D may be expected to yield a fair representation of the actual y_t.

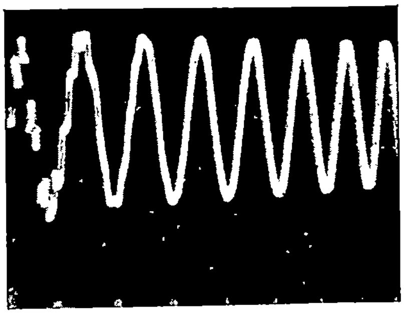


Fig. 1. Oscilloscope trace, timing pulse spacing 20 msec.

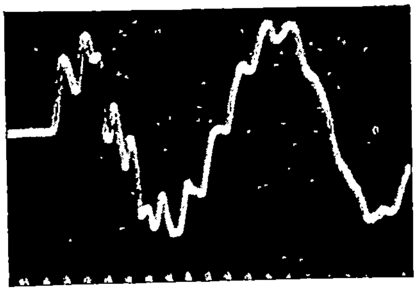


Fig. 2. Oscilloscope trace, timing pulse spacing 2 msec.

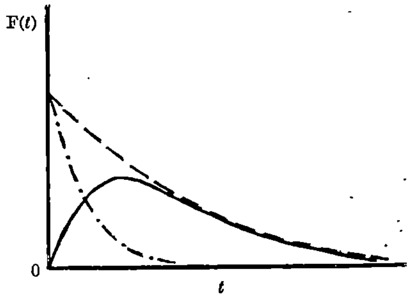


Fig. 3. Graphs of exp(−*at*), exp(−*bt*) and exp(−*at*) − exp(−*bt*). — —, exp(−*at*); —·—, exp(−*bt*); ——, exp(−*at*) − exp(−*bt*)

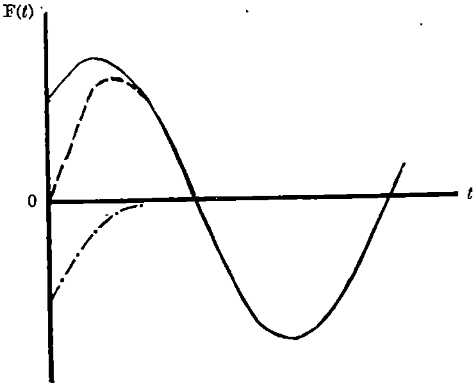


Fig. 4. Graphs of $A_1\cos(kt) + B_1\sin(kt)$, $D\exp(-bt) - C\exp(-at)$, and $A_1\cos(kt) + B_1\sin(kt) + D\exp(-bt) - C\exp(-at)$. ——, $A_1\cos kt + B_1\sin kt$; —·—$D\exp(-bt) - C\exp(-at)$; - - -, $A_1\cos kt + B_1\sin kt + D\exp(-bt) - C\exp(-at)$

Such a synthesis is given in Fig. 4 in which the sine and cosine terms have been combined. This figure shows a marked similarity to Figs. 1 and 2 so that the assumption of the form of P_t is further justified. Additional justification could perhaps be obtained by analysing wave-forms such as those shown in Figs. 1 and 2, but the analysis is made difficult by the presence of the anomalous first half-cycle.

R. V. Sharman
D. White

Physics Department,
Brunel College of Technology,
London, W.3.

[1] George, W. H., *Proc. Roy. Soc.*, **108**, 284 (1925).

[2] Cliffe, P., Ph.D. thesis, University of London (1951).

[3] Carslaw, H. S., and Jaeger, J. C., *Operational Methods in Applied Mathematics*, 145 (Oxford Univ. Press, 1947).

[4] Goland, M., Wickersham, P. D., and Dengler, M. A., *J. App. Mech.*, **22**, 1 (1955).

Ultrasonic Absorption of Polymer Solutions

It is well known that the presence of a small amount of low-absorption solute in a highly absorbing solvent will reduce the absorption of the solution by much more than would be expected from simple proportionality. This is the case for mixtures of benzene (high absorption) with methyl methacrylate (low absorption). Wada and Shimbo[1] have shown that if polymethylmethacrylate is added to a benzene–methylmethacrylate mixture the resultant absorption is higher than in the absence of the polymer, although

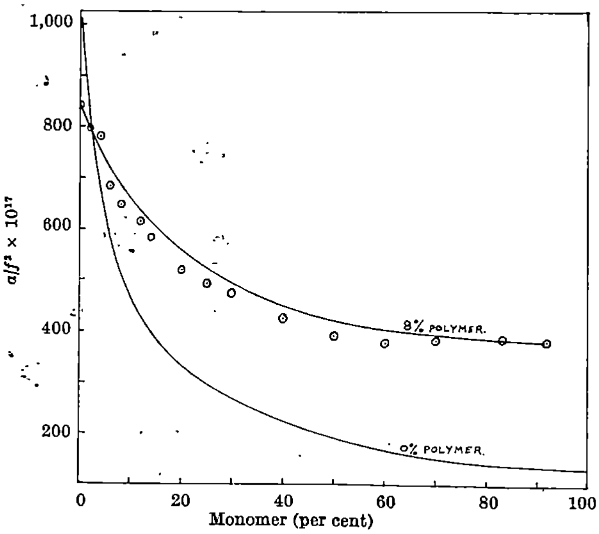


Fig. 1. α/f^2 as a function of monomer concentration. Full line, labelled 8 per cent polymer, predicted curve; dotted circles, experimental points

remaining less than that for the pure benzene. They suggested that this rise in absorption is caused by some of the monomer becoming 'enclosed' within the polymer chains so that it is no longer effective in reducing the solution's absorption. It is possible, however, to account for this rise in absorption without assuming 'enclosure'.

It is assumed that the absorption of a polymer – monomer – benzene mixture is made up of: (a) the absorption of a monomer – benzene mixture in which the polymer behaves in a similar manner to monomer in reducing the absorption; (b) an absorption due to the viscous motion of the solvent relative to the polymer chains[2,3]. This viscous absorption is assumed to be directly proportional to the mass of polymer present and the viscosity of the solvent. It is further assumed that these absorptions are additive. On these assumptions it is possible to account for the effects of the polymer found by Wada and Shimbo.

The ultrasonic absorptions of the mixtures have been measured at 26° C. and 5·19 mc./s. by a standard pulse technique. The measured absorption of 'Analar' benzene (expressed as α/f^2) is 1,010 × 10^{-17}, which is higher than the generally accepted value of 900 × 10^{-17}. However, for the present purposes, this discrepancy is unimportant since all the absorptions were measured under identical conditions with the same equipment.

The absorption due to the viscous motion of the solvent relative to the polymer chains was measured by comparing the absorption of a solution of polymethylmethacrylate in methylmethacrylate monomer with that of the pure monomer. Using monomer as solvent should probably eliminate any impurity effect which the polymer might have on the absorption. Expressed in terms of α/f^2 the viscous absorption of the polymer in monomer is 31 × 10^{-17} per gm. of polymer in 100-ml. solution. Thus, allowing for the higher viscosity of benzene compared with monomer, the expected value of α/f^2 for an 8 per cent (w/v) solution of polymer in benzene, in the absence of an impurity effect lowering the absorption, should be 1,310 × 10^{-17} while the measured value is 850 × 10^{-17}. The difference of 460 × 10^{-17} is that which would be produced by 6 gm. of monomer in 100 ml. of benzene solution. For 6 per cent and 4 per cent polymer solutions the corresponding equivalent monomer concentrations are found to be 3·8 gm. and 2·6 gm. of monomer in 100 ml. of solution. It appears, therefore, that the efficiency of the polymer, in so far as reducing the absorption is concerned, is about two-thirds that of the monomer (the actual values are 0·75, 0·63 and 0·65 respectively). This seems to be reasonable since the reduction in absorption by an impurity is due to collisions between it and the benzene molecules. If the monomer molecule is regarded as a cube there are six faces available for a collision with a benzene molecule, but when the monomer is connected into a polymer chain the number of faces available for collision drops to four for each monomer unit.

From a knowledge of the variation in α/f^2 and viscosity with monomer concentration in benzene – monomer mixtures and the amount of monomer equivalent to the polymer so far as reduction of absorption is concerned, it is possible to predict the variation in α/f^2 with monomer concentration for polymer – monomer – benzene mixtures. The results of one such calculation for an 8 per cent (w/v) solution of polymer are given in Fig. 1. The predicted curve lies consistently above the experimental points, showing that although the theory gives moderately good results it still requires improvement.

G. GOOBERMAN

Department of Electrical Engineering,
Royal College of Advanced Technology,
Salford.

[1] Wada, Y., and Shimbo, S., *J. Acoust. Soc. Amer.*, **25**, 549 (1953).
[2] Gotlieb, Y. Y., and Volkenshtein, M. V., *Doklady Akad. Nauk., S.S.S.R.*, **89**, 821 (1953) (Nat. Sci. Found. Translation 116).
[3] Mikhailov, I. G., and Shagalova, L. A., *Doklady Akad. Nauk., S.S.S.R.*, **89**, 829 (1953) (Nat. Sci. Found. Translation 117).

Molar Sound Velocity in Molten Hydrated Salts

FROM a systematic study of the temperature-variation of ultrasonic velocity and density in organic liquids, Rao[1] has found that the ratio of the temperature coefficients of the ultrasonic velocity and density is nearly equal to 3 for most organic liquids. From this result he has deduced the relation:

$$R = \frac{M}{\rho} (V)^{1/3}$$

where M is the molecular weight, ρ is the density, V is the ultrasonic velocity and R, known as molar sound velocity, is characteristic of the liquid and is independent of temperature. Although Rao established this relation in the case of organic liquids, later workers[2] have extended it to several inorganic liquids and have shown that R is independent of temperature. In the present communication, results are given of the variation with temperature of ultrasonic velocity in five molten hydrated salts.

The substances studied were lead acetate, sodium acetate, sodium–potassium tartrate, aluminium nitrate and calcium nitrate. For these substances the ultrasonic velocities in the molten state have been measured at various temperatures by using the fixed path interferometer method[3]. The variation of ultrasonic velocity with temperature for all salts is shown in Fig. 1. It will be seen that the variation of ultrasonic velocity with temperature is non-linear in all the salts except sodium acetate, which shows a linear decrease with increase of temperature. While investigating the variation of ultrasonic velocity with temperature in aqueous solutions of certain inorganic sulphates, Marks[4] found that the velocity–temperature curves are similar to those of water but displaced towards lower temperatures with increase in concentration. In the light of this earlier observation, the curves for the melts of sodium and lead acetates and calcium nitrate appear to be similar to the latter half of velocity-temperature curves of highly concentrated aqueous solutions, the maxima of which have been displaced considerably towards lower temperatures. It is interesting to note that the velocities in molten lead acetate are less than that of water. This behaviour is similar to that of other aqueous solutions of lead salts, as reported by Bärthel[5] and Krishnamurty[6]. The most unusual variation of ultrasonic velocity with temperature is observed in the case of double salt sodium–potassium tartrate, which shows a maximum at 75° C. which is very near the temperature of maximum velocity of water. A similar maximum is observed less prominently in the case of melt of aluminium nitrate. The behaviour of both these salts is unusual and needs further investigation in detail.

The values of molar sound velocity *R* are calculated at different temperatures, and it is found that for each salt *R* is independent of temperature. The maximum change, which is about 2 per cent, is observed only in the case of sodium–potassium tartrate. It may be noted that this temperature-independence of molar sound velocity has not been reported previously. Considering the molten hydrated salt as a highly concentrated aqueous solution of the salt in its own water of hydration and assuming linear variation of *R* with concentration at least in the higher concentration-range, the values of *R* for each salt are obtained by extrapolating to 100 per cent concentration and these values are given in Table 1. Computed values of *R* for the first three salts using previously estimated[7] values of *R* for the individual elements showed good agreement with the experimental values, thus justifying the procedure adopted here for calculating *R* for the pure salts from those of molten hydrated salts. The values of *R* for the last three salts in Table 1 are utilized for calculating *R* values for the metals aluminium, calcium and lead and have been given in the last column. It may be noted that the values of *R* for these three metals are obtained for the first time.

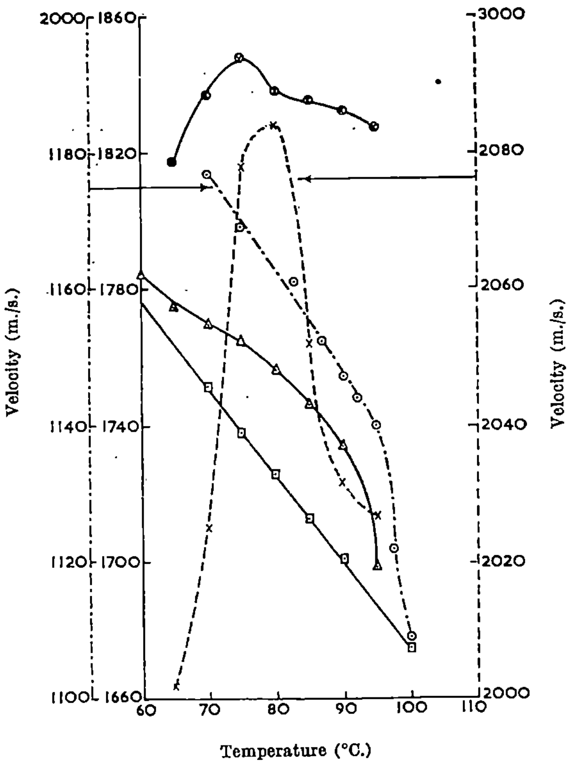


Fig. 1. Variation of ultrasonic velocity with temperature in molten hydrated salts. ⊗, Aluminium nitrate; ×, sodium potassium tartrate; △, calcium nitrate; ⊙, lead acetate; ⊡, sodium acetate

Table 1

Salt	Cm. (per cent)	*R* for hydrated salt	*R* for dehydrated salt	Computed value for salt	Computed value of *R* for new element
Sodium acetate	25	315	634	680	—
Sodium–potassium tartrate	20	438	1,350	1,329	—
Aluminium nitrate	10	295	1,060	—	Al = 319
Calcium nitrate	25	391	934	—	Ca = 436
Lead acetate	25	412	1,018	—	Pb = 127

We are indebted to the Council of Scientific and Industrial Research for financial assistance of this research work.

P. R. K. L. Padmini
B. Ramachandra Rao

Ultrasonic Research Laboratories,
Physics Department,
Andhra University,
Waltair.

---

[1] Rao, M. R., *Ind. J. Phys.*, **14**, 109 (1940).

[2] Rao, H. S., D.Sc. thesis submitted to Andhra University.

[3] Padmini, P. R. K. L., and Rao, B. R., communicated to *J. Phys. Soc.* (Japan). Rao, K. S., and Rao, B. R., *J. Acous. Soc. Amer.*, **31**, 431 (1959).

[4] Marks, W., *J. Acous. Soc. Amer.*, **31**, 936 (1959).

[5] Bärthel, R., *J. Acous. Soc. Amer.*, **26**, 227 (1954).

[6] Krishnamurty, Bh., *J. Sci. Indust. Res.*, **17**, 216 (1958).

[7] Padmini, P. R. K. L., and Rao, B. R., communicated to *Trans. Farad. Soc.* (London).

Neutron 'Flux'

Mr. Stretch[1] objects to the use of the term 'neutron flux' because the entity specified is a scalar, the movement of neutrons involved being random instead of a directed flow; he contrasts this with the usage of the word 'flux' for a vector quantity. This usage, as in 'heat flux', is, however, already a degenerate form. Though now often used with a vectorial meaning, 'flux' was originally a scalar, albeit derived from a vector. The original meaning, which is still the customary one in electrical theory, is 'the surface integral of the normal component of a vector', and this is a scalar. It is this mathematical concept, surely, that should be the standard of orthodoxy.

The suggestion of replacing 'neutron flux' by 'neutron potential' is open to the objection that it already has a different meaning when considering the interaction of a neutron with another particle.

W. R. Harper

67 Burton Court,
London, S.W.3.

---

[1] Stretch, K. L., *Nature*, **191**, 60 (1961).

MINERALOGY

Basic Dyestuffs in Clay Mineralogy

BASIC dyestuffs are adsorbed on clay minerals by cation-exchange reaction. The amount of dye adsorbed[1] and the resulting colour transformations[2] provide a possible basis for identifying clay minerals, but they are not very reliable. We have found that clay mineral – dye complexes exhibit characteristic thermograms and this method may possibly be applicable to the identification of clay mineral. Certain clay minerals which exhibit similar thermograms can be differentiated by the thermograms of their dye complexes.

Four types of clay minerals, namely, kaolinite, illite, nontronite and montmorillonite, were each treated with excess of aqueous solution of malachite green, methylene blue, methyl violet or piperidine. The unadsorbed organic material was removed by filtration and thorough washing. Thermograms were obtained by carrying out the differential thermal analysis of the complexes semi-automatically, using a Leeds and Northrup programme controller. The rate of heating was maintained at 10 deg. C./min.

Thermograms of kaolinite, illite, nontronite and montmorillonite with three basic dyestuffs, namely, malachite green, methylene blue and methyl violet, have been investigated. Differential thermal analyses of piperidine complexes of clay minerals have also been carried out for comparison. All the complexes exhibit low-temperature exothermic peaks at about 100° C. smaller than those of the untreated samples. The organic matter probably occupies the spaces normally held by the water molecules.

Kaolinite–dye complexes exhibit a small unmistakable exothermal dent between 350° and 435° C. due to the oxidation of a small amount of dye adsorbed by base-exchange reaction. The exothermic peak of kaolinite–malachite green complex is much more intense than the other kaolinite complexes, because malachite green is adsorbed to a greater extent than other dyestuffs.

Illite – dye complexes (Fig. 1*a*) show two intense exothermic peaks between 200° and 500° C. Nontronite and montmorillonite – dye complexes (Fig. 1*b*) show three exothermic peaks, two of pronounced intensities.

The second exothermic peak in illite-complexes at 460°–470° C. is less intense than that of nontronite-complexes occurring at about 600° C. Illites can be differentiated readily from nontronites by the second exothermic peak.

In montmorillonite – dye complexes, the higher-temperature exothermic peak at 650°–670° C. serves to differentiate montmorillonites from nontronites and illites, in which it occurs at about 600° C. and 460°–470° C. respectively.

From differential thermal analysis and thermogravimetric analysis it is concluded that low-temperature exothermic peaks are caused by oxidation of the dye molecules adsorbed on the edges of the clay minerals, while the high-temperature exothermic peaks are due to oxidation of dye molecules adsorbed in the interlayer.

Thus differential thermal analysis of clay mineral – dye complexes provides a more efficient method of identifying clay minerals than that of the clay mineral – piperidine complexes suggested by Allaway[3], Byrne[4] and Carthew[5]. Such complexes exhibit pronounced exothermal inflexions. In addition, the kaolinite – dye complex also shows a distinctive exothermal dent.

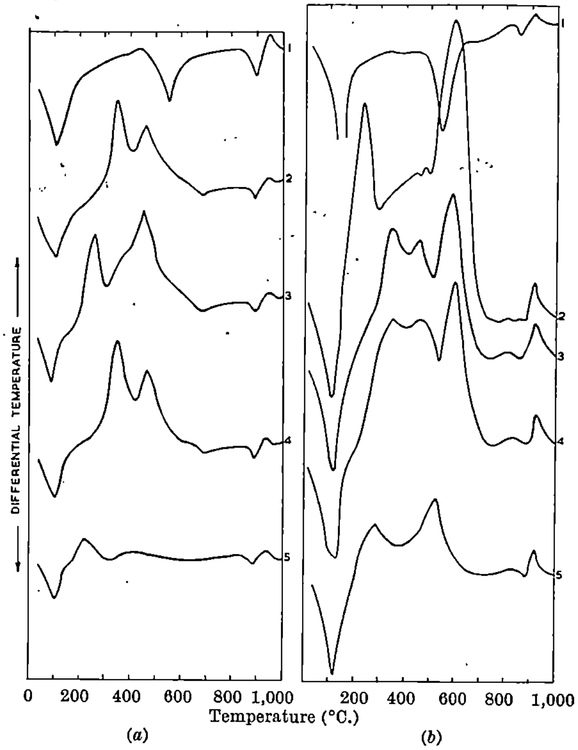


Fig. 1. (*a*) Thermograms of illite and its complexes: (1) illite; (2) malachite green complex; (3) methylene blue complex; (4) methyl violet complex; (5) piperidine complex. (*b*) Thermograms of nontronite and its complexes: (1) nontronite; (2) methylene blue complex; (3) malachite green complex; (4) methyl violet complex; (5) piperidine complex

This communication is published by permission of the Director, Central Building Research Institute, Roorkee, India.

V. S. RAMACHANDRAN
K. P. KACKER
N. K. PATWARDHAN

Building Materials Division,
Central Building Research Institute,
Roorkee, U.P.

[1] Bossazza, V. L., *Amer. Mineral.*, **29**, 235 (1944). Emodi, B. S., *Brit. Clay Min. Bull.*, **3**, 76 (1949). Robertson, R. H. S., and Ward, R. M., *J. Pharm. Pharmacol.*, **3**, 27 (1951). Worall, D., *Trans. Brit. Ceram. Soc.*, **57**, 210 (1958).

[2] Faust, G. T., U.S. Dept. Interior, Bureau of Mines, R.I. 3522, 21 (1940). Hauser, E. A., and Leggett, M. B., *J. Amer. Chem. Soc.*, **62**, 1811 (1940). Page, J. B., *Soil Science*, **51**, 133 (1941). Mielenz, R. C., King, M. E., and Schieltz, N. C., Amer. Pet. Inst., Project 49, *Preliminary Rept.*, No. 7, 135 (1950). Mielenz, R. C., and King, M. E., *Amer. Soc. Test. Mat. Proc.*, **51**, 1213 (1951).

[3] Allaway, W. H., *Proc. Soil Sci. Soc. Amer.*, **13**, 183 (1948).

[4] Byrne, P. J. S., *Proc. Second Nat. Conf. Clay and Clay Minerals, Washington*, 241 (1954).

[5] Carthew, A. R., *Soil Sci.*, **80**, 337 (1955).

GEOCHEMISTRY

Hot Spring Areas with Acid–Sulphate–Chloride Waters

WHITE[1], in a review of the thermal waters of volcanic origin, pointed out the problem of springs with a large flow of acid–sulphate–chloride water, such as Frying Pan Lake (or more correctly, Echo Crater) in the Waimangu area of the Rotorua-Taupo graben. This has a daily discharge of 800 kgm. of sulphuric acid. White remarked that the surface oxidation of hydrogen sulphide to sulphuric acid in the type of porous ground favourable to this process could account for only an insignificant part of the total production of sulphuric acid. Typical hot underground chloride water, as at Wairakei, is nearly neutral in reaction, and it seems unlikely that water appreciably acid could be stored underground without attack on the country rock. This communication suggests an explanation for the high-output acid–sulphate–chloride type of spring.

The second dissociation constant of sulphuric acid falls rapidly with rise of temperature. From data given by Noyes[2] from conductance determinations on 0·01 molar sulphuric acid up to 218° C., it appears that the second dissociation constant for 0·01 molar acid rises from approximately 5×10^{-6} at 250° C. to 0·019 at 20° C. If the water is neutral at 250° C., it will become acid at ordinary temperatures owing to increased dissociation of bisulphate by the reaction $HSO_4^- = H^+ + SO_4^{2-}$. The fall in *p*H due to this will outweigh the effect of the loss of carbon dioxide in the steam escaping from water reaching the surface. This means that sulphate waters which are neutral in *p*H underground at elevated temperatures can become waters of low *p*H at the surface.

Recently, chemical data have become available for the Rotokawa thermal area, about five miles east of Wairakei. This is an area with waters of acid character (*p*H about 2·5) containing both chloride and sulphate. Analyses of a number of hot-spring waters from Rotokawa indicate that there are two water types having chloride contents of 1,600 and 750 p.p.m. and sulphate contents of 400 and 1,000 p.p.m., respectively. The ionic ratios, chloride/sulphate, are about 10 and about 2 respectively. If the sulphate were of surface origin a random distribution of ratios would be expected. It is thought that both waters are supplied from deep underground sources.

It can be calculated for the Rotokawa examples that the *p*H of deep neutral waters (*p*H 5·65) at 250° C., containing 270 p.p.m. and 700 p.p.m. of total sulphate, would, after losing 30 per cent of steam at the surface, and cooling to 20° C., change in *p*H to 2·95 and 2·6 respectively. This is on the assumption that the content of bicarbonate in the underground water can be neglected. These *p*H values can be compared with those for a number of Rotokawa spring waters of low ammonia content. Three of the low-sulphate type had an average *p*H of 2·9 ± 0·4, and seven of the high-sulphate type had an average *p*H of 2·3 ± 0·15.

It seems, therefore, that some areas of acid springs of mixed chloride–sulphate water do not differ essentially in their origin from areas with springs of so-called 'alkaline' water (better termed chloride water). As there has not been any drilling at Rotokawa, it is not possible to obtain the ratio of chloride to total sulphur (that is, to sulphur both as hydrogen sulphide and as the sulphate) in the underground water but from the sulphate content alone it is at most 10. At Wairakei the ratio is 60. From other areas it is known that there are large differences in the ratios of chloride to boric acid, carbon dioxide, etc.

Hence the occurrence of waters of high sulphate content and low *p*H involves two problems: first that of the presence of a large amount of sulphur as sulphate (though not necessarily a larger proportion of the total sulphur), and secondly that of the low ratio of chloride to total sulphur.

Two possible mechanisms for producing chloride water high in sulphate can be suggested. First, the waters could result from the reaction of hydrothermal solutions with old sulphur deposits formed by oxidation of hydrogen sulphide near the surface, and buried by later volcanic activity. Disproportionation of sulphur in neutral solutions to sulphate and sulphide is predicted by thermodynamic information[3] and occurs readily in the laboratory.

The second mechanism is that hydrogen sulphide is oxidized to sulphate by ferric iron in the rocks. If the partial pressure of hydrogen in water in contact with iron-containing rocks is defined by the co-existence of hæmatite and magnetite, for equilibrium at temperatures over 250° C. in waters of neutral *p*H the sulphate/sulphide ratio is high[3]. This assumes that conditions prevailing correspond to a point on the phase diagram[4] where iron oxides rather than sulphides are the stable phases. For this to be the case at the likely hydrogen sulphide pressures of around 10^{-2} atmosphere, high temperatures probably more than 300° C. are necessary. Although iron sulphides become stable at lower temperatures as the water approaches the surface and loses steam, reversion of sulphate to sulphide is a slow process, and will not cause the loss of much sulphur from solution.

The first process would explain the low ratio chloride/total sulphur but is a less-likely occurrence. The second explanation makes the problem, apart from the possible requirement of high temperature, part of the general problem of the occurrence of deep chloride waters with great differences in the ratios of chloride to total sulphur, to carbon dioxide, to boric acid and to fluoride.

The analyses of the waters were made by Mr. W. A. J. Mahon, of the Dominion Laboratory.

A. J. Ellis

Dominion Laboratory,

S. H. Wilson

Institute of Nuclear Sciences,
Department of Scientific and
Industrial Research,
Wellington, New Zealand.

[1] White, D. E., *Bull. Geol. Soc. Amer.*, **68**, 1637 (1957).

[2] Noyes, A. A., *The Electrical Conductivity of Aqueous Solutions*, 279 (Washington, D.C., 1907).

[3] Barnes, H. L., *Carnegie Inst. Washington Year Book*, **57**, 237 (Washington, D.C., 1958).

[4] Foster, P. K., *N.Z. J. Sci.*, **2**, 426 (1959).

BIOPHYSICS

An Invariant Casein Substrate for Rennet Standardization, derivable from Diverse Sources of Cows' Milk

When rennin or commercial rennets (impure rennins) cause milk to coagulate, it is well known that the primary enzyme action consists in decomposing a part only of the casein, known as κ-casein[1]. Later, either through the activity of the products of decomposition or through loss of protective action, the casein aggregates and finally a gel (curd) is formed.

We have recently shown[2] that the initial decomposition as indicated by the fall in viscosity (Ostwald viscometer) follows a first-order equation under normal conditions. (At the concentrations used, all solutions are Newtonian.) The logarithmic reaction constant (k) is proportional to enzyme concentration when pure crystalline rennin is used, but varies as a power ($N < 1$) of the concentration for commercial

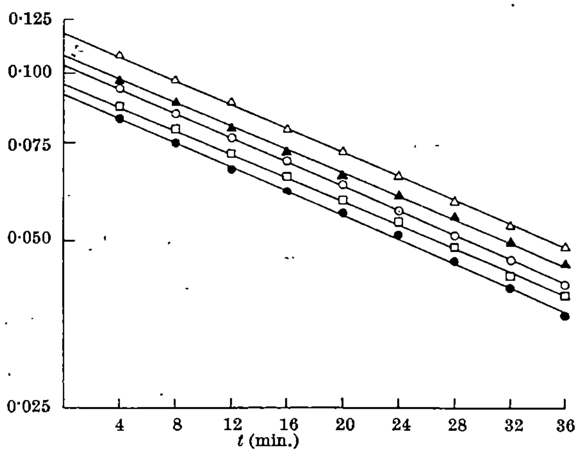


Fig. 1. Guggenheim curves for fall in viscosity of sodium caseinate from various milk sources: rennet A. △, Jersey; ▲, Friesian; □, Ayrshire; ○, bulk milk A; ●, bulk milk B

rennets. It seems likely that N reflects the purity of the rennet whereas k should give a measure of its activity. This latter may be high even for impure rennets, due to the presence of enzymes other than rennin. The problem is to find a reproducible substrate against which to compare different rennets, since milk, being a biological fluid, does not have a fixed composition; for example, milks may well differ in their proportions of κ-casein. The question is whether the composition of the κ-casein also varies.

We have found it possible to prepare sodium caseinates from milks not only from different herds but also from different breeds of cow, which give, within the limits of experimental error, the same value of k for any one rennet but, in general, different values for different rennets. These caseinates have the additional advantage that they show no appreciable aggregation.

The caseinate is prepared by finely precipitating it from fat-free milk at the isoelectric point (*p*H 4·7) with N hydrochloric acid, and then removing as much as possible of the soluble milk components by frequent washing and filtering. The acid casein is dissolved by the slow addition of 0·5 N sodium hydroxide, and vigorous stirring, taking care that the mixture does not become alkaline. Stirring is continued for at least 2 hr., with excessive acid casein present, before the concentrated sodium caseinate solution, which should now have a *p*H of 6·2–6·4, is filtered and freeze-dried. The experimental samples, made up from the freeze-dried sodium caseinate, are adjusted for *p*H and then heated to 80° C. for 5 min. to destroy the natural proteases. They are then kept for another 24 hr. in the cold to regain their normal viscosity, reduced during the heating. Before use it is also desirable to filter the substrate through suitable sinter-glass without suction. Unless these precautions are taken in preparing the caseinates, the values of k for different samples, using the same rennet, may differ considerably.

To determine k, a smooth curve is drawn by inspection through the graph of η_{sp}/time (where η_{sp} is the specific viscosity). There are normally some 20 points on each experimental curve. The time zero is the moment when rennet is added. Differences of η_{sp} ($\Delta\ \eta_{sp}$) corresponding to a standard time-interval ($\Delta\ t$) are determined, following Guggenheim's method[3]. The slopes of the Guggenheim curves, plotting log ($\Delta\ \eta_{sp}$) against t, give the values of k, only with a change of sign.

The positions of the curves would depend on the proportional falls in viscosity produced by the enzymes, that is, on the concentrations of κ-casein, except that, since viscosity does not vary linearly with caseinate concentration, an empirical adjustment is made to give the different samples approximately the same initial viscosities. Since the contribution of the products of decomposition of κ-casein to the total viscosity is not known, the proportion of κ-casein cannot be directly calculated, though it appears to be at least 20 per cent, and often more, that is, considerably higher than was previously supposed[1]. In Fig. 1 are shown a number of Guggenheim curves for sodium caseinates prepared from milks from different breeds and from mixed herds. It is clear that for some 50 min. at least, the reaction is very close to first-order. If the experiments are prolonged, the curves tend to deviate from linearity.

Table 1

Source of caseinate (cows' milk)	$k \times 10^4$ sec.$^{-1}$	
	Rennet A	Rennet B
Jersey	4·06	3·83
Ayrshire	4·01	3·87
Friesian	4·09	3·89
Bulk A	4·19	3·94
Bulk B	4·14	3·80
Bulk C	4·05	3·90
Bulk D	4·09	3·87
Mean	4·09	3·87
Standard deviation	0·0597	0·0458

In Table 1 are given values for k, together with their means and standard deviations, for caseinates from seven milks for two well-known brands of commercial rennet. Rennet A has a higher value of N and is known to be purer in rennin than is rennet B, but both are considered satisfactory for cheese manufacture.

We conclude that sodium caseinate, prepared as described, may be reproduced from milks from widely different sources and hence probably over long periods of time. The value of k for a particular rennet should thus provide a stable index of its activity.

A full account of this work, including details of experimental conditions, will be submitted, in due course, for publication (*J. Dairy Res.*).

We are indebted to our colleagues at the Institute for many helpful discussions.

G. W. Scott Blair
J. C. Oosthuizen*

National Institute for Research
in Dairying,
University of Reading.

---

* Permanent address: Glen College of Agriculture, Orange Free State, South Africa.

[1] Waugh, D. F., and Hippel, P. H. von, *J. Amer. Chem. Soc.*, **78**, 4576 (1956).

[2] Scott Blair, G. W., and Oosthuizen, J. C., *J. Dairy Res.*, **28** (2), 165 (1961).

[3] Guggenheim, E. A., *Phil. Mag.* (7th Ser.), **2**, 538 (1926).

Changes of Permeability of Yeast with Sodium Deoxycholate

Understanding of the phenomenon of cell permeability depends on detailed knowledge of the structure of the cell membrane. But the morphological and chemical organization of the cell membrane is still largely unknown. Study of the mechanism of action of detergents on the cell may represent one of

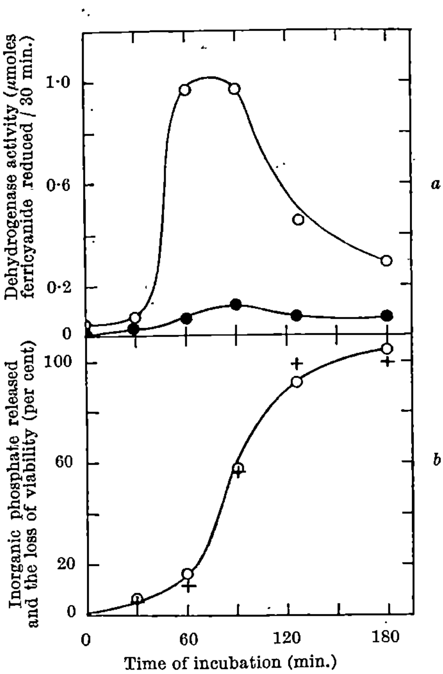


Fig. 1. Effect of deoxycholate on the dehydrogenase activity, inorganic phosphate released and viability in a suspension of yeast. Baker's yeast (11·7 mgm. dry weight/ml.) was incubated in 0·04 *M* *tris*-hydrochloric acid *p*H 7·5 with 0·006 *M* sodium deoxycholate at 27° C.; at suitable intervals, the medium was analysed for inorganic phosphate and the cells for succinic dehydrogenase activity. A portion of the cells was diluted and plated on Petri dishes with a synthetic medium; from the number of colonies the fraction of dead cells was calculated. *a* : ●, Dehydrogenase activity without added substrate; ○, succinic dehydrogenase activity; *b* : ○, inorganic phosphate released from the cells (per cent) (released by extraction with 10 per cent trichloracetic acid = 100 per cent); +, dead cells (percentage of total)

the suitable ways for the solving of this problem. From this point of view, the mechanism of action of deoxycholate on the yeast cell was investigated.

The intact yeast cells are not permeable to ions of dicarboxylic acids or to ferricyanide, hence no succinic dehydrogenase activity with ferricyanide as an electron acceptor can be demonstrated in the suspension of intact yeast cells. If deoxycholate is put into such a suspension, succinic dehydrogenase activity can be measured after a certain lag period; this means that in the presence of deoxycholate the cells become permeable. This effect of deoxycholate on the permeability is strongly dependent on temperature, increasing at higher temperature. The effect is enhanced by the lowering of *p*H (down to the point where the insoluble acid is precipitated) and by increasing ionic strength.

The dependence of the effect on the concentration of deoxycholate has a sigmoidal character. Under the experimental conditions used (see legend to Fig. 1), concentrations lower than 0·002 *M* were ineffective; at 0·005 *M* the effect was already maximal. At lower *p*H or higher ionic strength, the deoxycholate was effective even at lower concentrations.

Fig. 1 shows a time-course of the change in permeability of the cells incubated in the presence of deoxycholate, as measured by succinic dehydrogenase activity. The inorganic phosphate released from the cells and the viability of the cells are also plotted. Activity of succinic dehydrogenase reaches a maximum and then diminishes because of irreversible inactivation. Most striking is the fact that, while the loss of inorganic phosphate runs parallel to the loss of viability, which indicates that inorganic phosphate is released from the dead cells only, in a certain definite phase only about 20–30 per cent of cells are dead, while the succinic dehydrogenase activity measured is already a maximum. This suggests that part of the cells with the loose permeability barrier may be viable. This is supported, too, by the following observation: when the cells were pre-incubated with deoxycholate for such a time as to show almost a maximal or just a maximal succinic dehydrogenase activity and then washed with water, they did not show any, or only a very weak, succinic dehydrogenase activity, if deoxycholate were omitted from the test mixture. Thus, the action of deoxycholate may be, at least partly, reversible.

Recently, evidence has been provided by X-ray diffraction investigations[1], and direct observation by electron microscopy[2], that, under appropriate conditions, deoxycholate aggregates in solution to form a complex of macromolecular dimensions, the molecules assuming an elongated helical configuration. The dependence of the formation of these complexes on *p*H, on ionic strength and on the concentration of deoxycholate[3] is strikingly similar to the dependence of the changes of yeast permeability with deoxycholate on the above factors. This suggests strongly that the marked biological effect of deoxycholate, at least so far as its effect on yeast permeability is concerned, may be due to its ability to form helical fibre complexes. In these fibres, the hydrophobic steroid part of the molecule appears to be located inwardly, negatively charged carboxyl groups being on the surface. This seems to exclude the possibility that the molecules of deoxycholate would be oriented with their hydrophobic parts to the surface of the cell membrane and that the primary action of deoxycholate would be related to lowering of the surface tension. Rather there might exist another type of interaction, for example, similar to that which has been described recently by Lawrence[4], to explain the cleaning action of ionic detergents; that is, the interaction of the polar part of the deoxycholate complex with polar groups on the cell surface which would result in the formation of the ternary mixture, namely, polar part of deoxycholate/water/polar groups of the cell surface, followed by loosening of some components of the membrane, which are responsible for its permeability barrier.

L. Kováč

Department of Organic Chemistry
and Biochemistry,
Komensky University,
Bratislava, Czechoslovakia.

[1] Rich, A., and Blow, D. M., *Nature*, **182**, 423 (1958).

[2] McCrea, J. F., and Angerer, S., *Biochim. Biophys. Acta*, **42**, 355 (1960).

[3] Blow, D. M., and Rich, A., *J. Amer. Chem. Soc.*, **82**, 3566 (1960).

[4] Lawrence, A. S. C., *Nature*, **183**, 1491 (1959).

CHEMISTRY

Discreteness-of-Charge Effect and the Stability Theory of Colloids

According to the stability theory of hydrophobic colloids of Derjaguin and Landau[1] and Verwey and Overbeek[2] (D.L.V.O. theory) the electrolyte concentration required to produce coagulation increases with the surface potential of the colloidal particles. However, for silver halide sols, the electrolyte concentration increases with surface potential for univalent flocculating ions, either decreases or remains approximately unchanged for divalent ions and decreases for trivalent ions[3,4]. This can be explained by incorporating into the D.L.V.O. theory the so-called discreteness-of-charge effect[5–8].

Using the electric double-layer model of Grahame[9] and Devanathan[10] for a plate-like particle, an 'inner region' of oriented water dipoles, of thickness $d(\sim 5$ Å.) and dielectric constant ε_1 ($\sim$10–20), is assumed to lie between the particle medium and the dispersion medium, of dielectric constants ε_p and ε ($\sim$80), respectively. The volume density in the dispersion medium of the potential-determining ions, which constitute the charge on the particle wall and are negative, is small compared with the corresponding density n of the coagulating electrolyte of a z-z valency type. A layer of cations, valency z, is absorbed on the inner Helmholtz plane (I.H.P.), inside the inner region at a distance $\beta < d$ from the plate wall. The outer Helmholtz plane (O.H.P.), at distance d from the wall, is the boundary between the inner region and the diffuse layer. The two zones of the inner region of thicknesses β and $\gamma = d - \beta$ act as condensers of capacities Kd/β and Kd/γ per unit area, where $K = \varepsilon_1/4\pi d$. The mean electrostatic potentials at the wall, the I.H.P. and the O.H.P., are denoted by ψ_0, ψ_β and ψ_d respectively. Also σ_0 and σ_β are the mean surface densities at the wall and the I.H.P. Then we have the relations: $\psi_0 - \psi_\beta = \beta\sigma_0/Kd$, $\psi_\beta - \psi_d = \gamma(\sigma_0 + \sigma_\beta)/Kd$, $\sigma_0 + \sigma_\beta + \sigma_d = 0$, where σ_d is the total charge in a column of the diffuse layer of unit cross-section. Applying the Gouy–Chapman theory to the diffuse layer:

$$\sigma_d = -\left(\frac{2n\varepsilon kT}{\pi}\right)^{1/2} \sinh\left(\frac{1}{2}\,\frac{ze\psi_d}{kT}\right)$$

where k, T and e have their usual meanings.

In the Stern theory of the double layer the energy of adsorption of the cation consists of a chemical term and an electrostatic term $ze\psi_\beta$ and the discrete nature of the ionic charge is ignored. In allowing for this effect, the Soviet authors[5,6,8] neglect the thermal motion of the adsorbed cations and replace the continuous charge distribution by a hexagonal array of discrete ionic charges. We adopt a simpler 'cut-off' approximation, suggested by Grahame[7], which is equivalent in principle. Each adsorbed cation is regarded as the centre of a circular area in the I.H.P. from which the uniform charge density σ_β is removed, of radius r_0 such that $\pi r_0^2\sigma_\beta = ze$. By considering the multiple electrostatic image reflexions in the plane of the plate wall and in the O.H.P., where the dielectric constant is discontinuous, the resulting change in the potential at the centre of the adsorbed cation can be expressed very nearly as:

$$\varphi_\beta = -\frac{\beta\gamma}{Kd^2}\,g\sigma_\beta$$

where g is a factor depending on ε, ε_1, ε_p and n. If $\varepsilon_1 \ll \varepsilon, \varepsilon_p$ then $g \approx 1$, but in general $g > 1$. The potential φ_β is a two-dimensional analogue of the self-atmosphere potential in the Debye–Hückel theory of electrolytes. The contribution $ze\varphi_\beta$ to the energy of adsorption is not small and is responsible for the apparent variation of the specific adsorption energy with surface charge at the mercury-aqueous electrolyte interface[9].

If each adsorbed ion, retaining a portion of its hydration shell, occupies s adsorption sites, then employing the volume fraction statistics of Flory[11] and Huggins[12], the Stern adsorption isotherm is modified to read:

$$kT\ln[\sigma_\beta/(1 - s\sigma_\beta/N_s ze)^s] = -ze(\psi_\beta + \varphi_\beta) + kT\ln n + C$$

where N_s is the number of adsorption sites per unit area and C is a constant which includes the chemical term in the adsorption energy. From these relationships maxima in both $|\psi_\beta|$ and $|\psi_d|$ are found at $\sigma_\beta = \sigma_\beta^{(m)}$, where:

$$\sigma_\beta^{(m)}[1 - s\sigma_\beta^{(m)}/N_s ze]/[1 + s(s-1)\sigma_\beta^{(m)}/N_s ze] = \frac{kT}{zeg}K\frac{d^2}{\beta\gamma}$$

The electrostatic work of adsorption of a cation is $-ze(\psi_\beta + \varphi_\beta)$, which increases steadily with plate potential. When the ratio $r = \sigma_\beta/\sigma_\beta^{(m)} < 1$, both $|\psi_\beta|$ and $|\psi_d|$ increase with $|\psi_0|$, but at larger coverages ($r > 1$) the variation of φ_β with ψ_0 predominates, causing $|\psi_\beta|$ and, therefore, $|\psi_d|$, to diminish. A maximum in $|\psi_d|$ was found experimentally at the mercury-electrolyte interface[13].

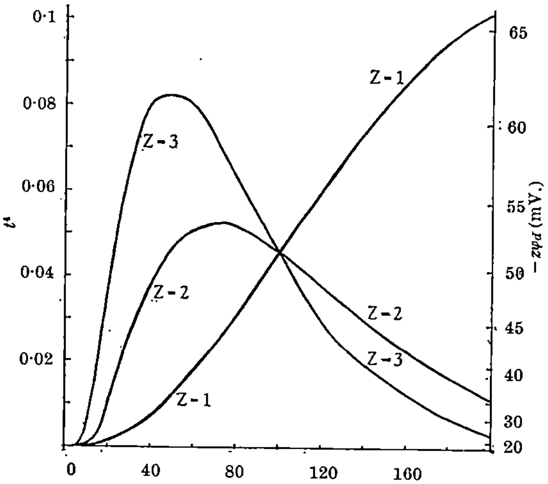


Fig. 1

Consider now two parallel plates of infinite thickness. If the potential distributions in the interpenetrating diffuse layers are linearly superposed, the coagulation condition becomes:

$$n = 107\,\frac{\varepsilon^3(kT)^5}{(ze)^6A^2}\,t^4,\quad t = \tanh\left(\frac{1}{4}\,\frac{ze\psi_d}{kT}\right)$$

where A is the constant in the van der Waals interaction energy. In the D.L.V.O. theory ψ_d is replaced by ψ_0 and, for large enough ψ_0, n is proportional to z^{-6}, a simple version of the Schulze–Hardy rule. In the typical plot of t^4 vs. ψ_0, by proper choice of C for $z = 1$, 2 and 3 this inverse sixth power rule is obeyed at $ze\psi_d/kT = 2$ and $r = 2$ for $z = 2$, where the three curves have a common intersection point ($\psi_d = 51{\cdot}4$ mV. if $z = 1$ and $T = 25°$ C.). Also $g = 1{\cdot}5$, $A = 2 \times 10^{-12}$ erg. cm.^{2}, $K = 30$ µF./cm.^{2}, $N_s = 5 \times 10^{14}$/cm.^{2}, $\gamma/d = 1/4$, $\varepsilon = 78{\cdot}5$ and $s = 1$, 3 and 5 respectively for $z = 1$, 2 and 3; the corresponding values of $\sigma_\beta^{(m)}$ are 2·84, 1·49 and 1·01 µcoul./cm.^{2}. The general trend of the dependence of coagulating concentration on plate potential is reproduced theoretically when $r < 1$ for $z = 1$, $r \gtrsim 1$ for $z = 2$ and $r > 1$ for $z = 3$.

Furthermore, deviations from the inverse sixth-power rule can occur, in agreement with experiment. The maximum in the $\psi_d - \psi_0$ curve, which is essential to the theory, is not obtained if the self-atmosphere potential φ_β is omitted. Various refinements, such as variation of K and γ/d with z and ψ_0 and a correction to the van der Waals energy due to the difference between the medium of the inner region and the particle medium, should not affect the general argument.

Part of the work by one of us (S. L.) was carried out at the National Research Council, Ottawa, Canada.

S. Levine
D. Calvert

Department of Mathematics,
University of Manchester.

G. M. Bell

Department of Mathematics,
Manchester College of Science
and Technology.

[1] Derjaguin and Landau, *Acta Physicochim., U.S.S.R.*, **14**, 633 (1941).

[2] Verwey and Overbeek, *Theory of the Stability of Lyophobic Colloids* (Elsevier, New York, 1948).

[3] Kruyt and Klompé, *Koll. Beih.*, **54**, 484 (1943).

[4] Težak, Matijević and Schulz, *J. Phys. Chem.*, **55**, 156 (1951).

[5] Esin and Shikov, *Zhur. fiz. Khim.*, **17**, 236 (1943).

[6] Ershler, *Zhur. fiz. Khim.*, **20**, 679 (1946).

[7] Grahame, *Z. Elektrochem.*, **30**, 508 (1954).

[8] Levich, Kirjanov and Filinovsky, *Dokl. Akad. Nauk, S.S.S.R.*, **135**, 1476 (1960).

[9] Grahame, *Chem. Rev.*, **41**, 441 (1947).

[10] Devanathan, *Trans. Farad. Soc.*, **50**, 373 (1954).

[11] Flory, *J. Chem. Phys.*, **10**, 51 (1942).

[12] Huggins, *J. Phys. Chem.*, **46**, 151 (1942) ; *Ann. N.Y. Acad. Sci.*, **43**, 1 (1942).

[13] Grahame and Sederberg, *J. Chem. Phys.*, **22**, 449 (1954).

Permeation of Gaseous Oxygen through Vitreous Silica

THE permeation of gaseous oxygen through vitreous silica has been measured over the temperature-range 950°–1,080° C. in a permeation cell constructed with two portions separated by a thin-walled spherical bulb with a short neck. The bulb was 25 mm. outside diameter, and served as the membrane of vitreous silica ; its area was 18·9 cm.^{2} and thickness 0·27 mm. The thickness was determined from electrical capacitance measurements. The silica was used as blown ; and no cleaning was done with hydrofluoric acid. The oxygen pressure, 300–800 mm. mercury, was applied to the convex side of the bulb. The low-pressure side led directly to a mass spectrometer with no leak intervening. The rate of permeation of gas was measured[1], in this case by the peak height of mass 32. It was assured that true permeation, and not merely degassing, was being measured by : (1) observing the slow rise of the oxygen peak after application of oxygen to the high-pressure side, (2) attainment of steady-state flow and then, (3) slow decay of the peak after the gas was removed from the high-pressure side.

The experimental values of rate of permeation P on an Arrhenius-type plot of log P against $1/T$ yielded a good straight line, giving the results shown in Table 1. The units of P are those used by Barrer[2] ; cm.^{3} gas (S.T.P.) per sec. for 1 mm. thickness per cm.^{2} area per cm. mercury difference of gas pressure. From the slope of this plot an activation energy of permeation of 22,000 cal. per mole was derived.

The lag method of Daynes[3] and Barrer[2] is ideally adapted for the mass spectrometer to measure the rate of attainment of steady-state flow. By this method the diffusion constant D, in cm.^{2}/sec., was found to be $1·2 \times 10^{-8}$ at 1,078° C. and $4·2 \times 10^{-9}$ at 950° C., yielding diffusion activation energy of 27,000 cal. for oxygen in vitreous silica. The lag time at 950° C. for the steady state was slightly more than 8 hr. in this particular cell with a wall 0·27 mm. thick.

The solubility was derived from the relationship $S = 7·6\,P/D$, and found to be $1·9 \times 10^{-3}$ at 1,078° C. and $2·3 \times 10^{-3}$ at 950° C. The units are cm.^{3} gas (S.T.P.) per cm.^{3} solid for 76 cm. gas pressure applied.

The fact that molecular oxygen is the diffusing species is indicated by the observation that the rate of permeation was proportional to the first power of the pressure.

Table 1

Temperature (° C.)	Rate of permeation P
900	$9·00 \times 10^{-13}$
950	$1·25 \times 10^{-12}$
1,000	$1·85 \times 10^{-12}$
1,050	$2·50 \times 10^{-12}$
1,100	$3·50 \times 10^{-13}$

Francis J. Norton

General Electric Research Laboratory,
Schenectady, New York.

[1] Norton, F. J., *J. Amer. Ceramic Soc.*, **36**, 90 (1953). Swets, D. E., Lee, R. W., and Frank, R. C., *J. Chem. Phys.*, **34**, 17 (1961).

[2] Barrer, R. M., *Diffusion in and through Solids*, 133 and 217 (Camb. Univ. Press, 1951).

[3] Daynes, H., *Proc. Roy. Soc.*, A, **97**, 286 (1920).

Aniline Oxidation initiated by Ultrasonic Waves

IN order to explain the chemical processes occurring in the ultrasonic field, Lindström and Lamm[1] assumed that the principal reaction occurring during the ultrasonic treatment of water is its decomposition into radicals:

$$H_2O = H + OH$$

By means of this reaction it is possible to explain a number of chemical processes[2–6], the oxidation of aniline included.

Aniline solutions (0·5, 1, 2 and 3 per cent, Merck) were used. All the solutions were prepared in redistilled water. The ultrasonic treatment was carried out in identical chemical vessels, containing 50 ml. of the desired aniline solution. A magnetostrictive transducer of 5·1 cm.^{2} radiation area was cooled by air in such a manner that the initial solution temperature (17°–18° C.) rose only by 4°–7° C., in accordance with the duration of the ultrasonic treatment. All the solutions were treated with the waves of 21-kc./sec. frequency and electric power of about 4·5 W./ml. of the solution.

All the aniline solutions of the appropriate concentration were treated with ultrasonic waves during 2 min. In the course of the treatment minute oxygen bubbles were let through every solution (about 35–40 bubbles per min.). After the treatment the solutions were poured into small calibrated cylinders with ground-in stoppers.

In the course of the observations it was found that the aqueous aniline solutions form different oxidation products after treatment as shown in Table 1.

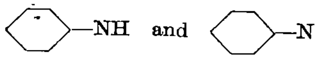
Table 1

Aniline solution concentration (per cent)	Ultrasonic treatment duration	Colour of dye
0·5	2 min.	violet
1·0	,,	violet-blue
2·0	,,	orange
3·0	,,	black shot with green

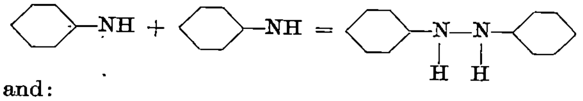
The violet dye obtained does not change under the influence of hydrochloric acid. Potassium hydroxide solution changes the colour of the dye to brown, but after acidification with hydrochloric acid it regains its violet colour. The violet-blue dye showed no changes under the influence of 6 per cent hydrogen peroxide solution. It separates on a filter paper into two layers: an external one of violet colouring and an internal one of black colouring shot with green. Neither of the dyes, violet and violet-blue, has been identified, but it is possible that they are derivatives of *p*-benzoquinone of an undetermined structure.

The orange dye has been identified as azobenzene, easily soluble in alcohol and especially in ether.

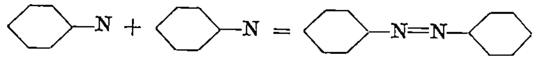
Concerning the azobenzene-forming mechanism in the ultrasonic field, it is necessary to assume first that aniline in aqueous solution decomposes into radicals:



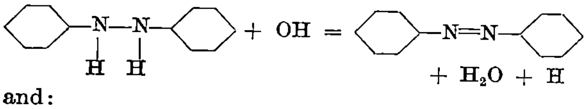
which, being in an unsaturated state, react with each other according to the equations:



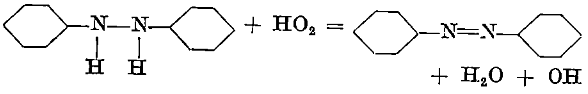
and:



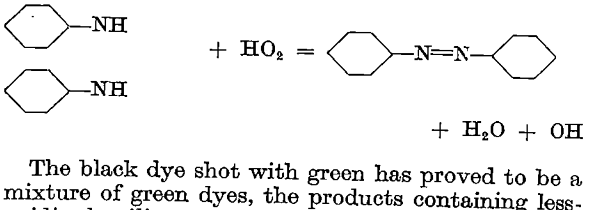
It is most probable that during the ultrasonic treatment of the aniline solutions in the presence of oxygen, hydrazobenzene is formed first, and is then readily oxidized by means of —OH or (OH_2) radicals, according to the equation:



and:



Reaction between the radicals is also possible, thus:



The black dye shot with green has proved to be a mixture of green dyes, the products containing less-oxidized aniline, and aniline black, not turning green when treated with hydrochloric acid.

The same quantities of the 3 per cent aniline solution were treated with ultrasonic waves during 1, 2, 3 and 4 min. respectively, at the temperature 17°–18° C. During the treatment, oxygen was let through the solution at the rate of about 35–40 bubbles per min.

In the course of the observations it was found that aqueous aniline solutions, during a short treatment (1–2 min.), formed mainly green products, probably emeraldine, nigraniline, and pernigraniline. After a longer treatment (3–4 min.) aniline black (not turning green) was mainly obtained as a polymolecular product containing numerous quinonoid bonds.

In the course of oxidation of aqueous aniline solutions it was found that an increase in the molecular pressure of oxygen did not lead to a rise in the oxidation effect.

Wiktor Wawrzyczek

General Chemistry Department,
Higher School of Agriculture,
Olsztyn, Poland.

[1] Lindström, O., and Lamm, O., *J. Phys. Colloid Chem.*, **55**, 1139 (1951).

[2] Grabar, P., and Prudhomme, R. O., *C.R. Acad. Sci., Paris*, **226**, 1821 (1948).

[3] Henglein, A., and Schulz, R., *Z. Naturforsch.*, **8b**, 227 (1953).

[4] Guéguen, H., *Bull. Soc. chim.* (France), **2**, 302 (1959); **7–8**, 1254 (1959).

[5] Rust, H. H., *Angew. Chem.*, **64**, 162 (1952); **65**, 249 (1953).

[6] Lindström, O., *J. Acoust. Soc. Amer.*, **27**, 654 (1955).

Electron Affinity of the Acceptor Molecule in Charge-Transfer Complexes

Whereas the influence of the structure of the donor molecule in charge-transfer complexes on the stability and spectroscopic behaviour of these complexes has been investigated extensively, the role of the electron acceptor has been much less studied. One reason for this emphasis is the lack of measurements of a parameter related to the electron affinity of the acceptor molecule, in contrast to the fairly readily available ionization potentials of the donors.

It has been shown for alternant aromatic hydrocarbons that the one-electron polarographic half-wave potentials measured under conditions such that the mono-negative ions are virtually unprotonated are directly related to the energy of the lowest unoccupied molecular orbitals and hence the electron affinities of these molecules[1]. In general this may be expected to hold for any π-system, and the half-wave potential will at least be a measure of the readiness of the system to accept an electron[2].

A series of measurements has been carried out on well-known acceptors using acetonitrile and dimethylformamide as solvents; the polarographic characteristics of hydrocarbons and quinones are well established under these conditions[2]. The results given in Table 1 show the potential of the first one-electron addition ; in each case it was confirmed by a.c. polarography and conventional means that the electron addition is sufficiently rapid not to require a measurable over-voltage and that no proton addition occurs. These compounds show a second one-electron step, some 0·8 V. more negative than the first; for simplicity this has not been included in Table 1. The figures in Table 1 are, in general, clearly in the expected order for complexing ability[3,4], the stronger complexing agents having the more positive potentials. The results for the two solvents are practically identical. The difference in complexing ability between chloranil and quinone is well brought out in contrast with a comparison of their conventional redox potentials (689 and 700 mV.). This method would thus appear to be at least a useful means of comparing acceptor-strengths. An indication that the series may provide more than a qualitative comparison of acceptor strengths is provided in Fig. 1 in which the change in free energy for complex formation with pyrene, determined spectroscopically[4], is plotted against the polarographic half-wave

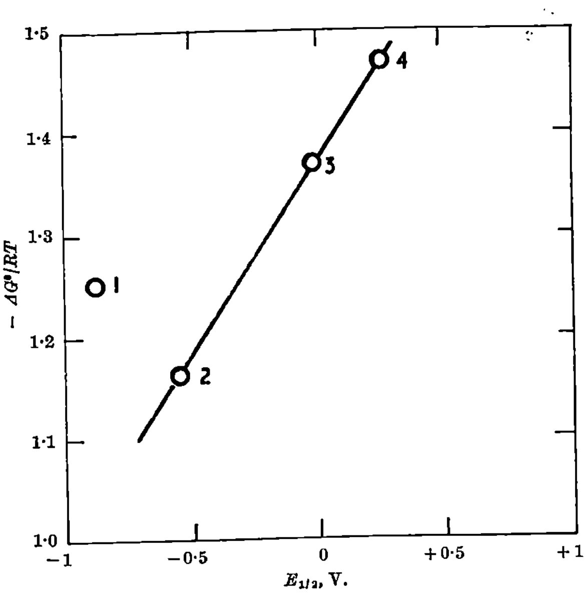


Fig. 1. Change in free energy of a series of complexes with pyrene (ref. 4) as a function of the half-wave potential of the acceptor. 1, Maleic anhydride; 2, *p*-benzoquinone; 3, chloranil; 4, tetracyanoethylene

potential of the acceptor. Other factors being equal, one would expect that the change in free energy would be directly related to the energy required to place an electron in the acceptor molecule. From Fig. 1 it would appear that the maleic anhydride complex has a greater stability than would be expected from the half-wave potential of this acceptor; pyromellitic dianhydride is also exceptional in this respect as it is claimed from spectroscopic measurements[5] that its complexing ability is close to that of tetracyanoethylene. An investigation into the reduction processes of anhydrides is in hand to see if further light can be thrown on this apparent anomaly.

The potentials of the second one-electron steps of the first three compounds in Table 1 lie at − 0·31, − 0·75 and − 0·80 V., in the region of the first steps for moderately strong acceptors. This suggests that it may be possible for a second electron to be shared with these molecules. This would apply more readily to compounds with even more positive half-wave potentials.

Table 1. HALF-WAVE POTENTIALS AT 25° OF THE FIRST ONE-ELECTRON ADDITION FOR SOME ORGANIC ACCEPTOR-MOLECULES. SUPPORTING ELECTROLYTE 0·1 *N* NEt_4ClO_4

Substance	Half-wave potential, *V*, versus S.C.E.*	
	Acetonitrile	Dimethylformamide
2 : 3 Dicyano 5 : 6 dichlorobenzoquinone	+0·51	—
Tetracyanoethylene	+0·25	+0·24
Chloranil	+0·02	+0·02
Picric acid	−0·26†	—
p-Benzoquinone	−0·52	−0·54
Pyromellitic dianhydride	−0·60	−0·60
p-Xyloquinone	−0·67	−0·68
9 : 10 Phenanthrenequinone	−0·66	−0·70
2-Methyl naphtho 1 : 4 quinone	−0·78§	—
Duroquinone	−0·84§	—
Maleic anhydride	—	−0·88‡
Anthraquinone	−0·94	−0·98
Phthalic anhydride	—	−1·36†

* Including liquid junction potential, acetonitrile–water.
† Some irreversibility, true $E\frac{1}{2}$ may be ± 0·05 V.
‡ Two-electron change.
§ From Wawzonek, S., Blaha, E. W., Berkey, R., and Runner, M. E., *J. Electrochem. Soc.*, 103, 456 (1956); potentials converted to S.C.E. (saturated calomel electrode).

Further work is planned on the quantitative aspects of this approach to the problem of donor–acceptor complexes. If a quantitative correlation were established, it would provide a basis for a greater understanding of the factors contributing to the stability of the complexes. It would also appear feasible to investigate donor–acceptor equilibria by polarographic means in a manner analogous to that for metal-ion complexes[6] in which factors such as contact charge-transfer would not interfere.

M. E. PEOVER

National Chemical Laboratory,
Department of Scientific and Industrial Research,
Teddington, Middlesex.

---

[1] Maccoll, A., *Nature*, **163**, 178 (1949). Pullman, A., Pullman, B., and Berthier, G., *Bull. Soc. Chim. France*, 591 (1950). Hoijtink, G. J., *Rec. Trav. Chim.*, **74**, 1525 (1955). Hoijtink, G. J., de Boer, E., van der Meij, P. H., and Weijland, W. P., *ibid.*, **75**, 487 (1956). Balk, P., de Bruyn, S., and Hoijtink, G. J., *ibid.*, **76**, 860 (1957). Given, P. H., *Nature*, **181**, 1001 (1958).

[2] Given, P. H., and Peover, M. E., *Advances in Polarography*, 948, edit. by Longmuir, I. S. (Pergamon Press, London, 1960).

[3] Andrews, L. J., *Chem. Revs.*, **54**, 713 (1954).

[4] Merrifield, R. E., and Phillips, W. D., *J. Amer. Chem. Soc.*, **80**, 2778 (1958).

[5] Ferstandig, L. L., Toland, W. G., and Heaton, C. D., *J. Amer. Chem. Soc.*, **83**, 1151 (1961).

[6] Irving, H., in ref. 2, p. 42.

Determination of Indium in High-purity Zinc

THE activation analysis of 5 parts in 10^{12} of indium in zinc has been carried out, using a method of separation investigated by Kosta and Hoste[1]. 1-gm. samples of zinc were irradiated in a neutron flux of 8×10^{11} n./cm.^{2} sec. in the *BR.I* reactor. After irradiation, the samples were dissolved in 10 ml. of 6 *N* hydrobromic acid with the addition of 3–4 drops of nitric acid (sp. grav. 1·40) in order to accelerate the dissolution. By addition of 5 ml. of 6 *N* hydrobromic acid to the solution, its concentration was increased to about 4 *N*. After two extractions with 5 ml. of isopropylether, the organic layer contained all the trivalent indium. Small amounts of zinc-65, which were present in the organic layer, were washed out with 10 ml. of 6 *N* hydrobromic acid.

The purified indium fraction contained the radioisotope indium-116*m* (*t*1/2 = 54 min.) and traces of zinc-65 (*t*1/2 = 240 days) and of zinc-69*m* (*t*1/2 = 14 hr.). Since discrimination of the gamma transitions of zinc-65 (E_γ = 1·114 MeV.) and of indium-116*m* (E_γ = 1·09 MeV.) is impossible by gamma-spectrometry, the activity of the former was determined by measurement of its 1·27-MeV. γ-ray. A single-channel gamma-spectrometer with a 3 in. × 3 in. sodium iodide (thallium) well-detector system was used for these measurements. The 438-keV. γ-ray from the isomeric transition of zinc-69 is used for measuring the purity of the indium fraction.

In order to avoid shadow effects of epithermal neutrons, the indium standards must be of very small size. Therefore one drop of an indium solution (0·2 mgm. indium/ml.) is weighed in a quartz tube and used as the standard. After irradiation and hundred-fold dilution, its level of activity is comparable to that of the indium in the samples. Results indicate the presence of 4·9–5·0 parts in 10^{12} of indium in three zinc samples of different purity in respect to other impurities.

NAÏM KOŠARIC

"Natron", Maglaj, Yugoslavia.

(The late) G. LELIAERT

Centre d'Étude de l'Énergie Nucléaire,
Mol-Donk, Belgium.

[1] Kosta, L., and Hoste, I., *Microchimica Acta*, 1-6, 790 (1956).

Reactivity of Calcium Sulphate Hemihydrate

CALCIUM sulphate hemihydrate reacts with water to yield calcium sulphate dihydrate. The rate of this reaction differs considerably from sample to sample, the variations in reactivity being due, at least in part, to conditions prevailing during the dehydration of the parent dihydrate. We have investigated the reactivity of calcium sulphate hemihydrate prepared by calcining various samples of finely powdered reagent grade calcium sulphate dihydrate (44 per cent −100 mesh and +200 mesh, 54 per cent −200 mesh) spread as a thin layer (120 gm. per 500 cm.^{2}) on a tray in an air oven. After calcination the material was exposed to the atmosphere at room temperature to allow absorption of moisture until the composition of hemihydrate was attained[1]. The reactivity of the product was assessed from the rate of increase in temperature of mixtures of 100 gm. of hemihydrate with 60 gm. of water, held in a Dewar flask[2]. The mixtures were made at 20° C. Two parameters of the reaction were studied, namely, the induction period defined as the time taken for the rate of increase in temperature to exceed 0·1 deg. C./min., and k the slope of the approximately straight part of the plot against time of $\log_{10}\Delta T/\Delta T_f$, where ΔT deg. C. is the increase in temperature and ΔT_f is the increase in temperature corresponding to complete hydration. The constant k is a measure of the self-acceleration of the reaction[1-3]. The induction period is inversely related to the reactivity.

With the samples of calcium sulphate dihydrate which we have used the induction period of the derived hemihydrate has initially increased rapidly with the length of calcination and has finally become independent of it. With one particular sample calcined at 143° C. the induction period showed a pronounced maximum at 20 hr. (Fig. 1). With calcination of this sample at 203° C. the peak occurred at 5 hr., and at 106° C. there was no maximum in the induction period (that is, minimum in reactivity). There were corresponding but less-pronounced variations in k with length of calcination (Fig. 2). Similar changes in reactivity seem to occur when some samples of gypsum plaster are recalcined[1]. The induction periods for long times of calcination at 106° C. and 203° C. were considerably less than for calcination at 143° C. The magnitude of the increase in temperature during hydration showed that all the samples of calcined gypsum consisted essentially of calcium sulphate hemihydrate.

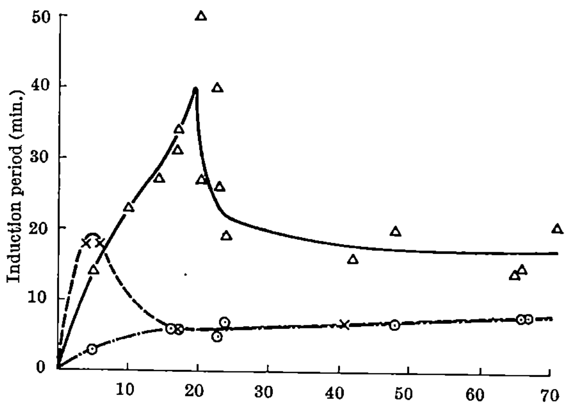


Fig. 1. Induction period plotted against period of calcination for samples prepared at 106° (⊙), 143° (△) and 206° C. (×)

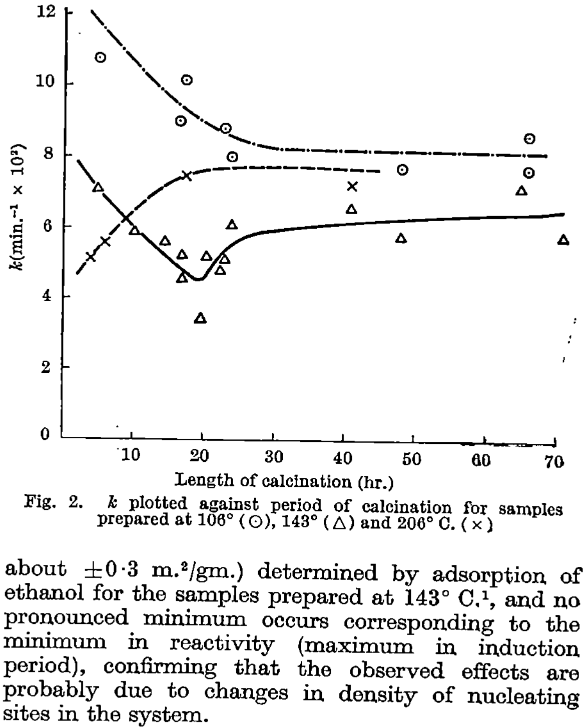


Fig. 2. k plotted against period of calcination for samples prepared at 106° (⊙), 143° (△) and 206° C. (×)

Variations in the reactivity of comparatively pure preparations of calcium sulphate hemihydrate, particularly when associated with sympathetic variations in k, are most likely to be due to differences in the density of seeding nuclei[1-3]. Large variations in surface area are reflected in the reactivity of calcium sulphate hemihydrate, particularly in the values of k[1]. However, Fig. 3 shows the surface area (error about ±0·3 m.^{2}/gm.) determined by adsorption of ethanol for the samples prepared at 143° C.[1], and no pronounced minimum occurs corresponding to the minimum in reactivity (maximum in induction period), confirming that the observed effects are probably due to changes in density of nucleating sites in the system.

The variations in reactivity described here can be explained by assuming the occurrence of two reactions that involve nuclei for the growth of calcium sulphate dihydrate. The first is the decomposition of the dihydrate lattice, and appears to be the only one occurring at 106° C. (Fig. 1). As calcination continues the product becomes less and less reactive as seeding particles of residual dihydrate disappear. As the temperature of calcination is raised this reaction becomes more rapid. However, there intervenes a

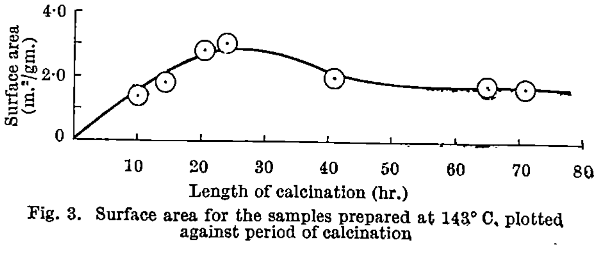


Fig. 3. Surface area for the samples prepared at 143° C. plotted against period of calcination

second reaction (presumably with a higher activation energy) that produces new nuclei so that at higher temperatures the reactivity first falls and then increases again. The nuclei produced by the second reaction are most unlikely to consist of fragments of dihydrate lattice, and the reaction probably involves some type of re-arrangement of the surfaces of the particles. The values of the surface area show no large variations, but the slight variations shown by the values for samples prepared at 143° C. may suggest a type of slow sintering after 20 hr. (Fig. 3). If the new sites favourable for nucleation of dihydrate do result from a re-arrangement of the surface of the grains of hemihydrate, they must be due to geometric features rather than to regions of strain.

The behaviour of some other samples where the peak is absent is due to the rates of these reactions, particularly the second, being so fast that the peak occurs so early that it is lost in the drop of reactivity caused by the first reaction. That an inhibitor which disappears at higher temperatures is not responsible for the effects described in this communication is indicated by the comparatively high reactivity of the samples prepared at 106° C.

This work forms part of a programme of research carried out within the Commonwealth Scientific and Industrial Research Organization and sponsored by the Colonial Sugar Refining Co., Ltd., and Australian Plaster Industries Pty., Ltd.

M. J. Ridge
H. Surkevicius
Angela A. Milne

Commonwealth Scientific and
Industrial Research Organization,
Division of Building Research,
Graham Road, Highett,
Victoria, Australia.

[1] Ridge, M. J., and Hill, R. D., *Austral. J. App. Sci.*, **11**, 180 (1960).
[2] Ridge, M. J., *Rev. Pure and App. Chem.*, **10**, 243 (1960).
[3] Ridge, M. J., *Austral. J. App. Sci.*, **10**, 218 (1959).

BIOCHEMISTRY

Prevention of Ammonia Toxicity by Amino-acids concerned in the Biosynthesis of Urea

Relationships have been pointed out by several investigators[1] between high levels of blood ammonia and various clinical syndromes, for example, some psychoses, mental disturbances associated with heart failure, tumours, jaundices, hepatic encephalopathies, etc. In some cases a reduction in the level of ammonia has resulted in considerable amelioration of these pathological symptoms; the various procedures for this purpose are of considerable interest[1].

In 1956, Greenstein and co-workers[2] emphasized the antitoxic action of L-arginine in experimental hyperammonæmia produced by intraperitoneal injections of ammonium salts or high amounts of certain amino-acids. This work was followed by clinical studies, and some investigators[3] obtained beneficial results after administration of L-arginine; in all these cases the authors observed a striking reduction of blood ammonia which paralleled the alleviation of toxic symptomatology.

Different procedures for the same purpose have been employed with as yet indecisive results[1,4]. While the action of glutamic acid might be due to removal of the toxic ammonia via glutamine synthesis, the effect of L-arginine may involve a mobilization or enhancement of the Krebs–Henseleit urea-synthesizing cycle, resulting in a decrease of the ammonia-level; this hypothesis is supported by the observation of appreciable increases in the levels of blood urea[2,3].

Table 1. Effect of Prior Administration of Amino-acids on Rats given LD_{50} Doses of Ammonium Acetate

Protective compounds	Dose (m.moles/kgm.)	No. of animals	Survivors (per cent)	Incidence of convulsion (per cent)
Phosphate buffer	—	25	48	100
L-Ornithine + L-Aspartic acid	0·5 0·5	20	95	20
L-Ornithine + L-Aspartic acid	1·0 1·0	25	100	0
L-Ornithine + L-Aspartic acid	1·5 1·5	20	100	0
L-Ornithine	3·0	20	100	0
L-Aspartic acid	3·0	19	58	85
L-Aspartic acid + L-Ornithine	2·5 0·5	31	90	20
L-Lysine	3·0	10	50	100
L-Isoleucine	3·0	17	65	88
L-Isoleucine + L-Ornithine	2·5 0·5	27	63	80
L-Ornithine	0·5	32	93	43

The animals were Long–Evans male rats weighing about 250 gm., starved for a period of 12 hr. with water *ad libitum*. Both ammonium acetate and amino-acids dissolved in phosphate buffer (final *p*H 7·4), were administered intraperitoneally; the amino-acids 1 hr. before ammonium salt. Control animals were treated only with phosphate buffer before the ammonium acetate.

We have tested other substances concerned in the synthesis of urea for a similar effect. For this, some amino-acids, mainly L-ornithine and L-aspartic acid, have been tested in chronic carbon tetrachloride poisoning in rats[5]; their protective efficiency was evaluated on the bases of increase in weight and of the total lipid and glycogen content of the livers; the efficiency reaches 96 per cent if an ornithine–aspartic acid mixture (2·5 m.moles/kgm.) is used. The present communication describes the protective effects of prior administration of this mixture against experimental hyperammonæmia produced by intraperitoneal injection of LD_{50} quantities of ammonium acetate (8·2 ± 0·8 m.moles/kgm.) into rats.

The first set of experiments established the protective effects of some amino-acids against the mortality of rats suffering from ammonia intoxication (Table 1). The mixture of L-ornithine and L-aspartic acid, after intraperitoneal administration, gives optimum protection at levels of 2·0 m.moles/kgm. body-weight; at levels of 1·0 m.mole/kgm., 95 per cent of the animals survived. It is noteworthy that aspartic acid alone (3·0 m.moles/kgm.) has almost no effect, whereas the addition of ornithine in rather low concentration (0·5 m.mole/kgm.) suffices to raise the survival-rate to 90 per cent. We have also tested other amino-acids which are not involved in the synthesis of hepatic urea. L-Lysine alone (3·0 m.moles/kgm.) is completely inefficient, whereas L-isoleucine shows a protective effect slightly greater than that shown by aspartic acid alone; the addition of L-ornithine does not produce any increase of efficiency.

Levels of ammonia and urea in the blood of these animals were determined after various protective treatments (Table 2). In protected rats the rise of blood ammonia was quickly checked and, in animals treated with the ornithine–aspartic acid mixture (1·5 m.moles/kgm. of each), the values fell rapidly to normal levels. Simultaneously, the levels of blood-urea showed a marked increase.

These results suggest that ornithine and aspartic acid exhibit a specific detoxifying action toward

Table 2. Ammonia- and Urea-Levels in Blood of Rats protected against LD_{50} Doses of Ammonium Acetate by the Prior Administration of Mixtures of L-Ornithine and L-Aspartic Acid

No. of animals	Ammonium acetate	Protective mixture	Dose (m.moles/kgm.)	Blood ammonia (μmoles/ml.)		Plasma urea (μmoles/ml.)	
				Time after injection of ammonium-acetate (min.)			
				15	60	15	60
50	—	Phosphate buffer		0·76 ± 0·11*	0·76 ± 0·11	5·98 ± 0·73	5·98 ± 0·73
30	—	L-Ornithine + L-Aspartic acid	1·5 1·5	0·96 ± 0·12	0·71 ± 0·11	7·11 ± 0·54	7·81 ± 0·69
50	LD_{50}	Phosphate buffer		6·28 ± 0·33	2·59 ± 0·52	7·45 ± 0·76	9·87 ± 0·76
40	LD_{50}	L-Ornithine + L-Aspartic acid	0·5 0·5	3·77 ± 0·71	1·72 ± 0·72	7·66 ± 0·53	10·42 ± 1·34
50	LD_{50}	L-Ornithine + L-Aspartic acid	1·0 1·0	2·19 ± 0·39	1·39 ± 0·35	9·83 ± 0·90	12·76 ± 1·89
40	LD_{50}	L-Ornithine + L-Aspartic acid	1·5 1·5	2·38 ± 0·34	0·64 ± 0·39	10·53 ± 0·68	14·71 ± 1·81

The experimental conditions were as given in Table 1. Blood samples were drawn by cardiac puncture at the times indicated; the time of 15 min. was selected to coincide with the death of 50 per cent of the intoxicated animals. The survivors of this group were examined along with the other animals after 1 hr. Ammonia was determined in whole blood by Seligson's method (ref. 6); urea was determined in plasma by Kitamura's method (ref. 7).

* Mean values ± standard deviations.

blood ammonia. Their effect may be attributed to an enhancement of the Krebs–Henseleit mechanism of synthesis of urea.

The results of a more detailed investigation in progress will be published elsewhere.

Francesco Salvatore*
Vincenzo Bocchini

Institute of Biological Chemistry,
Via Costantinopoli 16,
University of Naples.

* Present address: Argonne National Laboratory, Division of Biological and Medical Research, 9700 South Cass Avenue, Argonne, Illinois, U.S.A.

---

[1] Bessman, S. P., *Advan. Clin. Chem.*, **2**, 135 (1959).
[2] Greenstein, J. P., Winitz, M., Gullino, P., Birnbaum, S. M., and Otey, M. C., *Arch. Biochem. Biophys.*, **64**, 342 (1956).
[3] Najarian, J. S., and Harper, H. A., *Amer. J. Med.*, **21**, 832 (1956). Fahey, J. H., *J. Clin. Invest.*, **36**, 1647 (1957).
[4] Walshe, J., *Lancet*, i, 1075 (1953). MacDermott, W. V., Wareham, J., and Riddell, A. G., *New Eng. J. Med.*, **253**, 1093 (1955).
[5] Salvatore, F., Scoppa, P., and Cozzolino, D., *Clin. Chim. Acta*, **4**, 728 (1959); *Biochim. App.*, **6**, 434 (1959).
[6] Seligson, D., and Hirahara, H., *J. Lab. Clin. Med.*, **49**, 962 (1957).
[7] Kitamura, M., and Iuchi, I., *Clin. Chim. Acta*, **4**, 701 (1959).

Chemistry of a Mucopolysaccharide produced by Guinea Pig Lymphocytes

The guinea pig lymphocyte has long been known to contain a special inclusion body. Such lymphocytes, called Kurloff cells after their discoverer, increase greatly in number in pregnancy and were shown by Ledingham[1] to be produced in both males and females by administration of œstrogens. Marshall and Swettenham[2], by histochemical methods, have shown that the inclusion body is composed of a mucoprotein–sulphated mucopolysaccharide complex. Further work on the chemistry of a mucopolysaccharide fraction by extraction of spleens of œstrogen-treated guinea pigs gave the following results:

(1) No linked amino-acids are present in the polysaccharide fraction; (2) the electrophoretic mobility in phosphate buffer at *p*H 7 on cellulose acetate is the same as chondroitin sulphate; (3) the bulk of the amino-sugar is composed of galactosamine (Gardell's method[3]); (4) the molar ratio of hexosamine, uronic acid and sulphate is the same as chondroitin sulphate *A* or *C*, that is, if hexosamine = 1 (sulphate = 1·29, uronic acid = 1·43 and sodium = 3·43); (5) the material is susceptible to testicular hyaluronidase. Some of the technical methods used in these estimations will be described later in a separate publication.

These results confirm the conclusion of Marshall and Swettenham[2] that the inclusion body of the Kurloff cell contains a material similar to the ground-substance of cartilage or connective tissue. The formation of large amounts of such material in the pregnant animal, however, remains of unknown significance and attempts to trace the fate of this polysaccharide in the pregnant guinea pig by labelling with sulphur-35 were unsuccessful. Furthermore, it is uncertain whether the formation of such material is peculiar to guinea pig lymphocytes or whether it is only the formation of the inclusion body which is unique to this species.

Helen Muir

Medical Unit Laboratories,
St. Mary's Hospital, W.2.

A. H. E. Marshall

Bernhard Baron Institute,
The London Hospital, E.1.

---

[1] Ledingham, J. C. G., *J. Path. Bact.*, **50**, 201 (1940).
[2] Marshall, A. H. E., and Swettenham, K. V., *J. Anat.*, **93**, 348 (1959).
[3] Gardell, S., *Acta Chem. Scand.*, **7**, 207 (1953).

Influence of Plasma Proteins in the Control of Mitosis-rates in Generating Liver

Many experiments have been done to elucidate mechanisms of control over growth-rates of liver, and in a comprehensive review of the regenerating liver Harkness[1] suggests that variations in the levels of plasma proteins, as shown by Glinos[2] and Gey[3], offer the most likely cause of changes in mitosis-rates after partial hepatectomy. Lately new evidence in favour of the removal of a normally present inhibitor has been offered from mouse skin[4] and from rat and guinea pig liver[5].

It was shown that adrenal corticosteroids could be the inhibitor normally present[5,6], and I now suggest control of mitoses in at least two ways: First, by a change in concentration of the plasma corticosteroids as shown by several workers[6–8] and to which normal cells are sensitive. Secondly, by a possible alteration of redox potential around each cell which has the effect of preventing corticosteroid influence on mitosis[5]. This effect has been shown by withdrawal of ascorbic acid from guinea pigs, but similar conditions could result from the output of oxidizing substances from damaged cells, such as tumours, or in wound healing where the levels of circulating corticosteroid may be high yet mitosis-rates are not inhibited.

In the experiments of Glinos[2] alterations are made in the concentrations of plasma proteins believed to control mitosis; he demonstrates induction of mitosis in resting liver by dilution of plasma proteins and conversely a suppression of mitosis in regenerating liver by hæmo-concentration following water restriction.

Plasma proteins have been shown to carry corticosteroids[9,10], and it seems likely that the plasma protein influence is attributable, at least in part, to the associated corticosteroids; changes of protein-levels automatically altering the corticosteroid inhibition. To test this, Glinos's hæmo-concentration experiment has been repeated, but a comparison has been made between the degree of inhibition of liver regeneration in a dehydrated rat with intact adrenals and one with adrenals removed. If corticosteroids were the effective controllers of mitosis, adrenalectomy would allow a high rate of liver regeneration approximately double that found in rats with intact adrenals[5]. This has been demonstrated, even though plasma protein-levels have been maintained near those found in intact rats, which have no liver mitoses in a resting state.

Three groups of Wistar male rats weighing 200 gm. were compared. The first group of 60 rats were adrenalectomized and given food *ad lib.* but no water: 24 hr. later partial hepatectomy was done and the liver allowed to regenerate for 28 hr., when the rats were killed and a slice of the growing liver remnant taken for mitotic counting. The rats were bled by withdrawal of blood from the inferior vena cava and plasma protein estimated by the copper sulphate specific gravity drop method. Total time deprived of water was 52 hr.

The second group of 30 rats with intact adrenals were partially hepatectomized after deprivation of water for 24 hr. and were killed after 28-hr. liver regeneration, water being withheld also for a total of 52 hr. The third group of 10 rats with intact adrenals were given food and water *ad lib.* and partially hepatectomized. After 28 hr. liver regeneration the rats were killed and liver slices and blood collected for examination as in the preceding groups.

Mitotic counts of regenerated liver and plasma protein-levels are shown in Table 1 (mitoses per 1,000 cells).

Table 1. PLASMA PROTEIN AND MITOTIC COUNTS OF RAT LIVER REGROWING 28 HR. AFTER PARTIAL HEPATECTOMY

	Group 1	Group 2	Group 3
	Dehydrated adrenalectomized rats	Dehydrated intact adrenals (gm.)	Water allowed, adrenals intact (gm.)
Plasma protein (gm.) (per cent)	7·9 ± 1·3	9·1 ± 1·1	5·9 ± 0·8
Mitotic rate	62 ± 8	16 ± 5	38 ± 6

(± *S.D.*)

In group 1 the adrenalectomized dehydrated rats showed a higher liver regeneration-rate almost double that of the intact hepatectomized rats having full access to water which formed the third group. In the former case plasma protein concentration has been maintained but absence of corticosteroids has removed all inhibition on mitosis.

Groups of dehydrated rats with intact adrenals show an inhibition of mitosis in the regenerating liver as reported by Glinos. Here plasma protein-levels have been maintained and in addition it is likely that corticosteroid output is high due to the stress of water deprivation.

The third group shows the plasma protein and mitotic rates in partially hepatectomized rats not deprived of water. Plasma protein-levels of intact rats average 8–9 gm. per cent.

It appears, therefore, that plasma proteins are only partly responsible for mitosis regulation in liver, and that they form a vehicle for the carriage of corticosteroids which are the effective control. The reasoning of Glinos is not invalidated by this extension as the homeostatic mechanism of maintenance of plasma proteins carries with it a built-in control, the cortical hormones, which only achieve an inhibiting effect on liver growth when this organ is of a size sufficient to maintain full plasma protein-levels and thereby a proper concentration of corticosteroids.

J. T. HEMINGWAY*

Department of Radiotherapeutics,
Downing Street,
Cambridge.

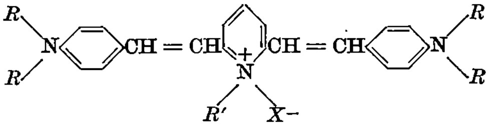
* Present address: Department of Physiology, Queen's College, Dundee.

[1] Harkness, R. D., *Brit. Med. Bull.*, **13**, 87 (1957).
[2] Glinos, A. D., *Science*, **123**, 673 (1956).
[3] Glinos, A. D., and Gey, G. O., *Proc. Soc. Exp. Biol. and Med.*, **80**, 421 (1952).
[4] Bullough, W. S., and Laurence, E. B., *Proc. Roy. Soc.*, B, **151**, 517 (1960).
[5] Hemingway, J. T., *Nature*, **185**, 106 (1960).
[6] Hemingway, J. T., and Cater, D. B., *Nature*, **181**, 1065 (1958).
[7] Roberts, K. B., Florey, H. W., and Joklik, W. K., *Quart. J. Exp. Physiol.*, **37**, 239 (1952).
[8] Horvath, E., and Kovacs, K., *Z. ges. exp. Med.*, **127**, 236 (1956).
[9] Sandberg, A. A., Slaunwhite, W. R., and Antoniades, H. N., *Rec. Prog. Horm. Res.*, P, 209 (1957).
[10] Petersen, R. E., *Ann. N.Y. Acad. Sci.*, **82**, 846 (1959).

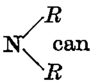
Pyridine Derivatives with Anthelmintic Activity

WE should like to report a new series of chemical compounds which are effective against certain nematode parasites of mammals. Some of these compounds show a broad anthelmintic spectrum, being active against representatives of a number of families of nematodes.

These compounds can be illustrated by the general structural formula (I):



(I)

in which R can be, for example, alkyl or benzyl, or  can consist of a cyclic ring, such as pyrrolidine, piperidine, morpholine, etc., and R' can be alkyl or benzyl and X^- can be a suitable anion, most commonly iodide. These and numerous other structural variations have been prepared and tested, and details of syntheses will be published shortly.

A number of members of this series were found quite active against natural infections of the mouse pinworm, *Syphacia obvelata*, but two of the series (A: (I) $R = CH_3$, $R' = CH_3$, $X = I$; (I) $R,R = (CH_2)_4$, $R' = C_2H_5$, $X = I$) gave superior and almost equal activity with single doses of as low as 5 mgm. of base per kgm. of body-weight. Although B was slightly better against *Syphacia*, it was less effective than A against various nematodes of dogs and cats.

Compound *A* was tested extensively against nematodes of dogs and cats, and, to a limited extent, against nematodes of monkeys, using single doses of 25 mgm./kgm. or less for most parasites or multiple doses of 25 mgm./kgm. for whipworm infections. It exhibited a broad anthelmintic spectrum, showing good to excellent activity against natural infections of *Ancylostoma caninum*, *Toxocara canis* and *T. cati* in dogs and cats; *Uncinaria stenocephala*, *Toxascaris leonina* and *Trichuris vulpis* in dogs; and *Trichuris trichiura*, *Oesophagostomum apiostomum* and *Physaloptera* sp. in monkeys.

Inasmuch as rats, dogs and cats have tolerated single doses as large as 500 mgm. per kgm. of body-weight without evidence of ill effects, there is a wide margin of safety between the effective and toxic doses and, in addition, the compounds are tasteless and appear to have negligible emetic activity.

A. P. Phillips
R. B. Burrows

Burroughs Wellcome and Co.,
United States, Inc.,
Wellcome Research Laboratories,
Tuckahoe, New York.

Comparison of the Serum Protein Fractions of the Newly Hatched Chick with those of Adult Birds using Starch-gel Electrophoresis

It has been established by several investigators that serum protein components of chick embryo undergo qualitative as well as quantitative changes throughout the developmental period until the time of hatching[1-4]. The serum protein composition of the newly hatched chick, however, resembles that of the adult bird, as revealed by moving-boundary and paper electrophoresis. The technique of starch-gel electrophoresis suggested by Smithies[5] is promising in that it enables one to detect numerous fractions in the pattern of the serum proteins, particularly in the α- and β-globulin regions, which cannot be detected by the conventional method of Tiselius or paper electrophoresis. By using the starch-gel method, I have been able to demonstrate differences in the patterns of serum proteins of the day-old and week-old chicks when compared with pooled sera of one- and two-year-old roosters, as shown in Fig. 1. The electrophoresis was performed on a vertical tray[6] for 18 hr. using borate buffer of *p*H 8·6 and a field-strength of 6 V./cm. Repeated electrophoretic runs gave patterns similar to those shown in Fig. 1, for the four age groups. Twelve fractions could be distinguished on the patterns of the adult roosters (two pre-albumin components are not seen on the photographs). The same number of fractions could also be seen on the electrophoretic patterns of the young chicks. The mobility and the number of fractions of α-globulins of the newly hatched chicks are distinctly different from those of adult birds. The fraction *b* of the serum of a day-old chick does not seem to be present in the serum of an adult bird, and of fraction *d* only traces are observed. Very little γ-globulin is present in day-old and week-old chicks. A distinct narrow band which is immediately behind the albumin (a post-albumin ?) in the adult bird cannot be seen in the young chicks.

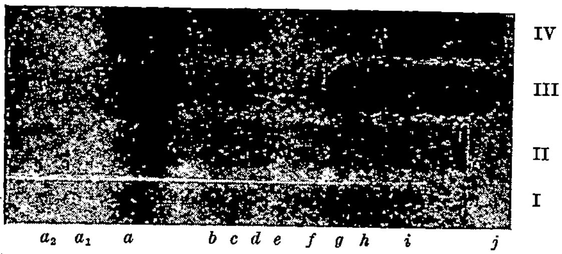


Fig. 1. Starch-gel electrophoresis of the sera of chicken at various ages. I, Day-old chick; II, week-old chick; III, one-year-old rooster; IV, two-year-old rooster

A. Amin

Razi Institute,
Hessarak,
Karaj, Iran.

[1] Moore, D. H., Shen, S. H., and Alexander, C. S., *Proc. Soc. Exp. Biol. Med.*, 58, 307 (1945).

[2] Marshall, M. E., and Deutch, H. F., *J. Biol. Chem.*, 185, 155 (1950).

[3] Hradec, J., and Lemez, L., *Ceskoslov. Morfol.*, 2, 260 (1954).

[4] Vanstone, W. E., Maw, W. A., and Common, R. H., *Canad. J. Biochem. Physiol.*, 33, 891 (1955).

[5] Smithies, O., *Biochem. J.*, 61, 629 (1955).

[6] Smithies, O., *Biochem. J.*, 71, 385 (1959).

Cholesterol in the Tapetum Lucidum of the Eye of the Opossum, *Didelphis virginiana*

The eye of the opossum *Didelphis virginiana* has a tapetum formed from the retinal pigment epithelium[1,2]. Investigation of the eyes of three specimens of *Didelphis virginiana* and of one specimen of the crab-eating opossum obtained through the kindness of Dr. S. Halbert, College of Physicians and Surgeons, New York, and of Dr. Osman Hill, Zoological Gardens, London, has shown that the tapetum of these animals is rich in cholesterol, cholesterol esters and lipids and it is tentatively suggested that these form the reflecting material *in vivo*.

The tapetum of the opossum, viewed ophthalmoscopically immediately after death, appeared as a triangular dead-white area. Dissection of the tapetal area was unsatisfactory, both in unfixed and in eyes fixed with formol, as it was difficult to separate it from overlying retina and underlying choroid. The roughly separated tapetum was dried, extracted with chloroform–methanol 1 : 1 (v/v), and both extract and residue were examined. Microscopical examination of the residual material showed that birefringence of the tapetum had been removed. The chloroform–methanol extract was dried and the residue extracted with acetone. The acetone-soluble fraction consisted largely of cholesterol as judged by estimation[3]. Paper partition chromatography[4], using 95 per cent methanol as solvent, showed that cholesterol esters and/or fat were present as well as free cholesterol. In this system cholesterol esters and fat remain at the origin and cholesterol has R_F 0·50. Markers of cholesterol, cholesterol oleate and cholesterol stearate were run in parallel. To determine whether the material in the tapetum which remained at the origin on the chromatogram contained cholesterol esters or was entirely fat, an aliquot was saponified and re-chromatographed alongside the original material. The spot at the origin disappeared on saponification and the spot corresponding to cholesterol became more intense and larger, which suggests that cholesterol esters are present.

One eye was examined histologically. After fixation with formol, the eye was opened and the lens, which is large and spherical, was removed. The back part of the eye was then embedded in gelatin and frozen-sections were cut and floated on gelatin-coated slides. When the Lieberman–Burchardt reagent was dropped on the sections, a strong positive cholesterol reaction developed in the area of the tapetum, and a weaker one was given by the optic nerve. Retina and choroid were negative. Staining with sudan III showed that the tapetal area was also rich in lipid. A few lipid globules were present in the choroid and orbital tissues.

These experiments showed that the opossum tapetum contained cholesterol, cholesterol esters and lipid, and that when these were extracted the birefringence of the original material was lost. An attempt was therefore made to estimate the total cholesterol of the different parts, dissected from a single eye of *Didelphis virgiana* (Table 1).

Table 1. CHOLESTEROL CONTENT OF DIFFERENT PARTS OF THE EYE OF *Didelphis virginiana*

Part of eye	Dry weight (mgm.)	Total cholesterol (per cent dry weight)
Sclera	84	0·4
Choroid	7·3	5·8
Retina	2·8	18·2
Tapetum	1·0	50·0

Table 1 shows that the dissected tapetum is richer in cholesterol than the other parts. Chromatography of this material before and after saponification showed that the cholesterol was largely esterified. It is probable that most of the cholesterol found in the dissected retina and choroid comes from contaminating tapetal tissue. Cattle retina contains only 2·0 per cent cholesterol and cattle choroid 0·80 per cent[5].

Roughly dissected tapetum from the unfixed eye was birefringent but crystals were not clearly detected. Crystals (Fig. 1) could be brushed off the tapetum of the eye fixed with formol and similar crystals were deposited from the chloroform–methanol and subsequent acetone extract of tapetal material. The appearance of the tapetum seen ophthalmoscopically or in the opened eye is chalk-white, without the brilliance which one sees from the crystals of guanine in the argentea and tapetum of fish. There is no iridescence.

Different animals solve the optical problem of providing a mirror backing to the retina in widely different chemical ways. The crystalline tapetum in fish and reptiles is made of guanine[6], in carnivores of zinc-cysteine[7,8], in lemurs of riboflavin[9], and in the opossum of cholesterol esters and lipid. In fish, guanine is also laid down in the scales, and in lemurs Dr. Osman Hill (personal communication) has detected large amounts of a yellow water-soluble pigment in the skin, which is probably riboflavin. Whether zinc-cysteine occurs in other tissues of carnivores and whether there is particular concentration of cholesterol and lipid in other organs in the opossum are not known.

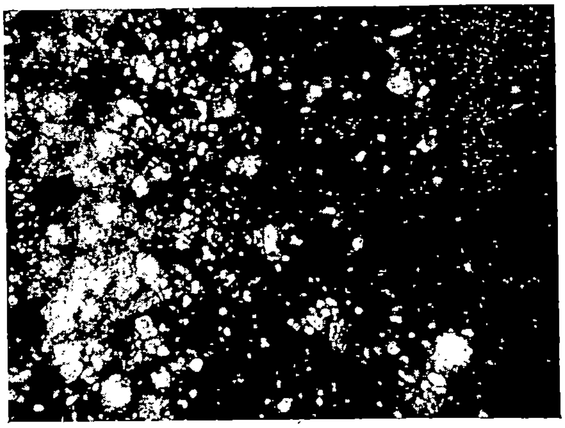


Fig. 1. Crystals brushed off tapetum of *Didelphis virginiana*

ANTOINETTE PIRIE

Nuffield Laboratory of Ophthalmology,
University of Oxford.

[1] Johnson, G. L., *Phil. Trans. Roy. Soc.*, **194**, 35 (1901).

[2] Walls, G. L., *J. Morph.*, **64**, 67 (1939).

[3] Colowick, S. P., and Kaplan, N. O., *Methods in Enzymology*, 3, 392 (Academic Press, New York, 1957).

[4] Kodicek, E., and Ashby, D. R., *Biochem. J.*, **57**, xii (1954).

[5] *Tabulæ Biologicæ* XXII, 2 (Oculus) (Vitgeverij Dr. W. Junk, Den Haag, 1951).

[6] Walls, G. L., *The Vertebrate Eye*, Cranbrook Inst. of Sci., Bull. 19 (1942).

[7] Weitzel, G., Buddecke, E., Fretzdorff, A. M.. Strecker, F. J., and Roester, U., *Hoppe-Seyl. Z.*, **299**, 193 (1955).

[8] Bernstein, M. H., and Pease, D. C., *J. Biophys. Biochem. Cytol.*, **5**, 35 (1959).

[9] Pirie, A., *Nature*, **183**, 985 (1959).

PHYSIOLOGY

Localization of the 'Sugar Pump' in the Intestinal Epithelium

GLUCOSE is actively transported across the intestinal epithelium from the lumen of the gut into the blood *in vivo* or into the fluid surrounding the serosal mesothelium *in vitro*. Because part of the glucose is metabolized during transport, it is not possible to determine the concentration of the unaltered sugar in the various layers of the intestine. However, 3-O-methylglucose, though transported by the same mechanism which acts on glucose[1], is not metabolized in animal tissue[2,3]. The synthesis of 3-O-$^{14}CH_3$-glucose in this laboratory[3] offered a method of following with reasonable accuracy the route of this sugar during transport.

Using surviving preparations of the small intestine of frogs (*Rana pipiens*), the following experimental procedure was employed[4]. 3-O-methylglucose (mixed with 3-O-$^{14}CH_3$-glucose) was dissolved in a Ringer's solution isosmotic with frog's blood and containing sulphate instead of chloride in order to minimize net water transport. The frogs were pithed, the abdomen was opened and the small intestine was ligated at both ends and injected with 1 ml. of the 3-methylglucose solution. The intestine was then excised and transferred to a flask containing 2 ml. of the solution identical with that which was injected into the lumen of the gut. The preparation was briefly gassed with a mixture or 95 per cent oxygen plus 5 per cent carbon dioxide and shaken in a 30° water-bath for 6 hr. Then the intestine was removed, carefully blotted on the outside and emptied of its contents. Thereafter, the intestine was split open, the mucosal surface gently blotted with a piece of cotton and the mucosa scraped off, using a coverglass. The mucosal scrapings were weighed, placed in a test-tube containing a known volume of hot water and refluxed in a hot water-bath for 10 min. The remaining portion of the intestinal tissue ('submucosal tissue') was similarly weighed and extracted with boiling distilled water.

Thus, at the end of the experiment, there were four different solutions : the contents of the lumen

Table 1. DISTRIBUTION OF 3-O-$^{14}CH_3$-GLUCOSE IN THE SMALL INTESTINE OF THE FROG

Exp. No.	Initial 3-methylglucose		Distribution of radioactivity at 6 hr. 10^3 counts/min./ml.			
	m.mol./litre	10^3 counts/min./ml.	Inside	Mucosal	Sub-mucosal	Outside
1	10	20·00	3·96	19·41	21·52	24·29
2	10	20·00	7·68	19·41	24·14	22·08
3	10	20·00	6·14	21·48	23·00	24·96
		Average	5·79	20·10	22·89	23·78
4	2·5	20·00	6·63	17·10	22·47	21·54
5	2·5	20·00	9·93	14·95	18·22	17·03
6	2·5	20·00	4·85	18·19	19·39	16·98
		Average	7·14	16·75	20·03	18·52

('inside fluid'), the extract of the mucosal scraping, the extract of the submucosal tissue, and the 'outside fluid' bathing the serosal mesothelium. From each solution samples were plated on to planchets and the radioactivity was counted in a windowless flow counter. The results were corrected for self-absorption. The water content of the tissue samples was calculated on the basis of an average value obtained in a series of determinations on both mucosal scrapings and submucosal tissues. The activity was expressed as counts/min./ml. of tissue water and of inside and outside fluids.

The results are shown in Table 1. The two series of experiments differ only in the initial concentration of 3-methylglucose; the amount of the tracer was the same in both series. As the principal anion was sulphate, which in turn kept the net movement of water across the intestine to a minimum, the change in concentration of the tracer in any compartment reflects the actual movement of 3-methylglucose regardless of its original concentration. It is evident from the results in Table 1 that during the experiment the concentration of 3-methylglucose decreased considerably in the lumen of the intestine, and that the concentration of 3-methylglucose in the water of the mucosal epithelial cells increased to a level two to three times higher than that in the lumen of the gut. The differences in concentration of 3-methylglucose between the other layers of the intestine and the serosal compartment are not so striking, and it is questionable if they were of any significance.

These results would suggest the following hypothesis: membrane facing the lumen of the intestinal epithelium is relatively impermeable to the sugar, and it is the site of the active transporting 'pump'. This would allow the sugar to be transported into the cell against a gradient and at the same time prevent a retrograde movement out of the cell into the lumen. The serosal-facing membrane of the epithelial cell is relatively permeable to sugar and so are the other tissues lying between the epithelial cells and the free serosal compartment. Thus, no appreciable concentration changes occur across these barriers.

These experiments do not indicate directly whether or not 3-methylglucose is present in the mucosal epithelial cells in the free or bound form. The concentration of 3-methylglucose is almost identical in the epithelial layer and in the subepithelial tissue as well as in the serosal compartment. If part of the sugar were bound in the mucosal cells, one would have to assume that a second concentration gradient of free sugar across the serosal-facing membrane occurred.

Attempts to demonstrate the presence of protein-bound 3-methylglucose in the extracts of intestinal mucosal epithelium were not successful. In these experiments the mucosal scrapings of 3-O-$^{14}CH_3$-glucose-absorbing guts were extracted with either cold saline or cold 0·6 *M* potassium chloride. Both extracts were centrifuged at 100,000*g* for 8 hr. More than 90 per cent of the total nitrogen of the extract was in the sediment, but the radioactivity in the supernatant did not change.

This work was supported by a research grant from the U.S. Public Health Service.

T. Z. CSÁKY
G. W. FERNALD

Department of Pharmacology,
University of North Carolina,
School of Medicine,
Chapel Hill, N.C.

[1] Csáky, T. Z., *Supp. Int. Abstracts Biol. Sci.*, 6 (1958).
[2] Csáky, T. Z., and Glenn, J. E., *Amer. J. Biol.*, **188**, 159 (1957).
[3] Csáky, T. Z., and Wilson, J. E., *Biochim. Biophys. Acta*, **22**, 185 (1956).
[4] Csáky, T. Z., and Fernald, G. W., *Amer. J. Physiol.*, **198**, 445 (1960).

Increase in Elastin Content of the Human Aorta during Growth

It is generally assumed that the elastic properties of the gross arteries, such as the aorta, are essentially due to elastic fibres of the arterial wall. However, there are few reports of quantitative studies of elastin in aorta; and often statistical proof of significance is omitted and conflicting statements have resulted. In particular, more detailed investigations on the elastin behaviour of the human aorta during growth are required.

Smith *et al.*[1] referred to increases in elastic tissues of the media with ageing, whereas no variation of the elastin content was found by Hass[2] or by Lansing *et al.*[3]. In agreement with the considerable decrease of elasticity of the aorta observed in ageing, Myers and Lang[4], Faber and Møller-Hou[5] and Bertolin[6] reported elastin as being highest in early life and gradually declining in the last decades of life. Kraemer and Miller[7], however, the only authors who reported statistical analysis of their own results, demonstrated an absence of changes with age in the percentage of aortic elastin.

All methods hitherto used in the determination of elastin consist basically of destroying materials other than elastin and gravimetric determination of the remaining insoluble substance. The availability of a new and specific method that permits the direct colorimetric estimation of elastin without destructive processes led me to review the behaviour of elastin in human aorta during growth and ageing.

This preliminary communication discusses the results of work on 16 normal young subjects up to 20 years old.

The direct method previously described by me[8], later further improved[9], has been used. The sample of tissue is treated with orcein. All free stain is removed and the stained orcein-elastin compound hydrolysed with elastase (pancreatin). Orcein-elastin, which is brought in this way into solution, is then estimated photometrically.

Fresh specimens were removed at the time of autopsy from the aortæ of apparently normal male subjects between 0 and 20 years of age who died from trauma. Specimens free from atherosclerotic lesions were always taken from the inferior part of thoracic aorta, stripped of adventitia and treated

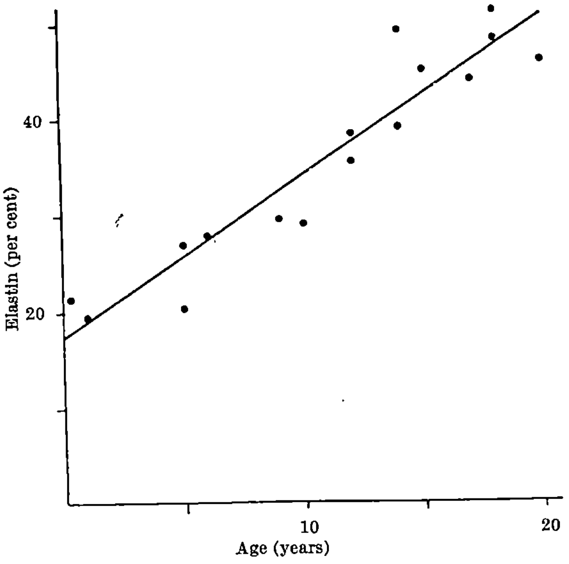


Fig. 1

as already described by me. 30 mgm. of the dry triturated matter were taken for analysis.

In Fig. 1 the results of analyses are expressed as percentage of elastin in the dry weight of the sample and plotted against age at time of death. Statistical analysis of this graph shows a highly significant increase in elastin content of the human aorta with age between 0 and 20 years (correlation coefficient: $r = 0{\cdot}877$; Student's $t = 6{\cdot}81$; $P < 0{\cdot}001$). The equation of the regression line is $y = 17{\cdot}5 + 1{\cdot}65\,x$.

Further investigations in progress in this laboratory seem, on the other hand, to demonstrate a significant decrease of elastin content in the aorta after 20 years until old age.

V. Scarselli

Instituto Biochimico Italiano,
Via Brembo 65,
Milano.

[1] Smith, C., Seitner, M. M., and Wang, H. P., *Anat. Rec.*, 109, 113 (1951).
[2] Hass, G. M., *Arch. Path.*, 34, 971 (1942).
[3] Lansing, A. I., Alex, M., and Rosenthal, T. B., *J. Gerontol.*, 5, 112 (1950).
[4] Myers, V. C., and Lang, W. W., *J. Gerontol.*, 1, 441 (1946).
[5] Faber, M., and Møller-Hou, G., *Acta Path. Microbiol. Scand.*, 31, 377 (1952).
[6] Bertolin, A., *Chir. Pat. Sper.*, 6, 240 (1958).
[7] Kraemer, D. M., and Miller, H., *Arch. Path.*, 55, 70 (1953).
[8] Scarselli, V., *Giornale di Biochimica*, 7, 20 (1958).
[9] Scarselli, V., *Nature*, 184, 1563 (1959).

Lack of Effect of Coenzyme Q_{10} on Coumarin-induced Hypoprothrombinæmia

Several coumarin and indanedione drugs are capable of inducing hypoprothrombinæmia, presumably by interfering with the cofactor role of vitamin K in prothrombin synthesis. This role of vitamin K, probably involving electron transport, can be competitively inhibited by coumarins, and the inhibition competitively overcome by large doses of vitamin K (ref. 1).

Recently[2] another group of quinones, the coenzymes Q, has been shown to be involved in electron transport. Numerous similarities of coenzymes Q to the vitamin K group[3] have led to the following comparison of vitamin K_1 and coenzyme Q_{10} as regards inhibition of the prothrombin response to a coumarin anticoagulant.

Table 1. Effect of Coenzyme Q_{10} on Prothrombin Response to Acenocoumarin in Normal Guinea Pig*

	Prothrombin time (sec.)	
	Range	Average
Q_{10} vehicle (1 or 2 c.c.)	31–34	33
Q_{10} (10 or 20 mgm.)	31–36	34
Acenocoumarin (1 dose) 4·5 mgm. in 1 c.c. H_2O	49–62	55
Acenocoumarin (1 dose) + Q_{10} 10 mgm.	53–130	79
Acenocoumarin (2 doses 24 hr. apart) each 4·5 mgm.	99–> 180	> 149
Acenocoumarin (2 doses 24 hr. apart) + Q_{10} 10 mgm.	78–177	134
Acenocoumarin (2 doses 24 hr. apart) + vitamin K_1 ('Mephyton') 10 mgm.	35–50	43

* All drugs were given intraperitoneally. Each group consists of at least four animals.

Guinea pigs were treated with sodium acenocoumarin, vitamin K_1, coenzyme Q_{10} (10 mgm./c.c.) in solvent vehicle (consisting of 'Emulphor' and dimethylacetamide, and the co-enzyme Q_{10} kindly provided by Dr. Karl Folkers of Merck, Sharp and Dohme, Rahway, New Jersey) and solvent vehicle as indicated in Table 1. Blood specimens were obtained 24 hr. after the last dosage, and the prothrombin time estimated by techniques previously reported[4]. The relatively long normal prothrombin time by the method employed is characteristic of the species. The results in Table 1 demonstrate that coenzyme Q_{10} has no vitamin K-like protective effect against acenocoumarin-induced hypoprothrombinæmia under the experimental conditions used.

Table 2. Effect of Coenzyme Q_{10} on Response to Acenocoumarin in Acutely Starved Guinea Pigs

	Prothrombin time (sec.)	
	Range	Average
Acenocoumarin 4·5 mgm. + 1 c.c. saline	40–55	48
Acenocoumarin 4·5 mgm. + coenzyme Q_{10} 10 mgm.	60–75	68
Acenocoumarin 4·5 mgm. + 1 c.c. vehicle	50–87	63

To test this observation further, the experiment was repeated in 12 animals deprived of food the day prior to and after dosage. The results are presented in Table 2. It is evident that coenzyme Q_{10} had no protective effect.

It is concluded that in the guinea pig, exogenous coenzyme Q_{10} has no vitamin K-like antagonistic action against the hypoprothrombinæmia induced by acenocoumarin.

This work was aided by grants *H*–4347 and *A*–4724 of the U.S. National Institutes of Health.

Murray Weiner
Theodore Chenkin
Peter G. Dayton

New York University Research Service,
Goldwater Memorial Hospital,
Welfare Island, New York.

[1] Link, K. P., *Harvey Lectures*, 39, 162 (1944).
[2] Green, D. M., and Lester, R. L., *Fed. Proc.*, 18, 987 (1959).
[3] Sastry, P. S., Jayaraman, J., and Ramasarma, T., *Nature*, 189, 577 (1961).
[4] Chenkin, T., Dayton, P. G., Weisberg, L. G., and Weiner, M., *Exp. Med. and Surg.*, 17, 219 (1960).

Pinnal Anaphylaxis: an Additional Anaphylactic Site

In some respects the mouse is a desirable experimental animal for immunological studies. Because of its small size and high reproductivity it can be used in statistically meaningful numbers. Availability of highly inbred strains minimizes variation in individual response and makes it possible to select strains of maximal sensitivity[1].

In other respects the mouse has certain drawbacks. One is its relative refractoriness to anaphylactic challenge. Another is the limited body-area available for local anaphylaxis.

Ovary[2] demonstrated the possibility of producing passive cutaneous anaphylaxis in the skin of the back of the mouse. He found that about 100 times as much rabbit antibody is necessary to induce local sensitization of the mouse skin as compared with the guinea pig. With high concentrations of reagents he was effectively limited to two injection sites, one on each side. His intravenous challenging dose of antigen was 1·2-mgm. hen ovalbumin.

The present investigation was undertaken with the objective of increasing the number of available sites for local anaphylactic challenge in the mouse and, perhaps, introducing more sensitive sites than the skin of the back. The ear was selected for several reasons. It is a translucent structure, in which collection of dye released by local anaphylactic reaction would be readily apparent. Though preferential inducement of capillary permeability in the ear, as compared with the skin of the back, is less marked in the mouse than in the rat and guinea pig, Feldberg and Miles[3] did find a distinctly greater response to 48/80 over the head, shoulders and limbs than elsewhere on the mouse skin. Finally, as a paired structure, one ear can, if desired, serve as a control to the reaction in the other. This initial communication reports primarily on the production of passive anaphylaxis in the ear of the mouse, using a partially purified duck ovalbumin and the rabbit antiserum to it.

The skin over the ear is very fine, and it is impracticable to carry out intradermal injection. Therefore, it was decided to instil the sensitizing serum subcutaneously. The mouse was lightly anæsthetized with ether. Using 26 gauge × ½-in. needles, a tenfold dilution of the antiserum was injected between the skin and cartilage on the outer aspect of one ear, while saline was injected similarly in the other. Volume was controlled by sight, a small bleb being raised. This approximates to 0·025 ml. of fluid per site.

At intervals varying from 30 min. to 24 hr. after sensitization the mice were injected intravenously in a tail vein with 0·25 ml. of a solution containing 1·25 mgm. of 'Pontamine' sky blue and 0·1 mgm. of the duck ovalbumin. Control mice were challenged with dye alone. A positive reaction, as exhibited by the local extravasation of blue dye in the ear, generally appeared within 10 min. and was complete after 30 min. The saline-injected control ears in antigen-challenged mice were uncoloured (apart from an occasional pin-prick of blue at the point of passage of the needle through the skin), as were both ears of the mice challenged with dye alone. Fig. 1 shows the complete results of a single experiment: no selection or elimination has been made. Positive reactions have been obtained when antigen challenge has been carried out as early as 30 min. and as long as 24 hr. after passive sensitization of the ear, 2–3 hr. being the optimal time for production of uniformly strong reactions.

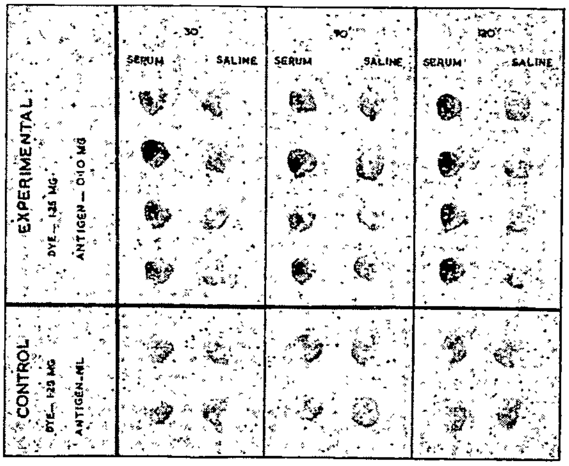


Fig. 1. Passive pinnal anaphylaxis in the mouse. Ears seen from inner aspect

The term pinnal anaphylaxis seems most appropriate to the phenomenon as the entire thickness of the pinna appears to be permeated with the dye: the skin on both the inner and outer aspects of the ear, the subcutaneous spaces and the cartilage.

Passive pinnal anaphylaxis has also been produced in intravenously blued mice on subcutaneous challenge with as little as 0·5 mgm. of duck ovalbumin. It has also been demonstrated in the guinea pig both on intravenous and subcutaneous challenge with duck ovalbumin and grass pollen extracts, using appropriate antisera. Quantitative studies, active pinnal anaphylaxis and other extensions and applications of the phenomenon are under investigation.

I wish to thank Miss Janet Dewdney for technical assistance, and Douglas F. Lawson for photographing the results.

J. G. Feinberg

Bencard Allergy Research Unit,
Beecham Research Laboratories, Ltd.,
Betchworth, Surrey.

[1] Rothberg, R. M., and Talmage, D. W., *Fed. Proc.*, 19, 215 (1960).
[2] Ovary, Z., *J. Immunol.*, 81, 355 (1958).
[3] Feldberg, W., and Miles, A. A., *J. Physiol.*, 120, 205 (1953).

Cytochrome *c* in the Heart of the Turtle

In a thesis on the anaerobic metabolism and mechanical efficiency of the heart of the turtle, by R. Blake Reeves[1], there is evidence that this heart is able to work efficiently for any length of time without access to oxygen (nitrogen anoxia). In view of this remarkable quality we found it interesting to investigate the content of cytochrome *c* in the heart of the turtle (*Testudo graeca*). To our knowledge, no such data are available in the literature[2–4], although reports on the cytochrome *c* content of the heart of the frog have been published by Keilin[5].

Six turtles were killed by ether and the hearts were weighed (3·5 gm.). The cytochrome *c* was extracted according to a method described by one of us (G. B.[4]); instead of 'Amberlite *XE*-64', however, 'Duolite *CS*-101' was used in the present investigation.

On analysis of the final eluate (reduced state) there was a typical cytochrome *c* spectrum with absorption maxima at 417, 521 and 550 mμ. The ratio $\frac{550\text{ m}\mu\text{ red.}}{280\text{ m}\mu\text{ ox.}} = 0{\cdot}3$ was very low compared with those of previous investigations[5]. Using the value of $E^{1\text{ cm.}}_{1\%}$ 550 mμ = 22·7 for crystalline bovine cytochrome *c*[6], the concentration of cytochrome *c* in turtle heart muscle was 2·7 mgm./100 gm. wet weight, which is about the same content as that of heart muscle of the frog[3].

We have found it of some interest to report this observation in connexion with the concept of the electron transport particle advanced by Green[7]. Further investigations on respiratory enzymes in poikilotherms are in progress.

Gunnar Biörck
Department of Medicine at Serafimerlasarettet,
Sven Paléus
Medical Nobel Institute,
Karolinska Institutet,
Stockholm.

[1] Reeves, R. B., thesis, Harvard University, Cambridge, Mass, 1959.
[2] Barcroft, J., *The Respiratory Function of the Blood*, Pt. 2 (Camb. Univ. Press, 1928).
[3] Lemberg, R., and Legge, J. W., *Hematin Compounds and Bile Pigments*, 377 (Interscience Pub. Inc., New York, 1949).
[4] Biörck, G., *Acta Med. Scand.*, 154, 305 (1956).
[5] Keilin, D., *Proc. Roy. Soc.*, B, 98, 312 (1925).
[6] Paléus, S., *Acta Chem. Scand.*, 14, 1743 (1960).
[7] Green, D. E., *The Harvey Lectures*, 52, 177 (1956–1957).

RADIOBIOLOGY

Failure of a Dog to discriminate between Strontium-90 and Calcium given Orally

The ability of a mammal to discriminate against strontium-90 has been the subject of intensive study in recent years. A recent summary of the results of several experiments using a variety of species and experimental approaches indicated that bone discrimination against strontium-90 in favour of calcium can vary from 2 to 4 (ref. 1). This means that the ratio of strontium-90 to calcium in bone as compared with the diet, referred to as the observed ratio, ranges from about 0·25 to 0·50 (ref. 2). These observations have led to the general belief that the mammal always discriminates against strontium-90 deposition to some significant extent.

There are a few reports of a lack of discrimination against orally administered strontium-90. Comar observed a ratio of 1·0 in cattle given a single oral dose of strontium-90 or calcium-45 (ref. 3). No work has been reported in which large animals received strontium-90 orally for long periods of time. However, rats were fed throughout their lifetime on a constant strontium-90/calcium diet and exhibited a ratio of 0·28 (ref. 4).

This investigation is concerned with beagles which were fed a constant diet of strontium-90 (1·8 μc. strontium-90/gm. dietary calcium) from the beginning of fœtal ossification (20–30 days after mating) until the pups reached early adulthood (540 days of age). It had previously been noted that the observed ratio between the fœtus and the dam's diet was 0·28, but that the growing pup, when maintained on the same diet, appeared to exhibit a ratio of about 1·0 (ref. 5). One of these pups was maintained on this same diet, first, through the dam during both the last half of gestation and the period of lactation, and secondly, from weaning until 18 months of age, at which time it was killed. The diet fed the dam and her litter was composed of 60 per cent dry kibbled ration to which were added 20 per cent beef and 20 per cent (by weight) water, including the strontium-90 dose. Dietary calcium was essentially all in the dry ration, which, by the classic permanganate method, was found to contain 3·3 per cent calcium by weight. Strontium-90 was mixed into the complete ration so that there were 1·8 μc. strontium-90 per 1·0 gm. calcium at the date of killing. The diet was fed *ad libitum* throughout the experiment to ensure a physiological approach.

The beagle grew normally and was in excellent condition throughout the experiment. It exhibited no lesions either radiographically or on gross and sub-gross examination of the organ systems. The humerus, a rib, and one half of the mandible including teeth were removed, stripped of their muscular attachments, and stored in acetone. The bones were dried later and the humerus was sawn into the eight pieces which are specified in Table 1. Each sample was ashed and then analysed for strontium-90 and calcium. The β-detector used was a well-type plastic scintillator; details of the strontium-90 measurement have been reported in ref. 6. Calcium in the bone samples was determined complexometrically with ethylenediamine tetraacetate (EDTA), which contained a small amount of magnesium chloride, at $p\text{H} > 10$ using eriochrome black *T* as the indicator. Magnesium in these samples comprised about 1 per cent of the calcium on a molar basis. The results are given in Table 1.

Table 1. CONCENTRATION OF STRONTIUM-90 IN BONE FOLLOWING CONTINUOUS EXPOSURE BEGINNING *in utero* TO 18 MONTHS OF AGE

Samples	gm. calcium	gm. calcium/ gm. ash	μc. strontium-90*	μc. strontium-90*/gm. calcium
Proximal epiphysis	0·260	0·391	0·50	1·9
Proximal epiphyseal plate	0·724	0·398	1·2	1·7
Proximal metaphysis	0·744	0·392	1·3	1·8
Proximal diaphysis	0·541	0·393	1·0	1·8
Mid-diaphysis	0·475	0·388	0·97	2·0
Distal diaphysis and metaphysis	0·476	0·388	0·93	1·9
Distal epiphyseal plate	0·689	0·389	1·3	1·9
Distal epiphysis	0·207	0·392	0·43	2·1
Entire humerus	4·12	0·390	7·7	1·9
Rib	0·492	0·386	0·94	1·9
Left mandible with teeth	3·68	0·381	7·4	2·0
Diet				1·8

* At date of killing.

We conclude then that the integrated effect of an exposure of a beagle to an essentially constant dietary strontium-90/calcium level which began *in utero* and continued to young adulthood is that the relative strontium-90/calcium content of the bone is the same as that of the dietary environment. Furthermore, we can compare the distribution along the length of the humerus and in different bones, and the variation is not significant at the 95 per cent confidence-level. Thus the macroscopic distribution in bone does not vary from bone to bone or from trabecular to cortical bone.

Our observation of a value of unity for the ratio in contrast with other reports of lower values serves to emphasize the complications in measuring the over-all effect of many variables, namely, species, age, nutrition, duration of exposure. Finally, we recognize that 1·8 μc. strontium-90/gm. calcium is quite a high level, and, although the skeleton of the dog appeared normal in all respects, our observations do not preclude the possibility of microscopic alterations in bone growth and remodelling processes which may have affected the results.

We thank Dr. J. Luick and Dr. H. Parker for treating the dog, and D. R. Atherton and F. W. Bruenger. This work was supported by the United States Atomic Energy Commission.

BETSY J. STOVER

Radiobiology Division,
Department of Anatomy,
University of Utah,
Salt Lake City.

M. GOLDMAN
A. C. ANDERSEN

School of Veterinary Medicine,
University of California,
Davis.

[1] *Report of the U.N. Sci. Comm. on the Effects of Atomic Radiation, Thirteenth Session*, Supp. 17 (*A*/3838), Annex *D* (New York, 1958).

[2] Comar, C. L., Wasserman, R. H., and Nold, M. M., *Proc. Soc. Exp. Biol. and Med.*, **92**, 859 (1956).

[3] Comar, C. L., *U.S. Atom. Ener. Comm. Rep. ORO*-110, 11 (1954).

[4] Comar, C. L., Whitney, I. B., and Lengemann, F. W., *Proc. Soc. Exp. Biol. and Med.*, **88**, 232 (1958).

[5] *U.S. Atom. Ener. Comm., Project* No. 6, Univ. Calif., Davis, *Progr. Rep., AECU*-4163, 21 (1958).

[6] Stover, Betsy J., and Atherton, D. R., *Proc. Soc. Exp. Biol. and Med.*, **99**, 201 (1958).

Carbon-14 from Nuclear Explosions as a Short-term Dating System: Use to determine the Origin of Heartwood

THE concentration of carbon-14 dioxide in the atmosphere has been increasing since 1954 in a manner shown (for the southern hemisphere) in Fig. 1 (refs. 1,2). This results from carbon-14, produced in nuclear explosions and carried up into the stratosphere by the associated heat, slowly diffusing into the atmosphere. This phenomenon can be used as a short-term dating system. Its use to study the mechanism of heartwood formation is described here.

It is not known if the heartwood extractives present in the heartwood of a tree are the result of 'waste' products of metabolism of the tree at the time of formation of the heartwood, or whether they represent material which has always been present in the sapwood ring and which is converted into heartwood extractives when the wood is converted from sapwood into heartwood. In general, the first hypothesis is favoured. To examine this point, the three outer heartwood rings (the 12th, 13th and 14th rings counted from the outside) of a *Pinus radiata* (total number of rings, 24) felled on August 4, 1959, in the Kaingaroa Forest, New Zealand, were taken and the heartwood extractives removed by acetone and ethyl acetate extraction in a Soxhlet apparatus.

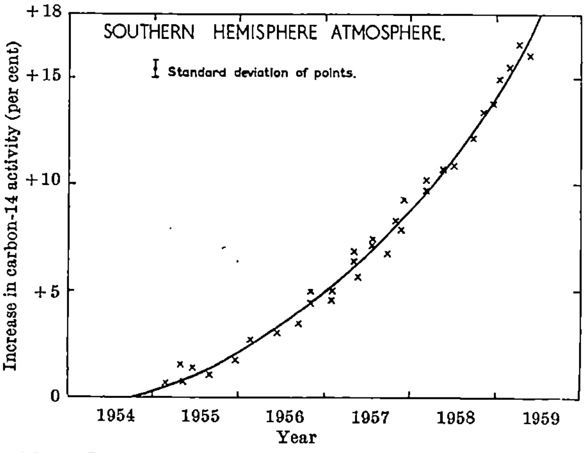


Fig. 1. Increase of carbon-14 in southern hemisphere atmosphere (after Ferguson, ref. 1)

Cellulose was then extracted from the residue. The cellulose and heartwood extractives were then converted to carbon dioxide and counted in the Institute of Nuclear Science low-level carbon-14 counter. The results (all corrected to the same carbon-12/carbon-13 ratio to allow for any isotope effect in the tree or in the experimental manipulation) are given in Table 1.

Table 1. ACTIVITY OF SAMPLE IN COUNTS PER MIN. FOR A 1-ATMO. COUNTER FILLING

All samples corrected to same carbon-12/carbon-13 ratio to correct for isotope effect in the metabolism of the tree and in laboratory manipulation. σ = Standard deviation of the counting

	Cellulose	Heartwood extractives	For comparison, the atmosphere at the time of formation of heartwood is given
Outer ring of heartwood	39·5 $\sigma = 0{\cdot}17$	39·7 $\sigma = 0{\cdot}17$	47·2
First inside ring	39·7 $\sigma = 0{\cdot}17$	39·8 $\sigma = 0{\cdot}17$	44·8
Second inside ring	39·4 $\sigma = 0{\cdot}17$	39·1 $\sigma = 0{\cdot}23$	42·6

It is seen that for a given ring of heartwood, the extractives correspond in activity to the cellulose and not to the atmosphere at the time of their conversion from sapwood to heartwood. The activity of the cellulose corresponds to the activity of the atmosphere when it was the outer ring of the tree (before nuclear explosions).

More specifically, it might be said that (at the 99 per cent statistical confidence-level) less than 6 per cent of the carbon in the heartwood extractives in the outer ring of the heartwood comes from the atmosphere in the year of its conversion from sapwood to heartwood. This might suggest that the sapwood of a pine tree always contains the material which will be converted to the heartwood extractives when that portion of the tree is converted from sapwood to heartwood. This raises the interesting theoretical possibility of artificially inducing the formation of heartwood.

It is hoped to publish later a detailed account of this and related work.

It should be pointed out that this phenomenon of increase of carbon-14 in the atmosphere provides a short-term dating system of great value for biological and other problems[2]. Due to exchange with the oceans, this unique situation will not last for more than a few years[3]. Thus, full use should be made of this effect by as many workers as possible in the immediate future.

The experimental work described in this communication was carried out when I was on the staff of the Institute of Nuclear Science, Lower Hutt, New Zealand. I thank the Forest Research Institute, Rotorua, for supplying the samples.

A. T. WILSON

Chemistry Department,
Victoria University of Wellington,
New Zealand.

[1] Ferguson, G. J., paper presented at the Int. Oceanographic Congress, New York (September 1959).

[2] Rafter, T. A., and Ferguson, G. J., *N.Z. J. Sci. Tech.*, **38**, B, 871 (1957).

[3] Broecker, W. S., and Olson, E. A., *Science*, **132**, 712 (1960).

Removal of Internally Deposited Radionuclides by Triethylenetetraamine-hexaacetic Acid

Increasing the number of donor groups in chelating agents can give rise to the formation of more stable chelates[1–3] and, therefore, to a higher mobilization of internally deposited trivalent and other metal ions of higher valency. This has been demonstrated with ethylenediamine - NNN′N′ - tetraacetic acid (EDTA) and diethylenetriamine-NNN′N′N″-pentaacetic acid (DTPA)[4,5], that is, with chelating agents with 6 and 8 donor groups respectively. For this reason extension of this work to the 10-dentate triethylenetetraamine - NNN′N′N″N‴ - hexaacetic acid (TTHA) seemed to be worth while.

Carrier-free cerium-144, yttrium-91 or strontium-85 was injected intravenously in rats 7–9 weeks old averaging 165 gm. in weight. The chelating agents (by courtesy of J. R. Geigy, S.A., Basle) were given immediately or at different time-intervals after the injection of the nuclide. As shown in Table 1, TTHA reduces the radiocerium content of the skeleton more effectively than DTPA, when given simultaneously or immediately after the administration of the nuclide. In the liver, however, under the same conditions, DTPA is more effective than TTHA. The comparative higher effectiveness of DTPA in mobilizing deposited radiocerium from the liver becomes still more pronounced if the chelating agent is administered after longer time intervals, whereas the superiority of TTHA over DTPA in the removal of radiocerium from the skeleton vanished. Analogous results have been obtained for radioyttrium (Table 2); but neither TTHA nor DTPA had any effect on the mobilization of radiostrontium.

The causes for the observed differences in the behaviour of these two chelating agents are not yet understood. It may be assumed tentatively that TTHA forms to some extent polynuclear colloidal chelates which may be metabolized differently from the smaller molecules of the 1 : 1-DTPA chelates. The greater effectiveness of TTHA as compared with DTPA in the removal from the skeleton—the critical organ for most radionuclides—of radiocerium and radioyttrium can be of therapeutical value in certain cases and justifies further investigations on other radionuclides, especially plutonium.

A. Catsch
D. Schindewolf-Jordan

Institut für Strahlenbiologie,
Kernforschungszentrum,
Karlsruhe 1.

---

[1] Anderegg, G., Nägeli, P., Müller, F., and Schwarzenbach, G., *Helv. Chim. Acta*, **42**, 827 (1959).

[2] Durham, E. J., and Ryskiewich, D. P., *J. Amer. Chem. Soc.*, **80**, 4812 (1958).

[3] Harder, R., and Chaberek, S., *J. Inorg. Nucl. Chem.*, **11**, 197 (1959).

[4] Heller, H. J., and Catsch, A., *Strahlentherapie*, **109**, 464 (1959).

[5] Catsch, A., *Fed. Proc.*, Supp. 1961 (in the press), which includes further references.

Table 1. Influence of DTPA and TTHA on the Distribution of Cerium-144. The Animals were killed 3–12 Days after the Intraperitoneal Administration of the Calcium Chelates. The Dosage, if not otherwise stated, was 250 μmoles/rat

Treatment	No. of animals	Per cent of control Mean and fiducial limits ($P = 0{\cdot}05$)		
		Liver	Kidneys	Skeleton
100 DTPA simult. (intravenous)	10	0·11 (0·08– 0·14)	14 (9·5– 21)	1·3 (0·9 – 1·8)
„ TTHA	10	0·23 (0·17– 0·31)	14 (9·1– 21)	0·45 (0·33– 0·62)
DTPA after 2 min.	28	1·00 (0·78– 1·30)	19 (16·0– 23)	10·00 (9·00–12·00)
TTHA	27	1·50 (1·30– 1·70)	16 (14·0– 19)	5·20 (4·50– 6·10)
DTPA after 24 hr.	22	21·00 (19·00–24·00)	54 (43·0– 68)	72·00 (64·00–80·00)
TTHA	12	33·00 (27·00–40·00)	64 (53·0– 77)	68·00 (62·00–74·00)
DTPA on 3rd day	13	28·00 (23·00–33·00)	86 (63·0–117)	72·00 (67·00–82·00)
TTHA	13	55·00 (48·00–63·00)	86 (68·0–109)	75·00 (67·00–84·00)
DTPA on 5th, 7th, 9th day	18	11·00 (8·40–14·00)	70 (53·0– 91)	61·00 (53·00–70·00)
TTHA	18	29·00 (23·00–36·00)	74 (58·0– 95)	58·00 (52·00–65·00)

Table 2. Influence of DTPA and TTHA on the Distribution of Yttrium-91. The Animals were killed 3–6 days after the Intraperitoneal Administration of the Calcium Chelates. The Dosage, if not otherwise stated, was 250 μmoles/rat

Treatment	No. of animals	Per cent of control Mean and fiducial limits ($P = 0{\cdot}05$)		
		Liver	Kidneys	Skeleton
DTPA after 2 min.	12	36 (26–51)	23 (18– 30)	5·7 (4·9– 6·6)
TTHA	12	42 (28–62)	25 (18– 33)	3·9 (3·6– 4·3)
100 DTPA after 24 hr. (intravenous)	8	57 (45–71)	89 (54–149)	81·0 (74·0–87·0)
„ TTHA	10	54 (44–65)	68 (43–107)	83·0 (77·0–89·0)
DTPA on 3rd day	13	50 (39–66)	76 (46–126)	76·0 (70·0–83·0)
TTHA	14	61 (47–79)	88 (54–144)	79·0 (74·0–85·0)

Incorporation of Phosphorus-32 into Urinary Concretions forming on Zinc Implants in Rat Bladders

It has been proposed that labelling with radioisotopes might be useful in studying the rate of formation of urinary calculi in experimental animals as a function of environmental conditions. The proposal was based on the fact that in siliceous urinary stones of bovine origin, which contain traces of phosphorus and calcium, diffusion and subsequent exchange of phosphorus-32 and calcium-45 with the non-radioactive counterparts of the stone material do not readily occur across the mucoprotein laminations[1]. It was felt that if stone formation occurs layer by layer, short-term massive administration of phosphorus-32 to an experimental animal forming a laminated calculus would result in incorporation of the radionuclide into a single or limited number of laminations, and that subsequent exchange into other laminations would be negligible. Further, if administration of phosphorus-32 were then stopped, and exchange from other parts of the body did not cause significant levels of phosphorus-32 in urine, subsequent laminations would be relatively free of label. Therefore periods of exposure to various environmental conditions could be alternated with periods of administration of isotope, and the amount of material deposited between the laminations containing the isotope would serve as a measure of the effect of environment. The results reported here are from preliminary experiments designed to assess certain aspects of the usefulness of this technique in studying calculi formation.

Rats were chosen as the experimental animal since it has been possible to form artificial calculi on zinc implants in this species readily[2,3]. It was deemed necessary to determine the urinary excretion of phosphorus-32 following massive doses of that isotope in order to establish whether or not the urinary level would subside rapidly enough to ensure that incorporation would become negligible between doses. Results from typical determinations of the excretion of urinary phosphorus-32 after a massive dose (40 μc./rat) are shown in Fig. 1. The results indicated that excretion of that isotope in the urine following administration decreased rapidly and was largely terminated after 8–10 days. The possibility of labelling single or a few laminations seemed feasible.

Thirty rats were therefore implanted with zinc pellets by techniques previously described[3] to induce calculi formation. The rats were fed 15 gm. of ground laboratory rat chow per rat per day. One group received 0·40 gm. of $CaHPO_4.2H_2O$ per rat per day as a supplement and other groups received 0·38 gm. of K_2HPO_4, or 0·086 gm. of tetraethyl silicate. An additional group served as controls.

The stones removed at autopsy (10 days after the second administration of phosphorus-32) were washed, dried and embedded in epoxy resin to serve as a mount. The mount was ground to expose the stones. Further careful grinding removed enough material to allow examination of a central transverse plane of the specimens. Microautoradiographs were prepared as previously described[1].

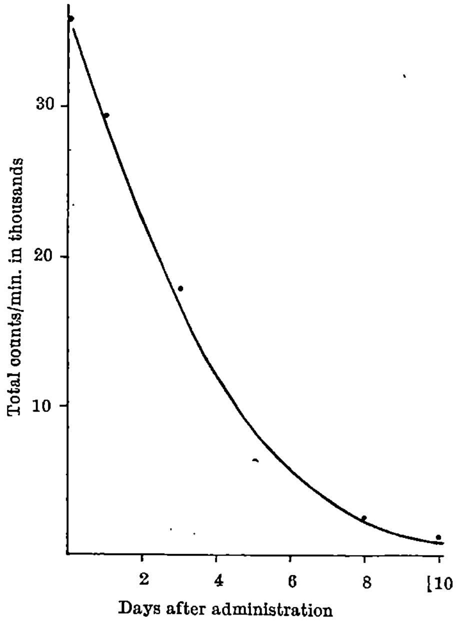


Fig. 1. Excretion of phosphorus-32 in the urine

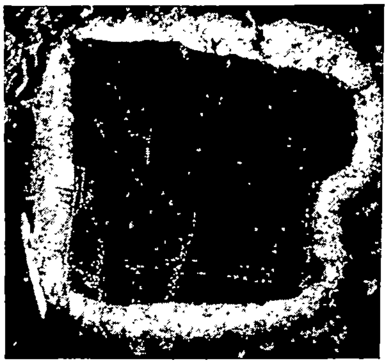


Fig. 2. Typical mounted rat calculus showing the zinc implant in the centre with the concretion surrounding the implant

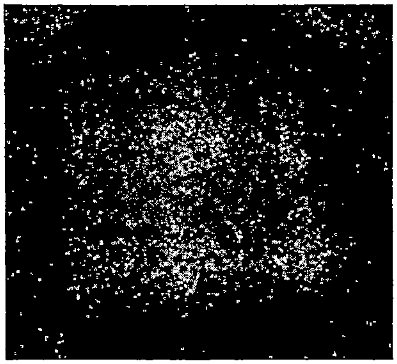


Fig. 3. Microautoradiograph of the stone shown in Fig. 2

Fig. 2 shows an example of the mounted stones from the group receiving calcium phosphate supplement and Fig. 3 shows a microautoradiograph of the same stone. The results were encouraging. There was sufficient phosphorus-32 incorporated to provide adequate exposure for the nuclear-track plate in 4–5 days, in spite of the relatively short half-life of phosphorus-32. The apparent lack of incorporation into two separate concentric rings corresponding to each dosage period was due to the fact that these stones, as shown by diffuse, low-intensity mucicarmine staining, were not grossly laminated with alternate inorganic and mucoprotein layers. The lack of mucoprotein layers apparently allowed random exchange of phosphorus to occur throughout the stone. Stones which were removed from other rats of the other groups and which contained enough deposit to yield a satisfactory microautoradiograph were similar to that shown.

Incorporation of phosphorus-32 was more than adequate for microautoradiography. It was somewhat lower in the rats receiving a tetraethyl silicate supplement than in the others. With silicate supplement, the stones were probably of the high-silicon but low-phosphorus type[3], and hence less incorporation would be expected. None of the stones was grossly laminated, and therefore showed only slight separation into distinct rings labelled with phosphorus-32.

If present experimental work on formation of siliceous calculi on zinc implants in the bovine species provides an adequate means of producing such calculi, then it is anticipated that the phosphorus-32 labelling technique will be very useful. Most siliceous bovine stones are strikingly laminar[1]; this is not the case with stones from rats, and bovine stones do contain enough phosphorus to ensure that sufficient incorporation of phosphorus-32 would be expected with little inter-laminar exchange. These preliminary results suggest that the method offers promise in the study of the rate of formation of bovine calculi under experimental conditions.

This investigation was supported in part by research grant *A*–2811 from the Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

Richard F. Keeler
Stuart A. Lovelace
Montana Veterinary Research Laboratory,
Montana State College,
Bozeman, Montana.

[1] Keeler, R. F., *Amer. J. Vet. Res.*, **21**, 428 (1960).

[2] Vermeulen, C. W., Grove, W. J., Goetz, R., Raggins, H. D., and Correll, N. O., *J. Urol.*, **64**, 541 (1950).

[3] Keeler, R. F., and Lovelace, S. A., *J. Exp. Med.*, **109**, 601 (1959).

HÆMATOLOGY

Haptoglobin Patterns in Cord Blood Serums

Although haptoglobin is almost always present in normal adult serums, it is demonstrable in only about 10 per cent of cord blood serums by the technique of starch-gel electrophoresis[1]. The factors accounting for this large percentage of ahaptoglobinæmic new-borns are obscure. The present communication is based on a survey of cord-blood serums undertaken in an attempt to evaluate some of the factors accounting for the high frequency of ahaptoglobinæmia in the new-born. At the same time, work was carried out to substantiate that the new-born baby with detectable haptoglobin produced it himself.

Two hundred and twelve cord blood serums were subjected to the horizontal starch-gel electrophoresis technique of Smithies[2], using Poulik's discontinuous buffer system[3]. Before electrophoresis, sufficient hæmoglobin was added to each serum to saturate completely any haptoglobin present. One half of each resultant gel pattern was stained with amido black 10*B* to detect protein bands, and the other half with a dilute benzidine stain to locate the haptoglobin–hæmoglobin complexes[4].

Of the 212 cord blood serums examined, 27 (13 per cent) had detectable haptoglobin. As had been noted[5], the patterns, though characteristic, were frequently faint or not detectable with the amido black, and clearly definable only with the more sensitive benzidine stain. Haptoglobin typing of these new-born babies' parents revealed no exceptions to the genetic hypothesis of Smithies and Ford-Walker[6]. No unusual haptoglobin types were encountered.

Comparison of hæmoglobin, hæmatocrit and serum bilirubin-levels between the ahaptoglobinæmic new-borns and those with detectable haptoglobin revealed no evidence to suggest that *in vivo* hæmolysis caused ahaptoglobinæmia (Table 1).

Of fourteen cord blood serums examined from new-born babies with birth-weights less than 2,500 gm., three showed haptoglobin. The smallest tested, with an estimated gestational age of 28 wk. and a weight at birth of 1,106 gm., had haptoglobin of the 2–2 type. The mother had haptoglobin type 2–2 as well.

Table 1. Comparison of New-born Babies with and without Haptoglobin

Determinations*	Ahaptoglobinæmic new-born babies No. tested	Mean	Range	New-born babies with haptoglobin No. tested	Mean	Range
Hæmoglobin, gm. per cent	182	17·3	12·0–23·2	27	16·9	13·6–21·2
Hæmatocrit, per cent	180	58	40–78	26	59	45–80
Serum bilirubin, mgm. per cent	182	6·4	1·0 –18·0	26	5·7	2·0–14·0

* Performed at 2 days of age.

A higher number (13 of 27) of new-born babies with *Hp* 1–1 was found than would be expected in a randomly selected, predominantly Caucasian population such as this series represented. This was probably due to the greater technical ease of detecting the single band *Hp* 1–1 type when the total serum haptoglobin concentration is low.

In 12 of the 27 instances of cord blood serums with detectable haptoglobin, the mother's type differed from that of her offspring. This finding is indicative of the production of haptoglobin *in utero* by these twelve new-born babies. Of these twelve heterospecific baby–mother haptoglobin combinations, nine new-born babies showed *Hp* 1–1. For these nine it might be argued that the smaller molecular weight haptoglobin 1 species crossed the placenta from their *Hp* 1–2 mothers, to give *Hp* 1–1 patterns in the new-born serum. However, in the remaining three heterospecific baby–mother combinations, the new-born was *Hp* 1–2. The mothers of two of these had *Hp* 2–2 patterns, so their offspring must have produced the *Hp* 1 species of molecule *in utero*. The mother of the third new-born had *Hp* 1–1, giving evidence that her *Hp* 1–2 offspring must have produced the *Hp* 2 species of molecules under its own genetic control. This evidence for the fœtal production of haptoglobin corroborates the findings of Galatius-Jensen, who reported four mother new-born haptoglobin combinations with differing types in mother and baby[5].

We thank Dr. Stewart H. Clifford, project director at the Boston Lying-in Hospital, for making the hæmoglobin, hæmatocrit and bilirubin values available to us.

This work was supported by grants from the John A. Hartford Foundation, Inc., and the National Heart Institute (No. *H*–4706 *C*1).

A. R. Rausen
P. S. Gerald
L. K. Diamond
Department of Pediatrics,
Harvard Medical School,
and Department of Medicine,
Children's Hospital Medical Center,
Boston 15,
Massachusetts.

[1] Galatius-Jensen, F., *Acta Genet.*, **8**, 248 (1958).

[2] Smithies, O., *Biochem. J.*, **61**, 629 (1955).

[3] Poulik, M. D., *Nature*, **180**, 1477 (1957).

[4] Smithies, O., *Adv. Protein Chem.*, **14**, 65 (1959).

[5] Galatius-Jensen, F., *Proc. Sixth Europ. Cong. Hæmat.*, 269 (1957).

[6] Smithies, O., and Ford-Walker, N., *Nature*, **178**, 694 (1956).

Serum Factor Gm^a among the Iranians

Of all the genetically determined serum constituents which have been called *Gm* factors, the factor Gm^a, discovered by Grubb[1], is the most frequently studied from the point of view of its distribution among different populations. Hitherto, the proportion of $Gm(a+)$ subjects found among persons of the white race has varied between 67 and 39 per cent (Table 1).

We have examined 296 Iranian subjects, coming from different regions, whose blood samples were taken at the Transfusion Centre of the Iranian Army, at the time of their medical examination for military service. These subjects were twenty years old and apparently in good health.

Table 1. The Serum Factor Gm^a among the Various Populations of the White Race

Population	No. tested	*Gm*(*a*+) (per cent)	*Gm*(*a*−) (per cent)	Ref.
Lapland	110	67·3	32·7	1
Finland	477	65	35	4
England	133	61	39	5
Norway	320	60·6	39·4	6
Sweden	360	59·7	40·3	7
Australia	100	58	42	Kirk, 1948
Denmark	1,084	55·6	44·4	8
France	300	57·3	42·7	3
France	1,000	51·7	48·3	9
France	300	54·3	45·7	10
Italy (south)	125	40·2	59·8	5
Greece	308	39	61	9
Poland	167	39·5	60·5	12

The sera were separated from the clots at the Transfusion Centre, put in sterile bottles, sealed and sent by air to the laboratory for study.

The determination of the factor Gm^a was carried out by the tube technique with the same anti-*Rh* serum as that used in our previous work[2,3], and with the same serum from patients suffering from rheumatoid arthritis. These reagents, as well as the erythrocyte samples coming from the same donors, had thus been previously selected and controlled.

Out of the 296 sera examined, we found 163 *Gm*(*a*+) and 133 *Gm*(*a*−), that is, 55·4 per cent *Gm*(*a*+) and 44·6 per cent *Gm*(*a*−) respectively.

As the subjects examined came from different parts of Persia, the distribution was calculated separately for each of the 13 groups which could be defined.

The results for seven of them are presented in Table 2. The number of individuals coming from other regions (Mazadran, Kermanchah, Pharse, Kurdestan, Gorgan, Kerman and Yazd) was too small to allow the calculation of the frequency to have any significance.

Table 2. Distribution of the Gm^a Factor in the Different Provinces of Iran

	No. tested	Number *Gm*(*a* +)	*Gm*(*a* +) (per cent)
Azerbaijan	48	26	54·1
Gilan	45	25	55·5
Tehran	57	31	54·3
Isfahan	38	21	55·2
Kashan (QOM)	27	16	59·2
Hamadan	19	9	47·3
Khurasan	19	12	63·1

Table 3. Relation between the Gm^a Factor and the Blood Groups

	No.	Per cent	*Gm*(*a*+)		*Gma*(−)	
			No.	Per cent	No.	Per cent
A	89	30·1	44	27·0	45	33·8
B	71	24·0	45	27·6	26	19·6
O	115	38·8	63	38·6	52	39·1
AB	21	7·1	11	6·8	10	7·5
Total	296		163	55·4	133	44·6

So far as the relations between the factor Gm^a and the antigens of the blood groups *A*, *B*, *O* are concerned, the distribution gave us a value of χ^2, which was not significant.

A quantitative determination of the γ-globulins was carried out on each of the samples of serum and, as in previous work, no notable differences were observed between the average percentages of γ-globulins of *Gm*(*a*+) and *Gm*(*a*−) subjects.

Thus, the distribution of Gm^a factors among the Iranians is nearly the same as that previously found for persons of the white race.

Luba Podliachouk
A. Eyquem

Laboratory of Hæmatology
and Blood Groups,
Institut Pasteur,
Paris.

R. Choaripour
M. Eftekhari

Transfusion Centre
of the Iranian Army,
Tehran, Iran.

[1] Grubb, R., *Acta Path. Microbiol. Scand.*, **39**, 195 (1956)

[2] Podliachouk, L., Eyquem, A. and Jacqueline, F., *Ann. Inst. Pasteur*, **99**, 224 (1960).

[3] Podliachouk, L., Jacqueline, F., and Eyquem, A., *Ann. Inst. Pasteur*, **94**, 590 (1958).

[4] Mäkelä, O., and Tiilikainen, A., *Ann. Med. Exp. Biol. Fenn.*, **37**, 180 (1959).

[5] Lawler, S., *Brit. Med. J.*, **i**, 1538 (1958).

[6] Harboe, M. H., and Lundevall, J., *Acta Path. Microbiol. Scand.*, **45**, 357 (1959).

[7] Grubb, R., and Laurell, A. B., *Acta Path. Microbiol. Scand.*, **39**, 390 (1956).

[8] Linnet-Jepsen, P., Galatius-Jensen, F., and Hauge, M., *Acta Genet. Statist. Med.*, **8**, 164 (1958).

[9] Ropartz, C., and Hurel, R., *Transfusion*, **1**, 20 (1958).

[10] Moullec, J., Kherumian, D., Sutton, E., and Espagnon, P., *Rev. Hemat.*, **2**, 512 (1956).

[11] Podliachouk, L., Angelopoulos, G., and Eyquem, A., *Vox Sanguinis*, 1960 (in the press).

[12] Podliachouk, L., Krawczynska, Z., and Eyquem, A. (unpublished results).

HISTOCHEMISTRY

Histochemical and Homogenization Studies of Mitochondrial α-Glycerophosphate Dehydrogenase in the Nervous System

An insoluble brain enzyme capable of oxidizing α-glycerophosphate[1] has been shown to be part of a purely mitochondrial system which resembles succinate dehydrogenase in that pyridine nucleotides do not participate in electron transfer[2]. Histochemical localization of this activity in mammalian nervous tissue was made difficult by the fact that tetrazolium salts are ineffective electron-acceptors in preparations which oxidize α-glycerophosphate. Maximal reaction-rates, however, were obtained by using 2-methyl-1,4-naphthoquinone (menadione, K_3) as intermediate acceptor. Histochemically, this effect was first demonstrated by Wattenberg in liver slices[3]. We found that menadione could be substituted by the sodium bisulphite derivative, but the diphosphoric acid ester ('Synkavite'), vitamin K_1, and 1,4-benzoquinone were ineffective.

In the presence of reduced menadione (K_3H_2), tetrazolium salts are reduced to formazan pigment. This reaction, which is non-enzymic[4], renders histochemically visible the reduction of K_3 by its reductase. Formazan formation by K_3H_2 occurred above *p*H 6·1 and at neutral and slightly alkaline *p*H; this reaction could compete successfully with both auto-oxidation of K_3 and the activity of K_3H_2-oxidase (compare ref. 5). At *p*H 7·4 we found the rate of tetrazolium reduction by rat brain mitochondria in the presence of K_3 to be about 50 per cent higher with α-glycerophosphate than with succinate as substrate. This result compares favourably with measurements of respiration-rate in the presence of oxygen[6].

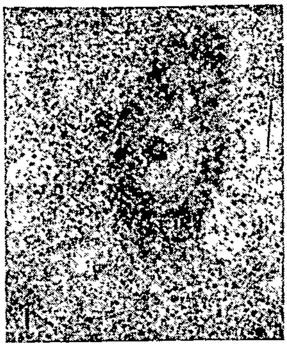


Fig. 1. Rat spinal cord. α-Glycerophosphate–menadione reductase activity in oligodendrocytes. The inactive motor ganglionic cell is counter-stained with thionine. (× 560)

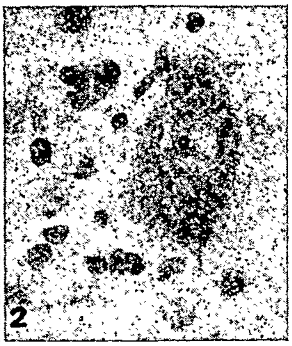


Fig. 2. As Fig. 1, but activity inhibited by addition of phenyl-indane-dione. (× 560)

Histochemical reactions were carried out on 2–8μ fresh frozen sections of rat tissue which were incubated for 15 min. at 22° in media containing substrate (10^{-2} M), 3-(4·5-dimethylthiazolyl-2)-2·5-diphenyl tetrazolium bromide (MTT) (10^{-3} M), cobalt chloride (10^{-3} M), *tris*(hydroxymethyl)aminomethane buffer *p*H 7·4 (0·1 M), sucrose (0·25 M), polyvinylpyrrolidone (1 per cent). High activity with α-glycerophosphate as substrate was observed in neuroglia (Fig. 1) in cells of choroid plexus, in ependyma and in vascular structures. Moderately strong activity was observed in ganglionic cells in certain areas (for example, spinal ganglia); motor neurones were inactive.

Quantitative studies were carried out on rat brain homogenate using the histochemical systems and the results are shown in Table 1. The specific activity of washed brain mitochondria (prepared according to ref. 7) was compared with the activity of the remainder of the cytoplasmic fraction containing soluble flavoproteins.

Table 1. TETRAZOLIUM REDUCTION BY RAT BRAIN FRACTION IN PRESENCE OR ABSENCE OF MENADIONE*

Substrate	Addition	Mitochondria		Cytoplasmic fraction
		a†	*b*†	
α-Glycerophosphate (10^{-2} M)	none	0·15	0·06	—
,, ,,	K_3	0·95	0·91	—
Succinate (10^{-2} M)	none	0·22	0·26	—
,, ,,	K_3	0·47	0·47	—
DPNH (10^{-3} M)	none	0·42	0·40	0·20
,, ,,	K_3	1·16	1·50	1·10
TPNH (10^{-3} M)	none	0·22	0·22	0·25
,, ,,	K_3	1·24	1·10	1·15

* Activity expressed as μgm. formazan/mgm. protein/min.
† *a*: Freshly prepared; *b*, frozen-thawed twice.

All the enzyme reactions tested were sensitive to both dicoumarol (10^{-4} M) and *p*-chloromercuribenzoate (10^{-5} M), thus resembling the reactions of a purified brain diaphorase[8]. Selective reduction of the K_3-dependent increase in mitochondrial activity was produced by 2-phenyl-indane-1 : 3-dione (PID) (5 × 10^{-4} M), both *in vitro* and histochemically (Fig. 2). It is likely that PID exerts a similar action on various mitochondrial systems in other organs, since an antagonistic effect of K_3 and PID on uptake of iodine-131 by rat thyroid slices has been observed by Williams and Doniach (unpublished results). Wosilait[9] has demonstrated different inhibitory activity of dicoumarol and PID on vitamin K_1-reductase from dog and rat liver.

Amobarbital can act as inhibitor of both reduced diphosphopyridine nucleotide (DPNH)–coenzyme *Q* reductase[10,11] and purified brain diaphorase[8]. In our experiments its sole effect (at 5 × 10^{-4} M concentration) was to abolish the K_3-induced increase in mitochondrial tetrazolium reduction with succinate and α-glycerophosphate as substrates. On the other hand, mitochondrial activity with pyridine nucleotide-linked systems was not affected. Antimycin *A* partially inhibited direct tetrazolium reduction with α-glycerophosphate and succinate as substrates, but did not impair formazan production via K_3 as the intermediate electron carrier. With DPNH and reduced triphosphopyridine nucleotide (TPNH) as substrates, no effect of antimycin *A* on the rate of formazan formation could be demonstrated in the presence or absence of K_3.

The extensive glial activity of mitochondrial α-glycerophosphate dehydrogenase is of interest in view of the important part played by mitochondrial glycolysis in brain metabolism[12]. The histochemical reaction involving K_3 and α-glycerophosphate probably uses a pathway different from that leading to direct enzymic tetrazolium reduction. In accordance with Wosilait and Nason[13], the present enzyme should be termed α-glycerophosphate-menadione reductase.

R. HESS
A. G. E. PEARSE

Postgraduate Medical School,
Ducane Road,
London, W.12.

[1] Green, D. E., *Biochem. J.*, **30**, 629 (1936).
[2] Ringler, R. L., and Singer, T. P., *Arch. Biophys. Biochem.*, **77**, 229 (1958).
[3] Wattenberg, L. W., and Leong, J. L., *J. Histochem. Cytochem.*, **8**, 296 (1960).
[4] Slater, T. F., *Nature*, **183**, 50 (1959).
[5] Colpa-Boonstra, J. P., and Slater, E. C., *Biochem. Biophys. Acta*, **27**, 122 (1958).
[6] Sacktor, B., Packet, L., and Estabrook, R. W., *Arch. Biophys. Biochem.*, **80**, 68 (1959).
[7] Abood, L. G., and Alexander, L., *J. Biol. Chem.*, **225**, 717 (1957).
[8] Giuditta, A., and Strecker, H. J., *Biochem. Biophys. Res. Comm.*, **2**, 159 (1960).
[9] Wosilait, W., *J. Biol. Chem.*, **235**, 1196 (1960).
[10] Hatefi, Y., Lester, R. L., Crane, F. L., and Widmer, C., *Biochim. Biophys. Acta*, **31**, 490 (1959).
[11] Redfearn, E. R., and Pumphrey, A. M., *Biochem. J.*, **76**, 64 (1960).
[12] Abood, L. G., Brunngraber, E., and Taylor, M,. *J. Biol. Chem.*, **234**, 1307 (1959).
[13] Wosilait, W., and Nason, A., *J. Biol. Chem.*, **206**, 255 (1954).

HISTOLOGY

Ethylene Glycol Monomethyl Ether, a New Fixative for Histological Work

WHILE studying the guinea pig mast cell[1], we have discovered a new chemical family of histological fixatives, namely, the glycol ethers. These alcohol derivatives, possessing both an ether and an alcohol group, are miscible with most liquids and are excellent solvents for a variety of substances completely insoluble in water. Marketed under the trade names of 'Cellosolves' and 'Carbitals', they have found wide use as solvents for cellulose esters in the lacquer industry. Conversely, they do coagulate and precipitate proteins as well as mucopolysaccharides.

Our initial observations were made on the guinea pig mesentery, with special reference to the labile water-soluble mast cell. Excised specimens were immediately placed in the test solutions (100 per cent concentration, ice cold) and remained there for 18 hr. Afterwards, staining of this naturally thin membrane was achieved with methylene blue (0·25 per cent aqueous, 3 min.), dehydration in absolute ethyl alcohol (2 min., twice), clearing in xylene (5 min., twice), and whole mounts made in a synthetic resin medium (*HSR*).

Ethylene glycol monomethyl ether acetate proved to be the best coagulant fixative of seventeen members of the glycol ether family which were studied. It not only regularly gave solid blue-black mast cells, but also produced a highly desirable intensification of the general staining. The other mesenteric cells showed fine nuclear detail, and collagen fibres were distinct. Significantly, the parent substance, 'Cellosolve' (ethylene glycol mono-ethyl ether), was also an excellent fixative of the mast cell and its use led to almost selective staining of the mast cell. The mesentery and its other cells stood as a pale almost non-staining background for the vivid blue-black mast cells. Here the distinct basophilic granules were in evidence and at times the nuclear space was apparent as a non-staining vacuole. The polymorphism of the mast cell was particularly striking. In the control specimens degranulation was not seen. When such mast cell discharge was induced pharmacodynamically, the fixative faithfully captured the free granules.

The excellence of these fixatives for the guinea pig mesentery led us to explore the general usefulness of the glycol ethers in fixation of other mammalian tissue, using standard paraffin embedding and staining procedures. Using normal as well as diseased human skin, as a test object, it was found that ethylene glycol monomethyl ether produced superior fixation of the cellular components even at room temperature. Nuclear detail, colour brightness and contrast, and glycogen fixation were particularly attractive attributes of the slides prepared from 100 different biopsies. However, in some specimens polarization or streaming of the cytoplasm was seen. A variety of stains were used with consistently fine results and it was found that washing out of the fixative was not necessary.

Despite the rather awesome list of available fixatives[2], Baker has pointed out that the number of substances really useful in chemical fixation is still very small[3]. Accordingly, ethylene glycol monomethyl ether and certain of its congeners are recommended as new non-aqueous fixatives deserving of further trial in histological and histochemical work. They may prove particularly rewarding in fixed tissue enzyme assays of water-soluble components. In our laboratory they have been of continuing value in the fixation and staining of mast cells.

This work was conducted under the sponsorship of the Commission on Cutaneous Diseases of the Armed Forces Epidemiological Board and was supported by the office of the Surgeon General, Department of the Army.

W. B. SHELLEY
RALPH FLORENCE

Department of Dermatology,
University of Pennsylvania,
School of Medicine,
Philadelphia.

---

[1] Shelley, W. B., and Florence, R., *A Technique for the Study of Mast Cells: The Guinea Pig Mesentery* (in the press).

[2] Gray, P., *The Microtomist's Formulary and Guide* (Blakiston Co., New York, 1954).

[3] Baker, J. R., *Principles of Biological Microtechnique* (John Wiley and Sons, Inc., 1958).

BIOLOGY

Adaptive Value of Regenerative Ability

THE view that the distribution of regenerative ability among and within animals is not correlated with the incidence of loss or damage to their organs, and so with survival value, rests very heavily on the work of Morgan[1] on the hermit crab, *Eupagurus longicarpus*. He concluded that the regenerative ability of its various appendages was not correlated with their liability to loss: in particular, the last two pereiopods (thoracic walking legs) and the abdominal appendages, all of which are protected inside the host shell and are rarely lost, regenerate well. He also found that limbs regenerate not only at their autotomy plane, classically regarded as an adaptation to facilitate both escape and regeneration, but also when amputated proximally or distally to the plane. Recent work[2,3] has tended to confirm in some detail the neo-Darwinian view that regenerative ability is correlated with its utility, as measured by the incidence of natural losses. However, since considerable emphasis has again[4] been placed on Morgan's conclusions, it seems worth re-examining his results in the light of a survey made a few years ago (August 1955) on *E. bernhardus*.

Eighty-two individuals, 42♂ and 40♀, were collected at Seaton, south-east Cornwall. They were removed from the shell after fixation in alcohol, and examined under a low-power binocular microscope for fresh and long-standing (natural) losses of eyes, first and second antennæ, pereiopods and abdominal appendages. Long-standing losses include those in the course of regeneration. Recent losses are due mainly to irritation by the fixative. Of the abdominal appendages, the pair of uropods and the left-side members of segments, 3, 4 and 5 are normally present in both sexes. In addition, the left appendage of segment two is normally present in the female, all others being absent in both sexes.

The flagellum of the second antenna fractures readily at any point and the distal end was found missing in most individuals. In addition, eleven showed a recent loss. Losses to the other appendages were:

Table 1

Appendages	♂		♀	
	Fresh	Long-standing	Fresh	Long-standing
Eyes	0	2	0	3
Antennules	0	1	0	1
Pereiopods				
1 (chelæ)	7	5	7	2
2	0	0	3	0
3	0	1 (tip)	2	0
4	0	0	0	0
5	0	0	0	0
Abdominal				
2	normally	absent	0	0
3	3	0	0	0
4	1	2	0	0
5	0	1	0	0
6 (uropods)	0	0	0	0

The incidence of loss is seen to be related to the degree of exposure of the appendages, but with one outstanding exception, the abdominal appendages of the male. Morgan also found some of these appendages missing, but dismissed the matter lightly . . .

"it is possible that the (abdominal) appendages may be occasionally lost". His percentage of losses (4 in 100 crabs) was somewhat less than half that among the pereiopods (29 in 288 crabs), both percentages being similar to those of the present analysis. It was therefore unwarranted to conclude that the ability of the abdominal appendages to regenerate has no useful biological significance.

Morgan claimed that these appendages regenerate virtually as well as the anterior pereiopods, but in fact he had only 10/47 (21 per cent) successful regenerations compared with 10/12 (92 per cent) for the latter. Similar quantitative differences are evident between his records for regeneration at the autotomy plane and those for regeneration elsewhere in a limb. These last results are in essential agreement with more recent results for the thoracic limb of *Asellus*[5], in which the distribution of regenerative ability seems adaptive in every detail. Morgan tended to minimize quantitative differences in ability and to magnify differences in susceptibility to loss.

If he had measured all as carefully as his data permitted, he could have brought out more clearly the unique properties of the uropods and the last two pairs of pereiopods. These properties are confirmed by the present results and by themselves present a far more serious challenge to the 'adaptation' theory. These appendages all regenerate very well, yet are rarely found missing or regenerating. The right-hand pereiopods 4 and 5 appear to be usually shorter and paler than those on the left and the short right uropod is sometimes paler and possibly thinner-shelled than its partner, but these may be normal ontogenetic aspects of the general asymmetry of the posterior part of the body and there was no completely convincing case, in the present results, of natural losses in the process of regeneration.

In explanation of the behaviour of these limbs, it is important to appreciate their indispensable role in movements in and out of the gastropod shell and in anchoring the hermit inside it. The loss of one limb renders its partner also useless, and probably all three pairs are individually indispensable. This would be an adequate adaptive basis for both their high regenerative ability and their lack of autotomy mechanisms. Incidence of loss is related directly to exposure, but also inversely to the indispensability of the appendage. This may explain also why the female, unlike the male, does not lose her other abdominal appendages, which are used to carry the 'berry' after oviposition. A parallel case is the fifth thoracic limb of the mature male *Asellus*[6], both members of the pair being essential to hold the female during the (considerable) period before coition. The limbs autotomize readily in females and immature males. Like the *Eupagurus* limbs in question, the limb of the mature male regenerates well if experimentally amputated.

A. E. NEEDHAM

Department of Zoology,
University Museum,
Oxford.

---

[1] Morgan, T. H., *Zool. Bull.*, 1, 287 (1898); *Anat. Anz.*, 17, 1 (1900).
[2] Needham, A. E., *Proc. Zool. Soc. Lond.*, 123, 111 (1953).
[3] Tufail, M., Ph.D. thesis, University of Exeter (1960).
[4] Vorontzova, M. A., and Liosner, L. D., *Asexual Propagation and Regeneration* (Pergamon Press, Oxford, 1960).
[5] Needham, A. E., *J. Exp. Biol.*, 24, 220 (1947).
[6] Needham, A. E., *Quart. J. Micro. Sci.*, 84, 47 (1942).

Simultaneous Eradication of Three Ectoparasitic Species from a Colony of Laboratory Mice

THE mange mites and blood-sucking louse commonly infesting laboratory mice (to the almost certain detriment of survival and fecundity) can be entirely eradicated by modern topical and systemic pesticides. The present communication gives details of a proved, if time-consuming, procedure whereby a one-room colony of four hundred cages was cleared of three of the following ectoparasites (Fig. 1):

Mange mites. (i) *Myocoptes musculinus*[1], off-white; massive brown claspers on third and fourth pairs of legs; concentrates on the body, especially neck and rump; may thickly encrust the skin; clusters of pink eggs on each hair often discolour the coats of albinos. Eradicated.

(ii) *Myobia musculi*[1], all-white; claspers on first pair of legs; concentrates on the head, especially at the base of whiskers and eye-lashes; sometimes causes weeping sores; white eggs, one at the base of any hair. Eradicated.

(iii) *Psorergates simplex*[2], white; no claspers; all stages found together within pustules under the skin, especially facial skin; severe manifestation[3] rare. Virtually eradicated.

Blood-sucking louse. (iv) *Polyplax serrata*, 1 mm. long; highly mobile; silver eggs attached half-way along the hairs. Eradicated.

Prospects of disinfestation. Residual infestations remained after sulphur dust[4], lindane[5,6], tetmosol[6] and dibutylphthalate[3] had been used as whole-body treatments, and none seemed capable of disinfesting an entire colony. Only tetmosol caused no distress to the mice; it was also observed to kill *Polyplax* instantly.

However, at least four pesticides untried by me have some prospect of eradicating all the above mouse pests. The simplest to use would be the systemic

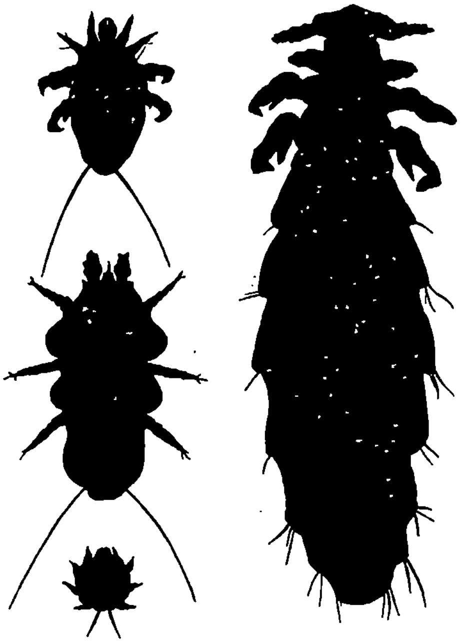


Fig. 1. Representations to scale of the four ectoparasitic species on laboratory mice. The largest is the louse *Polyplax*; the others, from top to bottom, are the mites *Myocoptes* ♀ (the abdomen of the male scarcely protrudes beyond the last legs), *Myobia* and *Psorergates*

pesticides tedion[7,8] and trolene[9], both widely applied to farm animals, if suitable doses for their addition to the food or water can be found for mice. Otherwise, the topical pesticides chlordane or malathion[10] could be tried. A single application of chlordane to fresh bedding is known to eradicate *Polyplax* from mice[4], and the murine mites might be equally susceptible, since those on hamsters were considerably reduced in numbers by the remote action of chlordane painted on to the trays of their cages[11]. Similarly for malathion: a single dip killed all *Myocoptes*[12]; routine treatment of bedding cured a colony of myocoptic and myobial manges where mice were frequently introduced from outside sources[5]; and it might prove effective against *Polyplax*, having been used successfully against lice on birds[13].

The present method uses an aqueous dip of tetmosol and DMC. DMC had already been used in 50 per cent alcoholic solution, first to free a small colony from *Myocoptes*[14], and afterwards a much larger colony from *Myocoptes*, *Myobia* and *Polyplax* (Carter, T. C., and Lyon, M. F., personal communication). In these cases, each mouse was bathed three times at five-day intervals, and very great care was needed to ensure survival. For this reason, after the first room of the larger colony had been disinfested, mice from the remaining rooms were treated at birth by removing the mites with forceps, swabbing the mice once only with the DMC solution, and then fostering them immediately on to already disinfested mothers (Carter, T. C., and Lyon, M. F., personal communication). But disinfestation can be more casual if DMC and tetmosol are presented together in aqueous emulsion, using the tetmosol both to augment the miticidal action of DMC and to be the effective insecticide in the absence of alcohol.

A dip was made by mixing: 2 gm. DMC dissolved in 3 gm. ethanol; 67 gm. tetmosol, and 1 litre warm tap-water. The gift of the DMC, or di-(*p*-chlorophenyl)methylcarbinol, by Sherwin Williams Inc., Cleveland, Ohio, is gratefully acknowledged; the tetmosol, a 25 per cent solution of tetraethylthiuram monosulphide, was supplied by I.C.I. (Pharmaceuticals) Ltd., Wilmslow, Cheshire. The dip was maintained at 30°–37° C. in a water-bath no hotter than 40° C., otherwise mice falling into it could be killed. Mice were given two dippings 1 hr. apart and two further dippings 1–3 weeks later. The first and third dippings were prevented by air pockets from killing every parasite but made the skin thoroughly receptive to the dip on the next occasions; the third and fourth dippings mopped up rare survivors. On each occasion the mice swam for half a minute and were submerged about six times, were drained for three-quarters of a minute while the next mouse was swimming, and then returned to their cages—to their old cages after the first and third dippings, and to clean cages after the second and fourth. A few severely chilled mice had to be specially revived in warm air.

Disinfestation was started when no new litters were immediately expected and the mice could be treated as adults or well-grown weanlings. All mice had the first two dippings within 5 weeks, and many stocks were by then completely disinfested. About a quarter of the mice were required for breeding before their final dippings could be given, and complete disinfestation of these stocks was deferred up to six months and applied to later generations. During this time the disinfested stocks were isolated so far as possible on the upper shelves, and hands were scrubbed before handling them. But no lice and very few mites survived the ancestral second dipping, and recontamination was not observed. The full treatment averages 3 min. per mouse, but much time might be saved by dipping only once in a more concentrated and penetrating emulsion to which a harmless wetting agent such as 1 per cent sodium alkylaryl sulphonate[2] had been added, and by treating several mice together in baskets constructed for the purpose.

Altogether, 1,700 mice were treated without loss. About one thousand of these became parents and were minutely inspected under a stereoscopic microscope at autopsy. In subsequent generations a similar number, representing 10 per cent of sires, have been inspected on the face and back. No *Myocoptes*, *Myobia* or *Polyplax* have been seen in the 2½–3 yr. since treatments were completed, nor have *Psorergates* pustules been recognized in the past half year.

NIGEL BATEMAN

Agricultural Research Council's
Animal Breeding Research Organisation,
Edinburgh, 9.

[1] Gambles, R. M., *Brit. Vet. J.*, 108, 194 (1952).
[2] Flynn, R. J., *Amer. J. Vet. Res.*, 20, 198 (1959).
[3] Cook, R., *Brit. Vet. J.*, 112, 22 (1956).
[4] Madden, A. H., Tozloski, A. H., and Sweetman, H. L., *J. Econ. Ent.*, 47, 442 (1954).
[5] Webb, jun., J. E., and Shepherd, E. L., *J. Econ. Ent.*, 52, 790 (1959).
[6] Cook, R., *Brit. Vet. J.*, 109, 113 (1953).
[7] March, R. B., *Ann. Rev. Biol.*, 3, 355 (1953).
[8] Ferguson, W. C., *J. Econ. Ent.*, 51, 352 (1958).
[9] Peterson, D. G., and Kingscote, A. A., *Canad. J. Biochem. and Physiol.*, 37, 1105 (1959).
[10] Boocock, D., *Pest Tech.*, 1, 120 (1959).
[11] Scott, H. G., *J. Econ. Ent.*, 51, 412 (1958).
[12] Davis, R., *J. Ent. Econ.*, 50, 695 (1957).
[13] Rodriguez, J. L., and Riehl, L. A., *J. Econ. Ent.*, 52, 774 (1959).
[14] Stoner, R. D., and Hale, W. M., *J. Econ. Ent.*, 46, 692 (1953).

A Natural Hybrid of *Tilapia nigra* and *Tilapia leucosticta* from Lake Naivasha, Kenya Colony

CICHLID fish of the genus *Tilapia* are of considerable importance in both natural fisheries and pond culture in many tropical and subtropical climates, particularly throughout Africa. Certain species have proved to be adaptable to varied environmental conditions, and consequently there has been a considerable artificial dispersion of species beyond their natural geographical range, as far afield as Malaya and Pacific islands, and including high-altitude waters in several parts of Africa. This transportation has brought into contact species which were hitherto geographically isolated, and due, at least in part, to this, there is an increasing number of records of hybridization. Lowe[1] first directed attention to possible hybridization between *T. nilotica* (Linné) and *T. nigra* (Günther), and between *T. nilotica* and *T. esculenta* Graham in Uganda. Yashouv and Chervinsky[2] have crossed *T. nilotica* and *T. galilea* (Artédi) in Israel, and Bard[3] reports a hybrid between *T. nilotica* and *T. machrochir* Boulenger in culture ponds in the Cameroons. More recently, Hickling[4,5] crossed a Malayan strain of *T. mossambica* Peters with another of the *T. mossambica* group from Zanzibar, and Mortimer[6] has hybridized *T. mossambica* and *T. andersoni* (Castelnau) in culture ponds in Northern Rhodesia.

Excluding Lowe's[1] unconfirmed observations, none of these hybrids has occurred in 'natural' waters. The crosses recorded by Hickling[5] and Mortimer[6] were

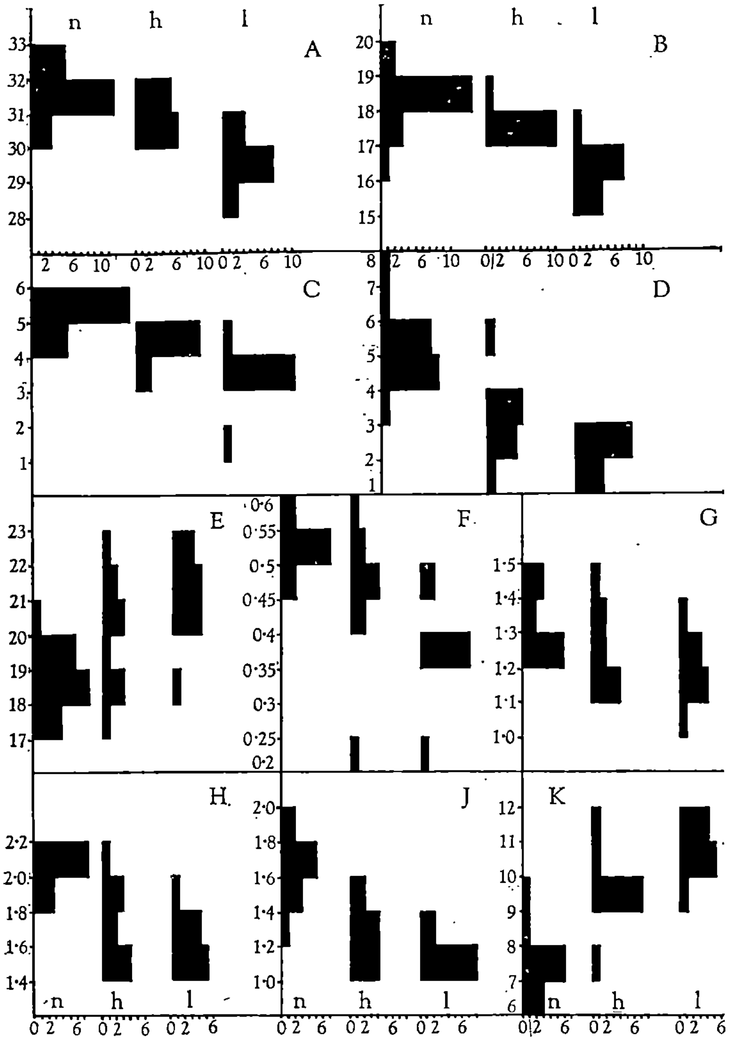


Fig. 1. *Abscissæ:* all figures from left to right, (*n*) *T. nigra*, (*h*) hybrids, (*l*) *T. leucosticta*, as numbers of fish.

Ordinates: (*A*) Lateral line scales. (*B*) Dorsal fin spine number. (*C*) Anal fin spine number. (*D*) Extent of pectoral fins: (1 < vent, 2 < papilla, 3 < anal spine I, 4 < spine II, 5 < spine III, 6 < spine IV, 7 < spine V, 8 < spine VI). (*E*) Gill raker count. (*F*) Ratio of length/width of tooth pad on lower pharyngeal bone. (*G*) Ratio of length/depth of premaxillary. (*H*) Ratio of length/depth of dentary. (*J*) Ratio of length/width of dentary. (*K*) Ratio or standard length/length of dentary bone

the results of deliberate attempts to produce improved strains for fish culture work.

In all the instances mentioned, the species belong to the 'maternal brooder' group of *Tilapia*, in which the female normally broods the eggs and young in her mouth.

In East Africa, *T. zillii* (Gervais), a macrophyte feeder, has been more widely dispersed than most other species, in the hope that it will clear weed-choked waters; and Whitehead[7] has described a cross between the female mouth-brooding species *T. nigra* and the 'substratum-spawning' *T. zillii* in Lake Naivasha and also in a flooded rice paddy field at the Tebere Rice Irrigation Scheme, Kenya. Lake Naivasha is a shallow basin of some eighty square miles, which originally contained only one species, *Aplocheilichthys antinorii* (Boulenger), endemic to Lake Naivasha and southern Ethiopia. During the past forty years, various other species have been introduced: *T. nigra* was stocked to provide forage for the introduced black bass (*Micropterus salmoides* (Le Seur)). Rainbow trout (*Salmo gairdnerii* (Richardson) have also gained access from the affluent rivers and *Lebistes* and *Gambusia* were introduced for mosquito control. Most recently, *T. zillii* was stocked from ponds in Kisumu, Kenya.

In postulating hybridization beween *T. nigra* and *T. zillii*, Whitehead (unpublished work) noted certain similarities between a proportion of his hybrids and *T. leucosticta* Trewavas, although the agreement was not complete. On the basis of Whitehead's[7] communication and some of his unpublished results, Trewavas and Greenwood[8] suggested that the hybrids were in fact *T. leucosticta*, another mouth-brooding species, which had gained access to Lake Naivasha by accidental inclusion in the *T. zillii* stocking. In addition, they did not exclude the possibility of some hybridization having occurred between *T. nigra* and *T. leucosticta*.

The occurrence of both pure *T. leucosticta* and hybrids between *T. leucosticta* and *T. nigra* has been confirmed by more recent work, on Lake Naivasha.

During a routine analysis of some five thousand fish, numerous specimens were collected, the taxonomic characters of which agree in detail with the description of *T. leucosticta* given by Lowe[9]. In addition, however, eleven specimens, of 28–31 cm. total length, were recognizably distinct from both *T. leucosticta* and *T. nigra*, and showed a variable complex of intermediate characters. The field characteristics on which they were originally distinguished were depth of body, profile of head, shape and size of mouth, count of anal fin spines and coloration. Further examination of the meristic and morphometric characters of these specimens confirmed their intermediacy, and a summary of the principal characters is given in Fig. 1.

Confirmation of the hybrid identity has not yet been attempted by artificially crossing *T. nigra* and *T. leucosticta*, but there can be little doubt that hybridization has occurred, and that Whitehead's[7] original description was based on a mixture of pure *T. leucosticta* and *T. nigra* × *T. leucosticta* hybrids. The fact that all these hybrids were mature males and the low proportion in the total population may invite speculation regarding their viability. This cannot be established until a very much wider size-range has been examined, since sexual dimorphism occurs in the growth of both *T. nigra* and *T. leucosticta*, the males being somewhat larger.

This investigation was undertaken as a necessary preliminary to a study of the fishery potential of Lake Naivasha. It is now possible to identify *T. nigra*, *T. leucosticta*, *T. zillii* and *T. leucosticta* × *T. nigra* hybrids in a population formerly considered to be pure *T. nigra* and *T. zillii*. However, the exact taxonomic identity of a large proportion of the Naivasha *Tilapia* still remains to be determined, owing to the very wide and variable range of colour, meristic and morphometric characters which has been encountered.

H. Y. Elder
D. J. Garrod

East African Freshwater Fisheries Research Organization,
Jinja, Uganda.

[1] Lowe, R. H., *Rev. Zool. Bot. Africa*, **57**, 1 (1958).
[2] Yashouv, A., and Chervinsky, J., *Nature*, **184**, 1739 (1959).
[3] Bard, J., *CCTA/CSA* Symposium, Hydrobiology and Inland Fisheries (Lusaka, 1960) (in the press).
[4] Hickling, C. F., *Nature*, **183**, 287 (1959).
[5] Hickling, C. F., *J. Genet.*, **57**, 1 (1960).
[6] Mortimer, M. A. E., *CCTA/CSA* Symposium. Hydrobiology and Inland Fisheries (Lusaka, 1960) (in the press).
[7] Whitehead, P. J. P., *Nature*, **187**, 878 (1960).
[8] Trewavas, E., and Greenwood, P. H., *Nature*, **188**, 868 (1960).
[9] Lowe, R. H., *Rev. Zool. Bot. Africa*, **55**, 3 (1957).

Further Observations on a Filamentous Saprophyte from Wookey Hole Caves

Work now completed on this unusual organism, found growing in darkness in rather stagnant water of Wookey Hole Caves, Somerset, has yielded some information additional to that reported earlier[1]; but the identity of the saprophyte remains unknown.

The organism, although slow-growing, may be successfully cultured in the light or in darkness on a range of solid and of liquid media, and can readily utilize glucose, sucrose and mannose. A notable feature is its great plasticity of form, which may vary, dependent on the cultural conditions, from septate, branching filaments to chains of rounded cells which almost separate from one another. X-ray diagrams of the cell wall show some similarity to those of yeasts. Usually there are several very small, obscure nuclei per cell, and photosynthetic pigments are lacking. On certain media the organism produces large amounts of oil, especially in old cells; thick-walled chlamydospore-like bodies, which give rise to fine filaments on germination, are formed in some cultures. On media low in glucose, anastomoses are frequent; however, no sexual organs have been observed, despite the wide range of cultural conditions and treatments investigated.

A full, illustrated account of this saprophyte, which seems to show more affinity to the fungi than to the algae, will be given elsewhere[2].

A. J. Willis

Department of Botany,
University of Bristol.

[1] Round, F. E., and Willis, A. J., *Nature*, **178**, 215 (1956).
[2] Willis, A. J., *Proc. Univ. Bristol Spelæological Soc.*, **9**, No. 2 (in the press).

Axenic Seedlings of *Drosera*

Carnivory in higher plants fascinated Darwin[1] and later students of evolution, yet a century-long debate[2] on the extent of *Drosera's* dependence on, and specialization for, insect prey remains inconclusive. A technique for obtaining axenic *D. intermedia* (a sundew) is reported here—a step forward in analysing its biochemical capabilities.

Büsgen[3] preferred seedlings to winter buds as more uniform experimental subjects. Vegetative plantlets are better, as they circumvent genetic variation. However, for purposes of aseptic cultivation, seeds—easier to handle and protected by their hard coat—are best, assuming that vegetative propagation follows. Seeds were collected in September (between ripening and shedding) at Bethel, New York, and stored dry at room temperature for 8 months. For sterilization, seeds were immersed in an aqueous solution of 'Clorox' (5 per cent) plus 'Aerosol' *OT* (0·02 per cent) for 10 min. with constant shaking, then washed three times in sterile distilled water and inoculated in batches of 10–12 from the water to agar slants (composition in Table 1).

Table 1. Composition of Slant Media

Compound	mgm./100 ml. final medium
NH_4NO_3	12·0
KH_2PO_4	6·8
$MgSO_4.7H_2O$	12·0
$Fe(NH_4)_2(SO_4)_2.6H_2O$	0·56
$CaCO_3$ (dissolved in HCl)	2·0
Sucrose	2,000·0
Trypticase (Baltimore Biological Lab.)	50·0
'Complete Supplement No. 10'*	0·5 ml./100
Agar	1,000·0
*p*H 6·0	

* An aqueous solution of *B* vitamins, amino-acids, hydrolysed nucleic acids, and an acid hydrolysate of casein (Hycase, Sheffield) quite similar to 'complete supplement No. 9' given in ref. 4.

For uniformity, seeds for each experiment were sterilized together. Viability of unsterilized seeds was not determined. Of 3,200 seeds on slants stored 3 months at room temperature in the dark, only two germinated. Exposure to indole-3-acetic acid, gibberellic acid, dilute sulphuric acid, high temperature (37° C.), or freezing followed by either high or room temperature, did not enhance germination.

However, several slants left on a west-facing window-sill in September showed 6 per cent germination after one month, suggesting physical germination stimuli. *D. intermedia* in southern New York State normally germinates in March and April on the moist surface of acid bogs. Temperature- and light-alternation, reported stimulatory to germination in various other plants[5], were tried. Freshly inoculated slants were subjected to a cycle of 12 hr. at 15° C. and 12 hr. at 38° C., and either illuminated by two 40-W. 'cool-white' fluorescent lamps or kept in the dark when desired, by wrapping the tubes. Germination was observed after 5 weeks of treatment. As shown in Table 2, seeds subjected to a temperature-cycle plus light showed significant germination, but not when exposed to constant temperature or placed in the dark, at any temperature. No dark period is necessary.

Table 2. Effect of Light and Temperature Alternation on Germination of *D. intermedia*

	Percentage germination*		
	Constant		Alternate Light and dark
	Light	Dark	
15° C. constant	0	0	0
38° C. constant	2	0	0
15°–38° C. alternation	44	2	31†

* Each sample contained about 100 seeds.
† In the temperature–light alternation, the illuminated phase was at 38° C., the dark at 15° C. The reciprocal experiment (light at 15° C., dark at 38° C.), was not done.

Growth is erratic, some seedlings dying quickly, others putting forth several small but well-developed leaves. Media and conditions for sustained growth are not yet satisfactory. As seedlings (Fig. 1) are now readily available, this step is accessible to experiment.

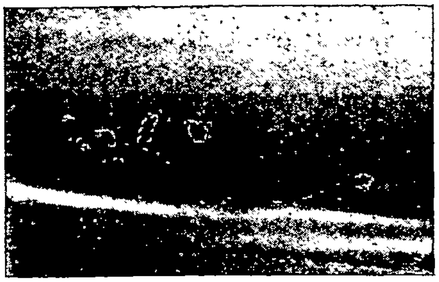


Fig. 1. An axenic *Drosera intermedia* seedling at 1 month (× 4). Black ovals are ungerminated seeds.

Some of this work was done at Brandeis University, Waltham, Massachusetts.

RICHARD M. BURGER*

Haskins Laboratories,
305 East 43rd Street,
New York 17.

* Present address: Adelphi College, Garden City, New York.

[1] Darwin, C., *Insectivorous Plants* (D. Appleton and Co., New York, 1897).

[2] Schmucker, T., and Linnemann, G., in Rhuland, W., *Handbuch der Pflanzenphysiologie*, 11, 198 (Springer-Verlag, Berlin, 1959).

[3] Büsgen, M., *Bot. Zeitung*, 41, 569, 585 (1883).

[4] Hutner, S. H., Baker, H., Aaronson, S., Nathan, H. A., Rodriguez, E., Lockwood, S., Sanders, M., and Petersen, R. A., *J. Protozool.*, 4, 269 (1957).

[5] Crocker, W., and Barton, L. V., *Physiology of Seeds* (Chronica Botanica, Waltham, Massachusetts, 1957).

Spore Liberation and Dispersal of Coffee Rust *Hemileia vastatrix* B. et Br.

Nutman, Roberts and Bock[1] have produced evidence recently that *Hemileia vastatrix* uredospores are not liberated into the air from fructifications by wind alone at speeds up to 12·5 miles/hr. and only in small quantities if the supporting leaf flutters violently. They infer that contact of water with the pustules is the principal method of liberation.

However, investigations in the field made during rainstorms at the Coffee Research Station, Ruiru, Kenya, by me in 1956 had shown that wetting of the lower leaf surfaces was usually not very marked and was principally by upward splash from lower leaves. Rust lesions are confined to the lower surfaces and would be too infrequently hit for this to be the predominant method of spore liberation. Microscopic observations of lesions brought in from the field showed that spores were not dislodged by air currents alone to any marked degree. However, with plants grown in the still air of a greenhouse, a light tap on a rusted leaf produced a visible spore cloud. Recently, rust lesions resulting from the inoculation of a coffee plant kindly supplied by the Royal Botanic Gardens, Kew, and kept in a laboratory were found not to liberate spores under calm conditions, but abundant liberation occurred when they were tapped lightly. A lesion tapped at 2–3 week intervals liberated 366,100 spores during three months, and only some further 50,000 could be washed off at the end of this period.

Water droplets allowed to fall on leaves at points situated above fructifications, previously kept in calm conditions, produced abundant spore liberation; for example, ten 5-mm. diameter droplets dropped from a height of 4 ft. liberated 396,600 spores. Further, from such fructifications, currents of air at speeds estimated as less than 1 mile/hr. produced marked liberation. A current not greater than 2 miles/hr. deposited 23,440 spores on 2·5 cm.^{2} of sticky surface held 1 cm. behind a fructification. These observations contrast strongly with those of Nutman[1].

Thus, under field conditions, liberation of spores into the air is probably considerable, though in accord with their rate of fall[1], and as liberation is probably most marked during rainfall, the majority may well be deposited near their point of origin as indicated by Burdekin's[2] observations. The epidemic spread may thus approximate to that expected for a fungus disseminated by rain-splash.

In fructifications kept under calm conditions, stocks of spores are built up by the fascicles of hyphæ which erupt through each stoma forming short, irregular filaments several spores wide. Small mechanical impulses cause these to break off and fall away. Under field conditions, few spores will be ready for liberation at any one time and this may explain Nutman's[1] observations, since his experimental material was brought in from the field.

R. W. RAYNER

12 St. Peter's Square,
Hammersmith,
London, W.6.

[1] Nutman, F. J., Roberts, F. M., and Bock, K. R., *Trans. Brit. Mycol. Soc.*, 43, 509 (1960).

[2] Burdekin, D. A., *Kenya Coffee*, 25, 212 (1960).

Production of Symptoms of Barley Leaf-Spot Disease by Culture Filtrate of *Helminthosporium sativum*

It has been reported by several investigators[1–4] that symptoms of some diseases could be reproduced by the action of the culture filtrate on the host plant, instead of using the infecting spores.

During 1957–58 a series of experiments were carried out at the Phytopathological Laboratory, Willie Commelin Scholten, Baarn, Holland, on the susceptibility of the different Dutch barley varieties, namely, Agio, Balder, Piroline and Wisa, to the leaf-spot disease caused by *Helminthosporium sativum* P. K. and B. Pathogenicity tests were carried out under glasshouse conditions where seedlings of the four barley varieties were sprayed with a standardized suspension of *Helminthosporium* spores. The typical brownish necrotic spots appeared on all seedlings. The most resistant variety, as indicated by the size of the necrotic spots, was Balder, followed by Agio, then Wisa, while Piroline was the most susceptible. The same experiments were repeated, but under field conditions, where identical results on mature barley plants were obtained.

It was thought interesting to study the response of these different barley varieties to the probable toxic action of *Helminthosporium* filtrate. Accordingly, the fungus was grown on modified Fries No. 3 medium[4] for 4 weeks at 25° C. The filtrate was passed through a glass filter to get rid of spores and tiny mycelial fragments. The filtrate was distributed into sample tubes. For each of the four barley varieties, four tubes were prepared in each of which three cut shoots were dipped. The cut shoots dipped in the filtrate were left afterwards for 48 hr. at room temperature ranging from 15° to 20° C. Brownish necrotic spots, similar to those induced by spore infection, were clear on the four varieties. In addition to the necrotic symptoms, cut shoots showed wilting and twisting, and they became dark green in colour. Necrotic lesions, which appeared due to the toxic action of the filtrate, had almost the same size in the four varieties, denoting that the resistant, as well as the susceptible, varieties of barley responded similarly to the toxic action of *Helminthosporium* filtrate. It was also possible to reproduce symptoms of oat blight by using the culture filtrate of *Helminthosporium victoriae*[4]. Dilution of the filtrate with water 1 : 100 did not affect symptom reproduction, while filtrate diluted to 1 : 1,000 failed to reproduce symptoms on any of the barley varieties.

During 1958–1959 pathogenicity of the Dutch strain of *Helminthosporium sativum* to the Egyptian barley variety Baladi 39 and to the Sudanese variety Baladi was tested. The two varieties proved to be moderately susceptible to infections by *Helminthosporium* spores. The degree of susceptibility of both varieties was more or less comparable with that of the Dutch variety Agio. When cut shoots of the Egyptian variety Baladi 39 and the Sudanese variety Baladi were dipped in *Helminthosporium* filtrate, prepared exactly under the conditions mentioned, the shoots wilted, twisted and became darker in colour, but no necrotic symptoms could be detected at all. Hence, reproduction of natural symptoms on these African varieties was unsuccessful by using the filtrate of the Dutch strain of *Helminthosporium sativum*. Such differences between symptom production by the fungal pathogen and symptom reproduction by its filtrate emphasize the specificity of the host variety on which natural symptoms can be reproduced. Further work on this point in which African strains of *Helminthosporium* are involved, and on the nature of the toxins produced by the fungus, is in progress, and full details will be published later.

S. K. Gayed

Botany Department,
Faculty of Science,
University of Khartoum,
Sudan.

[1] Zentmeyer, G. A., and Horsfall, J. G., *Science*, **95**, 512 (1942).
[2] Dimond, A. E., (Abs.) *Phytopath.*, **37**, 7 (1947).
[3] Wheeler, H. E., and Luke, H. H., (Abs.) *Phytopath.*, **44**, 334 (1954).
[4] Pringle, P. B., and Braun, A. C., *Phytopath.*, **47**, 369 (1957).

Effect of Excess Supply of Heavy Metals on Barley during Germination, with Special Reference to Catalase and Peroxidase

The injurious effect of the excess supply of heavy metals on plant growth, among other factors, has been attributed to induced iron-deficiency[1–3]. Recently[4–7], it has been shown that heavy metals such as cobalt, nickel, copper, molybdenum and chromium, when supplied in excess, depress chlorophyll and catalase; in some instances peroxidase is also depressed. The repeated application of iron–ethylenediamine tetra-acetic acid to plants receiving the heavy metals mitigates the effect of heavy metals[6] on chlorophyll, catalase and peroxidase.

In order to test the effect of heavy metals on catalase and peroxidase before chlorophyll had developed, some preliminary investigations were made on barley (*Hordeum vulgare*) and 'kharbooza' (*Cucumis melo*) during germination. Contrary to the results obtained with adult plants, in seedlings the synthesis of catalase seemed to be stimulated at 1 m*M* concentration of cobalt. The investigation was extended to other heavy metals, and the effect of cobalt, nickel, molybdenum and chromium supply, each at 1, 5 and 25 m*M* concentrations, on barley during germination was studied. Each of the excess heavy-metal treatments was superimposed on a basal solution[6]—the full nutrient solution. The seeds were soaked in the appropriate nutrient solution for 12 hr. and then transferred to Petri dishes containing filter paper liberally moistened with the solution in which the seeds had been soaked. The Petri dishes were maintained in diffused light at room temperatures which varied between 19° and 23° C.

Table 1. Effect of the Excess Supply of Heavy Metals on Germination and Catalase and Peroxidase.
Results expressed as percentage increase (+) or decrease (−) over values obtained with seedlings given basal solution (ref. 6)

Treatment		Catalase		Peroxidase		Germination (length of first formed root)
		a	*b*	*a*	*b*	
Cobalt	1 m*M*	+44	+53	−20	−15	+21
	5 m*M*	+53	+33	−11	−22	−35
	25 m*M*	+61	−10	−39	−64	−92
Nickel	1 m*M*	+30	+24	−6	−10	−27
	5 m*M*	+147	+61	−21	−49	−77
	25 m*M*	+105	+24	−64	−78	−96
Molybdenum	1 m*M*	+2	+27	−7	+13	+1
	5 m*M*	+25	−25	−11	−47	−86
	25 m*M*	−22	−57	−57	−76	No emergence
Chromium	1 m*M*	+36	−3	−28	−49	−89
	5 m*M*	+152	+35	−47	−72	−96
	25 m*M*	+112	+26	−71	−76	No emergence

a, Expressed on fresh weight basis; *b*, expressed on per-seedling basis

At 1 m*M* supply germination was slightly stimulated by cobalt and depressed by nickel and chromium. At 5 m*M* and 25 m*M* concentrations germination was depressed by cobalt, nickel, molybdenum and chromium.

There was active synthesis of catalase during germination—eight-fold increase in 120 hr. from the soaking of the seeds in the basal solution. Excess supply of cobalt, nickel and chromium stimulated the synthesis of catalase, the effect being very marked at 5 and 25 m*M* supply each of nickel and chromium. No stimulation of the synthesis of catalase was found with molybdenum at 5 and 25 m*M* concentrations. In the germinating seeds, the main region for the synthesis of catalase was the growing embryo, that is, the developing root and shoot tissue. At 5 and 25 m*M* supply of nickel and chromium, although the formation of catalase-synthesizing tissue was severely restricted, yet the catalase expressed on the per seed basis was found to be higher than in seedlings raised without the excess supply of heavy metals.

As compared with catalase, peroxidase synthesis was slow—less than two-fold increase in 120 hr. from the soaking of seeds in the basal solution. Excess supply of heavy metals depressed peroxidase. Uptake of oxygen by the seedlings, as measured in a Warburg apparatus at 32° C., and total dehydrogenase activity, as measured by the reduction of triphenyl-tetrazolium chloride, were also depressed by the heavy metals, the effects being more pronounced in the growing embryo than in the rest of the seedling.

The uptake of heavy metals continued even when the germination was severely depressed and in general the heavy metals depressed the uptake of iron.

The stimulation of the synthesis of catalase by the excess supply of heavy metals would mean not only the increased synthesis of the protein moiety of catalase but also increased synthesis or at least availability of iron-porphyrin for the formation of catalase. This result cannot be explained in terms of the hypotheses advanced to date to explain the role of the excess supply of heavy metals in plants.

NEW VERSATILITY IN Liquid Scintillation Counting

With the Packard Tri-Carb® Spectrometer

Now you can count assortments of carbon-14 and tritium samples interchangeably without readjusting controls . . . perform double labeling at the true balance point for each isotope . . . or do both. In addition, the new Tri-Carb Spectrometer with its expander-amplifier circuitry can be used for dual-channel gamma counting merely by adding a well detector. This versatility is a Packard exclusive—made possible by two separate channels of pulse height analysis with individually variable amplifiers and four adjustable discriminator levels.

Other features are all-transistorized circuitry and convenient mounting of all controls at eye level above the freezer. Width of the instrument is reduced to a space-saving 3½ feet.

Three models in various combinations offer a total of nine liquid scintillation counting systems—refrigerated or non-refrigerated . . . for automatic or semi-automatic operation. From this selection you can readily meet the requirements of your budget and provide for present and future needs. You'll get the satisfaction and performance which only a Tri-Carb Spectrometer—with its world-wide reputation for accuracy and reliability—can provide. Call your Packard representative or write for Bulletin AD-1002.

INSTRUMENTS FOR RADIOACTIVITY MEASUREMENT AND CHROMATOGRAPHY

PACKARD INSTRUMENT INTERNATIONAL S.A.

Packard

Talstrasse 39, Zurich 1, Switzerland
3 Square Grange, Paris VIII, France

Packard Instrument Co., Inc., La Grange 60, Illinois, U.S.A.

LEEC

BRUSHING MACHINE

for cleaning Glassware in Laboratories and Hospitals

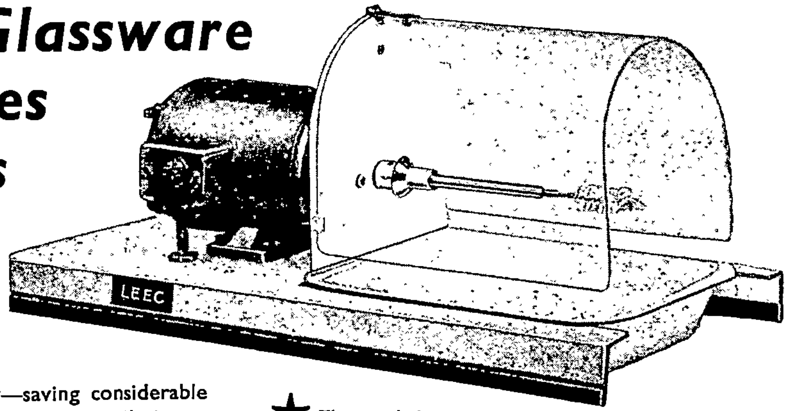
Especially suitable for Test Tubes, Syringes, Vials, Bottles and other small Glassware—

Cleans thoroughly with speed and safety—saving considerable time in the modern busy laboratory. The brush is easily interchangeable, and the durable plastic Spray Shield can be tilted for cleaning. The Drip Tray simply slides into the base. A strongly built machine provided with a special "on/off" switch for independent control. The base is nickel plated and finished in white stove enamel. Light and portable. **£17.2.0.**

★ *Thousands in use in numerous Hospitals and Laboratories throughout the country. Enquiries to-*

LABORATORY & ELECTRICAL ENGINEERING CO.
71–73 Goldsmith Street — Nottingham
Tel. Nottm. 48331

LEEC



Pickstone

NEW IMPROVED
GENERAL PURPOSE
THERMOSTATIC OVEN

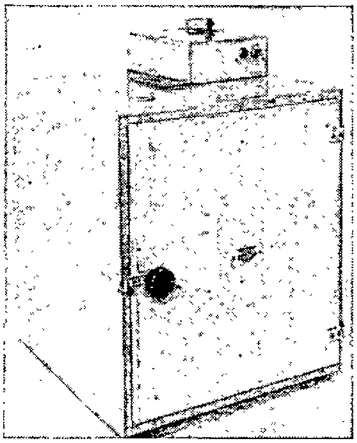
£26 complete carriage paid U.K.

Exterior : 15 in. × 15 in. × 21 in.
Interior : 12 in. × 12 in. × 14 n.
Operating Temp. : 30° C to 260° C
Temp. variation : at 100° C about ± 2.5° C
Heating up time : to 260° C in one hour
Temp. Constancy : better than ± 1° C
Overshoot : not more than app. 5° C

The Oven is 1½ in. fibreglass-insulated on all sides and door, and is fitted with 3 weldmesh trays, vent, thermometer aperture, and calibrated thermostat. Mild steel construction, finished Cloud Grey enamel.

Order with confidence or request detailed leaflet :

R. E. PICKSTONE LTD.
36 Avon Trading Estate, Mornington Avenue, London, W.14.
FULham 4558



ZENITH
(REGD. TRADE-MARK)

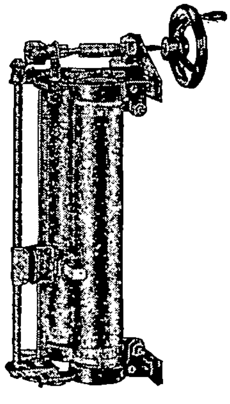
TUBULAR SLIDING RESISTANCES

Back-of-Board Types

Rugged, robust, reliable rheostats, ratings from 200 to 2,000 watts. Clutch fitted to prevent over turning. Laminated phosphor bronze contacts fitted as standard to avoid residual resistance at all-out position. Carbon contacts available when desired.

Illustrated catalogue of all types free on request

The ZENITH ELECTRIC CO. Ltd.
ZENITH WORKS, VILLIERS RD., WILLESDEN GREEN
LONDON, N.W.2
Phone : WILlesden 6581-5 *Grams :* Voltaohm, Norphone, London
MANUFACTURERS OF ELECTRICAL EQUIPMENT
INCLUDING RADIO AND TELEVISION COMPONENTS



Detailed results of the investigations will be presented elsewhere.

Acknowledgment is made to the Council of Scientific and Industrial Research, India, for financial assistance.

S. C. Agarwala
A. Kumar
C. P. Sharma

Department of Botany,
University of Lucknow,
Lucknow.

[1] Hewitt, E. J., *Ann. Rep. Long Ashton Res. Sta.* (1948).
[2] Smith, P. F., and Specht, A. W., *Plant Physiol.*, **28**, 371 (1953).
[3] Crooke, W. M., *Ann. App. Biol.*, **43**, 465 (1955).
[4] Wallace, J. M., Dissertation Abstr., **17**, 2398 (1957).
[5] Agarwala, S. C., and Kumar, A., *Proc. Forty-seventh Indian Sci. Congr.*, Pt. 3, 399 (1960).
[6] Agarwala, S. C., and Kumar, A., *J. Indian Bot. Soc.* (in the press).
[7] DeKock, P. C., *et al.*, *Plant. Physiol.*, **35**, 599 (1960).

ENTOMOLOGY

An Abnormal Form of Female Rat Flea, *Xenopsylla cheopis* Roths.

Rothschild, in 1911, separated the three species of fleas, *X. cheopis*, *X. astia* and *X. brasiliensis* of the genus *Xenopsylla* found on rats. *X. cheopis* is widely distributed in the tropics and is the principal plague flea in India. The three species (♀♀) mentioned here can be easily identified by the shape of the spermatheca. Until now, it has been reported that *X. cheopis* has a single spermatheca[1-3] (Fig. 1).

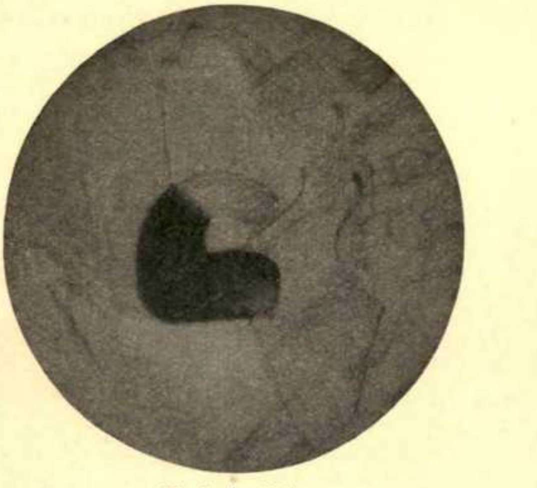


Fig. 1. (× 132)

During a routine collection of rat fleas from Delhi City for conducting insecticide susceptibility tests, a single specimen of *X. cheopis* (♀♀) was observed which has the normal typical spermatheca and another spermatheca-like structure (Fig. 2). The normal spermetheca has all the characteristics of a typical specimen: (1) the tail is much longer and at the base it is slightly wider than at the head; (2) the terminal part of the tail is longer and narrows gradually; and (3) the lower margin of the head and tail-end are at about the same level (Fig. 2, *A*). The additional spermatheca-like structure (the tail-end of which is just close to the terminal part of the tail of the typical form (Fig. 2, *B*) is pear-shaped and has a small blunt tail, but no distinct head portion.

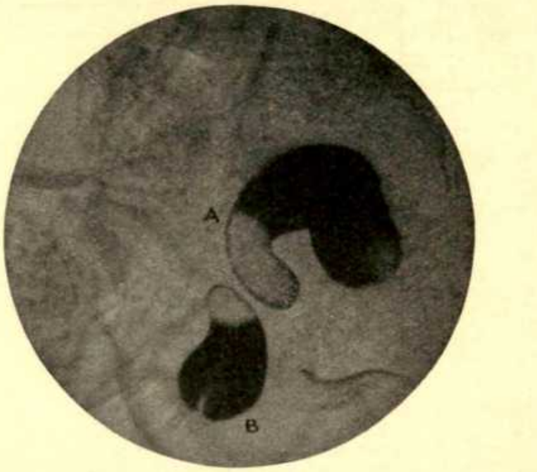


Fig. 2. (× 132)

There has been no previous record in the literature, so far as we know, of the occurrence of a similar abnormality in *X. cheopis* (♀♀).

M. I. D. Sharma
G. C. Joshi

Malaria Institute of India,
Delhi.

[1] Jordan, K., *A Handbook for the Identification of Insects of Medical Importance* by Smart, J. (printed by order of the Trustees of the British Museum, 1956).
[2] Pollitzer, R., *Plague*. World Health Organization, Monograph Series No. 22 (World Health Organization, Palais des Nations, Geneva, 1954).
[3] Roy, D. N., and Brown, A. W. A., *Entomology* (*Medical and Veterinary*) (Excelsior Press, Calcutta 12, 1954).

MICROBIOLOGY

Role of the Pentose Phosphate Pathway in *Pasteurella multocida*

It is acknowledged that in a large number of bacteria the main path for carbohydrate oxidation is the pentose phosphate cycle or other metabolic sequences not involving the Embden–Meyerhof pathway[1]. In the genus *Pasteurella*, work on *P. pestis* has shown that growing cells oxidize glucose through the pentose phosphate pathway[2], but resting cells oxidize glucose through the combined action of glycolysis and the citric acid cycle[3]. In contrast with this metabolic duality, in *P. multocida* the pentose phosphate cycle is the main path for carbohydrate oxidation, both in growing and resting cells, and in regard to the citric acid cycle its principal role seems to be to supply carbon skeletons for the synthesis of amino-acids. These conclusions are borne out by experiments carried out with *P. multocida* (strain Beaufort No. 28 from the Institut Pasteur cultured aerobically for 4 hr. (growing cells) or 24 hr. (resting cells)) at 37° and *p*H 7·2 in a medium made of meat extract, 5 gm.; yeast extract (Difco), 1 gm.; peptone, 30 gm.; sodium chloride, 5 gm.; glucose, 20 gm.; agar, 25 gm. and water up to 1 litre. Our observations can be summarized as follows.

(*a*) Resting *P. multocida* oxidizes glucose, fructose, gluconate and ribose. The Qo_2 values (μl. oxygen consumption/mgm. dried cells/hr.) of the cells incubated with 5 m*M* substrate, in m*M* phosphate buffer *p*H 7·2 at 37°, were 24 with glucose or fructose, 18 with gluconate and 12 with ribose (control, 2·3–2·6).

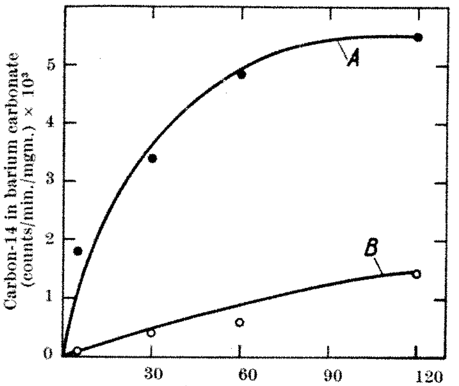


Fig. 1. Radioactivity of carbon dioxide evolved during the oxidation of [1-^{14}C] and [6-^{14}C] glucose by *P. multocida*. Incubation in double side-arm Warburg manometers. 23·2 mgm. (dry weight) of bacterial cells suspended in m*M* phosphate buffer, *p*H 7·2 with 5·3 m*M* [^{14}C] glucose (3·70 × 10^6 counts/min.); total volume, 2·65 ml.; 0·15 ml. 2·7 *N* potassium hydroxide in the centre well and 0·20 ml. 3 *N* sulphuric acid in one side-arm; air in the gas space; temperature, 37°. At the intervals stated, the acid is tipped into the main compartment of the flask and the incubation continued for 15 min. The carbonate fixed in the alkali is precipitated as barium carbonate for carbon-14 counting as described previously (ref. 5). Line *A*, [1-^{14}C]-glucose; line *B*, [6-^{14}C] glucose. Qo_2, 22·2; control (without glucose), 1·3

(*b*) The specific activity of the carbon dioxide produced by oxidation of [1-^{14}C] glucose with resting cells of *P. multocida* is much higher than with [6-^{14}C]-glucose (Fig. 1). An approximate evaluation of the contribution of the pentose phosphate cycle to glucose oxidation can be calculated[4] by extrapolating to zero time the ratio of the specific activities of the carbon dioxide produced from [6-^{14}C] glucose and [1-^{14}C] glucose, respectively. From the results shown in Fig. 1, about 85 per cent glucose should be oxidized through the pentose phosphate cycle. With growing *P. multocida* the results are similar.

(*c*) After oxidation of carbon-14 glucose (1, 6 or *G*) by resting or proliferating *P. multocida*, the cells incorporate carbon-14. Paper chromatography and radioautography of methanol–water extracts[5] of the cells show the labelling of: (1) glucose, glucose-6-phosphate, fructose-1,6-diphosphate, 6-phosphogluconate, ribose-5-phosphate, and sedoheptulose-7-phosphate; (2) lactic, succinic, fumaric and malic acids; and (3) glutamic and aspartic acids, alanine, threonine, leucine, tyrosine and valine-tryptophan.

During the oxidation of [6-^{14}C] glucose by resting *P. multocida*, the kinetics of distribution of carbon-14 (per cent) in the sugar phosphates (and glucose) fit into the pattern of the shunt pathway. In fact, in the 0–3 min. interval, carbon-14 appears in the sugar phosphates in the order (1) glucose-6-phosphate, (2) 6-phosphogluconate and (3) ribose-5-phosphate, sedoheptulose-7-phosphate and fructose-1,6-diphosphate. After incubation for 3 min., variations in 6-phosphogluconate, ribose-5-phosphate and sedoheptulose-7-phosphate run closely parallel. On the other hand, the percentage of carbon-14 in glucose remains nearly constant. With [1-^{14}C] glucose as substrate, glucose-6-phosphate and 6-phosphogluconate again are labelled first, but the percentage of carbon-14 in the glucose reservoir decreases very rapidly. Similar kinetics are found with growing cells.

(*d*) In the presence of radioactive bicarbonate, gluconate oxidation promotes fixation of carbon-14 by resting *P. multocida*. It appears in glucose-6-phosphate, as would be expected from the reversibility of 6-phosphogluconate decarboxylation[6].

(*e*) Resting *P. multocida* oxidize pyruvate and the intermediates of the citric acid cycle. With substrate concentration 10 m*M*, the Qo_2 values (measured as in *a*) were 24 (pyruvate), 3·7 (citrate), 6·2 (DL-isocitrate), 19 (α-oxoglutarate), 26 (succinate), 13 (fumarate), 5 (L-malate) and 3·5 (control). After oxidation of [2-^{14}C] pyruvate, carbon-14 appears in the carbon dioxide evolved, the intermediates of the citric acid cycle and amino-acids. Growing cells incorporate more carbon-14 into the amino-acids (Table 1). It is to be noted that the labelling of cysteic acid shows that the bacterium is able to fix sulphur into organic substances.

Table 1. DISTRIBUTION (PER CENT) OF CARBON-14 IN AMINO-ACIDS AFTER OXIDATION OF [2-^{14}C] PYRUVATE BY *P. multocida*

Growing cells: 65 (initial) and 78 (final) mgm. *P. multocida* (dry weight) suspended in 22·4 ml. culture medium (see text) with 25·2 μmoles [2-^{14}C] pyruvate (17·76 × 10^7 counts/min.).
Resting cells: 142 mgm. bacteria suspended in 10 ml. m*M* phosphate buffer, *p*H 7·2, with 112 μmoles [2-^{14}C] pyruvate (8·8 × 10^7 counts/min.). Temperature, 37°. After 60 min. incubation, the suspensions were spun off and the cells suspended in 9 ml. methanol for paper chromatography, radioautography and carbon-14 counting (ref. 5).

Amino-acid	Carbon-14 fixed (percentage of total in the methanol–water soluble substances) Growing cells	Resting cells
Aspartic	0*	0·3
Glutamic	2·5	3·3
Alanine	5·6	0·4
Serine	2·1	0·3
Glycine	1·6	0·6
Threonine	6·6	0
Histidine	5·3	0
Lysine–Arginine	5·3	0·7
Leucine	2·8	0·2
Tryptophan–valine	6·0	0
Cystine	0	0·2
Cysteic	0	11·6

* Not detectable on the radioautogram.

In proliferating *P. pestis*, the shunt pathway provides pentose phosphates for nucleotide synthesis[3], and this can also be readily accepted in *P. multocida*; on the other hand, the pentose phosphate cycle also has a synthetic role in resting *P. multocida* as source of the pentose and heptose components of the polysaccharides of the bacteria[7].

INDA M. DE ISSALY*
A. ISSALY*
A. O. M. STOPPANI

Institute of Biochemistry,
School of Medicine,
University of Buenos Aires.

* On leave from the Instituto Nacional de Microbiologia.

[1] Wood, H. G., *Physiol. Revs.*, **35**, 841 (1955). Elsden, S. R., and Peel, J. L., *Ann. Rev. Microbiol.*, **12**, 145 (1958).
[2] Santer, M., and Ajl, S., *J. Bact.*, **69**, 713 (1955).
[3] Santer, M., and Ajl, S., *J. Bact.*, **69**, 298 (1955). Englesberg, E., and Levy, J. B., *J. Bact.*, **69**, 418 (1955).
[4] Korkes, S., *Ann. Rev. Biochem.*, **25**, 685 (1956).
[5] Stoppani, A. O. M., Conches, L., de Favelukes, S. L. S., and Sacerdote, F. L., *Biochem. J.*, **70**, 438 (1958).
[6] Horecker, B. L., and Smyrniotis, P. Z., *J. Biol. Chem.*, **196**, 135 (1952). Moses, V., Holm-Hansen, O., and Calvin, M., *J. Bact.*, **77**, 55 (1959).
[7] Briefman, L., and Yaw, K. E., *J. Bact.*, **75**, 236 (1958). MacLennan, A. P., and Rondle, C. J. M., *Nature*, **180**, 1045 (1957).

Spontaneous Development of *L* Forms of Streptococci requiring Secretions of Other Bacteria or Sulphydryl Compounds for Normal Growth

CERTAIN strains of non-hæmolytic streptococci, isolated in our laboratory from blood cultures of cases of subacute bacterial endocarditis and from otitis media, were found to require for their growth a substance secreted by most bacteria. They were isolated only due to the incidental presence of

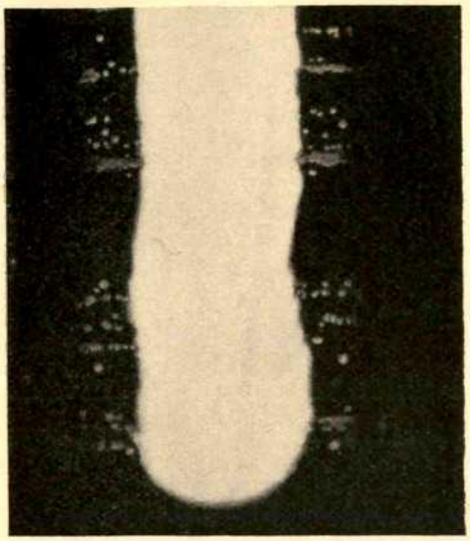


Fig. 1. Incomplete satellite growth zone of *Streptococcus* 6626. (Polarized light. × 5)

colonies of contaminating bacteria, around which they grew in satellite colonies. The bacterial secretion could be replaced by some sulphydryl compounds. In the absence of such substances, these strains tend to spontaneous development of *L* forms if an appropriate osmotic stabilizer is present in the agar medium. The sulphydryl compounds as well as the bacterial secretion seem to play a part in the formation of the cell wall of these streptococci.

The zone of satellite growth of the strains as shown on plates of *B.B.L.* soy trypticase agar or Difco heart infusion agar containing 5 per cent serum differed significantly from that known from *Haemophilus influenzae*, being much narrower and ending with a rather sharp edge (Fig. 1) which was accentuated if by incubating for a further 24 hr. the unused nutrients of the surrounding agar medium caused the outer colonies to grow in size.

A great variety of bacteria—Gram-positive and Gram-negative, catalase-positive and catalase-negative—including bacteroides, as well as actinomycetes and yeasts promoted growth.

The first substance found to be capable of substituting for the bacterial secretions was cysteine in concentrations of 0·5–1 mgm./ml. Its growth-promoting influence could not be explained by change of redox potential only, as anaerobic conditions did not alter the growth requirements of the strains.

Sodium thioglycollate, reduced glutathione and thiomalic acid were effective. Cystine, cysteic acid, methionine, sodium thiosulphate (up to 1 mgm./ml.) and elementary sulphur were not effective. It was therefore concluded that growth promotion by those substances depends on the sulphydryl groups.

The morphology of the streptococci was normal if growth was optimal. With lower concentrations of sulphydryl group substances, or at the outer limit of the satellite growth zone, the cocci showed extreme pleomorphism with swellings, global forms and elongations of different degrees. This suggested that the substance secreted by other bacteria, or the cysteine and like substances, had some function in the formation of cell walls, and that growth in *L* colonies might be produced without growth-promoting substances if support were given by adding to the medium substances known to have an osmotically stabilizing effect on bacterial cells the wall of which developed incompletely or not at all.

On plates of heart infusion agar + 5 per cent pooled human serum + 2 and 5 per cent sucrose respectively, two of the streptococcal strains grew in typical *L* colonies. The addition of sucrose to the medium had a remarkable influence also on the satellite growth of the streptococcus. Satellite colonies farther off were gradually diminished in size down to small transitional colonies, containing normal streptococcal forms together with swollen forms and net-like structures. Still farther off, beyond the influence of the bacterial secretion, there was pure growth of typical *L* colonies (Figs. 2 and 3). Many of these *L* forms remained stable even in the presence of other bacterial colonies or of cysteine.

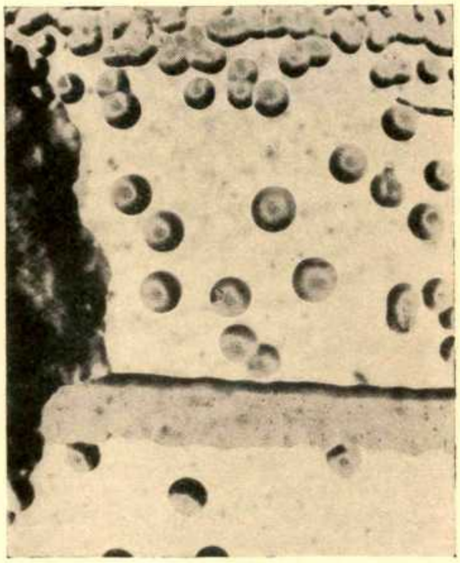


Fig. 2

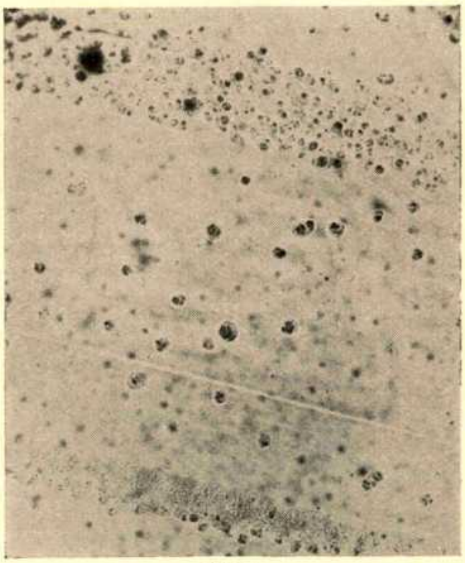


Fig. 3

Figs. 2 and 3. Development of normal satellite colonies (near growth of *E. coli* (Fig. 2) and of *L* colonies (beyond influence of bacterial secretion) (Fig. 3) from the same plating of *Streptococcus* 3554 on heart infusion agar containing 5 per cent human serum and 2 per cent sucrose. (× 35)

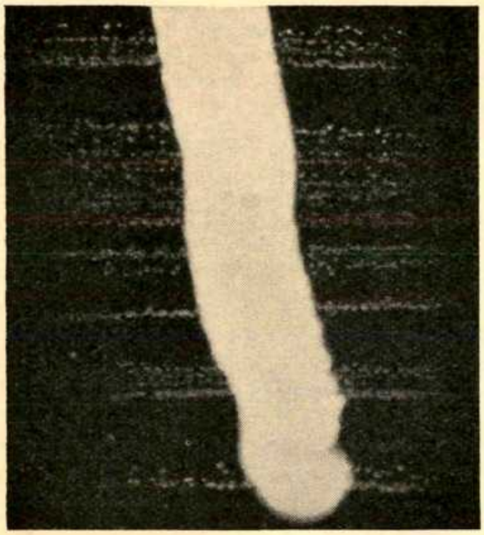


Fig. 4. 'Normalized' satellite growth zone of *Streptococcus* 6626 obtained under anaerobic conditions. (Polarized light. × 5)

Another of the streptococcal strains described here did not produce typical *L* colonies if sucrose was used as stabilizer, but in the presence of 1·5 per cent sodium chloride it grew colonies consisting of 'large bodies', swollen forms not stained by the Gram technique, and net-like structures.

A fourth strain also did not grow in typical *L* colonies, but on either of these media colonies developed which may be described as transitional stages containing a mixture of globular forms, swollen and elongated cocci stainable by the Gram technique, and of net-like structures. Possibly we have not yet found the appropriate osmotic stabilizer for the support of the *L* forms of these two strains.

Only living bacteria had an effect in promoting growth. The secreted substance passed through 'Cellophane'. It lost its growth-promoting property rapidly by oxidation. Obviously it was this lability which accounted for the abnormal appearance of the satellite growth zone, the growth-promoting substance losing its activity while diffusing into the more distant parts of the agar. Under anaerobic conditions, satellite colonies developed not only in a zone much broader than that obtained aerobically but also gradually diminished in size in the outer parts of the zone (Fig. 4).

Cysteine (1 mgm./ml.) or other sulphydryl compounds are known to be essential for growth from small inocula of hæmolytic streptococci[1]. Although our strains require cysteine even for growth from large inocula, basically the requirement might be the same. Nickerson and van Rij[2] found that sulphydryl compounds promoted cell division.

The effect of penicillin in inducing growth of *L* forms and the resistance of *L* forms to penicillin is well known. According to Dufrenoy and Pratt[3], penicillin inhibits the reduction of —S—S bonds of the bacterial cell constituents, whereas cysteine is known as a neutralizer of penicillin[4]. Nickerson[5] showed that a mutant of *Candida albicans* lacking an enzyme capable of reducing —S—S bonds of the cell wall grew in filaments instead of growing in the yeast form.

In our strains it may be assumed that we are dealing with an inability to reduce —S—S bonds required for the formation of the cell wall as a genetic metabolic defect, which is not necessarily lethal if appropriate stabilizers are present enabling them to continue life as *L* forms. There is an obvious analogy to the *L* form which Lederberg and St. Clair[6] succeeded in obtaining in sucrose agar from a mutant of *E. coli* deficient in diaminopimelic acid.

The possibility of using the specific growth requirement of these streptococci for development of a bioassay for sulphydryl groups is being studied.

AYALA FRENKEL
WILLY HIRSCH

Department of Bacteriology,
Central Laboratory of the Kupat Holim,
Haifa.

[1] Ginsburg, I., and Grossowicz, N., *Proc. Soc. Exp. Biol. Med.*, **96**, 108 (1957).

[2] Nickerson, W. J., and van Rij, N. J. W., *Biochem. et Biophys. Acta*, **3**, 461 (1949).

[3] Dufrenoy, J., and Pratt, R., *J. Bact.*, **53**, 657 (1947).

[4] Chow, B. F., and McKee, C. M., *Proc. Soc. Exp. Biol. Med.*, 58, 175 (1945). Hickey, R. J., *Science*, **101**, 232 (1945). Muir, R. H., and Valley, G., *Science*, **101**, 390 (1945).

[5] Nickerson, W. J., abstracts of papers, 130th Meeting, Amer. Chem. Soc., Atlantic City, 15 *C* (1956).

[6] Lederberg, J., and St. Clair, J., *J. Bact.*, **75**, 143 (1958).

Specificity of the Mutagenic Action of the Alkylating Agents

ETHYL methanesulphonate is mutagenic when applied to mature bacteriophage particles in suspension[1]. Methyl methanesulphonate is either non-mutagenic or results in the induction of mutants at a frequency not much greater than the spontaneous frequency[2]. Krieg (quoted by Bautz and Freese[2]) has reported that ethyl methanesulphonate will not induce reversion of bacteriophage mutations induced by itself. Mutants induced by ethyl methanesulphonate can be induced to revert with 2-aminopurine[2]. Methyl methanesulphonate is much more lethal on a molar basis than is the ethyl derivative[1].

Freese accounts for the specificity of the ethyl derivative by supposing that both ethyl and methyl methanesulphonates combine with guanine residues within the deoxyribonucleic acid (DNA), but that only the ethylated guanine breaks down to form an apurinic acid at an appreciable rate. The apurinic acid is assumed to lead to errors in pairing, resulting in mutation during vegetative growth of phage.

Treatment of bacteriophage *T*2 with ethyl methanesulphonate labilizes the phage, so that the number of plaque-forming units diminishes when the treated phage is stored in the absence of the alkylating agent[1]. It is implied by the Freese hypothesis (but is not essential to it) that this post-treatment decay in plaque-forming units represents the progressive depurination of the alkylated DNA.

I have found, in confirmation of earlier results[1], that treatment of bacteriophage *T*2 with methyl methanesulphonate results in a post-treatment decay effect comparable to that induced by ethyl methanesulphonate. The degree of decay after treatment depends on the initial concentration of the alkylating agent used for treatment (Fig. 1). The methyl methanesulphonate effect occurs at a lower molarity but at a higher immediate inactivation than does the ethyl methanesulphonate effect. Treatment of bacteriophage with alkylating agents causes both an immediate and a delayed decrease in plaque-forming centres and may result in an appreciable frequency of induced mutation, depending on the agent. In this

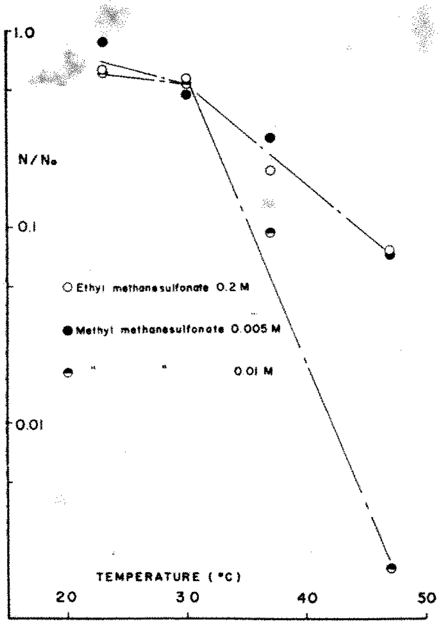


Fig. 1. Post-treatment decay of bacteriophage *T*2 following treatment with alkylating agents.
Approximately 10^{12} phage per ml. were incubated in *M*-9 buffer with the indicated alkylating agent for 40 min. at 37° C. The survivals immediately following treatment were: non-treated, 1·0; 0·2 *M* ethyl methanesulphonate, 0·97; 0·005 M methyl methanesulphonate, 0·57; 0·01 *M* methyl methanesulphonate, 0·16. Following treatment, the suspension was diluted by a factor of 10^3 with *M*-9 buffer and samples were stored 21 hr. at the temperatures indicated. All values are corrected for the decay of non-treated material kept at the same temperature. Plaque-forming centres were determined by the agar layer method. The results are not plotted as an Arrhenius curve in order to indicate lack of a theoretical interpretation for the concentration effect

experiment the frequency of *r* plus mottled mutants in the material treated with ethyl methanesulphonate was about 7×10^{-3} immediately after treatment, 8×10^{-3} after 21 hr. at 30° C. and 6×10^{-3} after 21 hr. at 47° C. This frequency was five times the spontaneous. The number of mutants scored was so small that the frequency of mutants in the material treated with methyl methanesulphonate was not significantly different from the spontaneous frequency. In all parts of the experiment, 20 mutants per 19,000 total plaques were scored in the non-treated set, 89 per 17,000 plaques in the set treated with ethyl methanesulphonate, and 9 per 12,400 and 19 per 15,000 plaques in the sets treated with 0·005 *M* and 0·01 *M* methyl methanesulphonate, giving frequencies of 1·0, 5·2, 0·7 and $1·3 \times 10^{-3}$ respectively.

Table 1. EFFECT OF METHYL METHANESULPHONATE IN INDUCING REVERSIONS OF *E. coli WP*-2

Treatment	Fraction surviving determined on: Minimal + tryptophan	Fraction surviving determined on: Minimal + 2·5 per cent broth	Total revertants per ml.*	Revertants/ 10^8 survivors
None	1·0	1·0	60	13
Ethyl methane-sulphonate (0·1 *M*)	0·87	0·87	3,120	780
Methyl methane-sulphonate				
0·025 *M*	0·43	0·5	710	308
0·05 *M*	0·0005	0·0096	150	3,400

* Revertants were scored after 2 days incubation at 37° C. on plates of minimal medium agar supplemented with 2·5 per cent Difco nutrient broth (8 gm./l.). Treatment was for 30 min. in 0·1 *M*, *p*H 7·2, phosphate buffer. The cells were collected and washed from exponentially growing cultures in minimal medium plus tryptophan before treatment and then collected, washed and re-suspended in buffer following treatment.

There were $4·6 \times 10^8$ viable organisms per ml. in the non-treated sample.

Methyl methanesulphonate is highly mutagenic for the tryptophan-requiring *WP*-2 derivative of *E. coli* strain *B*/*r* (Table 1). An increased frequency of both large colonies (presumed revertants) and small colonies (presumed suppressors) results from treatment with this alkylating agent. The frequency is comparable with that induced by ethyl methanesulphonate. Methyl methanesulphonate causes an effect similar to that of ultra-violet irradiation of *B*/*r*, since treated bacteria give higher viability counts on medium supplemented with broth than on a synthetic medium[3]. There seems to be no post-treatment decay effect in bacteria comparable to that observed with bacteriophage[4]. It is known that in *Neurospora* dimethyl sulphate is even more effective as a mutagen at certain loci than is either diethyl sulphate or ethyl methanesulphonate[5].

I conclude, therefore, that the post-treatment decay reaction following treatment of bacteriophage with alkylating agents is not directly related to the mutagenic reaction, and that either the mechanism of the mutagenic action of alkylating agents differs in mature bacteriophage from that in bacteria, or that the specificity of the ethyl derivatives for bacteriophage mutagenesis is not completely explained.

This investigation was supported in part by a research grant from the National Institutes of Health, U.S. Public Health Service.

BERNARD S. STRAUSS

Department of Microbiology,
University of Chicago.

[1] Loveless, A., *Proc. Roy. Soc.*, B, **150**, 497 (1959).

[2] Bautz, E., and Freese, E., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1585 (1960).

[3] Alper, T., and Gillies, N., *J. Gen. Microbiol.*, **22**, 113 (1960).

[4] Loveless, A., and Stock, J. C., *Proc. Roy. Soc.*, B, **151**, 129 (1959). Strauss, B., *J. Bacteriol.*, **81**, 573 (1961).

[5] Westergaard, M., *Experientia*, **13**, 224 (1957).

SOIL SCIENCE

Resistance of Humus to Decomposition

AN organic soil (previously under wattle followed by grass) containing 8·51 per cent organic carbon (Walkley and Black[1], corrected) has been submitted 204 times to the following cycle: (*a*) moistened to field capacity; (*b*) allowed to decompose for 5 days at 25° C. in the macro-respirometer[2]; (*c*) oven-dried at 100° C. for 48 hr. After 105 such treatments it was calculated[3] that no further decomposition would occur, on moistening, after about the 250th treatment and that 73 per cent of the organic carbon originally present would have been mineralized. After 204 such treatments, however, the soil had become completely structureless and on moistening formed cohesive lumps, a condition adverse to aerobic decomposition. The experiment was therefore concluded and the soil analysed. A comparison of the results for the soil before and after the 204 treatments is given in Table 1.

The agreement between the calculated value for carbon mineralization after 250 treatments (73 per cent) and that found after 204 treatments (63·5 per cent) is satisfactory, particularly since the calculated value (based on the first 105 treatments) did not allow for the subsequent loss of structure during the later treatments, leading to decreased microbial activity and mineralization of organic carbon.

The main feature of interest is the high proportion of organic carbon and nitrogen that remained un-

Table 1

	Before	After	Mineralized (per cent)
Organic carbon (per cent)	8·51	3·11	63·5
Organic nitrogen (per cent)	0·69	0·37	46·4
C : N ratio	12·33	8·41	
*p*H (using 0·01 *M* calcium chloride)	6·0	7·9	
p.p.m. inorganic phosphorus (cold *N* sulphuric acid)	133	570	
p.p.m. organic phosphorus (Walker and Adams, ref. 4)	437	Nil	100·0

mineralized in spite of the enhanced microbial activity following the frequent moistenings of the soil after oven-drying. This is in marked contrast to organic phosphorus, which was completely mineralized. Thompson and Black[5], however, have shown that soil organic phosphorus is susceptible to hydrolysis with hot water. A duplicate soil sample was therefore kept at field capacity at 100° C. for 16 days (a period equivalent to that in which the experimental soil was in a hot moist state during its 204 treatments) when it was found that 53 per cent of the organic phosphorus and 7 per cent of the organic nitrogen were hydrolysed, with no increase on longer heating. With the experimental soil, therefore, the non-hydrolysable organic phosphorus compounds must have been microbially decomposed together with at least part of the hydrolysable compounds which are likely to be even more susceptible to microbial attack. It appears that potentially the whole of the soil organic phosphorus is fairly readily decomposable microbially. Since organic phosphorus complexes of soil are more readily extracted than other fractions of soil organic matter[6] they may also be more accessible to micro-organisms.

The increase in *p*H probably results from the release of bases during decomposition and the absorption of carbon dioxide by the sodium hydroxide in the respirometer vessel, which would prevent the formation of carbonic acid. The increase in ammonia amounted to only 1 m.equiv./100 gm. soil instead of the 23 m.equiv. demanded by theory. Presumably much of the ammonia was driven off under the hot, moist slightly alkaline conditions occurring early in the successive drying periods.

H. F. Birch
M. T. Friend

East African Agriculture and
Forestry Research Organisation,
P. O. Box 21,
Kikuyu, Kenya.

---

[1] Walkley, A., and Black, I. A., *Soil Sci.*, **37**, 29 (1934).

[2] Birch, H. F., and Friend, M. T., *Nature*, **178**, 500 (1956).

[3] Birch, H. F., *Plant and Soil*, **12**, 81 (1960).

[4] Walker, T. W., and Adams, A. F. R., *Soil Sci.*, **85**, 307 (1958).

[5] Thompson, L. M., and Black, C. A., *Soil Sci. Soc. Amer. Proc.*, **12**, 323 (1947).

[6] Bremner, J. M., *J. Soil Sci.*, **2**, 67 (1951).

PSYCHOLOGY

Effect of Attention in Peripheral Vision

The facts adduced by B. Babington Smith[1] appear to be new. At first it seemed that they might be explained in terms of what Stout calls "the conditions of attention"[2]—conditions that include change as well as extensity, intensity, duration, contrast and interest. If Stout is right, the factor missing in the object was change. I therefore repeated the experiment with objects containing moving parts, like the second-hand of a watch and an oscillating compass needle, and was amazed to find that the peripheral fade-out was, if anything, more marked.

The explanation of the phenomenon is, I believe, the following. When one inhibits the natural tendency to focus an object to which one is attending, one inevitably tends to reconstruct its details from memory. Though perception and memory are not inherently incompatible, the simplest kinds of image are, in fact, antagonistic to their sensational counterparts. Hence the effort to reconstruct the object from memory inhibits the exact perception of it. The most important inference to be drawn from the experiment is not that attention inhibits the perception of peripheral objects, but that the instinct of curiosity in its simpler forms operates by means of a commutative mechanism, whereby it automatically switches over to memory, when it is frustrated in perception. Experiments might be devised to show under what conditions the mind automatically switches back to perception, and, once the main facts had been elucidated, considerable improvements in methods of teaching and learning might be effected.

Arthur Thomson

c/o Dr. C. B. Robinson,
Purdysburn Hospital,
Belfast 18.

---

[1] *Nature*, **189**, 776 (1961).

[2] Stout, G. F., *Manual of Psychology*, fourth ed., 167.

In my communication of March 4, I spoke of placing objects on a table. As Mr. Thomson has deduced, the situation I described was static, and thus change, which is, broadly speaking, one of the essential conditions for perception, was lacking. Mr. Thomson's observations with the second-hand of a watch and a compass needle are very interesting but puzzle me, for I have failed to find what he describes. Others as well as myself have found that objects in motion do not disappear; though one correspondent has spoken of 'flicker', seen peripherally, disappearing when attended to.

The second part of Mr. Thomson's letter also puzzles me; while I can conceive that "the effort to reconstruct an object from memory inhibits the exact perception of it", it seems to me that switching over "by means of a commutative mechanism", as he suggests, ought to produce a memory image in place of the percept which disappears. This is not, however, what seems to happen.

Finally, Mr. Thomson writes that the facts I adduced appear to be new. Having failed to locate similar observations in Aubert[1], Baird[2] or Helmholtz[3], I had hoped this was so. Since publication, however, further search suggests that observations by Troxler[4], who was studying reports of a blind spot, and (according to Helmholtz's reference to their work) by Aubert and Förster[5], who were studying the sensitivity of the retina, refer to differential disappearance of objects in the peripheral field in a way conformable to what I have described.

Even nearer to the mark is a note by J. H. Hyslop[6], to which Prof. R. C. Oldfield has directed my attention, in which the following occurs: "When mentally preoccupied and having the eyes fixated on a given point or object I often notice the disappearance of a part of the indirect field of vision.

I have tried to see whether it might not be due to the falling of the object on the blind spot; but uniformly discover that it is not, as the disappearing object may be on the side of the retina opposite the blind spot. On careful experiment and observation I find that the disappearance is directly related to the degree of abstraction, and that I can reproduce it artificially, if I am successful, as I sometimes am, in effecting the abstraction necessary and at the same time the proper adjustment of attention. It is difficult to produce the artificial abstraction required, but when I am successful I effect the disappearance of the object, which immediately reappears the moment attention is given to it without altering the fixation of the eyes. The effect seems to be that of making clear an actual impression, while attention in the previous experiment seems to destroy an after image."

Hyslop's use of the term 'abstraction' in contrast with 'attention' is interesting, and supports the view of several correspondents, particularly Dr. N. Moray of Sheffield, that the word 'attention' in my communication in *Nature* is a source of difficulty. It was of course with this possibility in mind that I had written "attend to one of the objects and try to see or describe it in detail".

Prof. O. L. Zangwill has suggested that the mechanism of disappearance is related to Hering's local adaptation, but while this may be so, I find no evidence of Hering having associated local adaptation with attention.

B. BABINGTON SMITH

Institute of Experimental Psychology,
University of Oxford,
1 South Parks Road,
Oxford.

---

[1] Aubert, H., *Moleschotts Untersuchungen zur Naturl.*, B2V, 279 (1858).

[2] Baird, J. W., *The Color Sensitivity of the Peripheral Retina* (Wash., Carnegie Inst., 1905).

[3] Helmholtz, H. von, *Physiological Optics*, 1924 ed., **2**, 42 and elsewhere (1866).

[4] Troxler, D., Himly and Schmidt, *Opthalmologische Bibliothek*, **2**, 2 (1804).

[5] Aubert, H., and Förster, O., *Allg. med. Zentralzeitung, Berlin*, No. 33, 259 (as summarized in a sentence by Helmholtz) (1957).

[6] Hyslop, J. H., *Psychol. Rev.*, **10**, 296 (1903).

[7] Hering, E., *Grundzuge der Lehrer von Lichtsinn*, 265 (1920), and *Archiv für Ophthalm. (Graefe)*, **37**, Abt. III, 31 (1891).

Centrally Produced Geometrical Illusions

So little is known about the mechanism responsible for the well-known geometrical illusions that it is not possible to decide on general theoretical grounds whether they are the result of retinal processes, central processes or both. An experimental procedure to demonstrate that they are at least partly of central origin is therefore of interest.

The procedure consists of constructing a visual target, in which the illusion figure is seen in stereoscopic vision but not in monocular vision. The simplest way of achieving this is as follows. A sheet of glass is covered with an array of randomly placed dots. On a second glass sheet the well-known Müller–Lyer illusion, for example, is constructed out of dotted lines, the separation of the dots being about the same as those on the first sheet. The two are then mounted parallel to one another, separated by a few inches. When viewed monocularly under conditions which prevent head movements, shadows and other sources of monocular distance clues, the two planes are seen as merged and the Müller–Lyer figure cannot be distinguished. In binocular vision the two planes are easily separated and all subjects report the illusion.

The rigour of the experiment can be improved in two ways: (1) if the three vertices A, B, C, of the figure are marked by dots of a different colour, judgment of distance AB compared with distance BC can be made, and in fact turns out to be significantly different, under monocular and binocular conditions; (2) instead of using two real planes, the situation can be simulated by constructing stereoscope diagrams using an electronic computer[1]. This enables a rigorous control of factors such as dot density, and ensures that the signal received by each eye is a thoroughly pseudo-random array of dots; the information used by the stereoscopic mechanism is entirely contained in the relation between the two arrays.

A quantitative comparison of illusions produced in this way with their more familiar form involves intricate statistics and will be reported elsewhere. Figural after-effects can be produced under similar conditions, but are always weak, though I believe that this is due to incidental aspects of the procedure.

The work described here is part of the research programme of the National Physical Laboratory and this communication is published by permission of the Director of the Laboratory.

S. PAPERT

Autonomics Division,
National Physical Laboratory,
Teddington, Middlesex.

---

[1] Julesz, B., *Proc. Fourth London Symp. on Information Theory*, edit. C. Cherry, 1961.

MISCELLANY

The National Reference Library of Science and Invention

THE leading article in *Nature* of July 1 presents a timely challenge to all organizations concerned with the use of scientific and technical knowledge to think deeply about the plans for the National Reference Library of Science and Invention. This is certainly a project needing imaginative and sympathetic consideration, not only by librarians but also even more, perhaps, by the scientific societies and institutions and by technical interests in industry. It is earnestly to be hoped that, as representatives of potential users of the new library, they will study the plans and give their considered views soon, lest the preparatory work becomes too far advanced for them to be taken into account.

The success of the undertaking will, however, depend primarily on the degree to which the Government itself demonstrates its determination to treat the communication of research results, and technical knowledge generally, with the same seriousness and sense of urgency as are devoted to the acquisition of them. In this, the new Library could prove a most potent instrument. But on its two fundamental needs—adequate space to house the necessary documents and adequate staff to exploit them—Mr. Freeth's reply to the adjournment debate in the House of Commons on March 14 was anything but reassuring. The decision to split the holdings of the new Library between the British Museum site in Bloomsbury and the new building on the South Bank, while regrettable, is understandable. But it can only be regarded as a workable compromise on two conditions: First, the South Bank building must be large enough to ensure that the bulk of the documents in frequent demand can be housed there, not only in 1965, but also for at least the following twenty years when, with the projected development of the Bloomsbury site, the situation might well be reviewed; and, secondly, arrangements must be made rapidly to transport or transmit documents held in the British Museum, so that the reader on the South Bank can have what he wants within minutes rather than hours. Much of the work of a reader in a reference library involves consulting many documents in conjunction with each other, and following up references found in them: delay in obtaining documents may make it impossible to complete a search and necessitate further visits. The obvious way to minimize this danger is to accommodate the greatest possible stock on the South Bank, and it is earnestly to be hoped that some way of increasing the space available will be found.

The Government seems to recognize that the new Library will ultimately need a staff which is well equipped professionally, particularly with scientists, and more numerous; but it is not enough to promise more staff when the Library becomes operational. In a lending library, records can give a reliable indication of users' habits; but virtually nothing is known as established fact about the use of reference material on open shelves. Surely now is the time to appoint a nucleus of the future staff, free of operational duties, to determine users' needs and to establish data on which detailed plans can be based.

Mr. E. M. Nicholson is doubtless wise to urge that now that a start has been made one should not jog the arm which lifts the cup. No one in his senses would wish to do so; the project must be given every chance to succeed. This means not only that critics should criticize constructively, but also that the Government should provide powerful support for the practical implications of its own proposals. Unless it does, the National Reference Library of Science and Invention may be little more than a less dingy Patent Office Library inconveniently and perhaps ineffectively linked with the British Museum.

LESLIE WILSON
(Director)

Aslib,
3 Belgrave Square,
London, S.W.1.

THE leading article in *Nature* of July 1, on the National Reference Library and other issues, calls for comment in regard to the problems of scientific information. Miss Webb is certainly to be supported in her aim of recruiting graduate staff who will assist readers in exploiting the resources of the new Library. The National Lending Library, which will not be in such direct contact with its users, is in a much more difficult position to assist in information retrieval (so long as it remains primarily a library). It is not to be expected, however, that such national libraries should undertake large tasks of information retrieval for readers, and hence they are not the correct authorities to handle research on the problems of the collation and dissemination of scientific information. Furthermore, although the needs of users must, of course, be studied and so far as possible met, the organization of such libraries is best left in the hands of scientifically trained librarians, and should not be considered a matter for dictation by any body of users who are only too often unable to specify their questions clearly and are mostly unacquainted with the intricacies of such problems as classification and information techniques. In some respects, however, this position may be changing, since an increasing proportion of the users of the Patent Office Library comprises information officers.

The problems of information retrieval arise mainly, however, in special libraries and, more particularly, in information departments in industry, research associations, and the like. Many smaller (and perhaps even some larger) researches into information techniques are being carried out, with little publicity, in information departments of the larger industries, and steady progress has been made by the Classification Research Group; most of such groups are in regular contact with Aslib. The collation of such work and the promotion of new researches would, therefore, be far better undertaken by Aslib, with its new research department, than put under the vague ægis of the national libraries. The research being undertaken by Mr. Cleverdon on retrieval by different methods of classification, at the College of Aeronautics, Cranfield, under the auspices of Aslib (on a grant from the National Science Foundation, and not, be it noted, from the British Government) is an excellent example of pioneering research in this field. The Advisory Council on Scientific Policy does not appear to be adequately acquainted with the nature of the problems to be solved.

Mr. Nicholson is quoted on the need for "senior people trained professionally both as scientists and librarians" and reference is made to the "perennial discussions on training intelligence and information officers". I would point out that the Institute of Information Scientists, formed in 1958, is now concerned with education in information work and a two-year postgraduate evening course, based on the Institute's full syllabus, was started last January at the Northampton College of Advanced Technology, London. Examinations based on this syllabus, and leading to the granting of its certificate, will be held by the Institute. The provision of this course was arranged in consultation with Aslib. If sufficient students are forthcoming, it is hoped to start a repeat course as early as next September. It may also be of interest to mention that Dr. G. Malcolm Dyson, now research director to *Chemical Abstracts*, served as president of the Institute for three years, and this office has now been undertaken by Sir Lindor Brown, who is also president of Aslib. The training of information scientists (the science graduate plus information officer really needed by Mr. Nicholson) is thus being actively and constructively promoted.

Finally, it needs to be stressed that information techniques and the problems of information retrieval, vital to modern scientific work, are not just matters of empirical trial and error, either in library organization or with complex computers, but require steady theoretical and practical scientific investigation. For this, the subject must ultimately become a recognized branch of postgraduate studies at universities and technical colleges. Much greater Government support and encouragement are necessary, however, before this final stage of respectability is achieved.

J. FARRADANE
(Honorary Secretary)

Institute of Information Scientists,
"Torran",
Crofton Road,
Orpington, Kent.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Spectroscopist (preferably with a degree in physics or chemistry, and experience in spectroscopy) in the Department of Chemistry, to have general responsibility for the maintenance and use of a range of spectroscopic equipment in the Department, including N.M.R., infra-red, Raman and U.V., and to collaborate with members of the teaching staff on research problems, and supervise routine measurements required—The Registrar, The University, Manchester, 13 (August 21).

Gordon Jacobs Fellow (with research qualifications) at the Royal Marsden Hospital for work in collaboration with the Institute of Cancer Research on the effects of ionizing radiations on nerves and the nervous system—The Secretary, Royal Marsden Hospital, Fulham Road, London, S.W.3 (August 25).

Sub-Librarian (graduate, with professional qualifications in librarianship) in the University Library—The Registrar and Secretary, The University, Bristol (August 25).

Assistant in Zoology in the Department of Natural History—The Secretary, The University, Aberdeen (August 31).

Assistant Lecturer or Research Assistant in Endocrine Biochemistry, to assist in the analysis of steroids and other hormones as part of the research programme of the unit—The Registrar, The University, Liverpool, quoting Ref. CV/N (August 31).

Assistant Librarian (with a good honours degree and preferably experience and professional diplomas)—The Librarian, University College of Sussex, Stanmer House, Stanmer, Brighton (August 31).

Horticultural Advisory Officers (with a degree in horticulture, agriculture or science, and preferably practical experience in the production of horticultural crops and in field advisory and experimental work) in the Agricultural Department, New Zealand, to advise growers chiefly on fruit and vegetable production, and to conduct field experiments, surveys and special investigations—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. B13/2/4 (August 31).

Lecturer (with a university degree or equivalent qualification) in the Department of Textile Chemistry—The Registrar, Manchester College of Science and Technology, Manchester, 1 (August 31).

Scientific Assistant, Abstractor (with a degree in agriculture or natural science, and able to read, or willing to learn, Russian)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire (August 31).

Senior Lecturer (with a degree with honours in engineering, and preferably teaching experience) in Engineering; a Senior Lecturer (with an honours degree in mathematics and postgraduate qualifications and experience in university teaching) in Mathematics; a Lecturer (with an honours degree in mathematics and preferably some postgraduate study) in Mathematics; and a Lecturer (with an honours degree with special interest in inorganic and/or physical chemistry, and preferably experience in university teaching) in Chemistry at the University College of Townsville, University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 31).

Lecturer or Assistant Lecturer (medically or non-medically qualified candidate) in the Department of Histology—The Registrar, The University, Liverpool, quoting Ref. CV/N (September 4).

Graduate Research Assistant in the Department of Mechanical Engineering to work on problems of vibration and lubrication of machine tools—The Secretary and Registrar, The University, Southampton (September 9).

Chemists and Bacteriologists (with a degree in chemistry and bacteriology) in the Agriculture Department, New Zealand, for the investigation, analysis and research into dairy products, raw materials and allied by-products—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. B13/2/A (September 15).

Lecturer/Senior Lecturer in Horticulture at the University of Sydney, Australia, to undertake teaching and research in fruit and vegetable production—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

Chemical Engineer, Physical Chemist or Physicist (honours graduate with at least two years' research experience) in the Unit Operations Group, Chemical Engineering Section, C.S.I.R.O., Melbourne, to be engaged on either of the following projects: (a) a study of the dynamic characteristics and controllability of closed circuit grinding, and other solid-handling processes, using modern techniques, including frequency response; or (b) investigations within the general field of water desalination—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 608/14 (September 16).

Principal Physicist (with a first- or second-class honours degree in physics) in charge of the Department of Medical Physics—The House Governor, King's College Hospital, Denmark Hill, London, S.E.5 (September 16).

Reader (preferably with a science degree or a degree in architecture, and relevant research experience) in Building Science in the Newcastle Division of the University of Durham—The Deputy Registrar, University Office, 1a Kensington Terrace, Newcastle-upon-Tyne, 2 (September 21).

Senior Lecturer (with a medical qualification and good experience in clinical biochemistry) in Chemical Pathology at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1, or The Registrar, University of Otago (London and New Zealand, September 30).

Reader in Human Genetics in the Department of Zoology, University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (October 15).

Director of the Poultry Research Centre, Edinburgh—The Secretary, Agricultural Research Council, Cunard Building, 15 Regent Street, London, S.W.1 (October 31).

Lecturer (with a degree in science or agricultural science, with some experience in research and teaching in plant pathology) in Botany at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (October 31).

Assistant Biochemist, Basic grade (preferably holding the A.R.I.C., the Grad.Mem.R.I.C. or B.Sc.(Hons.) in chemistry or biochemistry) in the Group Laboratory at Crumpsall Hospital—The Hospital Administrator, Crumpsall Hospital, Manchester 8.

Assistant Experimental Officer (with a pass degree, preferably in zoology and chemistry) for biochemical research on fisheries problems—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berks, quoting Ref. 61/24.

Assistant, Grade B (with qualifications and experience to teach mathematics for Diploma in Technology courses and for degree courses in engineering and general science) in Mathematics—The Registrar (Dept. F), Bradford Institute of Technology, Bradford 7.

Assistant Physicist (with at least an H.N.C. in physics, or equivalent qualification) on the staff of the Area Chief Chemist located in Birmingham, for duties involving interesting work on instrumentation in a modern laboratory, together with development and maintenance of instruments associated with gas making plant—The Industrial Relations Officer, West Midlands Gas Board, 6 Augustus Road, Edgbaston, Birmingham, 15.

Assistant Professor (with a Ph.D. or equivalent, and preferably a background and experience in marine biology) of Biology to give instruction primarily in animal physiology—The Head, Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

Graduate Research Assistant (with an interest in organic chemistry) in the Department of Chemistry—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

Histologist (with an honours degree in physiology or zoology or equivalent qualification, and preferably previous experience in histological methods) in the Physiology Department to be in charge of the histological work of the department, and to collaborate in research mainly in the field of endocrinology—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berks, quoting Ref. No. 61/25.

Lecturer (preferably with experience in the field of infra-red spectroscopy) in Organic Chemistry for work up to Dip.Tech. and Grad.R.I.C. Part II level—The Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. No. CST/61/32.

Lecturer (well qualified, and preferably with both teaching and research experience) in Inorganic Chemistry—The Academic Registrar, Loughborough College of Technology, Loughborough, quoting Ref. 21/2/AF.

Lecturer (with appropriate qualifications and experience of carrying out practical research work in the field of clinical psychology) in Psychology—The Secretary, Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E.5.

Physicist/Physical Chemist (with a university degree and some experience in the conduct of research) to undertake research in connexion with problems in food manufacture—The Director of Research, British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey, marking envelope "Private and Confidential".

Postgraduate Research Assistants (with a degree in biochemistry, chemistry, physiology, physics or zoology) in the Department of Physiology and Biochemistry—The Secretary and Registrar, The University, Southampton.

Radiobiologist (with a knowledge of dosimetry, and preferably a background in entomology and/or invertebrate zoology) for research and development on biological effects of ionizing radiation for application in a long-range biological control programme—The Director, Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas, Venezuela.

Research Assistant (with an honours degree in botany) to collaborate in compiling a Flora of Turkey—Dr. P. H. Davis, University Department of Botany, Royal Botanic Gardens, Edinburgh, 3.

Research Fellow (honours graduate with special qualifications in botany, biochemistry or microbiology, and preferably experience of radiochemical techniques) in Plant Physiology in the Department of Biological Sciences, to undertake research in algal metabolism—The Registrar (Department F), Bradford Institute of Technology, Bradford 7.

Research Students; Research Assistants (with a good honours degree or Graduate Members of the Royal Institute of Chemistry) and Postdoctoral Fellows in the Organic Chemistry Section of the Department of Chemistry for work concerned with (1) photochemical reactions of aromatic compounds, or (2) the chemistry of unsolvated organomagnesium halides—Dr. D. Bryce-Smith, The University Reading.

Scientific Officer, Chemist (not above 45 years of age, with a first- or second-class B.Sc. honours degree in chemistry, or A.R.I.C. or F.R.I.C., with postgraduate experience preferably in food chemistry and insecticide analysis) in the Federal Ministry of Commerce and Industry, Federal Government of Nigeria, to undertake chemical work pertaining to the deterioration and protection of raw foodstuffs during storage in Nigeria—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. E/C3239/1.

Senior Laboratory Technician, Grade I (with appropriate qualifications and experience)—The Head Master, King Edward VI School, Southampton.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Aspects of Co-existence. By Sir William Hayter. (Nineteenth Montague Burton Lecture on International Relations.) Pp. 18. (Leeds: The University, 1961.) 6*d*. [96

The Grassland Research Institute. Experiments in Progress No. 13: Annual Report for 1959–60. Pp. 124. (Hurley, near Maidenhead: The Grassland Research Institute, 1961.) 7*s*. 6*d*. [96

Department of Scientific and Industrial Research. Scientific Research in British Universities, 1960–61. Pp. xiii+513. (London: H.M. Stationery Office, 1961.) 32*s*. 6*d*. net. [96

National Union of Journalists. Freelance Directory, 1961. Pp. ii+86. (London: National Union of Journalists, 1961.) [96

John Innes Institute. Fifty-first Annual Report, 1960. Pp. 48. (Bayfordbury, Hertford: John Innes Institute, 1961.) 3*s*. [96

The Royal Institute of Chemistry. Register of Fellows and Associates, 1960, with a Supplementary List of Graduate Members. Pp. xx+643. (London: The Royal Institute of Chemistry, 1960.) [96

Department of Scientific and Industrial Research: Geological Survey and Museum. British Regional Geology: East Anglia and Adjoining Areas. By C. P. Chatwin. Fourth edition. Pp. vi+101+8 plates. (London: H.M. Stationery Office, 1961.) 6*s*. net. [96

Ministry of Agriculture, Fisheries and Food. Bulletin No. 123: Diseases of Vegetables. By Lawrence Ogilvie. Pp. v+101+12 plates. (London: H.M. Stationery Office, 1961.) 7*s*. 6*d*. net. [96

Department of Scientific and Industrial Research. Memoirs of the Geological Survey of Great Britain—England and Wales. Geology of the Country around Monmouth and Chepstow. (Explanation of Sheets 233 and 250.) By Dr. F. B. A. Welch and Dr. F. M. Trotter. With contributions by T. R. M. Lawrie, J. D. D. Smith and R. W. Elliot. Pp. viii+164+2 plates. (London: H.M. Stationery Office, 1961.) 22*s*. 6*d*. net. [96

British Society for the Promotion of Vegetable Research. Eleventh Annual Report, 1960 (Oct. 1959–Sept. 1960.) Pp. viii+72. (Wellesbourne, Warwick: British Society for the Promotion of Vegetable Research, 1961.) [126

Agricultural Research Council. A Bibliography of Farm Buildings Research, 1945–1958. Part 5: Buildings for the Drying and Storage of Grain. Pp. 43. (London: Agricultural Research Council, 1961.) 3*s*. [126

Ministry of Agriculture, Fisheries and Food. Domestic Food Consumption and Expenditure: 1959—Annual Report of the National Food Survey Committee. Pp. vi+142. (London: H.M. Stationery Office, 1961.) 8*s*. 6*d*. net. [126

The Carnegie United Kingdom Trust. 47th Annual Report, 1960. Pp. viii+98. (Dunfermline, Fife: The Carnegie United Kingdom Trust, 1961.) [126

Freshwater Biological Association. Twenty-ninth Annual Report for the year ended 31 March 1961, including Reports of the Council and of the Directors, and Accounts for 1960–61, with Lists of Officers, Council, Staff and Members. Pp. 88+1 plate. (Ambleside: Freshwater Biological Association, 1961.) 4*s*. [126

British Electrical and Allied Industries Research Association. Technical Report L/T395: Factors Influencing Intrinsic Electric Strength. Breakdown of Alkali Halide Crystals. By R. Cooper. Pp. 21+23 figures. (Leatherhead: British Electrical and Allied Industries Research Association, 1960.) 21*s*. [126

Institute of Fuel. Origin, Objects and Activities of the Institute. List of Officers, Council and Committees. Alphabetical Lists of Corporate Members (Chartered Fuel Technologists) and of various classes of Non-Corporate Members. Pp. 290+57. Careers in Fuel. Pp. 31 (3 plates) (London: Institute of Fuel, 1961.) [146

Department of Scientific and Industrial Research: Building Research Station. National Building Studies: Research Paper 34: A Study of Space and Water Heating in Local-Authority Flats, 1956–59. By J. B. Dick, J. H. Madge and C. N. Craig. Pp. vi+110. (London: H.M. Stationery Office, 1961.) 6*s*. net. [146

Women Engineers in the U.S.S.R. (A Study of Women Engineers in the U.S.S.R. carried out under the auspices of the Caroline Haslett Memorial Trust.) Pp. 28. (London: The Caroline Haslett Memorial Trust, 25 Foubert's Place, W.1, 1960.) [146

Department of Scientific and Industrial Research: Building Research Station. Factory Building Studies No. 8: Colouring in Factories. By H. L. Gloag. Pp. iv+19+9 plates. (London: H.M. Stationery Office, 1961.) 7*s*. 6*d*. net. [146

Tuberculosis in Animals: Proceedings of a Symposium held at the Zoological Society of London, May 24th, 1960. Edited by J. N. Ritchie and W. D. Macrae. (Symposia of the Zoological Society of London, No. 4). Pp. v+94. (London: Zoological Society of London, 1961.) 2*s*. 6*d*. [146

Other Countries

Panjab University, Lahore. Nutritive Value of Food-Stuffs and Planning of Satisfactory Diets in Pakistan. Part 1: Composition of Raw-Food-stuffs. By Dr. M. I. D. Chughtai and Dr. A. Waheed Khan. Pp. vi+34. (Lahore: Division of Biochemistry, Institute of Chemistry, Panjab University, 1960.) Rs. 1–8–0; 2*s*. 6*d*. [126

Regional Research Laboratory, Hyderabad. Annual Report, 1959–60. Pp. xiv+217. (Hyderabad: Regional Research Laboratory, 1961.) [126

Publications de l'Institut National pour l'Etude Agronomique du Congo. Série Technique No. 64: Les Buffles Domestiques d'Asie Importés au Congo—Observations Faites après sept Années de Multiplication. Par Dr. M. Mammerickx. Pp. 97+26 photographies. (Bruxelles: Institut National pour l'Etude Agronomique du Congo, 1961.) 180 francs. [126

Organization for European Economic Co-operation: Office for Scientific and Technical Personnel. New Thinking in School Mathematics. Pp. 246. (Paris: Organization for European Economic Co-operation, 1961.) 10 N.F.; 15*s*.; 2.50 dollars; 8.50 D.M. [126

Tropical Fish Culture Research Institute, Malacca. Report for year ended August 31st 1960. Pp. 28+4 plates. (Batu Berendam, Malacca: Tropical Fish Culture Research Institute, 1961.) [126

Annual Report for the year 1959 of the Museums Trustees of Kenya and of the Coryndon Memorial Museum, Nairobi. Pp. 24+1 plate. (Nairobi: Museums Trustees of Kenya and Coryndon Memorial Museum, 1961.) 1*s*. [126

Ministère de l'Agriculture de la Province de Québec. Le Conseil des Recherches Agricoles. Recherches Agronomiques: Sommaire des Résultats, 1958–1960. Pp. 32. (Québec: Ministère de l'Agriculture, 1960.) [126

Smithsonian Institution, Washington, D.C. Publication 4466: The Victorian American. By Anthony N. B. Garvan and Peter C. Welsh. (Lithographs from the Harry T. Peters "American on Stone" Collection.) Pp. 30. (Washington, D.C.: Smithsonian Institution, 1961.) [126

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 60–22: Ground-Water Resources of Plum Coulee Area, Manitoba (Townships 1 to 6, Ranges 1 to 5 West of Principal Meridian.) By J. E. Charron. Pp. iii+81. 50 cents. Paper 60–23: Preliminary Report on Hydrogeology, Ottawa-Hull Area. Ontario and Quebec. By L. V. Brandon. Pp. ii+17. 50 cents. (Ottawa: Queen's Printer, 1961.) [126

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 103 (April 1961): *Bacillus thuringiensis* Berliner: Über seine Biologie, Pathogenie und Anwendung in der Biologischen Schädlingsbekämpfung. Von Dr. A. Krieg. (In memorian Dr. Ernst Berliner 1880–1957.) Pp. 79. (Berlin-Dahlen: Biologischen Bundesanstalt für Lind- und Forstwirtschaft, 1961.) 12.40 D.M. [126

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 74: The Jurassic Faunas of the Canadian Arctic: Middle and Upper Jurassic Ammonites. By Hans Frebold. Pp. ix+43+21 plates. (Ottawa: Queen's Printer, 1961.) 2 dollars. [126

University of California Publications in Geological Sciences. Vol. 34, No. 7: Paleoecologic Molluscan Geography of the Californian Pleistocene. By James W. Valentine. Pp. iv+309–442. 2.50 dollars. Vol. 37, No. 3: Foraminifera from the Sacate Formation South of Refugio Pass, Santa Barbara County, California. By Gordon R. Hornaday. Pp. 165–232 (13 plates). 2 dollars. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1961.) [126

Organization for European Economic Co-operation. Fertilizers in Europe: Production, Consumption, Prices and Trade—10th Study, 1958–1961. Pp. 97. (Paris: Organization for European Economic Co-operation; London: H.M. Stationery Office, 1961.) 6 N.F.; 9*s*.; 1.50 dollars. [126

Smithsonian Institution, Washington. Contributions from the Museum of History and Technology, Paper 16: Drug Supplies in the American Revolution. By George B. Griffenhagen. (United States National Museum Bulletin 225.) Pp. 109–133. (Washington, D.C.: Government Printing Office, 1961.) 25 cents. [126

United States Department of Commerce: Coast and Geodetic Survey. Publication 65–1, Part 14: Plane Coordinate Intersection Tables (2½-Minute), Kansas. Pp. iii+124. 65 cents. Publication 65–1, Part 20: Plane Coordinate Intersection Tables (2½-Minute), Michigan. Pp. iii+187. 1 dollar. (Washington, D.C.: Government Printing Office, 1961.) [126

Smithsonian Institution: Bureau of American Ethnology. Bulletin 180: Symposium on Cherokee and Iroquois Culture. Edited by William N. Fenton and John Gulick. Pp. vi+292. (Washington, D.C.: Government Printing Office, 1961.) 1.25 dollars. [146

World Health Organization. Technical Report Series. No. 193: Teacher Preparation for Health Education—Report of a Joint WHO/UNESCO Expert Committee. Pp. 19. 1 Swiss franc; 1*s*. 9*d*.; 0.30 dollars. No. 210: Standardization of Methods for Conducting Microbic Sensitivity Tests—Second Report of the Expert Committee on Antibiotics. Pp. 24. 1 Swiss franc; 1*s*. 9*d*.; 0.30 dollars. No. 214: Molluscicides: Second Report of the Expert Committee on Bilharziasis. Pp. 50. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars. No. 215: Planning of Public Health Services—Fourth Report of the Expert Committee on Public Health Administration. Pp. 48. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars. European Standards for Drinking Water. Pp. 52. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars. International Work in Health Statistics, 1948–1958. By H. S. Gear, Y. Biraud and S. Swaroop. Pp. 56. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars. (Geneva: World Health Organization; London: H.M. Stationery Office, 1960 and 1961.) [146

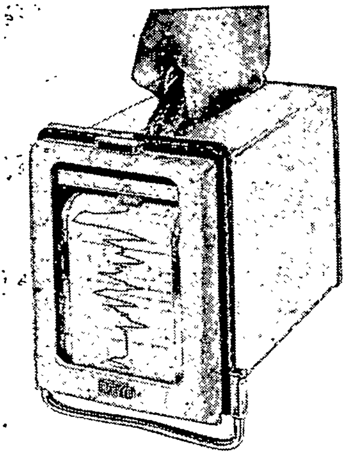
Fonds National de la Recherche Scientifique, Bruxelles. Trente-troisième Rapport Annuel, 1959–1960. Pp. 284. (Bruxelles: Fonds National de la Recherche Scientifique, 1961.) [146

Transactions of the American Philosophical Society. New Series, Vol. 51, Part 3: The Monguors of the Kansu-Tibetan Frontier. Part 3: Records of the Monguor Clans—History of the Monguors in Huangchung and the Chronicles of the Lu Family. By Louis M. J. Schram. Pp. 117. (Philadelphia: American Philosophical Society, 1961.) 3 dollars. [156

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office



The Battery Operated POTENTIOMETER RECORDER that is *COMPLETELY PORTABLE*

Available with Ministry of Power Certificate of Intrinsic Safety

The applications listed are examples of Potentiometric measuring and recording hitherto impossible in the industrial field. That they *are* now possible is due to this transistorised instrument which, with its built-in battery power supply is completely independent of mains current and leads (with their consequential limitations). Very few instruments can claim to have had such a marked record of technical success in such a wide field of industrial and scientific achievement. The MERVYN Battery Operated Potentiometer Recorder claims exclusively to be both *completely portable and self contained*.... it weighs only 17 lbs.

- Internal Battery Power Supply has a life of 250 hours.
- Low Drift Transistor Chopper operating at a frequency chosen to minimise 50-cycle interference
- Battery-driven motor for the combined Potentiometer and Writing Drive
- Transistorised amplifier and voltage reference from Zener Diode
- Inkless Recording using 7-day clock mechanism

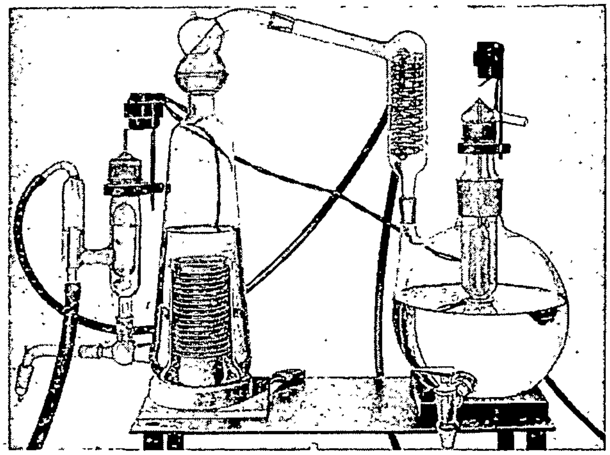
The ranges acceptable are from 0-5 mV up to 0-1 volt, by Interchangeable Range Cards

Write for further information mentioning 'Nature' to:

P841

Mining
Road Construction
British & Foreign Electrical Boards
Health Physics
Tyre Manufacture
Paper Production
Stove Enamelling
Low Temperature Control
Weather Statistics
Oceanographic Problems
Motor Research

MERVYN INSTRUMENTS WOKING · SURREY · ENGLAND · WOKING 5200/3



This unique water still is in use in a number of research establishments and hospitals where water of the highest purity is essential

PRICE, complete with heater and all electrical equipment 230 volt (a.c. only)—**£30-0-0**

Inclusive of carriage and packing in U.K.

Obtainable only from

L. V. D. SCORAH, M.Sc.
(Manufacturer of the "SCORAH" Blowpipe)

44 NORTHFIELD ROAD, KINGS NORTON,
BIRMINGHAM, 30. KINGS NORTON 1885

The SCORAH Automatic Electric all glass WATER STILL (patented)

Automatic Can be left on without attention. Heater current cuts off if water supply is interrupted, or inadequate or forgotten. When receiver (5 litres capacity) is full, current cuts off. When distilled water is drawn from receiver, the still resumes operation until receiver is refilled.

Purity Electrostatically charged condenser droplets can pick up fumes, dust and air-borne organisms from the laboratory atmosphere. The design of the receiver vessel ensures absence of such contamination.

Efficiency Heater of Scorah design and manufacture, consumption 1,700 watts, output 2.4 litres per hour (the full theoretical yield). Cost 3½d. per gallon at 1d. per unit.

Safety The heating element is out of contact with the feed water, thus eliminating danger from "live" water supply or electrolytic effects or "burn out".

Assembly No retort stands or clamps are required. The 2½-in. diameter ball joint gives a strainless set-up. Outlet cap on boiler for quick periodical drain-off of sediment. The still may be installed on a shelf or platform about 1 ft. above bench level near a sink and enclosed in a transparent plastic cover to keep out dust.
Phoenix heat-resisting glass. Interchangeable ground glass joints.

APPOINTMENTS VACANT

UNIVERSITY OF SYDNEY
NUFFIELD FOUNDATION RESEARCH FELLOWSHIPS—SCHOOL OF ELECTRICAL ENGINEERING

Applications are invited for two positions of Nuffield Foundation Research Fellow in the School of Electrical Engineering. Candidates should be well qualified in electrical engineering or physics with general experience in electronics and considerable detailed knowledge of solid state circuits. The successful applicants will be associated with the development of electrical engineering aspects of the design of a large radio-astronomy interferometer system under development by the Radio Astronomy Centre, a joint project of the Schools of Electrical Engineering and Physics. The main field of interest will be the application of solid state circuitry to radio-astronomy receiver systems. In each case, the salary will be £A.1,950 per annum and the appointment will be for a period of three years. No allowance will be made to appointees for their travelling expenses to Sydney.

Applications, giving details of qualifications and experience and the names of three referees, should be forwarded to reach the undersigned not later than September 30, 1961. In addition, overseas candidates are required to forward two copies of the application to the Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1, from whom further information may be obtained.

M. A. TELFER,
Registrar.

University of Sydney,
Sydney, New South Wales,
Australia.

UNIVERSITY OF NEW ENGLAND
LECTURER/SENIOR LECTURER IN BOTANY

Applications are invited for the position of either Lecturer in Botany or Senior Lecturer in Botany. Applicants for the Lectureship should hold the degree of Ph.D. and in the case of the Senior Lectureship, should have this degree as well as publications to their credit. Copies of publications can be sent with the application. The successful applicant will be required to lecture in plant physiology or in mycology-microbiology to advanced students in botany and to supervise research projects of postgraduate and final-year (botany double major) students. The successful applicants must be prepared to devote the remainder of his time to research in his particular field of interest. Commencing salary will be within the range £A.1,730 by £A.105 to £A.2,435 for a Lecturer and £A.2,550 by £A.95 to £A.3,000 for a Senior Lecturer, and in addition a cost of living allowance which is at present £A.42, is payable. Provision is made for superannuation, travel and removal expenses and assistance in buying or building a house.

Further information may be obtained from the Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close, in Australia and London, on October 6, 1961.

UNIVERSITY OF WESTERN AUSTRALIA
SENIOR DEMONSTRATORSHIP IN ZOOLOGY

Applications are invited from graduates for appointment to the above-named position, which has a salary range of £A.1,400 by £A.50 to £A.1,700 per annum with superannuation similar to F.S.S.U. The position offers primarily a teaching appointment and the appointee will be responsible for organizing and supervising laboratory classes. Facilities will be available for research provided it does not interfere with teaching duties.

The appointee is required to take up duties by January, 1962. Conditions of appointment are obtainable from the Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on September 30, 1961.

RESEARCH BIOCHEMIST

Bob Martin Ltd. seek the services of a young female Research Biochemist. The position would suit a recent graduate but some experience of vitamin and chromatographic techniques would be an added recommendation.

Apply, giving details of qualifications and experience, to the Chief Chemist, Bob Martin Ltd., Southport, Lancs.

COMMONWEALTH OF AUSTRALIA

DEFENCE STANDARDS LABORATORY

The Department of Supply invite applications for the following permanent positions at the Defence Standards Laboratories, MELBOURNE:

RESEARCH CHEMIST

POSITION.—Higher Experimental Officer.

SALARY.—£1,761/£1,981 (Australian currency). (A substantial increase may be awarded shortly.)

DUTIES.—Assist in the study of light-sensitive reactions, particularly the stabilization and inhibition of photo-chemical reactions in the presence of oxygen and ozone. Qualitative inorganic chemistry and a study of "photographic" processes form a large part of the work.

QUALIFICATIONS.—Degree or equivalent qualifications in Chemistry, with several years experience. Experience in general analytical, micro-chemical and photographic techniques an advantage.

POLYMER TESTING & ANALYSIS

POSITION.—Technical Officer Grade 1.

SALARY.—£1,390/£1,581 (Australian currency).

DUTIES.—(a) Development of test methods and investigation of material/performance relationships. (b) Micro-chemical analysis of complex materials by inorganic, organic, and physical methods.

QUALIFICATIONS.—Educational qualifications to be stated. Extensive suitable experience relevant to the studies.

CONDITIONS.—These positions offer permanent appointment to the Commonwealth Public Service, and the successful applicants will be required to contribute to the Commonwealth Superannuation Scheme on confirmation of appointment. Under specified conditions, air/sea transport for the appointees and dependants (wife and dependent children) will be provided by the Commonwealth.

APPLICATIONS.—Forms obtainable from the Senior Representative (AV.119/7), Department of Supply, Australia House, Strand, London, W.C.2, with whom completed applications should be lodged by September 4, 1961.

COMMONWEALTH OF AUSTRALIA

DEFENCE STANDARDS LABORATORIES

PHYSICAL CHEMISTS

The Department of Supply invite applications for the following two positions at the Defence Standards Laboratories, MELBOURNE:

POSITION.—HIGHER SCIENTIFIC OFFICER.

SALARY.—£2,036/£2,296 (Australian currency). (A substantial increase may be awarded shortly.)

DUTIES:

(Position No. 55.) Participate in the current programme of research directed to the correlation of chemical structure with biological activity. The work of the Group includes all aspects of absorption spectrometry and the appointee will be initially assigned responsibility for the infra-red field.

(Position No. 110.) Research into physical chemical aspects, in particular the mass spectrometry, of a variety of problems of interest to the work of the Laboratories. These include (a) absorbed layers on active surfaces, (b) high molecular weight compounds, (c) structure determination of physiologically active substances.

QUALIFICATIONS.—Honours degree in Science with Chemistry as a major subject and some post-graduate experience in physical chemistry. For **Position No. 55** familiarity with any branch of absorption spectrometry, including nuclear magnetic resonance, would be an advantage, and for **Position No. 110** familiarity with mass-spectrometry would be an advantage.

CONDITIONS.—Under certain conditions, air/sea transport for the successful candidates and dependants (wife and dependent children) will be provided by the Commonwealth. **ALTERNATIVELY**, consideration may be given to a three (3) or five (5) year contract of employment in Australia. In this case the Commonwealth will bear the cost of return fares together with the cost of removing the successful applicants' furniture and effects up to a maximum cost of £500 (Australian), £250 each way.

NOTE.—Applicants who do not possess the necessary experience, may initially be appointed as Scientific Officer Grade 1, £A.1,541/£A.1,926, and at a later date may be promoted to Higher Scientific Officer.

APPLICATIONS.—Forms obtainable from the Senior Representative (A.V.118/8), Department of Supply, Australia House, Strand, London, W.C.2, with whom completed applications should be lodged by September 4, 1961.

MICROBIOLOGY TECHNICIAN. APPLICATIONS are invited from men or women familiar with the techniques of microbiology. The work involves the investigation of this facet of the properties of the aqueous emulsions of thermoplastics we manufacture. A pension scheme, canteen and social club add to the interest of the post.—Please write to the Personnel Manager, Vinyl Products Limited, Butter Hill, Carshalton, Surrey.

APPRENTICESHIP AND TECHNICAL EDUCATION

THE debate in the House of Commons on apprenticeship and training, postponed from May 8, fell appropriately in the middle of Commonwealth Technical Training Week, to which reference was freely made in the debate. Both the Carr Report on the recruitment and training of young workers in industry and the later White Paper, *Better Opportunities in Technical Training* (see *Nature*, March 4, p. 701), were much in the minds of speakers in the debate, and while few new proposals were made, the debate showed the extent to which opinion on all sides has advanced in the past three years. The purpose of the Commonwealth Technical Training Week was to stimulate thought and action on technical education in all Commonwealth countries, and while the United Kingdom has no grounds for complacency in the matter, any deficiencies are the responsibility of both major political parties. On all sides of the House of Commons there are those who have sought to rouse public opinion to a sense of what is required, and have pressed constructive proposals on successive Governments, but nevertheless of 550,000 young persons who in 1960 in Great Britain entered employment, 420,000 went into unskilled jobs.

In his speech at Guildhall inaugurating the week, the Duke of Edinburgh said that he refused to believe that this is the right proportion either for the individuals concerned or for the country as a whole; and, in opening the debate in Parliament, Mr. F. Lee agreed, adding that there were many who wished to enter apprenticeship but who were denied the opportunity, and that new production methods demand new skills. A nation which does not acquire new skills condemns itself to obsolete production methods and continues to train young people in skills which have lost much of their value. The skill of many of our craftsmen is, Mr. Lee suggested, becoming outmoded, and we need to re-train many of our present craftsmen as well as to train more apprentices.

Mr. Lee, and other speakers in the debate on May 31 and in an earlier debate on apprenticeships a year earlier, on June 30, 1960, stressed the need for the trade unions to take account of these changes in technique, and while Mr. John Hare, the Minister of Labour, who followed, agreed with what Mr. Lee had said as to the importance of training, he emphasized that this is a matter for employers as well as trade unions, and that it is necessary to consider whether our present methods of training will stand the test of the working life of the boy who is being trained. He believes that the time has come for industry to have a fresh look at the content and methods of training, and suggested that the quality of training would be improved if employers made greater use of the facilities available for further education; the whole of Britain should benefit if employers made greater use of block release courses, but, particularly in Scotland, many apprentices do not get the day release recommended in apprenticeship schemes.

Mr. Hare, who rejected the charge that the Government has been inactive in the matter, referred to some progress which has been made since the Carr Report was discussed and the Industrial Training Council established, without suggesting that there is any ground for complacency. On the contrary, he agreed that the real testing time lies ahead, for the number of school-leavers will rise to 660,000 this year and to 720,000 next year. To impress this on both sides of industry, the Industrial Training Council had called a series of conferences throughout Britain, and, following these conferences, regional committees have been set up which will keep under review and encourage arrangements for recruitment and training during the peak years of the bulge. He believes that these committees will be a most valuable reinforcement to the measures already taken to encourage increased training.

The vital question, Mr. Hare insisted, is the quality of training, and this was the keynote in speeches which the Duke of Edinburgh made during Commonwealth Technical Training Week. In opening the week at Guildhall on May 29, he gave four basic reasons why every country should organize its technical training as efficiently as possible. First, the standard of intelligence, competence and workmanship in industry, commerce and agriculture controls absolutely the ability of any country to hold its own in the highly competitive business of international trade. Secondly, even in the least-developed countries, there is growing mechanization in all the public services, such as transport, communications, water, power and light. In the more developed countries, mechanization has gone much further. As a result, there is a growing need for technically competent persons capable of maintaining this increasingly complicated mechanical complex. Thirdly, technical training is essential if individuals are to make the best use of their aptitudes; and fourthly is the need for teachers, especially in technical subjects. Such teachers are not only needed in Britain. It should be obvious that the less-developed countries are less developed because the standard of technical knowledge is not very high, and the United Kingdom has the duty of providing more teachers not only for her own expansion but also for the developing countries of the Commonwealth.

One of the tasks of the week, however, the Duke of Edinburgh added, was to direct the attention of employers, trade unions and technical teachers to the need for bringing the whole complicated, confused, unco-ordinated system of apprenticeship and technical training in Britain up to date; or, where no system exists, to work out a sensible arrangement quickly. Until the Carr Report appeared, that was a controversial issue; but no feature of the debate on May 31 and the similar debate in June of last year has been more welcome than the evidence of a disposition to re-examine this question. Commonwealth Training Week should certainly encourage this development. In the House of Commons, not only was the rigid five-year period for apprenticeship challenged as irrelevant to modern conditions, but also some constructive suggestions were advanced. Mr. John Robertson believes that apprenticeship should be considered as a continuation of full-time education and transition from class-room to workshop made less abrupt. He would put training under the control either of education authorities or of joint apprenticeship committees representing both sides of industry and the education authorities.

The latter suggestion appears to be a move towards apprenticeship to an industry rather than to a firm, a suggestion which has already found expression in Parliament and was clearly in the minds of other participants in the debate. Mr. Robertson recognized that some measure of compulsion such as day-release might be necessary, and, coming from an experienced trade union organizer, intimately associated with skilled industrial workers and well aware of their problems and outlook, his speech is surely significant. He admitted frankly that long-established customs and practices in industry may need to be changed, and pointed specifically to the lack of knowledge of the numbers and categories of apprenticeships and to the widespread support on both sides of industry for a national register of apprentices; he also suggested in this connexion that raising the school-leaving age might be helpful.

Two other constructive speeches in the debate may be mentioned. Mr. R. L. Mawby looks for more mobility of skilled labour and advocated re-training of skilled workers to this end. While he was less concerned about the five-year period of apprenticeship—though he urged the need for greater flexibility—he was concerned about the quality of the training given during those years and rightly urged that this is what really matters. The importance of skill is increasing, and industry should see that the right incentive is given to new apprentices to acquire new skills. He seemed more hopeful than most speakers about the Industrial Training Council, if it is adequately strengthened and supported.

Mr. R. E. Prentice, whose speech was particularly welcomed by the Parliamentary Secretary to the Minister of Labour, Mr. P. J. M. Thomas, in concluding the debate, was concerned especially with the challenge of the bulge and the opportunity it affords, but urged that much more attention must be given to the question of what constitutes a proper apprenticeship or proper industrial training in the second half of this century. He began, like most speakers, by challenging the rigidity of a five-year period for apprenticeship, and urged that more attention must be given to standards of training and that we need to train many more people to handle a wide range of skilled work.

A feature of the debate was, in fact, this recognition of the need for greater flexibility, but Mr. Prentice made once again his suggestion for some kind of differential training levy rather than the pay-roll tax proposed in the Budget last April, which he thought is likely to discourage apprenticeship schemes. The differential training levy, as operated in France for many years, could penalize the firm which poached trained workers and did not train its own, and assist the firm which did its full share or more of training. He was doubtful as to the value of an apprenticeship council, but thought that the Industrial Training Council needs more staff and resources.

The Parliamentary Secretary welcomed the emphasis on flexibility and stressed that the key to the situation at the craft-level lies in the willingness of employers and unions to increase the intake of apprentices. He also said that the establishment of a national craft apprenticeship system is a long-term process; he did not comment on this proposal for a differential training levy. He welcomed many of the suggestions, however, and particularly the references made to the relation between training in technical colleges and industrial training, to which Miss J. M. Quennell had directed particular attention; he urged the need to consider the strains and stresses which the situation is placing on technical colleges in Britain and the importance of further inquiry into the field of technical education and industry's future requirements for technicians. But he was as firm as Mr. Hare that training should not be completely divorced from production and that industry must accept its training reponsibilities. The Government's efforts should be designed to reinforce and not supplant industry's own efforts.

When the pay-roll clause in the Finance Bill was debated in the House of Commons on June 13, Mr. Prentice did not press his plea for a training levy, though he again referred to it; but he strongly urged that surcharges should not be payable in respect of employees under eighteen. Mr. R. E. Carr also urged that the pay-roll tax should be dropped. But in replying for the Government, the Financial Secretary to the Treasury, Sir Edward Boyle, would go no further than admit that if the surcharge came to be imposed, there would be a strong case for special treatment for boys and girls under eighteen. Sir Edward declined to enter into a firm commitment to provide partial or total relief for boys and girls under eighteen, but promised that the arguments advanced would be kept in mind.

Miss Quennell, who was among those who challenged the appropriateness of a five-year apprenticeship, suggested that the technical colleges could play a greater part. First-year apprenticeship courses are indeed being introduced in some technical colleges and the proposals of the White Paper on technical education are intended to help generally here, not only by improving selection and broadening the content of courses, reducing wastage and filling in gaps in the existing arrangement, but also especially by stimulating co-operation between the education authorities and both sides of industry. The implication of the policy set forth in the White Paper and of the new routes to further education which it provides were indeed discussed in a series of four papers presented at a conference on April 19 arranged by the British Association for Commercial and Industrial Education*.

In the first of these, Mr. G. E. Manning stressed the importance of good teachers in order to make full use of the potentialities of the secondary modern schools and the technical schools and colleges of Britain. Here the questions of salary and status cannot be ignored. Prof. Boris Ford directed attention to the danger that an elaborate system of regional examining boards might destroy the individuality and diversity of our modern schools as well as depress the majority of children who are not good at working at subject examinations. It is important, he said, to encourage the maximum opportunity for independent experiment, and he thought we should work towards a national policy of an award for every school-leaver based on comprehensive assessment, and supported (where relevant) by regional certificates awarded on the basis of local examinations moderated regionally. He also insisted that the education of pupils of average or less than average ability between the ages of thirteen and sixteen, which the Minister has now asked the Central Advisory Council to consider, is the most difficult and worrying segment of the educational system of Britain.

Dr. Ethel Venables, who looked at the proposals from an educational point of view, said that the major problem of educating for industry is not one of selection or teaching methods, important as these are, but of how we could encourage the desire to learn. She suggested that the real way to tackle this problem is to recognize that able young people are a national asset, and that they cannot be educated adequately if a sense of purpose is lacking; society in general and industry (including the trade unions in particular) must be ready to reward achievement at all levels. The nature of the goal would vary throughout industry, but much more time must be devoted to general and social studies.

Mr. F. H. Perkins, speaking from the industrial and commercial point of view, stressed the importance of enabling a transfer from a national certificate to a craft course to be made as early and smoothly as possible and also of much closer co-operation between the technical colleges and industry. He, too, referred to the difficult problem of a sufficient supply of teachers, some of whom must come from industry, and to the need for flexibility if operative courses, following suitable selection, were to give useful service to industry. The need for clear thinking and for careful definition of terms was obvious in Mr. Perkins's paper, and some of the speeches in Parliament showed that even now the functions of the technical colleges and of the colleges of technology are liable to be confused.

Nevertheless, the debate on May 31 demonstrated that if, in his appeal for a fresh look at the system of apprenticeship in Britain, the Duke of Edinburgh was not exactly pushing at an open door, there is now a good deal of support for review. Commonwealth Technical Training Week could well go some way towards achieving its aim of getting industry to examine and improve its schemes of training, recruitment and allocation. If employers and trade unions can be induced to take a fresh look at this question of training, putting aside out-of-date attitudes and thinking only in terms of what is relevant in the second half of the twentieth century, it will have been well worth while. The publicity it has received should contribute to that end, for, as the Parliamentary Secretary to the Ministry of Labour rightly said, an interested and informed public opinion is essential; without it, the necessary measures are unlikely to be initiated or effectively implemented. Commonwealth Technical Training Week is at most only a beginning.

It is important, therefore, that full use should be made of the wider interest it has created and the clear public understanding of some of the issues that are involved. There may now perhaps be the less danger that necessary inquiries into methods of teaching already under way will tend to discourage the students themselves, though it is to be hoped that too much emphasis will not be placed on this aspect while the policies of the White Paper are being given effect. The new group in the Oxford University Department of Education which is studying the reasons why comparatively fewer students read for technological qualifications in Britain than in the U.S.S.R. or the United States, the sub-committee of the National Advisory Committee on Education for Industry and Commerce which is examining the organization of sandwich courses, and the investigation at Birmingham under Dr. T. Lupton, of the College of Advanced Technology, with the aid of a Nuffield grant, of the quality of industrial and college training, should provide some of the new knowledge for which Miss Quennell, for example, pleaded; but such investigations must not be allowed to give an impression that so much is wrong with existing teaching that it is not worth while for a student to enrol in a technical institute. Incidentally, in a written answer in the House of Commons on June 15, Sir David Eccles said that his Department is establishing a new branch to give emphasis to educational research generally.

Again, little good is likely to come of the meeting of the Minister of Education with representatives of English local authorities to discuss the status of the colleges of advanced technology unless a well-informed public opinion is available to support whatever decisions may be reached. For the Minister to succeed in making these colleges independent, on university lines, would not of itself suffice to solve all the many difficult problems, and indeed might raise fresh issues in regard to technical education because the regional principle has been insufficiently regarded in creating these problems. Indeed, one major asset of Commonwealth Technical Training Week has been the way in which it has directed attention to technical education as a whole, at all levels, from that of the university department and of the college of technology to that of the secondary modern and technical school. Furthermore, it should have indicated to many how important it is that Britain should play her full part in expanding technical training in the Commonwealth as a whole. It is not sufficient to fill the gaps in the existing system in Britain, to improve the quality of the training given and to extend its scale: we must both provide a reasonable number of places for overseas students in the colleges and universities of Britain, and also supply an increasing number of qualified and enthusiastic teachers for overseas, especially in the less-developed territories.

---

* British Association for Commercial and Industrial Education. New Routes to Further Education: Papers presented at the B.A.C.I.E. Spring Conference, Senate House, University of London, 19 April, 1961. Pp. ii+31. (London: British Association for Commercial and Industrial Education, 1961.)

That is sufficient reason for urging much closer co-operation between the Ministry of Education, industry, the universities and others concerned with technical training. Firm assurances were given in the House of Commons as to the co-operation between the Ministry of Labour and the Ministry of Education, and Mr. Robert Carr gave convincing reasons last June why technical training in industry should not be made a responsibility of the Ministry of Education, while at the same time he urged as strongly as Mr. Austen Albu, who made the suggestion, the need for closer co-operation between the two Ministries. The Minister for Science, however, is also vitally concerned, through his responsibilities for the Department of Scientific and Industrial Research alone, as is the Chancellor of the Exchequer, through his responsibility for the University Grants Committee.

It will be recalled that the Carr Committee itself recommended greater flexibility in the age of entry to apprenticeship as well as that opportunity for change of employment should not be limited on grounds of age. The strong support which has been indicated for giving the first year's training in the school rather than in the workshop, and the critical look at the quality of teaching in the workshop which was noticeable in the House of Commons, at least as much from the trade union side as from anywhere else, show the extent to which public opinion has moved since the Carr Report appeared, and how largely Lady Gertrude Williams's criteria are becoming accepted: the first—an adequate supply of training vacancies—was thoroughly discussed during the Commonwealth Technical Training Week. The importance of spreading the costs of training more justly over all firms benefiting from the employment of adult skilled men, of ensuring that men capable of learning to do a job are not permanently excluded simply because they missed the opportunity to train when they were sixteen, and of improving the quality of teaching in the workshop, were fully recognized in the recent debate. On exactly how best to achieve these ends there is still room for discussion and inquiry, and perhaps more especially on the further criteria that theoretical instruction should be graded to suit the capacities of pupils. The scientist and technologist must bear a large responsibility for seeing that matters are not allowed to drift and that the co-operation and new outlook made evident during Commonwealth Technical Training Week are maintained. It is welcome evidence of the acceptance of such responsibility that Mr. G. C. Eltenton should devote such a large part of his presidential address on May 23 to the Society of Instrument Technology to consideration of the education and training of the technologist and technician.

WILLIAM HARVEY

William Harvey : Englishman, 1578–1657

By Prof. Kenneth J. Franklin. Pp. 151+5 plates. (London: Macgibbon and Kee, 1961.) 18*s*. net.

FOR three hundred years, Harveian Orations have been delivered annually to the Royal College of Physicians, and several 'orators' have observed that there is little new to be said about Harvey's life and work. Yet within the past few years fresh information has been forthcoming. The discovery of the Harveian Oration for 1662, ascribed to Sir Charles Scarborough, is a pupil's testimony to Harvey's ideals, knowledge and inquiring mind[1]. Dr. Richard Hunter and Dr. Ida Macalpine have assembled data about Harvey's knowledge of neurology and psychiatry[2]. This aspect has been further extended by Mrs. Gweneth Whitteridge, who with great skill, ingenuity and patience has deciphered Harvey's notes on *De motu locali animalium* (1627), being his views on muscular contraction and its control by the brain[3]. Lastly, Sir Russell Brain, in his Harveian Oration for 1959, has given a comprehensive account of "William Harvey, Neurologist", not suggesting that he contributed anything new to neurology, but illuminating him in Sir Thomas Browne's phrase as the "ocular philosopher", "a great observer of man and of nature, and a thinker whose intuition saw when traditional ideas were outmoded, even though he did not always know how they were to be replaced".

A letter of Bishop Brian Duppa to Sir Justinian Isham[5] relates that Harvey paid attention to dietetics and nutrition and would sometimes fast two days together; that the bishop was with him the day before he died; and that Harvey's life "went out like a spark, without any violence or noise at all".

In respect of all this and further additional information, a new life of Harvey was desirable. Prof. Franklin, who has already rendered great service to Harvey's memory by his new English translations of *De motu cordis* and of *De circulatione sanguinis*, the two anatomical essays addressed by Harvey in 1649 to Jean Riolan, jun., has now written Harvey's biography.

In the first place, the author reviews earlier speculations on the circulation, and demonstrates that Ibn an-Nafis (about 1210–88), a learned doctor from Damascus[6], only foreshadowed to some extent the pulmonary or lesser circulation; Servetus discovered it, but this received little attention. The opinions of Columbus, Vesalius, Botallus, Cæsalpinus and Fabricius, who noted the valves in veins, are also discussed. Prof. Franklin's conclusion is that the observations and opinions of the pre-Harveian "circulators in no way diminish Harvey's fame as the independent discoverer of the circulation".

Harvey's life and work are next described, and attention is paid to the importance of Harvey's second great work, *De generatione animalium*. This would have been even more valuable had it not been for the pillage and destruction of Harvey's notes and papers—including a volume on the generation of insects—in his lodgings in Whitehall by the soldiers of the Parliament.

Prof. Franklin writes concisely and lucidly with scholarship and literary grace. The book is well illustrated and produced.

---

[1] Payne, L. M., *J. Hist. Med.*, 12, 158 (1957).

[2] Hunter, R., and Macalpine, I., *J. Hist. Med.*, 12, 126 (1957).

[3] *Harvey De motu locali animalium*, edited, translated and introduced by Gweneth Whitteridge (Cambridge, 1958).

[4] Brain, W. Russell, "William Harvey, Neurologist", *Brit. Med. J.*, ii, 899 (1959).

[5] Isham, G., *The Correspondence of Bishop Brian Duppa and Sir Justinian Isham, 1650–1660*. Northamptonshire Record Society, 17, for 1950–51, Letter LXXXIX (1955). See also MacNalty, A. S., "William Harvey; His Influence on Public Health", *J. Roy. Soc. of Health*, 77, 330 (1957).

[6] Meyerhof, M., "Ibn-an-Nafis (XIII cent.) and his Theory of the lesser Circulation", *Isis*, 23, 100 (1935).

SOUNDS USED BY ANIMALS

Animal Sounds and Communication

Edited by W. E. Lanyon and W. N. Tavolga. Pp. xiii + 443 + 1 12-in. long playing record. (Washington, D.C.: American Institute of Biological Sciences, 1960.) 9.50 dollars.

THE critical and analytical study of the sounds used by animals in communication is a relatively new branch of biology. The reason for this is that only in the past fifteen or twenty years have recording and analytical techniques been developed far enough for the analysis of the extremely complex sound signals which animals use. Moreover, as the technology of microphones, amplifiers and tape recorders improves (as it does year by year) new possibilities for the study and understanding of animal vocalizations emerge.

The present book, edited by Drs. Lanyon and Tavolga, constitutes a valuable and stimulating guide to those interested in this field. There is an introduction by J. T. Emlen, jun., followed by five chapters, each by a different author, these being largely a transcript of the papers presented at a symposium of the American Institute of Biological Sciences, 1958. Since the vast majority of work on animal sounds has so far dealt with birds, this volume is naturally still rather over-weighted on the bird side, but it is noteworthy for three chapters on other groups—one dealing with sound communication in Orthoptera and Cicadidae, by R. T. Alexander; another on sound production and underwater communication in fishes, by W. N. Tavolga; and a third on the influence of sound on the behaviour of amphibians and reptiles, by C. M. Bogart.

All these are valuable interim summaries in rapidly expanding fields. The book starts with a useful though rather too elementary discussion of techniques by P. P. Kellogg. D. J. Borror's chapter on the analysis of animal sounds is also rather slight and elementary for 1960, but it must be remembered that it was written before August 1958.

W. E. Lanyon's chapter on the ontogeny of vocalizations in birds, and that by P. Marler on bird songs and mate selection are both admirable and forward-looking contributions, suggesting new experiments and new lines of thought. N. E. Collias contributes a very stimulating chapter in which he sets forth an ecological and functional classification of animal sounds, based on wide knowledge and reading. He suggests that the various acoustic signals of animals fall readily into ecological and functional categories related to: (1) food, (2) predators, (3) sexual behaviour and related fighting, (4) parent–young interrelations and (5) aggregations and group movements. He points out that the same sound may have more than one specific use, depending on ecological context, and sounds with similar functions, even when produced by different mechanisms and different animals, are frequently characterized by certain common properties in their physical structure. The book concludes with a chapter by Charles F. Hockett devoted to logical consideration and the study of animal communication. This is a helpful contribution, but it is perhaps written rather too largely from the point of view of a student of human language.

A most important and valuable feature of the book as a whole is that a 12-in. long-playing record is issued with it. This provides precise audio-illustrations of the chief points raised by the seven main chapters and brings to life many of the diagrams. As a result the book is immensely more valuable and intelligible than it otherwise would have been. It is to be hoped that the practice of issuing records with books on this and similar subjects will be widely adopted.

W. H. THORPE

ANALYSIS OF ORGANIC COMPOUNDS

Organic Analysis

Vol. 4. Edited by John Mitchell, Jr., I. M. Kolthoff, E. S. Proskauer, and A. Weissberger. Pp. vii + 429. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 102*s*.

VOLUME 4 of this well-known series of publications on organic analysis gives further authoritative information regarding the application of the newer instrumental techniques and procedures.

H. Foster presents a detailed account of the principles and usages of nuclear magnetic resonance spectroscopy, and comments also on the limitations of the method. The basic principles utilized in interpreting high-resolution nuclear magnetic resonance spectra are discussed for the case of pure compounds, for example, ethanol, and also for hydrocarbon mixtures. Molecular association, gem splitting, chelation, geometrical isomerism are all illustrated by reference to typical resonance spectra; the effect of the too often ubiquitous presence of water impurity is commented on, and nuclear magnetic resonance investigations on a number of complex substances, for example, Feist's acid, steroids, alkaloids, etc., are detailed.

Nogare and Safranski have provided a most comprehensive review of the applications of gas-liquid chromatography. Theoretical principles, modern apparatus and techniques, detectors, etc., are described in great detail, and considerable reference is made to many varied and important investigations. Programmed temperature and high-temperature gas chromatography receive special treatment.

One rather unique chapter by Krc deals with crystallographic methods, and indicates the valuable information which is yielded by a combined microscopical and X-ray diffraction examination of a given substance. The value of morphological studies of polymorphic modifications, optical properties, fusion behaviour, etc., are all clearly illustrated. The experimental pitfalls associated with the application of the powder X-ray diffraction technique to the identification of organic crystalline materials are indicated. The chapter contains an excellent survey of fundamental crystal optics, morphology, use of Miller indices, etc.

Neilands contributes a welcome review chapter on enzymes, their availability and their varied analytical and clinical usages. He supplies a valuable bibliography of specific applications, for example, estimation of ethanol in blood, detection of peroxides, analysis of specified amino-acids, etc.

The wide use of organic peroxides and ozonides by many industries has resulted in the production of a timely review by Martin on the determination of these occasionally hazardous reagents. The adventitious formation of peroxides in ether solvents is discussed, and the use of an activated alumina column for solvent desensitization and purification is worthy of note.

Iodometric, colorimetric, polarographic, infra-red and other related techniques for peroxide determination are described in reasonable detail, and some attention is paid to the estimation of peroxide mixtures using chromatographic or counter-current distribution separation procedures. Analytical interferences produced by sulphur compounds, conjugated diolefins, aldehydes, etc., are defined: the polarographic method of analysis is considered the most widely applicable method for peroxides generally.

The present volume ends with a chapter by Ke on the applications of differential thermal analysis to the examination of high polymers. The apparatus is described fully, and the thermograms are interpreted in terms of conventional thermodynamic parameters. Applications of this powerful technique to work on synthetic and natural polymers are clearly and extensively demonstrated.

This new volume of the *Organic Analysis* series will undoubtedly be as eulogistically received as were its predecessors. It presents, in a small compass, a considerable amount of information on the theoretical and practical aspects of the six important subjects reviewed, and provides material which will undoubtedly be of the greatest interest to the industrial chemist, the research worker and the academic student.

D. T. LEWIS

STILL MORE NUCLEIC ACIDS

The Nucleic Acids

Vol. 3. Edited by Erwin Chargaff and J. N. Davidson. Pp. xvi + 588. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 18 dollars.

WHEN the first two volumes of this book were published, it was evident to the editors that the research on this subject was expanding rapidly, but even they did not anticipate the explosion which has since followed. In an attempt to bring their treatise up to date, they have now issued Volume 3, which has as its object the covering of certain aspects of the subject which had been neglected in the original volumes, or which have recently assumed particular importance. The editors should be congratulated on their decision to take this course, and the third volume of this series will certainly prove a valuable addition to many libraries. They might, however, consider at this time whether it would not be more useful to begin a new series, similar to *Advances in Protein Chemistry*, which would provide definitive articles on recent aspects of the subject and not involve so much delay in publication, rather than to continue this series. It is certainly unlikely that the compiling of a comprehensive and up-to-date text-book on nucleic acids will be feasible for many years.

The new volume is a sort of compromise between a text-book and a review. All the contributors are actively engaged in research on the subjects covered in their contributions, and while this certainly makes for authoritative articles, there is the concomitant risk of the authors giving a one-sided view of the subject. For example, someone reading Schuster's article on virus ribonucleic acids might get the impression that research on this subject began about 1954, whereas the pioneering work of Bawden and Pirie, who first discovered ribonucleic acids in a number of plant viruses some twenty-five years ago, is not referred to, though some of their later work is. Max Schlesinger, a neglected pioneer if ever there was one, is not even mentioned in an otherwise admirable chapter on bacteriophage nucleic acids written by Sinsheimer, though Schlesinger was not only the first person to purify a bacteriophage but was also the first person to identify nucleic acid in any virus. Sinsheimer devotes tantalizingly little space in this article to his own work on the single-stranded deoxyribonucleic acid of the bacteriophage $\varphi X174$, which demonstrates quite convincingly that complementary filaments of deoxyribonucleic acid are not essential for the transfer of genetic information.

Sadron's contribution is largely devoted to light-scattering and hydrodynamical methods of investigating nucleic acids which do not have enough resolving power for polydisperse solutions. However, some of the problems which he poses have since been solved by the elegant methods of Meselson and his colleagues, which unfortunately are too recent to have been included in this chapter, though they are touched on elsewhere. The photochemistry of nucleic acids is covered in considerable detail by Shugar, who also provides a useful list of recommended volumes for extra reading. Khorana reviews both the chemical and enzymic synthesis of polynucleotides, a subject to which he has contributed so much, both directly and by his synthesis of substrates, but surprisingly he omits mention of the enzymic synthesis of small polynucleotides by the reversal of the action of ribonucleases.

The chapter by Hoagland on nucleic acids and their involvement in protein synthesis is most welcome as an authoritative review of this important work, much of which has come from his laboratory, while Gros describes related happenings in bacteria.

The effects of 'antimetabolites' on nucleic acid metabolism, which a few years ago gave so much hope, unfortunately unrealized, of a rational approach to the control of many serious diseases, are covered by Handschuhmacher and Welch. Unexpectedly, this work has made a real contribution to our knowledge of nucleic acid metabolism which could scarcely have been obtained by other means.

A compendium of the analyses of microbial nucleic acids is given by Belozersky and Spirin (it is most encouraging to see a contribution from the U.S.S.R.), and the biosynthesis of purine and pyrimidine nucleotides is covered by Buchanan and Crosbie respectively. Finally, Lajtha gives a short review of the effects of ionizing radiations on nucleic acid metabolism.

ROY MARKHAM

KENNAWAY AND THE CARCINOGENS*

By Prof. ANTOINE LACASSAGNE
Institut du Radium, Paris

I FIRST met Ernest Kennaway in 1923 at the Cancer Hospital in London when he was working on the isolation of the active carcinogenic principles in tar. In the following year Kennaway began to publish the results of the researches; the importance had escaped me a year earlier, yet the discovery of a 'carcinogenesis factor', by the pyrolysis of various organic substances, was to prove one of the most fertile in the whole of cancer research. It supplied the solution to a riddle which for more than 150 years had puzzled clinicians confronted with 'industrial cancers', but unable to understand how they arose. It opened the important chapter of chemical carcinogenesis which was to be written over the ensuing years by Kennaway himself (as director of the Research Institute of the Cancer Hospital and professor of experimental pathology) and the team of brilliant co-workers with whom he had surrounded himself. Through this work, organic chemistry was to be enriched by hundreds of new substances, and the way opened to the prophylaxis of industrial cancers.

During this time my own attention had been directed towards the study of carcinogenesis by endogenous chemical substances, that is to say, by certain steroid hormones. The structural relationship of these substances with the polycyclic hydrocarbons, the carcinogenicity of which had been demonstrated by the workers at the Cancer Hospital, had finally brought home to me the outstanding importance of Kennaway's discovery. But when I came to spend some days in his company at Oxford, in 1950, he was already gravely ill. We were among the twenty or so participants in a colloquium on the geographical pathology of cancer, held during July 29–August 4, following the International Cancer Congress in Paris and the International Radiology Congress in London. His wife was also present, sustaining the sick man with that affectionate companionship and scientific collaboration which she had lavished on him for thirty years, combined with the tenderness of a sister of mercy. Thanks to her, he retained to the end of his life what for a man like Kennaway was a true consolation—the ability to continue his work.

Great scientific discoveries come about by two means. Some are born of almost magical intuition and good fortune, suddenly laying bare a whole unsuspected realm of Nature. Others are reached by patient work and devoted attention to a particular problem. Kennaway's discovery was of the latter kind.

The observation made at the end of the eighteenth century by the British surgeon, Percivall Pott, of a relationship between the development of certain cutaneous cancers and irritation of the skin by the combustion products of coal tar, had been followed by others. Experimental work on the suspected carcinogenicity of tar, pitch and paraffin was started at the end of the nineteenth century. Not until 1915, however, was a positive and conclusive result obtained, by the sensational discovery by Yamagiwa and Ichikawa. Work was at once started in various laboratories the world over to follow up their results. It was recognized that the mouse was the animal of choice, the most convincing results being obtained in this mammal in the shortest time. The lack of conformity in the results, however, led to the observation that the activity of tars varied according to their origin. Opinions differed as to which of the numerous constituents of coal tar might be responsible for its carcinogenic activity, suspicion being directed against arsenic, the phenols, the pyrrols, aniline, benzidine, anthracene and others. A first pointer came with the recognition that only the highest boiling fractions, distilling at temperatures above 300°, possess carcinogenic activity.

It was to the unravelling of this tangled skein that Kennaway had set himself. His patient and methodical work was to supply a thread which, a few years later, was to lead the workers at the Cancer Hospital, through a rapid succession of discoveries, to write one of the fairest pages in the history of science.

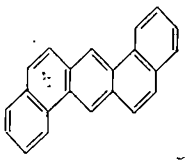
Having observed that compounds distilling at a very high temperature were for the most part hydrocarbons, he had the idea of making a synthetic tar, starting from a very simple organic substance consisting only of hydrogen and carbon. A pyrolysis product of isoprene was tested on the skin of a mouse, with a positive result, and Kennaway's well-reasoned hypothesis was confirmed[1]. Actually, he obtained a higher proportion of tumours than with ordinary coal tars.

In 1925, Kennaway confirmed the results which he had published the previous year, this time using acetylene. The activity of acetylene tars varied according to the temperature at which they were prepared[2]. Other synthetic tars prepared from different organic substances, both animal and vegetable, also proved carcinogenic. In 1930, Kennaway published an even more important observation[3], starting this time from a tetrahydrogenated derivative of naphthalene, tetraline. Under the action of aluminium chloride this pure hydrocarbon acquires new properties, being in fact transformed into carcinogenic compounds. Since the work of Berthelot in 1864[4] it had been known that the pyrolysis of acetylene gives aromatic hydrocarbons by the cyclization of acyclic hydrocarbons. It therefore seemed probable that carcinogenic polycyclic hydrocarbons had in fact been produced in the earlier experiments also.

This hypothesis was confirmed when, in collaboration with Hieger, Kennaway[5] found that carcinogenic tars, whether natural or synthetic, gave a common fluorescence spectrum (three bands at 4000, 4180 and 4400 Å.) to which Mayneord had already directed attention. The fact that a tetracyclic hydrocarbon, 1,2-benzanthracene, had a rather similar spectrum, pointed to another line of inquiry in the search for the causal agent. This was justified when, among the polycyclic hydrocarbons which these authors prepared synthetically, they found one, 1,2:5,6

---

* Substance of the first Sir Ernest Kennaway Memorial Lecture, delivered at the Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, on June 12.

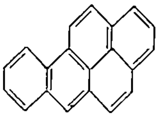


1,2 : 5,6-Dibenzanthracene

(I)

dibenzanthracene (I), which tested on mouse skin produced epitheliomas[6]. The way was open for fresh efforts on the part of the team at the Cancer Hospital, which had now been joined by J. W. Cook.

In 1932, Cook, in collaboration with Hewett and Hieger, had succeeded in isolating from coal tar a pentacyclic hydrocarbon, 3,4-benzpyrene (II), which



3,4-Benzpyrene

(II)

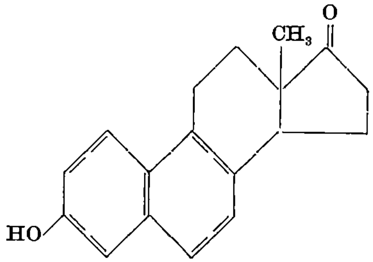
proved to be a much more potent carcinogen than the others previously synthesized[7]. Eight years of methodical work had thus supplied a solution to the problem posed a century and a half earlier.

3,4-benzpyrene seems to be the carcinogenic hydrocarbon produced most frequently and most abundantly in soot from coal fires, in the treatment of mineral oils, and, in certain circumstances, from the combustion of petroleum—all substances derived from the luxuriant vegetable and animal matter laid down during the primary era. 3,4-benzpyrene is also the principal hydrocarbon in cigarette smoke and in the vitiated air of industrial cities. It will be seen that the discoveries of which Kennaway was the author or initiator exercise a continuing repercussion on public life to-day.

They had an immediate influence on the trend of work in all the laboratories concerned with cancer research. While the team at the Cancer Hospital was widening the field of chemical carcinogens, demonstrating the induction of various types of cancer by a very large number of aromatic polycyclic hydrocarbons of differing structure (derivatives of anthracene and phenanthrene), similar teams of chemists and biologists were being formed in the United States, in Germany, Belgium, France, Japan, Norway, Switzerland and elsewhere. In a few years, numerous papers had been published on this subject and many new facts established. The time had come to classify these new data, and in 1937 [8], 1939 [9] and 1940 [10] the team at the Cancer Hospital published invaluable monographs analysing the principal papers and furnishing 877 references to earlier publications.

Since the induction of tumours by chemical means had now been amply demonstrated, it seemed that an understanding of the mechanism of carcinogenesis should soon lie within our reach. The relationship between the molecular structure and carcinogenic activity of chemical compounds was clear. The skeleton of the majority of them contains the phenanthrene ring system; oncogenic activity can be produced, enhanced, weakened or destroyed by simple substitutions on the molecule. It became possible to judge the carcinogenic potency of a given molecule simply from the ring configuration and the position of the substituents. For example, in 1933, Cook[11] predicted the carcinogenicity of a substance which had just been synthesized in Germany, and in the following year he demonstrated this experimentally, in collaboration with Haslewood[12]. The methylcholanthrene (see IV) molecule contains the phenanthrene structure common to numerous compounds the biological activity of which is nevertheless very dissimilar, such as alkaloids, certain cardiac poisons, vitamin D, steroid hormones and bile acids.

The similarity between the skeletons of the carcinogenic hydrocarbons and that of equilenin (III)

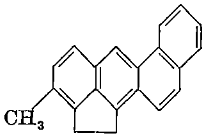


Equilenin

(III)

(a sex hormone discovered by André Girard *et al.* in 1932 in the urine of pregnant mares[13]) did not escape the attention of the research workers in Great Britain. In 1933, Cook and Dodds[14] published their finding of slight, but characteristic, œstrogenic activity of hydrocarbons such as 3,4-benzpyrene and 5,6-cyclopenteno-1,2-benzanthracene.

Was it not therefore conceivable that certain normal metabolites, of steroid structure, might be transformed into carcinogenic compounds in the body itself, as a result of one or more errors of metabolism? It was in fact not long before a new aromatic hydrocarbon, 3-methylcholanthrene (IV) was obtained and



3-Methylcholanthrene

(IV)

found to be even more active than 3,4-benzpyrene: it was produced synthetically from a bile acid, deoxycholic acid (see VIII) by dehydrogenation processes of which the organism is, however, known to be capable.

Kennaway at once perceived the connexion between this finding and an experiment of which he had published the results in 1928 with Sampson[15], and which had yielded tumours using the combustion products of cholesterol. The phenanthrene nucleus is also present in the cholesterol molecule, and it seems that steroid hormones, like bile acids, are built up from this molecule. Kennaway therefore, with his wife and Cook[16], put in hand experiments to demonstrate the carcinogenic activity of deoxycholic acid. It was two years before a single epithelioma was produced in the skin of a mouse; but subcutaneous injection produced several sarcomata *in situ*.

It was in this field of the relationship between carcinogens and hormones that collaboration started between the Research Institute of the Cancer Hospital (shortly to be named the Chester Beatty Research

Institute) and the Institut du Radium in Paris. The work which had been carried out in my laboratory since 1932 on the role of the steroid hormones in tumour induction probably influenced the workers at the Cancer Hospital in their new line of research. It is certain that the most recent publications on the part of these workers caused the line of research at the Institut du Radium to branch in the direction of the study of carcinogenesis by chemical agents.

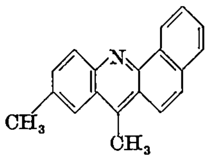
Meanwhile, Burrows, with his skill in histopathology, had proved a most valuable addition to the team at the Cancer Hospital studying induction of cancer by hydrocarbons. From 1935 onwards, he began to specialize in the study of hormonal cancers, and here too his contribution was invaluable. The recruitment of Horning to the Department enriched and accelerated its development. Henceforward I paid frequent fruitful visits to the Chester Beatty, and these two scientists became my firm friends; I cannot recall our cordial relationship without emotion.

I myself meantime had the good fortune to attract to my department a first-class organic chemist, Buu-Hoï. In collaboration with him, I was able to penetrate the vast field of exploration opened up by Kennaway, and to extend the investigations on the mechanism of carcinogenesis which I had pursued first with radiation cancers and later with hormonal cancers. I hope it will not be thought out of place if I now speak a little about the work we had been doing in Paris, which stemmed directly from Kennaway's discoveries.

It was inevitable that we should have to come to compare the action of the carcinogenic hydrocarbons with that of drugs, partly because of the close relationship between carcinogenicity and molecular structure demonstrated by Kennaway, and partly from the idea that the production of radiation cancers might be due to the probable attack on particular targets formed by intracellular organelles. In 1942 we conceived the idea of looking for proof along the lines of Ehrlich's postulate: *corpora non agunt nisi fixata*, already well established in chemotherapy. Thus the classic example of sulphanilamide and *p*-aminobenzoic acid had proved that the fixation of a pharmacodynamic agent on the cell may be disturbed by the presence of a molecule of similar structure. Using pairs of molecules consisting of a polycyclic carcinogen and an inactive or weakly active molecule, acting simultaneously on the skin of the mouse, Buu-Hoï, Rudali and I[17] found that such an antagonism between two hydrocarbons did in fact exist. The most reasonable explanation of the phenomenon seemed to us to be competition between two molecules to attach themselves to the organelle or organelles governing growth and cell division.

This fact has been more or less fully confirmed by several authors. American workers have even shown that certain hydrocarbons, such as methylcholanthrene, inhibit the production of cancer of the liver by carcinogens of very different structure, of the azo group. Two of the members of the active Madison school, the Millers, who have done so much to elucidate the problem of experimental liver cancer, have put forward a very tempting explanation of this observation[18]. With their co-workers, they were able to show that this fixation occurs at the level of certain cellular proteins[19], and thus to confirm that azo carcinogens behave in the same way as pharmacodynamic agents. It has since been found that, in the case of skin cancers, hydrocarbons possess the same capacity for attaching themselves to cellular proteins[20], and from the result of experiments with labelled hydrocarbons it is hoped to determine the quantitative relationship between this capacity and the carcinogenic potency of hydrocarbons. All these results form a solid basis for Kennaway's conception of chemical carcinogenesis.

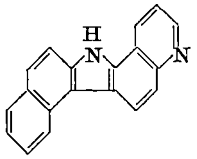
In the course of this work we were led to compare some polycyclic aromatic compounds with various nitrogen analogues. We found that the very large group of heterocyclic compounds analogous in structure to numerous classes of polycyclic hydrocarbons contained an even larger number of substances as active as, or even more active than, the corresponding homocyclic models. For example, the group of angular benzacridines, to which we devoted several years of study[21] includes an even larger number of carcinogens than the related benzanthracenes; a compound such as 3,10-dimethyl-7,8-benzacridine (V) is among the most active substances known.



3,10-Dimethyl-7,8-benzacridine

(V)

More recent work has brought to our notice a fresh class of heterocyclic nitrogenous compounds which promises to be at least as fertile in results as the benzacridine group. This is the azadibenzocarbazole group, some of the members of which are extremely potent, for example, compound (VI).



4′-Aza-1,2 : 5,6-dibenzocarbazole

(VI)

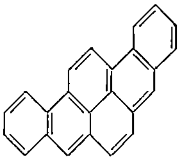
Here, as with the benzacridines, displacement of the heterocyclic nitrogen atom results in a considerable change in biological activity.

One might be tempted to believe that research of this kind, proving as it does that the already considerable number of known chemical carcinogens can be constantly increased, is of purely theoretical interest. In reality, the aim which we had set ourselves from the beginning was very different and very precise: it was to search for a relationship between carcinogenic activity and molecular structure, in the hope of reaching an understanding of the mechanism of chemical carcinogenesis. Earlier authors who had attempted to relate carcinogenic activity to certain molecular structures, such as the position of substituents or the number of rings, had come up against irreconcilable contradictions.

Now, during the Second World War, when France was occupied and only German publications reached us, my attention was attracted by an article by a chemist named Otto Schmidt[22] expounding a theory which appeared to furnish a mathematical explanation of carcinogenesis by polycyclic hydrocarbons, applicable equally to radiation carcinogenesis and even to virus production! The fact that I could not follow Schmidt's reasoning made me the more curious. I questioned the chemists among my friends; each referred me to another more versed in these matters; until finally I met a young theoretical chemist, Raymond Daudel, who was good enough to take an interest in this work. The author explained the differences in the activity of polycyclic hydrocarbons by calculations of wave mechanics. We were thus concerned with a quantum theory, and since, twenty years earlier, my friend the great physicist Holweck and I had been among the first to explain the biological action of radiations by the quantum theory, I was tempted to try to confirm, by experiment, Otto Schmidt's purely speculative idea.

Dare I say that I had fortunately not seen the latest monograph by Cook and Kennaway, published in 1940[10]. I should have been discouraged from undertaking any work in this field, had I read their judgment on Schmidt's work: "These conceptions of Schmidt are of interest, but in the absence of exact data concerning electron densities it is difficult to submit them to experimental test. Moreover, like many other theoretical interpretations they are apt to be more successful in explaining known phenomena than in predicting new ones."

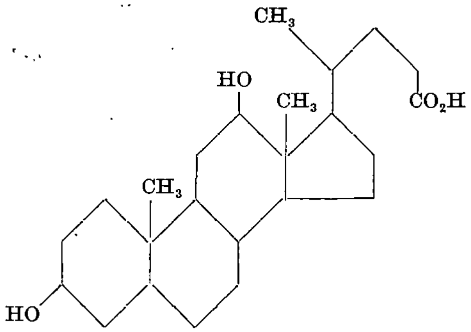
Yet in the twenty years which have elapsed since that time, the rapid progress in theoretical chemistry, aided by the powerful electronic computers, has consolidated the quantum theory of chemical carcinogenesis to the point where it is possible to predict the carcinogenic activity of hydrocarbons before they have been tested. I have neither the time nor the intention to go through the long stages by which the theoretical chemists of the Institut du Radium travelled along this arduous road, thanks to the abundant new material which our organic chemists and experimental biologists had been constantly supplying to the Daudels, the Pullmans and the Chalvets. I may simply recall that it was as a result of the latters' investigations of the electron densities in the molecule of 3,4:9,10-dibenzpyrene (VII), which suggested that this substance might be highly carcinogenic, that we undertook its synthesis and test on the mouse; and it proved to be one of the most potent carcinogens known[23]. The close relationship between this biological property and the molecular constitution of the compound indicates that there is a direct causal connexion between them, as Kennaway had assumed.



3,4 : 9,10-Dibenzpyrene

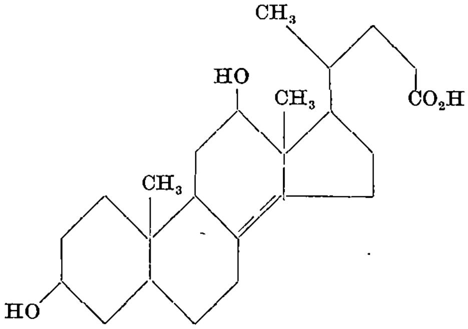
(VII)

Again, quite recently, we have confirmed another of Kennaway's important theses and found that the induction of certain spontaneous tumours may be due to certain metabolic variations in the biogenesis or the catabolism of natural steroids. I mentioned earlier the experiment he carried out with Cook and Lady Kennaway[16], which led to the recognition of oncogenic activity in deoxycholic acid (VIII). This result had not been confirmed by other workers, but in collaboration with Buu-Hoï and Zajdela[24] we have just established that another derivative of cholic acid, namely, apocholic acid (IX), is markedly sarcomagenic, and that this result is reproducible at will.



Deoxycholic acid

(VIII)



Apocholic acid

(IX)

In spite of all this brilliant work, one is obliged to admit that the accumulated research of half a century into the pathogenesis of tumours has still only brought us to the beginning of an understanding of the problem. Nevertheless, one can safely say that the results obtained from the study of chemical carcinogenesis still seem to stand out above all else in this difficult field—even in this era of virus research. They bear witness to Kennaway's worth and to the immortality of his work. His discoveries supplied the scientific bases necessary for the solution of many problems of occupational, preventive and social medicine to which this generous and forward-thinking man dedicated himself during the last years of his life.

I have wondered why I had been chosen to give the first lecture in memory of Sir Ernest Kennaway. Certainly one of the chief reasons must have been the way in which my researches tended in his direction, and the resulting bonds of friendship between the workers at the Chester Beatty Institute and those at the Institut du Radium. But I am inclined to think that account was also taken of the fact that we were contemporaries, he being a few years my senior. We came to maturity during the thirty years preceding the outbreak of the First World War, in a period when the cultivated middle classes, both in Britain and in France, enjoyed complete independence of mind and liberty of action.

It was a period when one could go round the world without a passport, stay at any hotel without filling up a form for the police, profess oneself an agnostic in the political as well as the religious sense without risk of being thought a revolutionary and suspected of a plot to endanger the safety of the State and of society.

We both grew up in an atmosphere where a respect for individual liberty and a veneration for scientific progress were looked on as articles of faith, and we must have been subjected to the same environmental conditions. The two provincial cities where we began our studies were certainly a great distance apart: Exeter, the 'Queen of south-west England', and Lyon, the 'Queen of south-east France'; and yet they are both hard-working industrial towns, with an equally ancient history—*Isca Dumnoniorum and Lugdunum.* In point of fact our scientific careers ran on parallel lines and were devoted purely to impartial research.

I should have failed in my duty if, after paying homage to Kennaway the scholar, servant of human progress, I had not also saluted Kennaway the thinker, who, in philosophy as in science, knew no other guiding star than reason.

[1] Kennaway, E. L., *J. Path. Bact.*, **27**, 233 (1924).
[2] Kennaway, E. L., *Brit. Med. J.*, ii, 1 (1925).
[3] Kennaway, E. L., *Biochem. J.*, **24**, 497 (1930).
[4] Berthelot, M., *Leçons sur les méthodes générales de synthèse*, 307 (Gauthier-Villars, Paris, 1864).
[5] Kennaway, E. L., and Hieger, I., *Brit. Med. J.*, i, 1044 (1930).
[6] Cook, J. W., Hieger, I., Kennaway, E. L., and Mayneord, W. V., *Proc. Roy. Soc.*, B, **111**, 455 (1932).
[7] Cook, J. W., Hewett, C., and Hieger, I., *Nature*, **130**, 926 (1932).
[8] Cook, J. W., Haslewood, G. A. D., Hewett, C., Hieger, I., Kennaway, E. L., and Mayneord, W. V., *Amer. J. Cancer*, **29**, 217 (1937).
[9] Cook, J. W., and Kennaway, E. L., *Amer. J. Cancer*, **33**, 50 (1938).
[10] Cook, J. W., and Kennaway, E. L., *Amer. J. Cancer*, **39**, 381 (1940).
[11] Cook, J. W., *Proc. Roy. Soc.*, B, **113**, 273 (1933).
[12] Cook, J. W., and Haslewood, G. A. D., *Chem. Indust.*, **52**, 758 (1933).
[13] Girard, A., Sandulesco, G., Fridenson, A., and Rutgers, J. J., *C.R. Acad. Sci., Paris*, **195**, 981 (1932).
[14] Cook, J. W., and Dodds, E. C., *Nature*, **131**, 205 (1933).
[15] Kennaway, E. L., and Sampson, B., *J. Path. Bact.*, **31**, 609 (1928).
[16] Cook, J. W., Kennaway, E. L., and Kennaway, N. M., *Nature*, **145**, 627 (1940).
[17] Lacassagne, A., Buu-Hoï, N. P., and Rudali, G., *Brit. J. Exp. Path.*, **26**, 5 (1945).
[18] Miller, E. C., Miller, J. A., and Brown, R. R., *Cancer Res.*, **12**, 282 (1952).
[19] Miller, E. C., and Miller, J. A., *Cancer Res.*, **7**, 468 (1947).
[20] Heidelberger, C., and Moldenhauer, M. G., *Cancer Res.*, **16**, 442 (1956).
[21] Lacassagne, A., Buu-Hoï, N. P., Daudel, R., and Zajdela, F., *Adv. in Cancer Res.*, **4**, 315 (1956).
[22] Schmidt, O., *Z. physik. Chemie*, B, **42**, 83 (1939).
[23] Lacassagne, A., Zajdela, F., Buu-Hoï, N. P., and Chalvet, H., *C.R. Acad. Sci., Paris*, **245**, 273 (1957).
[24] Lacassagne, A., Buu-Hoï, N. P., and Zajdela, F., *Nature*, **190**, 1007 (1961).

SEASONAL VARIATIONS OF CÆSIUM-137 IN AIR AT GROUND-LEVEL

By K. G. VOHRA, V. S. BHATNAGAR and C. RANGARAJAN
Air Monitoring Section, Atomic Energy Establishment, Trombay, Bombay-73

DURING 1956–58 the levels of radioactive fall-out showed substantial increase in the spring season at several places all over the world. This was attributed to the seasonal effects in the mixing between the stratosphere and the troposphere[1,2]. These conclusions were supported by the observed increase in ozone concentrations in the spring season in the northern hemisphere[3]. An alternative explanation of this spring increase in fall-out was given by Martell[4]. According to him, the spring maximum was observed because of the fact that the U.S.S.R. tests were carried out in the autumn of each year and this gave rise to a maximum of activity on the ground in late winter or spring, a period of 4–6 months being necessary for the matter from fall-out to diffuse from the stratosphere into the troposphere and the ground-level air. However, this explanation was based only on the weapon tests carried out in the polar regions and satisfied the data available at that time.

When the nuclear weapon tests were continuing, it was difficult to decide whether the spring increase was due to the weapons testing schedule or natural seasonal effects, or both. The cessation of nuclear weapon tests after October 1958 provided a period of about 15 months when the measurements could be carried out to study the seasonal variations.

We have been collecting samples of air-borne particulate matter on filter papers since early in 1956. These samples have been collected daily at several stations in India and analysed for gross beta-activity[5]. For finding out whether any seasonal variation existed, we started analysis of these samples for cæsium-137. Since cæsium-137 has a long radioactive half-life, the decay of activity on the filter paper during the period of storage was negligible. The daily samples were pooled for individual months, ashed at a temperature of 400° C., and counted for cæsium-137 using a low-level gamma-ray scintillation spectrometer. The cæsium-137 activity was estimated as monthly average concentration in $\mu\mu$c./m.^{3} of air.

Fig. 1 shows cæsium-137 concentrations, gross beta-activity, and ratios of cæsium-137 to gross beta-activity at Bombay for the years 1956–60. The dotted lines show the periods for which data were not available. The ratio of cæsium-137 to gross beta-activity gives an indication of the effective age of the

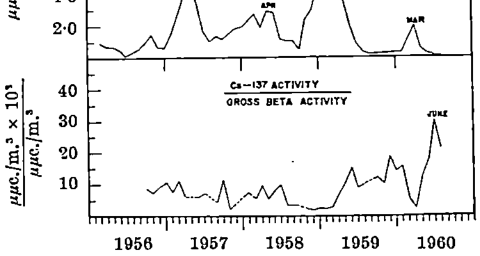


Fig. 1. Activity of cæsium-137 and gross beta-activity of ground-level air and the ratio cæsium-137/gross beta-activity at Bombay

Table 1. LOCATIONS OF THE SAMPLING STATIONS

Station	Altitude*	Latitude	Longitude
Srinagar	1,598 m.	34° 06′ N.	74° 55′ E.
Delhi	219 m.	28° 45′ N.	77° 20′ E.
Calcutta	Sea-level	22° 34′ N.	88° 25′ E.
Nagpur	311 m.	21° 12′ N.	79° 04′ E.
Bombay	Sea-level	18° 57′ N.	72° 55′ E.
Bangalore	922 m.	12° 57′ N.	77° 30′ E.
Ootacamund	2,235 m.	11° 23′ N.	76° 40′ E.

* Above mean sea-level.

fission products. Monthly cæsium-137 data for seven other stations in India (Table 1) for the years 1959 and 1960 is shown in Fig. 2, which also includes the levels averaged for all the stations.

Fig. 1 shows that the general trend of variations of the gross beta-activity and cæsium-137 in the air is similar. The peak in gross beta-activity in early 1960 is due to the first French atomic test[6]. The corresponding increase in cæsium-137 due to the French atomic test should be small. Therefore, the increase in cæsium-137 which started in the month of January 1960 and continued until March 1960 should be attributed to seasonal effects. The spring peaks observed in the years 1957, 1958 and 1959 could be due to the combined effects of spring increase and intensive testing of nuclear weapons a few months before the periods showing increase.

The ratio of cæsium-137 to gross beta-activity shows increasing age of the activity after October 1958, when tests were discontinued. However, the ratio shows a sharp decrease during February and March 1960, when the atmosphere contained fission products from the first French atomic test. After March 1960, the ratio increases rapidly, reaching a maximum in the month of June 1960. This also indicates an increase in the concentration of old debris during the months April, May and June 1960.

There are large variations in the levels of cæsium-137 at different stations (Fig. 2), but in general, there is a small increase from late 1959 to early 1960, followed by a decrease. This is more clearly seen in the graph showing the levels averaged for all the stations. This increase is not due to the French atomic test as the contribution of this test to cæsium-137 level in February and March has been estimated to be about 10–15 per cent of the total level from the previous tests. The period of increase at these stations is spread through January to May. These observations may therefore be explained by the seasonal effects in the mixing between the stratosphere and the troposphere as proposed by Stewart[1] on the basis of the Brewer model[7].

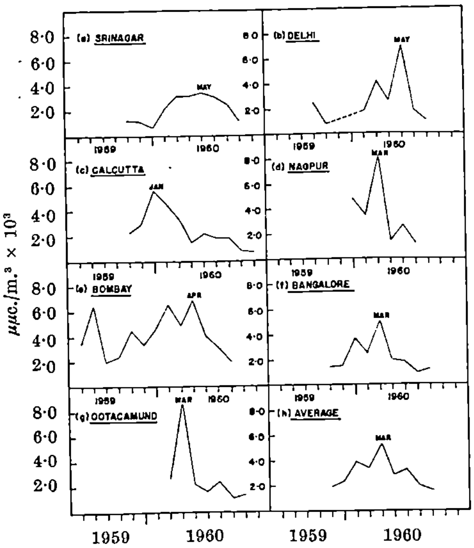


Fig. 2. Cæsium-137 concentration in ground-level air, 1959–60

Recently, there has been evidence from other sources as well on the existence of spring maximum in fall-out[8]. Gustafson has found increased levels of cæsium-137 in surface air during the period April–July 1960, from examination of air filter samples collected at the Argonne National Laboratory[9]. Kuroda, from his work on the concentration of strontium-90 in rainwater[10], and Lockhart, from the measurements of ground-level air-borne radioactivity[11], have also shown the existence of seasonal effects.

[1] Stewart, N. G., *et al.*, *The World-wide Deposition of Long-lived Fission Products from Nuclear Test Explosions*, Atomic Energy Research Establishment, *HP/R* 2354 (1957).

[2] Machta, L., *Discussion of Meteorological Factors and Fallout Distribution, Environmental Contamination from Weapon Tests, HASL*-42 (1958).

[3] Ramanathan, K. R., *et al.*, *Quart. J. Roy. Met. Soc.*, **86**, No. 368 (1960).

[4] Martell, E. A., *Science*, **129**, 197 (1959).

[5] Vohra, K. G., Shirvaikar, V. V., and Rangarajan, C., *Ind. J. Meterology and Geophys.*, **9**, No. 4 (1958).

[6] Vohra, K. G., Bhatnagar, V. S., and Rangarajan, C., *Ind. J. Meterology and Geophys.*, **12**, No. 1 ((1961).

[7] Brewer, A. W., *Quart. J. Roy. Meteorol. Soc.*, **75**, No. 351 (1949).

[8] Dyer, A. J., *Nature*, **189**, 905 (1961).

[9] Gustafson, P. F., Brar, S. S., and Kerrigan, M. A., *et al.*, *Science*, **133**, No. 3451 (1961).

[10] Fry, L. M., Jew, F. A., and Kuroda, P. K., *J. Geophys. Res.*, **65**, No. 7 (1960).

[11] Lockhart, *et al.*, *Science*, **132**, No. 3425 (1960).

A TAKE-OVER BID IN ACOUSTICAL TRAINING

By PROF. E. J. RICHARDS, O.B.E.
University of Southampton

SINCE noise is essentially related to horse-power, and since this latter commodity is increasing rapidly per man in this industrial community of ours, it was apt that the twelfth scientific conference held by the National Physical Laboratory on June 26–28 in conjunction with the Building Research Station and the National Engineering Laboratory on the "Control of Noise" should concern itself with 'unwanted sound', a topic of great current interest and controversy. In his opening remarks the director, Sir Gordon Sutherland, said that just as "beauty is in the eye of the beholder", then "noise is in the ear of the listener". The enormous width of coverage provided during the three days to the three hundred or so delegates followed as a natural corollary and included a series of papers on acoustical measurements and the various objective and subjective units, on the nature of sound sources and methods of

Appearing in August

POPULAR LECTURES IN MATHEMATICS

Translated from the Russian by **HALINA MOSS**

Edited by **IAN N. SNEDDON,** *Simson Professor of Mathematics, University of Glasgow*

I—**The Method of Mathematical Induction** *I. S. SOMINSKII*	7*s.* 6*d. net*
II—**Fibonacci Numbers** *N. N. VOROB'EV*	10*s. net*
III—**Some Applications of Mechanics to Mathematics** *V. A. USPENSKII*	10*s. net*
IV—**Geometrical Constructions using Compasses Only** *A. N. KOSTOVSKII*	10*s. net*
V—**The Ruler in Geometrical Constructions** *A. S. SMOGORZHEVSKII*	10*s. net*
VI—**Inequalities** *P. P. KOROVKIN*	10*s. net*

Each of these books covers a topic in elementary mathematics, starting from the kind of knowledge gained by a student in the early forms at school. Three of these—Inequalities, Fibonacci Numbers and The Method of Mathematical Induction—deal with algebra and cover the topics in a much fuller and more interesting way than is done in elementary text-books; they should be of great value to anyone who is continuing with the study of mathematics either for itself or as a tool in engineering and physics. The remaining three titles deal with geometrical topics and contain much material which is not available in existing text-books.

Although dealing with elementary topics these books approach them from an adult point of view and in addition to being of great interest to school teachers and university lecturers, they should be of interest to people who have long since stopped the study of mathematics, but who now want to learn something of the topics under discussion.

Each book contains problems worked by the authors, all distinguished mathematicians, and exercises to be worked by the reader. In each case there is a section on the solutions of the problems.

The above 6 Lectures are also available bound together in a special Library Edition. 50*s. net*

**Distributed in America by the Blaisdell Publishing Co.*

Also available in this New Series

One Hundred Problems in Elementary Mathematics

H. STEINHAUS

The author aims to stimulate independent mathematical thinking in young people between 14 and 17 years of age, giving examples of the application of mathematics to problems and situations encountered in life, in science, in sport and in business. 30*s. net* ($5.00)

Animal Hormones — A Comparative Survey

Part I—Kinetic and Metabolic Hormones

P. M. JENKIN, *Senior Lecturer in Zoology, University of Bristol*

This is an introductory survey of the sources and actions of vascular hormones in invertebrates, compared with those in vertebrates. Part I covers 'kinetic' hormones—controlling muscles, pigmentary effectors and glands, and 'metabolic' hormones—acting on respiration, diapause, and the balance of carbohydrates, proteins, salts, and water. Part II will cover 'morphogenetic' hormones—controlling growth, differentiation and reproduction. Detailed examples and figures from recent literature illustrate each type of hormonal action, so that the practice, principles and problems of endocrinology are clearly presented for University students, graduates starting research, and teachers. The logical approach to the subject provides a framework within which new material can quickly be assessed.

296 *pages* *numerous illustrations* 45*s. net* ($7.50)

All books gladly sent on 14 days' approval

PERGAMON PRESS

OXFORD LONDON NEW YORK PARIS

Headington Hill Hall, Oxford. 4 & 5 *Fitzroy Sq., London, W.*1. 122 *East 55th St., New York 22, N.Y.*

LIVERPOOL EDUCATION COMMITTEE
COLLEGE OF TECHNOLOGY
BYROM STREET, LIVERPOOL, 3

Post-Graduate Courses in
NUCLEAR SCIENCE
SESSION 1961–62

A. "**RADIATION SAFETY AND HEALTH PHYSICS.**"
(In consultation with the U.K. Atomic Energy Authority.)

1. Seven-months full-time course, leading to College Diploma in Health Physics. Fee: £25 for each part.
   Part 1, January 22 to April 13, 1962.
   Part 2, April 30 to July 20, 1962.

   Part 1 may be taken separately and complies with the U.K.A.E.A. Veale Committee recommendations for Category B courses.

   The Department of Scientific and Industrial Research has accepted the whole course as suitable for the tenure of its advanced course studentships.

   Applications for an award should be addressed to the College.

2. Two-weeks full-time courses, Fee: £12.
   RS16 September 18 to September 29, 1961.
   RS17 November 13 to November 24, 1961.
   RS18 May 28 to June 8, 1962.
   RS19 July 2 to July 13, 1962.

B. **GENERAL COURSES.**

1. RADIOCHEMISTRY, two-weeks full-time October 16–27, 1961. Fee: £12.
2. RADIOBIOLOGY, two-weeks full-time. Fee £12.
   RB2 August 28 to September 8, 1961.
   RB3 January 8 to January 19, 1962.
   RB4 May 7 to May 18, 1962.
3. RADIATION CHEMISTRY, one-week full-time, June 18 to 22, 1962. Fee: £5.

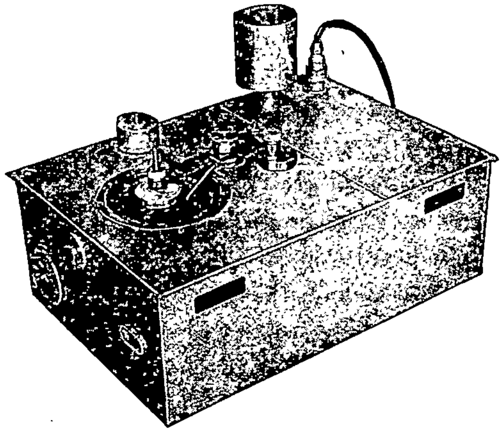
Further particulars and application forms for all courses may be obtained from the Head of Department of Chemistry, at the above address.

THOMAS ALKER,
Town Clerk and Clerk to the
Local Education Authority.

(J6937)

AUTOMATIC POLISHING MACHINE
for metallurgical specimens

New design with novel features essential for the modern works laboratory



Please write for details

R. & J. BECK LTD.
69/71 Mortimer St. London W.1

GREEN'S PURE FILTER PAPERS

Have you met Green's 904?

Very rapid, of enormous wet strength and resistant to acids and alkalis.

Write for price list N.G. and free samples.

J. BARCHAM GREEN LTD.,
MAIDSTONE ENGLAND

suppression, on the ear and hearing conservation, and lastly on community reaction criteria and the possibilities of legislation.

In a way, noise investigations have progressed beyond the stage where large scientific break-throughs are to be expected, and the tone of the conference was therefore one of quiet informativeness rather than belligerent assertiveness. Nevertheless, two items of interest arose time and time again.

The first concerned units, and the need for the establishment of satisfactory criteria of noise-levels which are in keeping with the true subjective reaction of the community. As one speaker lightheartedly put it, "the reason why acousticians are held in disrepute is because they are still arguing emotionally over units. If electrical engineers still regarded electrical current in terms of the nerve stimulus of a dead frog, they equally would be laughed at". Unfortunately, the subjective unit of noise is "emotional" and must include adequately, though maybe not completely, the stimulus of both the ear and the brain. The conference papers did a great deal to pinpoint these basic parameters ; indeed, the papers by D. W. Robinson and Prof. C. W. Kosten were most satisfactory, not so much for their detailed content, as for the impression conveyed that the corner had been turned, and that provided the scientific man-power was available, units acceptable universally to describe aircraft, motor-cycle and factory noises would be put forward during the next ten years.

The second item which needs airing and which was mentioned by many speakers is the provision of trained acoustical engineers. This has up to now been the prerogative of the university physics departments, and it can only be said that both in Great Britain and elsewhere they have taken this duty lightly, even though in fairness it must be said that there are isolated units fighting against the stream of atomic energy and cosmic research at such universities as Durham (Newcastle upon Tyne), London (Imperial College of Science and Technology), Liverpool and Southampton. However, it is significant that since the death of Prof. E. G. Richardson at Newcastle, there has been no single professor of acoustics in Great Britain, and even with the grandiose plans for university expansion there does not appear to be any rush to put right this deficiency. Even at the Imperial College acoustics appears in a lowly place in the expansion process.

Bearing in mind that physicists are being forced to introduce a greater and greater width of fundamental understanding to their students in the three years at their disposal, it may well be that physics departments are justified in this attitude since the basic physics involved in pure acoustics, if not in noise generation, has been established for many years, and the outstanding problems extend into physiology, psychology and engineering as well as into physics. The time has probably arrived therefore when the custodianship of acoustical training should be taken up by the faculties of applied science or engineering and that such a 'take-over bid' should be included in the plans for the next quinquennium. Only in this way can the gross shortage of acoustical and vibration engineers be met.

Such a step is not an unnatural one. Engineering has been defined as the efficient application of the sciences in the interests of the community. Thus a good engineering training, in addition to the teaching of the fundamental sciences and methods of application, also includes some training in economics and in the behaviour of the community. The engineer expects to be interested in 'human engineering', as it is called in the United States, and not only in the purely scientific aspects of his work.

What steps should be taken if the above point of view is accepted ? The establishment at Southampton of a year's postgraduate M.Sc. course in 'vibration and noise studies' to commence in October, and the provision of a readership and lectureship by the Hawker Siddeley Group to support this course is ample evidence of my view. However, a case can also be made out for a 'human engineering' option in the undergraduate stage of the degree in engineering. Such a course could well provide tuition in acoustics, vibration and elementary physiology in the third year in the way that aerodynamics and design, nuclear power and thermodynamics, and hydraulics and soil mechanics is provided now for the aeronautical, nuclear and civil engineer respectively.

Bearing in mind the ever-present practice of putting more and more in a pint pot, the universities themselves favour specialization in the fourth year rather than at the undergraduate stage. However, the pressure from industry for the services of even mediocre students is putting the success of fourth-year M.Sc. courses in jeopardy, and it is to be hoped that industry and the research establishments will themselves see fit to sponsor for such courses students coming into their employ, in order to put right the complaint regarding training so often made at this excellent conference.

OBITUARIES

Dr. Lee de Forest

Dr. Lee de Forest, a pioneer in the history of radio communications and, after much litigation, the acknowledged inventor of the three-electrode thermionic valve, died on June 30 in California at the age of eighty-seven. He was born in August 1873 in the parsonage at Council Bluffs, Iowa, his father being a Congregational minister in that town. Like his father, he graduated from the Yale Sheffield Scientific School in 1896, receiving his Ph.D. degree three years later. While at Yale his interest in electrical phenomena was stimulated by the work of Sir William Crookes and Nikola Tesla and by witnessing demonstrations of Hertz's experiments on electromagnetic waves. In 1899 he went to Chicago to work in the Dynamo Department of the Western Electric Co., and it was there that he became interested in the possibilities of a hot gas or flame detector as an alternative to the coherer then used in wireless receiving circuits.

From this he turned his attention to the Edison effect which he had learnt about at Yale, and which led J. A. Fleming to patent, in 1904, the first diode thermionic valve detector. Two years later, on October 25, 1906, de Forest applied for a patent for a three-electrode valve—a triode—as a device for amplifying weak alternating currents such as were obtained in the reception of radio signals. This was achieved by applying a voltage to the third electrode inserted between the hot filament or cathode, and the plate, or anode. A few months later, de Forest extended the patent to cover the use of the three-electrode valve as a detector.

With a true inventor's imagination, Lee de Forest foresaw some of the possibilities of radio telephony and broadcasting; and, using an arc transmitter at the Metropolitan Opera House in January 1910, he was the first to broadcast opera arias sung by Caruso and other noted artists.

In 1914 the three-electrode valve was introduced as an oscillator and quickly replaced the arc as a source of continuous oscillations for radiotelephony. It was during the First World War, with the great demands it made on radio communications, that the thermionic valve was rapidly developed and applied to the generation of electromagnetic waves at the sending station, and to the detection and amplification of signal currents at both radio and audio frequencies at the receiver. During the period 1919–24, de Forest devoted most of his attention to the problems of talking cinematograph films, and his phonofilm was an early development in this field.

Altogether he held some 300 patents in the United States and other countries, covering such subjects as the guidance of aircraft by radio beams, the wire transmission of news pictures, and the measurement of cosmic rays. He was awarded the Cross of the French Legion of Honour, and his work was recognized in the United States by the receipt of the Edison Medal and Elliott Cresson Medal of the Franklin Institute, and by Gold Medals at the St. Louis Exhibition in 1904 and the Panama Pacific Exhibition in San Francisco in 1915. He was a founder, Fellow and past-president of the Institute of Radio Engineers (New York), the Gold Medal of which he won in 1922. Apart from various scientific papers, Lee de Forest was the author of *Television Today and Tomorrow* and his autobiography entitled *Father of Radio*.

R. L. SMITH-ROSE

Prof. B. Hague

PROF. BERNARD HAGUE died on September 29, 1960, in his sixty-eighth year, having occupied the James Watt chair of electrical engineering in the University of Glasgow since 1946, on the retirement of Prof. G. W. O. Howe. Born at Barnsley, Yorkshire, he received his early education at the Grammar School, Eccles, and at the Central School, Rochdale. He started as a millwright apprentice in Rochdale at the age of fifteen and later served as a junior draughtsman with Messrs. Ferranti, Ltd., Hollinwood. He attended evening classes at Rochdale Technical School from where he obtained an exhibition enabling him to study at the City and Guilds College, London. He graduated with first-class honours in 1915, and in the following year obtained the diploma of the Imperial College of Science and Technology. During 1916–20 he was employed as a technical assistant at the Royal Aircraft Establishment, Farnborough, and he began his teaching career with an appointment as a lecturer at the City and Guilds College after being awarded an M.Sc. degree in 1919. His association with the late Prof. G. W. O. Howe, then a lecturer at the City and Guilds College, led to his appointment as a lecturer at the University of Glasgow in 1923, after the appointment of G. W. O. Howe to the James Watt chair of electrical engineering at that University in 1921.

In 1926 he was awarded the Ph.D. of the University of Glasgow, and became a D.Sc. in 1927 and a Fellow of the City and Guilds of London Institute in 1936.

Prof. Hague was a true scholar of wide knowledge and experience. His intellectual pursuits and admirable personal characteristics earned him the respect of many and the loyalty of his staff. He was the author of books on a.c. bridge methods, instrument transformers, electromagnetic problems in electrical engineering, an introduction to vector analysis and the method of symmetrical co-ordination in the theory of polyphase circuits. His *A.C. Bridge Methods* is still regarded as a reference text and has been translated into several languages.

Hague had a particular clarity of presentation both in his writing and in his teaching and this reflected a thoroughness and sincerity of personal endeavour. He appreciated the visual arts and had a deep interest in music, being himself an oboist of distinction. He was a member of the Royal Musical Association and previously a governor of the Royal Scottish Academy of Music.

Above all, his interest in his students was personal and genuine, and he was ever ready to give of himself to the benefit of others, thereby gaining great respect and affection from all with whom he came into contact.

J. LAMB

NEWS and VIEWS

Chemical Defence Experimental Establishment, Porton : Dr. E. A. Perren

DR. E. A. PERREN has just retired from the post of director of the Chemical Defence Experimental Establishment, Porton, after thirty-eight years service in the War Department and Ministry of Supply. He studied chemistry at the Royal College of Science and obtained his Ph.D. in 1922 for researches in Prof. Jocelyn Thorpe's laboratory on three-carbon tautomerism. He then continued to work with Thorpe, as his assistant, at Porton and was absorbed later into the permanent staff there. He was concerned for many years with research into methods of producing smoke-screens, and the results of this work were put to profitable use during the Second World War. His responsibilities afterwards widened and included spells of duty at headquarters and in supervisory work at the Establishment. During 1948–49 he returned to the laboratory to undertake some research in organic chemistry and was then loaned for two years to the Canadian Government to take charge of its Experimental Station at Suffield, Alberta. In 1955 he was appointed director at Porton and retires with the rank of chief scientific officer. Dr. Perren is to continue to serve the Chemical Defence Experimental Establishment in the research laboratories.

Mr. E. E. Haddon

Mr. E. E. Haddon, director of Chemical Defence Research and Development at the War Office, has succeeded Dr. Perren. Mr. Haddon was born at York in 1908. He was educated at Archbishop Holgate's School, York, and at Queen Mary College, London (then East London College), where he graduated with honours in chemistry. After a short period with the Admiralty, he joined the Chemical Warfare Research Department at the War Office in 1929, and during the Second World War became deputy to the Controller of Chemical Defence Development in the Ministry of Supply. In 1945, on promotion to senior principal scientific officer, he temporarily gave up his association with chemical defence to become secretary to the Scientific Advisory Council and assistant director of technical and personnel administration in the Ministry of Supply. He returned to the field of chemical defence in 1952, when he became assistant director, Chemical Defence Research and Development. Five years later he was promoted to the grade of deputy chief scientific officer on becoming director. Mr. Haddon has been closely concerned not only with the direction of chemical defence research in the United Kingdom but also with the framing and implementation of national defence research policy and with the furtherance of international collaboration in this field.

London Transport Directorate of Research : Mr. A. T. Wilford

Mr. A. T. Wilford, director of research, London Transport, is to retire on June 30. Mr. Wilford was educated at Wilson's Grammar School, Camberwell, and obtained his degree at the Imperial College of Science and Technology. During the First World War he served in the Special Brigade, Royal Engineers, and later with the Royal Field Artillery. He entered the service of the former Underground Companies in 1920 as chemist with the London General Omnibus Co. and became chief chemist in 1926, a post which he continued to occupy under the London Passenger Transport Board. He became superintendent of laboratories when the Laboratory Services of the London Transport Executive were centralized in January 1949, and, in May 1950, he was appointed to the new post of director of research. Mr. Wilford has been responsible for the general direction of research activities and scientific control in London Transport and for liaison in these matter with other sections of the British Transport Commission. The new Research Laboratory at Chiswick, which was officially opened in December 1960, was designed in accordance with his requirements. He has served on a number of committees and for several years was a member of Council of the Institute of Petroleum. He is the author of a number of papers concerning the use of fuels and lubricants in road service vehicles and for one of these he was awarded the Crompton–Lanchester Medal of the Institution of Mechanical Engineers.

Mr. H. E. Styles

Mr. H. E. Styles, who is succeeding Mr. Wilford as director of research, London Transport, is fifty-two. He was educated at Strand School, Brixton, and Battersea Polytechnic, where he graduated with first-class honours in chemistry. He joined the staff of the London General Omnibus Co. laboratory, the forerunner of London Transport's present Research Laboratory, in September 1927. In 1940 he became assistant to the chief chemist and, later, assistant to the superintendent of laboratories. Mr. Styles was appointed superintendent of laboratories in 1950, in charge of the Central Laboratory at Chiswick and three laboratories located at London Transport's Lots Road, Greenwich and Neasden electricity generating stations. During the time he has been superintendent, the activities of the Central Laboratory have expanded, both in volume and range, to such an extent as to warrant provision of the newly erected Research Laboratory at Chiswick, in the design of which Mr. Styles was closely concerned. Mr. Styles represents London Transport on the Standardisation Committee of the Institute of Petroleum, the Illumination Industry Standards Committee of the British Standards Institution and the National Illumination Committee of Great Britain. He is chairman of the sub-committee of the National Illumination Committee concerned with Lighting for Transport.

Mr. Isaac Shoenberg : Silver Jubilee of British Television

Following a recommendation of the Television Advisory Committee, an experimental television service began in London in August 1936, with two systems—those of Baird on 240 lines and of Electrical and Musical Industries, Ltd. (E.M.I.), on 405 lines (interlaced). Three months later a public service was formally opened by the Postmaster General; and in February 1937 the Television Advisory Committee recommended that henceforth only the E.M.I. system should be used. In this way the world's first public television service was inaugurated; and what has been sometimes termed the Shoenberg–E.M.I. system has been used by the B.B.C. ever since. Isaac Shoenberg was born in Pinsk, in Russia, in 1880. After taking his degree at the Technological Institute of Kiev, he joined a Russian company and was responsible for research, design and installation of wireless transmitting stations. He came to England in 1914, becoming a British subject five years later. In 1931 he became director of research of the newly formed Electrical and Musical Industries, Ltd. Mr. Shoenberg led the team of scientists and engineers which, in a remarkably short time, developed the 405-line television system, to substantially the same standards as are in use to-day. Mr. Shoenberg was awarded the Faraday Medal of the Institution of Electrical Engineers in 1954 in recognition of his outstanding technical contribution to the British television service. He was the guest of honour at a reception held recently by E.M.I. at Hayes to mark the silver jubilee of the E.M.I. television system.

Status of the Norfolk Broads

In a written answer in the House of Commons on August 1, the Minister of Housing and Local Government, Mr. H. Brooke, pointed out that the primary responsibility for the designation of National Parks in Britain rests with the National Parks Commission. The Commission had advised him that the unique character of the Norfolk Broads made them in no way comparable in type with the other areas which, in conformity with the general requirements of the National Parks and Access to the Countryside Act, had been designated as National Parks. Mr. Brooke concurred in the Commission's conclusion that designation under that Act would not be appropriate, and he said he had agreed that the Minister of Transport, in consultation with the Minister of Agriculture, Fisheries and Food and himself, will consider the views of the Great Yarmouth Port and Haven Commissioners, the East Suffolk and Norfolk Rivers Board, and other local bodies about the future of the Broads.

International Council of Scientific Unions

THE ninth general assembly of the International Council of Scientific Unions will be held at the School of Pharmacy, University of London, during September 25–28. The general assembly will be preceded by meetings of the bureau and of the executive board to be held in the rooms of the Royal Society, which is the British adhering body to the International Council of Scientific Unions. The last general assembly of the Council held in London was in 1946, the first after the Second World War. While the scientific results of the international effort put into the International Geophysical Year are gradually becoming available, plans are being made by the International Council of Scientific Unions and several of the Unions for the "International Year of the Quiet Sun", April 1, 1964–December 31, 1965. The results obtained during the maximum solar activity period of the International Geophysical Year can only be of full value with a complementary programme at the forthcoming period of minimum activity. The disciplines of geomagnetism, aurora and airglow, cosmic rays and space research are particularly affected. Preliminary work is taking place on an "Upper Mantle Project" proposed by the International Union of Geodesy and Geophysics. There is to be a meeting on the subject in Moscow in 1963. The study of the upper mantle and its influence on the Earth's crust is fundamental to the solution of the outstanding questions of geology and solid earth geophysics, and will include work on deep drilling, deep-sea seismographs, deep-focus earthquakes, magnetic and gravimetric investigations, tectonic and magnetic development of crust, work on changes in phase, thermal conditions and equation of state, and high-pressure laboratory work on behaviour of rocks.

The International Union of Biological Sciences has for some time been discussing plans for an "International Biological Programme". Preparation for the programme will take several years, and a preparatory committee of six members has been set up to establish communication lines with the United Nations Organization, national adhering bodies, and other interested groups, and to formulate a precise programme on the basis of information gained. The "Special Committee of Oceanic Research", set up by the International Council of Scientific Unions in 1957, is planning an intensive investigation of the Indian Ocean. A number of reconnaisance cruises has been carried out; but the main period of activity in the Indian Ocean will be 1962–63. With at least thirteen countries and some forty-three ships, the International Indian Ocean Expedition promises to be more ambitious than the oceanographic contribution to the International Geophysical Year.

First Pamphlet on Clean Air

THE year 1661 may be said to be the beginning of the campaign for clean air. In that year John Evelyn wrote his famous tract, or discourse, *Fumifugium, or the Smoake of London Dissipated.* The work is dated May 1661, and it was presented to Charles II in September 1661. King Charles, Evelyn records in his diary, "was pleased that I should publish it by his special commands, being much gratified with it". The National Society for Clean Air has now reprinted the tract. It is available either as a paper-back at 2*s.* 6*d.* or cloth-bound at 5*s.* (postage 3*d.* or 6*d.* extra) from Field House, Breams Buildings, London, E.C.4. In the Epistle Dedicatory to "The King's Most Sacred Majesty", Evelyn explains how the idea of the book was conceived: "It was one day, as I was Walking in Your MAJESTIES Palace at White-Hall that a presumptuous Smoake issuing from one or two Tunnels neer Northumberland house, and not far from Scotland Yard, did so invade the Court; that all the Rooms Galleries, and Places about it were filled and infested with it; and that to such a degree, as Men could hardly discern one another for the Clowd, and none could support, without manifest Inconveniency". In his preface to the tract, the author waxes eloquent: "That this Glorious and Antient City, which from Wood might be rendred Brick (like another *Rome*) from Brick made Stone and Marble; which commands the Proud Ocean to the *Indies*, and reaches the farthest *Antipodes* should wrap her stately head in Clowds of Smoake and Sulphur, so full of Stink and Darknesse, I deplore with just Indignation".

Measurement of Quality in the Chemical Factory

IN the latest Department of Scientific and Industrial Research booklet which has been produced to extend the application of science in industry, A. J. Clark and C. W. Munday, of the Distillers Co., Ltd., discuss the use of instruments to control processes in the production of chemicals. The authors sum up their attitude with the words, "All chemical plants should have skilled analysts for measuring product quality, and it is indisputable that a first-class analyst is far more versatile than any instrument. However, if it is found that the successful functioning of a process depends on a large number of routine analytical operations, it is worth considering whether an instrument would be a more effective way of doing the job". The advantage of such an arrangement is that the analyst can be released for more creative work, and that continuous control can be exercised without the delay inherent in human supervision. In this booklet, *Quality Measurement in the Chemical Factory*, the emphasis is on instruments of proved worth and relative cheapness, with as wide a range of applications as possible. The authors pay special attention to the use of gas chromatography. Process-stream gas chromatography analysers with a simple repetition time of 1 min. are now becoming available, and, linked to automatic controllers, are already controlling chemical processes. Copies of the booklet may be obtained free from the Department of Scientific and Industrial Research Library, Charles House, 5–11 Regent St., London, S.W.1.

A Soviet View of British Libraries

UNDER the title "A Soviet View of British Libraries", the Library Association has issued as *Pamphlet* No. 22 a report on a visit in October—November 1959 by V. I. Shunkov, G. G. Firsov and N. I. Tyulina, translated by A. Thompson (Pp. 32, The Library Association, London, 1961. 5*s.*). The pamphlet gives an interesting picture of the learned and public libraries as seen through Russian eyes, of co-operation between these libraries, their staffs, organization, buildings and equipment. Of the learned libraries, only the British Museum Library and the National Libraries of Scotland and Wales are mentioned more than in passing; of the university libraries only those of Oxford and Cambridge; and the special libraries are even more sketchily noted. There is no reference to any library maintained by a professional society such as the Chemical Society, and the description of the public library service is likewise extremely condensed, though it is obvious from the comments made that the writers of the report have seen and pondered a great deal they have been unable otherwise to mention. The slenderness of most library staffs is stressed, but in noting the favourable comments on buildings and equipment it should be realized that most of the libraries visited were in new buildings, not always completely finished or equipped.

Japanese Planetarium Equipment

MESSRS. CHIYODA KOGAKU SEIKO K.K., manufacturers of Minolta cameras, have now developed a series of Minolta planetarium projectors. These projectors are distributed in the United Kingdom by Japanese Cameras, Ltd., Tunstall, Stoke-on-Trent. There are four standard projector models in the series priced in the range £7,000—£69,000. Models *S* and III are remote controlled and require a dome the diameter of which is in the range 66–100 ft., while models *MS* and *M* are controlled from a position adjacent to the projector and require a dome the diameter of which is in the range 20–50 ft. Models *MS* and *M* project about 3,000 stars, and projectors are installed for the movements of the planets. These planetary projectors are quite separate in the case of model *MS*, but are coupled in the case of model *M*. Model *S* is similar to model *MS* except that it carries a projector giving 9,000 stars and a separate bright star projector. Model III, the most refined of the series, is similar to model *S* with regard to star projection, but incorporates the coupling of the planetary projectors as in model *M* as well as other devices. Each of the four standard projectors carries an equivalent set of special effects projectors, such as a Milky Way projector, a morning- or evening-glow projector, etc. Model III carries a constellation projector in addition. The standard projectors may be supplemented with auxiliary projectors to reproduce auroral phenomena, the solar system as viewed by an external observer, etc. It is interesting to observe that an auxiliary projector can be provided to illustrate the motion of artificial satellites—a choice of American or Russian being provided. It is to be hoped that the advent of cheaper projectors may encourage the establishment of more planetaria in Britain. Small instruments, not necessarily operating on a commercial basis, could be used to assist the teaching of astronomy in schools and colleges, especially if situated in large centres of population.

Medicine, Science and the Law

THREE papers, which appear in *Medicine, Science and the Law* (1, No. 4; July 1961), contain implications of considerable importance; each is a real advance in the development and refinement of scientific methods of identifying blood. Dr. R. A. A. Coombs and Barbara Dodd describe a microscopical technique which enables them to determine the nature of a stain on a single fibre of clothing or other material. They show that it is now possible to say with a high degree of certainty not only that the fibre is stained with human blood but also to determine, at least within the limits of the *ABH* system, the group to which the blood belongs. The method is applicable to stains of considerable age, certainly up to three years, in the case of human blood. Dr. H. H. Muller and his colleagues have examined the problem of whether stains consist of human or animal blood in a different way. They have evolved a method of testing for hæmoglobin in the stain. Hæmoglobin differs in its minute physical and chemical characteristics from species to species, but it has hitherto been difficult to identify the species from which it came. The method suggested does not give information about the group of the blood in question. Dr. I. Dunsford, R. Lodge and W. Spielmann describe an investigation into blood groups in a paternity case in which, after a detailed examination of the groups of the child and putative father using all the refinements in technique normally available, the blood groups of the putative father fitted the blood groups of the child in every aspect but one. The child's *Rh* constitution was shown to be *CDE*/*cde* and the putative father's more probably *CDe*/*cDE* but possibly *CDE*/*cde* (that is, as in the child). The technique described in their paper enabled the authors to show that the putative father's *Rh* constitution was *CDE*/*cde*, so that he was not excluded. Moreover, the 'fit' of his blood with the child's is so good that (although the authors do not claim it) the scientific evidence points to his being in fact the father.

Blindness in Africans in Northern Rhodesia

IN 1949 territorial ophthalmic services were started in Northern Rhodesia. Before that date very little was known of the incidence of eye diseases and even less about the extent and causes of blindness. In 1950 a simple blindness register card was produced and distributed to all administrative officers, together with a simple memorandum on eye diseases describing the four conditions which are the principal causes of blindness. Dr. C. M. Phillips, Government ophthalmologist, Lusaka, now shows (*Central African J. of Med.*, 7, No. 5; May 1961) that the overall incidence of blindness is considered to be 500–750 per 100,000; in some areas it rises as high as 2,300, with the unique feature of having a higher incidence of onset in childhood than in adulthood. The main causal lesions of 2,701 patients examined are corneal pathology, phthisis bulbi, senile cataract and trachoma. It is considered that the predominant factor responsible for the first two lesions is traditional African medicine. Taking all factors into consideration, it is believed that four out of every five persons who have lost their sight did so from a cause that is either preventable or curable.

Uses of Castorseed

A TWO-DAY seminar on Castorseed and its Products was held during August 11–12, 1960, at the Regional Research Laboratory, Hyderabad, under the sponsorship of the Council of Scientific and Industrial Research, India. In order to consider the various factors bearing on the present and future of the castor industry in India, a number of questions likely to arise were first formulated under suitable heads. These were sent to a large number of research institutions, organizations dealing with castor, and firms either possessing castor or utilizing its products, especially castor oil. Data or information illustrating and amplifying the questions raised were requested. The data, received in the form of brief notes, were arranged suitably under the different heads. These questions and notes were then sent round to all the organizations for thorough study, ensuring that the discussions at the venue would be well informed and fruitful. The entire material has now been brought together in a booklet, *Castorseed and its Products* (Pp. viii + 87. Hyderabad: Indian Central Oilseeds Committee. 1961). It includes the questions raised, the notes received and the discussions which later took place at the sessions. Though the seminar dealt primarily with castor in its Indian setting, the material in this booklet might well have a wider interest.

National Council for Technological Awards

THE report of the National Council for Technological Awards for the period April 1960 to March 1961 (London: National Council for Technical Awards. Pp. 37) records that nearly 5,000 students were following courses leading to the diploma in technology, compared with 3,800 a year ago, and 309 diplomas were awarded, bringing the total to 472. Approximately 83 per cent of the engineering students and 56 per cent of students of other technologies are working in industry, and the Council is impressed by the increase in the number of students following sandwich courses in technologies other than engineering, from 219 in November 1957 to 1,246 at the end of the year under review; for engineering sandwich courses the corresponding figures are 652 and 3,191. Of 1,769 students enrolled in first-year courses leading to the diploma in the current session, 63 were women. Almost 25 per cent of these students entered their courses by virtue of holding good Ordinary National Certificates, and the Council points out that but for the development of these courses few of these students would have had an opportunity of gaining a qualification equivalent in standard to a university honours degree, and many might never have been added to the nation's technological man-power. The diploma is now accepted as a qualification for entry to the Scientific Civil Service equivalent to a university degree, and most professional institutions exempt holders of the diploma from taking the whole of their own examinations for graduate membership. The Council expresses appreciation of two awards from the Nuffield Foundation in support of study of the nature of courses leading to the diploma: one at the Brunel College of Technology concentrates on the relation of college periods of study to industrial training periods, and the other at the Birmingham College of Advanced Technology is concerned with the spacing of industrial and college training with the object of assembling a body of thought and opinion as a basis for deciding whether changes are needed to improve the quality of industrial and college training, increase the numbers attending advanced courses, and mobilize more effectively the teaching and research resources of colleges and companies. List No. 13 issued also by the Council details 100 existing and 13 proposed courses leading to the diploma.

The Calouste Gulbenkian Foundation, Lisbon

THE trustees of the Calouste Gulbenkian Foundation, Lisbon, have announced the grants made during the first half of 1961, which total about £360,000, and, of which, grants in the United Kingdom and British Commonwealth total nearly £100,000. Again they include grants for three years to British undergraduate scientific expeditions, as follows: £200 to the Royal Geographical Society in aid of the expeditions; £200 to the University of Leicester expedition to East Greenland; £200 to the University of Wales expedition to Mount Sabalan; £150 to the Cambridge Arctic Canada expedition; £100 to the University of Oxford expedition to the Andes of Peru; £100 to the University of Oxford expedition to the Atlas Mountains of Southern Morocco; £100 to the Cambridge expedition to Nagir, Karakoram; £100 to the Oxford expedition to the South Caspian; and £50 to the University of Oxford women's expedition to the Madeiras. The grants made in aid of education in Great Britain and the Commonwealth total £41,800, including: £15,000 to the School of Oriental and African Studies, University of London; £4,500 to the Science Masters' Association; £3,900 for three years to the School Library Association. In Portugal, £56,241 has been granted in aid of science and medicine, including grants for the Santa Maria Hospital, Lisbon, for biochemistry at the University of Oporto, the Laboratory of Mass Spectrometry of the Nuclear Energy Study Commission and the Centre of Geological Studies, Lisbon. A total of £7,500 has been given to the Faculty of Engineering of the University of Baghdad. Other countries receiving grants include Brazil (£8,508) and France (£2,612). Grants have also been made to the World Federation for Mental Health and the International Union against Cancer.

Fellowships for Plankton Research

IN order to encourage research relating to plankton in the western North Atlantic, the Lamont Geological Observatory is offering several fellowships for independent studies in this field. These fellowships are open to qualified scientists of any nationality, and include transportation costs from home to Sandy Hook, New Jersey, and return, plus a stipend which will be limited ordinarily to 1,500 dollars. Applications and requests for further information should be addressed to the Director, Sandy Hook Marine Laboratory, P.O. Box 428, Highlands, New Jersey.

Carlsberg-Wellcome Travelling Research Fellowships

THE Carlsberg Foundation (Copenhagen) and the Wellcome Trust (London) have announced the award of Carlsberg-Wellcome Fellowships for 1960–61 to: Dr. E. Poulsen, senior Lecturer at the Royal Veterinary and Agricultural College, Copenhagen, who will work at the Medical Research Council's Toxicology Research Unit at Carshalton on the neurotoxic influence of alicyclic phosphates and similar compounds; Dr. A. F. Hayward, of the Anatomy Department of the University of Glasgow, who will work at the Carlsberg Laboratory in Copenhagen, under the direction of Dr. H. Holter, studying the electron microscopic appearances in pinocytosis; Dr. S. L. Rowles, a member of the external scientific staff of the Medical Research Council at the School of Dental Surgery in Birmingham, who will work with Dr. Tovborg Jensen of the Royal Veterinary and Agricultural College, Copenhagen, on the chemistry of the calcium phosphates with special reference to those occurring in oral tissues.

Society for Applied Bacteriology

AT the annual meeting of the Society for Applied Bacteriology held in Wye on July 11 the following were elected officers of the Society: *Honorary President*, Mr. G. Sykes; *Honorary Secretary*, Dr. Ella M. Barnes; *Honorary Treasurer*, Mr. G. Elis Jones; *Honorary Editors*, Dr. S. E. Jacobs and Dr. D. J. Jayne-Williams; *Honorary Publications Manager*, Mr. A. H. Walters; *Honorary Advertising Manager*, Mr. E. J. Mann; *Members of Committee*, Mr. B. M. Gibbs, Dr. N. D. Harris, Dr. Agnes A. Nichols, Dr. J. R. Norris, Dr. A. M. Paton, Dr. Patricia M. Scholes, Dr. A. Seaman, Dr. Joan Taylor, Dr. G. C. Ware.

Australian Mammal Society

The following officers and council members were elected for 1961–62 at the third general meeting of the Australian Mammal Society, held in Brisbane, Australia, on May 26: *President*, Dr. G. B. Sharman; *Vice-President*, Dr. W. D. L. Ride; *Secretary*, Dr. R. G. Chittleborough; *Treasurer*, Mr. J. A. Thomson; *Editor*, Mr. J. H. Calaby; *Council Members*, Dr. A. K. Lee and Dr. A. G. Lyne.

The Night Sky in September

New moon occurs on Sept. 10d. 02h. 50m. U.T. and full moon on Sept. 24d. 11h. 34m. The following conjunctions with the Moon take place: Sept. 1d. 23h., Aldebaran 0·3° S.; Sept. 7d. 00h., Venus 2° N.; Sept. 19d. 22h., Saturn 3° S.; Sept. 20h. 05m., Jupiter 3° S.; Sept. 29d. 07h., Aldebaran 0·4° S. In addition to these conjunctions with the Moon, Mars is in conjunction with Spica on Sept. 20d. 11h., Mars being 2° S., Mercury with Spica on Sept. 21d. 12h., Mercury being 0·1° N., Mercury with Mars on Sept. 22d. 21h., Mercury being 3° S., and Venus with Regulus on Sept. 22d. 23h., Venus being 0·4° N. Mercury is too close to the Sun for observation. Venus is a morning star, rising at 1h. 50m., 2h. 30m. and 3h. 10m. on September 1, 15 and 30, respectively. Its stellar magnitude on September 15 is – 3·4; the visible portion of the apparent disk increases during the month from 0·792 to 0·870 and its distance from the Earth increases from 115 to 131 million miles. Mars is too close to the Sun for observation. Jupiter is an evening star, setting at 1h. 30m., 0h. 30m. and 23h. 30m. on Sept. 1, 15 and 30, respectively. It is in Capricornus; its stellar magnitude on Sept. 15 is – 2·2, and its distance from the Earth is 410 million miles. Saturn is an evening star, setting at 1h. 10m., 0h. 10m. and 23h. 10m. at the beginning, middle and end of the month, respectively. It is in Sagittarius; its stellar magnitude is + 0·6 on Sept. 15 and its distance from the Earth is 880 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Sept. 1d. 23h. 46·7m., σ^3 Tau. (*R*); Sept. 16d. 19h. 54·7m., 24 Sco. (*D*); Sept. 26d. 21h. 25·6m., ξ^2 Cet. (*D*); Sept. 26d. 22h. 19·6m., ξ^2 Cet. (*R*); Sept. 28d. 1h. 40·4m., 5 Tau. (*D*); Sept. 28d. 2h. 46·6m., 5 Tau. (*R*); Sept. 28d. 22h. 50·1m., γ Tau. (*D*); Sept. 28d. 23h. 43·0m., γ Tau. (*R*); Sept. 29d. 3h. 32·9m., θ^1 Tau. (*D*); Sept. 29d. 3h. 45·1m., θ^2 Tau. (*D*); Sept. 29d. 4h. 30·6m., θ^2 Tau. (*R*); Sept. 29d. 4h. 43·2m., θ^1 Tau. (*R*); Sept. 29d. 7h. 57·7m., α Tau. (*D*); Sept. 29d. 8h. 53·8m., α Tau. (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The autumnal equinox occurs on Sept. 23d. 07h.

Announcements

Dr. L. R. G. Treloar, of the Shirley Institute, Manchester, has been awarded the Colwyn Medal of the Institution of the Rubber Industry. Mr. M. M. Heywood, director of P. D. Cow and Co., Ltd., has been awarded the Institution's Hancock Medal.

A symposium on "Forest Ecosystem" (definitions and classification of forest ecosystem) was held in Montreal in 1959 under the auspices of the Ninth International Botanical Congress. Dr. I. Hustich of Finland was chairman and organizer for the symposium. The papers have now been published in full in a special issue of the *Bulletin of the International Society for Tropical Ecology*. Copies can be had from Dr. G. S. Puri, General Secretary, International Society for Tropical Ecology, 10-Chatham Lines, Allahabad, India (price Rs. 25, £2, 6.00 dollars).

The Chemical Society is arranging a symposium on electron spin resonance, which will be held in Queen Mary College, London, on November 2. Further information can be obtained from the Secretary, Chemical Society, Burlington House, London, W.1.

The annual Pittsburgh Diffraction Conference will be held at the Mellon Institute, Pittsburgh, Pennsylvania, during November 1–3. Further information can be obtained from T. B. Massalski, Mellon Institute, 4400 Fifth Avenue, Pittsburgh 13, Pennsylvania.

The Universities Federation for Animal Welfare (UFAW) will hold its annual Summer Congress at Shipham, Winscombe, Somerset, during September 16–23, 1961. Further information can be obtained from UFAW (Summer Congress), 7a Lamb's Conduit Passage, London, W.C.1.

The Borough Polytechnic, London, is organizing a course of six lectures on "The Technique of Technical Writing". The lectures will be given on Friday afternoons commencing October 20. Further information can be obtained from the Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

A meeting of the British Pharmaceutical Conference will be held at Portsmouth during September 18–22. To be included in the programme is a lecture by Dr. A. Isaacs on "Interferon" and a symposium on "Skin Medication". Further information can be obtained from the secretary, British Pharmaceutical Conference, 17 Bloomsbury Square, London, W.C.1.

The Food Group of the Society of Chemical Industry is organizing a symposium on "Recent Advances in Processing Cereals" at the Royal Society of Medicine, London, during September 14–15. Further information can be obtained from the Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

An international symposium on "Photoelasticity" will be held at the Illinois Institute of Technology, Chicago, Illinois, during October 29–31. The symposium will consist of five sessions including: birefringent coatings; photothermoelasticity and photoplasticity; two- and three-dimensional photoelasticity; dynamic photoelasticity and three-dimensional photoelasticity. Further information can be obtained from Prof. M. M. Frocht, chairman of the International Symposium on Photoelasticity, Department of Mechanics, Illinois Institute of Technology, Technology Center, Chicago, 16.

Erratum. In the communication entitled "Effects of Injections of Some Intermediary Metabolites on the 'Insulin Response' of Normal Rabbits", which appeared in the issue of July 29, p. 491, for "trichloroacetic" on line 9 read "tricarboxylic".

THE CARNEGIE UNITED KINGDOM TRUST

THE forty-seventh annual report of the Carnegie United Kingdom Trust covers the year 1960, but also includes a review of the quinquennium 1956–60, in which grants-in-aid of £525,881 brought the total expenditure of the Trust since it was founded in 1913 to more than £6 million*.

Apart from grants which are virtually subscriptions to national bodies and which in 1961–65 will comprise only the National Councils of Social Service in England, Scotland, Wales and Northern Ireland, and Foras Eireann in the Republic of Ireland, the Trust's grants fall into two main categories: those used to finance special inquiries and experiments, and those which provide a wide range of equipment and services. The first category gives the Trust scope to support innovation and pioneering in the arts, in social services and in education, but during the past quinquennium the largest proportion of grant-aid has gone to beneficiaries in the second category, particularly for the re-equipment of village halls and for museums. During the quinquennium, grants for the arts accounted for 11·72 per cent, for community services for 56·58 per cent, physical and mental welfare for 6·99 per cent, education for 15·5 per cent, youth services for 0·62 per cent, and nature reserves and parks for 8·59 per cent, the last including a grant for the David Marshall Lodge, Aberfoyle, opened in 1960, in the Queen Elizabeth Forest Park, and a sum of £50,000 has been allocated for the provision of other suitable amenities in such special areas during the 1961–65 quinquennium, out of a total allocation of £416,500. Other allocations for 1961–65 include £45,000 for museums, £100,000 for new communities, £100,000 for youth service and £10,000 for field studies, which will cover grants for projects approved and recommended by the Council for British Archæology and by the Council for Nature, both for field work in natural history and for Conservation Corps projects.

During 1960 the Trust continued its policy of assisting the development of museums and their associated art galleries, and 16 museums were offered grants for modernizing their displays; but the Trust does not consider that the museum service generally receives the support it deserves, though it is watching with interest certain developments which promise to deal with the situation. During the quinquennium, grants amounting to £49,930 brought the total aid from the Trust during 30 years to nearly £150,000, not including £16,695 promised to 38 museums towards expenditure on reorganizing displayed collections and a special grant of £1,000 to the Royal Burgh of Culross for work on an attractive old building, "The Study", which the National Trust for Scotland has restored for use partly for residential purposes, partly as a small museum and partly as an information centre, and another of £10,000 towards the capital cost of a Shetland Museum and Art Gallery at Lerwick. For the next quinquennium, the Trust's grants for museums will continue to be devoted largely to encouraging modernization of displays.

Although the last quinquennial report recorded the completion of the Trust's policy of grant-aid for the building or adaptation of village halls in the United Kingdom, an inquiry in 1956 convinced the Trustees that there were needs not being met from public funds, and they decided to return to this field with an allocation of £100,000 for grants for refurnishing and re-equipping pre-war halls in villages of not more than 1,000 people. By the end of 1960 some 1,636 applications had been received and the allocation increased to £200,000. During 1960 promises numbering 216 brought the total to 1,267 and the commitment to £171,031. Two more schemes for film projectors in the Highlands were approved on the recommendation of the Highlands and Islands Film Guild and a set of equipment is being presented to the people living on the Skerries, off the coast of Shetland. The social problems of new communities have received special attention during the quinquennium, and a report by Dr. J. H. Nicholson on his study of four types of new community is being published this year under the title "New Communities in Britain: Achievements and Problems". The £100,000 allocated for grants in this field will be available for projects that are truly pioneer, and likely to serve as prototypes, if successful; a grant of £12,000 has already been promised for the building of the Family Welfare Centre of the Kirkby Council of Social Service, and a tapering grant of £3,000 over 5 years to Hartcliffe Community House, Bristol, for provision of a warden and a qualified social worker.

A grant of £1,000 was made to Gates Youth Club, Crawley, to assist in building a permanent youth club and one of £8,000 to Edinburgh University Settlement for the erection of a multi-purpose building to serve primarily as a youth centre and also as a training ground for youth leaders. The Trust has also promised half the total cost of £13,000 required to establish the Northern Ireland Association for Mental Health for its first five years, and a supplementary grant of £3,500 was offered to the National Association for the Paralysed, for connecting to mains drainage several buildings at Dorincourt for work among the young chronic sick. £5,000 on a £1 for £1 basis after the first £10,000 was assured was offered to the Edinburgh Cripple Aid Society for a scheme to cost £20,000 for establishing an up-to-date centre for all physically handicapped persons who require assistance, and a grant of £2,250 was promised to the Y.W.C.A. for the salary and expenses of a part-time research assistant, to work for three years under supervision from the head of the Social Science Department, London School of Economics, for an experiment in social work among adolescents based on a coffee bar in the Paddington area of London.

During 1960, 120 students from 75 local scientific societies received bursaries to attend residential courses in botany, zoology, geology, ecology, archæology and related subjects at a cost of £360, and 17 grants, totalling £1,091, were made for field work in archæology. Nine courses for instruction of amateurs in meteorology were arranged, five of which were vacation courses at the Field Study Centres of Malham Tarn, Preston Montfort and Dale Fort, and three week-end courses at Lasham, Longmynd and Dunstable. Grants for youth service in the new quinquennium will be substantially for refurbishing and improving existing youth clubs. A grant of £12,750 payable over 5 years from the beginning of 1960 has been promised to the Central Council for the Care of Cripples to establish and develop the Homecrafts Advisory Association for the Disabled.

---

* The Carnegie United Kingdom Trust. 47th Annual Report, 1960. Pp. viii+98. (Dunfermline, Fife: The Carnegie United Kingdom Trust, 1961.)

THE REGIONAL RESEARCH LABORATORY, HYDERABAD

THE annual report for 1959–60* of the Regional Research Laboratory, Hyderabad, is a substantial attractively produced volume printed on hand-made paper manufactured by the Laboratory. It includes lists of research staff, auxiliary technical staff and administrators' staff and of publications and patents, and gives fairly full details of research projects in progress. Most of the projects relate to some industrial process or product, but increasing emphasis is laid on the fundamental aspects of applied problems. A Specialists' Committee, under the chairmanship of Prof. M. S. Thacker, has been appointed to examine the research programmes and make recommendations broadly on the future scope of the research programmes undertaken by the Laboratory.

The work of the Laboratory continues to range over a wide field. In oils and fats, the keeping quality of castor oil and cottonseed oil claimed much attention, as well as the preparation of derivatives. A series of programmes on surface coatings dealt with dehydrated castor oil, its isomerization and styrenation, and the preparation of varnishes from dehydrated castor oil, as well as the preparation of alkyds, emulsion paints, the weathering of paints and the use of cashewnut shell liquid. Syntheses in the quinoline, isoquinoline and thieno-pyrimidine series were directed to the preparation of compounds of potential pharmacological interest, while a process for the manufacture of phenylacetic acid was re-examined, and 8-hydroxyquinoline and 5-chloro-7-iodo-8-hydroxyquinoline synthesized for treatment of amœbic dysentery. Schemes relating to essential oils and aromatic chemicals deal with palmarosa oil, Indian cinnamon leaf oil and the utilization of Indian turpentine. In addition to entomological work on insecticides, investigations have been carried out on the effect of X-rays on the biological and biochemical characteristics of the spermatozoa of insects.

In biochemistry, the microbiological production of calcium and ferrous gluconate and of citric acid, the effect of cell concentration on the biological properties of individual cells in cell suspensions, and the biosynthesis and metabolism of proteins and nucleic acids have been examined. Programmes dealing with fuel cover the low-temperature carbonization of non-caking Indian coals and the utilization of products of the low-temperature carbonization of coal. Activated carbons and bleaching earths continue to receive attention, and there are programmes dealing with titanium pigments, the manufacture of white cement from felspar and the preparation of catalysts for the low-pressure hydrogenation of tar. Physico-chemical investigations dealt with the mineralogical composition of the bleaching earths, spectro-photometric work on tar acids from low-temperature carbonization, the surface properties and heat of wetting of absorbents and catalysts.

Under chemical engineering is included work on the vapour–liquid equilibrium of fatty acids and tar acids; the fluidized activation of carbon; pilot plant production of lævulinic acid and lounginin (a synthetic flavouring agent); and pilot-plant investigations in the milling of cotton seed and castor seed.

---

* Regional Research Laboratory, Hyderabad. Annual Report, 1959–60. Pp. xiv+217. (Hyderabad: Regional Research Laboratory, 1961.)

BIORHEOLOGY AND MICROCIRCULATION

FOLLOWING a reception at the Hadassah Visitors' Club, a seminar on biorheology and microcirculation was opened on April 24 at the Medical School, Jerusalem, by Dr. K. J. Mann, the general director of Hadassah, and delegates were also welcomed by Prof. M. Rachmilewitz, dean of the medical school, Hebrew University.

Dr. E. Davis (Jerusalem) introduced the symposium with a paper on the general nature and problems of microcirculation as studied by optical and electron microscopy. A main problem is how the capillaries change in diameter—whether passively in response to changes in blood content, or actively in response to nervous or chemical stimuli. How red cells pass through the apparently intact capillary is also still a mystery, nor are the spontaneous changes in calibre and rate of flow of blood in the small vessels understood.

Introducing the biorheological aspect, Dr. G. W. Scott Blair (Reading, England) outlined briefly the history of the application of rheology, "the study of the deformation and flow of matter", to biological systems. Poiseuille, a pioneer of rheology, and Bingham, who named this branch of physics and organized the first society of rheologists, both worked on problems of blood-flow. Biorheologists now study many other systems, such as muscle, protoplasm, cervical mucus, synovial fluids, bronchial mucus, intraocular and cerebrospinal fluids, strength of bones, etc.

Dr. H. Harders (Hamburg, Germany) stressed the technical difficulties involved in direct microscopic observations of microcirculation *in vivo*. But the importance of such studies is undisputed. Many diseases have microcirculatory aspects, and some, such as sickle-cell disease, cold hæmagglutination syndrome, polycythæmia and hæmorrhagic thrombocythæmia, are primarily disturbances of microcirculation. The influence of vaso-active drugs and of smoking can be studied by microscopic examination of capillaries. Dr. Harders concluded by predicting that microvascular observations would, in future, play an essential part in routine examinations.

Dr. J. W. Irwin (Boston, Massachusetts) showed, with a film, the pulmonary microcirculation in the living rabbit. The arterioles, capillaries and venules could be seen, and the pulsating flow was readily observed. Pharmacological reactions were also demonstrated. A capacitance electromanometer was shown which could be used, provided that the tip of the microcannula was less than 20μ in diameter, but a more efficient manometer would be needed if true capillary pressures were to be studied.

Dr. J. Weinman and Dr. M. Manoach (Jerusalem) described photoelectric techniques for studying the peripheral circulation in man. Living tissue is transparent to those wave-lengths of red and infrared radiation at which measurements of extinction coefficients of blood are generally made. Quite cheap incandescent lamps and photoconducting cells may be used as radiation sources and detectors to follow pulsating flow of blood and vasomotor action. Photocells and light sources are combined in a small unit, attachable to any part of the body. Such stimuli as postural gravitational changes and smoking may be studied in this way.

The last session at Jerusalem was opened by Prof. A. Katchalsky (Rehovot), who described differences in osmotic fragility under normal and gradual conditions of hæmolysis. Under normal hæmolysis, stresses develop rapidly and rupture the cell membrane. Under slow hæmolysis a higher hypotonicity is required. The membrane behaves as a Kelvin body with a characteristic retardation time. Dr. D. Danon (Rehovot) showed two phase-contrast microcine films illustrating deformation, shearing and hæmolysis of normal human erythrocytes under gradually decreasing salt concentration. Mixed ion exchangers and also dialysis through a 'Cellophane' membrane were used to reduce salt concentrations. Dr. G. Segré and Dr. A. Silberberg (Rehovot) studied the radial distribution of rigid macroscopic spheres in a Poiseuille field of flow, showing that particles undergo a sideways displacement, both from the centre outwards and from the tube wall inwards. Although these results should be applied with caution to flow of blood, M. G. Taylor, scanning a capillary optically and measuring absorption along a diameter, has shown a similar 'tubular pinch' effect.

The second part of the programme, held at the Weizmann Institute of Science, Rehovot, opened with a paper by Dr. S. Lazarus, Dr. H. N. Munro and Dr. G. H. Bell (Glasgow) on capillary strength tests in scurvy and their reactions to vitamins C and P therapy. Both positive and negative pressure tests were used on three small groups of subjects of about the same age: (1) fifteen cases of scurvy; (2) twenty-nine hospital in-patients without scurvy; and (3) twenty well-nourished hospital visitors. Negative pressure tests were inconcuslive, but positive pressure tests showed some weakness in the capillaries of scurvy patients, though more than a quarter of them fell within the normal range of group (3) subjects. Treatment with vitamins C and P did not increase capillary strength. The authors tentatively suggested that it was not scurvy which produces capillary weakness, but that subjects with already weak capillaries are perhaps more prone to develop scurvy.

Dr. S. Ben Hador, Dr. E. Davis, Dr. J. Landau and Dr. M. Ivri (Jerusalem) discussed the effect of adrenaline on the small blood vessels of the conjunctiva and nailfold, concluding that these vessels are more sensitive to adrenaline in the presence of hypertension than when blood pressure is normal. As an example of the results presented: injecting 0·4 mgm. adrenaline produced severe thinning in the small vessels of the nailfold in two of seven healthy subjects, and appreciable thinning in two more cases; whereas, among eleven hypertensives, severe thinning appeared in six, and appreciable thinning in five, cases. Among ten arteriosclerotics, there was no severe thinning and there were six cases of appreciable thinning.

Dr. J. W. Irwin (Boston, Massachusetts) discussed microcirculation of the inner ear, and showed a film demonstrating this in living guinea pigs. A remarkable and unexplained finding is that the chemical components of endolymph and perilymph, which the author considered as part of the circulation of the inner ear, appear to differ, although these fluids are separated in one area only by a two-cell thick membrane (Reissner's membrane).

Dr. E. Davis, Dr. S. Ben Hador, Dr. J. Landau and Dr. M. Ivri (Jerusalem) then described observations and experiments on vasomotion in human capillaries. The number of spontaneous changes a minute in calibre or velocity of blood-flow in capillaries is defined as the 'vasomotor count'. In healthy subjects and in arteriosclerotics, the vasomotor count lies normally between 0 and 14, but in hypertension and in diabetes mellitus, two-thirds of the cases showed values greater than 14. Injections of 0·4 mgm. adrenaline showed no change in vasomotor count in healthy subjects, but increases in 7 out of 11 cases of hypertension and also 3 out of 9 arteriosclerotics. The increased vasomotion of hypertension may reflect increased sensitivity to adrenaline.

In the final paper, Dr. G. W. Scott Blair (Reading, England) briefly outlined the history of rheological theory, from Hooke's law of elasticity (stored energy) and Newton's concept of viscous flow (dissipated energy) to modern theories of entropy-high elasticity. Living organisms maintain themselves by preventing fatal rises in entropy through drawing 'negative entropy' from their surroundings. In a steady, as distinct from a static, state a system undergoing an irreversible process requires a rate of increase of entropy having the minimum possible value consistent with external conditions. Dr. Scott Blair predicted that it is along the lines of Onsager and Prigogine's 'irreversible thermodynamics' that rheological theory must develop if it is to deal with studies of living systems.

Prof. M. Reiner (Haifa), who presided at one of the sessions, discussed the function of models in biorheology and, in a final general discussion, views were expressed concerning the future international organization of biorheology, especially the part which might be played by the new international journal *Biorheology*, shortly to appear.

Apart from the technical sessions, there were many pleasant social activities at which the traditional Israeli hospitality was seen to its full advantage.

G. W. SCOTT BLAIR

PAPER CHROMATOGRAPHY

THE technique of paper chromatography was first described by Consden, Gordon and Martin in 1944. In 1948, Dr. Gordon was invited to Czechoslovakia for a year to set up and teach this technique. Since that time the work of the Czech Schools has become more and more important in this field and there can be little doubt that, at present, the work of Drs. I. M. Hais, K. Macek, C. Michalec and their co-workers is among the most important of the systematic and general research in paper chromatography. So far as we are aware, the Czech Chemical Society is the only chemical society which has a Chromatography Group, and it is therefore not surprising that it should have organized a conference on the subject of paper chromatography during July 21–24, 1961.

The conference, using Czech, English and German as official languages, was organized so that the first two days were given to the discussion of new techniques, reviews of particular fields, papers relating chromatographic behaviour to chemical and physical properties and to the paper chromatography of lipids—a subject to which the Czechs have devoted a very great deal of time—and the third day to a discussion of theoretical and practical problems. As many papers were read in Czech, books containing adequate summaries in various languages were handed to each participant in advance and interpreters were assigned to individual national groups. The first two days were considered as open days, and students and others interested were encouraged to attend. The third day was arranged as a closed meeting confined to about sixty participants, which was held in a hall with radio equipment and transistorized headphones on which were relayed simultaneous translations in English, German and Russian. For this the participants were transported to a small castle outside Prague which the Government has made available to the National Academy of Sciences as a holiday resort for scientists.

In the introductory review, Dr. R. Consden discussed briefly the origin and development of paper chromatography and went on to a consideration of the various new papers, now becoming available, and new techniques, such as centrifugal field methods. Other speakers then reviewed particular subjects. Thus Dr. C. Knight of Balston, Ltd. (Whatman filter papers), spoke on the new ion exchange papers in which the active exchange group is built into the cellulose molecule. He described the cation exchangers such as cellulose–phosphate and citrate and carboxymethylcellulose, together with their application to the separation of amino-acids and cations in general and followed with a similar discussion on the cellulosic anion exchangers including diethylaminoethyl cellulose. Similar papers were also discussed by Dr. Grüne, of Schleicher and Schüll, who went on to consider the value of new types of paper which have alumina or silica gel incorporated into the cellulose matrix at the manufacturing stage. She pointed out that these papers function as micro alumina or silica gel columns and yield results similar to the classical column procedures. They are thus useful for the examination of new solvents for column chromatography as well as for separations in their own right. Among many examples quoted we may mention only the separation of carotenoids and chlorophylls on these papers.

There followed reviews on the separation of particular families of compounds and a few may be taken for special comment. Dr. F. H. Pollard gave an interesting review of present work and advances in the inorganic field. In particular, the search for new general and specific location reagents and for faster solvents had been quite successful. In a second paper he described a method for the rapid systematic analysis of some twenty-four cations using a single solvent in conjunction with a large number of reagents for location and confirmation of identity. This method had been used most successfully by students in place of the classical inorganic group separations. Results of Australian workers in the inorganic field were discussed by Dr. M. Lederer. An excellent review of the chromatographic separation of some two hundred alkaloids on formamide-treated paper was given by Dr. D. Waldi (Germany), who directed attention to the possible errors due to variation in the formamide itself.

One whole session of the conference was devoted to the paper chromatography of fatty acids, esters and related compounds. Following a comprehensive review by Dr. Michalec on solvents, reagents and techniques useful in this field, other papers discussed particular series of compounds. Methods were described for the separation of *cis–trans*-isomers of the acids together with the application of this work in the determination of the mechanics of catalytic hydrogenation. Other speakers dealt with the use of coated papers, such as formamide or dimethylformamide-treated papers, for the separation of free fatty acids or their urea adducts, and the quantitative methods available for esters, glycerides, etc.

The only paper devoted to the theory of paper chromatography was that of Prof. V. V. Racinskij (U.S.S.R.), who discussed chromatographic separation in terms of the general theory of sorption dynamics. Like other theories, this theory requires the measurement of the cross-sectional areas of both phases on the paper. However, as it has not yet been found possible to determine these areas, we must agree with Prof. Racinskij himself, who concluded by directing attention to the lack of applicability of these calculations and theories to everyday paper chromatography.

Two films were shown near the beginning of the meeting. One entitled "Principles of Chromatography", by Drs. J. B. Jepson and I. Smith, illustrated the general procedures and techniques of paper and column chromatography, while the second, by Drs. V. Holeysovsky, V. Tomasek, K. Kochloefl and K. Setinek, was a complete description of the "Technique of Paper Chromatography of Amino-acids" as carried out in Prague. Taken together they formed a suitable introduction to the conference for the many students present. While the British film is available on loan or sale it is regrettable that the Czech film is not.

The original intention for the closed meeting on the third day was to have a symposium on the relationships between chemical structure and chromatographic behaviour in the morning followed by a symposium on systematic analysis in the afternoon. In a sense, the whole three days were devoted to these topics;

but, despite the very long hours put in each day, there was insufficient time for adequate and fruitful discussions.

The main discussion at the two symposia, at which Prof. Marini-Bettolo (Italy) presided, centred around the papers of Drs. Hais and Macek, and included contributions by Drs. Consden, Smith, Waldi, Racinskij and others. They emphasized the fact that the vast amount of chromatographic data in the literature was not being used to the full simply because of the lack of standardization of apparatus, methods, reagents, etc., and that this rendered comparisons and deductions between the results of different workers dangerous. They directed attention to the very wide and detailed systematic studies of Dr. I. Smith in the biochemical field and to the limited number of such studies in other fields. We were interested to learn that the Soviet Union does not produce a single piece of apparatus which is primarily intended for paper chromatography. The variety of types of apparatus used by workers in different countries is unfortunately manifold. Because of these differences it is not yet possible to correlate the data produced in different laboratories on anything like the scale which would be desirable. This is regrettable, as there can be no doubt that standardized and correlated data would be valuable in avoiding the present duplication and reduplication of labour. Moreover, far more important than this, standardized data could be used on electronic data processing machines based on suitable punched-card systems. Furthermore, the availability of such standardized data would be valuable for the determination of various other chromatographic data, such as R_m values, and for the correlation of such properties with other physicochemical measurements. This in turn might lead to a type of structural analysis based on the splitting of R_F and R_m values into functional group constants with the possible application of physical-organic chemistry to the general problems of paper chromatography. This latter point was discussed at some length by Ing. Prochazka and Ing. Gasparic. The Czech Chemical Society has attempted with some success to standardize methods in its own country and, in spite of difficulties, Drs. Macek and Hais have gone ahead and devised their own punched-card system suitable for machine operation. No doubt we can look forward to more interesting developments from their laboratories.

Participants were shown around two laboratories. Dr. Macek conducted a tour of the Pharmaceutical and Biochemical Research Institute, which is well equipped to study all aspects of paper chromatography, including the data-correlation problem. This was followed by a tour of the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Science, led by Prof. B. Keil. The main problem of Dr. Keil and his team is the amino-acid sequence of chymotrypsinogen and the laboratory is extremely well equipped for this purpose. The electronic data sorting approach to the study of overlapping sequences, etc., is also in an advanced stage. We are interested to note the close collaboration between this team and others in the United States working on the same problems.

I. SMITH
J. B. WEISS

FOREST PRODUCTS RESEARCH IN BRITAIN

THE extreme complexity of the principal product of the forest, timber, is well exemplified by the very varied contents of the report for 1960 of the Director of Forest Products Research*. In it reference is made to research into wood structure, wood seasoning, bending, mechanics and other properties of timber. Sections are devoted to woodworking, wood preservation and to the study of the fungi and insects which batten on timber. The chemistry and physics of timber are receiving attention and in this connexion surely more attention should be given to the chemistry of those timbers of trees which from a sylvicultural point of view ought to be much more widely grown, especially in England, than they are—namely, oak, birch, alder and other leaf-trees. It would be very helpful if timbers containing substances which are a nuisance in paper-making, but which might furnish materials for other uses, could be enhanced in value and grown more profitably. Recent progress in Japan is of interest.

The close association of the Forest Products Research Laboratory with the Forestry Commission no doubt accounts for the prominence given in the research programme to Sitka spruce and what is loosely called "Lodgepole pine". The former has been very extensively planted and the latter, after a period of neglect, is now also much in favour. Both are useful trees, and it is certainly important that everything should be done to justify their being grown, but surely not at the expense of work on other sylviculturally more useful trees with no less valuable timber. The Republic of Ireland, where the utility of *Pinus contorta* Doug. was appreciated earlier than in Great Britain, is indeed fortunate in being able to make use of the Laboratory, while the latter is fortunate in being able to obtain research material from abroad suited to its needs.

An investigation just completed into the strength and specific gravity of Sitka spruce grown in Great Britain proves fairly conclusively that the method of site classification adopted by the Forestry Commission is at fault. The samples tested were chosen from six geographical regions corresponding to 'Districts' of the Meteorological Office, in other words, climatic regions, and within these the stands of trees, whence the samples came, were classified into five groups based on rate of growth in height, called, unfortunately, quality-classes, but really productivity-classes. The interesting result of this investigation was to find no significant differences among the six climatic regions, nor among the productivity-classes as a whole, but that differences in quality tend to arise more from individual site factors. It thus seems that rate of growth is not a sound basis of site classification for this kind of work, as most foresters realize. A better classification is one based on the factors of geology and the availability to the trees of nutrients and soil moisture. It would be valuable to have the work repeated or the existing data re-analysed on such a basis.

---

* Department of Scientific and Industrial Research. *Forest Products Research 1960*: Report of the Products Research Steering Committee with the Report of the Director of Forest Products Research. Pp. v+60+8 plates. (London: H.M.S.O., 1961.) 5s. net.

The report does not say specifically what the value of the timber of *Pinus contorta* Doug. is compared with that of other trees, information which would be of the utmost importance to the forester. The structure of its wood is said to be intermediate between, for example, yellow pine and Scots pine. There is some slackness in nomenclature in the report; apart from leaving one in doubt about what yellow pine is, the terms 'redwood' and 'whitewood' are still used with no explanation that the former is wood of Scots pine and the latter that of Norway spruce. The laboratory should think of the grower as well as of the user.

It is almost certain that timbers will long remain among the most useful of raw materials, and the more we can learn about them the better. As the chairman of the Forest Products Research Steering Committee in his introduction says, there is no lack of work to be done, but why is all the work kept in the south of Britain ? Some could be done just as well at a properly equipped laboratory in the north. The Committee has a difficult task in avoiding the many reefs and in selecting the most profitable waters. The greater attention now being given to home- (and Irish-) grown timbers is, however, likely to be fully justified.

M. L. ANDERSON

DRUG SUPPLIES DURING THE AMERICAN REVOLUTION

INITIALLY, drug supplies for the American Revolutionary Army came from stocks largely in the hands of private druggists. This source of supply was totally inadequate for a war that attained such proportions as the Revolution. Even if stocks of drugs in the Colonies had been far greater than they were, there is little reason for believing that shortages would not have developed. Even the most patriotic pharmacists were faced with complete financial suicide, caught between a spiralling inflation and a Congress that had no money and only a promise for the future. In an interesting paper, George B. Griffenhagen, formerly curator of medical sciences, U.S. National Museum, Smithsonian Institution (*Bull.* 225), shows how the internal organization of the medical department of the army was so chaotic that, even if adequate supplies were available and if the almost unsurmountable problems of communications and transportation had been solved, it is almost certain that shortages would have developed at least during the campaign of 1776.

The encouragement which Congress, through its Secret Committee, gave to private shippers for the importation of vital war supplies offered little relief in the field of medical supplies. Importation was cut off from Great Britain, and France did not directly export any quantities of medical supplies, at least until 1778. American privateers found it much more profitable to prey on British shipping than to develop trade channels with countries which, prior to the Revolution, were prohibited from shipping directly to the Colonies.

Hence, the most immediate relief from medical supply shortages was provided by the American privateers. Drug cargoes from British prize ships, many of which were en route to New York, served as a most important source of supply, particularly during 1777 and 1778.

The number of individual drugs mentioned in various inventories was considerable. Of these, only about a dozen were critically short. Heading the list of these 'capital articles' was Peruvian bark, the same cinchona from which quinine was later discovered. Tons of bark were used during the Revolutionary War, and the price more than quadrupled between June 1776 and September 1777.

The most prominent group of drugs on the list of capital articles consisted of cathartics and purgatives. Jalap, ipecac, and rhubarb were the botanical favourites, while bitter purging salt (Epsom salts) and Glauber's purging salts were the chemical choices for purging. Tartar emetic was the choice for a vomit, and cantharides (Spanish flies) was the most important ingredient for blistering plasters. Opium was administered for its narcotic effects, while gum camphor, nitre (saltpetre or potassium nitrate), and mercury (pure metal as well as certain salts) were employed for a variety of purposes. Lint, a form of absorbent material made by scraping or picking apart old woven material, was also in short supply.

Equipment shortages included surgical instruments and mortar, and pestles for pulverizing crude drugs. Glass vials for holding compounded medicines were also a supply problem, especially after essential drugs again became available. Some of the shortages were eased by local manufacture. Lint was produced in large quantities in the Colonies, and glass vials were manufactured in numerous glasshouses. Local manufacture of purging salts and nitre aided in eliminating shortages of these essential items, and, at the same time led to the first large-scale pharmaceutical manufacturing in the United States.

π-ELECTRONIC EXCITATION AND IONIZATION ENERGIES OF CONDENSED RING AROMATIC HYDROCARBONS

By DR. J. B. BIRKS and M. A. SLIFKIN

Physical Laboratories, University of Manchester

THE condensed ring aromatic hydrocarbons form an interesting group of molecules. They possess delocalized π-electron systems, loosely bound by a potential defined by the shape of the planar molecular skeleton; they scintillate, fluoresce and phosphoresce; some of the higher members are carcinogenic; and they possess certain well-defined electronic energy-levels which vary systematically with molecular size. They have been the subject of many theoretical studies, including those based on the molecular orbital[1-3], valence-bond, and free-electron[4] models. These have led to the classification and computation of the π-electronic energy-levels in many of the simpler compounds, although because of the complexity of the many-electron systems and the resultant interactions it is normally necessary to introduce arbitrary parameters to achieve reasonable agreement with the experimental data.

The electronic ground-state S_0 of the molecules is singlet, and analysis of the visible and ultra-violet absorption spectra ($S_0 \rightarrow S_n$) of vapours or dilute solutions yields direct information on the singlet π-excitation energy-levels S_n. Complementary data on the energy and life-time of the first singlet excitation state S_1 are obtained from studies of the molecular fluorescence emission, $S_1 \rightarrow S_0$.

The triplet π-excitation energy-levels are less amenable experimentally, since singlet-triplet absorption transitions are normally forbidden. The first triplet π-excitation energy T_1 can be obtained from observations of the low-temperature phosphorescence emission ($T_1 \rightarrow S_0$) of glassy solutions[5], or by the solvent perturbation technique[6] which allows the $S_0 \rightarrow T_1$ transition to be observed in absorption at room temperature. Higher triplet states have been observed in transient $T_1 \rightarrow T_n$ absorption transitions, using the flash photolysis method[7].

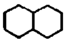
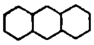
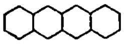
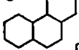
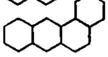
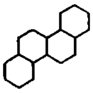
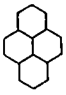
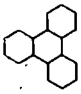
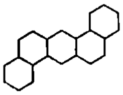
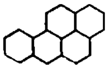
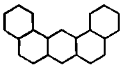
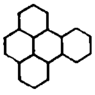
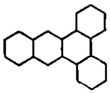
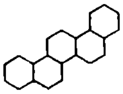
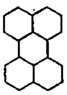
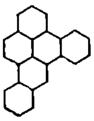
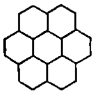
The ionization energies of a few of the smaller molecules and their derivatives have been obtained directly from photo-ionization measurements[8], electron impact studies[9] and vacuum ultra-violet Rydberg spectra[10] of the vapours. These methods are not applicable to the heavier, less volatile compounds, and an indirect technique[11,12], involving the measurement of the absorption spectra of the charge-transfer complexes formed by the compounds with an electron acceptor in solution, has been used to obtain the ionization energy I of the molecules.

In this article data are presented on I, T_1 and S_1 for 18 condensed ring aromatic hydrocarbons and for 10 methyl derivatives of 1 : 2-benzanthracene. The latter were included in the study in order to increase the number of carcinogenic hydrocarbons considered.

The energy E_{CT} of maximum absorption of the charge-transfer band of the complex with chloranil in carbon tetrachloride solution has been observed for each compound. Similar measurements on some of the compounds have been reported by previous observers[11,13]. Our values agree closely with these, except in the case of tetracene and chrysene, where they confirm the values of Briegleb and Czekalla[11], but are less by 0·11 eV. and 0·23 eV. respectively than those of Peticolas[13]. I has been evaluated from the linear relation established by Foster[12] from observations on compounds of known I, namely:

$$I = 5{\cdot}13 + 1{\cdot}12\,E_{CT} \quad \text{(eV.)} \qquad (1)$$

Table 1. CONDENSED RING AROMATIC HYDROCARBONS
(Energies in eV.)

			E_{CT}	I	T_1	S_1	$\overline{E}_1$	Carcinogenicity
1		Benzene	3·64	9·21	3·64 (ref. 6)	4·71	4·17	
2		Naphthalene	2·65	8·10	2·64 (ref. 6)	3·88	3·26	
3		Anthracene	2·00	7·37	1·82 (ref. 6)	3·32	2·57	
4		Tetracene	1·67	7·00	1·27 (ref. 14)	2·63	1·95	
5		Phenanthrene	2·64	8·09	2·68 (ref. 6)	3·58	3·13	
6		1:2-Benzanthracene	2·06	7·45	2·05 (ref. 16)	3·22	2·63	+
7		Chrysene	2·38	7·80	2·43 (ref. 15)	3·45	2·94	
8		Pyrene	2·16	7·55	2·09	3·34	2·71	
9		Triphenylene	2·64	8·09	2·88	3·54	3·21	
10		1:2:5:6-Dibenzanthracene	2·38	7·80	2·27 (ref. 16)	3·14	2·70	+ +
11		3:4-Benzpyrene	1·83	7·19	1·82 (ref. 6)	3·08	2·45	+ + + +
12		1:2:7:8-Dibenzanthracene	2·28	7·68	2·14	3·14	2·64	+
13		1: 2-Benzpyrene	2·17	7·56	2·30	3·22	2·76	
14		1:2:3:4-Dibenzanthracene	2·21	7·61	2·20 (ref. 6)	3·30	2·75	
15		Picene	2·38	7·80	2·62	3·30	2·96	
16		Perylene	1·70	7·03	1·55	2·85	2·20	
17		1:2:4:5-Dibenzpyrene	1·91	7·27	1·85	2·98	2·41	
18		Coronene	2·06	7·44	2·36 (ref. 16)	2·88	2·62	

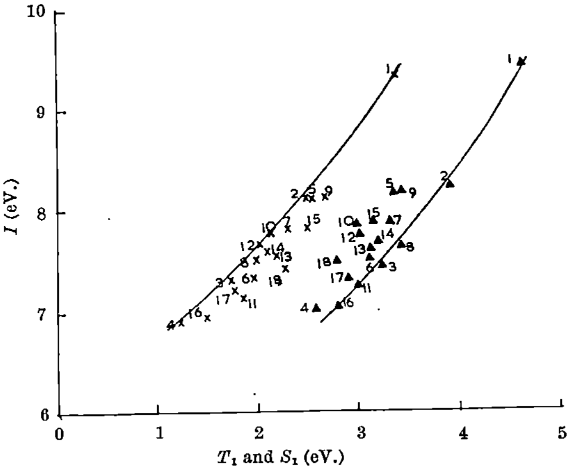


Fig. 1. Condensed ring aromatic hydrocarbons. T_1 (×) and S_1 (▲) against I

The triplet energy T_1 of seven of the compounds has been measured by the solvent perturbation technique[6] using oxygen under high pressure. The oxygen forms a contact charge-transfer complex with the hydrocarbon, and the absorption spectrum corresponding to the $S_0 \rightarrow T_1$ transition is observed superposed on the charge-transfer band. Values of T_1 for the other compounds have been obtained from the data of other observers who used solvent perturbation[6,14] or phosphorescence spectrometry[15-17]. Values of S_1 have been obtained from the solution absorption spectra[18,19].

The results are presented in Tables 1 and 2.

Table 2. METHYL DERIVATIVES OF 1:2-BENZANTHRACENE (Energies in eV.)

	E_{CT}	I	T_1 (ref.17)	S_1	$\overline{E}_1$	Carcinogenicity
1:2-Benzanthracene	2·06	7·45	2·05	3·22	2·63	+
2′-Methyl	2·02	7·39	2·06	3·22	2·64	
3′-Methyl	2·05	7·43	2·07	3·23	2·65	
4′-Methyl	2·05	7·43	2·07	3·20	2·63	
3-Methyl	2·05	7·43	2·08	3·20	2·64	+ +
4-Methyl	2·03	7·41	2·10	3·23	2·66	+ +
5-Methyl	2·02	7·39	2·06	3·23	2·64	+ +
6-Methyl	2·00	7·37	2·05	3·22	2·63	+
7-Methyl	2·00	7·37	2·06	3·21	2·63	+
10-Methyl	1·92	7·29	2·02	3·20	2·61	+ + + +
9:10-Dimethyl	2·05	7·43	1·92	3·26	2·59	+ + +

For many of the compounds E_{CT} and T_1 are practically equal. This feature, which has been noted previously by Peticolas[13], is considered to be accidental and not of general significance, since in several of the compounds there are clear differences between E_{CT} and T_1. E_{CT} depends on the particular charge-transfer acceptor, chloranil, and quite different values of E_{CT} are obtained with benzoquinone and trinitrobenzene, although these yield similar values of I from equations analogous to (1) (ref. 12).

I is plotted against T_1 and S_1 in Fig. 1. There is a very approximate linear relationship between I and S_1, corresponding to the simple Hückel-type equation:

$$I = \alpha + bS_1 \tag{2}$$

used by some authors[20] to estimate I. The degree of scatter in the experimental data indicates the inaccuracy of this method of obtaining I. There is a similar approximate linear relationship between I and T_1, which reflects the similarity between E_{CT} and T_1 in many of the compounds.

A more significant feature is that the curves through the values of S_1 and T_1 for the polyacenes (compounds 1–4) form upper and lower limits respectively for the values of S_1 and T_1 for the other compounds. For a given I, non-linear molecular arrangements of the aromatic ring systems yield lower or equal values of S_1 and higher or equal values of T_1 than the linear polyacene arrangement. Expressed alternatively, for a given T, the S_1–T_1 separation, corresponding to the spin-reversal energy, is greatest for the polyacenes.

These considerations suggest that the mean first π-excitation energy:

$$\overline{E}_1 = \tfrac{1}{2}(T_1 + S_1) \tag{3}$$

is a more meaningful quantity to compare with I, when considering condensed-ring molecules of different shapes. I is plotted against $\overline{E}_1$ in Fig. 2. A clear linear relationship is found between I and $\overline{E}_1$ given by:

$$I = 4{\cdot}39 + 1{\cdot}15\,\overline{E}_1 \quad \text{(eV.)} \tag{4}$$

which is obeyed, within experimental error, by all except three of the compounds. Moreover, the data extrapolate to give an intercept of 4·39 eV. for graphite, the infinite condensed ring 'molecule', at $\overline{E}_1 = 0$, in agreement with the experimentally determined work function[21].

The agreement of the experimental data with (4) is sufficiently striking to consider possible causes for the discrepancies in three of the compounds. The compounds in question, tetracene, 1 : 2 : 5 : 6-dibenzanthracene and 1 : 2 : 7 : 8-dibenzanthracene, possess the longest molecules of those considered, and it is possible that the deviations are due to

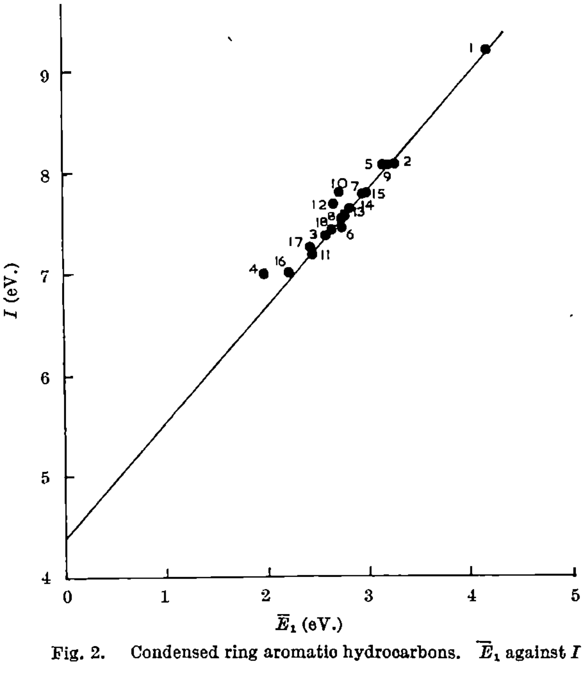


Fig. 2. Condensed ring aromatic hydrocarbons. $\overline{E}_1$ against I

the molecule being too elongated. It is of interest to note that molecular orbital theory[2] indicates that the lowest singlet state in the polyacenes changes from $^1B_{3u}{}^-$ to $^1B_{2u}{}^+$ on increasing the number of rings to 4 or more, and, while yielding reasonable values for the lower polyacenes, predicts a higher value for S_1 (3·11 eV.) for tetracene than that observed. The use of this value for S_1 would increase $\overline{E}_1$ to 2·20 eV. for tetracene and remove the anomaly. There has been some controversy over the value of T_1 for tetracene, but the experimental value taken is consistent with those of the lower polyacenes and with theory[2,14]. A further possibility is that deviations from the linear relation (1) may occur with elongated molecules.

The other two compounds have not been studied theoretically in any detail. They each have $S_1 =$ 3·14 eV. and $S_2 =$ 3·52 eV. The anomalies could be removed by using S_2 rather than S_1 in (3); but there appears little justification for this arbitrary procedure.

The measurements on the condensed-ring compounds and on the methyl derivatives of 1 : 2-benzanthracenes show that of the various energies observed only S_1 shows any degree of correlation with the carcinogenicity. This correlation has been discussed previously[22].

One of us (M. A. S.) wishes to acknowledge the tenure of a Department of Scientific and Industrial Research studentship.

[1] Hückel, E., *Z. Phys.*, **69**, 423 (1930); **70**, 204 (1931).

[2] Pariser, R., *J. Chem. Phys.*, **24**, 250 (1956).

[3] Pullman, A., and Pullman, B., *Cancérisation par les substances chimiques et structure moléculaire* (Masson, Paris, 1955).

[4] Platt, J. R., *J. Chem. Phys.*, **17**, 484 (1949).

[5] Lewis, G. N., Lipkin, D., and Magel, T. T., *J. Amer. Chem. Soc.* **63**, 3605 (1941).

[6] Evans, D. F., *J. Chem. Soc.*, 1351 (1954).

[7] Porter, G., and Windsor, M. W., *J. Chem. Phys.*, **21**, 2008 (1953); *Proc. Roy. Soc.*, A, **245**, 238 (1958).

[8] Watanabe, K., *J. Chem. Phys.*, **22**, 1565 (1954); **26**, 542 (1957).

[9] Morrison, J. D., and Nicholson, A. J. C., *J. Chem. Phys.*, **20**, 1021 (1952).

[10] Price, W. C., and Wood, R. W., *J. Chem. Phys.*, **3**, 439 (1935).

[11] Briegleb, G., and Czekalla, J., *Z. Elektrochem.*, **63**, 6 (1959).

[12] Foster, R., *Nature*, **183**, 1253 (1959); *Tetrahedron*, **10**, 96 (1960).

[13] Peticolas, W. L., *J. Chem. Phys.*, **26**, 429 (1957).

[14] Kasha, M., *J. Chem. Phys.*, **20**, 71 (1952). McGlynn, S. P., Padye, M. R., and Kasha, M., *ibid.*, **23**, 593 (1955).

[15] Lewis, G. N., and Kasha, M., *J. Amer. Chem. Soc.*, **66**, 2100 (1944).

[16] McClure, D. S., *J. Chem. Phys.*, **17**, 1905 (1949).

[17] Moodie, M. M., and Reid, C., *J. Chem. Phys.*, **22**, 252 (1954).

[18] Clar, E., *Aromatische Kohlenwasserstoffe* (Springer, Berlin, 1952). Friedel, R. A., and Orchin, M., *Ultra-Violet Spectra of Aromatic Compounds* (John Wiley, New York, 1951).

[19] *Organic Electronic Spectral Data*, **1** and **2** (Interscience, New York, 1960).

[20] Hedges, R. M., and Matsen, F. A., *J. Chem. Phys.*, **28**, 950 (1958).

[21] Brann, A., and Busch, G., *Helv. Phys. Acta*, **20**, 33 (1947).

[22] Birks, J. B., *Nature*, **190**, 232 (1961).

ELECTRICAL EFFECTS DURING DISSOLUTION AND PRECIPITATION OF ELECTROLYTES

By R. P. RASTOGI and B. P. BATRA
Chemistry Department, Punjab University, Chandigarh-3, India

AND

R. K. DASS
Physics Department, Government College, Hoshiarpur, India

EVIDENCE, although scant, has accumulated during the past three decades which indicates that potential differences are produced during phase-transformations[1-4]. Unfortunately, the phenomena associated with such transformations have not been subjected to systematic study. Hence, we have only doubtful explanations regarding the origin, build-up and the decay of potential difference. Difficulties in making reproducible measurements of potential differences are considerable. The purpose of the present article is to report the study of potential differences that are produced when the crystals of an electrolyte just appear or when these are allowed to dissolve. We will call the former the precipitation potential, and the latter the dissolution potential. So far as we are aware, this has not been reported earlier.

The initial experiments for measuring potential differences were performed in a simple way. A copper or silver tube was immersed in a bath kept at a certain temperature below the saturation temperature of the solution. This tube served as one electrode. A piece of wire of the same metal was kept in the centre of the tube, serving as another electrode. The two electrodes were connected through a Pye Scalamp galvanometer and a megohm resistance box having various steps in series. By adjusting the resistance and knowing the sensitivity of the galvanometer it could be converted into a direct-reading milli-voltmeter of high input impedance. This was considered to be sufficient for the purpose, since the saturated solutions of electrolytes are sufficiently conducting and the electrode area was quite large. The saturated solution was poured into the tube and the central electrode was immediately inserted. Potential difference was measured instantaneously. The experiments were performed with aqueous solutions of potassium chloride and potassium iodide saturated at 40° C. The asymmetry potential and thermo-e.m.f. were estimated and found to be negligible compared with the observed maximum potential differences. The decay of potential difference as a function of time was also observed. Smooth curves were obtained. The decay curves obtained by Workman and Reynolds for the freezing of ice were erratic, most probably due to difficulty in avoiding solidification on both the electrodes simultaneously. In our experiments one of the electrodes was always clean.

Difficulties in these experiments are numerous. First, the temperature corresponding to maximum potential difference is difficult to estimate, owing to the fact that nucleation may take place at any temperature intermediate between the saturation temperature and the bath temperature. Secondly, it was realized that owing to large electrode area, there was a distinctly greater possibility of nucleation occurring in crevices. This was quite clear, since great difficulty was encountered in cleaning the electrodes after each experiment so as to give negligible asymmetry potential.

These difficulties were removed to a great extent in subsequent experiments. The saturated solution was kept in a 'Pyrex' test-tube, closed at the upper end by a cork through which two electrodes, of good connecting wire, passed. One of the electrodes was in the form of a spiral. This almost touched the bottom of the tube. The other electrode just touched the surface of the solution. This was done to ensure that deposition of the crystals did not occur simultaneously on the two electrodes. If the nucleation or crystallization occurs on the two electrodes then the potential difference is neutralized or obscured. To guard against this, the second electrode was only inserted when the observations were to be made. Potential difference was measured with a vacuum tube voltmeter giving values that were correct to ± 5 mV. The 'Pyrex' tube was kept in a large beaker containing water. The outer bath was cooled in a controlled way. The cooling-rate was about 1 deg. in 5 min. The fall in temperature of the bath was followed closely by the aqueous solution of electrolytes in the 'Pyrex' tube. As soon as the first crystals appeared on the electrode, the potential difference was noted. The temperature of the bath was also noted at the same time. The crystallization at any desired temperature was induced by seeding. In this manner, the potential differences for a number of electrolytes were noted for different degrees of undercooling. To give an idea of the magnitude of the effect, only maximum values are shown in Table 1.

Table 1. PRECIPITATION AND DISSOLUTION POTENTIALS FOR ELECTROLYTES
(For precipitation potentials, the saturation temperature was 50° C.)

Substance	Precipitation potential		Dissolution potential	
	Temperature (°C.)	Potential difference (mV.)	Temperature (°C.)	Potential difference (mV.)
Potassium bromide	41	115	70	505
Potassium nitrate	44	120	80	184
Sodium acetate	42	460	70	350
Potassium acetate	47	640	70	440
Ammonium chloride	43	155	70	320
Potassium chloride	45	220	70	206
Potassium iodide	40	145	70	750
Ammonium bromide	40	350	70	450
Oxalic acid	45	450	50	196

The reverse effect involving measurement of dissolution potential was also studied in a simple way. Silver wires were used as electrodes. One of the electrodes was kept in a test-tube in which aqueous solution of the electrolyte saturated at 70° C. or 80° C. was kept. On cooling to room temperature or even lower, the electrode was covered with a thick deposit of freshly prepared crystals. The tube was broken at the bottom, so that excess of water was removed. Now, the broken tube along with the electrode was dipped in water or in the solution of the same electrolyte at a definite concentration. The other electrode was kept in water or in the solution beforehand. The two electrodes were connected to a direct-reading millivoltmeter made from a Pye Scalamp galvanometer as described earlier. As soon as the electrode embedded with crystals was dipped, an instantaneous deflexion in the galvanometer was observed. A large number of electrolytes were investigated. The effects of temperature, concentration and stirring were also investigated. The maximum values of dissolution potentials observed in water are also given in Table 1.

The following conclusions can be drawn from the data collected in the course of this work:

(1) The potential difference is developed as soon as a tiny crystal is formed at the electrode. However, we cannot say whether the potential difference is developed when the crystal nuclei is just formed or when it grows to certain dimensions. In the same manner, dissolution potential is developed as soon as the first crystals go into the solution.

(2) The electrode at which the crystallization takes place was always found to be positive. Similarly, in the case of dissolution potential, the electrode on which the dissolution took place was found to be positive.

(3) The precipitation potential increases with the degree of undercooling. The dissolution potentials also increase systematically in most cases as the temperature of the water is increased.

(4) The dissolution potential of an electrolyte in an aqueous solution of the same is a function of concentration. Its value increases as the dilution is increased.

(5) As the crystallization or dissolution proceeds, the potential difference diminishes. The decay curve of potential difference would be erratic when the crystals are deposited simultaneously on the two electrodes or when the dissolution takes place from both electrodes.

(6) Both phenomena are independent of the nature of the electrodes.

The exact mechanism of these phenomena is difficult to establish at present until more information is available. It seems reasonable to assume that an electrical double-layer is formed when the solid phase is just formed or a fresh double-layer is regenerated when the solid just dissolves. The concept of electrical double-layer may be useful here, since it is able to explain adequately a number of interfacial phenomena[5]. But, if so, the electrical potential difference should be developed at the appearance of first crystal nucleus. It is difficult to provide evidence for it.

The results show clearly that the greater the rate of transformation, the greater is the potential difference developed. B. Gross[6] has developed a theory of such phenomena which relates the potential differences to the rate of transformations. According to his model, as the interface advances, it leaves behind a trail of space-charge in the solid between the region deep in the solid and the liquid, which builds up a potential difference. However, it is difficult to imagine how these charges are dissipated since ultimately the solid must be electrically neutral. Moreover, in the present case the electrical conductivity of solid electrolyte and the diffusion of the electrolyte in the solution would be important factors which are not taken into account by this theory.

More detailed investigations are in progress and may be published in due course. This work forms part of a project sponsored by the Indian Council of Scientific and Industrial Research.

[1] Loeb, L. B., *Static Electrification* (Springer-Verlag, Berlin, 1958).
[2] Workman, E. J., and Reynolds, B. E., *Phys. Rev.*, **78**, 254 (1950).
[3] Gill, E. W. B., and Alfrey, S. A., *Nature*, **169**, 203 (1952).
[4] Costa Ribeiro, J., thesis, Univ. of Brazil (1945).
[5] Kruyt, H. R., *Colloid Science* (Elsevier Pub. Co., Amsterdam, 1952).
[6] Gross, B., *Phys. Rev.*, **94**, 1545 (1954).

METAL CHLORIN COMPLEXES IN RECENT SEDIMENTS AS INITIAL PRECURSORS TO PETROLEUM PORPHYRIN PIGMENTS

By G. W. HODGSON and ERIC PEAKE

Research Council of Alberta, Edmonton, Canada

THE presence of the isocyclic ring in the porphyrins found in crude oil is usually regarded as indicative of a chlorophyll origin for the petroleum porphyrin–metal complexes[1]. Since it appears, therefore, that pigments derived from chlorophyll probably accompany the general organic matter in its conversion to crude oil, it is of interest to establish the path by which chlorophyll becomes transformed to petroleum porphyrin pigments. In this manner it may be possible to define the chemical environments of the formation of crude oil more clearly.

Chlorophyll is readily converted to phæophytin by the loss of magnesium[2]. However, the path by which phæophytin is converted to metal–porphyrin complexes is not well defined; as discussed in an earlier communication[3], it may involve one of two major intermediates. In one, the phæophytin forms a metal–phæophytin complex, a chlorin complex, which is afterwards reduced and dehydrogenated to a metal-containing porphyrin. In the other, the phæophytin is reduced and dehydrogenated to a free porphyrin which afterwards forms a complex with a metal. Heretofore, however, neither chlorin complexes nor free porphyrins have been reported in recent sediments.

Recently, however, a porphyrin–metal complex has been found in post-glacial lake sediments, having light-absorption maxima at 410, 536 and 575 mμ (ref. 3). There is evidence that it is a carboxylated porphyrin complex, only one step removed from the decarboxylated porphyrin–metal complexes of crude oil.

The purpose of this article is to report the results of attempts to close the gap between the free chlorins, which are abundant in potential petroleum source sediments, and the porphyrin–metal complex, which exists in sediments geologically very little older. Present chemical work in this laboratory shows the likely initial steps, and these are confirmed by new pigment discoveries in recent lake and delta sediments.

From considerations of the reactivity of the peripheral groups on the chlorophyll molecule, it appears reasonable to believe that the main reaction path would include phæophytin, phæophorbide, phæoporphyrin, phylloerythrin, and deoxophylloerythrin, leading to deoxophylloerythroetioporphyrin of crude oil. A series of experiments subjecting phæophorbide to extended thermal action in a methanol–water system at 180° C. appeared to confirm such a sequence in principle. Compounds essentially similar to phæoporphyrin and phylloerythrin occurred within a few hours, followed shortly by two deoxophylloerythrin-like substances. Identification of the compounds was based on acid-solubility characteristics in addition to spectral configurations which have been established in this laboratory for the known compounds prepared according to Fischer[4]. Thus, the evolution of such compounds in a relatively simple thermal system indicates that such a pathway may be possible in this particular metal-free environment.

However, free porphyrins in potential petroleum source sediments have not been reported, and present work in this laboratory has as yet failed to reveal any. Such negative findings, therefore, tend to direct attention to the other path, namely, that involving the formation of a metal–chlorin complex which is afterwards transformed to a metal–porphyrin complex.

To investigate this point, various chlorophyll-containing mixtures of either spinach leaves or commercial chlorophyll were allowed to react with various vanadium salts in the presence of sodium sulphide in aqueous systems at temperatures ranging from 90° to 150° C. In most cases, starches, sugars and proteins were included as well, further to simulate source sediment environments. Vanadium complexes of various pigments appeared in the reaction systems after several hours, with the major pigment classes characterized by absorption spectra maxima at about 650 and 620 mμ for one, and 650 and 590 mμ for another. The first appeared to be a precursor of the second. A further reaction of phæophytin *a* with ammonium metavanadate in the presence of only sodium sulphide and an unsaturated vegetable oil in an aqueous system at 105° C. produced the same two classes of compounds in substantial yields in 60–70 hr. Part of each class of compounds showed the presence of free carboxyl groups, indicating at least partial hydrolysis of the phytyl alcohol group from the phæophytin. These compounds were insoluble in 6 *N* and 12 *N* hydrochloric acid and were, therefore, regarded as metal complexes of the pigments, presumably vanadium complexes. The location of spectral peaks indicated complexes of chlorins rather than porphyrins, since the latter absorb in a significantly lower spectral range[5].

Similar chlorin pigments were evident in recent sediments obtained from widely differing environments. For example, sediments from Lake Wabamun, a large, relatively shallow lake about 40 miles west of Edmonton, Alberta, Canada, were exhaustively extracted with aqueous acetone and the extracts transferred to ether. Uncomplexed chlorin pigments, principally phæophytin *a* and phæophorbide *a*, were acid-extracted from the ether, leaving an appreciable amount of chlorin-like material along with carotenoids and other substances. The acid-insoluble material, which would include any metal complexes of chlorins or porphyrins present in the sediments, was transferred to *n*-hexane and chromatographed on cellulose fibre.

Good spectral evidence was obtained for three pigments which were apparently metal complexes as indicated by both their acid-insolubility and spectral characteristics. Absorption maxima for the three compounds were: (*a*) 403, 598 and 648 mμ; (*b*) 413, 589 and 628 mμ; (*c*) 411 and 648 mμ.

The general spectral appearances were those of metal chlorins, similar to those of the products obtained in the vanadium–phæophorbide reactions reported above, and to that of the nickel phæophytin reaction previously reported at 410 and 635 mμ (ref. 6).

Analyses for vanadium, nickel and copper showed positive results for both vanadium and nickel. Material balances were difficult to test because of the lack of precise knowledge of the extinction coefficients of the pigments, and the incomplete separation of organic matter which was characterized by strong absorption below 500 mμ. Nevertheless, using the known extinction coefficient for the nickel complex of phæophytin[6], it was possible to show the presence of enough of both vanadium and nickel to account for the apparent amount of chlorin present. Thus, it is clear that recent sediments contain not only free chlorins but also metal-complexed chlorins. Accordingly, it is reasonable to suggest that the overall reaction path for the formation of petroleum porphyrin–metal complexes involves the entry of the metal into the pigment at a very early stage, the chlorin stage, in the general development of the pigment.

It is of some significance to note that the occurrence of metal–chlorins appears to be quite widespread. Metal–chlorin compounds very similar to those of Lake Wabamun, a plains lake, were found in a deep mountain lake, Pyramid Lake, in Jasper National Park, in the sediments of the delta of the Mackenzie River in the Canadian Arctic, and in the sediments of Laguna Madre in the Gulf of Mexico.

A further observation in the Lake Wabamun sediments is worthy of considerable attention. The sediments on the surface of the lake bottom contained not only metal complexes of chlorins but also, in addition, a metal complex of a porphyrin. It occurred in much smaller concentrations than the chlorin complexes, and had absorption maxima at 410, 530 and 570 mμ. Alkali solubility indicated the presence of a free carboxyl group. Another slightly different porphyrin complex had been found in a nearby lake as previously reported[3], but the significant difference is that in the present case the sediments were surface sediments, very recently deposited, in contrast with the deeper sediments which were probably several thousand years old.

In summary, there is much evidence to indicate that the reaction path for the formation of petroleum metal–porphyrin pigments involves the introduction of the metal into the pigment at a very early stage of development, the chlorin stage, and that almost the entire process is completed in a very short period of time; only hundreds of years are required for the deposition of a few inches of lake sediments.

We are pleased to acknowledge the assistance of R. H. Harvey and other skin-divers of the Edmonton Aquanauts for obtaining the lake-bottom sediments, G. H. Johnston, of the National Research Council, for the delta samples, Gene Rusnak, at present of the Marine Laboratory, University of Miami, for the sediments of Laguna Madre, and B. L. Baker, R. M. Elofson, and Brian Hitchon for general assistance and guidance.

[1] Treibs, A., *Chem. Zbl.*, **2**, 2831 (1936).

[2] Hodgson, G. W., and Hitchon, B., *Bull. Amer. Assoc. Petrol. Geol.*, **43**, 2481 (1959).

[3] Hodgson, G. W., Hitchon, B., Elofson, R. M., Baker, B. L., and Peake, E., *Geochem. Cosmochim. Acta*, **19**, 272 (1960).

[4] Fischer, H., *Chem. Rev.*, **20**, 41 (1937).

[5] Champlin, J. B. F., and Dunning, H. N., Preprints, *Div. Petrol. Chem., Amer. Chem. Soc.*, **3**, No. 4c, C17 (1958).

[6] Baker, B. L., and Hodgson, G. W., *J. Phys. Chem.*, **65**, 1078 (1961).

HISTOCHEMICAL DEMONSTRATION OF GAMMA-GLUTAMYL TRANSPEPTIDASE

By Prof. Z. ALBERT, Dr. MARIAN ORLOWSKI and Dr. A. SZEWCZUK

Polish Academy of Science, Department of Experimental Oncology and Department of Biochemistry, Institute of Immunology and Experimental Therapy and Third Medical Clinic, School of Medicine, Wroclaw, Poland

IN a previous paper two of us[1] described the occurrence of a specific peptidase in blood serum and some tissues. The enzyme hydrolysed some α-(N-γ-DL-glutamyl)-aminonitriles and was suspected to be a γ-glutamyl transpeptidase, similar to that described by Hanes *et al.*[2]. Further work confirmed this supposition and recently[3] a new method was described for the determination of γ-glutamyl transpeptidase, using γ-L-glutamyl-α-naphthylamide as substrate. The enzyme catalyses with this substrate a transpeptidation reaction according to the following scheme:

2γ-L-glutamyl-α-naphthylamide → α-naphthylamine + γ-L-glutamyl-γ-L-glutamyl-α-naphthylamide

The amount of α-naphthylamine liberated during the enzymic reaction was estimated by a modification of the Bratton and Marshall procedure[4] and used as a measure of enzyme activity. With this method, the enzyme activity was found to be very high in human kidney. Much lower activities were found in human liver, spleen and pancreas. Some activity was found in human serum and urine[3].

This article deals with the histochemical demonstration of γ-glutamyl transpeptidase in tissue sections of some animals and human beings. γ-L-glutamyl-α-naphthylamide was used as substrate and the liberated α-naphthylamine was coupled to an azo-dye with the aid of fast garnet *GBC* salt. The composition of the incubation mixture was as follows: 6 ml. substrate solution (5·44 mgm. γ-L-glutamyl-α-naphthylamide dissolved in 0·9 per cent sodium chloride), 4 ml. buffer solution (0·1 *M* phosphate buffer *p*H 6·7, or 0·2 *M* citrate–phosphate buffer at the same *p*H), 5 mgm. of fast garnet *GBC* salt per 10 ml. of incubation mixture. This incubation mixture was prepared fresh daily. Good results were also obtained using γ-L-glutamyl-β-naphthylamide. However, the solubility of this compound is very low at *p*H 6·7, and even 0·2 mgm. of this substrate per ml. of incubation mixture was difficult to dissolve. The hexazonium salt of pararosanilin[5] was also used as coupling agent instead of fast garnet *GBC*. Places with γ-glutamyl transpeptidase activity exhibit a brown-reddish colour with fast garnet *GBC* or a yellow colour with the hexazonium salt of pararosanilin as coupling agent.

The optimum *p*H for γ-glutamyl transpeptidase is about 8·8 and at *p*H 6·7 the enzyme activity amounts only to a few per cent of that measured in optimal conditions. Acceleration of the enzymatic reaction could be obtained at *p*H 6·7 by the addition of glycylglycine, which serves as an acceptor of the γ-glutamyl moiety according to the following reaction[3]:

$$\gamma\text{-L-glutamyl-}\alpha\text{-naphthylamide} + \text{glycylglycine} \xrightarrow{\text{enzyme}}$$
$$\rightarrow \gamma\text{-L-glutamyl-glycylglycine} + \alpha\text{-naphthylamine}$$

For acceleration of the histochemical reaction, we have added 20 μmoles of glycylglycine per ml. of incubation mixture. The addition of glycylglycine is particularly recommended for the demonstration of γ-glutamyl transpeptidase in tissues with low enzyme activity.

Healthy mice, rats, guinea pigs and rabbits were used for our experiments. Moreover, kidney sections were taken from human subjects some hours after death in street accidents. Sections, 3–4 mm. thick, were prepared from kidneys, spleen, liver, pancreas, testes, myocardium and lungs, and fixed in acetone, absolute ethyl alcohol or 6 per cent neutralized formalin. The fixation time was 11 hr. at 4° C. As freezing with carbon dioxide gave inferior results, the sections were embedded in paraffin. 5–10μ thick sections were incubated in the given mixture for varying periods of time. 5–20 min. were sufficient for kidney while for other tissues an incubation time of 1–3 hr. was necessary. Following incubation, the slides were immersed for 2 min. in 0·1 *M* solution of copper sulphate and then stained with hæmatoxylin for 1–2 min. Afterwards, they were rinsed with 0·9 per cent sodium chloride and mounted in glycerol-gelatin.

The following localization of the enzyme was observed. In the kidney, the lumen of the loop of Henle and to a less extent of the proximal convolutions of the tubulus shows homogeneous masses with a strong positive reaction. The enzyme was not visible in the glomeruli and collecting tubules. In mouse and rat kidneys the enzyme is distributed quite regularly all over the cortex (Fig. 1) whereas in guinea pigs and rabbits γ-glutamyl transpeptidase is located almost exclusively in medullary rays (Fig. 2). The localization of the enzyme in normal men is similar to that found in rabbits and guinea pigs. It is to be stressed that the homogeneous masses with a strong positive reaction adhere to the surface of epithelial cells not only in proximal convolutions of the tubulus, which possess a distinct brush border, but also in the loop of Henle devoid of this brush border.

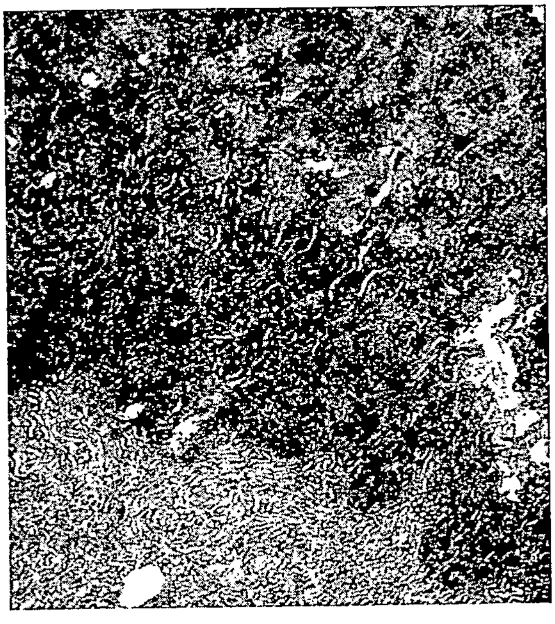


Fig. 1. γ-glutamyl transpeptidase in the cortex of the mouse kidney. (× 42)

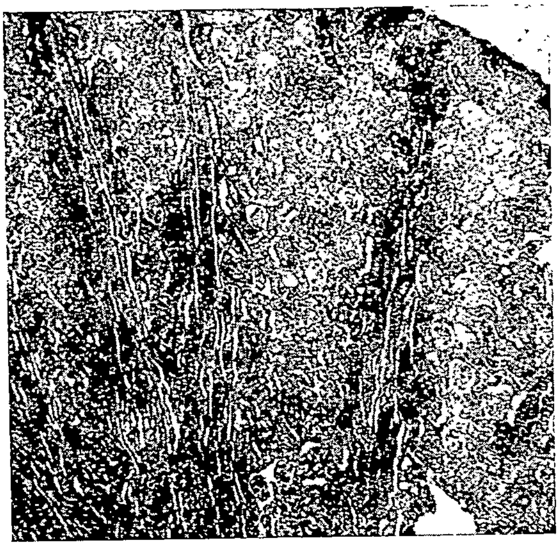


Fig. 2. The localization of γ-glutamyl transpeptidase in medullary rays in the kidney of the guinea pig. (× 42)

With the spleen, the enzyme is visible in guinea pigs, rabbits and rats in the walls of some capillaries of the red and white pulp, and moreover as granules in some cells of the reticulo-endothelial system.

With the liver, a positive reaction was seen in rats in the walls of bile canaliculi and in single cells of the reticulo-endothelial system in Glisson's capsule. In rabbits the enzyme was found in the form of granules in the cytoplasm of liver cells mostly around the nucleus. It was more abundant in the periphery than in the centre of lobules. The greatest enzyme activity was found in guinea pigs, taking the shape of confluent tiny granules on the periphery of liver cells and, as in rabbits, more profuse in the peripheral than the central part of the lobules.

With the pancreas, a relative abundance of the enzyme was seen in the cells of the outer secretory system in the mouse, rat, rabbit and guinea pig. The enzyme was not found in Langerhans's islands.

With testes, a positive reaction was observed in the testes of guinea pig within spermatozoa and spermatocytes. No activity was seen in the testes of the rat and the mouse. The myocardium and lungs of the mouse, rat and guinea pig did not exhibit enzyme activity.

[1] Szewczuk, A., and Orłowski, M., *Clin. Chim. Acta*, **5**, 680 (1960).
[2] Hanes, C. S., Hird, F. J. R., and Isherwood, F. A., *Nature*, **166**, 288 (1950).
[3] Orłowski, M., and Szewczuk, A., *Acta Biochimica Polonica* (in the press).
[4] Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, **128**, 537 (1939).
[5] Barka, T., *Nature*, **187**, 248 (1960).

REACTIONS OF HÆMOPROTEINS WITH HYDROGEN PEROXIDE AND THE SUPPOSED FORMATION OF HYDROGEN PEROXIDE DURING THE AUTOXIDATION OF HÆMOGLOBIN

By Prof. D. KEILIN, F.R.S.
Molteno Institute, University of Cambridge

IT is well known that many substances undergoing autoxidation, or oxidation catalysed by metals, or by some specific enzmyes, reduce molecular oxygen, directly or indirectly, to hydrogen peroxide, which can usually be detected by some simple reagents. However, the main limitations of these reagents are that they can seldom be used for the study of the continuous formation of hydrogen peroxide and that they may interfere with the primary oxidation reactions which generate the peroxide, especially if these reactions are brought about by some unstable catalytic systems. In such cases peroxidase, catalase and methæmoglobin can be used both for the detection of hydrogen peroxide and for following its gradual generation even by very unstable biological systems. These three hæmoproteins show striking resemblances in their structure and properties, including some of their reactions with hydrogen peroxide[1–4].

Reactions of Hæmoproteins with Hydrogen Peroxide

The following few examples illustrate the reactions of hæmoproteins with hydrogen peroxide generated by an oxidation system.

(1) *Catalatic decomposition of hydrogen peroxide.* The reduction of molecular oxygen to hydrogen peroxide by glucose oxidase (notatin), which catalyses the oxidation of glucose to gluconic acid, can easily be followed manometrically[5]. Thus, catalase added to this system, by liberating oxygen from the peroxide, halves the original uptake of oxygen and brings it down to the theoretical value corresponding to the reduction of oxygen to water. Similar results have been obtained with many other oxidizing systems.

(2) *Peroxidatic utilization of hydrogen peroxide.* Of special interest in this respect was the discovery that catalase, in addition to its well-known property of decomposing hydrogen peroxide to oxygen and water, is also capable of utilizing the hydrogen peroxide generated for the peroxidatic oxidation of alcohol to aldehyde[4,5]. The concept of the peroxidatic activity of catalase was later extended to some other hydrogen donors, including hydrogen peroxide itself[6–8]. A large variety of hydrogen donors were found to undergo peroxidatic or coupled oxidation catalysed by peroxidase, catalase and methæmoglobin. These hæmoproteins, however, show marked differences in their specificity and in their catalytic efficiency towards hydrogen donors[5]. As some of these donors, like benzidine, give highly coloured products on peroxidatic oxidation catalysed by peroxidase and methæmoglobin, the presence of hydrogen peroxide can easily be detected by this well-known colour reaction.

(3) *Compounds of hæmoproteins with hydrogen peroxide.* As these hæmoproteins form with hydrogen peroxide compounds which are easily recognized by their colour and absorption spectra, they can be used for the detection of hydrogen peroxide, as reagents acting in the absence of hydrogen donors. While peroxidase and methæmoglobin can be used for this purpose in their free state, catalase gives the best result when it is used as the azide-catalase complex which, when added to a solution containing hydrogen peroxide, changes its colour and the pattern of its absorption spectrum[1,3]. Moreover, azide catalase enables one to follow the formation of hydrogen peroxide even during the oxidation of sodium dithionite ($Na_2S_2O_4$) when other reagents cannot be used.

Is Hydrogen Peroxide formed during the Autoxidation of Hæmoglobin?

Oxyhæmoglobin, like hæmoglobin, is a compound with divalent iron atoms which may undergo slow autoxidation to the tervalent iron atoms of methæmoglobin. It was shown that up to 2 per cent of endoerythrocytic mammalian hæmoglobin *in vivo* may be present as methæmoglobin[9]. The hæmoglobin of *Gasterophilus* larvæ[10] and of certain parasitic nematodes[11], as well as myoglobin, are much more susceptible to autoxidation, especially during their purification, than are the mammalian blood hæmoglobins.

Of great interest, however, is the fact that while the oxidation of oxyhæmoglobin to methæmoglobin by potassium ferricyanide liberates all the bound oxygen, that is, 1 mole oxygen per gram-atom of iron, the autoxidation of oxyhæmoglobin liberates less than 50 per cent of the bound oxygen, instead of 75 per cent had some oxygen been used only for the oxidation of the divalent iron atoms of hæmoglobin. Brooks[12], who was the first to study the kinetics of the autoxidation of hæmoglobin, showed that the rate of this reaction at different oxygen pressures is proportional to the concentration of deoxygenated hæmoglobin. The observed first-order rate constant for the autoxidation of hæmoglobin is a function of the oxygen pressure, and has a well-defined maximum at about 20 mm. The kinetics and the possible mechanism of this reaction have recently been studied by several workers, both for hæmoglobin and myoglobin. The autoxidation is greatly accelerated by lowering the *p*H to about 5 and raising the temperature to 39°. Denaturation of oxyhæmoglobin by acid or alkali, which brings about the immediate oxidation of hæms to acid and alkaline hæmatin respectively, is also accompanied by the liberation of only a certain fraction of the bound oxygen.

It was suggested[13] that a part of the bound oxygen, which is not liberated from oxyhæmoglobin during its autoxidation, is activated and reacts with globin to form hydrogen peroxide which can be used by hæmatin for the catalysis of the peroxidatic oxidation of substances like ascorbate, bilirubin and biliverdin.

Unfortunately, no proper control experiments have been carried out on the oxidation of these substances by a solution of a parahæmatin or hæmochromogen in the absence of peroxide. The autoxidation of myoglobin was found to be accompanied by an uptake of 2·5 moles oxygen for each mole of metmyoglobin formed[14]. It is now generally accepted that the autoxidation of hæmoglobin and myoglobin to methæmoglobin and metmyoglobin respectively, brings about the simultaneous oxidation of some hydrogen-donor groups on globin itself. Although the mechanism of these autoxidation reactions is not yet properly understood, several workers[13–15] postulated the important part played in these reactions by hydrogen peroxide and related free radicals.

One of the objects of the present work was to find whether the postulated formation of hydrogen peroxide during the oxidation of oxyhæmoglobin on mild acidification can be demonstrated by some of the reactions of hydrogen peroxide with hæmoproteins which were discussed in the first part of this communication.

For this purpose experiments were carried out on horse-blood oxyhæmoglobin purified by crystallization which was repeated three times; the oxidation of oxyhæmoglobin was followed spectroscopically and the liberation of oxygen was determined manometrically.

The main results of these experiments can be summarized as follows:

(1) Contrary to claims made by previous workers[15], the addition of catalase to oxyhæmoglobin, prior to mild acidification, made no difference either to the rate of its oxidation to methæmoglobin, or to the amount of bound oxygen liberated from oxyhæmoglobin; ethanol added to this system failed to undergo oxidation to aldehyde.

(2) Benzidine added to the solution of oxyhæmoglobin did not undergo oxidation to a dark blue product on mild or strong acidification of this solution. The reaction failed even when a strong solution of purified peroxidase was mixed with oxyhæmoglobin. Yet, on the addition of traces of hydrogen peroxide to this solution, benzidine was rapidly oxidized to a dark blue product.

(3) Methæmoglobin formed during mild acidification never showed the presence of hydrogen peroxide by the formation of the methæmoglobin–peroxide compound, which could easily have been recognized by its red colour and the pattern of its absorption spectrum. The same applied to peroxidase added in a suitable concentration to an oxyhæmoglobin solution prior to its acidification.

(4) About 0·5 ml. of solution of pure peroxidase (0·37 × 10^{-3} *M*) enclosed within a small dialysing sac, and showing its characteristic absorption spectrum, was immersed in 20 ml. of solution of oxyhæmoglobin (1·4 × 10^{-3} *M* hæmin) which was acidified while being vigorously stirred. The dialysing sac, removed at different intervals of time for spectroscopic examination, failed to reveal any change in the absorption spectrum of peroxidase. Similar results were obtained when the dialysing sac contained a strong solution of azide catalase (in the presence of a large excess of azide) which is one of the most sensitive reagents for hydrogen peroxide.

All these experiments failed to give any indication of the formation of hydrogen peroxide in a solution of acidified oxyhæmoglobin undergoing autoxidation to methæmoglobin or to acid hæmatin. The problem of the mechanism of autoxidation of hæmoglobin and myoglobin therefore still remains open for further investigation.

[1] Keilin, D., and Hartree, E. F., *Proc. Roy. Soc.*, B, **121**, 173 (1936).
[2] Keilin, D., and Mann, T., *Proc. Roy. Soc.*, B, **122**, 119 (1937).
[3] Keilin, D., and Hartree, E. F., *Biochem. J.*, **49**, 88 (1951).
[4] Keilin, D., and Hartree, E. F., *Proc. Roy. Soc.*, B, **119**, 141 (1936).
[5] Keilin, D., and Hartree, E. F., *Biochem. J.*, **39**, 293 (1945); **60**, 310 (1955).
[6] Heppel, L. A., and Porterfield, V. T., *J. Biol. Chem.*, **178**, 549 (1949).
[7] Chance, B., *Nature*, **161**, 914 (1948).
[8] Chance, B., *J. Biol. Chem.*, **180**, 947 (1949).
[9] Fegler, G., *Quart. J. Exp. Physiol.*, **34**, 243 (1948).
[10] Keilin, D., and Wang, Y. L., *Biochem. J.*, **40**, 855 (1946).
[11] Rogers, W. P., *Austral. J. Sci. Res.*, B, **2**, 287 (1949).
[12] Brooks, J., *Biochem. J.*, **23**, 1391 (1929); *Proc. Roy. Soc.*, B, **109**, 35 (1931); B, **118**, 560 (1935).
[13] Lemberg, R., *Austral. J. Exp. Biol.*, **20**, 111 (1942). Lemberg, R., and Legge, J. W., *Hematin Compounds and Bile Pigments* (Intersci. Pub., New York, 1949).
[14] George, P., and Stratmann, C. J., *Biochem. J.*, **51**, 103, 418 (1952).
[15] Kikuchi, G., Shukuya, R., Suzuki, M., and Nakamura, C., *J. Biochem.*, Japan, **42**, 267 (1955).

RISE OF ANTIBODY TITRE IN CELL-FREE SPLEEN PREPARATIONS INCUBATED *IN VITRO*

By DR. DIETER H. SUSSDORF
Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena

AN initial attempt to demonstrate antibody-forming activity by cell-free tissue preparations was made by Šterzl and Hrubešová[1]. They injected cell-fractions and nucleoproteins from spleens of rabbits immunized with *Salmonella paratyphi B* vaccine into unimmunized neonatal rabbits and observed the appearance of specific agglutinins in the recipients. However, it is still not clear whether the antibodies appearing in the recipient animals were synthesized *de novo* after transfer or were initially bound to and afterwards released by the transferred materials[2]. With respect to the second possibility, a report by Feldman *et al.*[3] is of interest. They found that ribonucleoprotein-bound antibody was immunologically inactive but could be titrated following enzymatic degradation of the ribonucleoprotein.

The aim of the present work was to follow in an *in vitro* system the hæmolysin titres of extracts prepared from spleens of immunized rabbits. The animals were injected intravenously with standard doses of 0·8 × 10^9 washed sheep erythrocytes/kgm. (equivalent to 1 ml. of a 5 per cent suspension/kgm.). Primary stimulation consisted of a single injection on days 0, 1 and 3, and secondary stimulation of a single dose given 8 weeks after primary injection.

The preparation of an experimental extract (series *A*) was as follows: five rabbits were killed by an intravenous injection of air on day 4 or 5 following the first primary, or on day 3 following the secondary, injection. On these days, the serum hæmolysin titres of the animals ranged between 450 and 3,200 hæmolytic units/ml. The spleens of the rabbits were removed (average combined weight 9·4 gm.), cut into small pieces, and rinsed with about 5 ml. medium. The latter was a defined, complete tissue culture medium[4] with an amino-acid composition corresponding to that of rabbit γ-globulin[5]. For the purpose of these experiments, 0·7 μmole adenosine triphosphate[6] was added to the medium. The rinsed spleen pieces were combined with 1·0 ml. medium/gm. tissue and homogenized by 10 down–up excursions in a Potter–Elvehjem tissue grinder with a 'Kel-F' pestle. All procedures were carried out at 3° C. Probably not more than 75 per cent of the spleen cells were extracted by this method. This estimate is based on a count of the free nuclei present in the homogenate prior to centrifugation and the comparison of this number with the estimated number of cells representing 1 gm. of spleen tissue.

The homogenate was centrifuged at 500*g* for 10 min., and the resulting supernatant re-centrifuged at 1,700*g* for 15 min. The second supernatant (average volume 6·4 ml.), containing the large and small granule, and the soluble cell fractions, was transferred into a small Petri dish which was placed in a tissue culture incubator (37° C., 85 per cent humidity, 5 per cent carbon dioxide, 95 per cent air). The total time elapsed between homogenization and the beginning of incubation was 70–80 min. To avoid clotting, the extract was gently agitated with a magnetic stirrer during the second hour of incubation. Samples of 0·1 ml. were removed with ultramicro-pipettes at 0, 0·5, 1, 2, 3, 4, 6, 8, 12, 15 and 18 hr. Since it was found earlier that the samples did not contain detectable amounts of complement, complement inactivation was omitted.

The samples were diluted 1 : 50 with veronal buffer and cleared at 1,700*g* for 20 min. Their hæmolysin titres, determined according to the method of W. H. and L. G. Taliaferro[7,8], were expressed in photometrically determined 50 per cent hæmolytic units/ml. extract. A 50 per cent unit is defined as the reciprocal of the amount of extract lysing 50 per cent of 3·2 × 10^8 sheep erythrocytes in the presence of four 50 per cent units of complement. The over-all error of this titration method did not exceed 5 per cent.

Two types of controls were employed. The first (series *B*) involved the preparation of extracts from normal spleens to which hæmolytic serum was added immediately before homogenization. The amount of antibody added approximated the zero-time hæmolysin-level of the experimental extracts. The purpose of these controls was to determine the immunological stability of hæmolysins during *in vitro* incubation. The second set of controls (series *C*) was prepared from normal spleens alone.

Of nine experimental extracts, seven showed a significant elevation of hæmolytic activity, the net increase ranging between 21 and 99 hæmolytic units/gm. spleen. Because of 8-hr. intervals between samplings, one run could not be used for statistical evaluation, and only six of the positive experimental extracts are listed in Table 1 (*A*). Preparations from rabbits stimulated with primary or secondary antigen injections (four and two extracts, respectively) were considered together. The total incubation time was 8 hr. for four and 18 hr. for two of the series *A* extracts. Table 1 also lists the results obtained with five extracts from series *B* controls, three of which were incubated for 8 hr., and two for 18 hr. Not shown in Table 1 are three extracts of series *C* (normal spleens alone), since no hæmolytic activity could be detected during the 8-hr. incubation period.

Table 1. HÆMOLYTIC ACTIVITY DURING *in vitro* INCUBATION OF CELL-FREE SPLEEN PREPARATIONS

	A Experimental extracts (spleens of immunized rabbits)	*B* Control extracts (spleens of normal rabbits; immune serum added)
No. of extracts*	6	5
Combined spleen weight (gm.)	9·4 ± 0·4	7·0 ± 0·1
Volume of extract (ml.)	6·4 ± 0·7	5·5 ± 0·4
Hr. at beginning of titre rise above zero-time level	1·3 ± 0·4	(1·7 ± 0·9)†
Hr. of maximum titre (*A*) or of beginning of titre plateau (*B*)	7·3 ± 0·9	2·4 ± 0·2
Zero-time titre‡	128 ± 26	143 ± 34
Maximum titre‡	176 ± 32	148 ± 34
Net titre increase‡	48 ± 11	5 ± 5

Values given are means ± standard errors

* Each extract was prepared from the pooled spleens of five rabbits.

† The titres of only three of these extracts rose above zero-time level.

‡ In 50 per cent hæmolytic units/gm. spleen.

Mean titration curves for series *A* and *B*, covering 8 hr. of incubation, are shown in Figs. 1 and 2. The graphs were obtained by calculating the mean hourly titres for a given series from the hæmolysin curves of the individual extracts, and plotting these means against time. Differences between certain parameters listed in Table 1 and corresponding ones of the

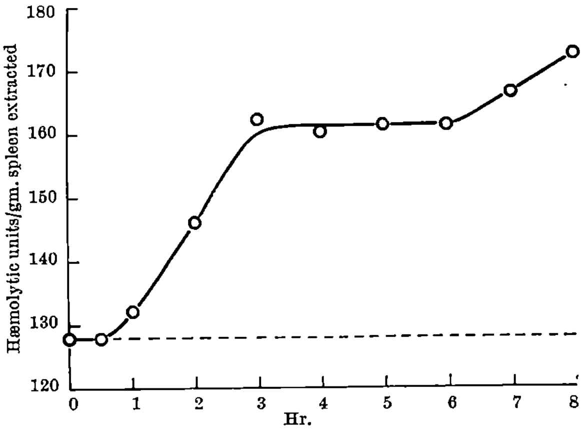


Fig. 1. Experimental extracts (series *A*). Mean hæmolysin curve of six *in vitro* incubated spleen extracts from rabbits immunized with sheep erythrocytes. (Each extract was prepared from five pooled spleens)

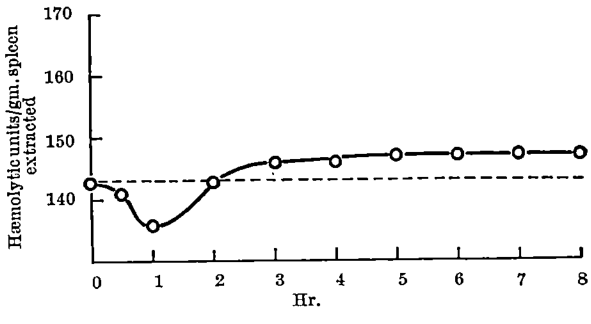


Fig. 2. Control extracts (series *B*). Mean hæmolysin curve of five *in vitro* incubated extracts from normal rabbit spleens to which hæmolytic serum was added before homogenization. (Each extract was prepared from five pooled spleens)

average curves are due to differences in the statistical approaches used to calculate the two sets of data.

The configuration of the mean hæmolysin curve for series *B* indicates a temporary immunological inactivation and subsequent reactivation of about 5 per cent of the added antibody. Inactivation may have been due to the adsorption of globulins to sub-cellular particles such as mitochondria and microsomes. The hæmolysins probably became detectable again after dissociation from these particles. Since adsorption may have begun during homogenization (70–80 min. prior to incubation), the initial titre of these extracts probably was at the level of the final titre plateau.

The two-step antibody rise shown by the mean hæmolysin curve for series *A* was displayed by five of the six extracts in this series. In some preparations, the initial antibody increase was followed by a shallow decline lasting 2 hr. or less. In the two extracts incubated for 18 hr., the second hæmolysin rise led to a plateau beginning at about 8 hr. which remained constant during the remainder of the incubation period.

The progressive increase in titre observed over a period of 8 hr. in spleen extracts from immunized animals suggests either the release of preformed antibody alone from a sub-cellular component, or the release of both preformed and newly synthesized antibody. The successive occurrence of these two processes may be indicated by the biphasic character of the mean hæmolysin curve.

I am indebted to Prof. Dan H. Campbell for his advice, and for supplying technical assistance and facilities.

This work was supported by fellowship *EF*-9543 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

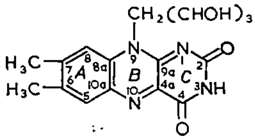
[1] Šterzl, J., and Hrubešová, M., *Fol. Biol.* (Prague), **2**, 21 (1956).
[2] Hrubešová, M., Askonas, B. A., and Humphrey, J. H., *Nature*, **183**, 97 (1959).
[3] Feldman, M., Elson, D., and Globerson, A., *Nature*, **185**, 317 (1960).
[4] Garvey, J. S. (personal communication).
[5] Smith, E. L., McFadden, M. L., Stockell, A., and Buettner-Janusch, V., *J. Biol. Chem.*, **214**, 197 (1955).
[6] Schweet, R., Lamfrom, H., and Allen, E., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1029 (1958).
[7] Taliaferro, W. H., and L. G., *J. Infect. Dis.*, **87**, 37 (1950).
[8] Taliaferro, W. H., and L. G., *J. Infect. Dis.*, **99**, 109 (1956).

BIOSYNTHESIS OF RIBOFLAVIN IN CELL-FREE SYSTEMS

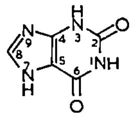
By Prof. T. W. GOODWIN and Dr. A. A. HORTON*

Department of Agricultural Biochemistry, University College of Wales, Aberystwyth

It has now been well established that ring *C* and N-9 and N-10 of ring *B* of riboflavin (I) are formed from a purine (II) derivative following the loss of C-8 of the purine[1,2]. For example, Plaut[3] and his colleagues observed that the labelling pattern in riboflavin synthesized by the mould *Ashbya gossypii* fed carbon-14-HCO'_3, -H.COO′ and -glycine was identical with that observed in purines synthesized in the presence of the same labelled substrates, and similar results have been obtained using carbon-14-L-serine[4] and carbon-14-L-threonine[5]. Experiments with generally labelled carbon-14 adenine showed that incorporation did not first involve the degradation of a purine and then re-synthesis of the metabolites into riboflavin[6], and these results have been confirmed using [^{14}C : ^{15}N] xanthine and guanine[7].



(I)

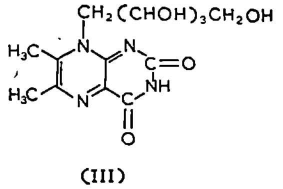


(II)

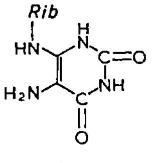
Under suitable cultural conditions up to 70 per cent of added carbon-14 guanine is incorporated into riboflavin by the yeast *Candida flareri*[8]. C-8 of the purine is lost during incorporation, which would indicate that 4,5-diaminouracil, or more likely a derivative, is the intermediate[9]. Support for this view came when 4,5-diaminouracil was identified as a metabolite of the flavinogenic fungus *Eremothecium ashbyii*, but the methods employed in these experiments would not preclude its existence *in vivo* as a ribose phosphate or ribitol derivative[10]. This raises a central problem in elucidating the mechanism of biosynthesis. At what stage and how does the ribitol side-chain become attached to N-9? The problem has not yet been settled; all that can be said at the moment is that as 6-7-dimethyl-8-ribityllumazine (III) is a precursor of riboflavin (see below), the ribitol residue appears before the completion of ring *A*. The only hint of mechanism comes from the work of Forrest and McNutt[11] with generally labelled carbon-14 guanosine; they found that the riboflavin arising from this substrate was much more strongly labelled in the ring system than in the ribitol side-chain; this observation strongly suggests that the ribitol did not arise by reduction of the ribose residue. However, this cannot be accepted out of hand, because guanosine could be in equilibrium with guanine and ribose-1-phosphate (reaction 1) in the presence of a nucleoside phosphorylase, and if the pool size of ribose-1-phosphate were much larger than that of guanine (and this is highly probable because it is 'active glycine', and thus purine, which is the limiting factor in flavinogenesis in *E. ashbyii*), then this would lead to the observed results:

$$\text{guanosine} + Pi \rightleftharpoons \text{guanine} + \text{ribose-1-phosphate} \quad (1)$$

On the assumption that 4-ribitylamino-5-amino-uracil (IV) might be the active precursor of riboflavin, a number of investigators synthesized this compound, which was found to be very unstable. Katagiri *et al.*[12]



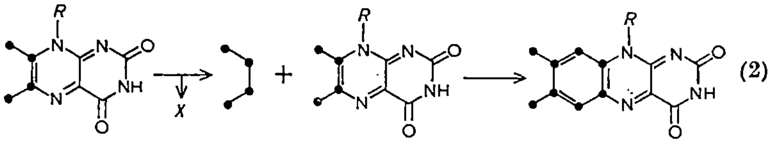
(III)



(IV)

---

* Present address: Department of Cell Physiology, Life Sciences Building, University of California, Berkeley 4, California.



(2)

have reported a slight conversion of this compound into riboflavin in *Aerobacter aerogenes*, but Plaut, who has given a full chemical synthesis of the compound[13], has not yet reported on its biological activity and has indicated, in a personal communication, that his results were most inconclusive; Kishi *et al.*[14] also failed to demonstrate any significant production of riboflavin when they used a crude enzyme preparation of *Eremothecium ashbyii*. With the collaboration of Dr. B. G. Audley, we synthesized (IV), using essentially Plaut's methods, and consistently failed to observe its conversion by cell-free extracts of *Candida flareri* into either 6,7-dimethyl-8-ribityllumazine or into riboflavin, although many variations in preparing the extracts and in incubation conditions have been tried[15]. If (IV) were a precursor of riboflavin, then the experimental conditions necessary for the demonstration of precursor activity are extremely elusive.

However, investigations on the final stages of riboflavin biosynthesis have met with a little more success. Masuda in 1956 isolated and identified 6,7-dimethyl-8-ribityllumazine (III) from the mycelium of *E. ashbyii*[16]. He finally proved the structure by total synthesis[17]. Thanks to the generosity of Dr. Masuda, who kindly provided us with a crystalline specimen of (III), we were able to demonstrate that cell-free extracts (produced by sonic disintegration), but not intact cell suspensions, of *Candida flareri* rapidly converted it into riboflavin. The activity resided in the supernatant fraction (not sedimentable at 100,000*g*) of the extract and the maximum conversion obtained under many varying conditions was always approximately 30 per cent. It is interesting to record here that, with Dr. B. G. Audley, we found that distilled water (*p*H 6·0) was an excellent solvent for separating (III) from riboflavin on paper chromatograms. The presence or absence of possible sources of the 4-carbon unit (for example, pyruvate, acetate, acetoin) required for the completion of ring *A* had little effect on the conversion, and riboflavin was synthesized from (III) alone in extracts which had been dialysed for 18 hr. against running de-ionized water (20 litres). The extent of conversion in such dialysed extracts was also unaffected by addition of possible 4-carbon precursors, by 10^{-3} *M* fluoride, or by the addition of a mixture of ATP, coenzyme *A*, thioctic acid, thiamine, diphosphopyridine nucleotide, and magnesium and calcium ions. While this work was in progress, similar results were reported for extracts of other organisms, including *E. ashbyii*[12,18], *Ashbya gossypii*[18,19], *Escherichia coli*[12], *Neurospora crassa*[12], *Lactobacillus plantarum*[12], bovine liver[12] and *Mycobact.* spp.[18]. Also in agreement with our results, it was found that intact cells of *E. ashbyii*[18,20] and *A. gossypii*[19] failed to carry out the conversion; furthermore, extracts of a riboflavin-requiring mutant of *Lactobacillus casei*[12,21] also lack the enzyme(s) necessary for the conversion.

Consideration of our results led us to the view that the conversion of (III) into riboflavin must be some type of 'dismutation' in which one molecule of (III) yields a 4-carbon unit, or two 2-carbon units, which can combine actively with a molecule of unchanged (III) to produce riboflavin. This can be formally visualized as indicated in reaction (2) (there are other possibilities which will give the same overall result).

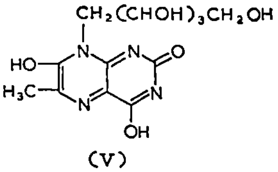
In order to test this hypothesis, we synthesized (III), labelled with carbon-14 in the positions indicated thus ● (see above), by treating 4-ribitylamino-5-aminouracil (IV) with generally labelled carbon-14 acetoin. The generally labelled acetoin was prepared enzymically by using a cell-free preparation (11,000*g* for 1 hr. at 4°) obtained from a sonate of *Acetobacter rancens* 23, which under appropriate conditions will convert pyruvate into acetoin in very high yield (80 per cent)[22]; when [2,3-^{14}C] pyruvate was used as substrate, generally labelled acetoin was obtained.

The 6,7-dimethyl-8-ribityllumazine, labelled with carbon-14 as indicated in reaction (2), was diluted with carrier 6 : 7-dimethyl-8-ribityllumazine and added to a dialysed cell-free preparation of *Candida flareri*; after incubation under optimal conditions for conversion (28° for 3 hr. with constant shaking) the reaction was stopped, carrier riboflavin added and crystalline riboflavin isolated. The radioactivities in this and in the acetic acid obtained from C-6 and C-7 and the attached methyl groups by Kuhn–Roth degradation were assayed as previously described[5]. The results obtained and the values expected if reaction (2) obtains are given in Table 1. They are clearly consistent with the view that (III) liberates enzymically an active 4-carbon unit (or two 2-carbon units) which will combine with (III) to form riboflavin. It is interesting to find that Plaut had come to the same conclusion independently regarding the formation of riboflavin from (III), and he has reported recently an investigation with a partly purified enzyme system which indicates that three molecules of (III) disappear for every one molecule of riboflavin produced[23].

Table 1. Enzymic Conversion of [^{14}C]-6,7-Dimethyl-8-ribityllumazine into Riboflavin in Cell-free Extracts of *C. flareri*. Dialysed cell-free extract of *C. flareri* used with no substrate other than [^{14}C]-6,7-dimethyl-8-ribityllumazine labelled as indicated in reaction 2; incubation for 3 hr. at 28° with continuous shaking

Compound	Specific activity (c.p.m./μmole) Expected	Observed
6,7-Dimethyl-8-ribityllumazine	—	8,000
Riboflavin	16,000	13,200
Acetic acid (from C-6 and C-7 and attached methyl groups)	3,300	3,000

During our experiments on the conversion of (III) into riboflavin, we encountered a secondary product, which shows up as a violet fluorescent spot on chromatography. This is clearly 'compound V' of Masuda *et al.*[24], which has the structure (V). There is general agreement that compound V is not an intermediate between 4-ribitylamino-5-aminouracil and riboflavin[25], but that it is formed as a side-reaction from (III), possibly by reaction of '*X*'



(V)

(reaction 2) with pyruvate[23,24]. If this is so, then it would appear that pyruvate as such is not the condensing unit for production of the aromatic ring of riboflavin. However, it is fairly clear that acetate is not immediately concerned[26], and shikimic acid, the other well-known precursor of aromatic rings[2], is also not involved[27]. It seems likely then that pyruvate gives rise to 'activated acetaldehyde'[28,29], which is incorporated step-wise into (III), or which condenses to form a 4-carbon unit such as acetoin, which combines with (IV) to yield (III). In support of this view it was found that [^{14}C] acetoin ([1-^{14}C]-3-hydroxy-2-butannone) is incorporated into ring *A* of riboflavin with the expected labelling pattern[27]. In this connexion it should be emphasized that the carbon-14 acetaldehyde liberated from carbon-14 threonine by the enzyme threonine aldolase in extracts of *E. ashbyii* is not incorporated into ring *A* of riboflavin[5]; this means either that the acetaldehyde liberated is never 'activated' or that 'activated acetaldehyde' is not concerned with ring *A* synthesis.

Conversion of 6,7-*dimethyl*-8-*ribityllumazine into riboflavin in rat liver.* The fact that riboflavin arises in micro-organisms from purines raises an interesting problem regarding the nature of the metabolic lesion which makes animals dependent on exogenous riboflavin, because all living systems can synthesize the purine skeleton for incorporation into their nucleic acids. It was reported that (III) could be converted into riboflavin by bovine liver preparations[12]; we decided to re-investigate this, using rat liver homogenates. We found that the supernatant from such homogenates centrifuged at 25,000*g* contained a substantial amount of flavin adenine dinucleotide; on incubation this disappeared and free riboflavin appeared. Thus the very high blanks obtained because of the presence of an active flavin adenine dinucleotidase in our preparation made it impossible to decide whether (III) was converted into riboflavin or not. This can be decided finally only by using [^{14}C]-6,7-dimethyl-8-ribityllumazine followed by enzyme purification. Katagiri *et al.*[12] did not comment on this possibility of riboflavin formation in the report of their work on bovine liver extracts.

We wish to thank Dr. T. Masuda for a gift of 6,7-dimethyl-8-ribityllumazine, Prof. J. de Ley for a culture of *Acetobacter rancens* 23, Dr. B. G. Audley for collaboration in certain experiments, the Agricultural Research Council for financial assistance (T. W. G.) and the University of Liverpool and University of Wales for personal grants (A. A. H.). The sonic disintegrator used was purchased with a grant from the Royal Society. Part of this work was carried out in the Biochemistry Department, University of Liverpool.

[1] Goodwin, T. W., *Prog. Indust. Microbiol.*, 1, 139 (1959).
[2] Goodwin, T. W., *Recent Adv. Biochem.* (Churchill, London, 1960).
[3] Plaut, G. W. E., *J. Biol. Chem.*, **208**, 513 (1954).
[4] Goodwin, T. W., and Jones, O. T. G., *Biochem. J.*, **64**, 9 (1956).
[5] Goodwin, T. W., and Horton, A. A., *Biochem. J.*, **75**, 53 (1960).
[6] McNutt, W. S., *J. Biol. Chem.*, **219**, 365 (1956).
[7] McNutt, W. S., *Fed. Proc.*, **19**, 24 (1960).
[8] Audley, B. G., Goodwin, T. W., and McEvoy, D., *Biochem. J.*, **72**, 8*P* (1959).
[9] McNutt, W. S., *J. Biol. Chem.*, **210**, 511 (1954).
[10] Goodwin, T. W., and Treble, D. H., *Biochem. J.*, **67**, 10*P* (1957).
[11] Forrest, H. S., and McNutt, W. S., *J. Amer. Chem. Soc.*, **80**, 951 (1958).
[12] Katagiri, H., Takeda, I., and Imai, K., *J. Vitaminol.* (Japan), **5**, 287 (1959).
[13] Maley, G. F., and Plaut, G. W. E., *J. Biol. Chem.*, **234**, 641 (1959).
[14] Kishi, T., Asai, M., Masuda, T., and Kuwada, S., *Chem. Pharm. Bull.* (Japan), **7**, 515 (1959).
[15] Horton, A. A., Ph.D. thesis, Univ. of Liverpool (1960).
[16] Masuda, T., *Pharm. Bull.* (Japan), **4**, 71, 375 (1956).
[17] Masuda, T., *Pharm. Bull.* (Japan), **5**, 28, 136 (1957).
[18] Korte, F., and Aldag, H. U., *Liebigs Ann.*, **628**, 144 (1959).
[19] Maley, G. F., and Plaut, G. W. E., *J. Amer. Chem. Soc.*, **81**, 2025 (1959).
[20] Korte, F., Aldag, H. U., and Schicke, H. G., *Z. Naturforsch.*, **134**, 463 (1958).
[21] Maley, G. F., and Plaut, G. W. E., *Fed. Proc.*, **17**, 268 (1958).
[22] de Ley, J., *J. Gen. Microbiol.*, **21**, 352 (1959).
[23] Plaut, G. W. E., *Fed. Proc.*, 19, 312 (1960); *J. Biol. Chem.*, **235**, *PC* 41 (1960).
[24] Masuda, T., Kishi, T., and Asai, M., *Chem. Pharm. Bull.* (Japan), **6**, 113, 291 (1958).
[25] Kuwada, S., Masuda, T., and Asai, M., *Chem. Pharm. Bull.* (Japan), **6**, 447, 618 (1958).
[26] Plaut, G. W. E., *J. Biol. Chem.*, **211**, 111 (1954).
[27] Goodwin, T. W., and Treble, D. H., *Biochem. J.*, **70**, 14*P* (1959).
[28] Holzer, H., and Beaucamp, K., *Z. angew. Chem.*, **71**, 776 (1959).
[29] Krampitz, L. O., Greull, G., Miller, C. S., Bickling, J. B., Skeggs, H. R., and Sprague, J. M., *J. Amer. Chem. Soc.*, **80**, 5893 (1958).

ESSENTIAL OIL FROM FRESH *CANNABIS SATIVA* AND ITS USE IN IDENTIFICATION

By L. MARTIN
The Commonwealth Laboratories, 415 Flinders Lane, Melbourne
AND
D. MORISON SMITH and C. G. FARMILO
The Laboratories of the Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario

THE two main natural narcotics found in the illicit international traffic are opium and *Cannabis*. Considerable research has been done to develop methods of identifying and estimating opium constituents, and correlating these results to determine the country of origin[1-3]. A similar collaborative research programme has been inaugurated under the auspices of the Commission on Narcotic Drugs of the Economic and Social Council of the United Nations at Geneva, as an aid to tighten international *Cannabis* control measures[4]. Present methods of identification of *Cannabis* are based on botanical features, and colour and ultra-violet absorption tests on petroleum ether extracts of the plant or its resin. Since the tests are not satisfactory for forensic purposes, investigation of methods of *Cannabis* identification were begun by Farmilo *et al.*[5,6].

On the basis of previous experience in this laboratory[7,8], essential oils have been shown to be useful in plant characterization and origin determination. For this work thirteen samples of male and female fresh *Cannabis sativa* L. (the samples were identified

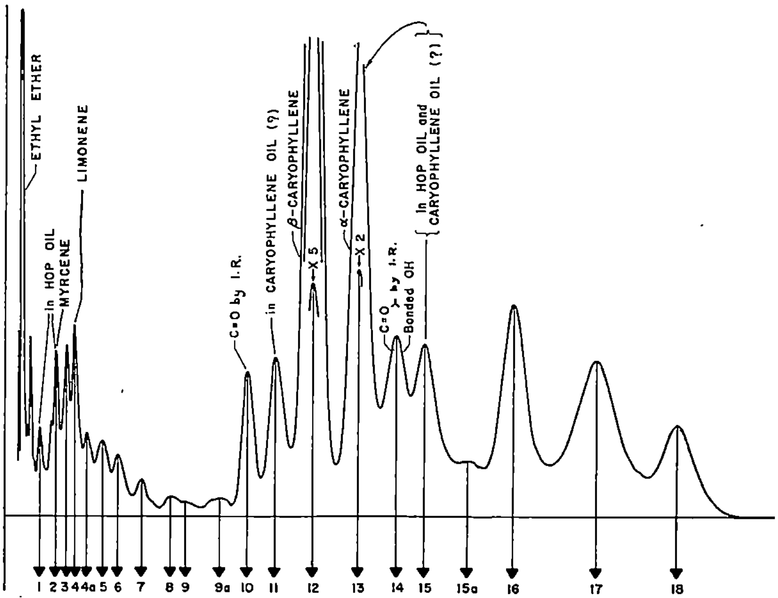


Fig. 1. Gas chromatogram. Oil from fresh large leaves of female *Cannabis sativa* (L.) Source: wild growth, Eastview, Ontario

botanically by Dr. D. Frankton, head of the Taxonomy Group, Weed Investigation Section, Plant Research Institute, Research Branch, Department of Agriculture; specimens are stored for future reference in the Arboretum of the Central Experimental Farm, Ottawa) found growing wild in ten different locations of the Canadian Provinces of Ontario and Quebec were used. (The samples of fresh *Cannabis* were provided through co-operation of the Narcotic Control Division, and the Royal Canadian Mounted Police, to whom we are indebted for this help.) The plants were stripped of leaves and inflorescences and the parts analysed separately. In addition, samples of dried *Cannabis*, hashish and charas of several different origins were studied. Hop oil and caryophyllene oil (courteously and generously supplied for this work by Fritzsche Bros., Inc., New York: the caryophyllene is produced from *Balsam copaiba* by washing and by fractionation) were used as references for characterization purposes.

Steam distillation of fresh male and female *Cannabis* plants at various stages of maturity yielded 0·05–0·11 per cent of oil (female and more mature specimens show highest yields). These results compare well with previous findings[9]. Gas chromatographic analysis of the oil (5 µl.) was carried out using a helium carrier gas-flow (75 ml./min.) through a column (2·5 m.) packed with 'Apiezon *M*' grease (20 per cent) supported on 'Chromasorb *W*' heated to approximately 220° C. More than thirty samples of oils have been assayed in this manner.

On one large oil sample, fractions corresponding to the constituents separated during gas chromatography were collected in small glass tubes packed loosely with glass wool, which were fitted on the exit end of the gas-chromatographic column. These fractions were dissolved in carbon tetrachloride and infra-red spectra were obtained using a Perkin Elmer Model 21, equipped with a scale expansion device and sodium chloride cavity cells (30 µl. capacity). After evaporation of the carbon tetrachloride solutions, the residues were redissolved in ethanol for application of colour and ultra-violet spectral tests. The spectra were taken in cells of 1 ml. capacity and 1 cm. path-length using a Beckman *DK*2 ratio recording spectrophotometer. The spectra of the neutral, acidic and basic ethanolic solutions were obtained. The colour tests, alkaline beam (ethanolic potassium hydroxide solution), Duquenois (vanillin and acetaldehyde in ethanol plus hydrochloric acid) and the Ghamrawy (*p*-dimethylaminobenzaldehyde in concentrated sulphuric acid) were carried out on a few drops of the ethanolic solution of each fraction.

The results of the above experiments are illustrated by the data given in Table 1 and a typical gas chromatogram is shown in Fig. 1. Table 1 lists the relative retention-time values (RRT-values) and the relative percentage composition of four oil samples obtained from leaves and inflorescences of fresh immature male and female plants picked in Eastview, Ontario. The illustration shows the gas chromatogram of the oil obtained from the large leaves of the female plant described.

A detailed examination of the chromatograms from the thirty *Cannabis* oil samples has shown remarkable consistency and reproducibility in the RRT-values of all peaks of the constituents 1–18. In comparing the RRT-values of one chromatogram with those of another a difference of 5 per cent in the values is taken arbitrarily as indicating a new component. Small peaks, such as those indicated by the numbers 4*a*, 9, 9*a*, and 15*a* are not always significant peaks in other chromatograms, and may vary in height and area depending on the part of the plant from which the oil is taken, for example, leaves, tip leaves, flowers, seed pods, etc. The roots and stems of the plants did not yield oil. The peaks 1–5 and those from 10 to 18 are thought to be most useful for the characterization of the oil of *Cannabis*.

Table 1. Results of Assay by Gas Chromatography of Oil from Fresh Immature *Cannabis* from Eastview, Ontario

Peak and fraction numbers	Relative retention times				Percentages			
	Leaves		Inflorescences		Leaves		Inflorescences	
	♂	♀	♂	♀	♂	♀	♂	♀
1	0·112	0·111	0·110	0·105	0·9	0·5	0·6	1·4
2	0·170	0·168	0·167	0·162	1·1	0·8	6·0	3·2
3	0·200	0·202	0·203	0·197	1·8	0·9	3·9	1·6
4	0·231	0·228	0·230	0·222	1·3	1·3	3·5	1·7
4*a*	0·269	0·265	0·266	0·258	0·5	1·6	1·3	0·6
5	0·323	0·319	0·307	0·300	0·9	1·1	1·6	0·5
6	0·375	0·369	0·366	0·357	1·2	0·8	1·4	0·5
7	0·448	0·443	0·435	0·435	*	0·5	*	0·4
8	0·555	0·540	0·535	0·530	0·7	0·2	*	0·3
9	0·590	0·586	0·586	0·575	*	*	0·4	*
9*a*	0·714	0·700	0·710	0·710	0·5	*	*	0·6
10	0·790	0·786	0·785	0·780	4·4	2·6	3·5	3·8
11	0·880	0·880	0·877	0·871	5·4	4·3	4·3	3·9
12	1·000	1·000	1·000	1·000	26·3	29·9	27·5	35·6
13	1·15	1·15	1·15	1·15	12·8	12·7	13·9	14·9
14	1·28	1·27	1·27	1·28	9·5	7·8	9·4	9·5
15	1·37	1·37	1·37	1·37	8·0	8·1	7·3	7·4
15*a*	1·51	1·51	1·50	1·50	*	*	*	*
16	1·66	1·66	1·65	1·67	9·0	9·4	4·5	4·5
17	1·93	1·92	1·93	1·97	10·7	11·8	5·2	5·1
18	2·20	2·12	2·20	2·29	5·0	5·7	5·7	4·6

* Peaks present, but too small to be measured.

The essential oil of *Cannabis* resin (called either hashish or charas, if the source is either Eygpt or India respectively) has long been known to contain terpenes and sesquiterpenes which boil at temperatures much lower than the physiologically active narcotic constituents, tetrahydrocannabinols. The complexity of the composition of this oil has never been realized before, nor would it have been practical to assay it without the aid of gas chromatography. Simonsen and Todd[11] state that the essential oil from hashish consists mainly of *p*-cymene, 1-methyl-4-*iso*propenylbenzene and humulene (α-caryophyllene). They were not able to identify the presence of myrcene in hashish, although Wood, Spivey and Easterfield[12] had much earlier found it in charas from *Cannabis indica* (syn. *sativa*) obtained from Etawah in the province of Uttar Pradesh in India. By comparison of the RRT-values from the gas chromatograms of the oil of hops, another member of the family Moraceae, and of caryophyllene oil, peaks 2 and 4 are identified as myrcene and limonene respectively. β- and α-caryophyllene are found to be peaks 12 and 13. A number of other bands, 1, 2, 11 and 15, may be present (the same RRT-values) in the hop oil chromatograms and should be relatively easily identified for this reason. Infra-red spectral data indicate that the other compounds of terpenoid character show distinct functional groups, for example, hydrogen-bonded OH and C = O vibrations are evident. Others have aromatic characteristics which are revealed by their infra-red and ultra-violet spectra. More rigorous characterization of the remaining unidentified bands is now being carried out.

Chromatograms of oils from dried *Cannabis*, hashish and charas show distinct differences from those of the fresh oils.

The alkaline-beam colour test is stated to be positive when cannabidiol is present[13] and is given by some of the crude oils prior to fractionation on the gas chromatograph. No positive beam-test was obtained with any of the fractions isolated by means of gas chromatography. The Duquenois test is given by both α- and β-caryophyllene fractions; however, the former may react more strongly with this reagent. The question of concentration differences remains to be settled. The Ghamrawy reagent appears to be the least selective in its reactivity, the orange-red colour being given by fractions 10, 11, 15, 16, 17, 18. After dilution with water the colours in these tests changed to the characteristic blue colour. When applied to the original petroleum ether extracts of the plants of *Cannabis*, the alkaline-beam test is not always positive. The Duquenois and Ghamrawy tests have always been positive. It is therefore quite possible that the latter indicate the presence of the two caryophyllenes.

In conclusion, we wish to state that the fourteen peaks which are constantly present in the gas chromatograms of the oil serve to characterize, beyond a reasonable doubt, fresh, green *Cannabis sativa* L. found in Quebec and Ontario. Dried *Cannabis*, charas and hashish may also be distinguished and characterized on this basis. There are further useful chemical differences which help to identify them.

A full report of this work will be published elsewhere.

[1] *U.N. Documents, ST/SOA/Ser. K*/1 to 97.

[2] *Chem. and Eng. News*, **30**, No. 8, 19*A* (1958).

[3] Pfeifer, S., *Die Pharmazie*, **15**, 322 (1960).

[4] Braenden, O., *et al.*, *U.N. Documents ST/SOA/Ser. S*/1 and 2 (1960).

[5] Farmilo, C. G., *U.N. Document*, *E/CN*.7/304 (1955).

[6] Farmilo, C. G. and Genest, K., *U.N. Document*, *E/CN*.7/337 (1959).

[7] Smith, D. M., and Levi, L., Abstracts of papers presented to the 138th meeting of the Amer. Chem. Soc., September 1960; Paper No. 17, Analytical Division.

[8] Smith, D. M., and Levi, L., Abstract, *Chem. in Can.*, **12**, No. 4, 53 (1960), presented at the Chem. Inst. of Can. Conf., Ottawa, Canada (June 1950).

[9] Gildemeister, E., and Hoffman, Fr., *Die Atherische Ole, Hanföl*, Band IV, 552, 4 Auflage (1956) (Wilhelm Treibs Akademie Verlag, Berlin).

[10] Guenther, E., *The Essential Oils*, **6**, 139 (D. Van Nostrand, New York, 1952).

[11] Simonsen, J. L., and Todd, A. R., *J. Chem. Soc.*, 188 (1942).

[12] Wood, T. B., Spivey, W. T. N., and Easterfield, T. H., *J. Chem. Soc.*, **69**, Pt. 1, 539 (1896).

[13] Adams, R., Hunt, M., and Clark, J. H., *J. Amer. Chem. Soc.*, **62**, 197 (1940).

INDUCTION OF MUTATIONS BY DEOXYRIBONUCLEIC ACID IN *DROSOPHILA MELANOGASTER*

By DR. O. G. FAHMY and DR. MYRTLE J. FAHMY

Chester Beatty Research Institute, Institute of Cancer Research : Royal Cancer Hospital, London, S.W.3

THE genetic activity of deoxyribonucleic acid (DNA) was conclusively demonstrated by the phenomenon of bacterial transformation[1,2], mainly in the genera *Diplococcus* and *Haemophilus*. When a genetically marked strain was exposed to DNA extracted from another with different genes, a new combination occurred in the recipient through the incorporation of one or more of the donor loci. A parallel phenomenon is that of transduction, which was demonstrated in *Salmonella*[3,4], where heritable changes of bacterial characteristics were brought about by bacteriophage-carried material assumed to be DNA. The consequences of administration of DNA in higher organisms has, so far, been a subject of conflicting reports. A phenomenon akin to bacterial transformation was reported in ducks by the Benoit group[5-7], but subsequent experimentation[7,8] failed to confirm these results. In mammals efforts to induce 'somatic transformation' have all failed[9,10].

For the investigation of the genetic consequences of DNA administration, *Drosophila* is a favourable test system. Techniques are available for the detection of mutations as well as induced transformation at specific loci. Both these aspects have been studied and the results are presented in this communication. While no specific gene replacement (locus transformation) was observed, the mutation-rate (especially as regards *Minutes*) was raised significantly above the control-level.

The DNA utilized in the present experiments was prepared in the Chester Beatty Research Institute; the first sample was extracted by Brookes from adults of the wild type strain (Oregon-*K*) of *Drosophila melanogaster*, according to Kirby's *p*-aminosalicylate/phenol method[11]. The DNA yield was extremely low and although free from RNA and protein, was in admixture with some unidentified impurities, probably polysaccharides. It was thought possible that the impurities might have been derived from the adult's chitinous exoskeleton, and accordingly attempts were made to extract DNA from embryonic tissue, namely, developing eggs. A method was devised for the production of eggs *en masse*, which enabled the collection of 2–3 gm. of pre-larval embryos per week, from a population of about 50,000 flies. The extraction of DNA from embryos was undertaken by Kirby by a modification of his previously published method[11]. This involved the breaking down of the tissues with diethyldithiocarbamate and phenolphthalein diphosphate before the application of phenol[12]. The percentage base ratio for a typical sample of the DNA separated by this method[12] was: guanine 23·8, adenine 29·3, cytosine 18·5, thymine 28·4. A sample of heterologous DNA from rat liver was also tested. This was prepared by the *p*-aminosalicylate/phenol method and gave nearly the same percentage base ratio[13] as the *Drosophila* sample (guanine 21·3, adenine 29·1, cytosine 20·1, thymine 29·5).

A cell-free extract of embryonic tissue was also tested for mutagenicity. This was prepared as follows: 1 gm. of eggs (pre-larval embryos) were mixed with 0·4 gm. of solid sodium chloride, and thoroughly ground in a glass homogenizer surrounded by solid carbon dioxide. The tissue homogenate was then diluted with 100 ml. of distilled water (to make the salt concentration isotonic) and filtered through glass wool. The filtrate was examined microscopically and proved to be a suspension free from whole cells and intact chromosomes. The concentrated salt treatment used probably disrupted the DNA/histone linkages, so that the above suspension might be looked upon as a dilute solution of DNA in admixture with other cell constituents.

The various samples of DNA were dissolved in isotonic saline (0·4 per cent sodium chloride) at the desired concentration (0·1–1 per cent) and the solutions were injected into the hæmocœl by our micro-injection technique[14]. For the mutagenicity tests wild-type (Oregon-*K*) males were treated and their progeny was scored for mutations. Chromosome fragments and visible mutations (autosomal dominants and sex-linked recessives) were tested for by the attached-*X* technique, and the sex-linked recessive lethals and visibles by the Muller-5 method[15]. In the transformation experiments, multiple recessive stocks were used carrying either 3 or 7 markers. Flies from these stocks were injected with a DNA solution extracted from the wild type, which would be derived from the full gene complement, including the normal alleles of the mutant markers. If any marked locus were substituted by the normal allele from the administered DNA, the phenotypic expression, corresponding to the substituted (transformed) locus, would be expected to revert to the wild type.

In both the mutagenicity and transformation experiments the progeny of the treated males was fractionated into 3-day broods by our standard technique[15]. Breeding was undertaken in mass-cultures with roughly equal male to female ratio.

The most convincing mutagenic effect of DNA treatment was the considerable increase in the rate of *Minutes* above the control-level (Table 1), which proved to be very highly significant in all experiments (Table 3). These mutations are a large class of non-allelic genotypic changes of comparable phenotype, expressed as various degrees of reduction in the length and thickness of the thoracic macrochætes. In extreme cases, the bristle effect is accompanied by various anomalies of the eyes as well as the wings (especially as regards venation). *Minute* mutants have a longer period of development, and are accordingly late in eclosion. They are dominant and lethal in the homozygous condition and are frequently demonstrable as deficiencies or small deletions in the salivary-gland chromosomes[16]. At the highest injected dose (1 per cent homologous sample, Table 1) the induced rate of *Minutes* was higher than the controls by a factor of nearly 12, while at the lower doses (with both the homologous and heterologous samples) the increase was by a factor of 2–3. The data from the homologous samples suggested that there might be a direct dose-effect relationship (Tables 1 and 3). In contrast to the situation with the *Minutes*, other induced F_1 autosomal dominants were rare, and were not significantly above the control-level; they were, accordingly, excluded from the present analysis.

The yield of the sex-linked recessive visibles in the treated series was also raised above the control-level both in the F_1 and F_2 experiments (Tables 1 and 2). For a statistical assessment of this trend, a certain amount of pooling of the data was undertaken to overcome the extreme rarity of visible mutations. The normal and mutant chromosomes in the F_1 and F_2 experiments for the control and treated series (from the same DNA sample and dose) were summed and compared in 2 × 2 contingency tables, and the results are given in Table 3. This analysis established the statistical significance of the increase in the visible rate with the high-dose experiments. The visibles from all experiments were subjected to allelism tests. The specific loci so far identified are listed here in their chromosomal order.

Control: *yellow* (*y*), *white* (*w*), *ommatoreductum* (*omm*), *dusky* (*dy*), *rudimentary* (*r*), *forked* (*f*).
Homologous DNA: *yellow* (*y*), *broad* (*br*), *stubs* (*sbs*), *pebbled* (*peb*), *tenuous-bristles* (*tnb*), *vesiculated* (*vs*), *smaller* (*sma*)-2, *vermilion* (*v*), *costakink* (*csk*), *miniature* (*m*), *narrow-scoop* (*nrs*), *fused* (*fu*).
Heterologous DNA: *brachymacrochaete* (*brc*), *white* (*w*), *rugose* (*rg*), *carmine* (*cm*), *copper* (*cop*), *rudimentary* (*r*)-2, *forked* (*f*)-2, *bronzy* (*bz*).

It would seem probable, therefore, that DNA treatment simply increased the incidence of mutation at the more responsive loci which occasionally mutated spontaneously. One point of interest, however, is that the *broad* (*br*), *rudimentary* (*r*), and *bronzy* (*bz*) mutants proved to be of the non-complementing[17] type, suggesting that the damage extended over all the cistrons of these complex loci. This may be an indication of the large size of genic damage by DNA, which of course is clearly suggested by the induction of *Minutes*.

The administration of DNA does not seem to result in a dramatic increase in the rate of the sex-linked recessive lethals (Table 2). Nevertheless, in one of the high-dose experiments (1 per cent DNA from adults, Table 3) there was a significant increase in the rate of these mutations above the control-level. It would seem probable, therefore, that larger scale experiments might permit a conclusive demonstration of the induction of recessive lethals by DNA.

Table 1. THE *Minutes* AND SEX-LINKED RECESSIVE VISIBLES (v) INDUCED BY DNA, DETECTED IN THE F_1 MALES FROM ATTACHED-X EXPERIMENTS; MOSAIC MUTANTS IN BRACKETS

	Control				Homologous DNA								Heterologous DNA			
					0·2 per cent (embryos)				1 per cent (embryos)				0·1 per cent			
		Minutes		v		*Minutes*		v		*Minutes*		v		*Minutes*		v
Broods	Males	No.	Per 10^3	No.	Males	No.	Per 10^3	No.	Males	No.	Per 10^3	No.	Males	No.	Per 10^3	No.
I	9,619	8	0·83	3 (1)	9,638	19 (3)	1·97	1	1,993	20	10·04	2 (2)	11,682	36 (4)	3·08	2
II	9,538	12 (1)	1·26	3 (1)	4,931	8 (2)	1·62	1	1,349	27 (1)	20·01	0	7,899	15 (3)	1·90	1
III	9,669	8 (1)	0·83	1	7,653	32 (2)	4·18	3 (2)	525	8	15·24	0	7,916	9 (1)	1·14	2 (1)
IV	10,074	10 (3)	0·99	0	4,345	7 (2)	1·61	3 (2)	839	2 (1)	2·38	2 (2)	6,885	17 (2)	2·47	3 (2)
V	7,338	5 (1)	0·68	0	1,987	4 (1)	2·01	0	521	2	3·84	0	7,379	11 (1)	1·49	3 (2)
VI+	28,176	28 (4)	0·99	4 (1)	1,782	5	2·81	1 (1)	—	—	—	—	7,172	8 (1)	1·12	2
Total	74,414	71 (10)	0·95	11 (3)	30,336	75 (10)	2·47	9 (5)	5,227	59 (2)	11·29	4 (4)	48,933	96 (12)	1·96	13 (5)

Table 2. THE SEX-LINKED RECESSIVE LETHALS (1) AND VISIBLES (v) INDUCED BY DNA, DETECTED IN THE F_2 PROGENY FROM MULLER-5 EXPERIMENTS; MOSAIC MUTANTS IN BRACKETS

Brood	Control			Homologous DNA						Heterologous DNA		
				1 per cent (adults)			1 per cent (embryos)			0·1 per cent		
	Chrm.	1	v	Chrm.	1	v	Chrm.	1	v	Chrm.	1	v
I	7,960	21	1	195	1	2 (1)	495	2	1	514	2 (1)	0
II	4,737	5	1	114	1	1	514	3 (1)	0	523	1	0
III	1,545	0	0	531	1	1 (1)	486	0	1 (1)	515	1	0
IV	1,078	2	0	573	4 (2)	1	485	0	1	486	1	0
V	487	0	0	88	1	0	2	0	0	473	1	0
VI+	—	—	—	122	0	0	141	0	0	1,394	1	1
Total	15,807	28	2	1,623	8 (2)	5 (2)	2,123	5 (1)	3 (1)	3,905	7 (1)	1
Mutants per 10^3	—	1·77	0·13	—	4·93	3·08	—	2·36	1·41	—	1·79	0·26

Table 3. MUTATION-RATES INDUCED BY DNA, AND THEIR STATISTICAL COMPARISON WITH THE CONTROL LEVEL

Experiment	Autosomal *Minutes*			X-recessive visibles			X-recessive lethals		
	Rate per 10^3	χ^2	P	Rate per 10^3	χ^2	P	Rate per 10^3	χ^2	P
Control	0·95	—	—	0·14	—	—	1·77	—	—
Homologous DNA									
1 per cent (embryos)	11·29	320·1	$\ll 10^{-9}$	0·95	21·7	10^{-5}	2·36	0·4	0·5
1 per cent (adults)	—	—	—	3·08	70·2	$< 10^{-9}$	4·93	7·1	< 0·01
0·2 per cent (embryos)	2·47	35·7	$< 10^{-8}$	0·30	2·9	0·1	—	—	—
Heterologous DNA									
0·1 per cent (rat liver)	1·96	22·2	$< 10^{-5}$	0·26	2·6	0·1	1·79	0·001	0·9

Mutagenesis studies of the fractionated progeny of males treated with various alkylating agents enabled the assessment of the time of sampling of the various stages of the testis in the brood-technique used in the present experiments. The post-spermatogonial stages (sperm, spermatids and spermatocytes) are predominantly utilized in the first four broods 0–12 days after treatment, whereas the spermatogonia are mainly recovered in the fifth and later broods (cf. ref. 18). In the light of the above conclusion, it is possible to infer the stages of spermatogenesis most susceptible to mutation by DNA. In practically all the experiments and with all the mutations scored (especially the *Minutes*), the maximal mutation-rate occurred in the first or second brood. There was one exceptional experiment (0·2 per cent homologous sample, Table 1) where the maximal rate (as regards *Minutes*) occurred in the third brood; but in no instance did the peak of mutability occur beyond the fourth brood. It is therefore certain that maximal mutagenicity by DNA occurs in mature sperm or late spermatids and minimal effect is on the earlier sperm mother cells, especially the spermatogonia. It should be pointed out, however, that even spermatogonia are not completely refractory to DNA; the rate of *Minutes* in the late progeny (fifth brood onwards) proved to be significantly greater than that in corresponding progeny fractions among the controls.

The DNA samples used in the above mutagenicity tests were extracted by procedures involving the utilization of compounds (such as phenol) which may not be mutagenically inert. Although it is virtually certain that none of these reagents was left in the purified DNA samples, it was felt desirable to ascertain the mutagenicity of DNA in the absence of such reagents. A cell-free tissue extract (in which the DNA/protein linkages were disrupted by concentrated salt) was accordingly subjected to an attached-X test. The injected suspension proved to be extremely toxic to the treated males, but it was possible to recover a single brood, containing 4,299 F_1 patriclinous males. In this sample 11 *Minutes* were scored—a rate of 2·55 per thousand, which proved to be significantly higher than the control-level ($\chi^2{}_{(1)} = 10{\cdot}0$; $P = 0{\cdot}001$).

The mutation-rate (as regards *Minutes*) induced by the tissue extract (1 gm. embryonic tissue in 100 ml. saline) was of the same order of magnitude as that induced by 0·2 per cent solution of pure DNA extracted from developing eggs (Table 1). If the mutagenic activity were entirely due to the DNA content of the tissue extract, the biological results would require the presence of 200 mgm. of DNA per gram of embryonic tissue; whereas in fact the extraction yield in Kirby's experiments was approximately 2–3 mgm. per gram of developing eggs[12]. This might be an indication that the mutagenic efficiency of 'native' DNA was partially impaired by the phenol extraction procedure. Alternatively, it could suggest that the cell extract contained ingredients (such as nucleohistones or RNA) which contributed to the induction of *Minutes*. This problem is now under investigation.

The experimental details of the attempts at transformation of specific loci are given in Table 4. A multiple recessive stock was injected with DNA extracted from the wild type and the F_1 progeny was scored for reversion (transformation to the wild-type phenotype) at the various loci in the stock. In one experiment (1 per cent DNA from adults, Table 4) three autosomal markers were used: *brown* (*bw*: II), *ebony* (*e*: III), *poliert* (*pol*: IV), and only the male parents were injected with the DNA solution. In later experiments a stock with seven markers was used; *X*-chromosome with four mutations—*yellow* (*y*), *vermilion* (*v*), *miniature* (*m*), *forked* (*f*)—was incorporated in the gene complement together with the above three autosomals. A high concentration of DNA (1 per cent solution from embryos, Table 4) was injected either into both parents or into the male parent only. When injection was restricted to the fathers, only the number of the F_1 daughters was taken to represent the frequency of the potential transformants at the sex-linked loci. This precaution was taken in case a close association between the DNA and the treated chromosome is a pre-requisite of transformation. The high DNA concentrations had strong toxic and sterilizing effects on the treated flies, which prevented the recovery of large progeny. To overcome this difficulty, a low DNA dose was used and only the males were injected (0·2 per cent solution from embryos, Table 4) and this permitted a much larger test.

Table 4. NUMBER OF F_1 PROGENY OBSERVED FOR TRANSFORMATION AT SPECIFIC LOCI, AFTER TREATMENT WITH DNA FROM THE WILD TYPE

Sex injected	Females and males	Males	
Loci tested	*y v m f bw e pol*	*y v m f*	*bw e pol*
DNA sample			
1 per cent (adults)	—	—	1,185
1 per cent (embryos)	3,397	1,596	2,954
1 per cent (embryos)	99	757	1,547
0·2 per cent (embryos)	—	32,182	63,053
Total	3,496	34,535	68,739

As can be seen from Table 4, an overall total of 72,235 individuals was examined for transformation at the three autosomal loci, and 38,031 at the four sex-linked genes, but none was transformed to the normal allele. Any further increase in the size of sample would probably have involved the occurrence of spontaneous back mutation, which of course would not have been distinguishable from transformation. The present data, therefore, could reasonably be considered as strong negative evidence against the occurrence of specific locus transformation in *Drosophila*.

The exact mechanism whereby the injected DNA produces small chromosome deletions (*Minutes*) is difficult to interpret. Direct action through the induction of chromosome breaks as occurs with radiation can be safely excluded. The experiments were designed to reveal any viable chromosome fragments induced, but none was actually observed (in a sample of 83,893 scored females), indicating that the DNA treatment does not disrupt the polynucleotide chain of the chromosome. With the highly reactive alkylating agents, it was possible to show[19] that the induction of small deficiencies was not always correlated with chromosome breakage, but could also result from gene inactivation *in situ*. With DNA, however, it is virtually certain that neither the molecules themselves nor their degradation products could possess any alkylating ability to induce direct damage in the hereditary nucleoproteins.

It would seem, therefore, that mutations induced by DNA are the result of an indirect mechanism involving errors in chromosome replication, either during germ-cell multiplication or subsequent to fertilization during cleavage. Mitoses in the germ line do not appear to influence the mutation yield, since the effect of DNA treatment was higher on sperm and spermatids than on proliferating spermatogonia. However, this could be due to secondary factors such as a lower initial uptake of DNA, or a more rapid rate of its degradation by the cytoplasmic DNase in spermatogonia. It is reasonable to assume, therefore, that DNA incorporated in the germ cells at the time of treatment (especially in mature sperm), is passively transferred in association with the male chromosome complement to the zygote, and mutant induction actually occurs during chromosome replication at the first cleavage. An important feature of the phenomenon is that it appears to be non-specific: both as regards the inducing DNA (whether homologous or heterologous) and the induced mutations (predominantly non-allelic *Minutes*). The induction process, therefore, does not necessitate a specific exchange (akin to crossing-over) between the exogenous DNA and a particular locus on the chromosome, as was postulated[2] for bacterial transformation.

We are grateful to Prof. A. Haddow for his interest, to Prof. P. C. Koller for reading and criticizing the manuscript and to Dr. K. S. Kirby and Dr. P. Brookes for the preparation of the DNA samples tested.

This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

[1] Avery, O. T., McLeod, C. M., and McCarty, M., *J. Exp. Med.*, **79**, 137 (1944).

[2] Hotchkiss, R. D., *J. Cell. Comp. Physiol.*, **45**, Supp. 2, 1 (1955).

[3] Zinder, N. D., and Lederberg, J., *J. Bac.*, **64**, 679 (1952).

[4] Zinder, N. D., *J. Cell. Comp. Physiol.*, **45**, Supp. 2, 23 (1955).

[5] Benoit, J., Leroy, P., Vendrely, C., and Vendrely, R., *C.R. Acad. Sci., Paris*, **244**, 2320 (1957).

[6] Benoit, J., Leroy, P., Vendrely, C., and Vendrely, R., *C.R. Acad. Sci., Paris*, **248**, 2646 (1959).

[7] Benoit, J., Leroy, P., Vendrely, C., and Vendrely, R., *Trans. N.Y. Acad. Sci.*, **22**, 494 (1960).

[8] Svoboda, J., and Häskova, V., *Folia Biol.* (Prague), **5**, 402 (1959).

[9] Perry, T. L., and Walker, D., *Proc. Soc. Exp. Biol. Med.*, **99**, 717 (1958).

[10] Bearn, J. G., and Kirby, K. S., *Exp. Cell. Res.*, **17**, 547 (1959).

[11] Kirby, K. S., *Biochem. J.*, **66**, 495 (1957).

[12] Kirby, K. S. (personal communication).

[13] Kirby, K. S., *Biochim. Biophys. Acta*, **36**, 117 (1959).

[14] Fahmy, O. G., and Fahmy, M. J., *Heredity*, **15**, 115 (1960).

[15] Fahmy, O. G., and Fahmy, M. J., *J. Genet.*, **53**, 563 (1955).

[16] Bridges, C. B., and Brehme, K. S., *Carnegie Inst. Wash. Publ.*, **552** (Washington, D.C., 1944).

[17] Fahmy, O. G., and Fahmy, M. J., *Nature*, **184**, 1927 (1959).

[18] Fahmy, O. G., and Fahmy, M. J., *Genetics*, **45**, 1191 (1960).

[19] Fahmy, O. G., and Fahmy, M. J., *Advances in Radiobiology*, 437 (Oliver and Boyd, Edinburgh, 1957).

PROPERTIES OF ISOLATED PLANT PROTOPLASTS

By E. C. COCKING

Department of Botany, University of Nottingham

LIBERATED bacterial[1] and fungal[2] protoplasts have proved to be of unique value in morphological, biochemical and genetic work on these micro-organisms. Following the recent demonstration in this laboratory[3] that plant protoplasts can be liberated from root cells of *Lycopersicum esculentum* after digestion of the cell walls by the enzyme cellulase, investigations into the properties of these liberated plant protoplasts have now indicated their unique potentiality for similar studies.

Protoplasts were liberated from root tips of tomato seedlings (*Lycopersicum esculentum* Mill. var. Sutton's Best of All) by treatment with *Myrothecium* cellulase[3]. Seedlings were grown under aseptic conditions and 3-mm. root tips from roots 20 mm. in length were used. The root tips were incubated at 27° C. for 2 hr. with freshly prepared *Myrothecium* cellulase in a 0·6 *M* sucrose modified White's culture medium[4] at *p*H 4·5. After treatment, the root tips were washed free of cellulase and mounted in special micro-culture chambers[5] in which liberated protoplasts could be examined microscopically, while freely suspended in the culture medium.

In the first experiment (exp. 1) root tips treated with cellulase were mounted in 0·6 *M* sucrose White's medium and microscopic measurements made of changes with time of protoplast and vacuole diameters. Most of the liberated vacuolated protoplasts were stable for about 12 hr. in this medium at room temperature; however, a few (approximately 1 per cent) burst after about 1 hr. Changes in protoplast and vacuole diameters of one of these rapidly bursting vacuolated protoplasts are shown in Table 1. The densely protoplasmic protoplasts which showed little evidence of vacuolation were stable over a period of several days, when maintained in a similar medium. During this time, they showed a slight tendency to shrink; they became opaque and the outer membrane became more clearly defined.

In exp. 2, 3-mm. root tips of tomato seedlings grown and treated with cellulase under similar conditions to exp. 1 were mounted in micro-culture chambers containing a 0·6 *M* sucrose White's medium at *p*H 4·5 with 10^{-5}, 10^{-7}, 10^{-9} and 10^{-11} gm./ml. of 3-indolylacetic acid (IAA, Kodak). There was a very rapid bursting of vacuolated protoplasts. The most rapid and extensive bursting took place at 10^{-11} gm. IAA/ml. of 0·6 *M* sucrose White's medium and within 1 min. of treatment no vacuolated protoplasts or vacuoles could be observed. The least rapid bursting took place with 10^{-5} gm. IAA/ml. and changes in protoplast and vacuole diameters of one of these vacuolated protoplasts are shown in Table 2. There was not only a very rapid increase in the size of existing vacuoles present in the protoplasts, but also formation of new vacuoles both in already vacuolated protoplasts and in densely protoplasmic protoplasts. At an early stage in their development these vacuoles were more gel-like in nature and less rounded than existing vacuoles. When these vacuoles were liberated at this early stage of development, it was noted that the tonoplast was less clearly defined than in previously isolated more mature vacuoles. No inclusions were present in these vacuoles induced by IAA. Figs. 1 and 2 show typical changes in vacuolar form induced by 10^{-5} gm. IAA/ml. over a period of 3 hr. at room temperature. Photomicrographs were taken using a Leica camera and attachment mounted on a Baker series 4 microscope with a fluorite (× 100) phase-contrast oil immersion objective and 'Trilux' condenser. More rapid changes in vacuolar form were induced by lower concentrations of IAA, but photography of these was more difficult, due to the fact that marked changes took place during the required exposure time of 25 sec. A typical gel-like, mobile vacuole is shown in Fig. 1 (*A*); its formation was associated with active movement of the protoplasm and its associated granules.

Table 1. CHANGES IN PROTOPLAST AND VACUOLE DIAMETERS OF A RAPIDLY BURSTING VACUOLATED PROTOPLAST

(0·6 *M* sucrose White's medium *p*H 4·5)

Time (min.)	Protoplast diameter in two directions at right angles (μ)		Vacuole diameter in two directions at right angles (μ)	
10*	32·8	33·6	29·3	29·3
20	33·6	32·8	29·3	29·3
30	34·5	34·5	30·2	30·2
40	34·5	34·9	31·8	31·8
60	37·3	37·3	36·3	30·7
80	38·0	38·0	37·3	31·1
	Burst			
92			25·9	27·6
96			24·1	24·1
98			24·1	24·1
101			24·1	24·1
104			24·1	24·1

* Timed from the initial washing to remove excess cellulase.

Table 2. CHANGES IN PROTOPLAST AND VACUOLE DIAMETERS OF A VACUOLATED PROTOPLAST

(0·6 *M* sucrose White's medium *p*H 4·5 with 10^{-5} gm. 3-indolylacetic acid/ml.)

Time (min.)	Protoplast diameters in two directions at right angles (μ)		Vacuole diameters in two directions at right angles					
			Vacuole *A* (μ)		Vacuole *B* (μ)		Vacuole *C*† (μ)	
2*	51·8	53·5	‡	‡	‡	‡		
4	55·2	54·5	34·5	34·5	‡	‡		
7	58·6	58·6	38·0	38·0	‡	‡		
8	58·9	58·9	‡	‡	12·1	12·1		
8½	Burst							
10			41·4	41·4	14·5	14·5	34·5	34·5
12			36·2	36·2	17·3	17·3	38·0	38·0
24			36·2	36·2	17·3	17·3	38·0	38·0

* Timed from the initial washing to remove excess cellulase.

† Gel-like, mobile vacuole. Measurements on this vacuole were only possible after bursting of the protoplast.

‡ No measurements taken.

Further cultural studies of protoplasts were carried out using specially prepared tomato root extract. This extract was prepared by homogenizing twenty 20-mm. roots in an all-glass homogenizer under aseptic conditions with 0·5 ml. 0·6 *M* sucrose White's medium at *p*H 4·5. Liberated protoplasts were cultured in this whole homogenate in the special micro-culture chambers previously described. Chambers were sealed with heavy mineral oil (paraffin liq.

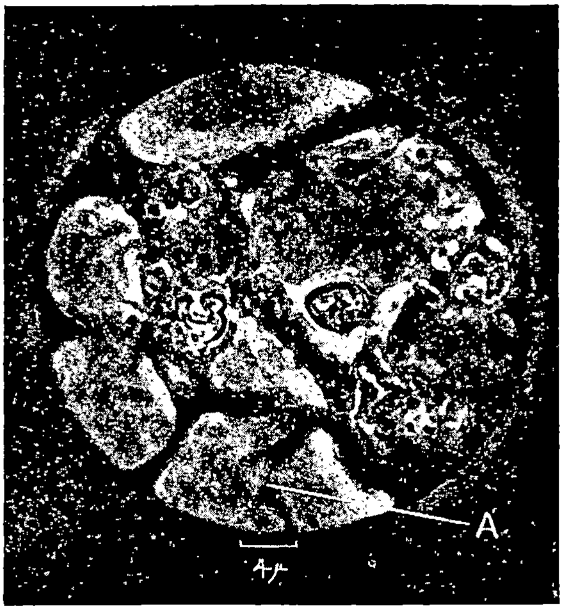


Fig. 1. Vacuolated protoplast (phase contrast). Treated with 10^{-5} gm. 3-indolylacetic acid/ml., 0·6 M sucrose White's medium for 15 min.

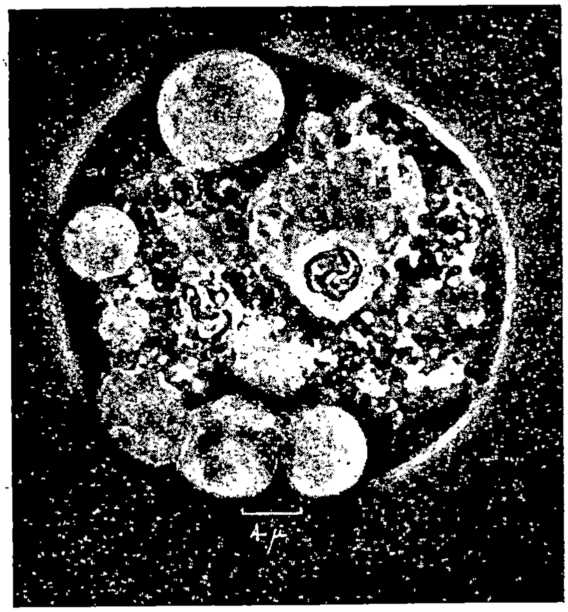


Fig. 2. Vacuolated protoplast (phase contrast). Treated with 10^{-5} gm. 3-indolylacetic acid/ml., 0·6 M sucrose White's medium for 3 hr. 15 min.

B.P. (Boots) to United States Pharmacopœia requirements), and under these conditions protoplasts could be maintained under aseptic conditions for at least one week (exp. 3). After culture for one day at room temperature, there was evidence of a more definite membrane around the more densely protoplasmic protoplasts. Furthermore, it was observed that several of these protoplasts had produced a bud-like protrusion (Fig. 3) after culture for two days in this tomato root extract; these protoplasts had by this time become highly opaque with particulate material massed near the surface of the now clearly defined outer membrane. These budding, densely protoplasmic structures closely resemble the budding cells observed[6] among single cells cultured in the presence of coconut milk. The implications of this finding will be discussed later.

It is known that mannitol[7] only very slowly penetrates into plant cells. Experiments were therefore carried out using mannitol as the osmotic stabilizer to determine whether the changes in opacity and membrane characteristics of these protoplasts observed in exps. 1 and 3 were being brought about by a toxic action of sucrose at the high molarity (0·6 M) necessarily used to maintain osmotic equilibrium in the culture medium. Protoplasts were cultured in 0·06 M sucrose, 0·54 M mannitol White's medium at pH 4·5. Changes in the opacity and membrane characteristics were observed similar to those in exps. 1 and 3. When comparable densely protoplasmic protoplasts were maintained in 0·6 M mannitol White's medium at pH 4·5 in the absence of sucrose, no such changes, however, could be observed. It would therefore seem highly probable that these changes are specific and are associated with sucrose metabolism and possibly cellulose synthesis.

These results indicate clearly the potentiality of the study of plant protoplasts in elucidating many of the present problems associated with growth and differentiation in plants. Owing to the absence of the cell wall, the response to nutrients and growth substances known to effect the growth and development of the whole root may be investigated at the protoplast-level with a greater attention to cytological detail and behaviour than has hitherto been possible with living cells. Moreover, the absence of the cell wall enables a more direct analysis of the mechanisms underlying these responses to be made. The protoplasmic effects of 3-indolylacetic acid and their possible association with increased water uptake by plant cells have recently been emphasized[8]. Other workers have focused their attention mainly on changes in the plasticity of the cell wall and possible chelation action of 3-indolylacetic acid[9-12]. Important as these effects on the cell wall may be, it is clearly possible to relate any effects of 3-indolylacetic acid observed with these protoplasts entirely to protoplasmic effects, and to effects on the plasmalemma and tonoplast. In view of the viscosity changes induced in vacuoles by 3-indolylacetic acid and observed in the present work, and the marked protoplasmic effects, it would seem that the application of simple osmotic relationships to explain the behaviour of these protoplasts in terms of vacuolar swelling may not be directly

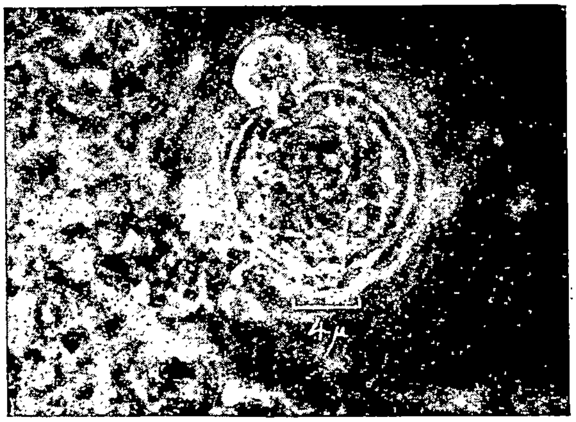


Fig. 3. Budding 'protoplast' (phase contrast)

possible. The study of the osmotic behaviour of mechanically isolated clearly defined vacuoles[13] may yield little information concerning the actual osmotic relationships of vacuoles *in vivo* as they are induced and develop under the action of 3-indolylacetic acid and other similar growth-substances.

Budding of both highly vacuolated[6] and densely protoplasmic[14] cultured single cells has been reported. It has also been established that these 'buds' can become independently growing cells either separating from, or remaining attached to, the 'mother cell'[6] provided that this cell is highly vacuolated. In the case of budding densely protoplasmic cells, division of the 'mother' cell nucleus has been observed, resulting in the formation of a binucleate cell but the 'buds' in this instance do not seem to have a separate existence from the 'mother cell'. It is of some significance that densely protoplasmic structures with buds have been seen to be formed from densely protoplasmic protoplasts, when these were cultured in a White's medium tomato root extract, which probably contains many of the growth-substances associated with the White's medium supplemented with whole coconut milk as used in the culture of budding cells[6,14]. It seems likely that these protoplasts first develop some form of primary cellulose wall and then, parallel to their later development, they acquire some aspects of the behaviour of freely suspended isolated cells[6]. Clearly, further work with coconut milk and culture media supplemented with mannitol will be required before more definite answers can be given to the many questions arising from these new findings.

McQuillen[15] has noted that the optical density and dry weight of *Bacillus megaterium* protoplasts increases during incubation in suitable media, but that reversion to a bacillary form rarely, if ever, occurs. It is suggested that reversion only occurs when a trace of original wall material is still present, which can act as a primer or 'starter' for synthesis of new cell-wall material. These densely protoplasmic plant protoplasts are known to arise from meristematic cells in which the cell wall is very intimately associated with the protoplasm[3], and it may be of some significance that signs of reversion to a cellular form mostly occur among protoplasts such as these.

During the course of the present investigations, it was repeatedly possible to observe separated vacuoles, nuclei and masses of granular protoplasmic material following osmotic bursting of protoplasts. The development of osmotic bursting as a mild procedure for the separation and ultimate fractionation of protoplasmic components has been rendered difficult by the instability of these components arising as a result of the osmotic changes. The demonstration in the present work of the bursting ability of 3-indolylacetic acid is being utilized for the separation of protoplasmic components, since it is now possible to induce bursting of protoplasts in 0·6 *M* sucrose White's medium without changing the osmotic environment.

Thanks are due to Miss O. Wright for expert technical assistance. The award of a grant-in-aid from the Royal Society and a research grant from the Department of Scientific and Industrial Research is gratefully acknowledged.

[1] Weibull, C., *Ann. Rev. Microbiol.*, **12**, 1 (1958).
[2] Bachmann, B. J., and Bonner, D. M., *J. Bact.*, **78**, 550 (1959).
[3] Cocking, E. C., *Nature*, **187**, 927 (1960).
[4] Boll, W. G., and Street, H. E., *New Phyt.*, **50**, 53 (1951).
[5] Jones, L. E., Hildebrandt, A. C., Riker, A. J., and Wu, J. H., *Amer. J. Bot.*, **47**, 468 (1960).
[6] Steward, F. C., Mapes, M. O., and Smith, J., *Amer. J. Bot.*, **45**, 693 (1958).
[7] Thimann, K. V., Loos, G. M., and Samuel, E. W., *Plant Phys.*, **35**, 848 (1960).
[8] Galston, A. W., and Purves, W. K., *Ann. Rev. Plant Phys.*, **11**, 239 (1960).
[9] Bennet-Clark, T. A., *The Chemistry and Mode of Action of Plant Growth Substances*, edit. by Wain, R. L., and Wightman, F., 284 (Butterworth, London, 1956).
[10] Cleland, R., *Plant. Phys.*, **35**, 581 (1960).
[11] Jansen, E. F., Jang, R., and Bonner, J., *Plant Phys.*, **35**, 567 (1960).
[12] Heath, O. V. S., and Clark, J. E., *J. Exp. Bot.*, **11**, 167 (1960).
[13] Mercer, F., *Ann. Rev. Plant Phys.*, **11**, 1 (1960).
[14] Mitra, J., Mapes, M. O., and Steward, F. C., *Amer. J. Bot.*, **47**, 357 (1960).
[15] McQuillen, K., in *The Bacteria*, edit. by Gunsalus, I. C., and Stanier, R. Y., **1**, 249 (Academic Press, 1960).

TUBER BULKING IN THE POTATO CROP

By R. W. RADLEY, M. A. TAHA and P. M. BREMNER
Department of Agriculture, University of Nottingham

YIELD in the potato crop is the resultant of the rate and duration of tuber bulking, the latter being a function of the time of tuber initiation and persistence of foliage. There is little precise information concerning the relative importance of these factors in accounting for differences in yield between varieties and different husbandry practices, and results obtained from field experiments at Sutton Bonington in the 1960 season may therefore be of interest.

Fig. 1 shows the course of leaf area development and tuber bulking, as revealed by weekly sampling, in Arran Pilot (the most widely grown early variety), Majestic (the most widely grown main crop) and Ulster Torch (a main crop, sometimes grown as an early, which is notable for its resistance to blight), planted on April 13.

The curve for increase in tuber weight with time, though similar in form, is displaced in time relative to that obtained by Borah and Milthorpe[1], and conflicts with the view that tuber initiation occurs near the time of maximum leaf area, and that the

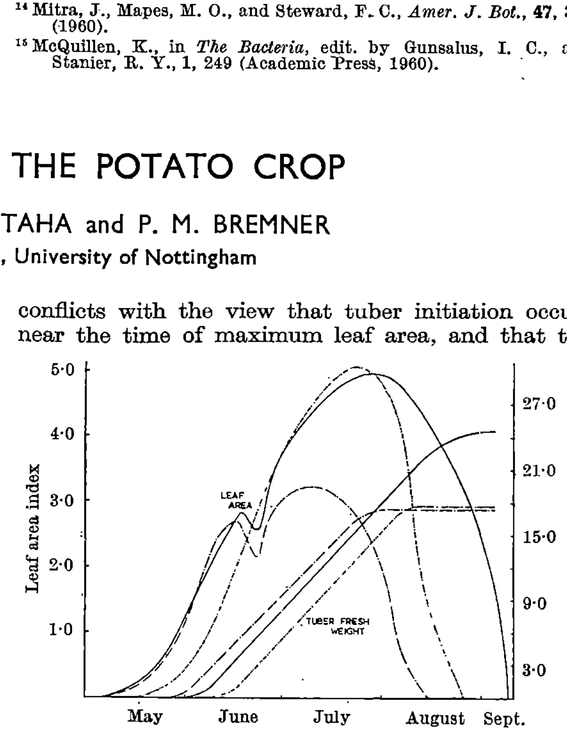


Fig. 1. Leaf area index and tuber growth in three varieties. ——, Ulster Torch; —···—···, Majestic; —·—, Arran Pilot

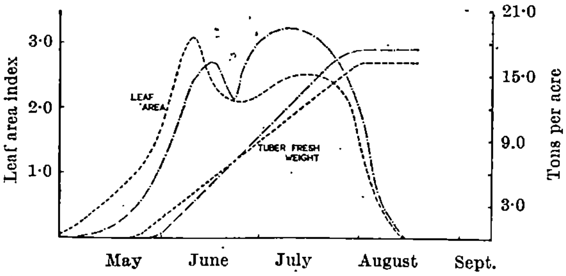


Fig. 2. Leaf area index and tuber growth in Arran Pilot planted on two different dates
– – –, Planted March 28; —·—, planted April 13

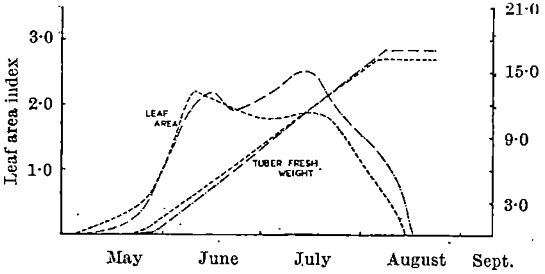


Fig. 3. Leaf area index and tuber growth in Ulster Chieftain planted on two different dates
– – –, Planted March 28; —·—, planted April 13

growth of tubers occurs at a time when leaf area is decreasing. In each variety the major part of tuber growth is made before maximum leaf area ; indeed, decline in leaf area by senescence (as in Arran Pilot) or by blight (as in Majestic, and later Ulster Torch) is followed in about 14 days by the cessation of tuber bulking. The same general picture emerges with other varieties included in these experiments (Figs. 2 and 3). It should be noted, too, that though departures from linearity during the main period of bulking are generally small ($r = 0·98$), they may be considerable when growing conditions are adverse : in the very dry 1959 season, particularly where inter-plant competition was intense, as with closely spaced plants, there was a mid-summer decline in leaf area and an almost complete cessation of tuber bulking. Subsequent recovery in leaf growth was associated with an increase in tuber weight.

Consideration of Fig. 1 suggests that tuber bulking-rate (here considered to be the slope of the curve during the linear phase) is, within fairly wide limits, independent of leaf area. On the other hand, Figs. 2 and 3 indicate that there may be a relationship between the two parameters, in so far as higher bulking-rate is associated with greater leaf area. The apparent discrepancy may possibly be due to leaf development in Ulster Torch and Majestic having surpassed the 'ceiling' at which crop growth-rate declines because of mutual shading of leaves. The pattern of total dry matter production in Arran Pilot and Ulster Torch is consistent with this hypothesis : there was no difference in this respect between the two varieties below leaf area index of 3, and little difference when the leaf area index of Ulster Torch increased to 5 while that of Arran Pilot remained at 3. This would suggest that there is no intrinsic difference between the varieties with regard to net assimilation-rate, but that the rate of Ulster Torch later declined relative to that of Arran Pilot because of the greater degree of mutual shading of leaves in the former variety.

Regarding the relative importance of the rate and duration of bulking in accounting for yield differences, Fig. 1 illustrates the paramount importance of the latter with these particular varieties in the 1960 season. By virtue of tuber initiation two weeks earlier, Arran Pilot had a yield advantage over Majestic of 4·4–4·8 tons per acre during the linear phase of bulking, but its final yield was slightly less because of earlier leaf senescence. Similarly, Ulster Torch had an advantage of 2·7 tons per acre over Majestic until the beginning of August, and gained a further 4·3 tons per acre afterwards, because of its blight resistance, which resulted in persistence of foliage for some 2–3 weeks after Majestic had virtually no green tissue. In contrast, differences in bulking-rate would appear to be of minor importance : with the exception of Ulster Chieftain (an early), which had a significantly lower bulking-rate than all others, varieties differed little in bulking-rate when planted at a time regarded as optimal for main crops (April 13.)

While duration of bulking is of great importance in determining final yield, it seems that attempts to prolong this by inducing early initiation, as may be done in the field by early planting of an early variety, may be ineffectual because of the reduced leaf area duration—a result of lower maximum leaf area and earlier senescence—associated with this practice. Thus, early planting may not in fact prolong the duration of tuber bulking and may result in lower bulking-rates. Figs. 2 and 3 show the increase in tuber-weight for Arran Pilot and Ulster Chieftain planted on two different dates (March 22 and April 13) and it is seen that appreciable yield advantage for early planting is short-lived, particularly in Arran Pilot. Planting before mid-April is, therefore, unlikely to increase yield unless early lifting is intended.

---

[1] Borah, N. H., and Milthorpe, F. L., *The Growth of the Potato Plant. Rep. Sch. Agric.* (Univ. Nottingham, 1959).

BIOCHEMICAL CHANGES IN LETHAL STAPHYLOCOCCAL INFECTIONS IN MICE

By GEORGE W. COUNTS, Dr. IAN M. SMITH, Dr. JOSEPH I. ROUTH, Dr. ELIA Ch. HAZARD and J. F. McTAVISH

Departments of Medicine and Biochemistry, University Hospitals and State University of Iowa, Iowa City

QUESTIONS regarding the specific cause of death in staplylococcal infections remain unanswered. *Staphylococci* are known to excrete a variety of toxic substances; but it is still uncertain that these are the major factors causing death. It has also been suggested[1] that endotoxins may be important and their effects could include: (1) direct toxic action on a process essential to life; (2) indirect action by the combination of a staphylococcal toxin with a normal body component to form a lethal compound; (3)

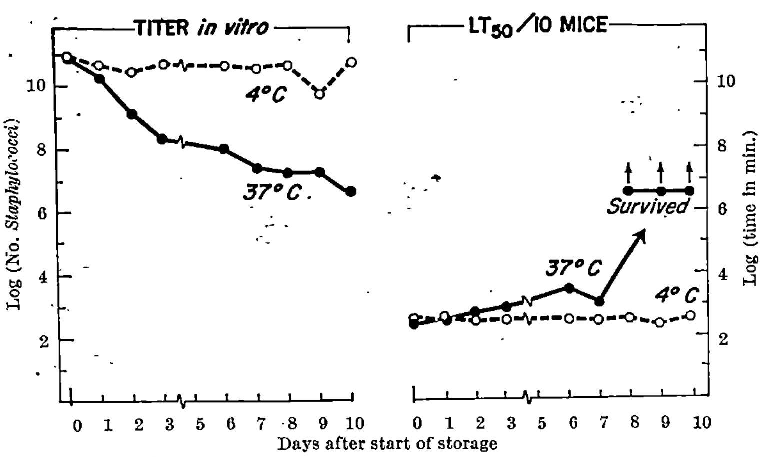


Fig. 1. Storage of staphylococcal culture at different temperatures

indirect action on the anabolism of a product essential to life and subsequent death due to deprivation; (4) the release, chemical cleavage or unmasking of a normal body component which, in excessive quantity, is toxic to metabolism.

The search for answers to this problem led to the following observations of the biochemical changes occurring in the blood of mice during a lethal staphylococcal infection.

We used the hæmolytic, coagulase-positive Smith strain of *Staphylococcus aureus*. This strain, originally phage-type *44A/42E* (ref. 2), was isolated from a patient with osteomyelitis. Using the standard phage(s) it is, however, now non-typable. It has been maintained in our laboratory by regular *in vitro* transfer. Our investigations have shown a reasonably constant number of *Staphylococci* in the bodies of moribund mice regardless of the inoculation route. Therefore, this work was confined to the use of heavy-dose (0·1 c.c. = 10^9 organisms) intraperitoneal infections. The methods have been described previously[1]. The mice used were Carworth Farms (New City, New York) White Swiss males. The animals weighed 15–18 gm. and were fasted for 12 hr. before use.

Blood was collected from the mice by beheading with scissors. Within 1 hr. after collection both serum and heparinized blood samples were transferred to the biochemistry laboratories, where blood values were established.

The values 'at death' are the result of injections of 10^9 organisms into groups of ten mice. When two of the ten animals died the remaining eight were beheaded. This was felt to be a true value since it was found earlier that, with this inoculum and this route, the animals died in 4–5 hr., and all 10 animals usually died within a span of 20–45 min.

In previous work with this strain, a particular inoculum of *Staphylococci* had to contain > 10^5 viable organisms to be lethal. In the new experiments equal parts of a culture with high numbers of organisms were allowed to age at 37° C. and 4° C. Aliquots of these two samples were tested daily for their potency *in vitro* and *in vivo*. With the culture stored at 4° C., there was little change in the titre of the culture during the period of observation, and also little change in the time required for a standard injection of this culture to kill a group of mice. With the 37° C. culture there was a direct correlation between the decreasing titre *in vitro* and the gradually declining ability to kill mice (Fig. 1).

Biochemical values for normal and infected *CFW* mice are given in Table 1. A comparison of the values in the normal and intraperitoneally infected mice showed statistically significant differences in these four values ($P \leqq 0·05$); increased transaminase, alkaline phosphatase, and inorganic phosphorus and decreased sodium. There may be important changes in the cholesterol and serum carbon dioxide values, but results so far have not been significant. Abnormal biochemical values of the same order were obtained following infection by several other routes (Table 2).

To follow the trend of these biochemical changes, blood samples were collected at hourly intervals following infection and subjected to analysis. Fig. 2 shows the large increase in alkaline phosphatase and inorganic phosphorus during a 4–5-hr. infection. While there was no significant difference between normal and terminal values for fasting blood sugar, there was a distinct hypoglycæmia occurring between the first and second hours. However, due to homeostatic mechanisms, the sugar values at death were within our established normal limits for the mouse.

Table 1. BLOOD BIOCHEMICAL VALUES FOR *CFW* MICE

	Normal	± *S.D.*	*S. aureus* at death*	± *S.D.*	*S. albus* at death
Fasting blood sugar mgm. per cent	52·7	12·20	35·8‡	14·50	56·0
Alkaline phosphatase s.u.	3·9	0·22	54·0†	24·40	4·8
Inorganic phosphorus mgm. per cent	9·7	0·71	12·8†	1·73	10·2
Transaminase s.f.u.	166·0	48·70	227·0†	64·30	299·0
Cholesterol mgm. per cent	139·0	33·80	106·0‡	28·30	181·0
Serum CO_2 m.equiv.	14·9	3·50	11·5‡	1·40	18·7
Sodium m.equiv.	149·8	3·10	145·1†	3·00	149·0
Potassium m.equiv.	8·9	1·27	8·8	1·07	
Chloride m.equiv.	110·5	5·40	109·6	2·40	
Blood urea nitrogen mgm. per cent	18·3	4·70	21·2	5·00	
Creatinine mgm. per cent	0·5	0·21	0·78	0·33	
Total serum protein gm. per cent	5·6	0·52	5·06	0·81	
Albumin gm. per cent	2·8	0·26	3·13	0·35	
Globulin gm. per cent	2·8	0·30	1·93	0·87	
Calcium mgm. per cent	11·1	1·72	10·8	1·27	
Uric acid mgm. per cent	2·8	0·61	2·7	0·58	
Acid phosphatase s.u.	1·6	0·45	1·97	0·22	
Total bilirubin mgm. per cent	0·0–0·2		0·1–0·3		
Cephalin flocculation	3+–4+		3+–4+		
Thymol turbidity u.	1·9	0·96	2·2	0·57	
Zinc turbidity u.	1·7	0·23	1·8	0·65	

* After 10^9 organisms intraperitoneally.
† Significant at $P \leqq 0·05$.
‡ Questionable significance (see text).
s.u., Sigma units; s.f.u., Sigma–Frankel units; u., Shank–Hoagland units.

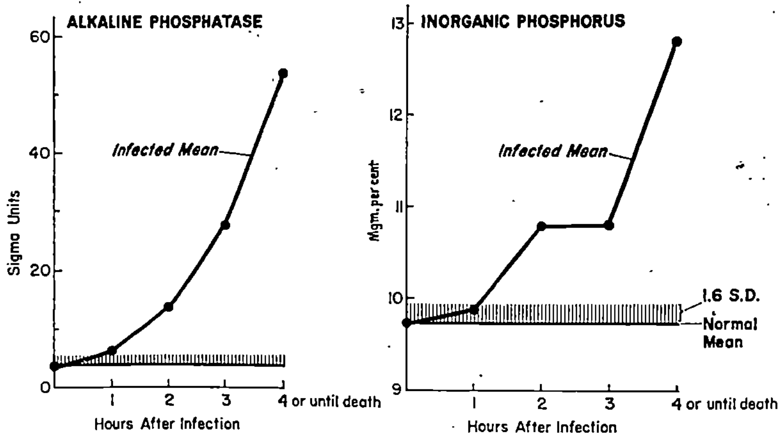


Fig. 2. Biochemistry of murine staphylococcal infections

The intraperitoneal injection of 10^{10} heat-killed *Staphylococci* produced early transient signs of illness, but no deaths, in the 14-day observation period. Blood samples collected at intervals of 8, 12, 24, 36 and 48 hr. and after 5 days showed no biochemical changes similar to those occurring during a lethal infection (also Table 2).

A strain of *S. albus* (*S. epidermidis* – coagulase-negative) obtained from a patient with septicæmia (case 6, ref. 3), although innocuous to mice in normal doses, was lethal after an inoculum of 10^{10} cells intraperitoneally. Blood values present at death failed to show the biochemical abnormalities produced by *S. aureus*. This was true with three other *S. albus* strains from patients with septicæmia.

Attempts to obtain a filtrable lethal factor from cultures of staphylococcal broth were unsuccessful. Intraperitoneal injections of 1·0 c.c. of sterile filtrate, obtained at various stages in the growth of the organism in brain–heart infusion broth were innocuous. Injections of 1·0 c.c. of sterile filtrate produced no deaths or obvious signs of illness in the animals, although it is possible that some toxic material was absorbed by the filter pad.

In addition, no filtrable lethal factor could be obtained from the serum of moribund animals when 0·5–2·0 c.c. were injected intraperitoneally into healthy recipient mice.

The results indicate that at the time of death from virulent *Staphylococci* in mice there is a high concentration of transaminase, alkaline phosphatase and inorganic phosphorus and a low sodium. A transient fall is noted in the blood glucose. These changes in the internal *milieu* are dependent on a heat-labile substance in the bacterial body. Blood serum with these biochemical changes, however, is not lethal intraperitoneally in an amount equal to an animal's circulating blood volume. *S. albus* does not produce these biochemical changes.

Various observations of changes in blood sugar, glycogen and phosphorus values occurring in experimental infections have been reported. Zeckwer and Goodell[4] studied anaphylaxis from killed bacteria injected into rabbits, and postulated that the hyperglycæmia present at death was the result of increased glycogenolysis rather than an interference with glucose metabolism. Injecting killed bacteria into rabbits, Delafield[5] showed that Gram-positive and Gram-negative organisms elicited different responses. Gram-negative bacteria caused an initial hyperglycæmia followed by a low content of sugar in the blood at 24 hr., and at the same time there were increased inorganic phosphorus values. Gram-positive bacteria, on the other hand, caused no significant changes in blood sugar or inorganic phosphorus. Kun and Miller[6], using *Salmonella* endotoxin in rabbits, reported essentially the same result, and in addition showed significantly reduced glycogen content of liver and muscle tissue. Similar blood sugar results were reported recently[7] following injections of anthrax toxin into rabbits. The hyperglycæmia was correlated with a decreased content of liver glycogen. A marked increase was also observed in the activity of alkaline phosphatase and transaminase.

An apyrase (adenylpyrophosphatase) can be extracted from several bacteria, but *Staphylococcus aureus* excretes apyrase into the culture media[8]. This enzyme liberated inorganic phosphorus from adenosine triphosphate, adenosine diphosphate, adenosine monophosphate and diphosphopyridine nucleotide. This work should be extended to *in vivo* tests.

We are studying at present the effects of accentuating or neutralizing these biochemical derangements in an attempt to devise a biochemical treatment for staphylococcal infections in the mouse[9]. This should

Table 2. BIOCHEMICAL VALUES 'AT DEATH'

Determinations	Control*	Live organisms					Dead organisms
		Intra-peritoneal* (0·1 c.c.)	Intravenous (0·1 c.c.)	Intracranial (0·02 c.c.)	Intramuscular (0·5 c.c.)	Subcutaneous (0·3 c.c.)	Intra-peritoneal† (1·0 c.c.)
Fasting blood sugar mgm. per cent	52·7	35·8	36·0	46·5	39·5	51·0	49·8
Alkaline phosphatase s.u.	3·9	54·0	27·9	11·0	33·0	6·8	1·6
Inorganic phosphorus mgm. per cent	9·7	12·8	14·0	12·0	13·9	13·4	10·2
Sodium m.equiv.	149·8	145·1	148·0	141·0	138·0	151·0	152·0
Transaminase s.f.u.	166·0	227·0	220·0	240·0	230·0	400·0	300·0
Cholesterol mgm. per cent	139·0	106·0	107·0	100·5	119·5	132·0	125·0
Serum carbon dioxide m.equiv.	14·9	11·5	21·0	21·6	11·1	19·3	21·4

* 5–6 determinations in these; 2–3 in the rest.
† Values present at peak of obvious illness (12 hr.).
Titre = 10^{10} organisms/c.c.

also indicate if these biochemical changes were an integral part of the death process, causal factors, or non-essential correlates.

This investigation was supported by research grant No. *E*4046 of the U.S. Public Health Service, National Institutes of Health. We thank Mrs. Betty Laske of the Infectious Disease Division Laboratory and the personnel of the Clinical Biochemistry Laboratory for assistance.

[1] Smith, I. M., Wilson, A. P., Hazard, E. Ch., Hummer, W. K., and Dewey, M. E., *J. Infect. Dis.*, **107**, 369 (1960).
[2] Smith, I. M., and Dubos, R. J., *J. Exp. Med.*, **103**, 87 (1956).
[3] Smith, I. M., Beals, P. D., Kingsbury, K. R., and Hasencleaver, H. F., *Arch. Int. Med.*, **102**, 375 (1958).
[4] Zeckwer, I. T., and Goodell, H., *J. Exp. Med.*, **42**, 57 (1925).
[5] Delafield, M. E., *J. Path. Bact.*, **35**, 55 (1932).
[6] Kun, E., and Miller, C. P., *Proc. Soc. Exp. Biol. Med.*, **67**, 221 (1948).
[7] Slein, M. W., and Logan, C. F., *J. Bact.*, **80**, 77 (1960).
[8] Sugiyama, H., and Dack, G. M., *J. Infect. Dis.*, **96**, 286 (1955).
[9] Smith, I. M., Counts, G. W., and Nelson, J. (to be published).

ELECTRON MICROSCOPE OBSERVATIONS OF THE RECEPTORS IN THE SENSORY VESICLE OF THE ASCIDIAN TADPOLE

By NOËL DILLY
Department of Anatomy, University College, London

THE free-swimming larvæ of sea squirts react to light and gravity in a manner that ensures their wide dispersal. The receptors sensitive to these stimuli are situated within the cerebral vesicle of the tadpole. In the species examined these consist of a unicellular otolith, and an ocellus made up from about ten cells. Since there are only about 60 cells in the whole of the cerebral vesicle the connectivity patterns of these receptors might yield important clues as to the manner in which they influence the motor systems of the tadpole.

Tadpole larvæ of *Ciona intestinalis, Phallusia mammillata* and *Ascidia nigra* were fixed in buffered osmic acid or potassium permanganate and embedded in 'Araldite'. Some specimens were stained in 1 per cent phosphotungstic acid in absolute acetone before embedding. The material was examined in a Siemens Elmiskop electron microscope.

The Otolith. This gravity receptor protrudes into the cavity of the vesicle from a small group of cells, which form a mound on the ventral wall (Fig. 1). The otolith itself is a single cell, consisting of an intracellular dense black mass of material, a narrow stalk, and an L-shaped 'foot'. The foot is situated in the group of cells forming the mound, whereas the narrow neck and dense body project into the cavity of the vesicle. This body is dense in the sense that it is opaque to light and electrons as well as being heavy. It is a sphere 15μ–25μ in diameter, and since its density is greater than that of the other cell contents it probably acts as the weight that distorts the otolith cell during rotation of the tadpole. The fact that it is densely black and is situated near the ocellus suggests that perhaps it may also function as a shutter, cutting off light from the ocellus intermittently as the otolith moves during rotation of the tadpole.

The remaining part of the otolith cell, which is free within the cavity of the cerebral vesicle, is conical in shape and contains the nucleus. The junction between fixed and free portions of the otolith cell is organized in a complex manner. The cell membrane is folded back on itself to form a collar around the stalk of the cell in such a way that the collar makes a socket around the stalk. The organization of this collar is further complicated because the cytoplasm is filled with distended vesicles of the endoplasmic reticulum. The collar is roughly cylindrical in outside shape, whereas the inner surface slopes inwards conically from a wide base above, to a narrow neck below. The stalk at the junction is only about 0·75μ in diameter. It seems remarkably thin to support the mass of the otolith. This is obviously the junction where maximum distortion will occur as the otolith moves in the cerebral vesicle and must therefore be suspected as the transducer region.

The foot region of the otolith cell, that is, the fixed region, extends at right angles to the long axis of the remainder of the cell, to give the whole structure an L shape. The right angle of the L is occupied by a further cell, which is probably a neurone. This cell differs from the other cells in the mound in that it is

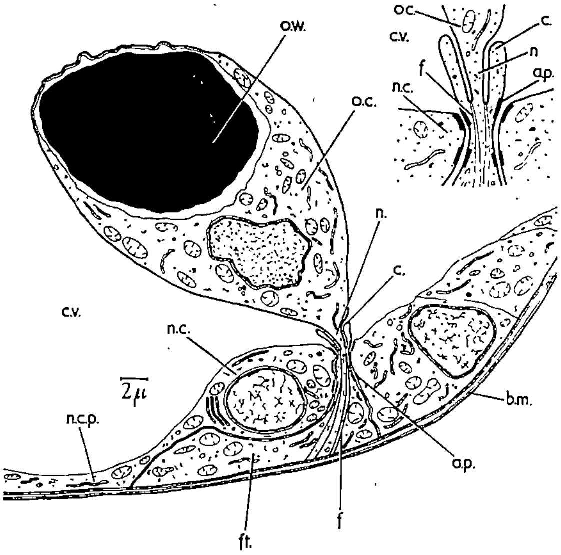


Fig. 1. Otolith (transverse section). Drawing from an electron-micrograph. The gravity receptor projects into the cavity of the cerebral vesicle, and is free to move within the cavity during rotation of the tadpole while swimming.

Inset, to show details of the neck region of the otolith cell. The vesicles of the endoplasmic reticulum have been omitted.

a.p., Attachment plaques; *b.m.*, basement membrane; *c*, collar of transducer; *C.V.*, cavity of the cerebral vesicle; *f.*, anchoring filaments; *ft.*, foot process of the otolith cell; *n.*, neck of the transducer region; *n.c.p.*, nerve cell process, probably an axon; *o.c.*, otolith cell body; *o.w.*, otolith weight

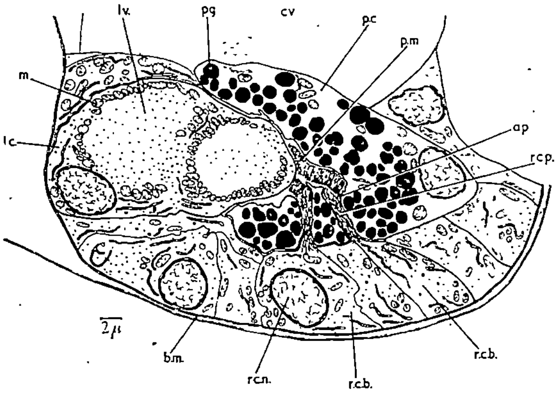


Fig. 2. Ocellus (transverse section). Drawing from an electron micrograph. The photoreceptor may be considered as made up of three parts, a lens system, a pigment cup, and a retina *a.p.*, Attachment plaques; *b.m.*, basement membrane; *C.V.*, cavity of the cerebral vesicle; *l.c.*, lens cell; *l.v.*, lens vesicle; *m.*, mitochondrion; *p.c.*, pigment cell; *p.g.*, pigment granule; *p.m.*, piled photoreceptor membrane; *r.c.b.*, retinal cell body; *r.c.n.*, retinal cell nucleus; *r.c.p.*, tubular process of the retinal cell

oval in shape and has a long cell process, which is probably an axon running initially transversely across the cerebral vesicle. The oval shape of this cell is such that the short arm of the L is pyramidal in shape whereas the long arm is narrow in the middle and wide at each end. The constant position and presence of this cell in the angle of the L, together with its process, suggest that it is probably the neurone that transmits the impulses generated by movements of the otolith. This may be effected by generator potentials in the junctional region causing action potentials to be generated in the neurone; or the neurone may be distorted mechanically by movements of the otolith.

Both sides of the vertical pyramidal region of the foot are attached to their surrounding cells by specializations of the cell membranes similar to the attachment plaques described by Odland[1], and probably serving to anchor the foot firmly to the surrounding cells.

There is a set of fibrils in the otolith running from the membrane folds of the fixed free junction through the cytoplasm of the foot region to the basement membrane. They arise from the narrowest innermost part of the neck region and are attached to the innermost membrane of the basement membrane. They are 150–250 Å. in diameter and perhaps serve as a mechanical anchor for the neck region so that it cannot be excessively distorted.

The Ocellus. The ocellus (Fig. 2), the photoreceptor, is made up of three parts, a pigment cup, a lens system and a retina. It is situated at the caudal end of the cerebral vesicle.

The pigment cup situated between the lens and the retinal cells is a single cell with the lens cell contained partly within its concavity. It contains a mass of pigment granules, varying in diameter between 0·5 and 2μ and probably all bound by a membrane. The granules are more concentrated in the thick central region than at the periphery of the cell. The nucleus is confined to the non-granular apex of the cell. The function of the pigment cell is obviously to keep stray light from falling on the photoreceptor endings, thus ensuring that only light that has been orientated by the lens falls on them.

The lens system is usually unicellular, but may consist of two or more cells. There are usually three lens vesicles, arranged in a line along the main axis of the concavity of the pigment cup. The lens vesicles vary in diameter between 6μ and 12μ. They are usually spherical in shape. Each vesicle is separated from the rest of the cytoplasm of the lens cell by a single-layered sphere of mitochondria. There is no membrane separating the lens vesicle contents from the rest of the cytoplasm of the lens cell. There are no cell organelles within the vesicles, which are filled with a granular material similar in composition to the background granular material of the extravesicular cytoplasm, but in a less-dense concentration. These granules, 150–200 Å. in diameter, are usually distributed homogeneously throughout the vesicle; however, occasionally they have a greater concentration at the centre of the vesicle than at the periphery. The nucleus of the lens cell is usually in that part of the cell distal from the pigment cup.

The retina consists of about eight cells arranged in a row outside the dorsal arm of the pigment cup. They are conical cells, resting on the basement membrane that separates the cerebral vesicle from the rest of the body. These cells narrow distally to form tubes that penetrate the pigment cup and end as piles of membranes closely applied to the lens cell in the base of the concavity of the pigment cup. The retinal cells can thus be considered to have three parts: the main cellular part outside the pigment cup, the tubular part, which penetrates the pigment-containing cell, and the piled membrane photoreceptor part.

The main cellular part is of a truncated cone shape, narrowing from a wide base resting on the basement membrane down to a narrow neck at the outer edge of the pigment cell. This part of the retinal cell contains the nucleus. As yet no synapse-bearing foot process has been found.

The single tubular part of each cell penetrates the pigment cell and is cylindrical, about 6μ long and 0·75μ in diameter. It extends from the apex of the retinal cell body to the piled membrane photoreceptor part of the cell. The tube contains numerous (50–100) filaments running along its long axis. These filaments are solid, some 150 Å. in diameter and without cross striations. They do not run parallel to one another but are tangled together along the length of the tube. These filaments do not penetrate into the retinal cell body cytoplasm. The retinal cell tubular part is separated from the cytoplasm of the pigment cup by its own cell membrane, and the cell membrane of the pigment cell. Along the lengths of these two membranes are membrane thickenings similar to attachment plaques. This tubular part probably corresponds to the connecting cilium described by De Robertis[2] in the retinal rods of the rabbit. It is unlike it in that in transverse section it does not contain tubules in the normal organization of the cilium (that is, nine peripheral and two central).

At their central ends each tube is continuous with a piled photoreceptor membrane. This corresponds well with the usual vertebrate rod pattern, consisting of piles of membranes arranged in disks 150–200 Å. thick and up to 2·5μ in diameter. The piles are several μ long and are arranged perpendicular to the direction of the light falling on them.

Thus the main morphological feature of vertebrate photoreceptors, a layered lamellar structure, is present in the sea-squirt tadpole.

I wish to thank Prof. J. Z. Young and Drs. E. G. Gray and J. D. Robertson for advice and criticism.

---

[1] Odland, G. F., *J. Biophys. Biochem. Cytol.*, **4**, 529 (1958).

[2] De Robertis, E., *J. Biophys. Biochem. Cytol.*, **2**, 319 (1956).

LIMITATIONS OF GEOMETRICAL MODELS FOR COMPLEMENTATION MAPPING OF ALLELIC SERIES

By Prof. ELOF AXEL CARLSON
Department of Zoology, University of California, Los Angeles

THE first attempt to construct a gene map from the functional relations between alleles was carried out in the scute series of *Drosophila melanogaster*[1-3]. This system of 'step-allelism' assumed, by analogy with 'deletion mapping', that the complementary relations expressed in the progeny derived from various allelic crosses represented spatially distinct sub-genes or regions of the scute gene. It was, however, based on the further assumption that each sub-gene controlled one specific character—in this case, a characteristic set of bristles. This latter assumption turned out to be erroneous because the map relations were no longer maintained in the same linear order when temperature differences[4] or genetic modifiers[5] were used in conjunction with the allelic crosses. Furthermore, a radiation breakage analysis[6] of this small region indicated discontinuous organization of the genetic units with many fewer elements than proposed in the step-allele analysis. Nevertheless, at least two separable elements (achæte and scute) were definitely established[7].

In recent years a similar use of functionally complementary relations among alleles has been made, and the process has been called complementation mapping[8,15]. In some systems the complementation units inferred from the phenotypically non-mutant heterozygotes are unambiguously distinct, spatially, as judged by recombination, as well as functionally. These systems require all the point mutations in one complementation unit to complement all the point mutations in the other complementation unit. Such a relation has been typically designated as non-allelism. In other cases, there are additional point mutations, not known to involve deficiencies or other re-arrangements of the genetic material, which cannot complement at all. Such mutants appear to extend their genetic effect to all the complementation units in an allelic series. This is the more typical expectation of complementation studies for allelic series, and it has been referred to as intralocus complementation. Further use of this technique has produced complementation maps involving four, six, or more complementation units (Table 2). In all these cases one or more complementation units may be affected by a point mutation. This expression of effect by the mutant is known as its complementation pattern.

In evaluating these complementation maps, several assumptions and variables must be explored. One requirement is a knowledge of the number of complementation patterns which are consistent with linearity. A second factor would be the number of other possible geometrical models which could satisfy a complementation mapping procedure. A third important problem is whether the maps derived from complementation studies are co-linear with those derived by recombination analysis (either by the use of associated outside marker genes or by use of matrices ordered for a topological correspondence to linearity[9]). Each of these criteria involves difficult genetic problems which defy a simple analysis. For example, it is now widely recognized that almost all allelic series (especially in micro-organisms) are bound to yield recombination among most of the members tested if large enough numbers of progeny are scored[10]. This might create a misleading sense of confidence in the correspondence of a complementation map to the spatial separability demonstrated by recombination. Although the complementation test often assumes and predicts spatial separability, it must be kept in mind that allelism *per se*, in present-day genetic thinking, also assumes the likelihood of a separability, by recombination, of the mutationally altered sites in different alleles. Another difficulty not always appreciated is the non-randomness of the mutations occurring within a genetic region[11]. The resulting mutational spectrum for a given gene may be interpreted to have arisen as a consequence of hot spots, varying length of the genetic sub-units, or the sensitivity of certain regions to mutational damage because their alterations involve a region critical to the specification of the active functional region of the gene product. Other areas of the gene, altered with the same frequency, may produce fewer mutations because the final product of the gene is not altered in a region critical to its function. Such apparent intragenic mutational differences may lead to inaccurate interpretations of the number of complementation units in an allelic series.

Mathematically, the relations between the complementation units and the complementation patterns possible for allelic series are summarized in Table 1. These show that a linear map involves only a portion of the patterns possible in abstract terms, especially as the number of complementation units increases. Two other maps are possible by use of this process—circular and conical models. The special geometrical cases, such as triangularity for three complementation units, the tetrahedron for four units, and pyramids with polygonal bases for larger numbers of units, all have properties topologically identical to either the circular or conical models mentioned. Notice that if four or more complementation units are proposed for a gene, all its complementation patterns can be satisfied by the conical model. If only one or two complementation units are proposed, both linearity

Table 1. Relation between Complementation Units, Patterns and Maps

Complementation units proposed	Maximum patterns for linearity	Maximum patterns for circularity*	Maximum patterns possible†
1	1	1	1
2	3	3	3
3	6	7	7
4	10	13	15
5	15	21	31
6	21	31	63
7	28	43	127
–	–	–	–
–	–	–	–
–	–	–	–
n	$n\left(\frac{n+1}{2}\right)$	$n(n-1)+1$	2^n-1

* Without radii.

† For four or more complementation units the maximum pattern can be achieved by a cone with (n) faces or a circle with n radii.

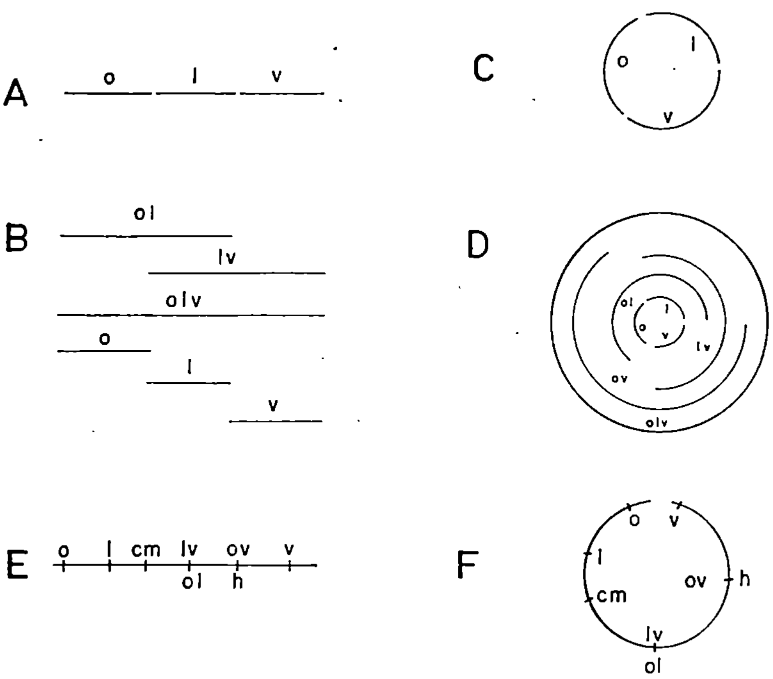


Fig. 1. *o*, Oblique wings; *l*, lethality; *v*, thoracic vortices; *cm*, comma, a weakly expressed mutant with *o* and *v* effects; *h*, humpy-like, an allele with exaggerated *o* and *v* effects. *A*, the linear complementation map with three complementation units. *B*, the six complementation patterns consistent for linearity. In this sequence, *o–l–v*, the omitted member is *ov*. If the sequence were *l–o–v*, the omitted member would be *lv*; for *o–v–l*, the omitted member would be *ol*. *C*, the circular complementation map with three complementation units. *D*, the seven complementation patterns consistent for circularity. *E*, the recombination map—all sites recombine with the other neighbouring sites and order is determined by positions of outside marker genes in the recombinants. *F*, the recombination map, if visualized as a circular map for correspondence with complementation map *D*. Note the discontinuities in *o* and *l* effects in both map *E* and *F*

and circularity can be satisfied. It is, of course, unlikely that these non-linear maps have any physical correspondence to gene structure.

An example of non-linearity in complementation mapping can be found in the dumpy series in *D. melanogaster*[12]. Here the three effects which are expressed by either the wing (*o*), thorax (*v*) or viability (*l*) produce a maximum of $2^n - 1 = 7$ complementation patterns. A linear map can be satisfied if $n\left(\frac{n+1}{2}\right) = 6$ patterns are obtained. However, the known phenotypes of the point mutations in the dumpy series suggest seven patterns: *o*, *l*, *v*, *ol*, *lv*, *ov*, and *olv*. This would correspond to a circular complementation map, since $n(n - 1) + 1 = 7$. Essentially similar arguments were proposed earlier to reject an interpretation of this series as a nest of three closely linked genes[13]. Notice that when the various complementation maps are compared with the directly determined recombination map, there is no obvious relation (Fig. 1).

It is, of course, possible that developmental complexities in *Drosophila* make allelic complementation an analogous, unrelated process to that found in viruses, bacteria and fungi. Nevertheless, all the intensively studied allelic series in micro-organisms must meet certain requirements for a critical demonstration of a spatially linear complementation map of the gene. These are, first, that the maximum permissible number of complementation patterns for the set of complementation units has been achieved and not exceeded. Because of the non-randomness of the allelic series collected, there is no easy way to eliminate the possibility of complementation patterns greater than $n\left(\frac{n+1}{2}\right)$, but certainly several alleles should be assigned to each of the complementation patterns. Secondly, the map obtained by the complementation technique, if it is intended to reflect gene structure, should be co-linear with the recombination map[14]. To date, this has not been achieved in a clear-cut way since, as examination of Table 2 indicates, the necessary maximum has not been achieved or the status of linearity is not an exclusive one. In the absence of these rigid criteria, it may be misleading to state that two genes or alleles are separated by a distance of several complementation units. It is entirely possible that the complementation units may have no real physical significance[15] for gene structure, and thus their maintenance for complementation mapping would require such *ad hoc* hypotheses as spreading effects[16], polymerization of gene products[17], or cytoplasmic recombination at the protein or ribonucleic acid template-level[8]. Additionally, it must be borne in mind that not all complementation patterns may exist in an allelic series; that co-linearity may exist for only certain portions

Table 2. Status of Complementation Mapping in Various Loci

Locus	Symbol	Organism	Comp. units proposed	Comp. patterns obtained	Comp. patterns needed	Comp. alleles used	Modal No. per pattern	Consistency with linear map	Ref.
Histidinol dehydrogenase	his D	*S. typhimurium*	2	3	3	33	6	Yes*	16, 21
Glutamic acid dehydrogenase	am	*N. crassa*	3	6	6	11	1	Insuff.	22
Dumpy	dp	*D. melanogaster*	3	7	6	31	3	No	12
Histidine *E*	his *E*	*S. typhimurium*	4	8	10	11	1	Insuff.	16
Histidine *B*	his *B*	*S. typhimurium*	4	9‡	10	24	1	Insuff.	16
Pantothenic acid	pan-2	*N. crassa*	6	12	21	23	1	Insuff.	13
Adenylosuccinase	ad-4	*N. crassa*	7	16	28	51	1	Insuff.	24
Isoleucine-valine	iv	*N. crassa*	†	18	§	19	1	Insuff.§	23

* Also consistent with circular complementation map.
† Three 'regions' proposed.
‡ Three additional patterns are redundant.
§ Must be 6 or more complementation units, with 21 patterns minimal for consistency with linear map.

of complementation and recombination maps; and that complementation may exist for two alleles which, by recombination analysis, are shown to occupy the same site.

Although it is not possible either to confirm or to invalidate the linearity of complementation mapping, the phenomenon of complementation is a real one and its value for understanding gene function, and indirectly, gene structure, is not denied[18]. Nor does the limitation imposed by the concept of the complementation unit rule out the cytoplasmic reassociation of component polypeptides of an enzyme or other protein[19,20]. Such demonstrated mechanisms of cytoplasmic complementation may be independent of the structural assumptions inherent in the complementation mapping of the particular gene involved. It seems, however, that the alterations in the gene molecule and the functional disturbances produced in its final product are more complex than can be accounted for on the assumption of mere co-linearity between genetic recombination and genetic complementation.

This work has been supported by grant *G*14222 from the U.S. National Science Foundation. I also wish to thank Drs. H. J. Muller and D. G. Catcheside for their criticisms of this manuscript.

[1] Agol, I. J., *Zh. Exp. Biol.*, **5**, 86 (1929) (in Russian).
[2] Dubinin, N. P., *Biol. Zentralbl.*, **49**, 328 (1929).
[3] Serebrovsky, A. S., *Pod. Znam. Marksisma*, 212 (1929) (in Russian).
[4] Child, G. P., *Genetics*, **20**, 109, 127 (1935).
[5] Sturtevant, A., and Schultz, J., *Proc. U.S. Nat. Acad. Sci.*, **17**, 265 (1931).
[6] Muller, H. J., and Prokofeyeva, A. A., *Proc. U.S. Nat. Acad. Sci.*, **21**, 16 (1935).
[7] Serebrovsky, A. S., *C.R.* (*Doklady*) *Acad. Sci., U.S.S.R.*, N.S., **19**, 77 (1938).
[8] Case, M. E., and Giles, N. H., *Proc. U.S. Nat. Acad. Sci.*, **44**, 378 (1958).
[9] Benzer, S., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1607 (1959).
[10] Pontecorvo, G., *Trends in Genetic Analysis* (Columbia Univ. Press, New York, 1958).
[11] Benzer, S., *The Chemical Basis of Heredity*, edit. by McElroy, W. D., and Glass, B., 70 (Johns Hopkins Press, Baltimore, 1957).
[12] Carlson, E. A., *Genetics*, **44**, 347 (1958).
[13] Muller, H. J., *Drosophila Inform. Serv.*, **12**, 39 (1939).
[14] Case, M. E., and Giles, N. H., *Proc. U.S. Nat. Acad. Sci.*, **46**, 659 (1960).
[15] Catcheside, D., *Symp. Soc. Gen. Microbiol.*, **10**, 181 (1960).
[16] Hartman, P., Hartman, Z., and Serman, D., *J. Gen. Microbiol.*, **22**, 369 (1960).
[17] Brenner, S., *Biochemistry of Human Genetics*, edit by Wolstenholme, G., and O'Connor, C. (J. and A. Churchill, Ltd., London, 1959).
[18] Muller, H. J., *Proc. Sixth Intern. Cong. Genet.*, **1**, 213, 255 (Ithaca, 1932).
[19] Itano, H., Singer, S., and Robinson, E., *Biochemistry of Human Genetics*, edit. by Wolstenholme, G., and O'Connor, C., 96 (J. and A. Churchill, Ltd., London, 1959).
[20] Schwartz, D., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1210 (1960).
[21] Hartman, P., Loper, J., and Serman, D., *J. Gen. Microbiol.*, **22**, 354 (1960).
[22] Fincham, J. R. S., *Proc. Tenth Intern. Cong. Genet.*, **1**, 355 (Montreal, 1958).
[23] Wagner, R., Somers, C., and Berquist, A., *Proc. U.S. Nat. Acad. Sci.*, **46**, 707 (1960).
[24] Woodward, D., Partridge, C., and Giles, N., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1237 (1958).

RELATIONSHIP BETWEEN SIMPLE AND SERIAL REACTION TIMES

By PROF. H. KAY and DR. A. D. WEISS*

Department of Psychology, University of Sheffield

IT is one of the oldest findings in experimental psychology that when a subject has to respond to a discrete signal some measurable delay will occur. But this delay is due to many variables which are not yet fully understood. Where a response is required to a second stimulus, closely following a first, the second reaction time (RT_2) has generally been shown to be much longer than the simple reaction time, so much so that a different kind of explanation has been proposed for the so-called 'psychological refractory phase'. It was the underlying hypothesis of these experiments that work on serial reaction times may have been handicapped by stemming primarily from Telford's[1] work and by ignoring the variations which occur in the simple reaction time itself. Delays in responding in the serial situation have been treated as if they were unique[2], but may in fact closely parallel fluctuations in the simple reaction time. This article reports some of the findings of an investigation designed to make an overall comparison of simple and serial reaction times.

Subjects sat in a sound-treated room wearing padded ear-phones and responded to an auditory signal by releasing a morse key. The design of the experiments, unfortunately complicated, manipulated fixed and variable intervals before both the first and second signals. Table 1 sets out these conditions. After a subject had pressed a 'ready' key there was a warning delay of either 1, 2, 3, or 4 sec. In the regular condition it was known which of these intervals would be used ; in the irregular it might be any one of the four periods. The first click occurred at the end of the warning delay and was followed by a fore-period terminated by a second click. There were eight fore-periods ranging from 25 to 1,000 m.sec. and again these might be regular (a known interval) or irregular (any one of the eight). Catch trials were used with all regular procedures.

Data were taken from five practised subjects. In all some 20,000 readings were recorded, excluding practice data, and, with the use of an electronic computor, were examined by analysis of variance. Any differences which are discussed were statistically significant.

At procedures I and II simple reaction times were examined after regular and irregular warning delays. The mean *RT*'s with the regular warning delay at the 1-, 2-, 3- and 4-sec. intervals were 134·3, 146·6, 146·3 and 152·6 m.sec., respectively. At the same intervals for the irregular procedure these means were 164·4, 146·4, 146·6 and 146·5, respectively. The 1-sec. warning delay produced the quickest response in the regular procedure and the slowest in the irregular.

To facilitate comparisons for the remaining procedures the results for all the fore-periods based on a mean of the four warning delays are presented in Table 1 and have been graphed in Fig. 1. The striking feature of the graph is the manner in which reaction time increases at the minimum fore-period with the increasing ambiguity of the experimental conditions. With procedure III, where both warning delay and fore-period were regular, the reaction time was almost the same for the fore-periods from 25

---

* Present address: Massachusetts General Hospital, Boston, Massachusetts.

Table 1

Procedure No.	Warning delay	Fore-period	Signal responded to	Fore-periods (m.sec.)							
				1 25	2 50	3 100	4 150	5 200	6 250	7 500	8 1.000
III	*R*	*R*	2	155	158	152	153	161	150	126	125
IV	*R*	*I*	2	175	166	154	155	156	159	147	131
V	*I*	*R*	2	189	176	159	154	151	144	123	115
VI	*I*	*I*	2	192	180	159	148	152	146	130	113
VII	*I*	*R*	1 and 2	244	234	199	185	173	163	126	108
VIII	*I*	*I*	1 and 2	265	246	218	195	197	180	136	119

Mean response times (m.sec.) to the second signal after a regular (*R*) or irregular (*I*) warning delay and fore-period. Warning delay intervals 1–4 sec. Fore-period, 25–1,000 msec.

Analysis of variance, using a random model as the conservative index of significance, gave *F*-ratios which were significant at the 0·01 level or above between the fore-period conditions of procedures V and VII, VI and VIII, and VII and VIII.

to 250 m.sec. For the irregular fore-period of procedure IV it increased slightly at the 25- and 50-m.sec. interval. With procedure V, where the 1–4 sec. warning delay was irregular, followed by the second click at a prescribed interval, subjects were slower in responding at the shorter fore-period than in procedure III where the warning delay preceding the first click had been regular. There was a further increase in reaction time at the shortest fore-periods for procedure VI, where both warning delay and fore-period were irregular. But it is obvious from the larger displacements for procedures VII and VIII that the delay resulting from perceiving an immediately preceding signal by no means accounts for all the interference of these responses. Procedures V and VI were identical with VII and VIII except that, in the two latter, subjects not only observed the first click but also manually responded to it. The slower reaction times which occurred to the second click as a result of this previous response were considerable and persistent. The results for procedures VII and VIII were not unlike those found by other work on the refractory phase, though the magnitude of the delay was not so great as in some earlier experiments.

If the results for procedures VII and VIII were considered in isolation, or against some so-called 'average' reaction time, it is apparent why an explanation of the 'psychological refractory phase' type would be necessary. There is a large increase as against some standard reaction time of say 140 m.sec. for an auditory stimulus. But in the present work the comparison is with reaction times produced under conditions which have varying degrees of similarity to those of the final procedures, VII and VIII. It is clear that with increasing uncertainty as to when either signal would occur there was an increasing reaction time at the shortest fore-periods. It is certainly the case that even the observation of a previous and irregular signal retarded the response to the critical signal, as in procedures V and VI. But this perception, contrary to the assertion of some workers, has been shown to be only one of many variables contributing to the delay. The manual response to the first click, in procedures VII and VIII, definitely increased further the delay in responding to the second click.

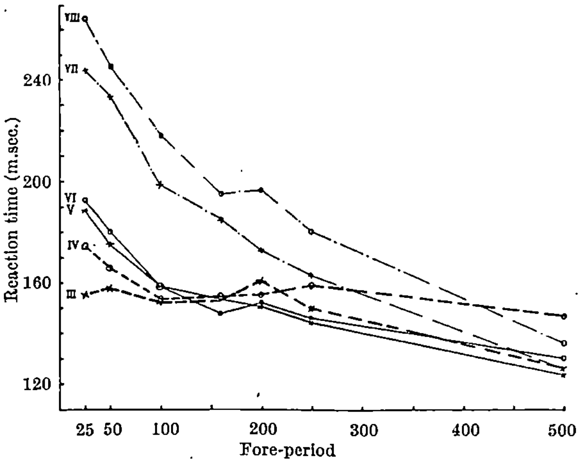


Fig. 1. Mean response times (m.sec.) to second signal at fore-periods 25–500 m.sec.

III: × – – ×, regular warning delay, regular fore-period.
IV: ○ – – ○, regular warning delay, irregular fore-period.
V: × — ×, irregular warning delay, regular fore-period.
VI: ○ — ○, irregular warning delay, irregular fore-period.
VII: × —●— ×, RT_2 irregular warning delay, regular fore-period.
VIII: ○ —●— ○, RT_2 irregular warning delay, irregular fore-period.

From this series of experiments it would seem that the variability in the simple reaction time, shown so clearly by Breitwieser[3] and Woodrow[4], is closely mirrored in the serial reaction-time situation. Theorists have been prone to postulate rigid fixed intervals of time between the completion of one response and the initiation of the next. The present work casts serious doubts on this approach. It would support previous work in so far as no two reaction times are overlapping, and there is the usual pronounced delay with the standard serial reaction-time situation of procedures VII and VIII. But this situation is only an extreme example of how RT_2 can be delayed. If we think of a minimal simple reaction time only occurring when the organism is optimally prepared to make a response to a signal, we have evidence from present and previous work how the reaction time will vary with uncertainty as to when the signal will occur. It would appear that in cases of such uncertainty the state of readiness of the organism cannot be raised to one critical peak point in time. In the case of the serial reaction time any signal which has to be perceived and which occurs immediately before the critical second signal, serves to lower the state of readiness of a subject. The disruption seems related to the degree of uncertainty of the first signal, and the extent of the response which is required. We might say that in the serial reaction-time conditions, the restriction where a subject has to observe a first signal and respond as quickly as possible to it is an optimally disturbing condition on the preparatory set for perceiving and responding to the second signal (RT_2). It would follow that one possible explanation for the delay in responding to the second signal is along lines identical with those for explaining the variability in the speed of responding to the first.

[1] Telford, C. W., *J. Exp. Psychol.*, **14**, 1 (1931).

[2] Welford, A. T., *Brit. J. Psychol.*, **43**, 2 (1952).

[3] Breitwieser, J. V., *Arch. Psychol.*, **2**, No. 18 (1911).

[4] Woodrow, H., *Psychol. Monogr.*, **17**, No. 5 (1914).

LETTERS TO THE EDITORS

RADIOPHYSICS

Trans-Equatorial Backscatter Observations of Magnetically Controlled Ionization

A ROTATING backscatter equipment operating at Brisbane on a frequency of 16 Mc./sec. with a highly directional aerial[1] has been modified to record echoes with ranges up to 15,000 km. It is found that on 95 per cent of afternoons echoes are returned from a north-easterly direction with ranges of 6–12,000 km. Typical sequences are shown in Figs. 1 and 2, where the plot is one of range versus magnetic azimuth from Brisbane.

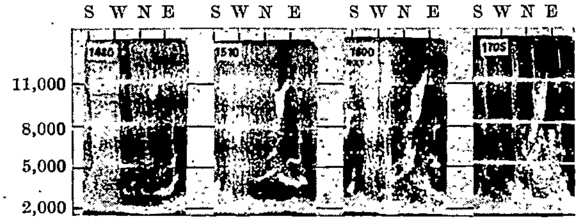


Fig. 1. Range–azimuth records of trans-equatorial backscatter. 6/10/60

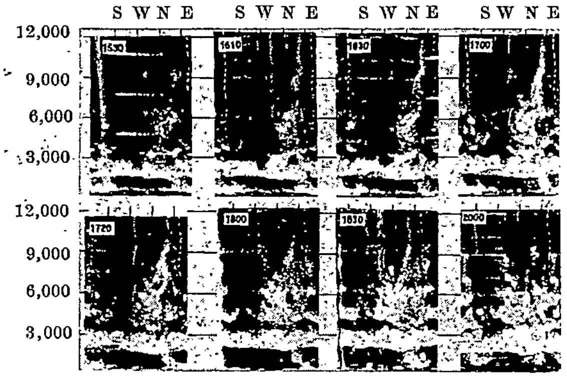


Fig. 2. Range–azimuth records of trans-equatorial backscatter. 2/1/61

It is well known that the electron density of the *F* region in the afternoon is less near the magnetic equator than in two sub-tropical belts at geomagnetic latitudes of about ± 20°, and several theories have been put forward to account for this phenomenon[2–5]. These theories involve the electrodynamic uplift of ionization at the equator and its subsequent downflow along the magnetic field lines to the sub-tropical regions near ± 20° M. In addition, Wright[6] has produced meridianal sections of the quiet day ionosphere, showing the extremely rapid variations of the ionization contours as a function of height and latitude.

If one compares the locations of the long-range echoes referred to here with the parallel of magnetic latitude 20° N. (that is, computed from the actual dip values), it is found that the echoes generally lie very close to this line. Two specific instances from the sequences of Figs. 1 and 2 are shown in Fig. 3.

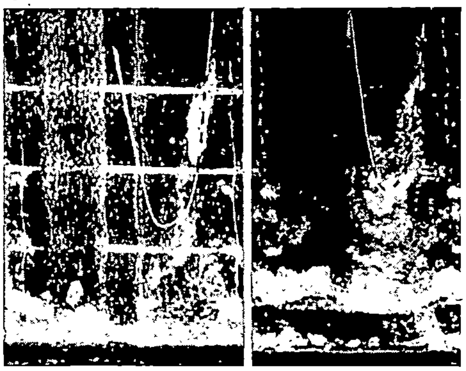


Fig. 3. Range-azimuth records for *a*, 1600/6/10/60 and *b*, 1700/2/1/61 with overlay plots of the 20° N. parallel of magnetic latitude

The question of the propagation mechanism is rather involved as the echoes come from regions in which the local time may be up to 6 hr. ahead of the local time of observation at Brisbane. It is believed, however, that the main mechanism is that of 'tilt-mode' propagation proposed by Villard *et al.*[7], sometimes with a single hop to the *F* region before the equatorial jump. This hypothesis is being examined further by computing the expected oblique profiles of ionization density at various times and in various directions from Brisbane.

The fact that the eastern limb of the echo is the predominant feature is probably due to the changing ionospheric conditions as one proceeds from east to west with changes in time of several hours. By the time the western limb should be visible at long ranges, the electron density south of the equator is too low for the necessary propagation conditions to be set up.

It appears probable that such observations of the whole length of the belt of high-density ionization are only possible with highly directive antennæ. The signal is often weak and scattered and with a broad beam antenna may well merge into the background noise. This point is to be checked shortly, and further tests will be made with a swept-frequency backscatter sounder operating at 15–30 Mc./sec. and with an additional fixed-frequency sounder operating at a higher frequency.

This work has been sponsored by the Electronics Research Directorate of the U.S. Air Force Research Division, Air Research and Development Command, under Contract No. *AF* 64(500)-9.

I thank Dr. R. W. E. McNicol for useful discussions.

J. A. THOMAS

Physics Department,
University of Queensland,
Brisbane.

---

[1] Thomas, J. A., and McNicol, R. W. E., *Nature*, **187**, 398 (1960).

[2] Mitra, S. K., *Nature*, **158**, 668 (1946).

[3] McNish, A. G., and Gautier, T. N., *J. Geophys. Res.*, **54**, 303 (1949).

[4] Martyn, D. F., *Physics of the Ionosphere*, 259 (Phys. Society, London 1955).

[5] Duncan, R. A., *J. Atmos. Terr. Phys.*, **18**, 89 (1960).

[6] Wright, J. W., *J. Geophys. Res.*, **65**, 185 (1960).

[7] Villard, O. G., Stein, S., and Yeh, K. C., *J. Geophys. Res.*, **62**, No. 3, 399 (1957).

RADIOASTRONOMY

Central Component of the Radio Source Centaurus *A*

The identification of the radio source Centaurus *A* with the peculiar galaxy *NGC* 5128 was first suggested by Bolton, Stanley and Slee[1]. The radio source consists of an extended component with dimensions of about 2° × 4° and a compact central component. The extended source has recently been shown by Bolton and Clark[2] to consist of two components. The compact central component was investigated in 1953 by Mills[3]. His observations indicated that the radio emission was associated with the wide dust band of *NGC* 5128. Recent measurements by Twiss, Carter and Little[4] have shown that the central source also consists of two components. This result was also obtained by Moffet and Maltby[5]. In a more recent radio investigation of *NGC* 5128, reported in this communication, I find that the radio source is not associated with the wide dust band.

Using the variable spacing interferometer at the Owens Valley Radio Observatory, Centaurus *A* has been observed with different antenna spacings and in different orientations. The measurements were taken at wave-lengths of 21·6 cm. and 31·3 cm. In order to determine the position of the minor axis, the source was observed for about 2 hr. on either side of upper culmination with a north–south interferometer. At the latitude of this Observatory the source is close to the horizon, and a relatively long horizontal antenna separation is therefore needed to investigate the source in the north–south direction.

In Fig. 1 the observed fringe amplitude is plotted against hour angle for two different antenna separations. Fig. 1*a* shows the observations at 31·3 cm. using a horizontal antenna spacing of 800 ft., while the curve in Fig. 1*b* is obtained at 21·6 cm. with the antennas 1,600 ft. apart. The accuracy of the individual points in the curves is ± 3 per cent of the maximum intensity of the source. In the following, we will neglect any change in the brightness distribution between these two frequencies. Changes in the fringe amplitude in Fig. 1 are due to changes in the effective antenna separation as well as to changes in the orientation of the source in the fringe pattern. From Fig. 1 we find that one maximum occurs at an hour angle equal to 1h. 10m. in both curves. This means that the minor axis is in a position angle of about 135°. Using the maxima and the minima in Fig. 1, it was found that the major axis is in position angle 46·5° ± 2·0°. Although the position angle of the minor axis deduced by Mills[3] was 40°, his observations would not be inconsistent with the position angle of 136·5° reported here. Indeed, all Mills's[3] observations are in reasonable agreement with the interpretation given here, if we take into account the fact that the source consists of two components.

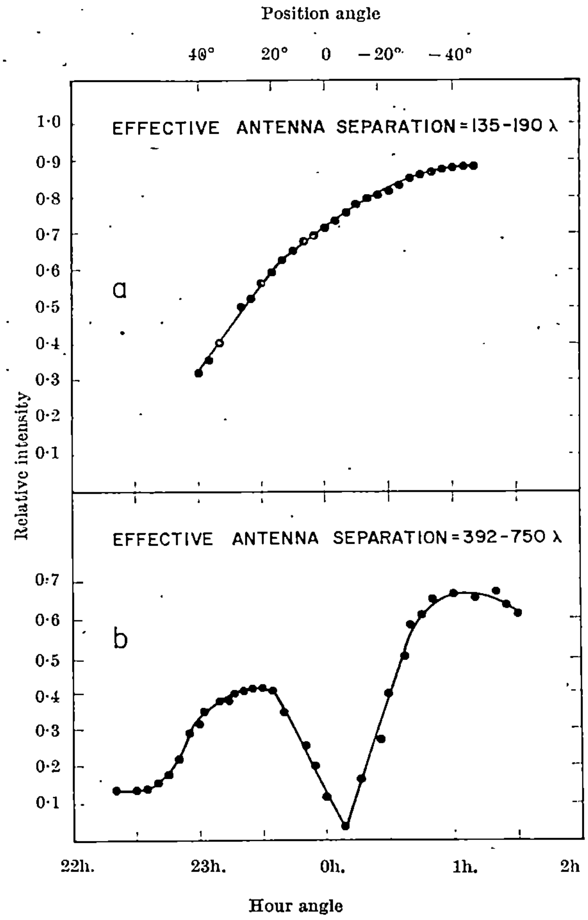


Fig. 1. Observed fringe amplitude plotted against hour angle. *a*, Observed curve at 31·3 cm. using a horizontal antenna spacing of 800 ft.; *b*, observed curve at 21·6 cm. with the antennas 1,600 ft. apart

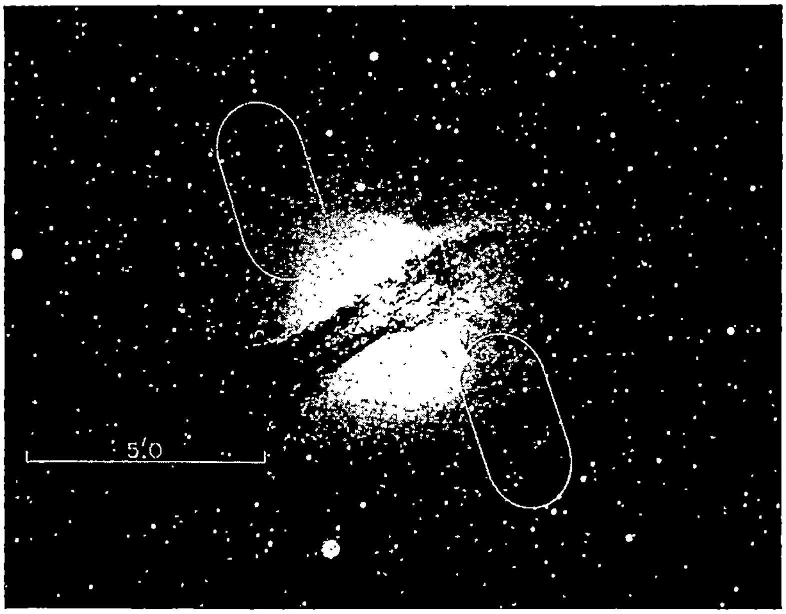


Fig. 2. Half-intensity contours of the two components in the central radio source of Centaurus *A*. The photograph of *NGC* 5128 is from the Mount Wilson and Palomar Observatories

Comparing the two maxima in Fig. 1*b*, I find that the maximum at 23h. 30m. is much lower. It may be shown that, if the source consisted of two circular components, the maximum at 23h. 30m. in Fig. 1*b* would have approximately the same value as the maximum at 1h. It is therefore reasonable to suggest that the components are elongated.

We are now in a position to derive a more detailed picture of the source. From the observations along an east–west base-line by Moffet[6], a diameter of about 2 min. of arc for each component is indicated. The curves in Fig. 1 were compared with those which would be produced by a model source consisting of two equal components 2·0′ in diameter, separated by 7·1′ along the major axis in position angle 46·5°. The elongation of the two components was found by comparing the observed and calculated curves. The interpretation was simplified by assuming that the two components have similar shapes. The shape of the components and their position is indicated in Fig. 2. A good fit to the observed curves was obtained. No significant deviation was present in Fig. 1*a*. In Fig. 1*b* the discrepancy between the calculated and the observed curve was found at the minima, where the calculated curve was equal to zero. Deviations between the curves were present between hour angles of 0 and 20m. and between 22h. 20m. and 22h. 50m. The deviation from the observed curve gave an upper limit of 1·3 to the ratio of the intensities of the components. This is in good agreement with Moffet's observations in the east–west direction[6].

From Fig. 2 it is evident that the radiation originates in a region outside the wide dust band. The major axis is approximately normal to the dust band. Each component is elongated approximately along the major axis of the extended source. It is interesting to note that the photographs of *NGC* 5128 in red light[7] show elongation of the galaxy along the same major axis as the central radio source.

It is significant to note that the sources lie on the polar axis of the galaxy. It is possible that the sources are associated with the escape of high-energy electrons from the galaxy through a polar fringing magnetic field. The non-existence of radio emission from the dust band may be explained as due to the presence of a high gas density in that region. Processes such as ionization loss will then dominate and the electrons will not lose energy by means of radio emission.

The programme of research in radio astronomy at the California Institute of Technology is supported by the U.S. Office of Naval Research under Contract *Nonr* 220(19). I am a recipient of a Fulbright travel grant and a grant from the Norwegian Research Council for Science and the Humanities.

P. MALTBY*

California Institute of Technology,
Pasadena,
California.

---

* From the Geophysical Institute, University of Bergen, Norway.

[1] Bolton, J. G., Stanley, G. J., and Slee, O. B., *Nature*, **164**, 101 (1949).

[2] Bolton, J. G., and Clark, B. G., *Pub. Astron. Soc. Pac.*, **72**, 29 (1960).

[3] Mills, B. Y., *Austral. J. Phys.*, **6**, 452 (1953).

[4] Twiss, R. Q., Carter, A. W. L., and Little, A. G., *The Observatory*, **80**, 153 (1960).

[5] Moffet, A. T., and Maltby, P., *Nature*, **191**, 453 (1961).

[6] Moffet, A. T. (in preparation).

[7] Baade, W., and Minkowski, R., *Astrophys. J.*, **119**, 215 (1954).

PHYSICS

Experiments on the Compression of Plasma in Cusp Geometry

CONTAINING plasma by cusp-shaped magnetic fields has for some time been considered a promising possibility because of the favourable stability properties predicted theoretically[1-4]. To investigate this configuration experimentally, an apparatus has been built in which shock pre-heated plasma is compressed by a rising cusped magnetic field.

The apparatus is shown in Fig. 1. The cylindrical 'Pyrex' discharge vessel is 20 cm. in diameter and 75 cm. long, and cone-shaped at each end to fit inside single-turn pre-heat coils. The two single-turn cusp field coils are of 22 cm. diameter with separation variable between 6 and 16 cm., and for the present experiments each coil was connected to a 600-μF. condenser bank rated at 40 kV. When charged to 24 kV., this provided an oscillating magnetic field of about 50 kilogauss at the line and point cusps, with a period of 50 μsec. The shock waves which ionize and pre-heat the initially neutral gas are produced by the simultaneous discharge of two auxiliary condenser banks into the pre-heat coils. The current in these coils rises to 200 k.amp. in a time of 1·5 μsec. Experiments have been carried out using hydrogen gas at pressures in the range 20–40 millitorr and argon and helium at somewhat lower pressures.

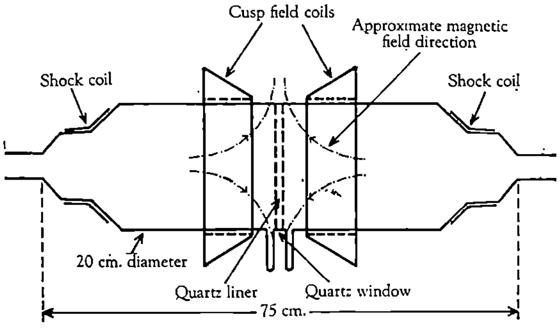


Fig. 1. Diagram of discharge vessel and associated magnetic field coils

With hydrogen at 22 millitorr, spectroscopic measurements on the bremsstrahlung and recombination continua (McWhirter, R. W. P., unpublished work) in the region of the Balmer series limit indicate that a plasma electron density of 2×10^{15} cm.$^{-3}$ and electron temperature of about 5×10^{4} °K. is produced by the shock pre-heating system.

The effect of the rising cusp field on the pre-heated plasma has been studied with a framing camera; examples of the observed phenomena are shown in Fig. 2. Fig. 2*a* shows the shocks approaching and meeting followed by the first half-cycle and part of the second half-cycle of the cusp field. Fig. 2*b* shows the growth of an instability after 2–3 μsec. in a typical case of the contraction velocity exceeding the estimated speed of sound. Fig. 2*c* shows an example of flute-type instability in helium in the very early stages of compression. Fig. 2*d* shows a condition in hydrogen in which no gross instability is observed and the full course of adiabatic compression can be seen.

It is suggested that the observed instabilities are of the Rayleigh–Taylor type[5], that is, resulting from the acceleration of the plasma by the magnetic field.

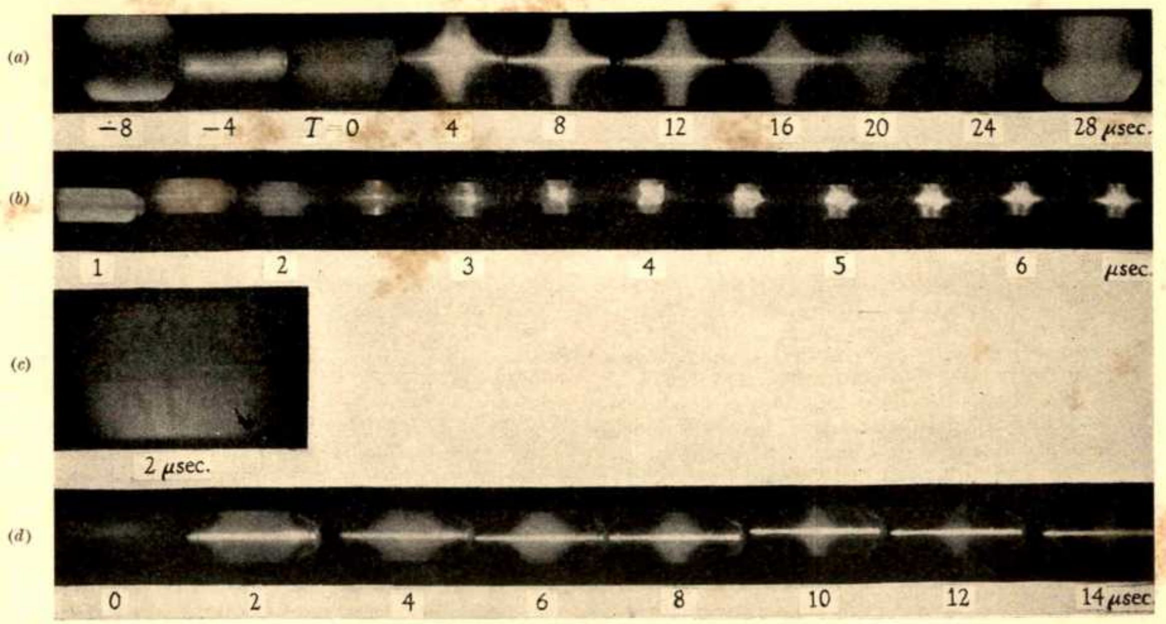


Fig. 2. Framing camera photographs of entire section of discharge tube between cusp field coils. The time at which the photographs are taken is marked. The cusp field starts rising at $T=0$. (*a*) Hydrogen–argon (5 per cent) mixture; 20 millitorr; coil separation, 16 cm.; exposure, 2 μsec. per frame. (*b*) Argon, 5 millitorr; exposure, 0·25 μsec. (*c*) Helium, 10 millitorr; exposure, 1 μsec. (*d*) Hydrogen, 35 millitorr; exposure, 1 μsec.

Such acceleration occurs during the initial stages of the compression (Fig. 2*c*) and, additionally, on the reflexion of complex strong shock patterns at the boundary in those cases where the contraction velocity exceeds the speed of sound in the pre-heated plasma (Fig. 2*b*). The criterion for stability in the relevant case of a plasma accelerated by a curved magnetic field is[1]:

$$\frac{B^2}{4\pi R} > nMf$$

where R is the radius of curvature of the magnetic field lines (taken positive for lines convex to the plasma), f is the plasma acceleration (positive for acceleration directed from the plasma towards the magnetic field), and nM is the plasma density. The observations are qualitatively consistent with this criterion in that the system is more unstable in heavier gases, and there is evidence of considerable stabilization during the later stages of compression when the sign of f changes. It should be emphasized that the instabilities discussed here are associated with the establishment of an equilibrium state, and no comprehensive results concerning the stability of such an equilibrium have been obtained in these preliminary experiments.

However, when operating conditions are such that these initial instabilities are not present, the cusp boundary appears to remain quite sharp during the first quarter-cycle. Analysis of framing camera pictures for some of the most stable and reproducible conditions, namely, 22 millitorr of hydrogen, gives volume compressions of about 15–20 after 8 μsec. Spectroscopic measurements at this time indicate an increase in density greater than 10 and an increase in electron temperature from 5×10^4 °K. to a value greater than the upper limit of 2×10^5 °K. measurable by the method.

We thank Mr. R. S. Pease for his helpful discussions, Mr. P. A. Worsnop whose engineering group built the apparatus, Dr. R. W. P. McWhirter for the spectroscopic measurements, and Mr. J. N. Burcham for much of the experimental work. The experiment profited from the initial design studies by Mr. J. G. Armstrong.

T. K. Allen
R. J. Bickerton

Atomic Energy Research Establishment,
United Kingdom Atomic Energy Authority,
Harwell, Didcot, Berkshire.

[1] Tuck, J. L., *U.S. Atomic Energy Comm. Rep., Washington*, **184**, 77 (1955).

[2] Grad, H., *U.S. Atomic Energy Comm. Rep., Washington*, **289**, 115 (1955); *U.S. Atomic Energy Comm. Rep., N.Y.O.* 7969 (1957).

[3] Berkowitz, J., *et al.*, *Proc. Second United Nations Conf. on Peaceful Uses of Atomic Energy*, **31**, 171 (1958).

[4] Kadmotsev, B. B., and Braginsky, S. I., *Proc. Second United Nations Conf. on Peaceful Uses of Atomic Energy*, **32**, 233 (1958).

[5] Taylor, G. I., *Proc. Roy. Soc.*, A **201**, 192 (1950).

A Proposal for a Tunable Source of Radiation for the Far Infra-red using Beats between Optical Masers

In this communication, the possibility is considered of generating radiation in the far infra-red by beats between optical masers.

Oscillations from optical masers have been detected at several different wave-lengths in the region of 7000 Å. using ruby[1,2], and also calcium fluoride doped with samarium. Calcium fluoride doped with uranium ions[3] has also been used to produce oscillations at 25,000 Å. So far these solid-state optical masers have only been operated in pulses, but seem to be potentially capable of continuous wave operation.

Recently, the dependence on temperature of the oscillation wave-length of a ruby optical maser has been investigated[4] and this has been found to vary from 6934 Å. at −180° C. to 6953 Å. at 210° C. If beats between a fixed frequency and a thermally tuned optical maser could be generated, then the difference signal could be tuned from centimetre to submillimetre wave-lengths. Indeed, there is no reason why the whole of the far infra-red spectral region could not be covered by selecting the frequency of oscillation of one optical maser and thermally tuning another. The spectral purity of the actual oscillation signal is determined chiefly by crystal imperfections, and typical values of oscillation line-width lie between 0·1 Å. and 0·02 Å. Thus a possible spectral width of about 1 Gc./sec. would be expected at the beat frequency, which would be narrow enough to be used as a tunable source of radiation in the far infra-red, even with the present state of the art of optical maser construction.

In a practical arrangement, one optical maser could be operated at, say, the temperature of liquid nitrogen, and its power output mixed with the output of a second optical maser temperature controlled to a pre-set value between 25° C. and 210° C. The wave-length of the beat signal would then be continuously variable over the range of 250μ–550μ. Such a wide tuning range (which undoubtedly could be extended by a larger variation of temperature or by using crystals with a larger temperature coefficient of oscillation wave-length) is unlikely to be equalled by a single maser operating directly in the far infra-red.

As the bursts of oscillation are only 0·5 msec. in duration, fairly exact synchronism of the oscillation bursts would be required if separate optical masers were to be used. However, two optical masers would not necessarily be required to produce oscillations at different wave-lengths, since experiments with concentrated ruby[2] have shown evidence of simultaneous oscillations at 7009 Å. and 7041 Å. Beats between these two signals would give an output signal of approximately 150μ wave-length. The problem of synchronism of oscillations would not arise provided that the energy from the ultra-violet flash-lamp is greater than the threshold-level necessary to maintain both oscillations simultaneously. Such an arrangement would be rather restricted in tuning range, since both oscillation wave-lengths would be shifted by approximately equal amounts on cooling or heating the crystal. On the other hand, this may be an advantage from the point of view of frequency stability of the beat signal.

Since the pulsed output of the optical maser is typically 10 kW., any system of optical mixing must be capable of handling this power without burning out. The non-linear properties of a mercury arc[5] or a cold cathode arc[6] have been exploited for the purpose of harmonic generation of submillimetre waves from a *Q*-band klystron, and it is suggested that the power handling capacity of the arc may be well suited to mixing optical maser signals. The output from each maser could be directed on to the cathode spot quite easily as the beam diameter is typically 2 mm. It seems conceivable that several watts of pulsed power could be generated in the far infra-red even if the mixing efficiency is only a few per cent.

In contrast to the ruby optical maser, the gas discharge type[7] is not continuously tunable over a wide band; nevertheless the exceptional spectral purity of the optical oscillation (which is many orders of magnitude narrower than in the ruby device) presents exciting possibilities as a source of beat frequency in the far infra-red. The gas optical maser has been operated at five different wave-lengths in the near infra-red and the strongest signal found at 11,530 Å. with a continuous wave output of 15 mW. The spectral purity has been measured to be in the region of 10–80 kc./sec. Thus beats only a few tens of kc./sec. wide between two optical masers operating at different spot frequencies should occur at wave-lengths of 180μ and 190μ as well as at several other wave-lengths between 15μ and 37μ. The beat frequency could probably be tuned over a narrow region by a small adjustment to the separation of the interferometer plates or by a variation of the gas pressure. Mixing of the optical signals could again be carried out by using a mercury or cold cathode arc as suggested before. Some sort of focusing would be necessary in this case. In this sort of way it should be possible to produce several tens of microwatts of continuous wave-power. Even this power-level, combined with the spectral purity of perhaps only a few kilocycles, presents a very attractive approach to the long-standing problem of suitable sources for high-resolution spectroscopy in the far infra-red.

D. C. LAINÉ

Department of Physics,
University College of North Staffordshire,
Keele.

[1] Collins, R. J., Nelson, D. F., Schawlow, A. L., Bond, W., Garrett, C. G. B., and Kaiser, W., *Phys. Rev. Letters*, **5**, 303 (1960).

[2] Schawlow, A. L., and Devlin, D. E., *Phys. Rev. Letters*, **6**, 96 (1961).

[3] Sorokin, P. P., and Stevenson, M. J., *Phys. Rev. Letters*, **5**, 557 (1960).

[4] Abella, I. D., and Cummins, H. Z., *J. App. Phys.*, **32**, 1177 (1961).

[5] Froome, K. D., *Nature*, **186**, 959 (1960).

[6] Froome, K. D., *Nature*, **188**, 43 (1960).

[7] Javan, A., Bennett, W. R., and Herriott, D. R., *Phys. Rev. Letters*, **6**, 106 (1961).

CHEMISTRY

A New Relation between the N—H Stretching Frequencies of Primary Amides

BELLAMY *et al.*[1], from the data obtained for a number of aromatic primary amines in dilute solutions, have shown that the N—H asymmetric and symmetric stretching frequencies are related by the expression $\nu_s = 345{\cdot}5 + 0{\cdot}875\ \nu_{as}$. The standard deviation of the calculated symmetric stretching frequencies from those of the recorded values is about 5 cm.$^{-1}$. These workers also mention that the same relation would not be obeyed in situations where the two N—H links are non-equivalent.

We[2,3] have recorded the N—H stretching frequencies of a number of primary amides in dilute solutions in the region of 3,530, 3,420, 3,350 and 3,200 cm.$^{-1}$ and assigned the first two absorption bands to the N—H asymmetric and symmetric stretching frequencies of the monomers and the other two to the same modes of vibration of associated molecules. Davies and Hallam[4], Davies and Evans[5], and Cleverley[6] have recorded the N—H stretching frequencies of a series of primary amides in dilute solutions. The data thus available for fourteen primary amides yield a simple linear relation between the asymmetric and symmetric stretching frequencies of the monomers recorded in the regions of 3,530 and 3,420 cm.$^{-1}$. A least-squares treatment shows that the frequencies of these two free N—H stretching vibrations are related by the expression $\nu_s = 1{\cdot}1214\ \nu_{as} - 542{\cdot}5$. In cases where the frequencies are reported by more than one group of workers, the mean values of the frequencies are used for the least-squares treatment. This relationship is obeyed for bands in the regions of 3,530 cm. and 3,420 cm.$^{-1}$, the average deviation of the calculated values from those of the recorded being about 3 cm.$^{-1}$. This relation is indicated in Fig. 1. But the same empirical relation does not hold for bonded N—H

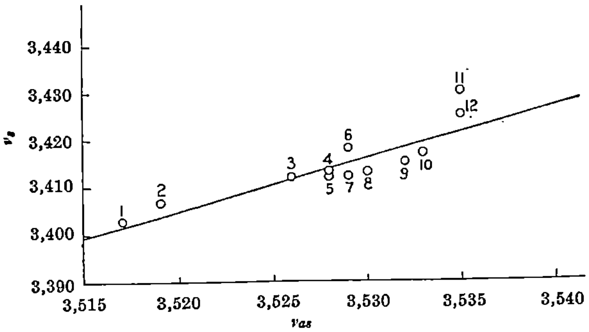


Fig. 1. 1, Trichloracetamide; 2, phenylacetamide; 3, *n*-valeramide; 4, *n*-butyramide; 5, *n*-decanamide and iso-butyramide; 6, acetamide; 7, *n*-dodecanamide and iso-valeramide; 8, *n*-octanamide; 9, *n*-heptanamide; 10, *n*-hexanamide; 11 benzamide; 12, formamide

asymmetric and symmetric stretching frequencies of the associated molecules in the region of 3,350 and 3,200 cm.$^{-1}$; the average deviation between the calculated and recorded ν_s frequencies is about 26 cm.$^{-1}$. This result is in agreement with our conclusions[3], since for the intermolecular associations proposed in our work on primary amides, it is considered that only one of the N—H links is bonded. The asymmetry thus introduced leads to the breakdown of this relationship.

The N—H stretching frequencies of four primary amides as recorded in solutions of dioxane[3] and the calculated ν_s values by the relation $\nu_s = 1{\cdot}1214\ \nu_{as} - 542{\cdot}5$ are given in Table 1.

Table 1. CALCULATED AND OBSERVED N—H STRETCHING FREQUENCIES OF PRIMARY AMIDES IN DIOXANE (CM.$^{-1}$)

Amide	N—H stretching bands		
	Observed		Calculated
	ν_{as}	ν_s	ν_s
Formamide	3,443	3,357	3,318
Acetamide	3,450	3,360	3,326
Benzamide	3,450	3,360	3,326
n-Butyramide	3,450	3,360	3,326

The smaller values for the N—H stretching frequencies of the primary amides in solutions of dioxane have been explained by us[3] on the basis that the intermolecular associations break up considerably in this solvent and the hydrogen atom of the amino group of the monomer amides form association with the donor group of dioxane. From the observations in Table 1, it is seen that the relationship proposed by us breaks down for the N—H stretching frequencies of the primary amides in dioxane. The difference between the calculated and observed ν_s frequencies is about 35 cm.$^{-1}$. This result indicates that it may not be sterically possible for two molecules of dioxane to bond to the same amino group of the monomer amides. Similar results were obtained by Bellamy and others[1] in their work on amines in solutions of dioxane.

P. G. PURANIK
K. VENKATA RAMIAH

Department of Physics,
University College of Science,
Osmania University, Hyderabad 7, India.

[1] Bellamy, L. J., and Williams, R. L., *Spectrochimica Acta*, 9, 341 (1957).

[2] Puranik, P. G., and Venkata Ramiah, K., *J. Mol. Spectroscopy*, 3, 486 (1959).

[3] Puranik, P. G., and Venkata Ramiah, K., *Proc. Ind. Acad. Sci.* (in the press).

[4] Davies, M., and Hallam, H. E., *Trans. Farad. Soc.*, 47, 1177 (1951).

[5] Davies, M., and Evans, J. C., *J. Chem. Phys.*, 20, 342 (1952).

[6] Cleverley, B., *Chemical Applications of Spectroscopy*, 514 (Interscience Pub., 1956).

Application of the Nickel Reaction and Thermal Isomerization of Alpha-Keto-Acid Hydrazones for their Chromatographic Analysis

THE nickel reaction, discovered by Katsuki *et al.*[1], produces a red colour with pure *cis* isomers of α-keto-acid-2,4-dinitrophenylhydrazones and differentiates them from their corresponding *trans* isomers. We have found that this reaction applies to isomeric differentiation of the keto-acid hydrazones on paper chromatograms by the following procedures:

After chromatography of the pyruvic acid hydrazones in the dark on Whatman No. 1 filter paper, using tertiary amyl alcohol/ethanol/water 5 : 1 : 4 as the solvent, the chromatogram was sprayed first with a 5 per cent (w/v) solution of nickel chloride in 70 per cent aqueous ethanol followed by a fresh 0·3 per cent suspension of zinc powder in aqueous ethanol. The paper was heated at 100° C. for a few minutes and the higher spot of the *cis* isomer turned reddish, whereas the lower *trans* spot did not. The *cis* spots of glyoxylic, phenylglyoxylic, phenylpyruvic and *p*-hydroxyphenylpyruvic acid-2,4-dinitrophenylhydrazones gave similar responses. Concentration of nickel chloride and zinc powder in alcohol and the time of heating may have to be changed depending on the intensity of chromatographic spots and the stability of the hydrazone isomers. As a matter of fact, even *trans* spots give colour responses for this reaction, if they are readily isomerized by heat. If the *cis* hydrazone of pyruvic acid is dissolved in ethyl acetate and dried on the paper, it will give an orange colour, after spraying with alcoholic nickel chloride without application of zinc powder. This does not occur if alcohol is used as the solvent, and the *trans* isomer gives no colour with either solvent.

A second useful method in distinguishing isomers involves thermal isomerization to the stable isomer. For example, a crude hydrazone from the products of anaerobic glycolysis of the sarcoma 37 solid tumour homogenates upon chromatography gave two spots for the *cis* and the *trans* isomers of pyruvic acid hydrazone respectively. However, if the crude hydrazone were heated at 120° C. for 15 min. before chromatography, only the *trans* isomer was observed, owing to thermal isomerization. Thus, it was clear that both original spots were due to pyruvic acid alone and not to two keto-acids. This identification could be confirmed by the use of the nickel reagents which coloured one of the spots in the original chromatogram and left the single spot of the second chromatogram uncoloured.

Knowledge of thermal decarboxylation of dinitrophenylhydrazones of certain β-keto-acids as well as of thermal isomerization may be combined with the nickel reaction advantageously for greater certainty. For example, from mouse urine isomeric spots for pyruvic and phenylpyruvic acid hydrazones were readily identified by such a combination. Possible misassignment of spots of pyruvic acid hydrazone as being due to acetoacetic acid hydrazone, which has an R_F close to that of pyruvic acid hydrazone isomers, was avoided, since heating the crude hydrazone before chromatography produced only a spot for *trans* pyruvic acid and none for acetone hydrazone which would have been formed from acetoacetic by thermal decarboxylation[2]. Similarly, confusion between the hydrazone of oxaloacetic acid which decarboxylates to pyruvic acid on heating, and of α-ketoglutaric acid, which does not decarboxylate, may be avoided. It should be noted that heating causes many aromatic keto-acid hydrazones to isomerize from *trans* to *cis*, the latter being the stable isomer in these cases, whereas the reverse behaviour is found with many aliphatic keto-acid hydrazones. The characterization of several aromatic keto-acid hydrazones will be presented elsewhere.

This investigation was aided by grant *C*-4342 from the National Cancer Institute, U.S. Public Health Service.

A. M. TOKUSHIGE

Division of Biochemistry,
Department of Biology,
Massachusetts Institute of Technology,
Cambridge,
Massachusetts.

E. S. COOK

Division of Chemistry and Biochemistry,
Institutum Divi Thomae,
Cincinnati,
Ohio.

---

[1] Katsuki, H., Sumizu, K., Moriwaki, T., Tanaka, S., and Hayashi, I., *Nature*, **181**, 639 (1958).

[2] Tokushige, A. M., and Cook, E. S., *Nature*, **184**, 267 (1959).

A Molecular Beam Reactor for examining the Rate, and Primary Products, of Reactive Bimolecular Collisions

A MOLECULAR beam technique has been developed which permits investigation of the primary products of reactive collisions between gas molecules or radicals. The principle of the technique can be described by reference to the schematic diagram in Fig. 1. A molecular beam of one gas, *A*, is formed at an aperture, 1, in the wall of the vessel, 2, and collimated by a second aperture, 3. The emergent beam from 3 crosses the reaction zone, 4, and leaves by a third aperture, 5, to enter a detector, 6. The latter may conveniently be a mass-spectrometer, so orientated that the molecular beam of *A* crosses the ionization chamber of the mass-spectrometer without impinging on the electrodes. With sufficient pumping speed for the collimating, reaction and mass-spectrometer compartments, the mass-spectrum peaks due to *A* are produced almost entirely from molecules which have experienced a collision-free transit from aperture 1 to the ionization region, and the contribution from 'background gas' molecules of *A* in these compartments can be made negligibly small. The second gas, *B*, is admitted into the reaction compartment at a pressure such that the mean free-path of *B* in *B* in the main body of this compartment is of the order of the molecular beam path-length in this compartment. Now let n_B be the average number of molecules of *B* crossing unit area within this compartment in unit time, and let n_A be the average number of molecules of *A* which cross unit area normal to the molecular beam path, in unit time, in the reaction zone. Two limiting cases are conceivable. If $n_A \ll n_B$, then molecules of *B* will effuse according to the cosine distribution law into the mass-spectrometer compartment. Thus the *B* molecules detected there will have arrived there as a result of collisions with other *B* molecules in the reaction zone. On the other hand, if $n_A \gg n_B$ then any molecule of *B* in the reaction zone has a much greater probability of collision with a molecule of *A* than with another molecule of *B*. Thus each *B* molecule detected in the mass-spectrometer will have arrived there by a collision-free path from a collision with an *A* molecule in the reaction zone. As a first approximation the rate at which such molecules reach the detector is the same as for the case of molecular effusion in the absence of the beam of *A* molecules. If, therefore, *A* and *B* are molecular species which react together, it is possible to detect the primary products of the reactive collisions in the reaction zone.

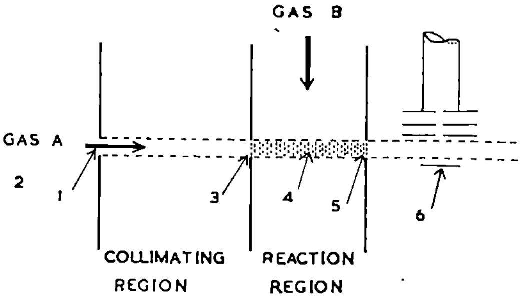


Fig. 1

This technique has been used for the study of the reaction of excited oxygen atoms with nitrogen dioxide:

$$O^* + NO_2 \rightarrow O_2 + NO \qquad (1)$$

The atomic oxygen was produced by an electrodeless radio-frequency discharge (10 Mc./sec.) through oxygen gas at 0·1 mm. mercury pressure in a tubular, hard-glass discharge tube. A round hole, 2·46 mm. diameter in the end of the tube, served as the first aperture, 1, of the molecular beam system. The molecular beam collimating system and the mass-spectrometer have been described elsewhere[1]. Mass-spectrometric study of the molecular beam showed that about 15 per cent of the oxygen molecules were dissociated into atoms, which were almost entirely in a metastable, electronically excited state with 2-eV. excitation energy. Nitrogen dioxide was introduced at a pressure about 10^{-4} mm. mercury into the reaction compartment (compartment C_2 of Fig. 3, ref. 1). Without the discharge operating, and using 70-eV. electrons for ionization in the mass-spectrometer, the ratio of the height of the $m/e = 46$ peak to that of the $m/e = 30$ peak from the nitrogen dioxide was 0·52. When the discharge was switched on, the ratio was reduced to 0·42, indicating formation of nitrous oxide by collisions of the atomic oxygen beam with nitrogen dioxide in the reaction zone. Exact quantitative assessment of the reaction efficiency of the collision process must await more precise measurements, but the preliminary figures suggest that the efficiency is considerably greater than the minimum limit of 1 reactive collision in 100 collisions proposed by Kistiakowsky and Kydd[2] for ground-state oxygen atoms.

While these preliminary results have served to demonstrate the potentialities of the technique, application is, at present, limited to collision processes having high reaction efficiencies. This restriction arises from the relatively small mass-spectrum peaks of reactant *B*, obtained with the technique described above. Preliminary experiments have shown that some improvement may be achieved by introducing reactant *B* also as a molecular beam aligned to intersect the beam of *A* in the reaction zone. Application of the technique to reactions with large activation energies may be possible by simulation of high-temperature conditions by the use of fast molecular beams of reactant *A*.

We record our appreciation of the helpful discussions and continued interest of Dr. W. J. Dunning and Prof. F. H. Garner during the development of the technique. One of us (A. J. B.) is indebted to the Department of Scientific and Industrial Research for a research scholarship.

C. W. Nutt
A. J. Biddlestone

Department of Chemical Engineering,
The University,
Birmingham 15.

[1] Nutt, C. W., *et al.*, *Trans. Farad. Soc.*, **55**, 1500, 1516 (1959).
[2] Kistiakowsky, G. B., and Kydd, P. H., *J. Amer. Chem. Soc.*, **79**, 4825 (1957).

Average Extinction Coefficient for Re-absorption of Fluorescence Radiation through Uranin Solution

The absorption and the fluorescence spectra of fluorescein in alkali solution overlap considerably. This overlap introduces a certain amount of error in the measurement of intensity of fluorescence in so far as it is not directly proportional to the incident radiation absorbed by the solution. If a fluorescent quantum is emitted somewhere deep in the solution, then during its passage from its point of emission to a measuring device like a photocell, there is a chance that it will be repeatedly re-absorbed and re-emitted by the solution. The consequence of this process is that the average life-time of the excited molecule is increased[1], as each absorption gives a fresh lease of life of about 10^{-8} sec. The effect will be greater, the higher the concentration of the fluorescing solution. Since intensity of fluorescence is directly proportional to the life-time, τ, of the fluorescent molecule under a given set of conditions $\tau_1/\tau_2 = F_1/F_2$, the intensity as measured by the photocell will not be the true intensity. The effect has been explained in different ways[1-3].

To assess the effect of the absorption–re-emission process, it is desirable to know ε_2, the extinction coefficient for the re-absorption of secondary radiation by the experimental solution. The usual method for the determination of the extinction coefficient cannot be applied in the determination of ε_2 because of the polychromatic nature of the fluorescence radiation, the band-spread of which is nearly 1μ in the green region. A plot of $\log I_0/I$ versus C for a constant solution thickness, l, does not give a straight line as obtained for a solution obeying Lambert–Beer's law using monochromatic radiation. Besides this, the secondary re-emission process may also have some effect. That the latter effect is not so important can be shown by comparing measurements for a quenched and an unquenched solution under identical experimental conditions.

The Phoenix light-scattering instrument model 1,000, with single photomultiplier, was adapted for fluorescence measurements by inserting a filter transmitting 3650 Å. in the incident light beam. The photomultiplier was set at 90° to the incident beam. An alkaline fluorescein solution of appropriate strength ($1{\cdot}0 \times 10^{-4}$ *M*) was placed in a cell, 2 cm. wide and 3·7 cm. long. It was placed so that the incident beam fell just near the edge of the shorter side of the cell, exciting fluorescence radiation along the length of the solution. The longer side of the cell, facing the photomultiplier, was covered with black paper except for a small rectangular opening (0·3 mm. wide) to allow the fluorescent radiation to escape. This acted as a source of fluorescence radiation.

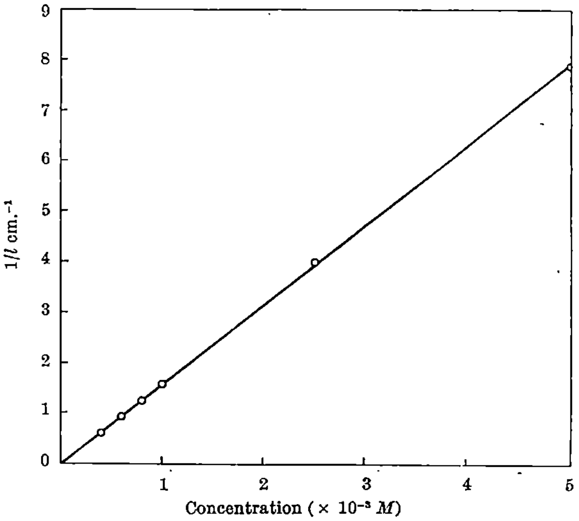


Fig. 1

Another cell of the same dimensions was placed in juxtaposition to the emitting cell, snugly between the latter and the photomultiplier. A yellow gelatine filter was placed in front of the photomultiplier to cut off any scattered primary radiation. The second cell contained alkaline fluorescein solution of various concentrations and the intensity of transmitted light was measured against water as the solvent blank. The plot of $\log I_0/I$ versus C was a curved line. The curvature was still there, when the same amount of potassium iodide solution was added to all the solutions to quench the fluorescence.

Now Lambert's law is known to have no exception. But for a diffuse, polychromatic and faint source like the fluorescence radiation, the use of cells of different dimensions to change the depth of the solution will mean introduction of an air gap between the source and the solution or between the solution and the photomultiplier. This will mean a loss of fluorescence radiation due to change in refractive index at the cell-air interface. To avoid this, the light-path was varied by inserting microscope glass-slides of known thicknesses into the 2-cm. cell placed next to the emitting cell. For each variation of light-path, l, readings were taken with the solvent blank (water) giving 100 per cent deflexion in the galvanometer. It was then replaced by the solution of known concentration, C, and the transmission of fluorescent light was measured in terms of galvanometer deflexion. For a given thickness, transmission was measured for different concentrations of the solutions ranging from 5×10^{-5} *M* to 1×10^{-2} *M*. By this technique, the thickness of the solution could be varied from 2 cm. to 0·05 cm.

For each concentration, the percentage transmission was plotted against the solution thickness. Curved lines were obtained, the curvature increasing with the concentration. The plot of $\log I_0/I$ against l was also curved because of the polychromatic nature of the radiation.

From a re-arrangement of Lambert–Beer's law we have :

$$\frac{1}{l} = \frac{\varepsilon_2 C}{\log I_0/I}$$

For a fixed percentage transmission, say 50 per cent:

$$\frac{1}{l} = \frac{\varepsilon_2 C}{\log 2} = \frac{\varepsilon_2}{0{\cdot}3010} C$$

Therefore, a plot of $1/l$ versus C should be a straight line passing through the origin. The slope of this line will be equal to $\varepsilon_2/0{\cdot}3010$ from which ε_2 can be calculated. The value so obtained will be the average value of the molar extinction coefficient over the whole wave-length range of fluorescence radiation.

The experimental data are plotted in Fig. 1. From the graph the slope of the straight line, $\varepsilon_2/0{\cdot}3010 = 1{,}600$ l.mole^{-1} cm.$^{-1}$ Therefore, $\varepsilon_2 = 481{\cdot}6$ l.mole^{-1} cm.$^{-1}$.

This method takes advantage of the fact that the total number of absorbing centres throughout the experiment remains constant, so that all the interfering effects cancel out. Virtually, the experiment is carried out at a constant value of Cl in accord with the general principle of visual colorimeters. Further calculations show that the value of ε_2 so obtained is a function of the optical density of the measured solution.

We thank the Council of Scientific and Industrial Research, India, for the award of Senior Research Fellowship to one of us (G. S. S.) and also for sponsoring the above scheme. We also express our gratitude to Prof. S. N. Mukerjee, head of the Department of Chemistry, Jadavpur University, for his encouragement.

K. K. ROHATGI
G. S. SINGHAL

Jadavpur University,
Calcutta 32.

[1] Rollefson, G. K., and Dodgen, H. W., *J. Chem. Phys.*, **12**, 107 (1944).

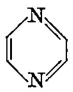
[2] Umberger, J. Q., and LaMer, V. K., *J. Amer. Chem. Soc.*, **67**, 1099 (1945).

[3] Bailey, E. A., and Rollefson, G. K., *J. Chem. Phys.*, **21**, 1315 (1953).

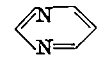
Polarization of Triplet→Singlet Emission Spectra

PHOSPHORESCENCE in homogeneous rigid media was identified by Lewis, Kasha and Calvin[1] during 1941–45 as triplet→singlet emission spectra. Simultaneously Weisman and Lipkin[2] were able to show by a wide-angle interference technique that these emissions are electric dipole transitions. Following these investigations, selection rules for triplet–singlet transitions, based on the triplet states undergoing spin-orbit coupling due to the spin magnetic moment of the electrons interacting with the molecular field, were given by McClure[3] and placed on a rigorous basis by Weisman[4]. Recently, Hutchison and Mangum[5], by observing paramagnetic resonance absorption in the phosphorescent state of naphthalene, have confirmed the triplet assignment.

The triplet–singlet transitions, forbidden by multiplicity selection rules, can be made allowed by the spin-orbit perturbations, which mixes states of different multiplicity. Thus the emitting triplet state, T, can be perturbed by one or more of the singlet states, S_k, through spin-orbit coupling and acquire a singlet character, the transition to the ground-state, S_0, being allowed by this singlet character. Since the components of the spin-orbit coupling operator, $(\text{grad } \mathbf{V} \times \mathbf{P}) \cdot \mathbf{S}$, transform like the components of the rotation operator, S_k should have the same symmetry as the product of symmetries of T and the rotation operator. According to this scheme the $T \rightarrow S_0$ spectrum should be polarized parallel to the $S_0 \rightarrow S_k$ absorption. Further, it should be parallel or perpendicular to other singlet–singlet transitions in the molecule depending on whether they are parallel or perpendicular to $S_0 \rightarrow S_k$. This can be confirmed directly, in favourable cases, by measuring the relative polarization of triplet→singlet bands with respect to any two mutually perpendicularly polarized singlet–singlet absorption bands in the molecule. While there is strong indirect evidence[1] from life-time measurements and heavy-atom effects for spin-orbit coupling perturbations in molecules, there is no direct confirmation of the mixing of the triplet with the singlet states, since polarization work up to now[6,11] has involved molecules where unambiguous theoretical prediction and experiment have not been possible.

Pyrazine, , is well suited for such an experiment for the following reasons. (1) the triplet→singlet emission has been assigned beyond doubt[7] as ${}^3B_{2u}(n,\pi^*)\rightarrow{}^1A_g$. (2) The two singlet–singlet transitions at 3300 Å. and 2600 Å. corresponding to ${}^1B_{2u}(n,\pi^*)\leftarrow{}^1A_g$ and ${}^1B_{3u}(\pi,\pi^*)\leftarrow{}^1A_g$ are well separated and their polarizations are known to be respectively along the axis perpendicular to the plane of the ring[8]; and perpendicular to the N—N axis but in the plane of molecule[9]. (3) It is predicted[10] unambiguously from the rotational nature of the spin-orbit coupling operator that all planar azine $\pi^*\rightarrow n$ triplet→singlet emissions should be polarized in the plane of the molecule, and in the case of pyrazine the nodal properties of the π^* and n orbitals lead to the prediction that the triplet→singlet emission should be polarized along the N—N axis[10], that is, perpendicular to both the singlet–singlet transitions at 3300 Å. and 2600 Å.

We have determined the polarization of the triplet→singlet emission spectrum throughout the band system relative to the ${}^1B_{2u}(n,\pi^*)\leftarrow{}^1A_g$ and to the ${}^1B_{3u}(\pi,\pi^*)\leftarrow{}^1A_g$ absorptions. The method consists in exciting into each of the absorption band systems using narrow band monochromatic polarized light at 77° K. in thin (1–3 mm. thick) rigid media and measuring the polarization of the resulting phosphorescence. Great care has been taken to reduce rotational depolarization, and under the experimental conditions this is small in spite of the long life-time of the phosphorescence. The degree of polarization, defined as $P = \dfrac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$, where $I_{\parallel}$ and $I_{\perp}$ are intensities of the emitted radiation parallel and perpendicular to the electric vector of the polarized exciting light, theoretically is $0{\cdot}50$ if the emission is polarized parallel to, and $-0{\cdot}33$ if it is polarized perpendicular to, the polarization of the exciting radiation[11]. In pyrazine for ${}^1B_{2u}(n,\pi^*)\leftarrow{}^1A_g$ and ${}^1B_{3u}(\pi,\pi^*)\leftarrow{}^1A_g$ excitations, P for the ${}^3B_{2u}(n,\pi^*)\rightarrow{}^1A_g$ phosphorescence emission is found to be $-0{\cdot}26$. The deviation from the ideal value of $-0{\cdot}33$ is probably due to depolarization factors in the optical system and in the rigid media and not due to the inherent nature of the emission. The above results leave only one possibility for the polarization of the triplet→singlet emission of pyrazine: along the N—N axis of the molecule, confirming the predicted spin-orbit coupling scheme in this molecule.

Further, experiments on the pyrimidine  $\pi^* \rightarrow n$ phosphorescence emission also conclusively yield in plane polarization, conforming to the spin-orbit coupling prediction. A detailed account of this work will be published in the *Journal of Chemical Physics*.

V. G. Krishna†
Lionel Goodman

Whitmore Chemical Laboratory,
Pennsylvania State University,
University Park, Pennsylvania.

---

† Esso Research and Engineering Predoctoral Fellow.

[1] Kasha, M., and McGlynn, S. P., *Ann. Rev. Phys. Chem.*, **7**, 403 (1956). Hameka, H. F., and Oosterhoff, L. J., *Molecular Physics*, **1**, 358 (1958).
[2] Weisman, S. I., and Lipkin, D., *J. Amer. Chem. Soc.*, **64**, 1916 (1942).
[3] McClure, D. S., *J. Chem. Phys.*, **17**, 665 (1949).
[4] Weisman, S. I., *J. Chem. Phys.*, **18**, 232 (1950).
[5] Hutchison, C. A., and Mangum, B. W., *J. Chem. Phys.*, **29**, 952 (1959).
[6] Lewis, G. N., Lipkin, D., and Magel, T. T., *J. Amer. Chem. Soc.*, **63**, 3005 (1941). Weisman, S. I., *J. Chem. Phys.*, **18**, 1258 (1950). Williams, R., *ibid.*, **30**, 233 (1959).
[7] Goodman, L., and Kasha, M., *J. Mol. Spec.*, **2**, 58 (1958).
[8] Innes, K. K., *et al.*, *Nature*, **187**, 500 (1960).
[9] Craig, D. P., *Revs. Pure and Appl. Chem.* (Australia), **3**, 207 (1953). Albrecht, A. C., and Simpson, W. T., *J. Amer. Chem. Soc.*, **77**, 4457 (1955).
[10] Clementi, E., and Kasha, M., *J. Mol. Spec.*, **2**, 297 (1958). Sidman, J. W., *ibid.*, **2**, 333 (1958).
[11] Pringsheim, P., *Fluorescence and Phosphorescence* (Interscience, 1948).

Deterioration of Solid-coated Capillary Columns on Standing

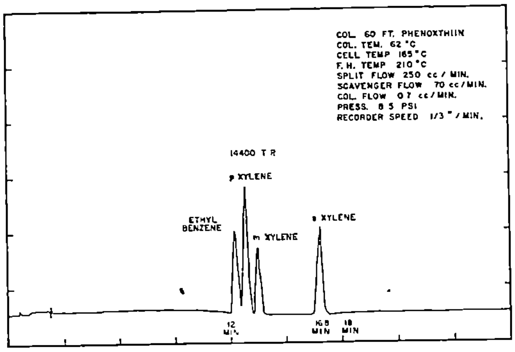
The recent introduction of capillary columns, which are able to give efficiencies of up to 1,000,000 theoretical plates[1], has given a great stimulus to the technique of gas chromatography. Many separations, heretofore impossible with packed columns, are extremely simple when capillary columns are used.

Although we have generally been successful with capillaries, we have recently noted a disturbing phenomenon when using them. The phenomenon referred to is the fact that at least some solid-capillaries will undergo a severe diminution of column efficiency on standing.

Fig. 1 shows the performance of a newly prepared 60 phenoxythiin capillary column. The column was prepared by coating a 0·010 in. internal diameter stainless steel capillary with 10–12 mgm. of phenoxythiin. 10 ml. of a 10 weight per cent solution of phenoxythiin in ethylene chloride were passed slowly through the column under a pressure of 10 p.s.i.g. of nitrogen. After the excess solution had all passed through the capillary, the flow of nitrogen was maintained overnight, at room temperature. The column was then conditioned for 4 hr. at 60° C. and then put into service, with the result shown in Fig. 1. The column continued to give equivalent performance for four weeks, during which time at least fifty samples were analysed on it.

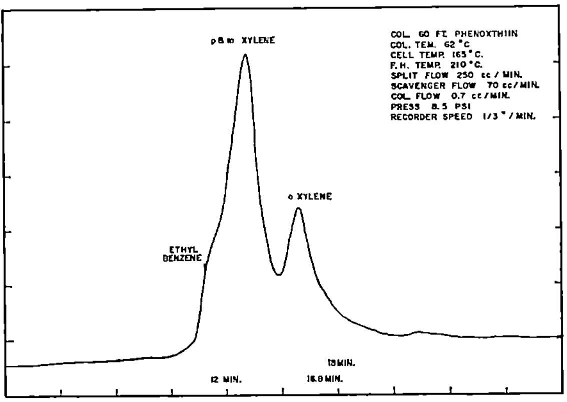
The column was then taken from the chromatograph and stored (at room temperature) for six months. Both ends of the column were opened to the atmosphere during storage.

On attempting to re-use this column, we noted the extreme deterioration of efficiency shown in Fig. 2, which is a run made under identical conditions, with the same column and the sample as the run shown in Fig. 1. While the lack of resolution in Fig. 2 makes it difficult to calculate column efficiency exactly, a rough calculation indicates that the efficiency has dropped from 14,400 theoretical plates in Fig. 1 to not more than 200 theoretical in Fig. 2.



Time

Fig. 1



Time

Fig. 2

A similar behaviour has been observed with columns coated with 7,8-benzoquinoline, another solid material. The deterioration of column efficiency was as extreme with this material as with the phenoxythiin.

We are at a loss to give a full explanation for this phenomenon. Two possibilities present themselves: (*a*) both the phenoxythiin and the 7,8-benzoquinoline were exposed to atmospheric oxygen during the standing period for at least six months and were hence subject to oxidation or other processes of decomposition; (*b*) on removal of the column from the heated chromatographic oven, and subsequent cooling, the substrates may have recrystallized in such a fashion that on re-melting they formed globules instead of a uniform coating. In support of the second possibility, we have noted that the appearance of Fig. 2 is strikingly similar to chromatograms made with glass capillaries, where we could observe the presence of substrate globules.

Fortunately, this behaviour appears to be restricted to solid-coated capillaries. We have not observed it in any of the liquid-coated (squalane, tri-cresyl phosphate, dioctyl sebacate) capillaries we have made. While it would be premature to predict such behaviour for all solid substrates, it does appear that this is a point worth further investigation, since phenoxythiin and 7,8-benzoquinoline are two of the most effective separators yet found for the lower aromatics.

GEORGE M. ROUAYHEB
WILLIAM C. HAMILTON

Research and Development Department,
Continental Oil Co.,
Ponca City,
Oklahoma.

---

[1] Zlatkis, A., and Kaufman, H. R., *Nature*, 184, 2010 (1959).

Adducts of Urea and Thiourea obtained by means of a Sublimation Technique

THE classical methods for obtaining adducts of urea and thiourea with some classes of organic substances imply the use of aqueous or alcoholic solutions of 'reagents' (urea or thiourea). Other methods have been described more recently by which adduction is obtained by mixing solid urea directly with the proper 'reactant'[1].

It has been found that the adduction of the urea-hydrocarbon type is actually a two-phase process involving urea in the solid state. The solvents for urea seem to have no function other than that of precipitating the reagent in the finely divided form suitable for interaction with the hydrocarbon[2].

All the adduction processes with solid urea usually require an appreciable induction time, which is necessary for the transition of the reagent from its normal crystalline form to that of the adduct including the reactant molecules. This induction time is usually shortened by reducing the urea in minute particles and adding small quantities of acetone or other simple ketones. The catalytic action of acetone is explained by way of having a part in the formation of an intermediate unstable adduct with urea[3].

In order to perform an adduction process with highly divided solid reagent, avoiding any mechanical stress on the adduct, we directly condense sublimated urea (or thiourea) on the liquid reactant placed on a cooled surface. The sublimated urea and thiourea, when in contact with an adductible material, crystallize around the matrix molecules as adduct, while, if some non-adductible material is placed on the cold surface, recondensation of urea and thiourea takes place in the normal crystalline form (tetragonal and orthorhombic respectively).

The process, which is actually rather fast, may be easily followed and successfully used for analytical purposes making use of the differences between the infra-red spectra of urea (and thiourea) in the normal crystalline form and in the adduct form[4–7]. The infra-red spectrum of the suspension obtained through adduction (in excess of reactant) clearly reveals whether an adduction has taken place. The spectrum may show also an excess of reactant, which in general does not disturb the detection of the main bands of the adduct. The excess reactant, anyway, can be washed away with pentane. In most cases the potassium bromide (sodium chloride for thiourea) pressed disks technique can be used without decomposition of the adducts.

The present technique of adduction 'via sublimation' may be performed with high-boiling adductible liquid molecules in a conventional laboratory sublimator using the vacuum supplied by a rotary oil pump. At a pressure of 0·1 mm. mercury, the experimental temperature is about 120° C. for urea and 140° C. for thiourea.

I have used this technique with a number of compounds. With urea I have adducted straight-chain paraffins, saturated and unsaturated fatty acids and esters as well as some alcohols and ketones. With thiourea I adducted several branched paraffins and naphthenic hydrocarbons.

The method is useful for the investigation of the adductibility of pure substances, thus providing information about their molecular shapes. The quantity of the product required is about 1 mgm. or less.

Furthermore, it is possible to detect small quantities of adductible materials in complex mixtures. For example, in a 1 per cent mixture of cetane in decaline the sublimated urea condensates in the hexagonal form (adduct), apparently until molecules of cetane are available. Then the condensation of urea takes place in the normal tetragonal form. This fact allows us to extend the investigation to solid materials (for example, higher hydrocarbons and their hydroxylated or carboxylated derivatives), when dissolved in a non-adductible high-boiling liquid.

Care must be taken to avoid in this case the deposition of a too thick layer of sublimated urea (or thiourea) on the liquid surface. As non-adductible solvents I used a high-boiling naphthenic oil for urea and an aromatic oil for thiourea. Although in this case we can find, besides urea or thiourea as adducts, even some reagent in the normal crystalline form, the complexing of the adductible fraction dissolved in the oil may be detected from the inspection of the shape of some characteristic band in the infra-red spectrum of the resulting suspension.

The regions which appear more characteristic for this inspection are near 3,400 cm.$^{-1}$ (ν(N—H) vibrations), near 1,650 cm.$^{-1}$ ($\delta(NH_2) - \nu(C=O)$ vibrations for urea and $\delta(NH_2)$ vibration for thiourea) and in the 700–800 cm.$^{-1}$ range (ν(C—C—N) for urea and $\nu(C=S) - \nu$(N—C—N) for thiourea), as can be seen

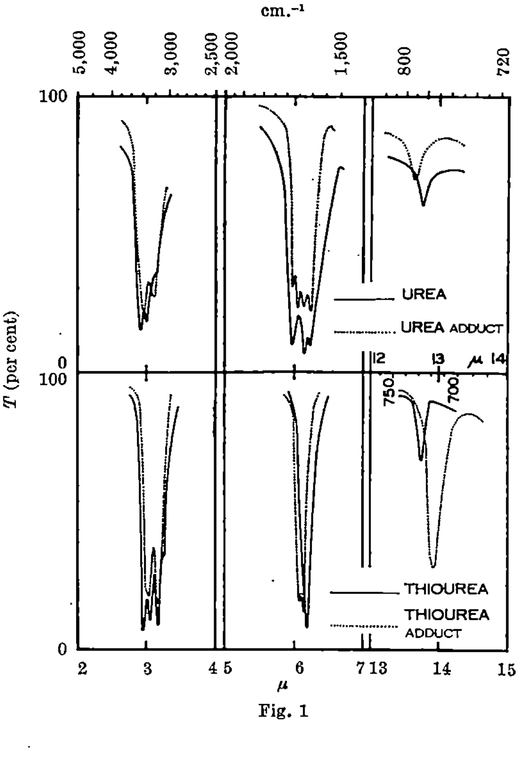


Fig. 1

from Fig. 1. The 3,400 cm.$^{-1}$ region is particularly suitable when hydrocarbons or any of their derivatives not containing —OH or —NH— groups are investigated about adductibility versus urea or thiourea or are employed as solvents for solid materials.

B. Casu

Infra-red Spectroscopic Laboratory,
Stazione Sperimentale Combustibili,
Istituto Scientifico di Chimica 'G. Ronzoni',
Milano.

[1] Schlenk, W., "Herstellung und methodische Verwendung von Einschlussverbindungen", in Houben-Weil's treatise *Methoden der Organischen Chemie*, **1**, 1, 391 (1958).

[2] Calderbank, P. H., and Nikolow, N. S., *J. Phys. Chem.*, **60**, 1 (1956).

[3] Rigamonti, R., Panetti, M., and Gay, R., *Riv. Combustibili*, **12**, 451 (1958).

[4] Caroti, G., and Casu, B., *Riv. Combustibili*, **12**, 461 (1958).

[5] Barlow, G. B., and Corish, P. S., *J. Chem. Soc.*, 1706 (1959).

[6] Mecke, R., and Kutzelnigg, *Z. Anal. Chem.*, **170**, 114 (1959).

[7] Fischer, P. H. H., and McDowell, C. A., *Canad. J. Chem.*, **38**, 187 (1960).

BIOCHEMISTRY

Chromatographic Separation of Biologically Active Components from Commercial Trypan Blue

Trypan blue has been shown to produce reticulosis and reticulo-sarcoma as well as congenital malformations when injected subcutaneously into rats[1,2]. Considerable differences in the biological activity of various commercial preparations of trypan blue have been found, and it has been suggested that these are due to variations in the amount and kind of impurities present[3,4]. Commercial trypan blue is known to contain a red impurity which is more diffusible than trypan blue itself and which is excreted rapidly in the urine[5-7]. This material has been separated from trypan blue by extraction with ethyl alcohol[8] or with acetone[7] or by passing through a cellulose column[7,9]. Although this red fraction causes some resorptions, when injected into pregnant rats, it does not appear to produce congenital malformations[4,9]. This suggests the possible presence of other active impurities in trypan blue.

We have fractionated commercial trypan blue (Merck) on alumina and have separated a purple impurity in addition to the red fraction. The former was highly active in inducing reticulosis in rats, whereas the latter was inactive. Purified trypan blue itself showed only slight activity. The crude dye (6 mgm. in 6 ml. methylethylketone/water/ethanol 75:25:10) was placed on a column (12 × 2·2 cm.) of alumina (B.D.H. or Merck, 50 gm.) and eluted successively with methylethylketone/water/ethanol (75:25:10), water and 0·01 *N* sodium hydroxide. The methylethylketone/water/ethanol eluted first a reddish fraction ($\lambda_{max.}$ 560mμ), followed by the main blue fraction ($\lambda_{max.}$ 600 mμ). Water eluted a single purple fraction ($\lambda_{max.}$ 570 mμ) as did 0·01 *N* sodium hydroxide. The contribution of the various fractions to the absorption of the crude dye at 580 mμ is shown in Table 1.

The red and blue fractions each gave a single band when rechromatographed on alumina. The former could, however, be resolved into two components, a faster moving red-violet dye and a slower blue-violet dye, by paper chromatography with 80 per cent aqueous ethanol as solvent. In this system the blue fraction moved very slowly and the purple fraction not at all. On rechromatographing the purple fraction on alumina, approximately half the material could be eluted with water; the remainder only came off with 0·01 *N* sodium hydroxide. It thus appears as if the sodium hydroxide fraction were derived from the water fraction as a result of irreversible adsorption of the dye on alumina.

Table 1. Relative Contribution of Various Fractions to the Optical Absorption of Crude Trypan Blue at 580 mμ

	Per cent
Red (violet)	12
Blue	76
Purple (water fraction)	5
Purple (sodium hydroxide fraction)	7

In order to prepare sufficient of the various fractions for biological testing 10 gm. of Merck's trypan blue was applied as a 0·5 per cent solution in methylethylketone/water/ethanol (75:25:10) to an alumina column (12 × 4·5 cm.) Under these conditions the violet components (red fraction) passed through the column without hold-up, while the blue fraction was retained until the column was saturated with blue dye. It was then eluted with methylethylketone/water/ethanol solvent. The purple fraction was finally eluted with water, and the material which remained on the column was discarded.

After removal of the solvent, each of these fractions was tested for its power to induce proliferation of the reticulo-endothelial system of rat liver, in a group of five female rats of the same age. The red fraction was used as a 0·5 per cent solution, and the blue and purple fractions as 1 per cent solutions in distilled water. Each rat received two subcutaneous injections of 1 ml. at an interval of a fortnight, and all rats were killed seven days after the last injection, that is 21 days after the beginning of the experiment. A further group of five rats served as controls and was treated with crude trypan blue (1 per cent) in the same manner.

No observable reticulosis developed in any of the five rats treated with the red fraction. The blue fraction caused a mild portal reticulosis which was very much less marked than that observed in rats treated with crude trypan blue. In each of the five rats the purple fraction produced an intense portal as well as an intralobular reticulosis associated with the formation of numerous small cysts in the portal tract. In some regions, there was increased vascularity between the small cysts, leading to collapse of the cysts and accentuation of the portal reticulosis. The reticulosis produced by the purple fraction was of far greater intensity than that observed in the animals treated with the unfractionated trypan blue.

From these results it appears that the activity, so far as proliferation of the reticulo-endothelial cells of rat liver is concerned, is associated with the purple fraction. The chromatographic behaviour of this active fraction differs considerably from that of the well-known red fraction, which was separated by 80 per cent ethanol into two components and which appears to be inactive with respect to induction of reticulosis as well as to production of congenital malformations. Trypan blue itself has only slight activity ; but the possibility that its activity is due to contamination with purple dye cannot be excluded.

Elucidation of the structures of the various components of trypan blue is in progress.

Our thanks are due to Dr. Christine Gilbert, who was responsible for the animal experimental aspects of the project, and to Dr. H. M. Schwartz and Dr.

F. J. Joubert for helpful discussions. This work was sponsored by the National Cancer Association of South Africa.

J. Dijkstra

National Chemical Research Laboratory,
South African Council for
Scientific and Industrial Research,
Pretoria.

J. Gillman

Department of Physiology, and
Joint Council for Scientific and Industrial Research,
University of the Witwatersrand Nutrition Unit,
Johannesburg.

[1] Gillman, J., Gilbert, C., Gillman, T., and Spence, I., *S. Afr. J. Med. Sci.*, **13**, 47 (1948).

[2] Gillman, J., Gillman, T., and Gilbert C., *S. Afr. J. Med. Sci.*, **14**, 21 (1949).

[3] Simpson, C. L., *Brit. J. Exp. Path.*, **33**, 524 (1952).

[4] Tuchmann-Duplessis, H., and Mercher-Parot, L., *Biologie medicale*, **48**, 238 (1959).

[5] Von Möllendorf, W., *Deut. med. Wochenschr.*, **40**, 570 (1914).

[6] Efskind, L., *Acta Medica Scand.*, **103**, 382 (1940).

[7] Kelly, J. W., *Stain Tech.*, **33**, 79 (1958).

[8] Hartwell, J. L., and Fieser, L. F., *Organic Synthesis*, **16**, 12 (1936).

[9] Beck, F., Spencer, B., and Baxter, J. S., *Nature*, **187**, 605 (1960).

Stimulation of Lipogenesis in Thyroid Tissue *in vitro* by Free Fatty Acids

Recently, it has been demonstrated that some of the *in vitro* actions of certain pituitary hormones on adipose tissue can be reproduced by simple supplementation of the suspending medium with albumin-complexed free fatty acids[1]. This has prompted a more generalized analysis of the effects of free fatty acids on the metabolism of surviving preparations of target endocrine glands. We have incubated slices of sheep and human thyroid tissue at 38° C. and *p*H 7·4 in modified Krebs–Ringer *tris*-phosphate or Krebs–Ringer bicarbonate[2] containing 2 mgm./ml. of uniformly labelled glucose (glucose-U-^{14}C), 10 mgm./ml. of fat-free bovine albumin[3], and varying quantities of sodium oleate. Direct chemical analysis[4] disclosed that such exogenous supplementation with oleate augmented the thyroidal pool of free fatty acids. The associated striking increases in lipogenesis from glucose-U-^{14}C form the basis of the present report.

Following 3 hr. incubation, thyroid slices were frozen with liquid nitrogen and extracted for total lipids as described previously[3]. Washed extracts were partitioned into neutral lipids and phospholipids with silicic acid columns[5] and aliquots of the fractions were assayed for radioactivity with an automatic Packard 'Tri-Carb' liquid scintillation counter (Packard Instrument Co., Inc., La Grange, Ill.). The remaining portions were hydrolysed for 6 hr. at 100° C. with 6 *N* hydrochloric acid–50 per cent ethyl alcohol. Fatty acids and non-saponifiable lipids were removed by three extractions with petroleum ether and pooled ether extracts were washed, evaporated to dryness and re-suspended for radioactive assay. The aqueous residues of the hydrolysates were evaporated *in vacuo* and re-suspended in water for resolution on paper by ionophoresis (*p*H 3·6; pyridine–acetic acid–water 1 : 10 : 89 v/v) and by chromatography (ethanol–water 8 : 1 v/v). Radioactive moieties on ionophoretograms and chromatograms were localized by radioautography and appropriate segments of paper were cut out for direct counting in the liquid scintillation counter. Recovery standards of glycerol-1-^{14}C were processed with each experiment to correct for losses of glycerol through volatilization and degradation[7] during preparative manipulations.

In Krebs–Ringer bicarbonate as well as Krebs–Ringer *tris*-phosphate, total lipogenesis from glucose-U-^{14}C by surviving thyroid slices was augmented two- to three-fold in the presence of 1 μM/ml. of albumin-complexed oleate. The stimulation was largely effected by a four- to five-fold increase in the radioactivity of the neutral lipid fraction (Table 1). Conjoint analyses to be presented elsewhere indicated that the enhanced lipogenesis was accompanied by a small rise in oxygen consumption, an inconstant alteration in glucose-assimilation, a reduction in the oxidative decarboxylation of glucose-U-^{14}C, and an increased formation of lactic acid-^{14}C from the labelled glucose. Less-prominent, albeit still significant, increases in the incorporation of glucose into thyroid lipids were obtained with higher concentrations of oleate (4–6 μM/ml.) which depressed Q_{O_2}. Analysis of aqueous residues following acid hydrolysis disclosed that glycerol-^{14}C (as glycerol or glycerophosphate) accounted for more than half the lipid radioactivity. Formation of glycerol from glucose-U-^{14}C was enhanced by 1 μM/ml. oleate; the increase was chiefly channelled into the neutral lipid fraction (Table 2). Oleate also stimulated the incorporation of radioactivity into neutral lipid components which retained solubility in petroleum ether following acid hydrolysis (Table 2). Alkaline hydrolysis disclosed that this increase consisted principally in fatty acids rather than non-saponifiable lipids.

From all the foregoing, it is apparent that exogenous supplementation of intra-thyroidal fatty acids results in striking changes in lipogenesis. The

Table 1. Effects of Albumin-complexed Oleate on the Incorporation of Glucose-U-^{14}C into the Lipid Fractions of Thyroid Slices

Exp. No.	Species	Medium †	μgm. glucose-U-^{14}C/gm. initial weight to:*			
			Phospholipid		Neutral lipid	
			A	*B*	*A*	*B*
1	Lamb	KRT-P	26·5	30·8	6·8	48·7
2	Lamb	KRB	31·6	38·7	9·2	42·1
3	Lamb	KRT-P	26·7	26·0	10·5	55·0
4	Lamb	KRT-P	41·4	42·6	14·8	86·0
5	Human	KRT-P	28·8	34·5	19·7	93·9
6	Human	KRB	24·9	19·1	17·4	48·2

* Results are expressed on the basis of the initial specific activity of the glucose-U-^{14}C in suspending media and the recovery of radioactivity as phospholipid of neutral lipid in thyroid slices following incubation.

† Thyroid slices were incubated for 3 hr. in modified Krebs–Ringer *tris*-phosphate (KRT-P) or Krebs–Ringer bicarbonate (KRB) containing fat-free albumin (10 mgm./ml.), uniformly labelled glucose (2 mgm./ml.), and no free fatty acids (*A*) or 1 μM/ml. of sodium oleate (*B*).

Table 2. Distribution of Radioactivity in the Hydrolysis Products of Thyroid Lipid Fractions

Exp. No.*	μgm. glucose-U-^{14}C/gm. initial weight to: Phospholipid				Neutral lipid			
	Petroleum ether		Glycerol		Petroleum ether		Glycerol	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
1	1·4	0·6	19·3	25·9	1·9	7·4	3·7	24·1
2	4·0	0·9	20·2	25·1	3·7	13·6	5·2	16·3
5	1·6	0·7	—	—	7·0	17·6	11·6	55·9
6	2·1	1·0	—	—	6·8	19·5	9·5	26·2

* Experiments are the same as in Table 1. Phospholipid and neutral lipid fractions were hydrolysed with 6 *N* hydrochloric acid–50 per cent ethyl alcohol. Acid hydrolysates were resolved into radioactive components which retained solubility in petroleum ether or which exhibited the chromatographic and ionophoretic properties of glycerol or glycerophosphate (glycerol). The (*B*) or (*A*) denotes the respective presence or absence of sodium oleate in suspending media during incubation.

phenomenon is somewhat similar to the "experimental aberrant lipogenesis" which has been described by Cogan and Kuwabara following 24–48 hr. of incubation of tissue 'buttons' with serum and sodium oleate[7]. However, in their system, increased sudanophilic material was not observed with thyroid preparations.

For the moment, the biochemical basis for the effects of albumin-complexed oleate must remain conjectural. The existence of intra-thyroidal mechanisms for the activation of fatty acids may be inferred from the finding in this Laboratory that surviving sheep thyroid slices can esterify and oxidize albumin-complexed palmitate-^{14}C (ref. 2). Unless such activation were rate-limiting, it is conceivable that an increased availability of free fatty acids could abet lipogenesis in several ways. First, it could provide long-chain coenzyme A derivatives for esterification of glycerophosphate[8]. Indeed, the plethora of acylated fatty acids might also compete successfully with cytidine derivatives for the unesterified carbon of the α,β-diglycerides[8] and thus account for the predominant impact on the neutral lipid fraction. Since phospholipid components were not fractionated in the present work, one cannot assess whether the occasional modest stimulation of phospholipid radioactivity proceeded beyond phosphatidic acid. Secondly, oxidation of an increased quantity of activated fatty acids could indirectly promote the synthesis of fatty acids as well as glycerophosphate from glycolysis. The generation of reduced diphosphopyridine nucleotide during catabolism of fatty acids by β-hydroxyacyl dehydrogenase[9] would provide co-factors for the former[10], and would enhance the formation of the latter from dihydroxy acetone phosphate since the equilibrium constant of soluble glycerophosphate dehydrogenase already favours this direction[11]. Differentiation between the above speculative possibilities (that is, metabolic changes secondary to the disposition of free fatty acids) and other potential actions of fatty acids[12] (that is, surface-active effects which may not entail prior activation) must await further investigation.

This work was supported in part by U.S. Public Health Service research grant *A*-1571 and training grant 2*A*–5060.

NORBERT FREINKEL*
AUGUSTO D. LITONJUA
EDWARD C. SAEF

Thorndike Memorial Laboratory,
Second and Fourth (Harvard) Medical Services,
Boston City Hospital,
and
Department of Medicine,
Harvard Medical School,
Boston, Mass.

---

* Investigator, Howard Hughes Medical Institute.

[1] Leboeuf, B., and Cahill, jun., G. F., *J. Biol. Chem.*, **236**, 41 (1961). Freinkel, N., *J. Clin. Invest.*, **40**, 476 (1961).
[2] Freinkel, N., *Endocrinol.*, **66**, 851 (1960).
[3] Raben, M., and Hollenberg, C. H., *J. Clin. Invest.*, **38**, 435 (1960).
[4] Dole, V. P., and Meinertz, H., *J. Biol. Chem.*, **235**, 2595 (1960).
[5] Borgstrom, B., *Acta Physiol. Scand.*, **25**, 101 (1952).
[6] Hanahan, D. J., and Olley, J. N., *J. Biol. Chem.*, **231**, 813 (1958).
[7] Cogan, D. G., and Kuwabara, T., *Arch. Path.*, **63**, 381 (1957). Cogan, D. G., and Kuwabara, T., *Arch. Path.*, **64**, 23 (1957).
[8] Kennedy, E. P., *Fed. Proc.*, **16**, 847 (1957).
[9] Wakil, S. J., Green, D. E., Mii, S., and Mahler, H. R., *J. Biol. Chem.*, **207**, 631 (1954).
[10] Porter, J. W., Wakil, S. J., Tietz, A., Jacob, M. I., and Gibson, D. M., *Biochem. Biophys. Acta*, **25**, 35 (1957).
[11] Young, H. L., and Pace, N., *Arch. Biochem. Biophys.*, **75**, 125 (1958).
[12] Pressman, B. C., and Lardy, H. A., *Biochim. Biophys. Acta*, **21**, 458 (1956).

Biosynthesis of Polyenoic Fatty Acids in Human Whole Blood

IT has been known for some time now that human blood cells are able to incorporate acetate-1-^{14}C into blood lipids[1,2]. James *et al.*[2,3] found the incorporation of radioactive acetate into the saturated as well as into the unsaturated fatty acids of blood lipids, including the 'essential fatty acids'. Based on these experiments, the authors concluded that a total synthesis of essential fatty acids in human blood would be possible. Klenk[4] and Mead *et al.*[5] showed that the C_{20}- and C_{22}-polyenoic fatty acids in rat liver originate from exogenous linoleic and linolenic acids by extension of the carbon chain by acetate units starting at the carboxylic group. In addition, new double bonds are introduced in the divinylmethane-rhythm in direction towards the acid group. This mechanism is valid for polyenoic acid synthesis in the human liver[6] as well as in the descending classes of vertebrates[7].

In order to find out if polyenoic acids can be synthesized by blood cells in the same way we have incubated human whole blood with acetate-1-^{14}C. The fatty acids were isolated and the unsaturated fraction degraded by oxidative ozonolysis. The resulting dicarboxylic acids were separated by column chromatography and the activity measured in a windowless flow-counter. Results are shown in Table 1 (for methods see refs. 4, 6, 7).

Radioactivity was found almost exclusively in glutaric acid (predominantly from Δ5,8,11,14-eicosatetraenoic acid = arachidonic acid), succinic acid (predominantly from Δ4,7,10,13,16,19-docosahexaenoic acid) and in the higher dicarboxylic acids, mainly azelaic acid (from oleic, linoleic and linolenic acids). On the contrary, malonic acid originating from the central parts of the polyunsaturated carbon chains showed only minute activity. This low activity seems to exclude the total synthesis of linoleic and linolenic acids which might be concluded from the activity of azelaic acid. Although degradation and re-extension reactions at the carboxylic end of linoleic and linolenic acids could contribute to the incorporation of this activity, it will originate mainly from oleic acid.

The results strongly support the mode of formation of polyenoic fatty acids outlined here, which seems also to be valid for their biosynthesis in human blood. We hope to obtain further support of these results by reductive ozonolysis of the unsaturated fatty acids

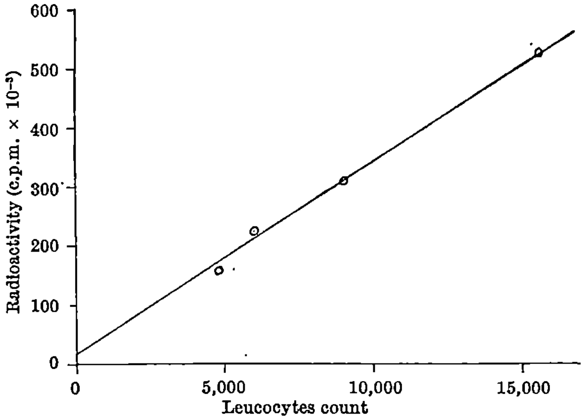


Fig. 1. Relation between total activity of fatty acids from human whole blood after incubation with acetate-1-^{14}C and leucocyte count

Table 1. SPECIFIC ACTIVITY OF DICARBOXYLIC ACIDS FROM OXIDATIVE OZONOLYSIS OF UNSATURATED FATTY ACIDS FROM HUMAN WHOLE BLOOD AFTER INCUBATION WITH ACETATE-1-^{14}C

In each case 50 ml. of freshly drawn blood were heparinized and after addition of 15 mgm. glucose and 0·23 mc. = 8·35 mgm. acetate-1-^{14}C incubated for 5 hr. at 37°. Yields in mole per mole polyenoic acid; specific activity in counts/min. and mgm.

Experiment	Higher M.W. dicarboxylic acids (azelaic acid)		Glutaric acid		Succinic acid		Malonic acid	
	Yield	C.p.m./mgm.	Yield	C.p.m./mgm.	Yield	C.p.m./mgm.	Yield	C.p.m./mgm.
1	0·54	1,275	0·12	655	0·07	532	0·15	28
2	0·51	4,230	0·12	1,240	0·08	1,195	0·43	63
3	0·60	5,640	0·12	2,890	0·06	2,290	0·36	85
4	0·59	3,810	0·10	1,980	0·08	1,620	0·32	68

and subsequent activity measurement of the degradation products derived from the methyl end[7].

It was suggested previously that the fatty acid synthesis in human blood occurs predominantly in erythrocytes. More recently several authors have given evidence for lipid synthesis mainly in leucocytes[8-10]. Besides, these platelets[10] and reticulocytes[9,10] seemed to be involved in fatty acid synthesis too. In the course of our investigations we obtained evidence for synthesis of fatty acids in human blood linked predominantly to leucocytes. Since our experimental conditions for the incubation of blood from subjects with different leucocyte counts were kept constant, we were able to compare leucocyte count and incorporation of radioactivity into the total fatty acids (Fig. 1).

Surprisingly enough we found good linearity between leucocyte count and total incorporated activity.

F. LEUPOLD

Medizinische Universitätsklinik,
Köln.

G. KREMER

Physiologisch-Chemisches Institut,
Universität, Köln.

[1] Altman, K. I., and Swisher, S. N., *Nature*, 174, 459 (1954).

[2] James, A. T., Lovelock, J. E., and Webb, J., *Biochem. J.*, 66, 60 (1957); *Essential Fatty Acids*, 72 (Butterworth, London, 1958).

[3] James, A. T., Lovelock, J. E., and Webb, J., *Biochem. J.*, 73, 106 (1959).

[4] Klenk, E., *Z. Physiol. Chem.*, 302, 268 (1955).

[5] Mead, J. F., Steinberg, G., and Howton, D. R., *J. Biol. Chem.*, 205, 683 (1953).

[6] Leupold, F., and Kremer, G., *Z. Physiol. Chem.*, 318, 251 (1960).

[7] Klenk, E., and Kremer, G., *Z. Physiol. Chem.*, 320, 111 (1960).

[8] Buchanan, A. A., *Biochem. J.*, 75, 315 (1960).

[9] Rowe, C. E., Allison, A. C., and Lovelock, J. E., *Biochim. Biophys. Acta*, 41, 310 (1960).

[10] Marks, P. A., Gellhorn, A., and Kidson, C., *J. Biol. Chem.*, 235, 2579 (1960).

Oxidation-Reduction Reactions of Plastoquinone in Isolated Chloroplasts

QUINONES have previously been implicated as oxidation-reduction carriers in the photosynthetic electron transport system on the assumption that chloroplasts of higher plants contained vitamin K_1[1-3]. It has now been shown that chloroplasts contain little or no vitamin K_1 but that another quinone, 2,3-dimethyl-5-solanesyl-benzoquinone, is present in relatively large amounts[4-6]. This quinone is known as plastoquinone, Q_{255} and Kofler's quinone.

Evidence that plastoquinone functions in the electron transport system associated with photosynthesis has been presented by Bishop[6], who has shown that it will restore photolytic activity when added to spinach chloroplasts which have been extracted with organic solvents. Recently, Crane *et al.*[7] have claimed that exposure of spinach chloroplasts to light resulted in an 80 per cent reduction of the endogenous plastoquinone. In the present communication we provide some further observations on the oxidation-reduction reactions of endogenous plastoquinone in isolated chloroplasts.

Chloroplasts were isolated from sugar-beet leaves (var. Sharp's Klein Wanzleben *E*) by the method of Mayer and Friend[8], except that the isolation medium was buffered to *p*H 7·8 with 0·05 *M* sodium/potassium phosphate instead of *tris*-hydrogen chloride. The chloroplasts (0·4–0·8 mgm. chlorophyll/ml.) were suspended in a medium containing 0·4 *M* sucrose, 0·01 *M* potassium chloride and 0·05 *M* sodium/potassium phosphate, *p*H 7·0.

Plastoquinone was extracted by a modification of the method[9] used for the extraction of ubiquinone from mitochondrial preparations, and the oxidation-reduction state of the quinone measured by the change in extinction at 255 mμ after the addition of sodium borohydride.

The concentration of plastoquinone in the chloroplasts was 9 μmoles/gm. protein or 0·1 mole per mole chlorophyll. This concentration agrees with that determined in spinach chloroplasts by Crane *et al.*[7]. There was no evidence for the presence of vitamin K_1. It is interesting to compare this concentration with that of another electron carrier in the chloroplast, cytochrome *f*. It has been shown[10,11] that the molar ratio of chlorophyll to cytochrome *f* in a number of chloroplast preparations is about 200–400. Thus the molar concentration of plastoquinone exceeds that of cytochrome *f* by a factor of 20–40. In this connexion, it is interesting to note that a related quinone, ubiquinone (coenzyme Q), in mitochondria is also present in a considerable molar excess over the individual cytochromes in mitochondria.

The oxidation-level of plastoquinone (that is, percentage of total in oxidized form) in freshly isolated chloroplasts varied between 45 and 90 per cent; the oxidation-level appeared to increase as the chloroplasts aged. Illumination (5 min. at 3,200 ft.-candles) resulted in a fall of the level to 35–65 per cent. Thus there had been approximately only 15 per cent reduction of the quinone. Crane *et al.*[7], on the other hand, have reported an 80 per cent reduction in a spinach chloroplast preparation incubated under similar conditions. The reason for this discrepancy is not apparent at the moment.

In order to obtain further information on the oxidation-reduction reactions of plastoquinone and its site of action in the electron transport system, the effect of certain natural and artificial electron carriers was studied.

Addition of 2,6-dichlorophenolindophenol (final concentration 0·5 m*M*) to the chloroplasts in the dark converted all the plastoquinone into the oxidized form. When this mixture was illuminated there was

Table 1. REDUCTION OF ENDOGENOUS PLASTOQUINONE IN CHLOROPLASTS BY TPNH

Addition	Incubation		Plastoquinone (per cent in oxidized form)
	Time	Light or dark	
Experiment 1			
None	0	dark	45·8
TPNH	5 min.	dark	26·0
TPNH	5 min.	light	34·3
Experiment 2			
None	0	dark	87·5
None	5 min.	light	73·5
TPN + chloroplast extract	15 min.	dark	80·5
TPN + chloroplast extract	15 min.	light	59·0

The incubation mixture contained 1 ml. chloroplast suspension, 1 ml. chloroplast extract or water, 0·625 mM TPN or TPNH.

5 per cent reduction to the quinol. Thus, under conditions where the Hill reaction is operative, the steady-state level of the plastoquinone is 95 per cent oxidized.

It was found that incubation of the chloroplasts with reduced triphosphopyridine nucleotide (TPNH) in the dark resulted in a reduction (20–30 per cent) of the plastoquinone. A similar reduction could also be brought about by TPNH formed photochemically in a reaction mixture consisting of chloroplasts, triphosphopyridine nucleotide (TPN) and a chloroplast extract containing photosynthetic pyridine nucleotide reductase[12] (Table 1).

These results provide further evidence that plastoquinone is a functional member of the photosynthetic electron transport system. The site of action of the quinone is now under investigation.

E. R. REDFEARN

Department of Biochemistry,
University of Liverpool,
Liverpool, 3.

J. FRIEND *

Low Temperature Research Station,
Downing Street,
Cambridge.

---

* Present address: Department of Botany, University of Hull.

[1] Wessels, J. S. C., *Rec. Trav. Chim.*, **73**, 529 (1954).
[2] Bishop, N. I., *Proc. U.S. Nat. Acad. Sci.*, **44**, 501 (1958).
[3] Arnon, D. I., *Nature*, **184**, 10 (1959).
[4] Kofler, M., Langemann, A., Rüegg, R., Gloor, U., Schwieter, U., Wursch, J., Wiss, O., and Isler, O., *Helv. Chim. Acta*, **42**, 1283 (1959).
[5] Crane, F. J., *Plant Physiol.*, **34**, 128, 546 (1959).
[6] Bishop, N. I., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1696 (1959).
[7] Crane, F. L., Ehrlich, B., and Kegel, L. P., *Biochem. Biophys. Res. Comm.*, **3**, 37 (1960).
[8] Mayer, A. M., and Friend, J., *J. Exp. Bot.*, **11**, 141 (1960).
[9] Pumphrey, A. M., and Redfearn, E. R., *Biochem. J.*, **76**, 61 (1960).
[10] Davenport, H. E., and Hill, R., *Proc. Roy. Soc.*, B, **139**, 327 (1952).
[11] Hulcher, F. H., and Vishniac, W., Brookhaven Symposia in Biology, No. 11, *The Photochemical Apparatus, Its Structure and Function*, 348 (Brookhaven National Laboratory, Upton, New York, 1959).
[12] Whatley, F. R., Allen, M. B., and Arnon, D. I., *Biochim. Biophys. Acta*, **32**, 32 (1959).

Metabolites of Vitamin B_{12} in Bile after Parenteral Administration of the Vitamin

SEVERAL investigations have shown that, in animals and in man[1], the major proportion of the total radioactivity excreted after an injection of radioactive vitamin B_{12} is excreted in the bile, and that in man at least two-thirds of the biliary radioactivity is re-absorbed from the intestine. This indicates that vitamin B_{12} is subject to an entero-hepatic circulation similar to other substances in bile.

Radioactive bile was collected via a bile duct cannula from rats injected with radioactive vitamin B_{12}. Vitamin B_{12} and radioactive cobalt-containing metabolites of B_{12} were extracted from the bile. The acidified bile (pH 2–3) was denatured with 95 per cent ethanol, and any bound B_{12} or B_{12} analogues freed by boiling in water for 15 min. The supernatant, after evaporation to a small volume, was subjected to repeated extractions with chloroform and then the water-phase (pH 2–3), saturated with ammonium sulphate, was extracted with secondary butanol. The secondary butanol phase, after adding ethyl ether, was extracted with water. Having thereby removed most of the pigment and impurities, compounds containing cobalt were adsorbed on activated charcoal, washed with 5 per cent phenol, and then eluted with hot 65 per cent acetone. The eluate was evaporated to a small volume to which some non-radioactive concentrated vitamin B_{12} solution was added for a control spot on the electrophoretogram and chromatogram. Throughout the extraction, the pH was kept at 2–3 and any cobalamines were maintained in the cyano form by the addition of 0·01 per cent sodium cyanide. Two-dimensional separation of the metabolites was attempted by electrophoresis at a potential of 8 V./cm. for 16 hr. on No. 1 Whatman filter paper in a buffer of 0·5 N acetic acid and 0·01 per cent sodium cyanide; followed by descending paper chromatography at right angles to the direction of electrophoresis in a system of water-saturated secondary butanol with 1 per cent acetic acid (w/v) and 0·01 per cent sodium cyanide. The resulting radioactive spots on the chromatogram were resolved by making autoradiographs using X-ray film. The radioactivity in the chromatogram spots was counted in a scintillation counter after elution with water.

Fig. 1 shows the results obtained from a sample of rat bile. The control spot contained only non-radioactive vitamin B_{12}, detected by its colour on the chromatogram, and is represented diagrammatically. The relative amounts of radioactivity contained in

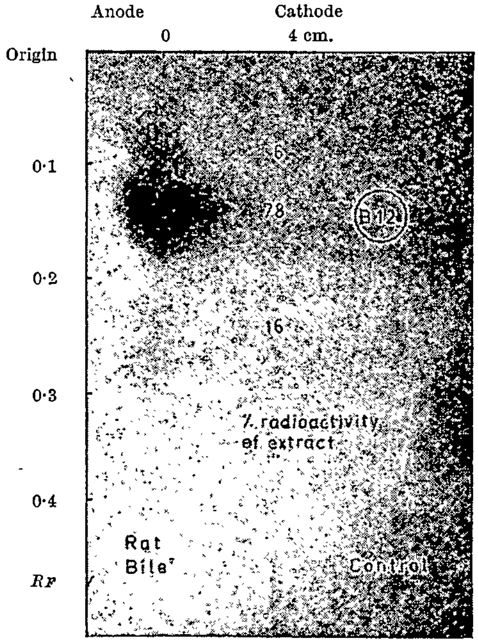


Fig. 1. Autoradiograph of a sample of rat bile following the injection of cobalt-56 vitamin B_{12}

each spot on the chromatogram is shown. A similar result was obtained from a different sample of rat bile.

Comparison with the work of others[2] indicates that the metabolites of vitamin B_{12} here demonstrated in bile are analogues of vitamin B_{12}, and like B_{12} have zero electrophoretic mobility. Free cobalt as cobalt chloride did not migrate in any of the areas shown on the chromatogram. The rat bile contained at least two analogues of vitamin B_{12}. The major portion, approximately three-fourths, of the radioactivity was present as vitamin B_{12}, the remaining fourth being in the B_{12} analogues.

It has been shown by others (see ref. 3) that cyanocobalamine is more readily absorbed in man than the other cobalamines and structural analogues of B_{12}, absorption of the latter being dependent on a strict chemical specificity of the B_{12} benzimidazole group[4]. It seems likely, therefore, that the metabolic products of B_{12} will not be absorbed, or will be poorly re-absorbed once they are excreted in the bile. For this reason, the presence of vitamin B_{12} analogues in bile offers a possible explanation of the appreciable amount of radioactivity sometimes found in the fæces of normal human subjects in the fæcal excretion test of B_{12} absorption[5]. Similarly, it offers an explanation of the finding[1] that re-absorption by the enterohepatic circulation of the radioactivity in human bile after parenteral cobalt-56 vitamin B_{12} was incomplete, being approximately two-thirds of the total radioactivity present.

JOHN B. STOKES*

Department of Medicine,
Massachusetts General Hospital
and the Harvard Medical School,
Boston, Mass.

---

* Eli Lilly Foreign Educational Fellow. Present address: c/o Royal Perth Hospital, Perth, Western Australia.

[1] Grasbeck, R., Nyberg, W., and Reizenstein, P., *Proc. Soc. Exp. Biol. and Med.*, 97, 780 (1958).

[2] Friedrich, W., and Bernhauer, K., *Z. Naturforsch.*, 10, B, 6 (1955). Porter, J. W. G., in Heinrich, H. C., *Vitamin B_{12}*, 43 (Ferdinand Enke, Stuttgart, 1957).

[3] Rosenblum, C., Davis, R. L., and Chow, B. F., *Proc. Soc. Exp. Biol. and Med.*, 95, 30 (1957).

[4] Heinrich, H. C., *Z. Vitamin- Hormon- u. Fermentforsch.*, 9, 385 (1958).

[5] Heinle, R. W., Welch, A. D., Scharf, V., Meacham, G. C., and Prusoff, W. H., *Trans. Ann. Amer. Physicians*, 65, 214 (1952). Mollin, D. L., and Baker, S. J., *Biochem. Soc. Symp.*, 13, 52 (1955).

Non-Enzymic Hydroxylation of Polycyclic Aromatic Hydrocarbons

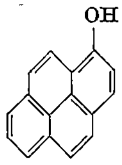
THE hydroxylation of aromatic compounds *in vivo* is well known[1], and polycyclic aromatic hydrocarbons[2] and aromatic amino-acids and related compounds[3] have been found to be hydroxylated by suitably fortified suspensions of rodent liver microsomes. Aromatic amino-acids and related compounds are also hydroxylated non-enzymically in phosphate buffer solutions containing iron and ascorbic acid in the presence of oxygen[4,5], or in hydrogen peroxide solutions containing Fe^{++} (ref. 6). In the case of polycyclic aromatic hydrocarbons slow conversion to quinones, by ascorbic acid[7], and more rapid oxidation by hydrogen peroxide[8], have been reported.

Udenfriend *et al.* claimed[4], and Dalgliesh confirmed[5], that the phenols produced by the non-enzymic hydroxylation of aromatic amino-acids, and related compounds, with a system involving iron, ascorbic acid and oxygen, were identical with the phenols produced during animal metabolism. In view of this and the possible connexion between *in vivo* metabolism of polycyclic aromatic hydrocarbons and carcinogenesis[9] the effect of the Udenfriend system on these compounds was examined. It was found (see Table 1) that a system involving Fe^{++}, a suitable complexing agent and oxygen, in a buffer solution, would readily convert pyrene to 3-hydroxypyrene (3 on Richter's system of nomenclature, but 1-hydroxy on Patterson's system), as follows:

Table 1. CONDITIONS FOR THE HYDROXYLATION OF PYRENE

Reaction system	Yield of phenol (mean of five experiments)
Complete system*	38γ
,, minus Fe^{++}	0
,, ,, EDTA	Trace (< 3γ)
,, ,, Ascorbic acid	28γ
,, ,, Fe^{++} and ascorbic acid	0
,, ,, EDTA and ascorbic acid	Trace (< 3γ)
,, ,, Fe^{++} and EDTA	0
,, but with nitrogen instead of oxygen	0
,, but with acetate buffer instead of phosphate	125γ
,, but with Fe^{+++} instead of Fe^{++}	Trace (< 3γ)

* A solution of 500 mgm. L-ascorbic acid, 250 mgm. ferrous sulphate and 500 mgm. of the disodium salt of ethylenediaminetetraacetic acid (EDTA) in 50 ml. of 0·1 *M* phosphate buffer *p*H 5·8 (or 0·1 *M* acetate buffer *p*H 5·8). Pyrene (2 mgm. in 0·4 ml. of ethanol) was added and oxygen gas bubbled through. The reaction was carried out at a temperature of 95° C. for 1 hr.



3-Hydroxypyrene

Ascorbic acid stimulated the reaction, but Fe^{+++} would not replace Fe^{++} even in the presence of ascorbic acid. This is in contrast to the Udenfriend system and with Mazelis's non-enzymic decarboxylation reaction[10]. Replacement of the phosphate buffer by an acetate buffer led to a three-fold improvement in yield. In the experiment at a lower temperature (38° C.) with the complete system, 8 μgm. of 3-hydroxypyrene were obtained from a reaction lasting 1 hr. With solutions containing the complete reaction system but with the Fe^{++} replaced by Cu^{+}, Cu^{++}, Co^{++}, Ce^{+++}, Mg^{++}, Mn^{++} Hg_2^{++} and Fe^{+++} (the valence state of the metal on introduction into the system is specified) either no, or only a trace of, 3-hydroxypyrene was formed.

The activity of the system was dependent on the choice of complexing agent, as shown in Table 2. The failure of cyanide to inhibit this system is in contrast with the behaviour of Warburg's model enzymes[11], based on iron-containing charcoals and the heavy-metal catalysed 'autoxidation' of ascorbic acid[12]. The effect of the complexing agent is undoubtedly to modify the oxidation-reduction potential

Table 2. EFFECT OF COMPLEXING AGENTS ON THE HYDROXYLATION OF PYRENE

Reaction system	Yield of phenol
Complete system	
,, plus potassium cyanide*	64γ
,, ,, riboflavin	55γ
,, ,, dipyridyl	Trace (< 3γ)
,, ,, *o*-phenanthroline	0
Complete system minus EDTA	
,, plus sodium citrate	26γ
,, ,, dipyridyl	Trace (?)
,, ,, *o*-phenanthroline	Trace (?)
Fe^{++} and riboflavin only	34γ

* 500 mgm. of each complexing agent was added, but a considerable part of the riboflavin failed to dissolve.

of the iron[13], and it is perhaps not without significance that both αα-dipyridyl and *o*-phenanthroline stabilize strongly the ferrous state[14]. Inhibition of the system by these two agents may, however, be due to hydroxylation of the complexing agent. The hydroxylating power of a combination of Fe^{++} and riboflavin must be of considerable biological interest.

3-hydroxypyrene is the phenol produced by the metabolism of pyrene[15] and the 3 position of pyrene is a site of free-radical attack[16]. It has been found during these experiments that naphthacene, chrysene, 1:2-benzanthracene and 3:4-benzopyrene also react under these conditions and work is now in progress to identify these products.

The 3-hydroxypyrene was identified by extracting the cooled reaction mixture with 3 × 50 ml. of ether, and chromatographing the dried ethereal extracts on alumina. On development with ether, any phenols present passed slowly down the column as bands fluorescing under ultra-violet illumination. Pyrene gave rise to a single bright-blue fluorescent band which after collection was evaporated to dryness under reduced pressure, and redissolved in spectroscopic ethanol. Its absorption spectrum (220–420 mμ) and that of its sodium salt proved identical with those of 3-hydroxypyrene and its sodium salt[17]. The yield was calculated from the absorption at 386 mμ and 366 mμ. Small losses occurred on the chromatography columns, and yields of less than 3γ could not be estimated satisfactorily.

F. Dewhurst
G. Calcutt

Department of Cancer Research,
Mount Vernon Hospital and the Radium Institute,
Northwood, Middlesex.

[1] Parke, D. V., and Williams, R. T., *Ann. Rep. Chem. Soc.*, 55, 376 (1959).

[2] Conney, A. H., Miller, E. C., and Miller, J. A., *J. Biol. Chem.*, 228, 753 (1957).

[3] Mitoma, C., Posner, H. S., Reitz, H. C., and Udenfriend, S., *Arch. Biochem. Biophys.*, 61, 431 (1956).

[4] Udenfriend, S., Clark, C. T., Axelrod, J., and Brodie, B. B., *J. Biol. Chem.*, 208, 731 (1954).

[5] Dalgliesh, C. E., *Arch. Biochem. Biophys.*, 58, 214 (1955).

[6] Raper, H. S., *Biochem. J.*, 26, 2000 (1932).

[7] Warren, F. L., *Biochem. J.*, 37, 338 (1943).

[8] Calcutt, G., *Brit. J. Cancer*, 4, 254 (1950).

[9] Harper, K. H., *Brit. J. Cancer*, 13, 732 (1959).

[10] Mazelis, M., *Nature*, 189, 305 (1961).

[11] Baldwin, E., *Dynamic Aspects of Biochemistry*, third ed., 160 (Camb. Univ. Press, 1959).

[12] Openheimer, C., and Stern, K. G., *Biological Oxidation*, 67 (W. Junk, The Hague, 1939).

[13] Emeléus, H. J., and Anderson, J. S., *Modern Aspects of Inorganic Chemistry*, second ed., 178 (Routledge and Kegan Paul, London, 1952).

[14] Emeléus, H. J., and Anderson, J. S., *Modern Aspects of Inorganic Chemistry*, second ed., 183 (Routledge and Kegan Paul, London, 1952).

[15] Harper, K. H., *Brit. J. Cancer*, 11, 499 (1957).

[16] Norman, R. O. C., Thompson, G. A., and Waters, W. A., *J. Chem. Soc.*, 175 (1958).

[17] Friedel, R. A., and Orchin, M., *Ultra-violet Spectra of Aromatic Compounds* (Chapman and Hall, London, 1951).

Kinetics of the Enzymic Degradation of Deoxyribonucleic Acid into Sub-units

Recent reports from this laboratory[1,2] described the enzymic degradation of deoxyribonucleic acid (DNA) into rod-like sub-units having a molecular weight of 5–6 × 10^5 and a Watson and Crick[3] double helical structure. No production of dialysable nucleotides and no increase of the ultra-violet absorption coefficient at 260 mμ were detectable during the digestion. It is the purpose of this communication to report further information obtained by using a purified enzyme preparation.

The crude enzymic preparation from chicken erythrocytes already described[1,2] was purified several hundred times according to a procedure which will be reported elsewhere[4], and used at activity concentrations about 100 times higher than those of the crude preparation used before. Degradation of chicken erythrocyte DNA (which was exclusively used in this work) by the more active enzyme was characterized by: (1) a first phase in which the molecular weight of DNA (as determined by light scattering[5]) dropped rapidly from its starting value of about 6×10^6 to about 5–6 × 10^5 (this phase showed all the features already described for the degradation of DNA by the crude enzymic preparation); (2) a second phase in which the molecular weight continued to drop at a slower rate. In this phase, which had not been detected when using the crude enzyme, there was formation of acid-soluble nucleotides and an increase in the ultra-violet absorption coefficient at 260 mμ. A calf thymus acid deoxyribonuclease (DNase) preparation, to be described elsewhere[6], degraded DNA in much the same way as the purified chicken erythrocytes preparation and was used almost exclusively in further work.

As our previous results[1,2] strongly suggested, a non-random breakdown of DNA by the enzyme, and as a similar suggestion had been put forward by Oth *et al.*[7] in their work on the degradation of DNA by acid DNase, the kinetics of the enzymic degradation was investigated in detail. Digestions were carried out in the light-scattering cell at 20°; to 30 ml. of 5–10 mgm./100 ml. of DNA (molecular weight = 6×10^6) in acetate buffer $pH = 5·4$, $\mu = 0·15$, 0·02 ml. of the enzymic preparation (generally containing 10γ protein) was added and molecular weights were determined at intervals of time. No time-lag was detectable, and the initial slope of log $(1 - R)$ versus log t ($R = M_w/M_w^0$; M_w = weight average molecular weight; M_w^0 = initial molecular weight; t = time) was near to 1. Therefore, making the reasonable assumption that $p_t = kt$ (p_t being the probability that any given bond will be cleaved during time t, and k a constant) for values of R between 1 and 0·8, it is concluded that the degradation occurs according to a 'single strand' degradation kinetics[8]. The same behaviour was displayed by DNA samples obtained by enzymic action and ranging in molecular weight from 6 to $1·1 \times 10^6$.

The degradation of DNA 'sub-units' was studied next. Several samples, obtained by enzymic action from different DNA preparations, and exhibiting molecular weights between 7 and 9 × 10^5 (in acetate buffer $pH = 5·4$, $\mu = 0·15$), were deproteinized by shaking with chloroform *iso*amyl alcohol and then digested in the light-scattering cells as before (DNA concentrations were 10–20 mgm./100 ml.). A time-lag was evident and the initial slope of log $(1 - R)$ versus log t was close to 2. Therefore, making the same assumptions as before, degradation occurred according to a 'double strand' kinetics.

Finally, the transition phase between the two degradation kinetics was investigated; as expected, this was characterized by a plateau region, in which the molecular weight did not change.

These results show that two different types of degradation are going on at the same time. The 'single strand' degradation is the only one apparent in the first phase, because it is acting during the time-lag of the 'double strand' degradation. Whereas the latter is a random degradation of the type already known for neutral DNase[8,9], the 'single strand' degradation as shown by this work is highly specific and unequivocally shows that (1) sites exist in DNA ($M_w = 6 \times 10^6$) where the enzymic action brings about the splitting of the molecule without any time-lag and (2) the number of these sites is discrete.

GIORGIO BERNARDI
CHARLES SADRON

Centre de Recherches sur les
Macromolécules,
Strasbourg.

[1] Bernardi, G., Champagne, M., and Sadron, C., *Nature*, **188**, 228 (1960).

[2] Bernardi, G., Champagne, M., and Sadron, C., *Biochim. Biophys. Acta*, **49**, 1 (1961).

[3] Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 737 (1953).

[4] Bernardi, G. (manuscript in preparation).

[5] Sadron, C., *The Nucleic Acids*, **3**, 1, edit. by Chargaff, E., and Davidson, J. N. (Academic Press, New York, 1960).

[6] Bernardi, G., *Biochim. Biophys Acta* (in the press).

[7] Oth, A., Frédéricq, E., and Hacha, R., *Biochim. Biophys. Acta*, **29**, 287 (1958).

[8] Schumaker, V. N., Richards, E. G., and Schachman, H. K., *J. Amer. Chem. Soc.*, **78**, 4230 (1956).

[9] Thomas, C. A., *J. Amer. Chem. Soc.*, **78**, 1861 (1956).

Relative Sweetness of α- and β-Forms of Selected Sugars

THE mechanism of taste perception has been approached through work on the relationships between chemical properties of taste stimuli and their resultant taste responses. Ferguson and Lawrence[1,2] reported that the D-configurations of leucine, *iso*leucine, valine, histidine, tryptophan and asparagine were sweet, whereas the L-forms were not. These authors observed that *iso*maltose (6-α-D-glucopyranosyl-D-glucose) was sweet, but its anomer, gentiobiose (6 - β - D - glucopyranosyl - D - glucose), was bitter. Cameron[3,4] found that a freshly prepared solution of 10 per cent α-D-glucose was definitely sweeter than a 16-hr.-old 10 per cent solution. The magnitude of the response (fourteen evaluations) suggested that the fresh solution had a sweetness equal to or more than that of a 10·5 per cent equilibrium solution.

The investigation discussed here was designed to repeat Cameron's work and to determine whether the α- and β-forms of other sugars differ in relative sweetness. The mutarotation-rates at room temperature of glucose, fructose, galactose, and lactose were determined by polarimetric measurements. Equilibrium between the α- and β-forms of these sugars was attained within 2·5 hr. after hydration. Consequently, to ensure having an equilibrium mixture, sugars were hydrated 3 hr. before tasting. Before this experiment, it was established that a freshly hydrated solution of sucrose, which does not undergo mutarotation, did not differ in sweetness from a 16-hr.-old solution.

The taste panel consisted of two women and three men, selected on the basis of their high acuity for detecting sweetness differences from an original group of eleven experienced tasters. Evaluations were made daily at 11.30 a.m. in individual, partitioned booths maintained at a controlled temperature. At each session every judge received four pairs of solutions, one of each pair having been hydrated 3 hr. previously, the other hydrated 100–200 sec. before tasting. The time between hydration of the fresh sample and tasting was kept to a minimum by adding freshly distilled water to the weighed compounds in individual volumetric flasks as each judge entered the booth. Judges were requested to circle the number of the sample within each pair which had the greater sweetness. The order of sample presentation was randomized. Distilled water was provided for oral rinsing at the judge's discretion. A minimum of forty separate evaluations per pair was collected. The method in ref. 5 was used to establish significant differences.

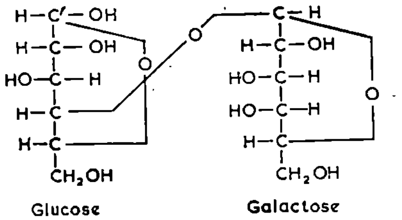


Fig. 1. α-Lactose (D-glucose-4(β-D-galactopyranoside))

As shown in Table 1, the differences in sweetness between the α- and β-configurations became greater as the concentration of the sugar increased. The α-configurations of fructose, glucose and galactose were sweeter than the β-forms, whereas the reverse was true for lactose. This observation is of interest, since the same asymmetric carbon atom (C–1) is responsible for the specific rotation of the lactose molecule as for the glucose molecule (Fig. 1). Rogers[6] suggested that the greater solubility of β-lactose ($55^{20^\circ\text{C.}}$ in 100 parts water) as compared with the α-form ($8^{20^\circ\text{C.}}$)[7] contributed to its greater sweetness. However, β-D-glucose is more soluble but less sweet than α-D-glucose ($154^{15^\circ\text{C.}}$, $82^{25^\circ\text{C.}}$, respectively)[8]. When 10 per cent α-D-glucose was used as a sweetener in peach nectar, 76 per cent of 66 comparisons showed

Table 1. RELATIVE SWEETNESS OF α- AND β-FORMS OF FOUR SUGARS

Compound	Concentration (per cent)	No. of evaluations	Sec. after hydration	Response considering (per cent)		
				Alpha sweeter	Equilibrium sweeter	Beta sweeter
α-D-fructose[1]	0·09	40	101	65·0*	35·0	
	0·20	48	103	87·5‡	12·5	
	0·30	40	124	85·0‡	15·0	
	5·00	40	120	90·0‡	10·0	
	7·00	48	126	68·8*	31·2	
α-D-glucose[2]	0·30	40	121	50·0	50·0	
	5·00	40	107	77·5‡	22·5	
	10·00	44	137	88·6‡	11·4	
β-D-glucose[3]	5·00	40	121		75·0†	25·0
	10·00	40	127		85·0‡	15·0
α-versus β-glucose	5·00	40	135	95·0‡		5·0
	10·00	40	148	100·0‡		0·0
α-lactose[4]	0·30	40	117	70·0†	30·0	
	5·00	40	270	40·0	60·0	
	7·00	44	222	13·6	86·4‡	
β-lactose[3]	0·30	48	99		72·9†	27·1
	5·00	40	124		45·0	55·0
	7·00	40	140		35·0	65·0*
α- versus β-lactose	5·00	40	184	25·0		75·0†
	7·00	40	266	12·5		87·5‡
α-D-galactose[3]	5·00	40	130	62·5	37·5	
	10·00	40	185	72·5†	27·5	

* Significant at $P = 0·05$. † Significant at $P = 0·01$. ‡ Significant at $P = 0·001$.

[1] Eastman Organic Chemicals, Lot 679. [2] Eastman Organic Chemicals, Lot 84. [3] Nutritional Biochemicals Corp. [4] Eastman Organic Chemicals, Lot 357.

the freshly sweetened nectar to be sweeter than that sweetened 6 hr. before.

In extensive work on the effectiveness of various carbohydrates in stimulating labellar and tarsal hairs of the blowfly, *Phormia regina*, Dethier[9] observed no significant differences in thresholds between α-D-glucose and an equilibrium mixture. On the other hand, Dethier demonstrated a difference between α-nitroglucose and β-nitroglucose, the former being somewhat more effective. The most striking difference was found in the great effectiveness of α-methyl glucoside in contrast to the ineffectiveness of β-methyl glucoside. The most effective carbohydrate stimuli for *Phormia regina* included compound sugars with an α-D-glucopyranoside link. Enzyme inhibitors such as azide, iodoacetate, phlorizin and fluoride did not prevent stimulation.

In his review on the relationship between molecular structure and odour, Stoll[10] stated that if optical isomers are distinguishable by their odour, molecules constituting the receptors may be optically active also, and thus exhibit a different adsorbent activity towards the odorous molecules in contact with them according to the sign of rotation of the latter. Lawrence and Ferguson[2] tested the theory that the stereospecific surface of the taste organ might be that of an enzyme, by measuring the adsorption of sweet and non-sweet isomeric pairs of compounds on charcoal, cut human hair, and tongue surfaces. No significant differences in adsorption were observed. In addition, these investigators found no obliteration of the sweet taste in the presence of the enzyme inhibitors, sodium azide, potassium fluoride, sodium iodoacetate, and sodium cyanide. Baradi and Bourne[11,12] reported that in the papilla foliata of the rabbit, quinine inhibited glycerophosphatase, 5-nucleotidase and esterase reactions, and greatly increased the ribonuclease reaction. Sucrose had no effect and saccharine inhibited esterase.

It must be concluded that the reason for differences in sweetness evoked by anomers of various compounds remains to be elucidated. The results reported here confirm Cameron's observations on glucose[3] and establish that the anomers of fructose, galactose, and lactose differ in intensity of sweetness also. Consequently, to assure equilibration of the α- and β-forms, sweetness investigations of asymmetrical sugars should involve hydration several hours before tasting.

ROSE MARIE PANGBORN
SANDRA C. GEE

Department of Food Science and
Technology,
University of California,
Davis.

[1] Ferguson, L. N., and Lawrence, A. R., *J. Chem. Ed.*, **35**, 435 (1958).

[2] Lawrence, A. R., and Ferguson, L. N., *Nature*, **183**, 1469 (1959).

[3] Cameron, A. T., *Trans. Roy. Soc. Canad.*, **37**, 11 (1943).

[4] Cameron, A. T., *Sci. Rep.* No. 9, 44 (Sugar Research Found., Inc., New York, 1947).

[5] Roessler, E. G., Baker, G. A., and Amerine, M. A., *Food Research*, **21**, 117 (1956).

[6] Rogers's Associates, *Fundamentals of Dairy Science*, 130, second ed. (Reinhold, 1935).

[7] Hodgman, C. D., *et al.*, *Handbook of Chemistry and Physics*, 947, thirty-eighth ed.

[8] Jenness, R., and Patton, S., *Principles of Dairy Chemistry*, 78 (John Wiley, 1959).

[9] Dethier, V. G., *Quart. Rev. Biol.*, **30**, 348 (1955).

[10] Stoll, M., *Molecular Structure and Organoleptic Quality*, S.C.I, 5 (Monograph No. 1, London, 1957).

[11] Baradi, A. F., and Bourne, G. H., *Science*, **113**, 660 (1951).

[12] Baradi, A. F., and Bourne, G. H., *Nature*, **168**, 977 (1951).

BIOPHYSICS

The Action Potential in Cells of *Chara*

IN the resting state of giant internodal cells of the fresh water algæ, *Chara* and *Nitella*, an electric potential difference exists between the interior of the cells and an external medium. The value of this potential difference appears to be determined by the permeability of the plasmalemma (outer cytoplasmic membrane) to potassium ions and, to a lesser extent, to sodium ions[1,2]. The resting resistance of this membrane is also related to the permeability to these ions and to their external concentrations[2].

The action potential, which is a reversible depolarization of the plasmalemma[3], occurs on stimulation of the cells by a pulse of current or by abrupt change in temperature, etc. It might be suggested that the action potential in the Characeae, which is qualitatively similar to the conducted impulse in a nerve axon, is similarly related to quick changes in permeability of the cell.

Osterhout[4] proposed a mechanism involving potassium ions and the inner cytoplasmic membrane (tonoplast), but this seems unlikely since, in fact, the action potential occurs across the plasmalemma and the potassium ion is almost in electrochemical equilibrium across it. Thus the potential difference would not be expected to change greatly if a change towards increased permeability to potassium occurred.

Gaffey and Mullins[5] reported that increased fluxes of potassium and chloride accompanied the action potential in cells of the corticated species *Chara globularis* and that the potential difference at the peak of the action potential was influenced by the concentration of chloride in the external medium.

The present investigation is concerned with the effects of changes in concentration of various ions on the action potential in single cells of *Chara australis*. An alternative explanation of some of the results of Gaffey and Mullins is offered.

Continuous records on a chart recorder were made of the potential difference between a micro-electrode in the vacuole and an external medium. A second electrode in the vacuole was used to pass current pulses to initiate action potentials. The threshold was generally about 30 mV. above the resting level. To investigate the effect of changes in concentration of a particular ion, the total concentration was kept constant and the concentration of one ion species varied at the expense of another, so that the cell wall potential remained constant[2].

Fig. 1 shows the resting potential difference and the potential difference reached at the peak of the action potential, as functions of calcium concentration, $[\mathrm{Ca}^{++}]_0$, in solutions of calcium and magnesium chloride of total concentration 6 m*N*. All solutions also contained 1 m*N* sodium chloride and 0·1 m*N* potassium chloride. While the resting potential was almost unaffected by changes in concentration of calcium or magnesium, the peak of the action potential was changed reversibly, a new value being reached within about 10 min. of changing the concentration. It is seen that the peak of the action potential approximately follows a line with a slope of 29 mV./(10 × $[\mathrm{Ca}^{++}]_0$), but with a tendency (observed in several experiments) for the peak to be more negative than the extrapolation of this line at low calcium concentration.

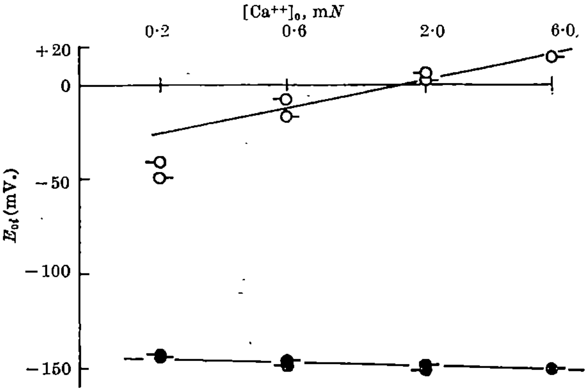


Fig. 1. Resting potential (●) and peak level of the action potential (○), plotted against calcium concentration on a logarithmic scale

Bars on the symbols show direction of increasing or decreasing concentration, starting and finishing at 0·2 m*N*. The line through some of the points for action potential has a slope of 29 mV. per ten-fold change in external calcium concentration

In the presence of calcium ions, changes in concentration of other ion species had no consistent effect on the peak of the action potential. In the absence of added calcium ions, the peak did not hold a steady value, but became more negative with time as a consequence of the removal by cation exchange via the cell wall Donnan phase[2] of the calcium in the immediate neighbourhood of the plasmalemma. Fig. 2 shows the decline in the magnitude ΔE of the action potential (the change in potential from resting-level to peak) in solutions of choline chloride. The rate of decrease of ΔE was greater in 10 m*N* than in 3·3 m*N* choline chloride. This agrees qualitatively with comparative rates of exchange in isolated cell walls of *Chara*[6].

In solutions not containing calcium, the cell eventually reached a stage where it could not be stimulated by pulses depolarizing the cell by as much as 100 mV.

It is now suggested that, had the changes in the experiments of Gaffey and Mullins[5] been made in the direction of increasing choline chloride concentration, the magnitude of the action potential would have appeared to decrease with increasing concentration of chloride (Fig. 3 in Gaffey and Mullins[5]), whereas in fact the decrease was a function of time. However, the reported extra fluxes of chloride during the action potential in *Chara globularis* remain unexplained.

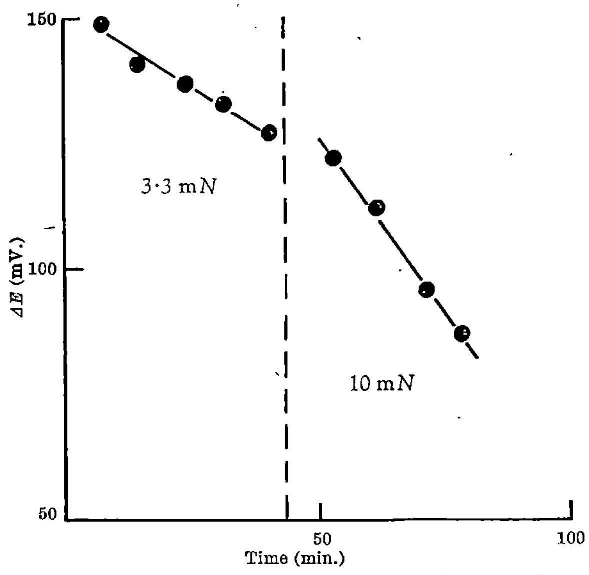


Fig. 2. The magnitude (ΔE) of the action potential in a cell in 3·3 m*N* and 10 m*N* choline chloride, plotted against time

The change in concentration of choline chloride was made at the time indicated by the dotted line

In the present experiments the action potential may be described in terms of a sudden increased permeability of the plasmalemma to calcium such that the potential approaches the ion equilibrium potential for this ion, given by:

$$E_{0i} = \frac{RT}{2F} \ln ([\mathrm{Ca}^{++}]_0/[\mathrm{Ca}^{++}]_i) \qquad (1)$$

$$= \text{const.} + 29 \log_{10} [\mathrm{Ca}^{++}]_0 \text{ at } 19^\circ \text{ C.} \qquad (2)$$

The effect of calcium was obtained in the presence of concomitant changes in magnesium, barium, or strontium concentration and is therefore specific to calcium. Changes in permeability to other ions besides calcium may be involved in the action potential. This may explain some observed deviations from the relation predicted by equation (1) or (2).

This work is part of the joint programme of the Division of Food Preservation, Commonwealth Scientific and Industrial Research Organization, and the Botany School, University of Sydney.

A. B. HOPE

Plant Physiology Unit,
Botany School,
University of Sydney,
Sydney.

---

[1] Walker, N. A., *Austral. J. Biol. Sci.*, **13** (4), 468 (1960).
[2] Hope, A. B., and Walker, N. A., *Austral. J. Biol. Sci.*, **14** (1) (in the press).
[3] Findlay, G. P., *Austral. J. Biol. Sci.*, **12** (4), 412 (1959).
[4] Osterhout, W. J. V., in *Electrochemistry in Biology and Medicine*, edit. by Schedlovsky (Wiley and Sons, Inc., New York, 1955).
[5] Gaffey, C. T., and Mullins, L. J., *J. Physiol.*, **144**, 505 (1958).
[6] Dainty, J., and Hope, A. B., *Austral. J. Biol. Sci.*, **12** (4), 395 (1959).

Voltage-Clamp Experiments with *Nitella*

HOPE[1] has suggested that in the large internodal cells of *Chara australis*, the depolarization of the plasmalemma during an action potential is caused by a sudden transient increase in the permeability of this membrane to calcium ions, and a consequent inward movement of these ions. This suggestion followed from an investigation of the effects of changes in concentration of various ions on the action potential in single *Chara* cells.

Recently, in this laboratory, it has been possible to examine in more detail the action potential process by applying a 'voltage-clamp' technique to the ecorticate internodal cells of a species of *Nitella*. The action potential in these cells has been described previously by Findlay[2]. In the voltage-clamping method a feed-back circuit is used to change the membrane potential to some pre-determined level and maintain it as close as possible to this level during the electrical activity of the cell. The current flowing across the membrane during the voltage-clamp is recorded. Fig. 1 shows the relationship between membrane current and time, for different membrane potentials. It is seen that the current may be divided into two components: a transient inward current and a steady outward current.

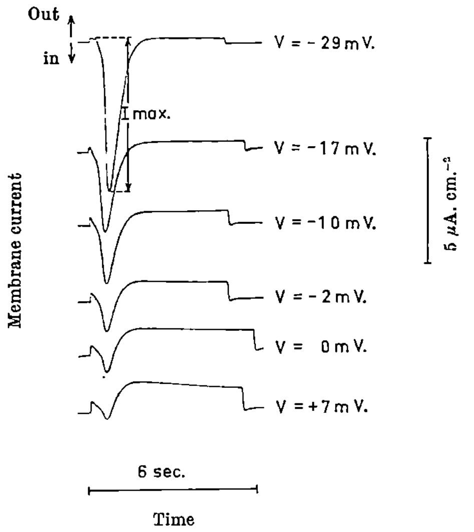


Fig. 1. Records of membrane current under a voltage-clamp. The membrane potential is shown at the right of each curve. The maximum inward current is shown as $I_{\text{max.}}$

Whereas the outward current increases as the membrane potential is changed in the positive direction, the inward current decreases, and eventually becomes zero.

The effect of external calcium concentration on these currents was investigated. The membrane potential – maximum inward current relationship for different concentrations of external calcium, $[\text{Ca}^{++}]_{\text{ext.}}$, was determined. It was also found that $[\text{Ca}^{++}]_{\text{ext.}}$ had little effect on the steady, outward-flowing current. Some results are shown in Fig. 2*a*. In this experiment, the cell was bathed in a solution of calcium and magnesium chloride and the calcium concentration varied at the expense of the magnesium. The total ionic concentration of the solution was kept constant throughout the experiment to eliminate changes in potential difference across the cell wall Donnan system[3]. In Fig. 2*b* the membrane potential for which the inward current was zero and the potential at the peak of an ordinary action potential are plotted against $[\text{Ca}^{++}]_{\text{ext.}}$

The role of the chloride in the action process was examined by determining the relationship between the clamped potential (V) and the maximum inward current ($I_{\text{max.}}$) for different $[\text{Cl}^{-}]_{\text{ext.}}$ and fixed $[\text{Ca}^{++}]_{\text{ext.}}$. The results are shown in Fig. 3. Curves (*a*) were obtained with $[\text{Ca}^{++}]_{\text{ext.}} = 0{\cdot}1$ mN and $[\text{Cl}^{-}]_{\text{ext.}} = 0{\cdot}1, 0{\cdot}3, 1{\cdot}0$ mN, and curves (*b*) with $[\text{Ca}^{++}]_{\text{ext.}} = 1{\cdot}0$ mN. The three curves in each of the two groups lie close to one another, and intersect the membrane potential axis at approximately the same position.

The results summarized in Fig. 2 show that the membrane potential for which the initial inward current is zero depends on $[\text{Ca}^{++}]_{\text{ext.}}$. The slope of the $V/I_{\text{max.}}$ curve, a measure of the minimum resistance of the membrane during activity, is of the order of 2–4 k.Ωcm.^{2}, while the resting resistance of the *Nitella* membrane is 30–80 k.Ωcm.^{2}. Fig. 3 shows that $[\text{Cl}^{-}]_{\text{ext.}}$, however, has little effect on the $V/I_{\text{max.}}$ relationship. These results indicate that the plasmalemma of *Nitella* during the initial stage of the action potential undergoes a transient increase in permeability to calcium. During this time calcium controls the $V/I_{\text{max.}}$ relationship. Hence, the membrane potential for which the calcium current is zero is the electrochemical equilibrium potential for calcium. Consequently, if $[\text{Ca}^{++}]_{\text{ext.}}$ is changed the intercept of the $V/I_{\text{max.}}$ graph with the V axis should vary according to the Nernst equation, namely a change of 29 mV. for each ten-fold change in $[\text{Ca}^{++}]_{\text{ext.}}$. However, it is found that in *Nitella* the change is usually 35–40 mV. One possible explanation of this larger value is that calcium is partly in the form of a monovalent complex, and both this and the divalent calcium together control the action potential. The 35–40 mV. change for ten-fold change in $[\text{Ca}^{++}]_{\text{ext.}}$ would represent the combined behaviour of the two forms of calcium. A plot of the potential at the top of the

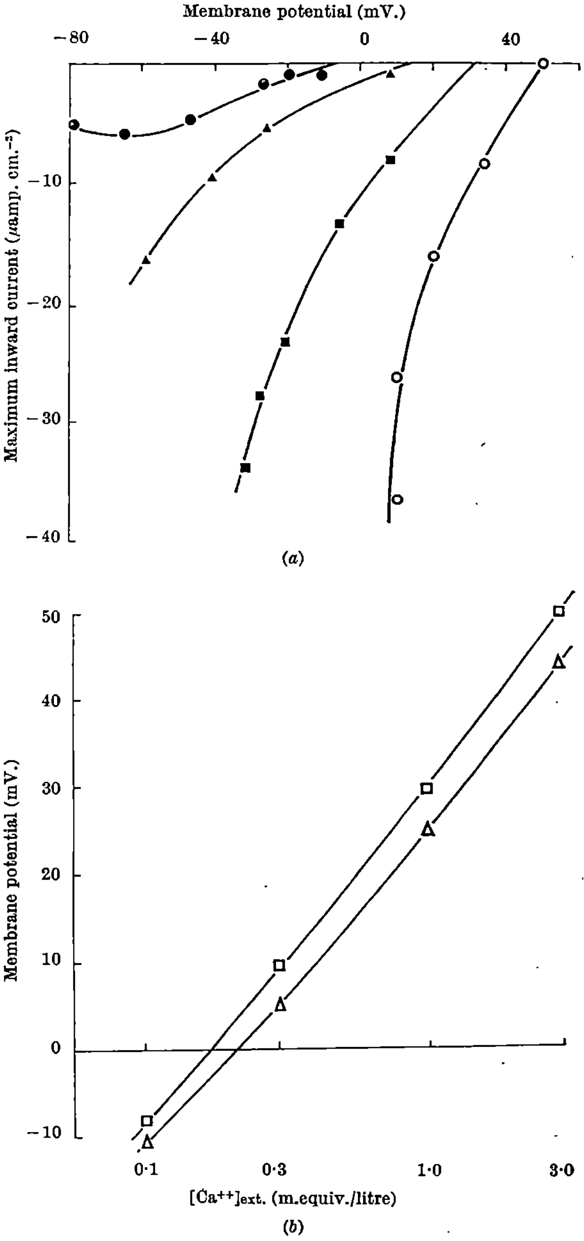


Fig. 2. (*a*) Relationship between maximum inward current and membrane potential for different $[\text{Ca}^{++}]_{\text{ext.}}$ ●, 0·1 mN; ▲, 0·3 mN; ■, 1·0 mN; ○, 3·0 mN. (*b*) Relationship between $[\text{Ca}^{++}]_{\text{ext.}}$ and membrane potential for $I_{\text{max.}} = 0$ (□), and potential at peak of ordinary action potential (△)

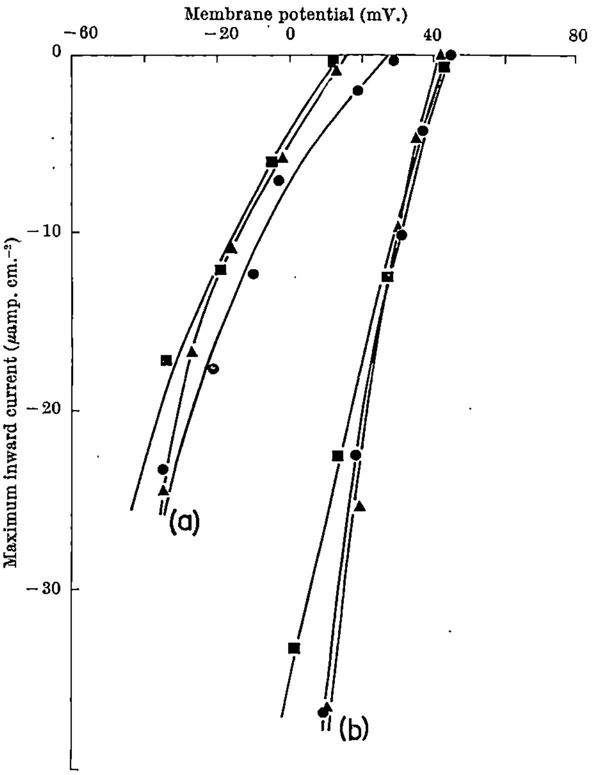


Fig. 3. Relationship between maximum inward current and membrane potential for varying $[Cl^-]_{ext.}$, and $[Ca^{++}]_{ext.}$ Curves (*a*) 0·1 m*N*; curves (*b*) 1·0 m*N* $[Ca^{++}]_{ext.}$ with $[Cl^-]_{ext.}$ = 0·1 (●); 0·3 (▲), and 1·0 (■) m*N*

spike of an ordinary action potential against $[Ca^{++}]_{ext.}$ also has a slope of 35–40 mV. per tenfold change in $[Ca^{++}]_{ext.}$. Furthermore, it is possible, as Hope[1] suggests, that other ions besides calcium may be involved to some extent in the action potential, and that the peak potential is not the electrochemical equilibrium potential for calcium alone.

Further evidence for an increase in membrane permeability to calcium during activity is afforded by the result that for any one membrane potential-level the maximum inward current increases as $[Ca^{++}]_{ext.}$ increases. This is not the case with chloride.

The foregoing experiments thus substantiate Hope's[1] conclusion that the calcium ion plays a major part in the action potential process. The voltage-clamp results show that the action potential (initiated when the membrane potential is raised above the threshold) results from a transient increase in membrane permeability to calcium. The membrane potential changes towards the electrochemical equilibrium potential for calcium. However, before this potential-level is reached (see Fig. 2*b*), the calcium permeability has decreased, and the membrane potential returns to the resting-level. The recovery is probably the result of an outward movement of potassium ions.

G. P. Findlay

Department of Physics,
University of Tasmania,
Hobart.

---

[1] Hope, A. B. (previous communication).

[2] Findlay, G. P., *Austral. J. Biol. Sci.*, **12**, 412 (1959).

[3] Hope, A. B., and Walker, N. A., *Austral. J. Biol. Sci.*, **14** (1) (in the press).

PHYSIOLOGY

Spontaneous Miniature Potentials from Insect Muscle Fibres

Recent reports[1,2] describe spontaneous potential changes at crustacean neuromuscular junctions which bear a close resemblance to vertebrate miniature endplate potentials[3]. Quantal 'packets' of acetylcholine, released from the pre-synaptic terminals of the vertebrate motor nerve, produce transient depolarizations at the post-synaptic muscle membrane of about 0·5 mV. Since reports of a similar type of activity at the insect neuromuscular junction have so far been lacking, it appeared of interest to submit the following account of its occurrence in this group.

Randomly occurring spontaneous potentials of up to 1 mV. have been recorded, in the giant South American cockroach, *Blaberus giganteus*, from single muscle fibres of the extensor tibiæ using internal micro-electrodes of resistance between 10 and 20 M.ohm. Considerable variations were observed (Fig. 1) in both amplitudes and rise times. These findings are consistent with the multi-terminal nerve innervation known to be present in insects[4,5]. The similarity of these transients to the spontaneous phenomena exhibited by the 'slow' fibres of the frog[6] is very evident. It is possible to visualize a series of junctions displaced along the muscle fibre, discharging randomly to produce a variety of potential types at the recording site.

Values for discharge frequencies collected from fibres of a large number of specimens followed a normal distribution curve ranging from 1 per sec. to 15 per sec. In general, the values were rather higher than those recorded for either the frog or the crayfish, and it is possible that the saline used[7] was slightly hypertonic to the body fluids. An increase in tonicity leads to a rise in the rate of discharge. For example, when the normal saline was replaced with one made five times hypertonic with sucrose, a marked increase in the frequency occurred over the first minute after application, followed by a gradual decline to zero over the next 30 min. Following return of the preparation to fresh normal saline, the normal discharge frequency returned (Fig. 2).

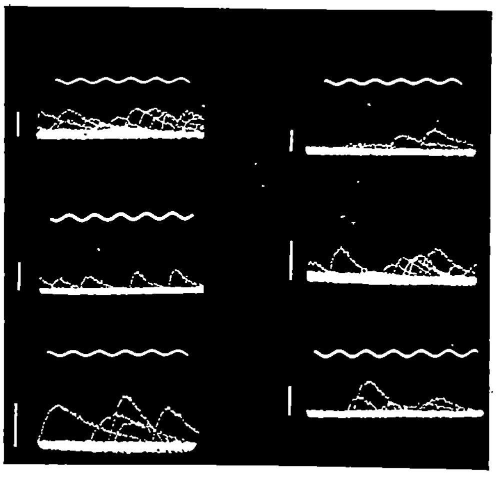


Fig. 1. Spontaneous potentials from six different muscle fibres of *Blaberus giganteus* with intracellular recording. Note variations in amplitudes and time-courses of potentials. Records were obtained by superimposition of a number of traces for each fibre. Vertical calibrations equal to 1 mV. Time calibrations, 400 c./sec.

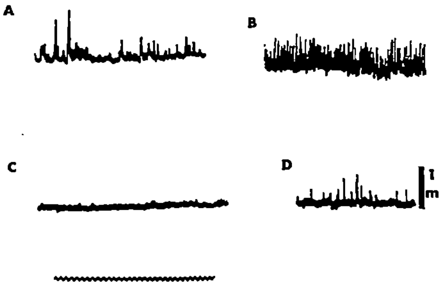


Fig. 2. Effect of tonicity on amplitude and frequency of spontaneous potentials. (*A*) Normal saline; (*B*) effect after 1 min. of increasing tonicity by a factor of five through the addition of sucrose to the saline; (*C*) effect after 30 min.; (*D*) return to normal tonicity. Voltage calibration same for *A*, *B*, *C* and *D*. Time calibration same for *A*, *B*, *C* and *D* at 30 c./sec.

A few recordings from the metathoracic extensor tibiae muscle fibres of the locust *Schistocerca gregaria* followed closely the pattern described for *Blaberus giganteus*.

It seems possible that the quantal transmitter discharge characteristic of the vertebrate nerve ending is also to be found in the insects, although the chemical identity of the transmitter substance is not yet known for any insect neuromuscular junctions.

P. N. R. Usherwood

Department of Zoology,
The University,
Glasgow.

[1] Dudel, J., and Orkand, R. K., *Nature*, **186**, 476 (1960).
[2] Reuben, J. P., and Grundfest, H., *Biol. Bull.*, **119**, 335 (1960).
[3] Fatt, P., and Katz, B., *J. Physiol.*, **117**, 109 (1952).
[4] Hoyle, G., *Proc. Roy. Soc.*, B, **143**, 281 (1955).
[5] Hoyle, G., *Proc. Roy. Soc.*, B, **143**, 343 (1955).
[6] Burke, W., *J. Physiol.*, **136**, 511 (1957).
[7] Becht, G., *Bijdr. Dierk.*, **29**, 7 (1959).

Thermodynamics and Osmoregulation

During a recent meeting concerning renal function one of the speakers suggested that the reason for the high metabolic rate of the mammalian kidney might be further investigated since very little energy seems to be used in forming the urine. The allusion was to a computation by Borsook and Winegarten[1] showing that the thermodynamic efficiency of the kidney is about 1 per cent. However, the inference, encountered not infrequently, and not only in connexion with renal function, is based on a misunderstanding of the nature of the computation.

The thermodynamic work performed by an organ like the kidney is the sum of a sequence of terms $RT \ln \frac{[a]\text{ blood}}{[a]\text{ urine}}$ for each of the components of the urine. Here $[a]$ is the activity of the substance, usually approximated by its concentration. It is important to note that this value depends solely on the initial and final states of the system (for example, blood and urine concentrations of the constituents), and on the temperature. It is completely independent of the mechanism by which the urine is formed. The actual energy expended by an organ like the kidney is a function of these mechanisms, and it is this parameter, and not the thermodynamic work done, that is reflected in the oxygen consumption of the organ. To return to the case of the mammalian kidney, if we take account of the manner in which the urine is formed, and use some recently published results on the energetics of sodium transport, it is easy to show that there is no mystery about the high oxygen requirement of this organ. It has been shown that about 16 equivalents of sodium ion are transported across the frog skin per mole of oxygen used[2,3]. In the course of a day the human kidneys filter about 30 equivalents of sodium, nearly all of which is re-absorbed. If we assume that all the re-absorption requires energy, and use Zerahn's value for sodium/oxygen (thereby assuming that the renal 'sodium pump' is the same as that in the skin of the frog) the rate of oxygen consumption would be about 25 ml./min. This is actually slightly higher than the total oxygen consumption for the organs, and no account has been taken of energy expended in re-absorbing other solutes. It is possible that some sodium is re-absorbed by systems with a lower energy requirement (for example, by forced exchange against H^+ and NH_4^+), or that sodium/oxygen < 16. The reasonable correspondence between observed and computed values, however, makes it obvious that a large fraction of the energy turnover must be devoted to solute re-absorption.

This type of misconception is not restricted to discussions on the mammalian kidney. Schlieper[4] has summarized much evidence showing that the oxygen consumption of aquatic organisms increases as their environment becomes more dilute. These data have been interpreted to mean that increased work is being done by the osmoregulatory organs in dilute media. This interpretation has been criticized, and one of the major objections has been based on the same misuse of the thermodynamic argument. Potts[5] has shown that the thermodynamic work done by the gills and kidneys of these organisms represents a very small proportion of their total energy turnover. Potts's data have been used as the basis for the argument that increased activity of the osmoregulatory organs could not account for the rise in oxygen consumption, which is often as large as 20–40 per cent[6,7]. As in the previous case the computation concerns thermodynamic work and is a minimal, not a realized value. The actual energy expended by the osmoregulatory organs depends on the mode of their operation; not on the initial and final states, but on the path by which the transformation is made. Thus, oxygen consumption of the crayfish antennal gland is about 5 per cent of the animal's total[8], certainly not a negligible fraction. If this organ, like the vertebrate kidney, functions on a filtration–re-absorption basis[9], it is likely that cessation of urine formation alone would cause a measurable fall in basal oxygen utilization. Such a cessation has been noted when the osmotic concentration of the external environment is raised. In addition, blood sodium rises when the concentration in the environment is increased. Since the rate of sodium uptake by the gills varies inversely with blood sodium-level[11], energy expenditure by the gills might also be expected to decrease in brackish water. The result would be just that noted by Schlieper: lower energy expenditure in the more concentrated medium. Thus, if the mechanisms are considered, an appreciable change in resting oxygen consumption is not only reasonable, but might have been predicted. However, it is not the chief purpose of this communication to defend Schlieper's thesis, but to point out that the thermodynamic argument against it is not valid.

While the thermodynamic work performed sets no upper limit on the amount of energy that may be expended it does provide an absolute minimum. Thus it is useful in evaluating models that are proposed for solute and solvent transport. In addition, use of a thermodynamic approach to osmoregulation has led to some interesting speculation about the evolution of these systems. If this approach is to be fruitful, however, the limitations of thermodynamics have to be observed.

This work was supported by funds for medical and biological research by the State of Washington Initiative Measure 171, and by grants from the National Science Foundation (*G*–12471), and the U.S. Public Health Service (*G*–4254).

LEONARD B. KIRSCHNER

Department of Zoology,
Washington State University,
Pullman,
Washington.

[1] Borsook, H., and Winegarten, H. M., *Proc. U.S. Nat. Acad. Sci.*, **3**, 13 (1931).

[2] Zerahn, K., *Acta Physiol. Scand.*, **36**, 300 (1956).

[3] Leaf, A., and Renshaw, A., *Biochem. J.*, **65**, 82 (1957).

[4] Remane, A., and Schlieper, C., *Die Biologie des Brackwassers* (E. Schweizbart, Stuttgart, 1958).

[5] Potts, W. T. W., *J. Exp. Biol.*, **31**, 618 (1955).

[6] Wolvekamp, H. P., and Waterman, T. H., *Crustacean Physiology*, edit. by Waterman, T. H. (Academic Press, New York).

[7] Robertson, J., *Crustacean Physiology*, edit. by Waterman, T. H. (Academic Press, New York).

[8] Peters, H., *Z. Morph. u. Okologie Tiere*, **30**, 355 (1935).

[9] Riegel, J. A., and Kirschner, L. B., *Biol. Bull.*, **118**, 296 (1960).

[10] Herrmann, F., *Z. vergleich. Physiol.*, **14**, 479 (1931).

[11] Shaw, J., *J. Exp. Biol.*, **36**, 126 (1959).

Effects of Drugs on Uptake of Isotopic Norepinephrine by Cat Tissues

PREVIOUS experiments have shown that catecholamines enter tissue slices both by simple diffusion and by a concentrating mechanism that can be demonstrated in brain, heart and spleen, but not in muscle or liver, when these tissues are incubated with physiological concentrations of isotopic epinephrine or norepinephrine[1,2]. The uptake by the concentrating mechanism is maximal in media containing 10–25 mμgm. of norepinephrine per ml. and is inhibited by reserpine and ouabain. At concentrations of norepinephrine greater than 200 mμgm. per ml., uptake by the concentrating mechanism becomes negligible in comparison to the amount entering by diffusion and the effect of reserpine is no longer demonstrable.

The present experiments were undertaken in the hope that the effects of several drugs on the uptake of norepinephrine may be related to their pharmacological effects. Slices of cat tissue were incubated for 45 min. in Krebs bicarbonate medium containing the appropriate drug and 5 mμgm. of *dl*-norepinephrine-7^{3}H per ml. The concentration of radioactivity in control slices of cortex, heart or spleen incubated without drug was usually about four times that in the medium. Approximately 80 per cent of the total radioactivity in the slices after incubation was accounted for chromatographically[3] as unchanged norepinephrine. The effects of a number of drugs on the uptake by the concentrating mechanism are listed in Table 1. In these experiments diffusion alone may be expected to give concentrations of norepinephrine in tissue approximately equal to that in the medium[2]. Percentage inhibition of the concentrating mechanism is therefore expressed as 100 × $(C_C – C_D)/(C_C – C_M)$, where C_C and C_D are the concentrations of isotopic norepinephrine in the tissue of the control and drug-treated slices respectively and C_M is the concentration in the incubation medium.

Table 1. EFFECT OF DRUGS ON THE CONCENTRATION OF RADIOACTIVITY IN SLICES OF CAT TISSUE INCUBATED 45 MIN. WITH *dl*-NOREPINEPHRINE-7^{3}H (5 MμGM./ML.)

Tissue		Molar concentration	Inhibition of uptake (per cent)
Cortex	Reserpine	10^{-6}	78
	Isoreserpine	10^{-6}	0
	Chlorpromazine	10^{-6}	72
	Cocaine	10^{-6}	100
	Mescaline	10^{-6}	32
	Lysergic acid diethylamide	10^{-8}	0
	Lysergic acid diethylamide	10^{-7}	0
	Lysergic acid diethylamide	10^{-6}	0
	Acetyl choline (with 25 μgm. eserine per ml.)	5×10^{-6}	0
	5-Hydroxytryptamine	5×10^{-6}	0
Spleen	Dibenamine	10^{-6}	0
	Dibenamine	10^{-5}	0
	Dibenamine	10^{-4}	0
	Ergotamine	10^{-6}	30
	Regitine	2×10^{-6}	14
	Chlorpromazine	10^{-6}	65
	Dichloroisoproterenol	10^{-6}	0
	Dichloroisoproterenol	5×10^{-5}	50
	Tyramine	5×10^{-6}	36
	Ephedrine	5×10^{-6}	32
	D-Amphetamine	5×10^{-6}	57
	Cocaine	10^{-6}	100
	Bretylium	3×10^{-5}	73
	Guanethidine	10^{-5}	78
	Tofranil	10^{-6}	77
Heart	Reserpine	10^{-6}	70
	Recanescine	10^{-6}	71
	Rescinnamine	10^{-6}	72
	Isoreserpine	10^{-6}	0
	Cocaine	10^{-6}	100

Norepinephrine uptake in heart slices was used for a comparison of the inhibitory effects of equimolar concentrations of several alkaloids structurally related to reserpine. The sedative agents, reserpine, recanescine and rescinnamine, were effective inhibitors. It is noteworthy that isoreserpine, which is without effect on uptake of norepinephrine, differs from reserpine only in the configuration at one carbon atom.

Both sympathomimetic and sympatholytic drugs are among the substances that inhibit the uptake of norepinephrine. Some of the former, including tyramine, ephedrine and amphetamine[4], may exert their effects by displacing norepinephrine from tissue stores near sympathetic nerve endings, thus making the amine available to receptor sites. The structural similarity of these amines to norepinephrine suggests that the displacement may result from competition by these agents for tissue storage sites.

Other inhibitory agents also release catecholamines from tissues, presumably by other mechanisms. The depletion of catecholamines by reserpine[5–7], for example, may result from some slowly reversible change in cell structure, since the effects of this drug persist for so long a time after its disappearance from the body. Guanethidine is unusual in that it depletes peripheral tissues of norepinephrine, but not of 5-hydroxytryptamine (5HT) (ref. 8). Experiments with blood platelets indicate that depletion of 5HT may result from interference by reserpine with an active transport mechanism that helps to maintain high intracellular levels of the former[9]. Depletion of norepinephrine stores in cells may be related to the effect of reserpine-like drugs on the uptake mechanism reported here.

The property most commonly encountered among these inhibitory drugs is the enhancement of the response of adrenergic organs to norepinephrine administered. This occurs with cocaine[10], reserpine[11], and bretylium[12] as well as with some sympathomimetic amines[5]. Sensitization can be demonstrated with chlorpromazine[13]. Thyroxine, which has been found in a separate investigation to block the uptake of norepinephrine (Dengler, H. J., unpublished results), is also a potentiator.

The uptake mechanism may well be a means for terminating the biological response to catecholamines by removing them from the site of activity. Should this be so, sensitization by these drugs would be expected as a consequence of their inhibitory properties. Evidence for the termination of biological effect by some sort of intracellular re-distribution rather than by metabolic alteration of the catecholamines has been reviewed recently[14].

HANS J. DENGLER*
HERBERT E. SPIEGEL
ELWOOD O. TITUS

Laboratory of Chemical Pharmacology,
National Heart Institute,
National Institutes of Health,
Bethesda, Maryland.

* Present address: Mediz Univ. Klinik, Chemisches Labor., Heidelberg.

[1] Titus, E., Wilson, C. W. M., and Dengler, H. J., *Fed. Proc.*, **19**, 297 (1960).
[2] Brodie, B. B., Dengler, H. J., Titus, E., and Wilson, C. W. M., *J. Physiol.* (in the press).
[3] Dengler, H., *Arch. Exp. Path. Pharmakol.*, **231**, 373 (1957).
[4] Burn, J. H., and Rand, M. J., *J. Physiol.*, **144**, 314 (1958).
[5] Holzbauer, M., and Vogt, M., *J. Neurochem.*, **1**, 8 (1956).
[6] Carlsson, A., Rosengren, E., Bertler, A., and Nilsson, J., in *Psychotropic Drugs*, edit. by Garattini, S., and Ghetti, V., 363 (Elsevier Pub. Co., Amsterdam and New York, 1957).
[7] Brodie, B. B., Olin, J. S., Kuntzman, R., and Shore, P. A., *Science*, **125**, 1293 (1957).
[8] Cass, R., Kuntzman, R., and Brodie, B. B., *Proc. Soc. Exp. Biol. Med.*, **103**, 870 (1960).
[9] Hughes, F. B., and Brodie, B. B., *J. Pharmacol. Exp. Therap.*, **127**, 96 (1959).
[10] Fleckenstein, A., and Bass, H., *Arch. Exp. Path. Pharmakol.*, **220**, 143 (1953).
[11] Bein, H. J., *Experientia*, **9**, 107 (1953).
[12] Boura, A. L. A., and Green, A. F., *Brit. J. Pharmacol.*, **14**, 536 (1959).
[13] Martin, W. R., Riehl, J. L., and Unna, K. R., *J. Pharmacol. Exp. Therap.*, **130**, 37 (1960).
[14] Koelle, G. B., *Pharmacol. Rev.*, **11**, 381 (1959).

Stimulation by Aldosterone of Sodium Transport in the Loop of Henle

Jones and de Wardener[1] have reported that normal subjects, when hydropenic, excrete a urine osmotically more concentrated than when given large quantities of vasopressin while hydrated. During dehydration, one element coming normally into play, besides antidiuretic hormone, is a hypersecretion of aldosterone by the adrenal cortex, as a response to the shrinkage of the liquid compartments of the body[2,3]. The possible influence of aldosterone on the concentrating capacities of the human kidney was therefore evaluated by comparing the urinary osmotic pressure after administration to hydrated subjects of vasopressin alone and of vasopressin associated with aldosterone.

Seven tests were performed on five normal young males. Two and a half units of 'Pitressin' tannate were injected subcutaneously at 8 a.m., first alone, then, one week later, in association with 0·5 mgm. of D-aldosterone-21-monoacetate in oil. The subjects emptied their bladders 2 hr. after the injections, and collected urine thereafter for a period of 8 hr. during which they were asked to drink about 1 litre of fluids in addition to their usual diet. The urine osmolality (m.osm./kgm. water) was determined with a Fiske osmometer.

The results are presented in Table 1. In the presence of aldosterone, there was a larger increase in urine osmotic pressure after injection of vasopressin.

The mean urine osmolality figure of 955 obtained after administration of vasopressin alone is comparable with the mean value of 972 reported by Jones and de Wardener, who indeed pointed out that the dose of 2·5 units of vasopressin sufficed to produce a maximal effect on the urinary osmotic pressure of their hydrated normal subjects.

Table 1. URINE OSMOLALITY IN NORMAL HYDRATED MALES AFTER VASOPRESSIN ASSOCIATED WITH OR WITHOUT ALDOSTERONE

Subjects	Vasopressin		Vasopressin + Aldosterone	
	Urine volume	Osmolality	Urine volume	Osmolality
J. C.	580	849	290	1,093
J. C.	460	1,098	305	1,175
J. C.	415	1,036	315	1,115
K. D.	310	933	350	907
M. V.	450	943	450	986
R. D.	430	885	160	1,024
R. B.	250	944	390	961

Mean $\Delta\pi$: 81·9. *S.E.* $\Delta\pi$: 33·4. $P < 0{\cdot}05$.

If aldosterone improves the concentrating ability of the human kidney, the conclusion may be drawn that this hormone stimulates the re-absorption of sodium (chloride) in the ascending limb of the loop of Henle. Sodium transport at that site, from the tubular lumen to the interstitium, is taken as the prime mover in the operation of the counter-current concentrating mechanism of the renal papilla[4,5]. Factors exerting an effect on the rate of sodium transport at that level would in turn influence the efficiency of the whole process of urine concentration. As suggested by the results presented here, aldosterone is one of these factors. They indicate, furthermore, that, even when stimulated by aldosterone, sodium transport in that part of the nephron is not linked to the secretion into the urine of other cations. It is pertinent to mention here that aldosterone has recently been shown to enhance active sodium transport in the isolated urinary bladder of the toad[6], a preparation in which sodium (chloride) movement is not coupled with cationic secretion[7].

I wish to express my gratitude to Mr. P. Denis, Parke, Davis and Co., for the gift of 'Pitressin' tannate, and to Prof. R. Gross, Ciba A. G., Basle, for the gift of D-aldosterone-21-monoacetate.

J. CRABBÉ*

Laboratorium voor experimentele Geneeskunde,
Rega Instituut,
Louvain, Belgium.

* Research Fellow of the Helen Hay Whitney Foundation, U.S.A.

[1] Jones, R. V. H., and de Wardener, H. E., *Brit. Med. J.*, i, 271 (1956).
[2] Bartter, F. C., Liddle, G. W., Duncan, jun., L. E., Barber, J. K., and Delea, C., *J. Clin. Invest.*, **35**, 1306 (1956).
[3] Falbriard, A., Muller, A. F., Neher, R., and Mach, R. S., *Schweiz. med. Wochenschr.*, **85**, 1218 (1955).
[4] Wirz, H., *The Neurohypophysis*, edit. by Heller, H., *Proc. Eighth Symp. Colston Res. Soc.*, 157 (Butterworths Sci. Pub., London, 1957).
[5] Gottschalk, C. W., and Mylle, M., *Amer. J. Physiol.*, **196**, 927 (1959).
[6] Crabbé, J., *Clin. Res.*, **8**, 227 (1960).
[7] Leaf, A., Anderson, J., and Page, L. B., *J. Gen. Physiol.*, **41**, 657 (1958).

PHARMACOLOGY

Inhibition of Phosphorylation by Atractyloside

IN previous papers it was shown that atractyloside, a glycoside (formerly called K-atractylate) extracted from the rhizome of *Atractylis gummifera*, the aglycone of which contains the perhydrophenanthrene nucleus[1], inhibits the uptake of oxygen by rat-liver mitochondria also at very low concentrations (0·0000036 *M*) and with a marked decrease of the P/O ratio[2].

Using hypotonically treated rat-liver mitochondria, with reduced diphosphopyridine nucleotide or ferrocytochrome-*c* as substrates, inhibition of phosphorylation was observed without decrease in the rate of oxygen uptake[3]. The 2,4-dinitrophenol-induced adenosine triphosphatase of intact mitochondria was inhibited by this drug, but the same was not true for Mg^{++}-activated adenosine triphosphatase of mitochondria disrupted by deoxycholate[4].

In this communication evidence is presented that inhibition of mitochondrial respiration by atractyloside might be due exclusively to interference with phosphorylation reactions.

Table 1 shows that : (*a*) atractyloside has no effect on the oxygen uptake of water pre-treated mitochondria oxidizing β-hydroxybutyrate ; (*b*) atractyloside fails to produce inhibition of respiration in an adenosine triphosphate-, glucose- and hexokinase-deficient system and in a phosphorus-deficient system; (*c*) the respiration of mitochondria, observed under phosphorylative conditions in the presence of atractyloside, is very similar to that of mitochondria incubated in the absence of adenosine triphosphate (ATP), glucose and hexokinase; this fact could indicate that the increase of uptake of oxygen due to phosphorylation is inhibited by the drug. Phosphorus was estimated by the Fiske and Subbarow method and a complete block of phosphorus uptake was observed in the vessels containing atractyloside.

Table 1. EFFECT OF ATRACTYLOSIDE ON MITOCHONDRIAL RESPIRATION
The oxygen uptake was measured manometrically at 26° C. in the Warburg apparatus. The vessels contained: (*a*) 0·03 *M* potassium phosphate buffer, *p*H 7·4, 0·005 *M* $MgSO_4$, 0·00036 *M* diphosphopyridine nucleotide, 0·001 *M* ethylenediamine tetraacetate, 0·09 *M* sucrose, 0·02 *M* β-hydroxybutyrate, 1 mgm. of nitrogen rat-liver mitochondria (isolated in 0·25 *M* sucrose and pre-treated with water for 30 min. at 0° C). (*b*) 0·015 *M* potassium phosphate buffer, *p*H 7·4, 0·03 *M tris* buffer, *p*H 7·4, 0·005 *M* $MgSO_4$, 0·03 *M* glucose, 0·0014 *M* ATP, 0·8 mgm. yeast hexokinase (Sigma type II), 0·01 *M* glutamate (or 0·02 *M* β-hydroxybutyrate), 0·001 *M* ethylenediamine tetraacetate, 0·09 *M* sucrose, 0·000005 *M* cytochrome-*C*, 1 mgm. of nitrogen rat-liver mitochondria (isolated in 0·25 *M* sucrose). Final volume, 2·8 ml.; time of incubation, 20 min.; exceptions are indicated; gas-phase, air; KOH and filter paper in the centre well. Inorganic phosphate was omitted by the Borst and Slater (ref. 5) procedure; P/O ratios are reported in parentheses.

Substrate	Omissions	Oxygen uptake (μatoms)	
		Without atractyloside	Atractyloside 0·00005 *M*
(*a*) Water pre-treated mitochondria			
β-Hydroxybutyrate	Nil	5·8	5·8
(*b*) 0·25 *M* sucrose mitochondria			
β-Hydroxybutyrate*	Nil	2·9 (2·6)	1·2 (0)
β-Hydroxybutyrate*	ATP, glucose, hexokinase	1·2	1·2
β-Hydroxybutyrate*	inorganic phosphate	1·1	1·1
Glutamate	Nil	14·1 (2·6)	2·0 (0)
Glutamate	ATP, glucose hexokinase	2·3	2·0
Glutamate	inorganic phosphate	1·4	1·3

* Time of incubation 10 min.

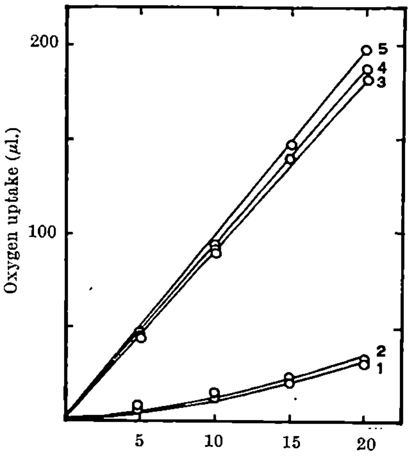


Fig. 1. The effect of atractyloside on the rate of glutamate oxidation under phosphorylative conditions and in presence of 2,4-dinitrophenol. The vessels contained 0·015 *M* potassium phosphate buffer, *p*H 7·4, 0·03 *M tris* buffer, *p*H 7·4, 0·005 *M* $MgSO_4$, 0·001 *M* ethylenediamine tetraacetate, 0·01 *M* glutamate, 0·09 *M* sucrose, 0·000005 *M* cytochrome-*c*, 1 mgm. of nitrogen rat-liver mitochondria (isolated in 0·25 *M* sucrose). Additions: 1, 0·0014 *M* ATP + 0·03 *M* glucose + 0·8 mgm. yeast hexokinase + 0·00005 *M* atractyloside; 2, nil; 3, 0·0014 *M* ATP + 0·03 *M* glucose + 0·8 mgm. yeast hexokinase; 4, 0·0014 *M* ATP + 0·00005 *M* 2,4-dinitrophenol + 0·00005 *M* atractyloside; 5, 0·0014 *M* ATP + 0·00005 *M* 2,4-dinitrophenol. Other conditions as in Table 1

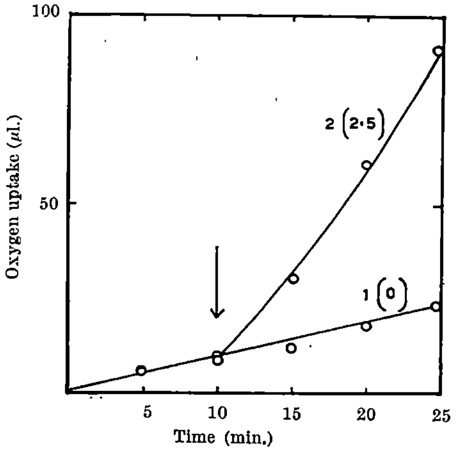


Fig. 2. Inhibition by atractyloside of the oxygen uptake stimulation induced by inorganic phosphate. The vessels contained 0·042 *M tris* buffer, *p*H 7·4, 0·005 *M* $MgSO_4$, 0·001 *M* ethylenediamine tetraacetate, 0·01 *M* glutamate, 0·09 *M* sucrose, 0·000005 *M* cytochrome-*c*, 0·8 mgm. of nitrogen rat-liver mitochondria (isolated in 0·25 *M* sucrose), 0·03 *M* glucose, 0·8 mgm. yeast hexokinase, 0·0014 *M* ATP. Additions at the arrow: 1, nil, or 25 μmoles potassium phosphate + 0·14 μmole atractyloside; 2, 25 μmoles potassium phosphate. Final volume 2·8 ml.; other conditions as in Table 1. The numbers in parentheses refer to the P/O ratios determined for the last 15 min. of the experimental period

Attempts were made to locate the action of the drug on the sequence of phosphorylation reactions. Fig. 1 shows the effect of atractyloside in a ∼P acceptors-deficient system activated by 2,4-dinitrophenol. A concentration of this substance, able to produce complete uncoupling of the respiratory chain phosphorylation, completely removes atractyloside inhibition on oxygen uptake with glutamate as substrate ; the same is obtained with β-hydroxybutyrate, succinate and malate as substrates.

Atractyloside blocks both uptake of phosphorus and the increase of respiration produced by addition of inorganic phosphate in mitochondria depleted of endogenous phosphorus by pre-incubation with adenosine triphosphate, glucose and hexokinase[5], with glutamate (Fig. 2) or β-hydroxybutyrate as substrate; consequently, it is likely that systems utilizing inorganic phosphate are particularly concerned with the inhibition of phosphorylation by atractyloside.

Because of its action on phosphorylation reactions, atractyloside appears to be a very suitable drug for work on oxidative phosphorylation; for example, according to Slater[6,7], these results could provide evidence that for the stimulatory effect of 2,4-dinitrophenol in $\sim$P acceptors-deficient systems, inorganic phosphorus is not necessary; indeed, the action of this uncoupling agent is present also with atractyloside, which inhibits phosphorus uptake. The various aspects of the antagonism between 2,4-dinitrophenol and atractyloside are under investigation. This work has been aided by a grant from the Consiglio Nazionale delle Ricerche.

A. Bruni
A. R. Contessa

Department of Pharmacology,
University of Perugia.

[1] Ajello, T., Piozzi, F., Quilico, A., and Sprio, V., *Lincei-Rend. Sci. fis. mat. e nat.*, **28**, 545 (1960); *La Chimica e l'Industria*, **42**, 876 (1960).

[2] Santi, R., *Nature*, **182**, 257 (1958). Bruni, A., Bistocchi, M., and Contessa, A. R., *Archivio Ital. Sci. Farmacol.*, Serie III, **10**, 86 (1960).

[3] Bruni, A., Bistocchi, M., and Contessa, A. R., *Archivio Ital. Sci. Farmacol.*, Serie III, **10**, 115 (1960).

[4] Bistocchi, M., and Millo, A., *Revista Espanola de Fisiologia*, **16**, Supp. 2, 259 (1960).

[5] Borst, P., and Slater, E. C., *Nature*, **184**, 1396 (1959).

[6] Slater, E. C., *Nature*, **172**, 975 (1953).

[7] Borst, P., and Slater, E. C., *Nature*, **185**, 537 (1960).

Comparison between Water and Alcohol Consumption in Six Animal Species in Free-choice Experiments

Great differences exist in the inter-individual fluctuation of the voluntary intake of alcohol by laboratory rats on free choice between 10 per cent alcohol and water[1]. This is also true of laboratory mice[2]. But on the whole the average alcohol consumption within a species of animal is constant. It can be said that each species has its own specific liking for alcohol.

In free-choice experiments the results are usually expressed as the total amount of alcohol consumed. But if the data are intended to give a picture of the preference for alcohol compared with water, it would be clearer to express the results in terms of the alcohol drunk in relation to the total amount of liquid consumed. In this way the preferences of animals of different weight and species can be directly compared.

In this work the consumption of 10 per cent alcohol and water in free-choice experiments in six animal species has been studied. The animal species were: hedgehog (*Erinaceus europeus*), golden hamster (*Cristatus auratus*), guinea pig (*Cavia porcellus*), rabbit (*Lepus cuniculus*), rat (*Mus norvegicus*, albino laboratory stock), and mouse (*Mus musculus*, Swiss albino Webster).

The hedgehogs were given only fish as food, the hamsters coarse oat meal and green forage, the guinea pigs were given hay and turnips, the rabbits hay or fresh grass, the rats a laboratory food mixture and the mice a commercial food mixture. The alcohol and water consumption was noted every day except for the mice, for which it was measured every 3–4 days. The position of the bottles was changed every 2–3 days. The results of the experiments are shown in Table 1.

The hedgehogs preferred water to alcohol solution. Sometimes, however, the animals drank considerable amounts of alcohol, and it could at times be judged from their movements that they were slightly drunk. The hamsters preferred alcohol. They seldom tasted the water and, when they did so, only until they found out that they were drinking from the wrong bottle. The guinea pigs preferred water. The comparatively large consumption of fluid and the wide variations in consumption showed that the animals spilled out some of the fluid; but it could be seen that they visited the water bottle more often and only seldom the alcohol vessel. The rabbits drank about as much alcohol as water. Individual rats differ in their choice between alcohol and water. Most of them prefer water to alcohol, but some prefer alcohol. In some individuals there was a change in preference while the experiments were in progress. What is said about rats is also true of mice.

If an animal cannot taste the difference between alcohol and water or has an equal liking for both, it does not discriminate between them, and the intake of the two drinks is about the same. Rabbits belong to this group of animals. Guinea pigs, rats, mice and hedgehogs discriminate between these solutions and prefer water to alcohol. It cannot be said from these experiments whether the preference for water depends only on an aversion to the taste of alcohol or whether any negative, physical effect of the alcohol is involved. Rats at least have an absolute aversion to alcohol and not merely a relative preference for water, since if alcohol is the only solution available the amount of fluid consumed is lower than when plain water is available[3].

In contrast to the animals mentioned above, hamsters have a pronounced preference for alcohol. This has earlier been shown to be true of deer mice (*Peromyscus c. californicus*)[4] and of the mouse strain Loiseleur and Petit used[5].

In this experiment there is no absolute measure of how much an animal likes or dislikes alcohol. It is only possible to measure how much the animal likes alcohol compared with its liking for water. A third alternative would have altered the results in relation

Table 1. Alcohol and Water Consumption in Six Animal Species in Free Choice Experiment

No. of animals	Animals × days	Species	10 per cent alcohol per day (ml.)	Water per day (ml.)	Percentage drunk as 10 per cent alcohol	Alcohol–water $P <$
12	420	Hedgehog	6·8 ± 0·53	11·9 ± 1·07	36·3	0·0002
10	340	Golden hamster	5·4 ± 0·19	0·7 ± 0·01	88·0	0·0002
8	256	Guinea pig	8·7 ± 0·97	71·2 ± 2·43	10·8	0·0002
8	168	Rabbit	34·8 ± 1·32	37·3 ± 1·54	48·3	0·5
12	300	Rat	7·4 ± 0·55	17·5 ± 0·98	29·8	0·0002
10	72	Mouse	10·0 ± 5·08	26·9 ± 5·09	27·1	0·0002

to the preference for the new solution. As Lester and Greenberg[6] have shown, a third bottle containing sugar solution strongly influenced the choice of alcohol. Only if the preference for a substance is strong in every set of alternatives can it be said that the animal has a specific craving for this substance.

The reason for the preference for, or aversion to, alcohol among animals is not known. It was shown earlier that a low original alcohol consumption in a rat can be changed into a strong preference in various ways: by a deficient diet causing metabolic block[7], by damaging liver function[8] or by inducing an experimental conflict situation[9]. In our investigation all these conditions could be excluded. According to one of us[10], the choice of alcohol can be explained by the metabolic type of the animal. Perhaps differences in metabolism can explain the differences in preference for alcohol in the various animal species.

ALPO ARVOLA
OLOF FORSANDER

Research Laboratories of the State
Alcohol Monopoly (Alko), Helsinki, and
Zoological Institute,
University of Helsinki, Finland.

[1] Mardones, R. J., Hederra, D. A., and Segovia, M. N., *Bol. soc. biol.*, Santiago, Chile, 7, 1 (1949).

[2] Williams, R. J., Berry, L. J., and Beerstecher, E., *Proc. U.S. Nat. Acad. Sci.*, 35, 265 (1949).

[3] Richter, C. P., in *Neuropharmacology*, edit. by Abramson, H. A., 3 (Josiah Macy Jr. Foundation, New York, 1957).

[4] Emerson, G. A., Brown, R. G., Nash, J. B., and Moore, W. T., *J. Pharmacol. Exp. Therap.*, 106, 384 (1952).

[5] Loiseleur, J., and Petit, M., *C.R. Soc. Biol., Paris*, 141, 568 (1947).

[6] Lester, D., and Greenberg, L. A., *Quart. J. Studies Alc.*, 13, 553 (1952).

[7] Mardones, R. J., and Onfray, B. E., *Rev. Chilena Hig. y Med. Prevent.*, 4, 293 (1942).

[8] Sirnes, T. B., *Quart. J. Studies Alc.*, 14, 3 (1953).

[9] Conger, J. J., *Quart. J. Studies Alc.*, 12, 1 (1951).

[10] Forsander, O., Kohonen, J., and Suomalainen, H., *Quart. J. Studies Alc.*, 19, 379 (1958).

RADIOBIOLOGY

Urinary Metabolites of ^{14}C-Tetrachloroethylene in Mice

IN recent years tetrachloroethylene (perchloroethylene) has rapidly gained in importance as an industrial solvent. Its toxic effects have not been investigated very much, however, and its metabolism is almost unknown. The present work was carried out in order to facilitate the estimation of health hazards caused by exposure to this solvent.

Starting with ^{14}C-acetylene, tetrachloroethylene was synthesized essentially according to the industrial method[1,2]:

$$CH{\equiv}CH + 2Cl_2 \longrightarrow CHCl_2CHCl_2$$

$$CHCl_2CHCl_2 + OH^- \longrightarrow CHCl{=}CCl_2 + H_2O + Cl^-$$

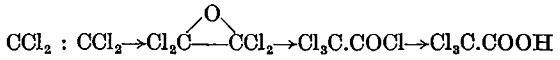
$$CHCl{=}CCl_2 + Cl_2 \longrightarrow CHCl_2CCl_3$$

$$CHCl_2CCl_3 + OH^- \longrightarrow CCl_2{=}CCl_2 + H_2O + Cl^-$$

The crude ^{14}C-tetrachloroethylene was purified by preparative gas chromatography, and the specific activity of the purified product was 0·7 μc./mgm.

Five female mice, one at a time, were exposed for 2 hr. in a sealed flask (2·7 l.) to the solvent vapour in doses of about 1·3 mgm./gm. body-weight. 70 per cent (42–87 per cent) of the solvent was absorbed. In four days about 90 per cent of the absorbed activity was excreted, namely, 70 per cent in the expired air, 20 per cent in the urine and less than 0·5 per cent in the fæces. By chromatographic, autoradiographic and isotope-dilution methods, in part by Jondorf's method[3], the following metabolites were identified in the urine (percentage of total urinary activity): trichloroacetic acid (52 per cent), oxalic acid (11 per cent) and dichloroacetic acid (traces). No monochloroacetic acid, formic acid or trichloroethanol was found. After hydrolysis 18 per cent of the urinary metabolites were still not extractable with ether.

These results indicate the formation of an epoxide further rearranged to trichloroacetyl chloride as one metabolic pathway for tetrachloroethylene:



$$CCl_2 : CCl_2 \rightarrow Cl_2C\overset{O}{\text{——}}CCl_2 \rightarrow Cl_3C.COCl \rightarrow Cl_3C.COOH$$

This reaction has been previously shown to occur *in vitro* with the oxidation of tetrachloroethylene in the presence of non-biological catalysts[4,5].

SVEN YLLNER

National Institute of Public Health,
Stockholm.

[1] *Fiat Final Rep.* No. 843 (1947).

[2] *Bios Final Rep.* No. 1056 (1947).

[3] Jondorf, W. R., *The Metabolism of Certain Chlorinated Hydrocarbons* (University of London, 1956).

[4] Kirkbride, F. W., U.S. Patent 2,321,823 (1943).

[5] Frankel, D. M., Johnson, C. E., and Pitt, H. M., *J. Org. Chem.*, 22, 1119 (1957).

Action of Tetracycline on the Level of Properdin

THE influence of antibiotics on the formation of antibodies has been studied by many authors. Recently, Patočka *et al.*[1] published a paper describing the inhibitory effect of tetracycline on the formation of specific antibodies of various types in infections or after immunization with brucellæ, the influenza virus and herpes in laboratory animals. Clinical experience shows that in certain infections caused by pathogenic agents (viruses) tetracycline may have a favourable effect on the course of the disease. We tried to ascertain whether in these cases this is not due to the action of tetracycline on a non-specific defence mechanism.

We therefore investigated the properdin-levels in rabbits daily for one month with intramuscular application of 25 mgm. tetracycline per kgm. body-weight. Determination of properdin was carried out by Isliker and Linder's[2] modification of Pillemer's method. Properdin was determined before the beginning of the experiment and at intervals of 10 days. Before the experiment the average properdin-level was approximately ten units, which is regarded as the normal level. During the first ten days, properdin activity increased to a varying degree in all laboratory animals, on the average to more than double its original value. On the twentieth day we noted a marked fall, in spite of the continued application of tetracycline; on the thirtieth day the level stabilized at about the normal value. Fig. 1 shows the curve of the properdin-level in a group of nine rabbits after statistical evaluation. The experiment was then repeated in a similar manner with six rabbits which were also exposed to X-rays (500 r.). The results obtained in this group were similar, except that the decrease in the properdin-level between the tenth and the twentieth day after irradiation was much more pronounced, frequently below the original level. In the control group of four rabbits the familiar decrease in properdin activity was

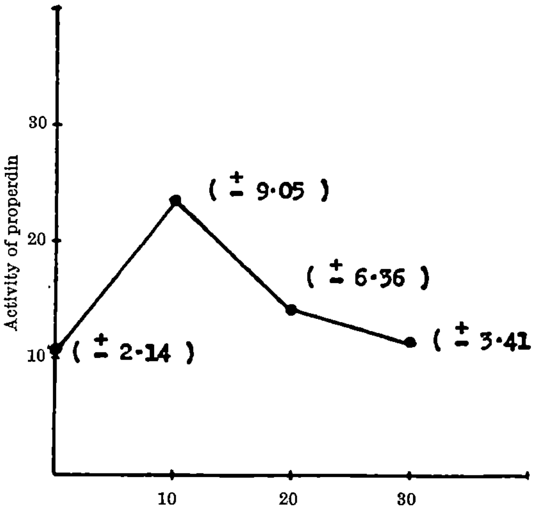


Fig. 1. Effect of tetracycline on the properdin-level in rabbits

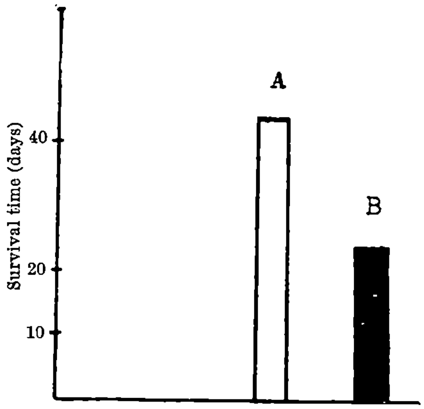


Fig. 2. Effect of tetracycline on the survival-time of irradiated rats. *A*, Group of ten rats treated with tetracycline; *B*, group of ten control rats

observed and normalization was reached on the thirtieth day.

To supplement this experiment, twenty white rats were irradiated (750 r.) and the effect of tetracycline on the survival-time was studied in ten rats against ten control animals. Fig. 2 shows a prolongation of the survival-time through the action of tetracycline. The relation between the prolonged survival-time of irradiated rats under the influence of tetracycline and the activity of the properdin system still remains a matter of conjecture; however, the results could serve as a basis for further work on the prevention of damage due to irradiation and the tetracyclines may be an effective co-factor in the suppression of the damaging effects of ionizing radiation.

VL. KUBELKA

Department of Epidemiology,
Medical School,
Charles University,

M. RYBÁK

Institute of Hæmatology
and Blood Transfusion,
Prague 2.

---

[1] Patočka, F., John, C., Kubelka, Vl., Korb, J., and Šrajbr, E., *Čas. lék. ces.*, 49, 1053 (1960).

[2] Isliker, A. C., and Linder, E., *Vox Sang.*, 3, 23 (1958).

HÆMATOLOGY

Thromboplastic Activity of Red Cells

WHILE a number of workers have reported thromboplastic activity exerted by hæmolysed red cells, intact red cells have been reported to display no such activity[1].

In experiments to be reported elsewhere, it has been found that under conditions more nearly approaching physiological, red cells exert a platelet-like thromboplastic activity of an order approaching that exerted by platelets.

The generation of thromboplastin was investigated first in diluted platelet-rich plasma. It was found that platelets which had thus been subjected to no manipulation exerted a much more sluggish action than that exerted by washed and re-suspended platelets. Twenty minutes might elapse after re-calcification before thromboplastic activity reached its maximum. If the plasma stood at room temperature for 2–3 hr. before re-calcification, this delay was shortened and the maximum activity developed was greater. A clot formed in the incubating plasma, if the ultimate concentration of plasma was more than 1 per cent, and it was found that thromboplastin formation was minimal until this clot formed and was separated. If the clot were allowed to stand for 2–3 min. before it was separated, thromboplastin was still minimal immediately after separation, but then developed rapidly and reached a higher maximum which approached that developed by washed platelet suspensions. Rapidly centrifuged normal plasma or slowly centrifuged plasma from thrombocytopænic patients treated in the same way developed little thromboplastic activity.

Blood was then used instead of plasma. Somewhat greater activity developed in diluted blood than in slowly centrifuged plasma, diluted to give the same plasma concentration. When blood from thrombocytopænic patients was used, diluted blood developed much greater activity than diluted slowly centrifuged plasma. There was little difference in the activities developed by normal blood and by thrombocytopænic blood.

This activity of red cells showed similar characteristics to those of platelets—in that maximum activity was displayed when the blood stood for 2–3 hr. first or when the clot was allowed to stand for 2–3 min. before it was separated; thromboplastin formation was minimal until the clot was separated. The activity was not due to hæmolysis, of which there was no evidence.

Certain discrepancies with previous observations could be resolved.

First, blood from thrombocytopænic patients displays poor prothrombin consumption. This is due to the fact that the red cells exert little thromboplastic activity until they have escaped from the clot. In the prothrombin consumption test centrifugation separates the cells from the serum before much action has been exerted. Little thromboplastic activity is then developed compared with the activity developed when the red cells are dispersed into the serum.

Other discrepancies are that the thrombin generation test of Macfarlane and Biggs[2] shows considerable difference between thrombocytopænic and normal blood, and that washed red cells have been found inactive in the thromboplastin generation test[1].

Investigation of these discrepancies revealed a difference between the behaviour of platelets and of red cells. While both develop marked activity only after coagulation has occurred, the activity exerted by red cells before coagulation has occurred is much weaker than is that of platelets. When, therefore, platelet activity of other source is minimal and when other conditions are unfavourable to coagulation, the development of red cell activity tends to be blocked. In the thrombin generation test coagulation is retarded, because the blood has not been exposed to surface activation. In the thromboplastin generation, test dilution of the constituents retards coagulation. In both cases when red cells are present, but no platelets, the red cell activity develops only slowly. By varying conditions, however, red-cell activity can easily be demonstrated in both types of test.

The difference in behaviour of platelets and red cells makes a quantitative comparison impossible, unless the conditions are specified. Moreover, since the removal of all platelets tends to block the development of red-cell activity, it might be thought that this activity would be unimportant under physiological conditions. This requires further investigation, but so far the evidence obtained tends to indicate that red cells contribute to coagulation under natural conditions and are partly responsible for the normal coagulation time found in thrombocytopænic conditions. Certain clinical differences between the hæmorrhagic states of thrombocytopænia and of coagulation defects, such as the difference in frequency of hæmarthroses, may be explicable in terms of this red-cell action.

JAMES A. INGLIS
JUNE W. HALLIDAY

Department of Pathology,
University of Queensland.

[1] O'Brien, J. R., *J. Clin. Path.*, **12**, 45 (1959).
[2] Macfarlane, R. G., and Biggs, R., *J. Clin. Path.*, **6**, 3 (1953).

Thalassæmia in an English and in a German Family

REPORTS of thalassæmia (major and minor) in British residents of the United Kingdom are few[1–6]. Equally few are reports of the occurrence of this abnormality in subjects of German extraction[7–14].

Recently, we have been studying the frequency of the carrier form of this abnormality (thalassæmia minor) in subjects of Greek, Egyptian, Syrian, Armenian, Italian, Ethiopian and Sudanese (Northern, 'Arab' and Southern, 'Negro') origin, using, as an absolute criterion, the presence of an increased erythrocyte osmotic resistance associated with abnormal morphological characteristics of the erythrocytes[15,16]. During our testing of healthy school children in Khartoum for this purpose we detected one English and one German girl whose erythrocytes repeatedly showed an increased osmotic resistance and, on unstained peripheral smears, anisopoikilocytosis and microcytes. Both girls (aged nine years and thirteen years respectively) were in good general health and appeared normal on clinical grounds. The abnormality was not found in fifteen other British and five other German children tested.

Similar studies carried out on the parents of the English girl (their only child) revealed that both carried the same abnormality and that both were in good general health. The mother was born in Cornwall and was of mixed Spanish–Irish–Cornish stock; she has a brother and a sister both of whom are in good health. The father was born in Malta of British parents. There is a possibility that there was some French admixture in his ancestry.

Examination of both parents of the German girl and of one brother showed that the father was carrying the abnormality and that the mother and brother were normal. The mother was born in Kothen, Anhalt, had a fair complexion and looked and felt well. She was of 'pure' German stock. The father was born in Korrig, Saarburg, and was of a dark complexion with heavy bushy eyebrows. He had suffered from malaria and had occasionally complained of easy fatigability. A recent hæmoglobin test yielded results within normal limits. His sister and his mother are said to have a similar dark complexion. His family had had close ties in Lorraine some two centuries ago.

The evidence of ties with continental Europe in the ancestry of the three parents having the thalassæmia abnormality in these two families raises the possibility that the abnormality entered both these families through their foreign antecedents. It is difficult to prove this point in the absence of any systematic work on the frequency of this abnormality in Spain and France as well as in Great Britain and Germany. Chernoff[17] lists only five references in the literature to thalassæmia in Spanish subjects, while there are only two reports of this abnormality in French subjects[18,19].

It is to be hoped that the significance of the thalassæmia abnormality in these countries will emerge in the not too distant future.

F. VELLA
S. A. IBRAHIM

Department of Biochemistry,
Faculty of Medicine,
University of Khartoum.

[1] Grinnan, A. G., *Amer. J. Roentgenol.*, **34**, 297 (1935).
[2] Bush, C. E., *Brit. J. Radiol.*, **10**, 491 (1937).
[3] Bywaters, E. G. L., *Arch. Dis. Childhood*, **13**, 173 (1938).
[4] Israels, L. G., Suderman, H. J., and Hoogstraten, J., *Lancet*, ii, 1318 (1955).
[5] Israels, M. C., and Turner, R. L., *Lancet*, ii, 1363 (1955).
[6] Havard, C. W. L., Lehmann, H., and Scott, R. B., *Brit. Med. J.*, i, 304 (1958).
[7] Graser, E., *Z. f. Kinderh.*, **62**, 698 (1941).
[8] Freudenberg, E., and Esser, M., *Ann. Paediat.*, **158**, 128 (1942).
[9] Rohr, K., *Helv. Med. Acta*, **10**, 31 (1943).
[10] Heilmayer, L., Muller, W., and Schubothe, H., *Klin. Wschschr.*, **29**, 333 (1951).
[11] Pribilla, W., *Deutsch. Arch. Klin. Med.*, **198**, 223 (1951).
[12] Martin, H., *Verhandl. Deutsch. Gesellsch. Inn. Med. Kong.*, **58**, 728 (1952).
[13] Schaefer, R., *Monetsschr. Kinderh.*, **102**, 108 (1954).
[14] Middlebrook, J. E., *New England J. Med.*, **255**, 815 (1956).
[15] Silvestroni, E., and Bianco, I., *Amer. J. Human Genet.*, **1**, 83 (1949).
[16] Silvestroni, E., and Bianco, I., *Abnormal Hæmoglobins*, 242 (C.I.O.M.S. Sympos., Blackwell Sci. Pub., Oxford, 1959).
[17] Chernoff, A. I., *Blood*, **14**, 899 (1959).
[18] Christiaens, L., Carlier, C., Crampon, and Goudemand, M., *Arch. Franc. Pediatr.*, **7**, 575 (1950).
[19] André, R., Bessis, M., Dreyfus, B., Jacob, S., and Malassenet, R., *Rev. Hémat.*, **13**, 31 (1958).

GERONTOLOGY

Expected Rate of Senescence and Age-dependent Mortality in Fish

IT is usual in fisheries investigations to assume that the mortality of wild fish from natural causes does not rise significantly with age, and calculations on this basis give reliable results for most economically important species[1–3]. Age is sometimes invoked to explain the disappearance of fish[4]: at high ages a number of species show a decline in numbers which might be due to natural ageing[5,6]; but for long-lived species the inference is that few individuals, especially in fished populations, live long enough for the effect of age to be important.

The rate of ageing has been measured actuarially in the guppy (*Lebistes*) on a large tank-bred population[7–9]. In females of this species, growth is 'indeterminate' as in larger fish, but the survival curves under conditions of medium growth are very like those of small mammals, with a limiting age of about 2,000 days. It is of interest to consider: (1) whether the time-relations of growth and senescence can be the same in a small as in a large species; (2) whether the eventual rate of senescence in long-lived fish can be inferred from the figures for *Lebistes*.

Taking the data of *Lebistes* it is possible to construct curves for other species, showing what would happen to ability to survive if hypothesis (1) hold good. Fig. 1 gives the growth-curves of 87 tank-bred female *Lebistes* by batches from an experiment still in progress, with the observed survival-curve for the whole sample to 1,100 days; the rest of the curve is supplied from previous series under similar conditions, up to 2,000 days. The median survival is about 1,140 days.

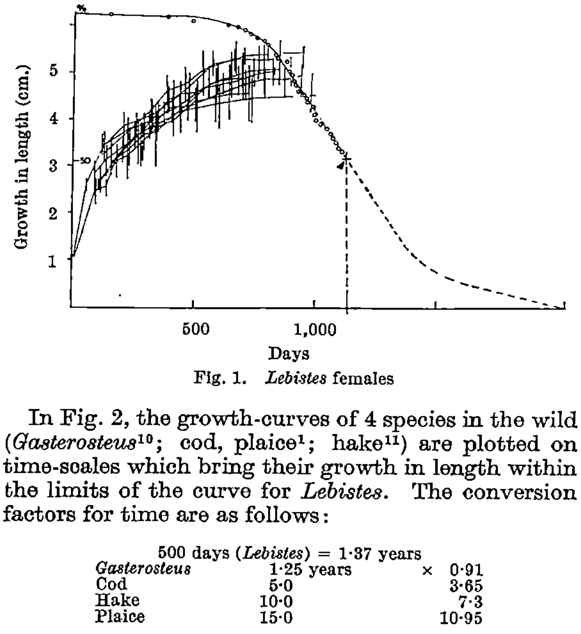


Fig. 1. *Lebistes* females

In Fig. 2, the growth-curves of 4 species in the wild (*Gasterosteus*[10]; cod, plaice[1]; hake[11]) are plotted on time-scales which bring their growth in length within the limits of the curve for *Lebistes*. The conversion factors for time are as follows:

500 days (*Lebistes*) = 1·37 years

Gasterosteus	1·25 years	× 0·91
Cod	5·0	3·65
Hake	10·0	7·3
Plaice	15·0	10·95

If these relationships hold good, populations with these growth-curves would be expected to have declined by a quarter, from senescence alone, by 2·5, 10, 20 and 30 years respectively, and the four species would have limiting ages of 5, 20, 40 and 60 years respectively. *Gasterosteus* in the wild does in fact live 2–3 years, with a few individuals surviving longer[10]. In the other species, if senescence were related to growth as in the guppy, senile mortality would not be significant within the normal expectation of life in fished populations—the modal age of plaice at catching being about 5 years[1]. The limiting ages seem likewise reasonable, except that on a basis of size one might expect the extreme life of cod to be more than 20 years. These approximations make no allowance for slower growth, however. The guppy population used in this experiment had a rather high rate of growth, and mortality at lower rates increases much more slowly. Since the relation between growth-rate and mortality increase may not be strictly linear, and since no wild population is likely to be as well fed as tank-reared fish in a laboratory, the actual survival-times in the wild may in fact be higher, but are unlikely to be less, than the approximation suggests.

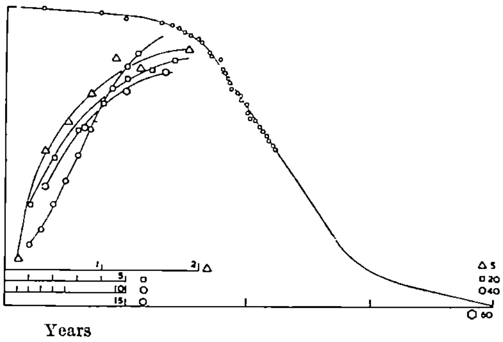


Fig. 2. Δ, Stickleback; □, cod; ○, hake; ◇ plaice

There seems accordingly to be no reason from existing data to postulate a different pattern of mortality for small as against large fish. The longest-lived freshwater fish appear to be the sturgeons, and for these the same transformation gives a limiting age of about 120 years, which agrees with the trend of recorded ages[12,13].

This work was undertaken during the tenure of a Nuffield research fellowship in gerontology.

ALEX COMFORT

Department of Zoology,
University College,
London.

[1] Beverton, R. J. H., and Holt, S. J., *Min. Agric. and Fish., Fishery Invest.*, Ser. II, **9** (London, 1957).

[2] Alm, G., *Rep. Inst. Freshw. Res. Drottningholm*, **40**, 5 (1959).

[3] Parker, R. R., and Larkin, P. A., *J. Fish. Res. Bd.*, **16**, 721 (1959).

[4] Ricker, W. E., *Trans. Amer. Fish. Soc.*, **77**, 114 (1949).

[5] Gerking, S. B., *Gerontologia*, **1**, 287 (1957).

[6] Gerking, S. B., *Animal Life Spans: Ciba Symp. on Ageing*, No. 5 (1959).

[7] Comfort, A., and Doljanski, F., *Gerontologia*, **2**, 266 (1958).

[8] Comfort, A., *Gerontologia*, **4**, 177 (1960).

[9] Comfort, A., *Gerontologia* (in the press).

[10] Jones, J. W., and Hynes, H. B. N., *J. Anim. Ecol.*, **19**, 59 (1950).

[11] Hickling, C. F., *Fish. Invest.*, Ser. II, xiii, No. 2 (1933).

[12] Pycha, R. L., *Calif. Fish Game*, **42**, 23 (1956).

[13] Milne, L., and Milne, M., *Nat. Hist. N.Y.*, **67**, 190 (1958).

Frequency of the 'Alpha' Rhythm, Reaction Time and Age

THE preliminary investigation described in this communication was suggested by the observations that (*a*) older people respond to a stimulus more slowly (simple reaction time is longer than in young people)[1–4], and (*b*) the dominant rhythm of the electroencephalogram, the 'alpha' rhythm, is slower in older people than in young[5–7]. These findings raised the question of whether 'alpha' frequency might be a factor responsible for differences in reaction time. The present experiment was designed to discover if simple reaction time is related to 'alpha' frequency of the electroencephalogram recorded during the time which elapses between a stimulus and a response.

Electroencephalograms were recorded from 18 subjects resting in a semi-reclined position, with eyes closed, in a darkened room. The subjects were asked to remain as alert as possible and told that, from time to time, they would hear a sound (250 cycle tone, well above threshold) to which they should respond as rapidly as possible by uttering a sound ('pow'). No forewarning was furnished to announce the presentation of this stimulus. A signal corresponding to the time the sound appeared was recorded on the chart of an Offner type *D* electroencephalogram machine along with the electroencephalogram tracings and a signal from a microphone which coincided with the subject's response. Bipolar electroencephalograms were recorded from left and right hemispheres. Each pair of surface electrodes was attached to the scalp so that one electrode was over the occipital and the other over the frontal area of the same hemisphere, with each electrode approximately 1¾ in. from the midline. An electrode on the arm served as a reference.

Thirteen of the subjects studied (18–72 years) yielded sufficiently regular and artefact-free 'alpha' rhythms to make measurement of frequency possible. The first peak or trough of a wave recorded immediately after the stimulus, and the last peak or trough recorded before the response were located, and the distance between them was measured and converted into time. Dividing this value by the number of cycles described yielded a number equal to the mean period of the 'alpha' rhythm. Only clearly defined waves were considered; cases where uncertainties arose regarding identification of peak or trough or number of cycles were excluded. In general, the mean values from left and right hemispheres did not differ. Whenever differences occurred the shortest 'alpha' period was arbitrarily used in the analysis. Reaction times were measured from the oscillograph chart and converted into time.

A total of 294 observations was obtained from the 13 subjects. The Pearson product-moment correlation (r) between reaction time and 'alpha' period was 0·38 ($P < 0{\cdot}001$). Of these observations, 78 were obtained under conditions where each subject was requested to exert maximum effort in staying alert and responding rapidly. In this case, the reaction times were shorter and showed a decrease (not statistically significant) in variability. These data are plotted in Fig. 1; in this instance $r = 0{\cdot}55$ ($P < 0{\cdot}001$).

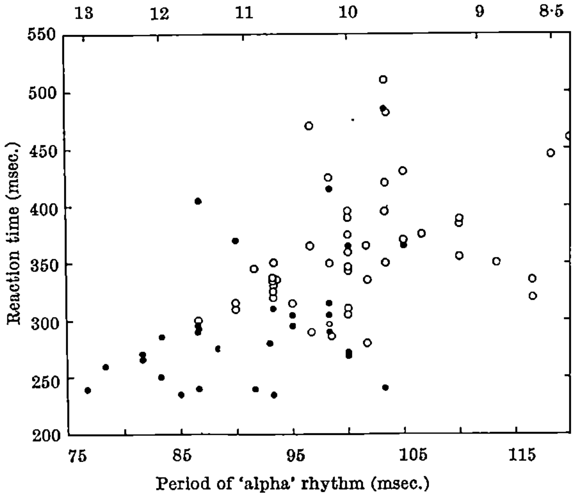


Fig. 1. Reaction time plotted against 'alpha' period. Numbers at the top of the graph refer to the corresponding 'alpha'-rhythm frequencies in c.p.s. Solid circles signify observations from subjects 18–49 years; open circles, subjects 53–72 years

Fig. 1, of course, is purely descriptive and does not distinguish between variances of individual subjects and the variance of the group. These data therefore were analysed by computing each subject's mean reaction time and mean 'alpha' period and correlating these measures. The rank-difference correlation was 0·81 ($P < 0{\cdot}01$). Finally, the subjects were split into two arbitrary groups, with 8 subjects, 18–49 years of age, in one, and 5 subjects, 53–72 years of age, in the other. Mean reaction time for the younger group was 295 msec., for the older group 367 msec. ; the difference of 72 msec. was significant ($P = 0{\cdot}05$). The mean 'alpha' period for the younger group was 90·9 msec., for the older group 102·7 msec., and the corresponding difference was also significant ($P < 0{\cdot}02$).

Thus, although data from only a small number of subjects were available, this investigation reveals the presence of a significant relationship between a person's average 'alpha' period and average reaction time: the longer the 'alpha' period (slower 'alpha' rhythm), the slower the response. It also suggests that older subjects with longer reaction times have correspondingly lower 'alpha' frequencies in the interval between stimulus and response than younger subjects with shorter reaction times. While no causal relationship is implied by the correlations, the results nevertheless lead to the speculation that the 'alpha' cycle, or some multiple of it, may serve as the unit of time in the programming of the nervous system. This possible implication is sufficient to warrant verification of our findings and further study. We need to know, for example, what specific part is played by age in this relationship, and whether the relationship holds in individual subjects.

WALTER W. SURWILLO

Gerontology Branch,
National Institutes of Health,
Baltimore City Hospitals,
Baltimore, Maryland.

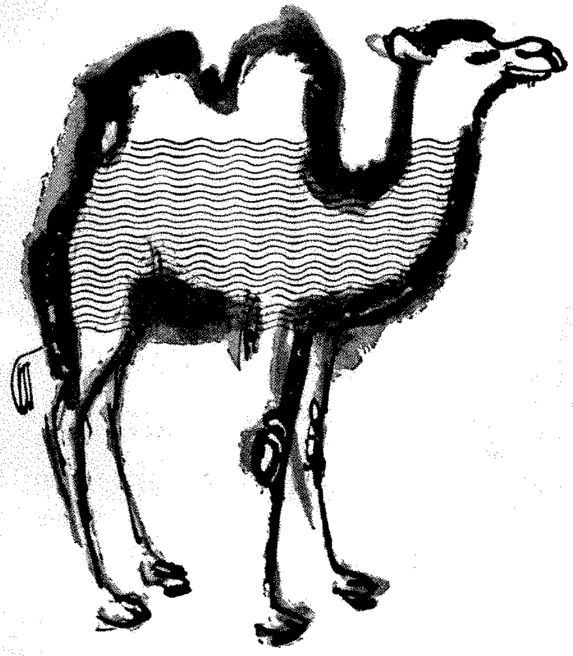
[1] Miles, W. R., *Amer. J. Psychol.*, **43**, 377 (1931).
[2] Bellis, C. J., *Proc. Soc. Exp. Biol., N.Y.*, **30**, 801 (1933).
[3] Obrist, W. D., *J. Psychol.*, **35**, 259 (1953).
[4] Birren, J. E., *The Process of Aging in the Nervous System*, edit. by Birren, J. E., *et al.* (Thomas, Springfield, Illinois, 1959).
[5] Mundy-Castle, A. C., Hurst, L. A., Beerstecher, D. M., and Prinsloo, T., *EEG Clin. Neurophysiol.*, **6**, 245 (1954).
[6] Obrist, W. D., *EEG Clin. Neurophysiol.*, **6**, 235 (1954).
[7] Harvald, B., *Acta Psychiat. Neurol. Scand.*, **33**, 193 (1958).

BIOLOGY

Effect of Gibberellic Acid on Growth and Indoleacetic Acid Oxidase Activity of *Lupinus albus* L. exposed to Different Photoperiods

THE leaves of 14-day-old *Lupinus albus* plants (variety *Gyulatanyai édes*) exhibit a high indoleacetic acid oxidase activity if grown under short-day conditions. In the leaves of plants exposed to long days and exhibiting more intense growth indoleacetic acid oxidase activity can be detected only after dialysis of the extracts[1]. The total phenol content of short-day exemplars is lower than that of long-day ones[2] (Table 1). Therefore, it is very probable that the indoleacetic acid oxidase activity of *Lupinus* seedlings is influenced by the phenol content of the leaves, and indirectly the growth response of plants to different photoperiods depends on the level of phenolics.

the
conservation
of water



Lack of water is enough to give anyone the hump, but when the shortage can affect the life of a community, the matter becomes serious. It is fortunate therefore that in the 1920's Rideal and others were led to consider — as an academic exercise — the effect of a non-volatile mono-molecular surface film on the rate of evaporation from a liquid substrate, and to establish that the rate could be reduced. Rideal used fatty acid films, but the Langmuirs in America showed later that cetyl alcohol films were even better.

So when the need to conserve water in semi-arid regions on a practical scale became a matter of concern — in Australia, in Africa, in the Americas, in Russia — the principle by which it could be conserved had already been established. Nevertheless it was not until 1955 that Mansfield, in Australia, was able to announce a successful process based on cetyl alcohol. The problem had been one of maintaining a durable film under adverse weather conditions, and Mansfield's solution for small reservoirs was to hold reserves of the alcohol in bead form in wire mesh rafts moored in the reservoirs. Methods for larger areas are now being actively studied.

This has led to further investigation into the spreading behaviour and durability of fatty alcohols, pure and commercial, and Unilever have collaborated in the work. For example, McArthur and Durham have studied commercial blends and shown that crystallinity is as important as composition in providing good spreading properties. Roylance and Jones have prepared pure cetyl alcohol in the stable beta form to investigate the mechanism and kinetics of film spreading, and Stewart of the C.S.I.R.O. in Australia has also used some of this material to study more particularly the effect of polymorphic change. Jones and Stephens have studied the causes of film attrition. Roylance and Jones are currently producing a range of pure alcohols for further studies.

So by contribution and collaboration Unilever is helping in the vital work of conserving water.

UNILEVER RESEARCH

UR 1-7049

LOST

On or about April 1st this year, between Lombard Street and the Metropolitan Police Boundary, a large quantity of Treasury Notes, Bonds, Sleep, Hope etc. by a Firm who forgot that **ASSOCIATED LEAD** supply: **LEAD, TIN, ANTIMONY AND THEIR ALLOYS; 99.999% PURE LEAD, TIN AND ANTIMONY; SOLDERS, INCLUDING SOLID AND CORED SOLDER WIRE; ANTIFRICTION MATERIALS; ANTIMONIAL LEAD; CABLE ALLOYS; LEAD SHEET AND PIPE; DENSE LEAD SHIELDING FOR NUCLEAR WORK** as well as every other form of Lead and Antimony, in any quantity. Firm concerned prefers to remain anonymous.

This announcement is issued for and on behalf of
ASSOCIATED LEAD MANUFACTURERS LIMITED
CLEMENTS HOUSE, 14 GRESHAM STREET, LONDON, E.C.2. CRESCENT HOUSE, NEWCASTLE. LEAD WORKS LANE, CHESTER
Export enquiries to: Associated Lead Manufacturers Export Co. Ltd., Clements House, 14 Gresham Street, London, E.C.2

Table 1. EFFECT OF TREATMENT WITH GIBBERELLIC ACID ON THE GROWTH OF 14-DAY-OLD *Lupinus albus* SEEDLINGS AND ON THE INDOLEACETIC ACID OXIDASE ACTIVITY AND PHENOL CONTENT OF THEIR LEAVES UNDER DIFFERENT PHOTOPERIODS

Photo-period	Treatment with gibberellic acid	Total heights of the overground parts (cm.)	Total phenols (expressed as 10^{-1} μgm. ferulic acid in mgm. dry matter)	Indoleacetic acid oxidase activity (μl. O_2/hr./mgm. dry matter)
Short day	–	61·6	2·57*	9·5
	+	90·4	2·40	9·3
Long day	–	107·1	3·23*	0
	+	136·8	3·28	0

* The difference between the phenol content of treated and control plants is significant only between short-day and long-day plants.

It has been shown that gibberellic acid *in vivo* reduces the activity of indoleacetic acid oxidase[3,4] probably by enhancing the synthesis of phenolic enzyme inhibitors[5,6]. Furthermore, according to Pilet, gibberellic acid exerts a direct inhibitory effect on indoleacetic acid oxidase and thereby creates a higher auxin level *in vivo*[3,7]. Therefore, a study of the effect of gibberellic acid on the indoleacetic acid oxidase activity, content of total phenolics and plant growth of 14-day-old *Lupinus albus* seedlings seemed to be warranted.

Seeds of this plant were germinated for 4 days at 25° C. in Petri dishes on filter paper moistened with gibberellic acid solution (10 mgm. in 1,000 ml.). The seedlings were then grown in pots and exposed to short-day (8 hr. daylight) or long-day conditions (8 hr. daylight + 16 hr. supplementary illumination by a 100-W. filament bulb; 2,000 lux) respectively. After the expansion of the cotyledons, the growing point of each plant was treated with gibberellic acid (2 μgm.). Controls were germinated on distilled water and the seedlings were similarly treated with distilled water. At the age of 14 days, the content of total phenolics[8] and the activity of indoleacetic acid oxidase[9] in the first leaf pairs were determined. The height of the plants was also measured. The results are summarized in Table 1.

Results similar to those shown in Table 1 were obtained with plants grown in nutrient solutions supplemented with 10 mgm. gibberellic acid/1,000 ml.

It may be seen that the growth of seedlings is promoted by gibberellic acid both under short- and long-day conditions; however, the stimulation is more pronounced with plants exposed to short days[10] and the effect is apparently additive[4].

The amount of total phenolics and the activity of indoleacetic acid oxidase is not altered by gibberellic acid treatment. The same results were obtained with gibberellic acid concentrations as high as 100 mgm./1,000 ml. The results clearly indicate that the stimulated growth of *Lupinus* seedlings evoked by gibberellic acid cannot be due to the direct or indirect (via the stimulation of phenolic inhibitors) inhibition of indoleacetic acid oxidase. It seems more likely that the effect is connected with the elevation of the endogenous auxin-level or with the neutralization of growth inhibitors[11–13]. Isotopic work of Fang *et al.*[14] also supports this idea.

The above results obtained with *Lupinus albus* might seem strange in the light of the available published data. However, we refer to observations by Hillman and Galston indicating that the indoleacetic acid oxidase of *Lupinus albus*, in contrast to that of the pea plant, cannot be influenced by red–infra-red irradiation[15].

Watanabe and Stutz[4] were also unable to show the effect of gibberellic acid on the indoleacetic acid oxidase of *Lupinus albus* leaves. However, they used 70-day-old plants and indoleacetic acid oxidase activity could not be detected in the leaves of control plants either. According to our results, this might be due to the high level of inhibitor(s)[2].

Thanks are due to Gy. Németh for the plant material and to G. L. Farkas for a sample of ferulic and gibberellic acid.

F. SÁGI
A. S. GARAY

Institute of Plant Breeding,
Fertőd, Hungary.

[1] Garay, A. S., Garay, M., and Sági, F., *Physiol. Plant.*, **12**, 799 (1959).
[2] Sági, F., and Garay, A. S., *Physiol. Plant.* (in the press).
[3] Pilet, P. E., and Wurgler, W., *Ber. Schweiz. Bot. Ges.*, **68**, 54 (1958).
[4] Watanabe, R., and Stutz, R. E., *Plant Physiol.*, **35**, 359 (1960).
[5] Galston, A. W., *Photoperiodism and Related Phenomena in Plants and Animals*, 137 (AAAS, Washington, 1959).
[6] Kögl, F., and Elema, J., *Naturwiss.*, **47**, 90 (1960).
[7] Pilet, P. E., *C.R. Acad. Sci., Paris*, **245**, 1327 (1957).
[8] Swain, T., and Hillis, W. E., *J. Sci. Food Agric.*, **10**, 63 (1959).
[9] Stutz, R. E., *Plant Physiol.*, **32**, 31 (1957).
[10] Wittwer, S. H., Bukovac, M. J., McVey, G. R., and Ballard, J. C., *Naturwiss.*, **46**, 117 (1959).
[11] Nitsch, J. P., *Proc. Amer. Soc. Hort. Sci.*, **70**, 512 (1957).
[12] Phillips, I. D. J., Vlitos, A. J., and Cutler, H., *Contr. Boyce Thompson Inst.*, **20**, 111 (1959).
[13] Brian, P. W., and Hemming, H. G., *Ann. Bot.*, N.S., **22**, 1 (1958).
[14] Fang, S. C., Bourke, J. B., Stevens, V. L., and Butts, J. S., *Plant Physiol.*, **35**, 251 (1960).
[15] Hillman, W. S., and Galston, A. W., *Plant Physiol.*, **32**, 129 (1957).

Relative Volumes of Cytoplasm and Vacuole in *Nitella*

IN order to understand the mechanism by which various organic molecules are accumulated by the vacuoles of the green alga *Nitella*, it was necessary to determine accurately the relative volumes of cytoplasm and vacuole in the cell. The unusually large size of these cells has made the genus popular as a tool for the study of biological transport mechanisms[1–5]. Single cells can be obtained up to nearly 2 mm. in diameter and more than 10 cm. in length. The contents of the large vacuole can be obtained relatively free of cytoplasm by cutting off one end and gently squeezing the solution on to a microscope slide or into a capillary para tube. There seems to be little agreement in the literature concerning the relative volumes of cytoplasm and vacuole. Thus Brooks[1] arrived at a value based on intracellular distribution of radioactive potassium and estimated 33 per cent cytoplasm, 67 per cent vacuole. Holm-Jensen *et al.*[2] measured the depth of the immobile chloroplast layer plus the streaming cytoplasm by differential focusing with a water-immersion objective, and the diameter of the cell with a micrometer eyepiece in a dissecting microscope. Calculations from their results give values of 10 per cent cytoplasm, 90 per cent vacuole. Recently, MacRobbie and Dainty[4], measuring the rate of loss of sodium-22 from *Nitellopsis* cells previously loaded with the isotope, calculated that the cytoplasmic compartment consisted of about 3 per cent of the cell. In a similar fashion, using potassium-42, Diamond and Solomon[5] arrived at values of 1·8 per cent cytoplasm.

For our experiments we used *Nitella opaca* Ag, gathered from a stream in southern Sweden and maintained in tap water. We used several direct methods to measure the volumes of the two compartments. In preliminary experiments we cut the end of a cell and squeezed out the total contents directly into a capillary tube. The tube was sealed and centrifuged. Volumes of the fractions were measured by projecting the image of the tube and contents into tracing paper, cutting out the paper, and weighing the areas. The total cytoplasm constituted 8·7 per cent of the recovered volume. Three distinct fractions were observed. The average volumes for the eight cells measured were: dense green chloroplast fraction, 3·8 per cent; translucent cytoplasm (probably the streaming layer), 4·9 per cent; clear supernatant vacuole, 91·3 per cent. There were two important sources of error in this technique which tend to give low values for the cytoplasm. It was impossible to get all the chloroplast fraction out of the cell, and some of the green material adhered to the walls of the capillary tubes.

In order to overcome these difficulties we placed intact cells in glass tubes, sealed one end, filled with water, and centrifuged at high speed with the tubes in a horizontal position. The three cellular fractions separated very distinctly. By projecting the image of the centrifuged cell into tracing paper, as above, we measured the following average values (per cent) for eight cells: chloroplast fraction, 4·1; streaming cytoplasm, 5·8; total cytoplasm, 9·9 (range, 8·8–11·8).

Since the technique of centrifugation probably compresses the *in situ* volume of the chloroplast and streaming cytoplasm fraction, we measured the dimensions of the sum of both the chloroplast and cytoplasmic layer with a calibrated focusing micrometer on a microscope with a water-immersion lens. Then, by measuring the diameter of the cell with a micrometer eyepiece, it is possible, assuming the solid geometry of right cylinders, to calculate the volume occupied by the two fractions. For eight cells the values are: range of diameter, 375–550μ; range of depth of cytoplasm, 11·2–16·1μ; average volume of cytoplasm, 12·2 per cent (range, 9·3–14·1 per cent).

In the fourth technique, we used the fixation of cells by various methods among which the Navaschin–Karpechenko technique appeared to cause the lowest degree of distortion, though invariably leading to some plasmolysis. Measurements were obtained from photomicrographs of microtome sections and suitable calculations made. The dimensions of a typical cell were found to be: thickness of cell wall, 7·9μ (sections of fresh material gave somewhat higher values); thickness of total plasmatic layer, 17·8μ; overall diameter of whole cell, 460μ. The relative volume of cytoplasm is therefore 14·4 per cent.

The last three methods used give values for cytoplasmic volume of 9·9–14·4 per cent, and are in rather close agreement. Both in living material and in the microtome sections the innermost part of the cytoplasm was seen to have a somewhat foamy appearance, which explains, at least in part, the difference between the values obtained by centrifuging and those obtained by direct measurements. We can say, with some degree of certainty, that the cytoplasm occupies at least 10 per cent of the physical volume of the cell, as had been suggested by Holm-Jensen *et al.*[2]. The value is at great variance with that of MacRobbie and Dainty[4] and Diamond and Solomon[5]. There is no doubt that there is some 'compartment' comprising 2–3 per cent of the cell which loses alkali metal ions in the fashion described by these workers. This compartment is not, however, the total cytoplasm. Rather, it might be the matrix of the immobile chloroplast fraction. As judged by qualitative microscopic observation, we estimate that the matrix occupies approximately 2–3 per cent of the volume of the cell.

Our work suggests that some compartments of the cell are not limited by the finite membranes of the tonoplast and plasmalemma, but should be considered as volumes of the cell which differ in physical and chemical properties which influence their permeability behaviour.

PAUL SALTMAN*
TYGE CHRISTENSEN

Institute for Biological Chemistry and
Institute of Thallophyta,
University of Copenhagen.

---

* Senior Postdoctoral Fellow of the U.S. Public Health Service. Permanent address: Department of Biochemistry, School of Medicine, University of Southern California.

[1] Brooks, S. C., *J. Cell. Comp. Physiol.*, **11**, 247 (1938).
[2] Holm-Jensen, I., Krogh, A., and Wartiovaara, V., *Act. Bot. Fenn.*, **36**, 3 (1944).
[3] Litwack, G., and Pramer, D., *Arch. Biochem. and Biophys.*, **68**, 396 (1957).
[4] MacRobbie, E. A. C., and Dainty, J., *J. Gen. Physiol.*, **42**, 335 (1959).
[5] Diamond, J. M., and Solomon, A. K., *J. Gen. Physiol.*, **42**, 1105 (1959).

Inoculation of Pine Stumps against Infection by *Fomes annosus*

RECENT trials have shown that inoculation of pine stumps with oidia of *Peniophora gigantea* prevents infection by spores of the fungus *Fomes annosus*, causing root-disease of conifers. *P. gigantea* is an active wood-rotting fungus which under natural conditions often competes with *F. annosus*, but for various reasons does not provide consistent control. It is a far more effective competitor than wood-inhabiting microfungi such as *Trichoderma viride* and *Penicillium* spp., which seldom colonize a fresh stump surface at all freely.

To prepare cultures of *P. gigantea*, pine wood-blocks of 3 cm. × 3 cm. × 1 cm. are autoclaved in batches of one hundred, inoculated with a suspension of oidia and incubated at 22° C. for 10 days. They are then sealed individually in small, sterile polythene bags, in which condition they remain usable for many months. The suspension of oidia is prepared by shaking a wood-block culture with about 100 ml. tap water for 1 min. It is then diluted 5–10 times and sprayed on to the stump surface by means of a large syringe directly after felling. According to the size of stump, 3–5 strokes suffice: these deliver 2–3 ml. suspension containing some 1×10^6 viable oidia. It is desirable to use a fresh suspension each day. In cold weather oidia may be suspended in 10 per cent ammonium sulphate, which considerably depresses the freezing point without greatly affecting spore viability.

In ten experiments, involving some 400 stumps of *Pinus sylvestris* or *P. nigra* var. *calabrica*, samples taken after 6–12 months showed that inoculation had consistently prevented infection by *F. annosus*, whether derived from natural sources or from adding up to 3,000 spores per stump. About 90 per cent of inoculated stumps produced sporophores of *P. gigantea* within a year, thus increasing local spore production of this useful competitor. The treatment was also effective in preventing growth of *F. annosus* into stumps, the roots of which were inoculated with the parasite soon after felling: in five stumps excavated a year later, no roots were found to contain *F. annosus*. By contrast, in root-inoculated stumps treated with creosote or urea at the time of felling, two-thirds and one-third of the roots, respectively, were occupied by the parasite. With naturally infected pine stumps, however, *P. gigantea* was rather less effective because it seldom replaced *F. annosus* in resinous roots already dead at the time of felling.

This method should prove useful in preventing entry of *F. annosus* into first-rotation pine stands of special value, such as experimental plots; it might also find wider application in young forests where *P. gigantea* is as yet uncommon. Despite the limitation mentioned, inoculation of stumps with *P. gigantea* may well be preferable to treatment with chemicals[1] in stands where *F. annosus* has already become established. Preliminary trials suggest that it may be possible, by selecting appropriate saprophytes, to protect stumps of other conifers against infection by *F. annosus*.

J. Rishbeth

Botany School,
Cambridge.

---

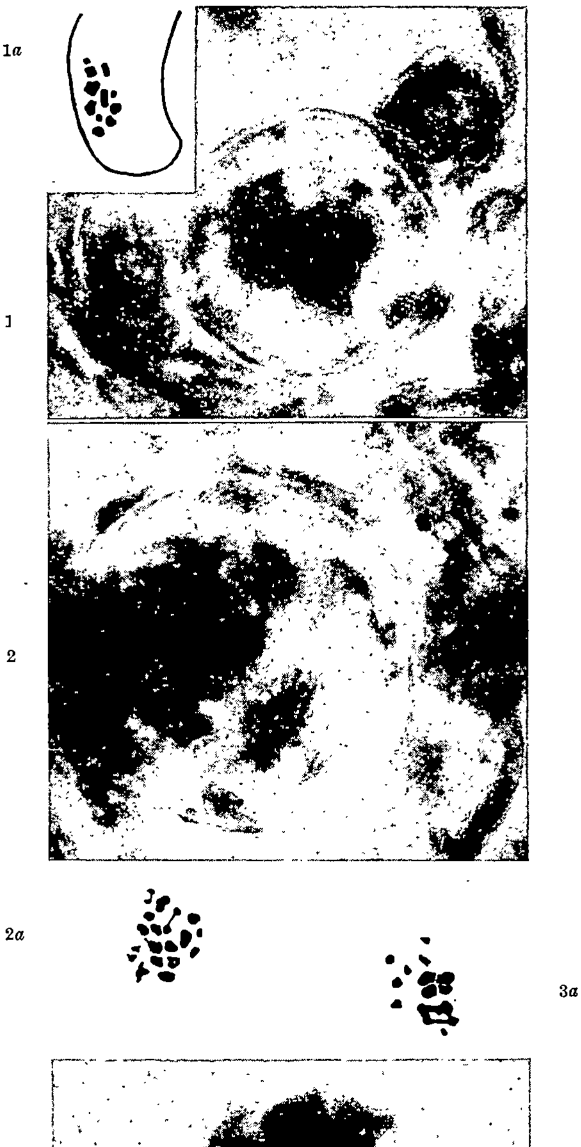
[1] Rishbeth, J., *Ann. App. Biol.*, 47, 519, 529 (1959).

Meiosis in the Oogonium and Antheridium of *Pythium debaryanum* Hesse

Aceto-orcein squash preparations of a culture of *Pythium debaryanum* Hesse were found to have nuclei in division in the oogonia and the antheridia (Figs. 1 and 2). The stage of division was usually rather more advanced in the oogonium than in the antheridium. The oogonia had 2–6 nuclei undergoing division, the antheridia usually one, occasionally two.

The haploid number of chromosomes is approximately twenty (Figs. 2, 2*a*), but an exact count has not been possible so far, partly because of the smallness of the chromosomes and partly because of certain irregularities. At first it was assumed that these divisions were mitotic, but a closer examination disclosed side views of configurations at early anaphase with loops such as would be expected to result from interstitial chiasmata. Also, certain irregularities such as early separation of failure of pairing indicated that these divisions were meiotic. No metaphases were seen in the hyphæ or sporangia, even after camphor treatment, and it is concluded that vegetative nuclei do not normally pass through an ordinary metaphase stage when dividing. The occurrence of metaphases in the oogonia and antheridia therefore indicates that these may be special divisions, that is, meiotic divisions.

Full confirmation of this was obtained when configurations of more than two chromosomes were obtained (Figs. 3, 3*a*). Such configurations could only occur in meiosis. This indicates that *Pythium debaryanum* is a structural hybrid, or a polyploid which may well account for the failure of previous attempts to germinate the oospores.



Figs. 1–3. 1. Oogonium and antheridium, metaphase I in antheridium; (1*a*) drawing of metaphase. 2. Oogonium and antheridium. Oogonium with 4 metaphases, 1 in focus. (2*a*) Drawing of metaphase plate. 3. Side view of metaphase I in oogonium showing a multiple configuration. (3*a*) Drawing of metaphase. (Figs. 1 and 2 × *c*. 2,600 ; Fig. 3 × *c*. 4,300)

The Peronosporales have generally been considered to be haploid with meiosis occurring in the first division of the oospore nucleus. However, there is little or no direct evidence for this view. It is very unlikely that *Pythium debaryanum* should have a different life-history from the rest of the group, and I feel sure that further work will show that the Peronosporales and probably the Biflagellatae as a whole are diploids in the vegetative stage with meiosis occurring in the oogonium and antheridium. It is very suggestive that two successive divisions have been reported to occur in the oogonium and antheridium of *Albugo* sp.[1]. The genetical work on the Biflagellatae is very scant, but according to Raper[2] the complex pattern of sexuality in *Dictyuchus* reported by Couch[3] could be accounted for by polyploidy or duplicate factors. This suggestion of Raper is in accordance with the present observations, in indicating that members of this group may be diploid rather than haploid as has been previously supposed.

I am indebted to my husband and Dr. B. J. Harris for helpful discussions and to the latter for the photomicrographs.

Note added in proof. Meiosis has been observed recently in the oogonium and antheridium in *Phytophthora cactorum,* which has approximately nine bivalents.

EVA SANSOME

Botany Department,
University College of Ghana,
Legon, Accra, Ghana.

[1] Stevens, F. L., *Bot. Gaz.*, **32**, 77 (1901).
[2] Raper, J. R., *Amer. J. Bot.*, **47**, 9, 794 (1960).
[3] Couch, J. N., *Ann. Bot.*, **40**, 848 (1926).

Effect of Varying Doses of Undiluted Semen on Fertility in the Domestic Fowl

SEVERAL reports[1-3] have appeared on the relationship between dose of undiluted semen and fertility in chickens, yet there is little evidence on the minimum volume of undiluted semen necessary (*a*) to produce any fertility, and (*b*) to establish the maximum or optimal level of fertility. The relationship between spermatozoa number and fertility has been investigated by using varying doses of semen diluted with synthetic diluents and seminal plasma[4,5]. However, recent work[6,7] indicates that synthetic diluents and even seminal plasma can alter the metabolic rate and perhaps even the life-span *in vivo* of fowl spermatozoa. This is a brief report of an experiment undertaken to establish the minimum volume of undiluted fowl semen required to produce any fertile eggs, and minimum volume necessary for optimal fertility.

Semen was obtained from 15 males selected randomly from each of two random-bred strains—the Ottawa S.C. White Leghorn control strain[9] (Leghorn control) and the Ottawa meat control strain[10] (meat control). All inseminations were made into random samples of females from two selected strains of S.C. White Leghorns[9] that were known to be similar in production traits. Both strains of females were highly fertile.

Semen was collected from each male and pooled by strain after rejecting samples which were contaminated and contained 'clear watery fluid'[7]. Fifteen doses of semen were used (Fig. 1) varying from 0·0002 ml. to 0·15 ml. The experiment was repeated for six days. Each day two hens were inseminated with each dose from each of the two strains of males. Twenty-four hens were inseminated with each dose for a total of 360 inseminations. Only hens that had laid already on that day were inseminated.

Glass pipettes fitted to a 1 ml. tuberculin syringe by rubber tubing were used to measure and deliver the semen. Four different-sized pipettes were used, the smallest graduated to 0·0002 ml. with a diameter of 0·25 mm. It was recognized that the error in measurement and delivery of the smallest dose was quite high, but not greater than ± 0·00005 ml.

Following the single insemination, all hens were trap-nested and the eggs were marked, dated and stored in a holding room at a temperature of 50° F. and with a relative humidity of 85–90 per cent. They were incubated weekly, until all hens were completely infertile.

Differences between the two strains in semen density were not significant. This confirmed our previous findings[10]. Therefore, the data on spermatozoa number from the two strains were pooled. Fertility was measured as the duration of fertility in days, following a single insemination[2]. The results are plotted in Fig. 1, showing both the size of the dose and the number of spermatozoa per dose.

The differences between the strains were highly significant. Differences between strains and between breeds in duration of fertility are a relatively widely spread phenomenon[2,11,12].

The duration of fertility for the Leghorn control and for the meat control increased with an increase in the semen dosage up to 0·01 ml. and 0·006 ml. respectively. Although there was a slight increase in fertility with doses above these levels, it was not significant. With doses as small as 0·0002 ml. some fertility resulted in both strains.

The relationship between fertility and spermatozoa number should be considered in the light of recent developments in the technique of semen collection[8,13]. Fowl semen collected free of contaminating fluids contains, on the average, six to seven million spermatozoa per ml. of semen[8,10]. This is double the concentration reported by most other workers who, no doubt, were using semen contaminated to some degree[4,5]. In this present experiment the minimum number of spermatozoa required for optimal fertility was 40 and 66 million for meat control and Leghorn control, respectively. These estimates are much lower than those reported by others[4,5]. There is little doubt that the use of careful semen collection and insemina-

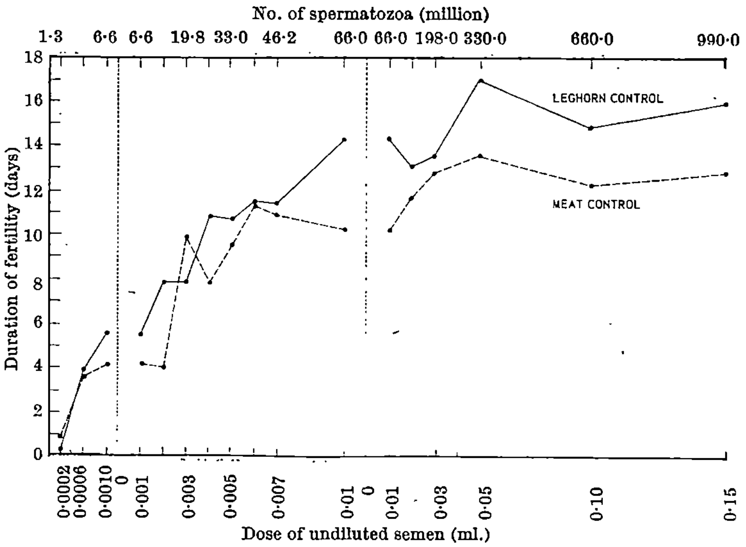


Fig. 1. Relationship between the dose of undiluted semen (number of spermatozoa) and the duration of fertility in two strains of domestic fowl

tion techniques have contributed to the results obtained.

G. C. Taneja*
R. S. Gowe

Animal Research Institute,
Canada Department of Agriculture,
Ottawa.

* Post-doctorate Fellow of the National Research Council, Canada.

[1] Burrows, W. H., and Quinn, J. P., *Poultry Sci.*, **17**, 131 (1938).

[2] Gowe, R. S., Johnson, A. S., and Merritt, E. S., *Prog. Rep.*, 1949–54, *Poultry Division, Canada Dept. Agric.* (1957).

[3] Cooper, D. M. and Rowell, J. G., *Vet. Rec.*, **71**, 715 (1959).

[4] Munro, S. S., *Canad. J. Res.*, **16**, 281 (1938).

[5] Rowell, J. G., and Cooper, D. M., *Poultry Sci.*, **36**, 706 (1957).

[6] van Tienhoven, A., *J. Agric. Sci.*, **54**, 67 (1960).

[7] Lake, P. E., *Proc. Third Intern. Congr. Anim. Repr.*, Sec. 3, 104 (1956).

[8] Lake, P. E., *J. Agric. Sci.*, **49**, 120 (1957).

[9] Gowe, R. S., Johnson, A. S., Downs, J. H., Gibson, R., Mountain, W. F., Strain, J. H., and Tinney, B. F., *Poultry Sci.*, **38**, 443 (1959).

[10] Taneja, G. C., and Gowe, R. S., *Poultry Sci.* (in the press).

[11] Howes, J. R., and Gowe, R. S., *Poultry Sci.*, **39**, 1259 (1960).

[12] Gowe, R. S., and Hutt, F. B., *Poultry Sci.*, **28**, 764 (1949).

[13] Lorenz, F. W., *Reproduction in Domestic Animals*, **2**, 366 (1959).

Copepod *Ommatokoita elongata* (Grant) in the Eyes of the Greenland Shark—a Possible Cause of Mutual Dependence

In July 1959[1], and in July–August 1960, I had the opportunity to take part in Greenland shark fishery with Norwegian commercial sealing vessels in east Greenland waters. Data on this shark were collected, supplemented with information received from experienced Greenland shark fishermen.

The sluggishness of this shark is proverbial, and it is to many a puzzle how such a sluggish fish is able to capture live prey.

The information received from the fishermen tends to show that, besides many other marine animals, the char, *Salmo alpinus*, has been repeatedly found in stomach contents of this shark, even so freshly swallowed that it could be used for human consumption on board. According to information received from several skippers, some of the chars found in shark stomachs had been cut by the shark's teeth when captured, and in such cases their tail parts were invariably missing in the stomach contents. This naturally leads to the assumption that the char has been swallowed head foremost by the shark. It is believed very strongly by Norwegian fishermen, who have been fishing for Greenland sharks, that its potential prey is lured to swim toward it by the 'white things', that is, the copepod *Ommatokoita elongata*, attached to its eyes. The copepods are said to be luminous by some fishermen.

The infestation with this copepod in south-east Greenland waters was found to be as follows :

Of the 1,505 sharks inspected, 1,270 (84·4 per cent) were infested in both eyes, 218 (14·5 per cent) were infested in one eye only, and only 17 sharks (1·1 per cent) were not infested. It is, however, possible that copepods have been lost during the haul or have been too small to be observed easily.

One copepod in each eye is the rule, but in some cases, included in the numbers given above, two, or rarely more, specimens were attached to one eye. Very rarely specimens were found attached to the skin near the eyes. The copepods are firmly attached to the cornea by a small disk. Numbers of small circular marks on the cornea are thought to be scars left by previous infections. The copepods were found to vary in length from 3–4 mm. to about 70 mm., including the egg sacks. The smallest copepods had evidently become attached recently, and there is thus reason to believe that uninfested sharks were only temporarily free of this copepod. The copepods are whitish-yellow in colour, and in particular adult specimens with large egg sacks are very conspicuous. The cornea was often found to be opaque, and in cases of heavy opacity, copepods were not found to be present.

Table 1

		O. elongata				
1959	Total	Both eyes	Right eye	Left eye	Absent	Not examined
Umivik Fjord	264	205	21	21	2	15
Umivik Bank	10	3	2		1	4
1960						
Umivik Fjord	664	519	49	50	9	37
Umivik Bank	244	173	11	12	2	46
Bank area east of Kangerdlugsuak Deep	2	2				
Off Deception Island	473	368	23	29	3	50
	1,657	1,270	106	112	17	152

According to the data collected, this copepod appears to be equally common in both small and large sharks. Differences in infestation relative to sex were very small. As is apparent from the list above, only small variations in infestation are observed between the different fishing localities.

It may be added that mainly small and medium sharks of both sexes were caught in the fjords and near land, while only large fish, from about 3·5 m. to nearly 5 m. in length, were caught on the banks. These were, with one exception, females only.

I have not been able to confirm the fishermen's belief that the copepods are luminous. No bioluminescence could be observed by the human eye in specimens kept in sea water in total darkness.

O. elongata is grouped in the family Lernaeopodidae which, according to Harvey[2], is not included in any of the two copepod sub-orders, Calanoidea and Cyclopoidea, to which bioluminescence seems to be restricted. It is therefore unlikely that *O. elongata* is able to produce light. However, histological examination has not been undertaken.

It may be argued that when the amount of light present in the sea is above a certain minimum, the whitish copepods will show up against a dark background. However, the effect of the tapetum lucidum of the shark's eyes in weak light conditions is not known.

It is generally accepted that many fishes, such as the angler fish (*Lophius piscatorius*) and ceratioid deep-sea fishes, use the first dorsal fin ray as a lure or 'fishing rod' for attracting prey[2]. Although the fishermen's assumption that the copepods in the eyes of the Greenland shark serve a similar purpose sounds rather far-fetched, it should not be entirely excluded. But if such should really be the case, of which I am so far not convinced, then the copepod is of some benefit to its host, and a mutual dependence, which may be classed as a case of mutualism, may be anticipated to exist between the two.

I am fully aware that this view is highly speculative, but it is felt to be of sufficient interest to attract biological attention, in order that other workers who have the opportunity to add further knowledge may do so.

Bjørn Berland

Institute of Marine Research,
Directorate of Fisheries,
Bergen.

[1] Berland, B., *Fauna* (Oslo), 13, 1 (1960).

[2] Harvey, E. N., *Bioluminescence* (New York, 1952).

Occurrence of *Eutrichocheles modestus* (Herbst) in the Near-shore Waters of Porto Novo, South India

Faunal studies of this region on the Coromandel Coast have been in progress for some years. Some interesting finds have been reported[1-3] recently, after the establishment of a marine biological laboratory at Porto Novo (11° 29′ N. and 79° 47′ E.), and this communication relates to the occurrence of the extremely rare decapod crustacean *Eutrichocheles modestus* (Herbst) (Fig. 1). A specimen 80 mm. in length (from the tip of the rostrum to the posterior extremity of the telson) was first obtained from a seine net operated by local fishermen at about the 10-fathom line (about 4–5 miles from shore) in February 1960. This area is well within the limits of the continental shelf, which extends to about 20 miles along this coast. Another specimen measuring 75·2 mm. in length was obtained in January 1961.

From a survey of the literature it would appear that only two specimens of this decapod have so far been obtained, and the exact source of neither of these is known with certainty. The first specimen described by Herbst (1794)[4] as *Cancer modestus* was probably taken in the East Indies, and the other specimen on which Wood-Mason (1875)[5] based the present generic name was caught somewhere off the Arakan coast of Burma. The latter specimen seems to be the only example in existence now[6]. With the present discovery of *Eutrichocheles modestus*, de Man's statement[7] "that this extremely rare form will probably prove to occur also in the Indian Archipelago" would appear prophetic.

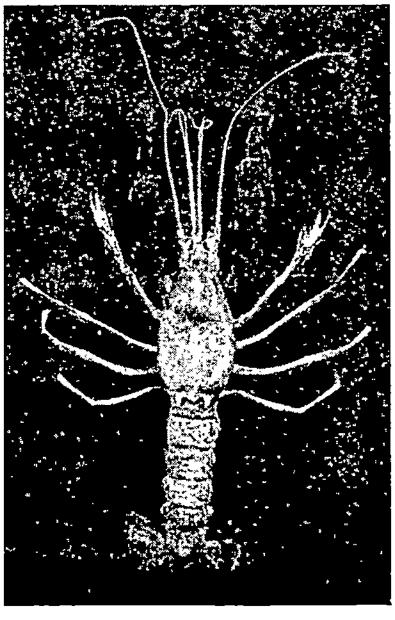


Fig. 1 *Eutrichocheles modestus* (Herbst)

The main features of the specimens from Porto Novo conform very closely to the earlier descriptions, but the following supplementary observations may be made. The fresh specimens appeared rather pink in colour and with preservation they have tended to become white. One is therefore reminded of the red pigment mentioned by Herbst[4] and the white colour referred to by Chopra[6]. Further, the cornea appears to be pigmented unlike what Chopra observed. Again, in our specimens the rostrum ends in a rather sharply pointed tip, and with regard to the abdominal segments, the teeth at the anterior margin of the fifth are rather indistinct compared with those on the third and fourth segments. There is a median groove in the telson running from the notch at its posterior margin, to the middle, where runs a transverse row of spines. The exopod of the uropod also has hairs. This feature was described by Chopra[6].

Unlike the Indian Museum specimen, the right cheliped and not the left is the bigger in our material and both the chelipeds are profusely covered with hair-like outgrowths. The nature and distribution of the tubercles and spines in the different regions of the chelipeds would also appear to vary from what has been described by the earlier authors.

When more specimens of this curious creature become available a detailed investigation of its anatomy will be undertaken, when it may also be possible to discuss its systematics in a more precise manner than has hitherto been possible.

We thank Mr. L. K. Sourirajulu, of the Zoology Department, for directing our attention to this animal in the net along with a collection of numerous other organisms. We also thank Mr. G. Ramakrishna, of the Zoological Survey of India, for help with a survey of literature. Our thanks are due to Prof. R. V. Seshaiya for his interest.

K. Balasubrahmanyan
J. Jacob*

Annamalai University,
Marine Biological Station,
Porto Novo.
South India.

* Present address: Institute of Animal Genetics, University of Edinburgh.

[1] Balasubrahmanyan, K., *Curr. Sci.*, 28, 371 (1959),

[2] Balasubrahmanyan, K., *Curr. Sci.*, 28, 495 (1959).

[3] Jacob, J., and Rangarajan, *K., J. Zool. Soc. India*, 12, 20 (1960).

[4] Herbst, J. F. W., *Krabben*, 2, Heft 5, 173 (1794).

[5] Wood-Mason, J., *Proc. Asiatic Soc. Bengal*, 231 (1875); *Ann. Mag. Nat. Hist.*, (4) 17, 264 (1876)

[6] Chopra, B., *Rec. Ind. Mus.*, 35, 277 (1933).

[7] de Man, J., *Siboga Exped. Rep.*, 39, a^2, 95 (1917).

LIMNOLOGY

A Lake in British Columbia containing Old Sea-Water

Stimulated by Strøm's reports[1,2], and by N. M. Carter's speculations (oral communication), we have made a preliminary examination of Powell Lake, British Columbia, and find conditions therein similar to those described by Strøm for Lakes Tokke and Botnvatn in Norway.

Powell Lake is a fjord lake about 50 km. long and 2 km. wide. The outline of the lake and the form of the mountains rising above it resemble the many fjord-like inlets along the British Columbian coast. However, the southern end of the lake (49° 53′ N., 124° 32′ W.) is separated from the adjacent

Strait of Georgia by a rocky sill 46 m. above sea-level.

A dam built fifty years ago downstream from the sill raises the lake-level to 56 m. above sea-level. Some 16 spot soundings made prior to our investigations indicated a maximum depth of 418 m., but the maximum which we recorded was 358 m. Of 10 soundings made by us in the southern part of the lake, a basin 10 km. long, 7 fell in the range 346–358 m. suggesting a nearly horizontal floor in this part. This lower part of the lake is separated from the upper part by two narrow straits which, judging from the few early soundings, are appreciably shallower than the basins above and below. The upper basins receive nearly all the important streams tributary to the lake. Turbidity currents which might develop directly from the muddy streams or by mud-slides from actively growing deltas are likely to be trapped in the upper basins, leaving the lower basin free, or nearly so, of invasions at depth by turbid water.

The salient water characteristics which make the Lake worthy of remark are shown in Fig. 1. Results were obtained on May 29 and 30, 1961, from the centre of the lower basin where the depths exceeded 350 m. The considerable rise in temperature of nearly 5° C. from 100 m. to the bottom was the first indication that the deeper water needed a significant salt content for stability. The chlorinity of the samples was determined by titration with silver nitrate and the salinity computed from Knudsen's relationship. It should be noted that this relationship is not entirely valid due to appreciable variations in the ratios of the ionic constituents of the water of the Lake from those found in modern sea-water. For example, the correction of the chlorinity titration for the presence of hydrogen sulphide (Table 1) reduces the salinity from 16·92 to 16·58 in the 334-m. sample. However, density and electrical conductivity values for the deep-water samples agreed closely with those for sea water of the same salinity. The presence of large amounts of dissolved gases in the bottom 50 m. of the water column was evident from the voluminous degassing which occurred while drawing the samples. These oxygen-depleted bottom waters had an *in situ* *p*H of 6·7–6·9 and showed a distinct yellow colour which increased in intensity with depth and was evidently due to dissolved organic matter. As shown in Table 1, the high hydrogen sulphide content of the bottom water results in the expected concurrent low iron (II) concentration. Curiously enough, this is a condition opposite to that found by Strøm[1] for Lake Tokke which in comparable chemical properties was otherwise similar to Powell Lake.

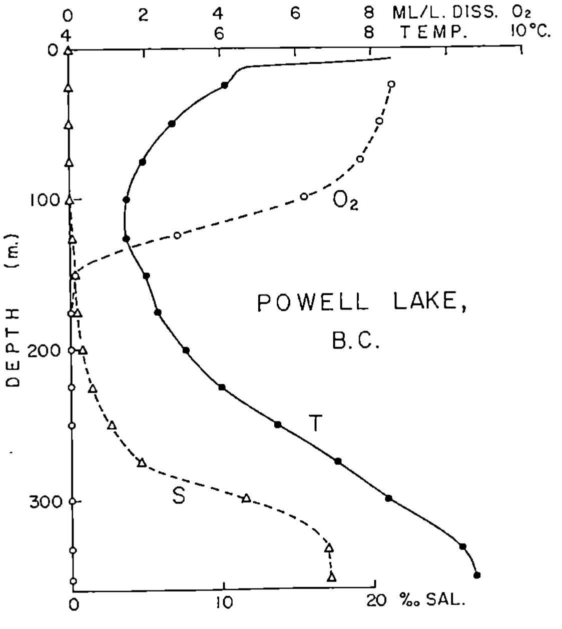


Fig. 1. Distribution of temperature, salinity and dissolved oxygen in Powell Lake, British Columbia

Table 1. Some Chemical Constituents of Powell Lake Bottom Water (334 m.)

Constituent	Concentration (mgm./l.)
Chloride	9,400
Sulphate	0
Bicarbonate	2,050
Total sulphide (H_2S, HS^-)	> 89
Total iron (dissolved and particulate)	< 0·5
Inorganic dissolved phosphate (P)	0·015
Inorganic dissolved silicate (Si)	10·4
Dissolved carbon dioxide	about 200
Dissolved methane	present*

* Identified by gas phase chromatography.

In the neighbouring inlets in which the stability of the water is high in the upper layers but decreases to the bottom a minimum temperature of 6°–8° C. is common at about 50 m. depth, while the deeper water is isothermal within 1° C. The minimum of lower value and greater depth in Powell Lake is attributed to the lower stability in the upper 150 m., which permits winter cooling to a greater depth. The considerable temperature gradient in the deeper water is associated with high stability of the deeper water due to the salinity gradient. It is possible that the temperature gradient is maintained by flow of heat through the bottom of the Lake. A flow of 40 cal./cm.^{2}/yr. would require the eddy heat conductivity in the water to be 3–4 times the molecular conductivity.

The saline water is presumed to have entered the Powell Lake Valley first during the disappearance of the last Cordilleran ice sheet close to 13,000 years ago. At this period the land was submerged in the vicinity of the Strait of Georgia at least locally to the present 175 m. contour[3]. Quaternary marine fossils reported from one point on the east side of Powell Lake may belong to this stage. With continued uplift, during and following the disappearance of the ice sheet, the rocky sill at the lower end of the Powell Lake basin shoaled, became emergent, and gradually rose to its present position above the sea. Radiocarbon dates from the opposite side of the Strait of Georgia, 70 km. to the south[4], indicate that the sea stood at the present 52-m. contour about 12,250 years ago and there is no suggestion that this level was afterwards submerged. In the lower Fraser Valley, 180 km. south-east of Powell Lake, the sea withdrew from the 60-m. contour before the last ice disappeared, some time between 11,000 and 7,300 years ago. In the Fraser Canyon, 220 km. east of Powell Lake, the ice sheet had disappeared and the Fraser River flowed below the present 75-m. contour 9,150 years ago. At the stage when Powell Lake was cut off from the Strait of Georgia it is possible that the waters of the Strait had a salinity less than that of the present 30–31$^{0}/_{00}$ due to dilution by large quantities of melt water from the dwindling ice tongues still present in parts of the lower Fraser valley[5,6] and at the head of at least one inlet[7] leading into the Strait.

We wish to acknowledge the assistance of Mr. J. Dice of Powell River, B.C., who made available and

operated his yacht, *Samarie*, from which our observations were made.

P. M. WILLIAMS
W. H. MATHEWS
G. L. PICKARD

Institute of Oceanography,
University of British Columbia,
Vancouver.

[1] Strøm, K., *Nature*, **180**, 982 (1957).
[2] Strøm, K., *Nature*, **189**, 913 (1961).
[3] Wilson, J. T., *et al.*, *Glacial Map of Canada* (1958).
[4] Terasmae, J., and Fyles, J. G., *Canad. J. Bot.*, **37**, 815 (1959).
[5] Armstrong, J. E., *Geol. Surv. Canad.*, Paper 57-5.
[6] Armstrong, J. E., *Geol. Surv. Canad.*, Paper 59-9.
[7] Mathews, W. H., *J. Geol.*, **59**, 357 (1951).

HISTOLOGY

Use of Veronal Buffers in Formalin Fixatives

FORMALIN 'buffered' with veronal (diethylbarbituric acid) has been used as a tissue fixative by Zacks and Blumberg[1] and by Dales[2], and as a storage fluid for fixed and stained specimens by Essner *et al.*[3]. It does not appear to have been realized, however, that veronal reacts rapidly with formaldehyde to produce a substance that has no buffering capacity within the physiological range of *p*H values.

This was observed when the osmium tetroxide in Palade's veronal–acetate buffered fixative[4] was replaced by sufficient formaldehyde to give a final concentration of 4 per cent. The composition of the mixture was 2·6 ml. of analytical reagent quality 36 per cent formaldehyde, 5 ml. each of *M*/7 sodium veronal and *M*/7 sodium acetate, and 5·5 ml. of *N*/10 hydrochloric acid in a total volume of 23 ml. By analogy with the corresponding osmium tetroxide fixative, the formalin variant was expected to have a *p*H of about 7·2. In fact, the measured *p*H was 9·8, indicating that the formaldehyde had reacted with the sodium salt of the monobasic veronal[5] to produce a neutral product, with concomitant liberation of alkali.

The experiment was repeated several times under carefully controlled conditions, and the resulting solutions were titrated with *N*/10 hydrochloric acid, using a Radiometer (Copenhagen) automatic titrator Type *TTT*1 and recording titrigraph type *SBR*2. The results indicated that one molecule of alkali was produced for every molecule of sodium veronal undergoing reaction, and that all the veronal was converted into the reaction product in the presence of the excess formaldehyde in the mixture. The shape of the titration curve was almost indistinguishable from that of a solution containing the same concentrations of sodium acetate and formaldehyde as those in the fixative, but from which the veronal salt had been omitted. It is clear, therefore, that the reaction product of veronal and formaldehyde has no buffering capacity. Any residual buffering action in formalin variants of Palade's fixative is due solely to acetate and, consequently, effective only in the range of approximately 3·6–5·8.

The *p*H of veronal-treated formalin fixatives may, of course, be adjusted to any convenient value near neutrality. The solutions will still act as fixatives, for in the case described here, there is a concentration of free formaldehyde of about 3 per cent, which is adequate for many fixation purposes.

It is not clear why the effect described here has not been observed before. It is possible that the magnitude of the change in *p*H, and that of the adjustment necessary to restore the *p*H to the required level, has been overlooked through the use of inadequate detection methods. The effect is immediately obvious, however, when a sensitive *p*H-meter is used, and such instruments are essential for the preparation of buffered solutions for cytological and cytochemical purposes.

While the reaction of unsubstituted barbituric acid with formaldehyde is known to produce C,C′-methylene*bis*barbituric acid[6], the reaction with disubstituted barbituric acids does not appear to have been described before. Since the possibility of methylene bridge formation between carbon atoms is excluded by the presence of the two ethyl groups in veronal, the observed effect is probably due either to the formation of a neutral N-hydroxymethyl-derivative, or of the corresponding N,N′-methylene*bis*-compound. Structural investigations have not been made to confirm which of these suggestions is correct.

We have commented elsewhere[7] on the value, for electron microscopy, of formalin-based fixatives to which veronal and other buffers have been added.

S. J. HOLT
R. MARIAN HICKS

Courtauld Institute of Biochemistry,
and Bland Sutton Institute of Pathology,
Middlesex Hospital Medical School,
London, W.1.

[1] Zacks, S. I., and Blumberg, J. M., *J. Histochem. and Cytochem.*, **8**, 337 (1960).
[2] Dales, S., *Exp. Cell Research*, **19**, 577 (1960).
[3] Essner, E., Novikoff, A. B., and Masek, B., *J. Biophys. and Biochem. Cytol.*, **4**, 611 (1958).
[4] Palade, G., *J. Exp. Med.*, **95**, 285 (1952).
[5] Fischer, E., and Dilthey, A., *Annalen*, **335**, 340 (1904).
[6] Gysling, H., and Schwarzenbach, G., *Helv. Chim. Acta*, **32**, 1484 (1949).
[7] Holt, S. J., and Hicks, R. M., *J. Biophys. and Biochem. Cytol.*, **10**, No. 4 (1961).

CYTOLOGY

Sub-Chromatid Structure and Chromosome Duplication in *Vicia faba*

A QUADRIPARTITE structure of chromosomes has been inferred from certain anaphase configurations in mitosis and meiosis, such as the two-side-arm bridges found in irradiated meiosis in *Uvularia*[1] and *Lilium*[2]. Similar bridges have been seen in endosperm mitosis[3,4]. Wilson *et al.*[5] have clearly demonstrated the sub-chromatid nature of such bridges in *Trillium*.

The present work has aimed at determining whether sub-chromatids are demonstrable in *Vicia faba*, the species used by Taylor *et al.*[6] for autoradiographic analysis of chromosome duplication.

Meiotic material was fixed at half-hourly intervals 2–5 hr. after irradiation at a dosage of 36 r. Root tips (laterals), grown in distilled water, were fixed 1–1·5 hr. after 108 r. irradiation. Dosage-rate was 36 r./min. at 6 m.amp., 200 kV., with a 0·85 mm. copper/0·5 mm. aluminium filter. Focal distance was 40 cm., half-value layer 1·55 mm. copper. Timing trials indicated that anthers in mid- and late-first anaphase at fixation would have been in stages of late diplotene to metaphase 2–2·5 hr. previously. Divisions in control material were regular except for a very low frequency of breakage and reunion.

Pollen mother cells in first and second anaphase showed a high frequency of two-side-arm bridges similar to those described for *Trillium*[5] (Fig. 1*a*).

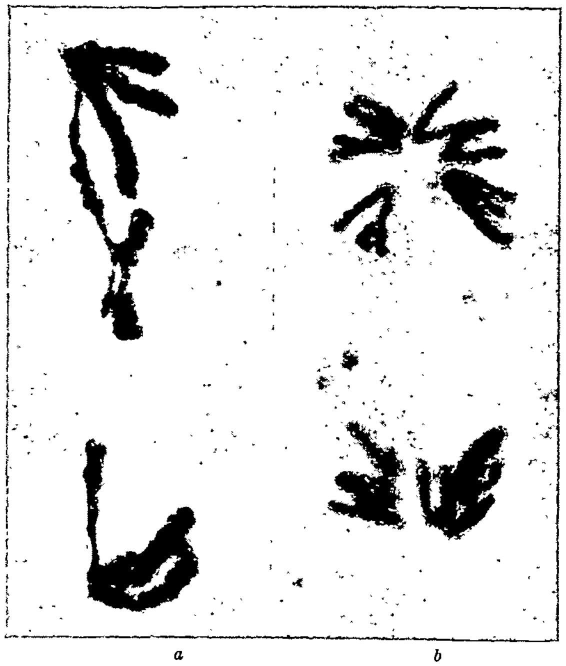


Fig. 1. (*a*) Metacentric chromosome of *V. faba* at anaphase I showing true two-side-arm bridge. (× 1,900). (*b*) Anaphase I showing *Y*-shaped chromatid with corresponding deficient chromatid at opposite pole. (× 1,000)

It was possible to trace sub-chromatids in these configurations, and to distinguish 'true' and 'pseudo' bridging, resulting respectively from non-sister sub-chromatid reunion and from non-sister sub-chromatid reciprocal restitution. Configurations predicted but not seen by Wilson *et al.*, the one-side-arm bridge and the *Y*-shaped chromatid with a corresponding deficient chromatid at the opposite pole, were also found (Fig. 1*b*).

All these configurations, together with the whole chromatid bridges and fragments which also occurred, may be explained as consequences of different patterns of sub-chromatid reunion and restitution. Since most of the configurations require non-sister sub-chromatid breakage or even 3- or 4-strand breakage, it must be inferred that breakage involves the whole chromosome, while reunion is a function of the half-chromatids. The view that the chromosome is the functional unit in breakage is supported by the fact that in all bridges examined the side-arms appear to be of equal length; breakage must have been at homologous or near-homologous points on the chromatids.

Mitotic configurations were similar and included one-side-arm bridges and *Y*-shaped chromosomes. The two-side-arm bridges closely resembled the 'point unions' described by Davidson[7].

To distinguish between the sub-chromatid hypothesis of two-side-arm bridge formation and 'matrix stickiness' as suggested by Östergren and Wakonig[8], irradiated seedlings were transferred to 0·05 per cent colchicine for 24–30 hr. immediately after 108 r. irradiation. If sub-chromatids are concerned in bridge formation, whole chromatid errors should be evident in tetraploid *c*-metaphases. Sub-chromatid reunion, normally resulting in a true two-side-arm bridge, would yield a quadriradial X_2 association composed of a dicentric chromatid and an acentric fragment, while sub-chromatid restitution would yield a quadriradial of normal chromatids. Although only a small proportion of cells showed tetraploid X_2 mitoses after the colchicine treatment, both these configurations were found.

The minimal hypothesis of sub-chromatid structure is that each consists of a single deoxyribonucleic acid (DNA) double helix. Meselson and Stahl[9] have shown that, in replication of DNA, a first-generation molecule contains one original helix and a newly synthesized complementary helix. Taylor *et al.*[6] found a segregation of labelled and non-labelled chromatids in the X_2 *c*-metaphase, but with the existence of half-chromatids (with the minimal single DNA structure) such a situation should not be evident until the X_3 division. It has been shown[10] that there may be a substantial delay either in the penetration or the action of 0·05 per cent colchicine in *V. faba*. This could lead to a mis-identification of X_3 cells as being X_2, because of their tetraploidy.

Taylor's results would be compatible with a quadripartite chromosome, if segregation of labelled from non-labelled sub-chromatids should occur. Relational coiling of sister sub-chromatids would seem to prevent this: if, as seems likely on electron-microscope evidence, more orders of sub-units are present, this difficulty would be further increased. La Cour and Pelc[11] also have questioned the significance of Taylor's work after failing to obtain similar results in a repeat of the experiment: they suggest that colchicine may interfere with replication.

The apparent disagreement of the chromosome duplication studies with the demonstrable half-chromatid structure, as well as the lack of repeatability of the autoradiographic work, indicate that a thorough study of the techniques used in such work is needed before interpretations will be of significance.

W. J. PEACOCK

Department of Botany,
University of Sydney.

[1] Darlington, C. D., and La Cour, L. F., *Heredity*, **6**, Supp., 41 (1953).
[2] Crouse, H. V., *Science*, **119**, 485 (1954).
[3] La Cour, L. F., and Rutishauser, A., *Chromosoma*, **6**, 696 (1954).
[4] Brock, R. D., *Heredity*, **9**, 199 (1955).
[5] Wilson, G. B., Sparrow, A. H., and Pond, V., *Amer. J. Bot.*, **46**, 309 (1959).
[6] Taylor, J. H., Woods, P. S., and Hughes, W. L., *Proc. U.S. Nat. Acad. Sci.*, **43**, 122 (1957).
[7] Davidson, D., *Chromosoma*, **9**, 39 (1957).
[8] Östergren, G., and Wakonig, T., *Bot. Not.*, **4**, 357 (1954).
[9] Meselson, M., and Stahl, F. W., *Proc. U.S. Nat. Acad. Sci.*, **44**, 671 (1958).
[10] Neary, G. J., Evans, H. J., and Tonkinson, S. M., *J. Genet.*, **56**, 363 (1959).
[11] La Cour, L. F., and Pelc, S. R., *Nature*, **182**, 506 (1958).

PSYCHIATRY

Barbiturates and Eye Vergence

It has been demonstrated[1] that barbiturates influence conjugate eye movements selectively in that they abolish the smooth tracking movements while leaving the saccadic movements intact. Disjunctive eye movements have time characteristics akin to the tracking movements; some effect of barbiturates on convergence has been mentioned[2].

In the following typical experiment a normal subject (age 21, height 6 ft. 3 in., weight 175 lb.) was given 390 mgm. amytal sodium ('Amobarbital Sod.', Lilly) by mouth. Measurements were taken, at suitable intervals over a 12-hr. period, of hetero-phoria with a target at 5 m., of the amplitudes of prism divergence and convergence with a target at

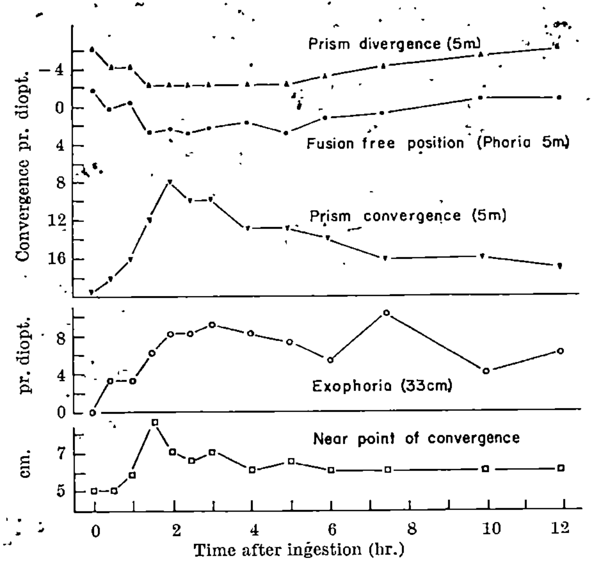


Fig. 1. Changes in a variety of vergence measures following ingestion of 390 mgm. amytal sodium

5 m., of heterophoria at 33 cm., and of the near point of convergence. The changes in the findings are illustrated in Fig. 1. The dose was moderate ; during the phase in which he was most affected (2–6 hr. after ingestion) the subject's ability to make responses was unimpaired, he exhibited only mild barbiturate nystagmus on extreme version of the eyes, and some tracking eye movements persisted. Vestibular nystagmus was unimpaired. The effects illustrated in Fig. 1 were : a change in distance heterophoria in the direction of esophoria, a reduction in the amplitude of the fusional range, a marked change in the near heterophoria in the direction of exophoria, and a recession of the near point of convergence. Experiments with ethanol and anoxia indicate similar effects[3].

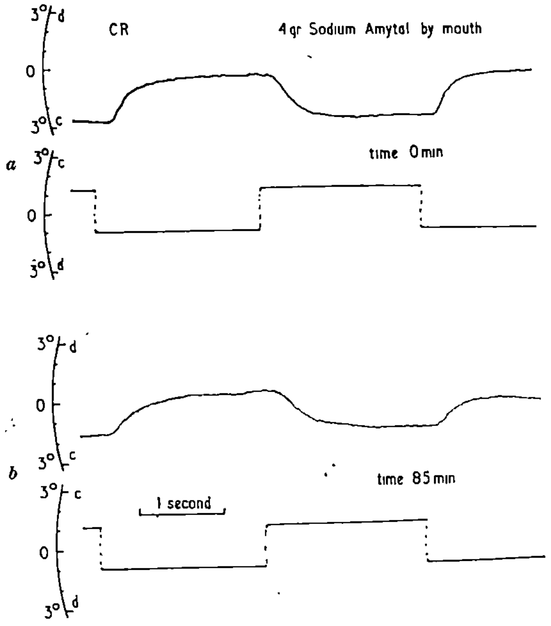


Fig. 2. Eye vergence response to 2° step change in target vergence. (a) Normal state; (b), 85 min. after ingestion of 260 mgm. amytal sodium

To establish whether or not those convergence responses that persisted in the limited fusional range were normal, eye movements records were obtained on one subject with a method described elsewhere[4]. The subject (age 33, height 5 ft. 7 in., weight 135 lb.) was given 260 mgm. amytal sodium by mouth and reached about the same level of intoxication as was demonstrated by the subject in the experiment in Fig. 1. In his normal state when the subject was presented with cathode-ray tubes at 2 m., one in front of each eye, on which spot targets were displaced suddenly so as to need a 2° vergence movement to re-establish binocular fixation, records such as those in Fig. 2*a* were obtained. During barbiturate intoxication the responses looked like those in Fig. 2*b*, demonstrating that, while the final level of convergence reached is the same as that in Fig. 2*a*, the time course of the response is slowed substantially. Records obtained from this subject during this phase showed that he still retains his capacity to make smooth pursuit eye movements during conjugate tracking of sine-waves.

To determine whether the change of the heterophoria at near, which has considerable clinical implications in view of the relatively small dose of the drug involved, could be accounted for in part by a sensory change, we have also measured accommodation. If it does not suffer a deficiency similar to that of convergence, one cannot maintain that the effect of barbiturates is to inhibit in some way the total near response which involves both accommodation and convergence. Measurements of accommodation were obtained by three methods during some of these experiments : (1) the near point of accommodation, (2) the amount of accommodation in play while the subject was fixating the near heterophoria target, using retinoscopy, and (3) the amount of accommodation exerted by the subject during monocular view of a near point target through a semi-transparent mirror, using stigmatoscopy. In each case there was essentially no change in the accommodation measurements throughout the experiment. Neither was there any change in the refractive state for infinity, as determined by retinoscopy.

These findings show that barbiturates interfere with the mechanism which organizes eye vergence movements. This occurs with quite moderate clinical doses which barely affect smooth tracking movements. Saccadic and vestibular eye movements and accommodation responses remain intact under these conditions and this helps to delimit the site of action of the drug.

This work was aided by grant *MY*3034 from the National Institute of Mental Health, U.S. Public Health Service, and by the Institute for Research in Vision, The Ohio State University.

GERALD WESTHEIMER*
CYRIL RASHBASS†

Columbus Psychiatric Institute
and Hospital,
The Ohio State University
Health Center,
Columbus 10, Ohio.

* Now at School of Optometry, University of California, Berkeley 4, California.

† On leave from the Institute of Psychiatry, University of London.

[1] Rashbass, C., *Nature*, **183**, 897 (1959).

[2] Bergman, P. S., Nathanson, M., and Bender, M. B., *Arch. Neurol. Psychiat.*, **67**, 357 (1952).

[3] Adler, F. H., *Physiology of the Eye*, third ed., 442 (The C. V. Mosby Co., St. Louis, 1959).

[4] Rashbass, C., and Westheimer, G., *J. Physiol.* (in the press).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Research Fellow (with a good honours degree in a biological subject, some research experience in genetics or preferably cytogenetics, experience in the tissue culture field would be an advantage) to work in the Cytogenetics Group of the Pædiatric Research Unit, Guy's Hospital Medical School—The Pædiatric Research Unit, Guy's Hospital Medical School, London Bridge, S.E.1 (August 25).

Lecturer (Grade II) or Assistant Lecturer (Grade III) in Mycology within the Department of Botany—The Registrar, The University, Bristol 8 (August 26).

Senior Lecturer (with a good honours degree, teaching experience, and either a higher degree or substantial research experience) in the Department of Chemistry, to be responsible for Physical Chemistry—The Principal, College of Further Education, Whitehaven, Cumberland (August 26).

Demonstrator in Psychology, to assist in practical classes and tutorial work—The Registrar, The University, Liverpool, quoting Ref. CV/T (August 28).

Geophysicist (with a degree in science or electrical engineering, and with a knowledge of physics) in the South Australian Department of Mines—The Agent-General and Trade Commissioner for South Australia, South Australia House, Strand, London, W.C.2 (August 31).

Lecturer or Senior Lecturer (qualified to lecture up to honours standard) in Chemistry (Organic or Physical) at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 31).

Librarian (professionally qualified and experienced) at the Nigerian College of Arts, Science and Technology, Zaria—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (September 1).

Principal Lecturer (preferably with an interest in the development of research projects) in Pharmaceutics—The Principal, College of Technology, Belfast 1, Northern Ireland (September 1).

Research Assistant (with a good honours degree in botany or agricultural botany and an interest in experimental ecology and/or genetics) in Agricultural Botany, for investigations on the evolution and nature of resistance to zinc and copper toxicity in natural populations of higher plants—The Registrar, University College of North Wales, Bangor, North Wales (September 1).

Research Assistant (with a qualification in chemistry and preferably previous experience in polymer chemistry) in the Isotope Unit, for work in the field of polymer reaction mechanisms—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (September 1).

Assistant Librarian (university graduate, preferably with library qualifications)—The Librarian, University Library (University of St. Andrews), Dundee, Scotland (September 2).

Lecturer and an Assistant Lecturer (graduates, with an interest in any of the branches of electrical engineering) in the Department of Electrical Engineering of the Faculty of Science—The Registrar, The University, Manchester 12 (September 2).

Experimental Officer, Grade I (with a degree in physics or equivalent qualifications) at the Weapons Research Establishment, near Adelaide, Australia, to develop optical equipment required for field and laboratory performance testing of lens systems, and to carry out necessary performance testing—The Senior Representative (AV. 120/5), Department of Supply, Australia House, Strand, London, W.C.2 (September 4).

Senior Lecturer, Grade I or II (with a good honours degree and teaching experience, and preferably some research experience) in Mathematics at the Nigerian College of Arts Science and Technology, Zaria Branch—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (September 4).

Lecturer (preferably with some teaching experience and a veterinary qualification) in the Department of Veterinary Physiology, Royal (Dick) School of Veterinary Studies—The Secretary, The University, Edinburgh (September 5).

Assistant Chief Inspector of Nuclear Installations (with a first- or second-class honours degree in engineering or physics, corporate membership of an appropriate professional institution, and wide experience of work on nuclear installations and the management of a team of professional engineers) in the Inspectorate of Nuclear Installations of the Ministry of Power to take charge of sections of the Inspectorate concerned with the safety assessment of nuclear installations, and with special problems in nuclear physics, chemistry, metallurgy and health physics—Civil Service Commission, 17 North Audley Street, London, W.1, quoting Ref. S/5355/61 (September 6).

Assistant (graduate in botany or agriculture or equivalent qualifications) to the Mycologist, for work involving testing the susceptibility to disease of crop varieties, and at present is mainly concerned with the cereals—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (September 15).

Lecturer and a Senior Lecturer in the Department of Medical Biochemistry, Monash University, Clayton, Victoria, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 15).

Director of Combined Studies in the Faculty of Science, to supervise admissions to, and the progress of. students in the courses leading to the newly instituted degree in which two science subjects are studied to a higher level—The Registrar, The University, Leeds 2 (September 18).

Senior Assistant Chemist (member of an appropriate chemical institution, extensive experience in large-scale sewage purification and trade effluent control, and preferably with a university degree and membership of the Institute of Sewage Purification) at Mogden Purification Works, Isleworth, Middlesex—The Chief Chemist, Middlesex County Main Drainage Department, P.O. Box No. 7, Isleworth, Middlesex, quoting H.23T (September 18).

Senior Research Fellow or Research Fellow (with a Ph.D. degree in organic chemistry, with additional experience in general synthetic organic chemistry) in the Biological Inorganic Chemistry Unit, John Curtin School of Medical Research, Institute of Advanced Studies, Australian National University, to work on the synthesis of multidentate organic ligands, as part of the programme of the Unit on the effects and utilization of metal complexes in biological systems—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 18).

University Demonstrator (with some acquaintance with population studies and biological statistics) in Physical Anthropology—The Secretary of the Appointments Committee, Faculty of Archæology and Anthropology, The University, Downing Street, Cambridge (September 18).

Senior Lecturer or a Lecturer in the Department of Electrical Engineering at Newcastle University College, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2, and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (September 22).

Experimental Officer or Senior Experimental Officer (preferably with previous experience of high voltage engineering, electronics, and nuclear physics, and a degree or diploma in physics or electrical engineering), to take charge of the maintenance and operation of the Manchester University 6-MeV. electrostatic generator—The Registrar, The University, Manchester 13 (September 30).

Full-time Lecturer or Senior Lecturer in Pathology—The Director, Department of Pathology, Institute of Ophthalmology (University of London), Judd Street, London, W.C.1 (September 30).

Lecturer in Theoretical Physics; a Lecturer in Physics; and Assistant Lecturers in Physics—The Registrar, The University, Manchester 13 (September 30).

Nuffield Foundation Research Fellows (2) (well qualified in electrical engineering or physics with general experience in electronics and considerable detailed knowledge of solid state circuits) in the School of Electrical Engineering, University of Sydney, Australia, to be associated with the development of electrical engineering aspects of the design of a large radioastronomy interferometer system under development by the Radio Astronomy Centre—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (September 30).

Senior Demonstrator (graduate) in Zoology at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

Lecturer in Applied Pathology—The Secretary, Queen's University, Belfast, Northern Ireland (October 1).

Lecturer/Senior Lecturer in Botany at the University of New England, Armidale, New South Wales, Australia, to lecture in plant physiology or in mycology-microbiology to advanced students in botany and to supervise research projects of postgraduate and final year (botany double major) students—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 6).

Assistant Experimental Officer (with H.N.C. or pass degree, or, if under 22, G.C.E. "A" level or equivalent) in the Physics Department, to assist in studies of physical properties of dairy products—butter, cheese, etc.—and to construct and modify experimental testing equipment in the departmental workshop—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 61/4.

Biochemist or Chemist, to study metabolism of ^{14}C-labelled steroid hormones—The Professor of Physiology, The Medical School, King's College (University of Durham), Newcastle-upon-Tyne 1.

Biochemist (qualified), for steroid work in an endocrine laboratory in the Obstetric Unit of the Medical School of University College Hospital—The Secretary, University College Hospital Medical School, University Street, London, W.C.1.

Grade B Assistant to teach Physics—The Principal, Huddersfield College of Technology, Huddersfield.

Graduate Assistant (science graduate, preferably with some dairy experience), to undertake a study of pipeline milking—Head of the Department of Dairy Science, University of Alberta, Edmonton, Alberta, Canada.

Graduate or Technician (A.M.I.L.T.), to assist with research on problems of fibrinolysis in relation to coronary thrombosis, etc., the work will be mainly hæmatological—The Assistant Secretary, Westminster Hospital, London, S.W.1.

Nuffield Research Assistant (with a good honours degree in either physics or a biological science) in Biophysics, to work under the direction of Dr. K. W. Keohane—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

Postgraduate Student (man or woman graduate with an honours degree in chemistry), for work involving research in boron chemistry—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey.

Research Assistant, to help with current research on an immunological project connected with rheumatoid arthritis—The Pathologist, Stoke Mandeville Hospital, Aylesbury, Bucks.

Research Assistant, to undertake programming investigation in connexion with a second college digital computer now under construction—The Principal, College of Technology, Broadway, Letchworth, Herts.

Research Assistant (with a B.Sc. degree) in the Department of Medicine, to join a small team working in cardio-renal physiology in its relation to human disease—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee.

Research Student (with an honours degree or equivalent qualification, for example, G.R.I.C.) in Electrodialysis, to carry out research for a period of three years under the direction of Dr. P. Meares on fundamental aspects of the transport of ions in charged membranes using radioactive tracers—Prof. G. M. Burnett, Department of Chemistry, The University, Old Aberdeen.

Research Technician (with some experience in a biochemical research laboratory), for work in the Department of Chemistry and Food Technology on chemical and fermentation studies of the cocoa bean—The Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

SENIOR TECHNICIAN (with at least A.I.S.T. or National Certificate), for electron microscope duties in the Department of Zoology—The Secretary, The University, Old College, South Bridge, Edinburgh.

TECHNICAL OFFICER (Utilization) (with a Diploma or City and Guilds Final Certificate in machine wood-working, wood technology or allied subject) IN THE DEPARTMENT OF FOREST RESEARCH, Nigeria, to take charge of one or more sections of the new Forest Products Research Laboratory, including wood-working, seasoning, impregnation and timber mechanics—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. 1.2/3.

TECHNICIAN (male or female) IN THE MICROBIOLOGICAL TEACHING LABORATORY—Mr. Edwards, The School of Pharmacy, 29/39 Brunswick Square, London, W.C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

IBM United Kingdom Limited. IBM Research Fellowships. Pp. 4. Endowed Research Time. Pp. 3. (London: IBM United Kingdom Limited, 1961.) [156

Department of Scientific and Industrial Research. Forest Products Research 1960: Report of the Forest Products Research Steering Committee with the Report of the Director of Forest Products Research. Pp. v+60+8 plates. (London: H.M. Stationery Office, 1961.) 5*s*. net. [156

The Manchester Joint Research Council. Sources of Scientific and Technical Information available to Local Industry. Pp. 22. An account of some of the Scientific and Technological Facilities which Manchester University and Manchester College of Science and Technology can offer to Industry. Pp. 20. (Manchester: The Manchester Joint Research Council, 1961.) [166

British Empire Cancer Campaign. Thirty-eighth Annual Report covering the year 1960. Part 1: The Chairman's Statement and the Accounts. Pp. xxxviii. Part 2: The Scientific Report of the Researches undertaken by the Central Organization and Its Autonomous Councils in the United Kingdom, and by some of Its Affiliated Organizations. Pp. xxxix+lviii+792. (London: British Empire Cancer Campaign, 1961.) [166

The National Anti-Vivisection Society (Incorporating London and Provincial Anti-Vivisection Society.) Eighty-sixth Annual Report for the year 1960. Pp. 16. (London: National Anti-Vivisection Society, 1961.) [206

Universities Council for Adult Education. Report on the year 1959-60. Pp. 28. (Bristol: W. E. Salt, Honorary Secretary and Treasurer, The University, 1961.) [206

Arthur D. Little Research Institute. The Acorn Lectures, Vol. 1. Edited by E. Jones. (Abridged versions on the originals.) Pp. v+46. (Inveresk: Arthur D. Little Research Institute, 1961.) [206

Third Report from the Estimates Committee together with part of the Minutes of Evidence taken before Sub-Committee A, and an Appendix. Session 1960–61—Form of the Estimates. Pp. vi+58. (London: H.M. Stationery Office, 1961.) 4*s*. 6*d*. net. [206

Ministry of Housing and Local Government. Alkali &c., Works Regulation Act, 1906; and Alkali, &c., Works Orders, 1928–1958; Alkali, &c., Works Regulation (Scotland) Acts, 1901 and 1951; and Alkali, &c., Works (Scotland) Orders, 1952 and 1958. Ninety-seventh Annual Report on Alkali, &c., Works by the Chief Inspectors, 1960. Pp. 68. (London: H.M. Stationery Office, 1961.) 4*s*. net. [206

Institute of Personnel Management. Job Analysis. By H. E. Roff and T. E. Watson. Pp. 40. (London: Institute of Personnel Management, 1961.) 7*s*. 6*d*. [206

Council for the Preservation of Rural England, Sheffield and Peak District Branch. Annual Report, April 1961. Pp. 28. (Sheffield: Council for the Preservation of Rural England, Sheffield and Peak District Branch, 1961.) [206

Sir John Cass College. Prospectus, Session 1961–62. Pp. 55. (London: Sir John Cass College, 1961.) [226

George T. Gurr, Ltd. Gurr's Commentary: Supplement on Fluorescence Microscopy. Edited by George T. Gurr. Pp. 16. (London: George T. Gurr, Ltd., 1961.) [226

Annual Report of the Yorkshire Philosophical Society for the year 1960. Pp. 15. (York: Yorkshire Philosophical Society 1961.) [226

Negretti and Zambra, Ltd. Publication No. APP/23: Grain Silo Temperature Measurement System. Pp. 3. Publication No. APP/25: Temperature Measurement in Grain Storage-Bins. Pp. 3. Publication No. APP/25: Grain Drying: Typical Control Scheme for a High Temperature, Electrically Heated, Single Chamber Grain Drying Installation. Pp. 4. (London: Negretti and Zambra, Ltd., 1961.) [226

Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1116–D: Geophysical Abstracts 183, October–December 1960. By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel and others. Pp. iii+457–636. 40 cents. Water-Supply Paper 1498-A: Flume Studies Using Medium Sand (0·45 mm.). By D. B. Simons and M. L. Albertson. Pp. viii+76. Water-Supply Paper 1593: Simplified Methods for Computing Total Sediment Discharge with the Modified Einstein Procedure. By B. R. Colby and D. W. Hubbell. Pp. vi+17+8 plates. (Washington, D.C.: Government Printing Office, 1961.) [156

Smithsonian Contribution to Astrophysics. Vol. 4, No. 2: Orbital Elements of Photographic Meteors. By Richard E. McCrosky and Annette Posen. Pp. ii+15–84. 40 cents. Vol. 5, No. 5: On the Motion of Satellites with Critical Inclination. Pp. ii+39–58. 20 cents. Vol. 5, No. 6: Gaps in the Distribution of Asteroids. By Yusuke Hagihara. Pp. ii+59–67. 20 cents. Vol. 5, No. 7: Major Flares and Geomagnetic Activity. By Barbara Bell. Pp. ii+69–83. 20 cents. Vol. 5, No. 8: An Annotated Bibliography on Interplanetary Dust. By Paul W. Hodge, Frances W. Wright and Dorrit Hoffleit. Pp. ii+85–111. 25 cents. (Washington, D.C.: Government Printing Office, 1961.) [156

Carnegie Institution of Washington. Publication No. 175, Vol. 21: Cosmic-Ray Results—Huancayo, Peru, January 1956—December 1959; Cheltenham, Maryland, January 1956—October 4, 1956; Fredericksburg, Virginia, October 5, 1956—December 1959; Christchurch, New Zealand, January 1956—June 1959; Godhavn, Greenland, January 1954—July 1959; Universidad Nacional de Mexico, July 1957—December 1958. Pp. v+226 (221 tables). Publication No. 620: Equatorial Electrojet in Peru. By Scott E. Forbush and Mateo Casaverde. Pp. v+135 (98 tables). (Washington, D.C.: Carnegie Institute of Washington, 1961.) [156

United States Department of the Interior: Geological Survey. Water-Supply Paper 1539–A: Exploratory Drilling for Ground Water in the Mountain Iron-Virginia Area, St. Louis County, Minnesota. By R. D. Cotter and J. E. Rogers. Pp. iii+13+2 plates. Water-Supply Paper 1539–D: Correlation of Ground-Water Levels and Air Temperatures in the Winter and Spring in Minnesota. By Robert Schneider. Pp. iii+14. Bulletin 1071–I: Surficial Geology of the Kingston Quadrangle, Rhode Island. By Clifford A. Kayne. Pp. iv+341–396+plates 32–34. Bulletin 1084–I: A Spectrochemical Method for the Semi-quantitative Analysis of Rocks, Minerals and Ores. By A. T. Myers, R. G. Havens and P. J. Dunton. Pp. iii+207–229. 15 cents. Bulletin 1100: Uranium and other Metals in Crude Oils By C. A. Horr, A. T. Myers, P. J. Dunton, and Harold J. Hyden. Pp. vii+99. 1 dollar. Bulletin 1115: Bibliography of North American Geology, 1958. By Ruth Reece King and others. Pp. iii+592. 2 dollars. (Washington, D.C.: Government Printing Office, 1960 and 1961.) [166

Population Reference Bureau, Inc. *Population Bulletin*, Vol. 17, No. 4 (June, 1961): Spotlight on Marriage. Pp. 61–80. (Washington, D.C.: Population Reference Bureau, Inc., 1961.) 50 cents. [166

New Zealand Forest Service. Information Series, No. 32: Development of the Exotic-Forest Resources as a Major Land Use. By Alex R. Entrician. Pp. 11. Forest Research Institute Technical Paper No. 30: Checking of Intergrown Knots during Seasoning of Radiata Pine Sawn Timber. By J. A. Kininmonth. Pp. 16. (Wellington: Government Printer, 1960 and 1961.) [166

United States Department of Health, Education and Welfare. Public Health Service Publication No. 824: Highlights of Progress in Mental Health Research. 1960—Items of Interest in Program Developments and Research conducted and supported by the National Institute of Mental Health. Pp. v+43. (Washington, D.C.: Government Printing Office, 1961.) 25 cents. [166

Organization for European Economic Co-operation: European Productivity Agency. Problems of Development: Olive Growing in Sardinia. By Roger Marcellin. Pp. 35. (Paris: Organization for European Economic Co-operation, European Productivity Agency, 1961.) [206

Smithsonian Miscellaneous Collections. Vol. 143, No. 2: Sixteen-day Weather Forecasts from Satellite Observations. By C. G. Abbot. Pp. 6. (Publication No. 4462.) (Washington, D.C.: Smithsonian Institution, 1961.) [206

United States Department of the Interior: Geological Survey. Bulletin 1081–D: Stratigraphy and Structure of the House Rock Valley Area, Coconino County, Arizona. By John D. Wells. Pp. iii+117–158+plates 4 and 5. 75 cents. Bulletin 1081–E: Geology of the Lloyd Quadrangle, Bearpaw Mountains, Blaine County, Montana. By Robert George Schmidt, W. T. Pecora, Bruce Bryant and W. G. Ernst. Pp. iii+159–188+plate 6. Bulletin 1089–B: Geology of the Rogers Lake and Kramer Quadrangles, California. By T. W. Dibblee, Jr. Pp. iv+73–139+plates 7–9. 1.25 dollars. (Washington, D.C.: Government Printing Office, 1960.) [206

Quaderno di Fisica, Serie 1, No. 2: Le Formule di Lorentz–Einstein. Da Guiseppe Antoni. Pp. 83. (Terni: Edizioni Thyrus, Casella Postale 52, 1960.) 750 lire. [206

Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig. Proceedings of the Fourth Conference on Potato Virus Diseases, Braunschweig, 12–17 September 1960. Edited by J. Brandes, R. Bartels, J. Völk and C. Wetter. Pp. 175. (Wageningen: H. Veenman & Zoonen, N.V., 1961.) [206

United States Department of Commerce: National Bureau of Standards. Monograph No. 19: Atomic Energy Levels in Crystals. By John L. Prather. Pp. iv+84. (Washington, D.C.: Government Printing Office, 1961.) 60 cents. [206

The Industrial Fund for the Advancement of Scientific Education in Schools. Report and Accounts for the year ended 30th December, 1960. Pp. 11. (Sydney: A. G. T. Carver, Chatsworth House, Bent Street, 1961.) [206

Australian Journal of Experimental Agriculture and Animal Husbandry, Vol. 1, No. 1 (May, 1961). Pp. 55. Published quarterly. Annual subscription £5 (Aust.). (East Melbourne: Australian Institute of Agricultural Science, 1961. Published for the Australian Agricultural Council.) [206

The Western Australian Museum. Annual Report for 1959–60. Pp. 33+4 plates. (Perth: Western Australian Museum, 1961.) [226

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office

GOVERNMENT RESPONSIBILITY IN SCIENTIFIC RESEARCH AND DEVELOPMENT

THE rather rambling debate in the House of Commons on July 10 on science was ostensibly concerned with the supply of services connected with science in the Civil Estimates for 1961–62, covering specifically the votes for the Office of the Minister for Science, Atomic Energy, the Department of Scientific and Industrial Research, the Ministry of Power, the Medical Research Council, the Agricultural Research Council, the Nature Conservancy, the Ministry of Education, the Scottish Education Department, and the universities and colleges, etc., of Great Britain. It is perhaps worth noting that, although referred to in the debate, the Overseas Research Council was not specified in the motion, although Sir David Eccles in particular stressed the vital importance of contributing an adequate proportion of scientists and technologists to the underdeveloped countries. The debate, which was opened by Mr. F. Peart, was, however, directed more specifically at the examination of Government scientific policy in general, and the adequacy of our present organization for determining priorities and formulating the policies needed to-day.

Policies are inevitably determined by the political objectives which are regarded as desirable, and to that extent scientific policy and the strategy and tactics by which policy is executed are influenced also by politics. Provided, however, political considerations are not allowed to overrule or dominate the strictly scientific or technical issues or possibilities, that should not prevent a constructive approach in which party politics are subsidiary, or even the formulation of particular policies on which the support of all political parties can be assured. Some such issues were manifest in the debate itself, but unfortunately, from the outset, party politics were allowed to play a dominating part. Speaking for the Government, the Minister of Education, Sir David Eccles, and the Parliamentary Secretary for Science, Mr. Denzil Freeth, had little difficulty in dealing with the charge of complacency. In consequence, few constructive suggestions were made, apart from those in Sir Hugh Linstead's sound and discerning speech, and the debate as a whole did little to explore the issues as to the relations between Government and science which were recently opened up in the concluding chapter of Sir Charles Snow's book, *Science and Government*. If speakers were aware of the issues which arise out of the relations between the scientist and Government, they made little attempt to discuss them, and the debate merely directed attention to some but not all, of the problems which arise in this field and which require examination before adopting any more definite pattern, or the authoritative intervention in the direction of science which Mr. T. F. Peart advocated and which was also put forward in the Labour Party's pamphlet, *Science and the Future of Britain**.

Broadly speaking, apart from seeking to sustain the charge of complacency brought by Mr. Peart, speakers from the Opposition were concerned to raise specific issues where greater effort appeared to be desirable rather than to elucidate the conditions which might promote greater understanding of the work of the scientist and the contribution of science in public affairs, or even more effective co-operation between the scientist and the administrator. Neither Government nor Opposition spokesmen for the most part noted that the contribution of a scientifically trained mind is a valuable and even a necessary corrective and check on the experience and intuition of the administrator; nor did they note Sir Frederick Brundrett's observation, in the tradition of a remark of Rutherford's that Sir Henry Tizard was wont to quote, that research does not flourish in an atmosphere of luxury but that it is best done when it has to justify itself.

Sir Frederick's remarks in the article which he contributed on "Government and Science" to the *Journal of Public Administration* some five years ago are entirely apposite to-day, and as worthy of note by participants in the recent debate as those of Sir Charles Snow in the book already noted. Sir Frederick was urging indeed greater support by the State for technology rather than for pure science, and pointing to the need which Sir Henry Tizard repeatedly emphasized for the effective use of existing knowledge. He stressed also the importance of direct contact between the research worker and the user, and expressed the view that the results achieved in civil research are not commensurate with the effort expended. Sir Frederick thought, indeed, that in civil research we are not sufficiently critical of the need for research as opposed to the need for applying existing knowledge. He suggested that our defence organization had gone a long way towards finding a proper solution to the problem of the relation between scientist and government, because in that field the scientist had comparatively clear objectives and his place had been firmly established on the policy-making bodies.

Recent moves by the Advisory Council for Scientific Policy have removed a little of the substance of some of Sir Frederick Brundrett's criticism, but it is still probably true that the Advisory Council is not sufficiently supported at lower levels and that its membership could include administrators from 'user' Departments. It is interesting to note how much in line with Sir Frederick's views are the conclusions reached by Sir Charles Snow. He emphasizes the importance of clear objectives and of an appropriate position within the structure of government, permitting of effective touch with working administrators and serving officers—the users—and also with Ministers and the highest Civil servants. He specifies, too, powers of action, but that is in relation to a committee responsible for dealing with a concrete problem: on such a body as the Advisory Council for Scientific Policy he has no specific comment, though he wants scientists to be active in all levels of government, and is as clear as Sir Frederick that the scientific mind has an essential contribution to make in the shaping of general policy.

---

* *Science and the Future of Britain*. Pp. ii+46. (London: The Labour Party, 1961.) 2*s*.

Sir Charles Snow may perhaps accept too readily the need for decisions, especially on technical matters, being made behind closed doors; but that only underlines the importance of the problem, to which Sir Henry Tizard directed attention in his Haldane Memorial Lecture, of ensuring that most of our future administrators, if not all, shall have had a broad scientific education. On one side, Sir Henry suggested, it may be recalled, forming a central scientific staff, engaged only on studying questions of policy, under the immediate direction of the Advisory Council, to which young scientists could be seconded for a year or two from the research establishments, to which they could be transferred back if they had not the necessary qualities for higher administration. On the other hand, Sir Henry lent the full weight of his experience and authority to any measures to ensure that future administrators had a broad scientific education. Sir Frederick has more recently confirmed these views and stressed not only the need for the administrator himself to possess sufficient scientific background to discriminate between the essential and non-essential elements in the scientific aspects of a problem but equally for many departments to include a scientific component as a permanent measure.

Sir Charles Snow's book was not overlooked in the Commons debate. Sir H. Legge-Bourke, stressing the point which Sir Charles Snow made about the contribution of foresight which scientists could make to our existential society, thought that this was one reason for increasing the number of those with scientific knowledge in the Civil Service who might not have any direct scientific responsibilities in most of their work. He was ready to support the argument for bringing in scientists at all levels of government, not to centralize the control of scientific policy, but rather to provide a bridge between the scientific and the political world and to lift our gaze to the horizons that lie ahead. It is essential, he believes, to ensure that at least one Minister has the time to view the whole field of national activity and to contrive ways of ensuring that Ministers, Civil servants, trade unionists, industrialists or others concerned are made aware of the need to consider the right scientific subject. For this reason he thought that the Minister should be the Minister for Science and not of Science, and he also urged that he should not disregard our national characteristics.

Sir Harry Legge-Bourke came nearest to the expression of the views of Snow as well as Brundrett and Tizard, but they also found some expression in Sir Hugh Linstead's speech. Fully supporting the idea of a Minister for Science, he thought the Minister's primary duty should be not to run science but to foster and encourage it independently—so far as may be possible—of the executive Government of the day. Sir Hugh saw the Minister's duty as being to define, on the best advice he could get, the problems on which the resources of the country should be concentrated, making certain that the fallow fields are cultivated, and that over-cultivated fields are allowed to lie fallow. He also believes that the best results would be obtained if the maximum use were made of instruments other than Government organizations, and although Sir Hugh referred to the use of project research, in which a Government Department financed research undertaken by a university on a particular project, he was obviously unconvinced as to the soundness of this technique. He stressed the importance of the Minister and his staff following up by personal contact rather than directive or Ministerial circular, and, having defined the framework and allocated the responsibilities, the Minister should be zealous to leave the scientists their freedom.

The Minister for Science has immense responsibilities; but, in urging that he would discharge those responsibilities best by the least possible use of the great power of his office and by the maximum possible use of its great influence, Sir Hugh took up a position much closer to that of Mr. Denzil Freeth in concluding the debate for the Government than to that of Mr. Peart in opening. The stress laid on the need for executive power on the part of the Minister and for a Scientific and Technical Planning Board, consisting largely of scientists and largely replacing the existing Advisory Council on Scientific Policy, which new Board would become the chief instrument for coordinating scientific activity, is the direct opposite of the present system and likely to lead to bureaucracy rather than freedom. As Mr. Freeth pointed out, with the present Advisory Council composed of scientists, industrialists and some official members, there is no political element in the advice given, or reports presented, to Parliament. Further, the Advisory Council makes scientific and not political judgments, considering the scientific needs of the nation as it sees them, and leaving Parliament to be the judge of policy. Further, the concept of research councils on which this system is based stems from the fact that scientific judgments can only be made by scientists, and the judgments on whether lines of research are worthy of public support are made by scientists familiar with the background of problems. The desirability of any project as part of the national, or international, picture of research is judged by leading scientists and expert laymen acting together.

Mr. Freeth, who himself also stressed the point that money is not necessarily the limiting factor in research, stoutly defended the scientists on the Agricultural Research Council from the charges of indolence levelled at them in *Science and the Future of Britain*. He pointed out also the value of alternative sources of finance for the research worker, and quoted Prof. Herbert Butterfield in refutation of the idea that Government and society should take command over the sciences. The future will not allow itself to be managed in this way. Government and society tend to expect the scientist to serve a utilitarian end, and we should be careful to continue to value science even more than technology. The traditional urbanity of the Government in Britain in respect of university autonomy is of greater long-term importance than is often realized, and Prof. Butterfield maintains that a world that gives science the greatest opportunity possible for autonomous development not only possesses freedom now but also has the promise of more flexible development in the future.

Prof. Butterfield, like Sir Hugh Linstead, was careful to reject the claim that the scientist—as scientist—should be allowed to govern. Science and technology in themselves are insufficient to teach the wisdom necessary for steering the ship of state or choosing educational objectives, or even indicating the proper use of scientific achievements. That much is not implied in Sir Charles Snow's emphasis on foresight: as Sir Hugh puts it, the scientist's place in the political scheme of things is rather as a referee than a player.

It would be unfair not to note that, in this highly constructive speech, Sir Hugh Linstead was also critical of the present balance of effort; but he lent no support to the view that because a certain amount was spent on the Agricultural Research Council or the Department of Scientific and Industrial Research, a certain amount must be spent on the Medical Research Council as well. He did not appear to dispute the Medical Research Council's own view that its present £5 million or so is inadequate, in that no worth-while scheme for medical research is rejected for lack of funds. Moreover, although this view has, in fact, been challenged by Prof. A. Haddow, Sir Howard Florey has emphasized the excessive time which may have to be spent in applying for funds. How far this adequacy of funds is true of biological science in general may be another matter, but Sir Hugh certainly did not accept Mr. Peart's choice of the Medical Research Council as an example of inadequate Government expenditure. He repeated that control of research and scientific effort in the universities and in the industry of Britain cannot be separated from control of the universities and of industry, and that undue extension of the planning and direction of university and industrial activities might dry up the very springs of original research and development we sought to foster.

As already noted, in opening the debate Mr. Peart had pleaded for the creation of a Scientific and Technical Planning Board, which he thought should be the main body to apply science in industry and to stimulate new activities, though he also urged greater use of the National Research Development Corporation, particularly in placing research or development contracts. An interesting speech by Mr. Aubrey Jones also advocated the adoption to some extent of the planning methods of the Soviet Union in science and technology, partly because of the present imperfection of communication in technology on the international plane, and partly because the most significant scientific and technological advances occur as a result of the marriage of scientific disciplines. Highly critical of the effectiveness of the research associations, he strongly advocated the use of civil development contracts, to be chosen deliberately and with strategy, so as to make industrial firms themselves more alert to research and development. He believes the old Ministry of Supply was a far better instrument for this purpose than the Department of Scientific and Industrial Research, and rejected outright the idea of extending the functions of the National Research Development Corporation to production. Instead, he would combine the Corporation with the industrial side of the Department of Scientific and Industrial Research, and make this the industrial and technological nucleus of the Ministry for Science.

Mr. Freeth made no comment on this specific suggestion in concluding for the Government; and he also ignored what was probably the most justifiable criticism advanced by Opposition speakers, namely, the Government's dilatoriness and parsimony in providing some of the conditions for effective scientific work—a responsibility which in fact all the post-war Governments share. Both Mr. S. Irving and Mr. A. M. Skeffington strongly urged a more generous policy in regard to scientific information services, referring to the needs of both the National Lending Library for Science and Technology and of the National Reference Library for Science and Information. Doubts as to the adequacy of the building and plans for the latter were freely expressed at a meeting arranged by the Association of Special Libraries and Information Bureaux on May 9, and the assurances then given by Sir Frank Francis, director of the British Museum, were manifestly unable to remove those fears. The need for more vision and greater generosity in finance and buildings was well put in the debate in the House of Commons by both Mr. Irving and Mr. Skeffington, and although on balance scientists and technologists may well think that the Government came out of the debate rather better than the Opposition on this specific issue, they should lend the strongest possible support to the efforts now being made to secure an adequate expansion of the proposals for the new Reference Library at least. The case for a scientific and technical planning board was perhaps not established, but the need for a continuing review of the efficiency of the whole administrative structure for scientific and technological activities was fully substantiated; this should include the supply of scientists and technologists, as well as technicians and craftsmen, the means of identifying areas calling for research and the fostering of those conditions which best encourage creative and effective work by the scientist. There is obviously still need for much constructive thought as to the relations between science and government, and the part the scientist, as such, should play, and the debate established a large measure of agreement among those who have thought deeply about the issues, independent of party political lines.

SPEECH, HANDEDNESS AND CEREBRAL DOMINANCE

Cerebral Dominance and Its Relation to Psychological Function

By Prof. O. L. Zangwill. (The Henderson Trust Lectures, No. 19.) Pp. vii+31. (Edinburgh and London: Oliver and Boyd, Ltd., 1960. Published for the William Ramsay Henderson Trust.) 10*s.* 6*d.* net.

PROF. ZANGWILL'S book embodies the William Ramsay Henderson Trust lectures, and it is pleasant to note, though he never alludes to the fact, that the object of the Trust is: "The advancement and diffusion of the science of phrenology". It could be argued indeed that the phrenologists are still with us, for there are still plenty of people ready to locate supposed psychological faculties in particular areas of the brain. Indeed, the idea of cerebral dominance and handedness implies some form of localization, if only in respect of the cerebral hemispheres, and Prof. Zangwill's object is to discover exactly what this means. The lectures are largely a summary of the work previously published by himself and his collaborators. As he frankly says, "these studies of speech and handedness unfortunately raise far more problems than they solve". Already, however, we must recognize that there is no clear-cut division into right-handed persons with a dominant left cerebral hemisphere and left-handers with a dominant right hemisphere. On the contrary, "in left-handed or predominantly left-handed patients, the dominant hemisphere as conventionally ascertained is more often the left than the right. Furthermore, left hemisphere dominance may even occur in sinistrals who have always written with the left hand". This has certain implications for the study of aphasia, for there is now evidence that recovery from aphasia tends to be more complete in patients who are left-handed or who have left-handed relatives than in those who are fully right-handed, perhaps because unilateral specialization of the language functions in the brain appears to vary between individuals, being as a rule far more pronounced in right-handed than in left-handed subjects.

Prof. Zangwill goes on to study the significance of these facts for the understanding of specific backwardness in reading and spelling. In 75 per cent of his cases there is evidence of some sinistral tendency in the individual or in his family. Mixed or inconsistent hand preference was common, and uncomplicated dextrality rare. He suggests, also, that so-called specific defects in reading and spelling are seldom present in isolation, and are commonly embedded in a more complex developmental syndrome.

For many years now evidence has been accumulating that even in clear-cut right-handedness there are important differences of function between the right and left cerebral hemispheres, and that the left hemisphere, which is dominant in such people, is not merely a mirror image of the right with the addition of functions concerned with symbolic formulation and expression. Some independent evidence of this has recently been provided by studies of sensory disturbances after penetrating brain-wounds in man (*Somato-sensory Changes after Penetrating Brain Wounds in Man*, by J. Semmes, S. Weinstein, L. Ghent and H. L. Teuber, Oxford University Press). It has been found that sensation of the left hand is more diffusely represented in the right cerebral hemisphere than is that of the right hand in the left. Similar observations have been made in many fields of discriminative activity. As yet these remain isolated observations, and we have not reached the stage at which any broad generalization can be inferred from them. Prof. Zangwill's book is a useful summary of the present state of our knowledge of the relationship between speech, handedness and cerebral dominance.

RUSSELL BRAIN

BACKGROUND TO ASTRONOMY

The Fabric of the Heavens

By Stephen Toulmin and June Goodfield. (Nuffield Foundation Unit for the History of Ideas. The Ancestry of Science, Vol. 1). Pp. 272+14 plates. (London: Hutchinson and Co. (Publishers), Ltd., 1961.) 25*s.* net.

HISTORY of science is at present expanding at university-level so rapidly that lectureships are sometimes left vacant for want of suitably trained lecturers to fill them. At school-level the subject is also expanding, but here another limiting factor is the shortage of suitable books.

The present work is that of a series which will do much towards alleviating the shortage. It is a survey of the interlinked history of astronomy and mechanics from earliest times to Newton, with a further outline bringing the story to the present day. It is to be followed in the next few months by no less than three more volumes, each dealing with a major field of history and philosophy of science. One might feel that a lifetime of teaching and research would alone qualify an author for attempting any one of these volumes, but there is a special urgency in history of science and a worthwhile place for general books which give some inkling of the excitement of the subject.

This *The Fabric of the Heavens* certainly does. It is written with fluency and conviction, and the reader never loses interest. There are, however, many signs of the haste with which it was written—along with the relevant illustrations we find a photograph of the port of Pythagoreon and snapshots of a Greek temple taken from different angles—as well as a number of confusions and serious errors of fact. Thus the authors ascribe the discovery of the planet Uranus in 1781 to John Herschel (born 1792) and quote William Herschel (died 1822) as congratulating Bessel on his discovery of stellar parallax in 1837; yet a philosopher of science like Dr. Toulmin must know John Herschel's 1830 *Preliminary Discourse on the Study of Natural Philosophy*, and in writing their promised *The Flux of Nature* the authors must surely be studying the cosmogony of William Herschel. Samuel Clarke, who in fact believed gravity to be a "connate and original law of all matter . . . We ought no more to enquire how bodies gravitate than how the world began to move" is described as protesting "that some push-and-bang mechanism must eventually be discovered for gravity". Galileo, we are told, "did not study freely falling bodies directly". Copernicus's elimination of the equant is discussed in the context of his general cosmology, although the question does not properly arise until we come to his detailed mathematical astronomy; here the role of neo-Platonism in Copernicus's thought is hopelessly underrated, and although highly disparate issues are involved the authors consider "whether its gains outweigh its losses".

These are but a few of the shortcomings of the book, to which must be added the lack of balance which emphasizes the prehistory of the subject at the expense of its more recent history. There is much that is competently and excitingly written; it is most regrettable that these qualities are not combined with the scholarship that has at last become usual in books on the history of science.

M. A. HOSKIN

CHEMICAL GENETICS

An Outline of Chemical Genetics

By Prof. Bernard S. Strauss. Pp. ix + 188. (Philadelphia and London: W. B. Saunders Company, 1960.) 35*s*.

THIS book grew out of a series of lectures delivered at the Faculty of Medicine in Osaka, Japan. It is meant as a brief account for scientists "who have heard snatches of the recent developments in genetics and find them fascinating". Its six chapters deal with: the genetic control of protein synthesis; the chemical nature of the hereditary material; the molecular meaning of genetic recombination; mutation as a chemical process; nucleo-cytoplasmic relationships; the biochemical genetics of man. A book of this kind need not be profound or original, and this book is neither. On the other hand, it should be clear and readable. On the whole, this book is both. It is, indeed, quite unusually readable and conveys to the reader something of the pleasure the original audience must have felt in hearing an interesting subject treated with ease and enthusiasm. Especially, the first two chapters are excellent reviews of the more purely chemical aspects of modern genetical theory.

In the treatment of strictly genetical questions clarity is less apparent. Dr. Strauss does nothing to destroy the superstition that formal genetics is an esoteric subject, difficult of approach even for scientifically trained minds. A few introductory pages of precise definitions and statements should have been sufficient to lay the genetical ground for the remainder of the book. Instead of this, snippets of genetic information are scattered all through the text, with a singular choice of the complex rather than the simple. Mendel's laws are nowhere explicitly stated, but several pages are devoted to the analysis of ordered tetrads in *Neurospora*.

As stressed in the introduction, such a short account of a vast subject must necessarily be selective; equally necessarily, selection will be subjective. The danger that this may slant the overall picture is not always avoided. This is particularly apparent—at least to me—in the chapters on nucleo-cytoplasmic relationships (where research on *Paramecium* is wholly disregarded), and on mutation. The latter deals almost exclusively with work on micro-organisms; yet even for these it is not correct to say that "selection methods are almost always part of any procedure for the detection of mutations". Formaldehyde, one of the most potent mutagens for *Drosophila*, is not mentioned. The ineffectiveness of purine-9-riboside in some experiments with bacteriophage is stressed; its high efficiency in the production of what appear to be point mutations in barley is not referred to. It is also scarcely fair to imply that only our new concept of the nature of deoxyribonucleic acid has resulted in the realization that "mutation is a chemical process since it presents a definite structural change within a molecule".

Critical appraisal, too, is more apparent in the chemical sections than in the genetical ones, where tentative conclusions—such as lack of mutagen specificity for forward mutations—are too readily presented as established facts. One gets the impression that the author is more at home in chemistry than in genetics and behaves like a well-bred foreigner who reserves criticism for the institutions of his native land. However, to be equally at home in both fields is a rare distinction, and, in spite of its shortcomings on the genetical side, this book can be recommended as an attractive and stimulating introduction to a subject that depends for development on close mutual understanding and co-operation between scientists of many branches.

CHARLOTTE AUERBACH

SOVIET POLAR RESEARCH

Problems of the North: No. 1, 1958

(Translation of "Problemy Severa"). Translated December 1960. Pp. iii + 376. (Translations Section, The Library, National Research Council, Ottawa, 1960.) Subscription price per issue, 7 dollars; Single papers, 1 dollar.

THE availability of Russian scientific literature in English translation has improved greatly in recent years. Scientists in some disciplines can now obtain cover-to-cover translations of the leading Soviet journals on their subject. The position is not as good as this in all fields, of course. One in which Soviet work is pre-eminent is that of polar studies, but here the research worker with no knowledge of Russian has had little help. There is thus good reason to welcome from Canada, the country which has fittingly been most active in preparing *ad hoc* translations of this material, the first English language version of a Soviet polar journal. *Problemy Severa*, a product of the Council for the Study of Productive Forces of the Academy of Sciences of the U.S.S.R., first appeared in 1958, and *Problems of the North* was published by the National Research Council in Ottawa in 1960.

The Soviet journal, of which three issues have appeared up to now, has the widest range of its polar contemporaries in the U.S.S.R.: wider, even, than its title implies, because it does not exclude the south. In the first issue, papers are grouped under the headings oceanology, geography and geology, geophysics, biology, economics, the north abroad, the Antarctic, and miscellaneous reports. The contributions are claimed to be the results of original research, and this is true of most of them (although, following what seems to be common Soviet practice, such results are often published several times over in different journals); but there are also a number of review articles. Certainly the papers in this volume hold much of interest to Western specialists: in particular, those on the movement of floating ice in the Arctic basin, the influence of sea-water temperatures in this connexion, polar wandering, the thawing process in frozen soil, the vegetation of northern Siberia at the time of the mammoth, wild life conservation, and Soviet economic organs functioning in the north. The two subsequent issues have continued to cover as wide a field. No. 4 is not yet out, although long expected, and the delay may be attributable to the transfer of the producing agency from the Academy to the State Economic Council.

The English version has been done well, and for an undertaking of this sort the editors probably deserve as much credit as the translators. The jargon of half a dozen disciplines has been comprehensibly rendered, and although much of the wordiness of the original remains, this is inevitable. The maps are well reproduced, and the half-tone illustrations are as good as the poor originals will permit. One criticism of editorial policy must be made, however. In a work so strongly geographical in tone, it is a pity that neither the transliteration nor the form of place-names is that used at the moment by government map-makers in the United Kingdom and the United States. The user who tries to locate a place in any official gazetteer or map may thus be in difficulties.

Terence Armstrong

WEEDS IN BRITAIN

Weeds and Aliens

By Sir Edward Salisbury. (The New Naturalist: a Survey of British Natural History.) Pp. 384+16 plates. (London: William Collins, Sons and Co., Ltd., 1961.) 30*s*. net.

IN this book, the latest in the New Naturalist series, Sir Edward Salisbury discusses, in sixteen chapters, the weed flora of Britain. Not only does he interpret the nature of weeds, and describe diminishing and spreading species and their association with differing ecological conditions, but he also considers characteristics of individual species, reasons for their survival as weeds and, briefly, their control by herbicides.

The author demonstrates clearly his unique knowledge of weeds in general and British weeds in particular. On many occasions, for example, in the genus *Chenopodium*, the reader is made aware of the several closely allied species forming a related group of weeds, clear line-drawings helping to pinpoint diagnostic morphological differences. Data on the reproductive capacity of many species show unmistakably the hallmark of Salisbury.

The case for detailed studies of the life-history, ecology and physiology of weeds, both for academic interest and for their control, is argued persuasively. Especially pleasing is the emphasis placed on the need for investigations on factors affecting establishment of weeds, a field which, until recently, has had less than its share of attention.

To substantiate his arguments, the author makes liberal use of interesting speculations, and logical deductions, not to mention, when available, up-to-date quantitative data. Indeed, the reader cannot fail to be stimulated by Salisbury's dynamic approach to the study of weeds, but he is left with the knowledge that much research is necessary before the adaptation of weed species to characteristic situations can be explained fully.

A disappointing feature of the book are the sections dealing with the association of weeds and specific ecological conditions. Many species are discussed in these chapters; they are briefly described but there are few diagrams to help in their identification. Several, often isolated, facts are presented in the consideration of species; in some, but by no means all, cases there are suggested control measures. These latter do not fall into any systematic pattern. Indeed, chemical control measures seem to be demonstrated empirically with few principles being expounded; for example, the author states that *Poa annua* is resistant to both 2-methyl-4-chlorophenoxyacetic acid and dichlorophenoxyacetic acid, but so are all Gramineae, and the chapter on chemical control, as presented, is of doubtful value.

There are a few surprising statements. For example, Salisbury considers *Allium vineale* more a grassland than an arable weed of heavy land, while bare fallowing is scarcely its most effective control measure. Exception can be taken to the use of common plant names. Some, for example tinker-tailor grass, are not only unaccompanied by specific names but are far from common; others, for example hungerweed and mousetail grass, are much less widely used to describe *Alopecurus myosuroides* than the unquoted synonyms blackgrass and slender foxtail.

In spite of these criticisms the book, containing some 150 references covering many subjects, will be as thought-provoking to the research worker as it is stimulating to the naturalist.

Alec Lazenby

PALÆOGEOLOGICAL MAPS

Palæogeologic Maps

By A. I. Levorsen. (A Series of Geology Texts.) Pp. viii+174. (San Francisco and London: W. H. Freeman and Company, 1960.) 43*s*.

THIS stimulating book deals not only with palæogeological maps in the strict sense but also with palæostructural, palinspastic and 'worm's eye' maps. These maps are quite easy to construct if the stratigraphical data are available; consequently the introductory chapters are short and the greater part of the book is devoted to their interpretation. To demonstrate the kind of information that can be extracted from these maps, about one hundred have been selected of areas ranging in size from a few square miles to whole continents. The maps have been well chosen and are clearly reproduced, the accompanying explanations are lucid and the deductions logical. Among the numerous uses that the author lists for these maps, the most important seem to be to distinguish between sedimentary and structural basins, to separate the structural effects of superimposed fold movements, to locate buried outcrops and to indicate the sources of ancient sediments. In one section the principles are applied to the problem of continental drift and clearly point against the conventional reconstruction of Gondwanaland.

It is disappointing to the British reader that by far the greater number of examples are chosen from North America. This is understandable in an American book, and in any event the technique is more suited to areas that have not been strongly folded. Nevertheless, of several good palæogeological maps published of British areas, the author selects only Buckman's map of the Cotteswolds, probably the first of all such maps. It is, evident, however that much more could be done in Britain along the lines developed in the book—perhaps with surprising results.

Study of the maps presented should provide excellent exercise for students at all stages and they are fortunately arranged in a graduated series. Their full appreciation requires an understanding of the four-dimensional scope of geology.

C. Downie

Commonwealth Universities Yearbook 1961

A Directory of the Universities of the British Commonwealth and the Handbook of their Association. Pp. xxxi+1590. (London: Association of Universities of the British Commonwealth, 1961.) 84*s*.; 13 dollars.

THIS edition of the *Commonwealth Universities Yearbook* is the thirty-eighth since it was first published in 1914. The Association of Universities of the British Commonwealth, which is responsible for the compilation of this directory, feels that "it should not only cover the principal features of each university but should also give accurate and up-to-date lists of members of the university teaching profession in each country of the Commonwealth". In 1,590 pages, including an index of more than 40,000 names, it does that and more, since it includes the universities in the Republic of Ireland, which are not actually in legal membership of the Association.

The continued expansion of systems for higher education has necessitated an 80-page increase in the length of this book as compared with its predecessor, and three new University Colleges (Fourah Bay College, the University College of Sierra Leone; the University of Nigeria at Nsukka; and the University College of Sussex) have been introduced this year.

In the preface, the editors state that due to a great deal of staff movement among local universities, and from one country of the Commonwealth to another, it is only by constant and complete revision that these lists can be made to serve the purpose of identifying particular teachers and research workers. However, the introduction in this issue of some notes on academic salaries, giving salary scales for different grades of staff at the various universities, may only serve to make the editors' task even more onerous in the future.

Journal of Helminthology

Supplement in Honour of R. T. Leiper on the occasion of his 80th Birthday, 1961. Pp. xxii+229. (London: London School of Hygiene and Tropical Medicine, 1961.) 30*s*.

THE eightieth birthday of Prof. R. T. Leiper, emeritus professor of helminthology in the University of London, has been celebrated by the issue by the *Journal of Helminthology* of this special supplement. The knowledge that Prof. Leiper is still actively at work will bring pleasure not only to the many biologists and medical men who have worked with him or have otherwise benefited from his kindly interest and help but also to many others who know or have admired his outstanding contributions to biology and medicine. These are discussed in this volume by Sir Philip Manson-Bahr, the late Sir Ian Clunies-Ross, Prof. B. G. Peters and Prof. T. W. M. Cameron. These contributions are so well known to parasitologists all over the world that they need no further comment here. Prof. J. J. C. Buckley and Dr. L. M. Russell write of Prof. Leiper's other activities, epitomized by his membership, or honorary membership, of many scientific societies and Government bodies, and of his long association with the London School of Hygiene and Tropical Medicine and the part he played in its development. A feature of this volume which will interest many is the record, covering eight pages, of the signatures of many of Prof. Leiper's pupils and associates. It will, one hopes, be a major source of satisfaction to Prof. Leiper that he has, as this record shows, taken, in one way or another, so many distinguished scientists under his wing; and the student of what is called graphology will no doubt find in these eight pages much to interest him and perhaps to refute the confident pronouncements in those engaging books which profess to deduce our characters from our battles with such pens as modern industry provides.

The rest of this interesting volume is occupied by 29 original papers contributed by leading parasitologists of many countries, so that the volume is, in addition to being a tribute to Prof. Leiper, an important issue of the *Journal of Helminthology*.

Prof. Leiper's appointment, on his eightieth birthday, as active consultant helminthologist to the Commonwealth Bureau of Helminthology, which he directed so efficiently for thirty years until his retirement in 1958, will ensure, all parasitologists will hope, that the highly experienced staff of this organization will be able to continue the invaluable work on which parasitology all over the world has for so long relied. No other Commonwealth organization could do this essential work so well. G. Lapage

Stochastic Population Models in Ecology and Epidemiology

By Prof. M. S. Bartlett. (Methuen's Monographs on Applied Probability and Statistics.) Pp. x+90. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1960.) 12*s*. 6*d*. net.

THIS small book by Prof. Bartlett is the fourth in the series of Methuen Monographs on Applied Probability and Statistics (edited by Prof. Bartlett), the series as a whole being intended to provide inexpensive introductions to the rapidly expanding field of applied probability and statistics. The book concerns itself with probabilistic models as they may be encountered in ecology and epidemiology, and does not attempt to deal with the extensive applications of probability in genetics. A sound basic knowledge of probability is certainly a necessity to appreciate the ideas discussed.

There is an introductory chapter dealing with the role of statistical theory in biology and the relevance and prognostic value of stochastic models. The next chapter deals with some of the well-known statistical distributions (Poisson, negative binomial, logarithmic) which are set up through probabilistic continuity arguments, and introduces basic ideas to encompass spatial distributions. The middle chapters are concerned with deterministic and stochastic models for the period to extinction (or equilibrium state) in populations subject to 'birth-and-death' processes and in which there may be competition, consideration being given to continuous and discrete mechanisms. The closing chapters give an account of models for recurrent epidemics, including the case where the spatial aspect is important and relevant. The basic stochastic models are in general complicated; nevertheless some assessments of the average association between weekly notifications and time to extinction are given in the case of measles epidemics.

The printing is good, the format is attractive and the price is reasonable.

A good deal of the monograph impinges on research which has been carried out in the past few years, a large share of this being due to Bartlett. It should be of considerable interest to ecologists and biologists, and there can be little doubt that every serious student of statistics should possess a copy.

METABOLIC STABILITY OF BODY CONSTITUENTS

By DR. A. N. DAVISON
Department of Biochemistry, Guy's Hospital Medical School, London, S.E.1
AND
DR. J. DOBBING
Department of Physiology, London Hospital Medical College, London, E.1

THIS article is concerned with an appraisal of some modern views on the collective metabolism of body constituents *in vivo*. Many of these ideas have already been expressed by other authors[1–4], particularly in the field of protein metabolism, and will be to that extent familiar. However, acceptance of the concept of metabolic heterogeneity has not always been accompanied by an appreciation of the influence of such ideas on the design of experiments. For this reason it was decided to state a simple working hypothesis, embracing the metabolism of body constituents as a whole, and to stress its influence on both the design and interpretation of metabolic experiments *in vivo*.

The widespread use of isotopes in biological research, which began rather more than twenty years ago, led to the demonstration of a number of important phenomena which had hitherto remained undetected by classical methods. Among these, perhaps the most striking was the discovery of the dynamic state of body constituents, prominently expounded by Schoenheimer[5]. Until this time, the metabolism of body constituents was generally thought to be divisible into two groups in the sense (for example) of the hypothesis of Folin[6] for the metabolism of proteins. Body constituents were either to be considered as structural, and therefore stable, or metabolic fuel, and therefore labile. Added to this was the concept of 'wear and tear' on structure accounting for a relatively small loss from this source and an equally small replacement. The storage of some energy sources in depots was also appreciated.

With the use of isotopes came the demonstration that even the constituents of some anatomical structures appeared to be in an extraordinarily rapid and free dynamic state. Constant rapid synthesis and degradation was postulated as the means by which body structure was held in its apparently static state, and even in such conditions as starvation, when the relative failure of the synthetic side of the equilibrium resulted in net loss of body substance, there was still demonstrable synthesis taking place.

These new revelations led to a general rejection of the older views in favour of the more acceptable hypothesis that the apparent heterogeneity of behaviour of body tissues concealed a hitherto unsuspected activity in all of them. In metabolic terms, this meant both structural constituents and 'fuel' were undergoing dynamic metabolism. The 'fuels' were as labile as had always been thought, but there was also a similar lability of the components of anatomical structure. Research workers began to base their thinking and the design of their experiments on the assumption of the dynamic state of all body constituents. In the field of experimental pathology it was widely suspected that the degeneration of structure in such degenerative conditions as, for example, disseminated sclerosis, might be the result of an imbalance of the equilibrium. Such assumptions have profoundly affected the direction taken by many experimentalists in their investigations.

However, even from the earliest times in the history of metabolic work using isotopes, there have been results which have challenged the simplicity of the new idea. Almost from the outset it was realized that although bone calcium and phosphorus were to a large extent in equilibrium with the body fluids, there was nevertheless a proportion of bone constituents which was apparently metabolically inert[7]. This inertness did not depend on the nature of the substance, since both calcium and phosphorus were at the same time labile and stable in the same tissue. It was therefore assumed that the observed degree of stability represented those constituents which were buried deep in the apatite crystals and were, for these mechanical reasons, inaccessible to the blood stream. In other tissues, notably in the case of muscle-protein, collagen and later of deoxyribonucleic acid in some situations, it gradually became clear that the lability of body constituents was not uniform and that some of them 'turned over' much more slowly than others. The important results of Thompson and Ballou[3], appearing under the title of "The Predominantly Non-Dynamic State of Body Constituents", summarized a growing impression that there was a considerable range of rates of metabolic activity throughout the body. It was even suggested that in the unique case of collagen, the constituent proteins were turning over so slowly that their biological half-life exceeded the life-span of the animal.

As a result of these observations it became clear not only that the metabolic activity of body constituents was heterogeneous, but also that the different organs were themselves heterogeneous in the relative proportions of highly active and less highly active components they contained. The liver was the best example of a tissue which was predominantly highly labile; tendon was at the opposite end of the scale, consisting, it was suggested, predominantly of materials which turn over very slowly; and all other tissues and organs could be arranged in order between these two according to the proportion of their constituents which were stable.

The very important question now arises whether there are body constituents which can be demonstrated to be absolutely inert and have no metabolic activity whatever. This state of complete inertness could possibly be suspected in collagen, at least in some parts of the body, both from the experimental evidence already available and from the very nature of the tissue and its relatively poor blood supply. However, the evidence for absolute inertness is in fact just as good for deoxyribonucleic acid even in such relatively active tissues as liver[8,9], and this is altogether more unexpected on the basis of the older hypotheses.

It is also important that the degree of metabolic stability should be considered in relation to the duration and conditions of any particular experiment. Thus, in a 4-hr. experiment, a given constituent or some proportion of it with a biological half-life of months is relatively stable; whereas in an experiment extending over a year the same constituent may be regarded as metabolically active. While absolute metabolic inertness is admittedly extremely difficult to demonstrate, such stability would have an extremely important influence on the design and interpretation of metabolic experiments. This article is therefore concerned with stating a working hypothesis which assumes an infinite heterogeneity in the metabolic activities of body constituents, amounting to complete and absolute inactivity of some of them; and in the application of this hypothesis to some recent observations on metabolic behaviour *in vivo*. It will be convenient to enunciate its main features and some of its implications in general terms before proceeding to discuss some of the evidence in its favour.

Hypothesis of Infinite Metabolic Heterogeneity

The basic principles of the hypothesis are as follows:

(1) The metabolic behaviour of all tissue constituents can be arbitrarily divided into two main types, present in varying proportion. These are the non-structural components involved in metabolic processes on one hand, and structural components on the other.

(2) The first group, henceforth referred to as the metabolic constituents, are heterogeneous with respect to their metabolic activities. There is a very wide range of turn-over rates to be found among them. Some of the shorter half-lives may be even shorter than can be measured by contemporary techniques; and some of the less-active members of the group are turning over so slowly that, for all practical purposes relative to the life-span of the animal, they may merge imperceptibly with the members of the second, absolutely stable group.

(3) The factors that decide to which group any given substance belongs in any particular tissue are only in a very limited sense dependent on the chemical identity and properties of that substance. Indeed, the same substance can belong, in varying proportion, to both groups in the same tissue.

(4) The relative proportions in which a single chemical substance is distributed between the two main compartments in any tissue will be decided by such factors as the nature and function of the tissue, its state of maturity, and its metabolic state which may vary considerably from time to time even in the adult animal. The chemical properties of a substance may often play a subordinate part in influencing this differential distribution.

If this hypothesis be accepted, a number of important consequences emerge, and these will now be discussed.

Consequences of the Hypothesis

The structural constituents in these terms will never exhibit any metabolic activity. They will be built up in incremental fashion into anatomical structure during the process of growth, and, once incorporated, will remain in that structure as long as it survives. This aspect of complete metabolic stability must be considered on a sub-cellular plane at first, but in those tissues such as the central nervous system, consisting of cells which are not normally renewed during adult life, there is likely to appear some complete stability at the higher tissue-level. Even the most labile epithelial cell which is constantly being lost and renewed contains structural components which are absolutely stable within the lifetime of that cell. The impression of turn-over of structural components of such labile whole tissues will be spurious and will simply represent the rate of loss and regeneration of cells which in the above sense contain stable structure.

Structural stability would, according to this hypothesis, be absolute at all times, even during the course of incremental growth. In those cells and tissues which develop over an extended period of time each molecule, as it is laid down in structure, would be permanent and stable, its persistence there only being restricted by the survival-time of the structure concerned. The principal effect of growth will be on the relative sizes of the metabolic and structural compartments, and it may even be incorrect to consider growing tissues as exhibiting greater turn-over (see below).

Structural metabolic stability will be determined only in a limited sense by the intracellular or extracellular location of the anatomical unit concerned. While it may be true that extracellular matrices such as collagen, inorganic and organic matrix of bone and myelin sheaths are likely to be metabolically stable because of their extracellular position, and while intracellular, cytoplasmic constituents may be largely members of the metabolically active functional compartment, nevertheless important exceptions make it necessary to avoid such misleading concepts as are implied in the expressions 'extracellular structure' and 'intracellular fuel'. The apparent stability of deoxyribonucleic acid and the obvious lability of many constituents of body fluids are the most notable exceptions.

Growing Tissues

One important application of this hypothesis concerns the widely held belief that the turn-over of body constituents is faster in developing animals than in the adult. It is well known that many body constituents appear to turn over to a greater extent in young developing tissues than in the adult when turn-over times are calculated from rates of uptake of precursor. However, it is possible that this apparently greater turn-over may only reflect the influence of incremental growth on the total uptake of a substance, a factor which may play no part in the total uptake into mature, fully developed tissues. In the case of such substances as phosphate, for example, which play a prominent part in both labile metabolism and permanent structure, it is clear that some of the high total uptake into some developing tissues may be ascribed to incremental growth of a structural compartment which, as it is laid down, may thereafter remain inert. This is not to deny the possibility of increased turn-over in the metabolically labile compartments of young animals; but any claim that young tissues have a more active metabolism than old tissues must take the influence of this increasing size of the structural compartment into account.

The theoretical criteria which must be satisfied before turn-over times may be calculated from rates of uptake of precursor into product have been appreciated for many years[10-12], but this has not prevented their unfortunate and imprecise application to highly complex biological systems, in which such rigid criteria can rarely be satisfied. If the entry of a metabolite into a developing tissue be considered partly in terms of entry into inert structure and partly into a labile metabolic compartment, it is clear that no valid estimation of turn-over rates will be possible by extrapolation of rates of total uptake. The assumption by some workers that, following a single dose, the rate of loss from a tissue will be a mirror image of the rate of entry, is often unwarranted, since it implies metabolic homogeneity of that substance in the tissue. Furthermore, any results in terms of specific activity are inevitably misleading. This is due to the fact that the specific activity expresses radioactivity per unit weight, including metabolically inert as well as labile substance in the denominator of the expression. The specific activity is therefore reduced or 'diluted' by any quantity of metabolically inert material present. The specific activity will be diluted to a varying extent, changing especially during development, and this will greatly complicate comparisons of old and young. The same artefact of expression will also have the effect of depressing the apparent rate of entry into tissues, and the greater the size of the inert compartment (as in the adult), the greater will be the apparent depression of these rates of entry. This point also has important general implications for work on blood-tissue relationships such as the 'blood-brain barrier'[13], in tissues where the proportion of inert constituents is high.

Before leaving the topic of the complicated metabolic differences between old and young tissues, it must be stressed that there are circumstances in many adult tissues which are analogous to those in the developing animal. Cells were originally classified by Bizzozero on the basis of the degree to which they have preserved the power to divide by mitosis into post-natal life. 'Labile' cells, which normally continue to multiply, and 'stable' cells which retain the capacity for mitotic division are to be distinguished from 'permanent' cells which lose this ability at about the time of birth. Tissues containing cells of the first two types are likely to show an apparent metabolic activity on the part of their structural components which may simply be due to the loss of whole cells and their replacement by regeneration. Inasmuch as regeneration is taking place in a tissue, to this extent there will be an apparent turnover of structural components. The structural constituents of the cells concerned will, on the basis of the present hypothesis, be metabolically inert, the inertness being limited only by the life span of the unit to which they belong. A special example of this is to be found in the proteins of the evanescent red cell[14,15]. There is, in this way, a metabolic analogy between regenerating tissue, normal or otherwise, neoplastic tissue and the normal developing animal. The truth of the analogy is often obscured by a failure to appreciate its basis in the varying life-span of morphological structure.

Implications for the Design of Metabolic Experiments

If the above hypothesis and its theoretical implications be accepted, it must follow that metabolically inert morphological structure can only be labelled during the course of its growth and development, either in the developing animal, or, in the case of regenerating tissues, during regeneration. In the case of the central nervous system, for example, the structural compartment presumably consists mainly of the myelin sheaths, and these must therefore be labelled in the course of myelination or not at all. The period of development during which inert structure can be labelled naturally depends on the tissue and on the species.

Incorporation of calcium, which is to become part of the inaccessible calcium compartment of adult bone, will occur only during the development of that compartment. Moreover, in the adult rat, the epiphyses of which never fuse, and the skeleton of which is therefore always 'developing', a different general picture will be seen from that in the human and other species which normally achieve a state of maturity in which there is little further incremental growth. The process of remodelling of adult bone blurs the distinction, and a new situation analogous to early development arises during repair. It is not known to what extent normally inert calcium leaves the skeleton under the many disturbed metabolic conditions of decalcification because, at present, experimental methods have not successfully distinguished in this case between inert and labile compartments.

Myelination of the rat central nervous system begins about ten days after birth, and is virtually over when the animal is seven weeks old; thus it is only during this period that labelled substances can be incorporated into the lipids and protein which make up the sheath. The baby guinea pig is virtually fully myelinated at birth, and similar incorporation in this species could only be accomplished in the fœtus. However, the situation is greatly complicated by the fact that constituents of the myelin sheath are not unique to that structure, and none is known which does not to some extent also take part in the metabolic activities of the brain. Therefore, although myelin may be labelled during myelination, every label will to some extent also enter the metabolic compartment; and until sufficient time has elapsed to allow the dynamic turnover to dispose of this labile portion, the extent to which label is incorporated into inert structure will be obscured. The length of time required to lose cholesterol labelled with carbon-14 and phospholipids labelled with phosphorus-32 from the metabolic compartment of the brain following single-dose administration during early life is at least five months after injection, and it is not until this labile material has disappeared from the total radioactivity that the metabolic inertness of the labelled structure is revealed.

Since experiments are usually performed on adult tissues, and for much shorter periods than this, it is not surprising that direct labelling of inert structure has only recently been demonstrated. Metabolic inertness can, however, be inferred or suspected in much shorter experiments from the failure to label such structures as these in adult tissues. Since nearly all the cholesterol in adult brain is in metabolically inert structure, it has been suspected for a long time that the failure of labelled cholesterol (or of labelled precursors) to enter adult brain might be due to the metabolic stability of this lipid in this organ. However, widespread misconceptions over the nature of the 'blood-brain barrier' were more readily available as an explanation, and it required the direct demonstration of inertness by means of the long-term experimental design mentioned above to provide the first direct evidence. Recently, it has been demonstrated that cholesterol labelled with carbon-14, given either as the intact lipid[18] or as a suitable precursor[19], can indeed be incorporated in small quantities into the central nervous system. This labelled cholesterol undergoes very slow turnover and on the basis of the present hypothesis may be taken to represent a small pool of metabolically active cholesterol. Myelin sheath cholesterol is, of course, only one example of a structural constituent of a tissue the inertness of which has been demonstrated by an experimental design of this kind. Other examples include the bone minerals[7,20], deoxyribonucleic acid in non-dividing cells[8,9], the glycine incorporated into collagen[21] and some of the structural proteins in muscle[22].

Single-dose Experiments

Experiments involving the single-dose administration of a precursor of a tissue constituent during the course of that tissue's development produce the kind of result illustrated in Fig. 1. The values of the ordinate are better expressed in terms of the anatomical unit when growing animals are being used, a device which avoids complications of the single-dose experiment due to incremental growth progressively diluting the labelled constituent. The fate of injected precursor can be considered in two phases. During the first (phase *A*), the gradual incorporation into a structural compartment is added to the amount taken up more rapidly into the metabolic compartment. The latter, however, has a finite biological half-life, and towards the end of phase *A* most of it has been lost. It is not until this loss is virtually complete that the structural, metabolically stable material is revealed (phase *B*). In most cases, phase *A* lasts several months. The two phases must also overlap considerably for a very long time, during which interpretation will be exceedingly difficult. A semilog plot helps to distinguish some of the grossly different components of the curve and could in some circumstances also lead to an estimate of compartment size[3].

It can also be seen that a mean turnover-rate in a heterogeneous system calculated only from rates of uptake is likely to be meaningless and often misleading. Metabolism can then only be properly investigated by prolonging the experiment to describe directly the rate of loss as well as the rate of incorporation. There is virtually always a serious restriction, therefore, on the value of *in vitro* experiments for these purposes. Even when the 'surviving' tissues are from adults, and particularly when they are from young animals, few remain viable long enough to follow the loss of incorporated label which is likely to be much more prolonged than the period necessary for uptake. An exception to this general limitation on the value of *in vitro* experiments might be found in tissue culture, the employment of which for these purposes has scarcely begun.

A further complication of *in vivo* metabolic experiments is the varying relationship between the changing blood-levels of labelled precursor and the amount of isotope in the tissue. Thus phosphorus-32 persists in the blood for at least two months after injection into neonatal rats, and considerably longer in adults[17]. Similarly cholesterol labelled with carbon-14 remains in the blood for a month after injection into day-old chicks[23]. This effect corresponds to a continuous perfusion of precursor, and in this way label may enter compartments of low metabolic activity. This could account for the slow and very small incorporation of [4-^{14}C]-cholesterol into the adult central nervous system and for the very slow loss of labelled sterol thereafter (Fig. 2).

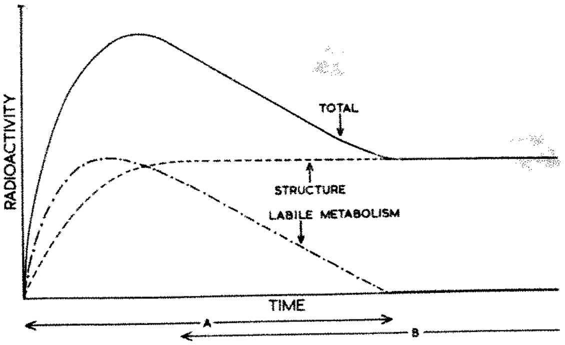


Fig. 1

Multiple-dose Techniques

The usefulness of single-dose experiments *in vivo* is, however, severely restricted in other ways which have been appreciated almost since the first use of isotopes in biology. The level of label at any time after injection is the resultant of so many metabolic processes, each of which has such a variable influence at different times, that meaningful interpretation is often impossible. One of the most variable factors is the size of the precursor pool being presented to the tissues in the early stages, since it usually drops precipitously soon after administration. The fall is due to equilibration with extracellular space, excretion and the preferential uptake which sometimes occurs rapidly into certain special tissues. Various techniques have been devised to overcome the difficulties of single-dose experiments by achieving a constant level of labelled precursor. This may be artificially accomplished by mathematical correction of the level of label in the product to the level in precursor at the same point in time, the ratios of product to precursor often being referred to as relative specific activities. Alternatively an attempt may be made to keep the precursor constant by repeated injection for a prolonged period until all active constituents have become equilibrated with it. Administration is then stopped, and the rate of loss of label studied. The mathematical correction to relative specific activities may be very misleading when slow synthetic and degradative processes are being studied in an animal in which precursor values are changing rapidly, and this is usually particularly the case in the period immediately after injection[24]. Another difficulty is that the relationship can only be calculated if the immediate precursor is known[10,12]. Rates of change of relative specific activities become even more difficult to interpret when the product in the numerator is metabolically heterogeneous in its relationships

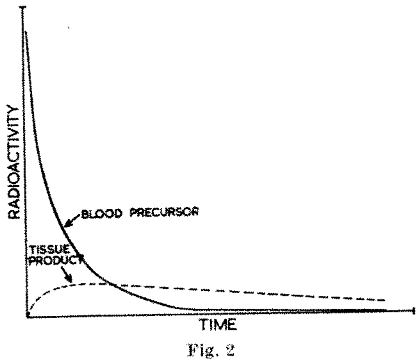


Fig. 2

with its precursor: that is to say, when there is a heterogeneity of turnover-rates of a substance in the different metabolic compartments of the same tissue.

A much more useful technique for investigating heterogeneous metabolism *in vivo* is that employed by Thompson and Ballou[3]. They exposed rats to tritiated water from the time of conception, by repeated administration to the mothers before and throughout pregnancy and lactation. Even after weaning, the water supply was constantly labelled, and so, when the animals were mature, they had presumably incorporated tritium fairly evenly into most body constituents. At this stage, tritium administration was stopped, and the rate of decline of tritium in different tissues and tissue constituents was studied for many months. Components of the biological decay curves were identified, extrapolated backwards, and the sizes of different compartments as well as their biological half-lives estimated. This design of experiment is capable of very great biochemical and physiological variation, and its extension would yield many important quantitative data concerning metabolically heterogeneous compartments. It has many advantages over the single-dose *in vivo* experiment for these purposes.

Areas consisting of inert structure can sometimes be separated from others predominantly containing rapidly metabolizing cells owing to the anatomical arrangement of some tissues. For example, brain white matter and grey matter can be crudely separated, and although there is no complete division into neuronal cells, glial cells, and myelin sheaths, significant metabolic differences can be studied which are partly based on the relative amount of inert structure present[16,25]. It is far more useful for many purposes to dissect brain this way than to separate it into brain stem, cerebellum and cerebrum. A similar gross separation is quite impossible in the case of the more homogeneous tissues like liver. However, differential centrifugation offers further possibilities of separating the different anatomically based components of tissues[26,27]. By these means it is possible to obtain information separately about the different roles of the same substance within the same tissue, and about the infinite range of metabolic rates involved.

Conclusion

A revolution occurred twenty years ago as the result of the application of isotopes to biological research. A new generation of thought quickly adopted the doctrine of the dynamic state of body constituents. In the past two decades, however, the new doctrine has been substantially modified by an increasing appreciation of the wide range of rates of metabolism taking place in tissues. The realization that the same material may be metabolized at different rates in the same tissue, and that many important tissue constituents may even be metabolically inert, has come relatively recently. This new evidence has been only slowly recognized by those who have already accepted that all body constituents engage in the dynamic equilibrium. An awareness of the concept of extreme and even infinite metabolic heterogeneity is necessary to a more complete understanding of tissue metabolism *in vivo*, and without it, it is not possible to make meaningful comparisons between different tissues, or between the same tissue at different stages of development. It is suggested that much that is puzzling about existing results may become clearer in the light of these new ideas. They should be considered in any investigation of *in vivo* metabolic turnover problems in the future.

[1] Neuberger, A., *Brit. Med. Bull.*, **8**, 210 (1952).

[2] Rittenberg, D., *Harvey Lectures*, 200 (Thomas, Illinois, 1950).

[3] Thompson, R. C., and Ballou, J. E., *J. Biol. Chem.*, **223**, 795 (1956).

[4] Tarver, H., *The Proteins*, **2*B***, 1199 (Academic Press, Inc., New York, 1954).

[5] Schoenheimer, R., *The Dynamic State of Body Constituents* (Cambridge, Mass., 1946).

[6] Folin, O., *Amer. J. Physiol.*, **13**, 117 (1905).

[7] Manly, R. S., Hodge, H. C., and Manly, M. L., *J. Biol. Chem.*, **134**, 293 (1940).

[8] Hecht, L. I., and Potter, V. R., *Cancer Res.*, **18**, 186 (1958).

[9] Fresco, J. R., and Bendich, A., *J. Biol. Chem.*, **235**, 1124 (1960).

[10] Reiner, J. M., *Arch. Biochem. Biophys.*, **46**, 53 (1953).

[11] Arnoff, S., *Techniques of Radiobiochemistry* (Iowa State College Press, Iowa, 1956).

[12] Zilversmit, D. B., *Amer. J. Med.*, **29**, 832 (1960).

[13] Dobbing, J., *Physiol. Rev.*, **41**, 130 (1961).

[14] Muir, H. M., Neuberger, A., and Perrone, J. C., *Biochem. J.*, **52**, 87 (1952).

[15] Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, **166**, 627 (1949).

[16] Davison, A. N., Dobbing, J., Morgan, R. S., and Payling Wright, G. P., *Lancet*, i, 658 (1959).

[17] Davison, A. N., and Dobbing, J., *Biochem. J.*, **75**, 565 (1960).

[18] Dobbing, J. (in preparation).

[19] Kabara, J. J., and Okita, G. T., *Fed. Proc.*, **18**, 408 (1959).

[20] Bauer, G. C. H., Carlsson, A., and Lindquist, B., *Radioactive Isotope in Klinik und Forschung*, **3**, 25 (Urban, Berlin, 1958).

[21] Neuberger, A., Perrone, G. C., and Slack, H. G. B., *Biochem. J.*, **49**, 199 (1951).

[22] Davison, A. N., *Biochem. J.*, **78**, 272 (1961).

[23] Davison, A. N., Dobbing, J., Morgan, R. S., and Payling Wright, G., *J. Neurochem.*, **3**, 89 (1958).

[24] Davison, A. N., and Dobbing, J., *Biochem. J.*, **73**, 701 (1959).

[25] Davison, A. N., and Dobbing, J., *Biochem. J.*, **75**, 571 (1960).

[26] August, C., Davison, A. N., and Maurice Williams, F. E., *Biochem. J.* (in the press).

[27] Korey, S., *Metabolism of the Nervous System*, edit. by Richter, D., 87 (Pergamon Press, London, 1957).

AFRICA'S FIRST SCIENCE MUSEUM

By MURRAY SCHOONRAAD

South African Council for Scientific and Industrial Research, Pretoria

ON August 26, 1960, Dr. P. J. du Toit, F.R.S., former president of the Scientific Council in Africa South of the Sahara, opened Africa's first Science Museum in Pretoria. The Permanent Exhibition of Modern Science was established under the auspices of the Pretoria Centre of the South African Association for the Advancement of Science to commemorate the Union Festival 1960. It has been arranged in the form of an exposition of contemporary science in which that in South Africa is seen against the background of world science. Its aim is not only scientific but also to demonstrate agricultural, mining and manufacturing processes; for to-day's science is to-morrow's technology. Furthermore, this will serve as an institution where the noteworthy achievements of South African scientists could be explained and their original instruments and equipment preserved for posterity.

R&D

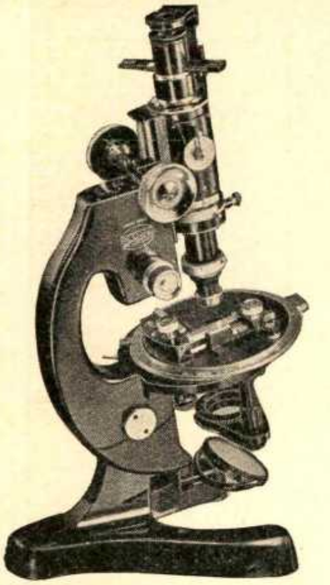
A new monthly technical magazine for the 103,000 scientific workers engaged in research and development

Although there may be little common ground between the products of one industry and another the techniques of research and development are universal. It is the purpose of 'R & D' not to report the results of research programmes but to pass on useful information about the means by which results were obtained—in short, to assist the cross-fertilisation of scientific ideas in the field of research and development.

In addition to information about new methods and new techniques, 'R & D' will provide details of new equipment and apparatus as it comes on the market and which forms no small part of the £655 million which it is estimated will be spent on research and development this year. 'R & D' has already had the co-operation of many of the research associations, of independent sponsored research organisations and of the research and development departments of many great companies.

'R & D' is available to bona fide scientists, engineers and technicians engaged in research and development on payment of an annual registration fee of 10/- (12 issues post free). Applications for registration together with the remittance for 10/- should be sent to the Circulation Manager, 'R & D', Tower House, Southampton Street, London, W.C.2.

No. 5000 PETROLOGICAL MICROSCOPE



Advanced student type of highest quality and workmanship.

Incorporating modern features.

Please write for full particulars.

R. & J. BECK LTD.

69/71 MORTIMER STREET, LONDON, W.1

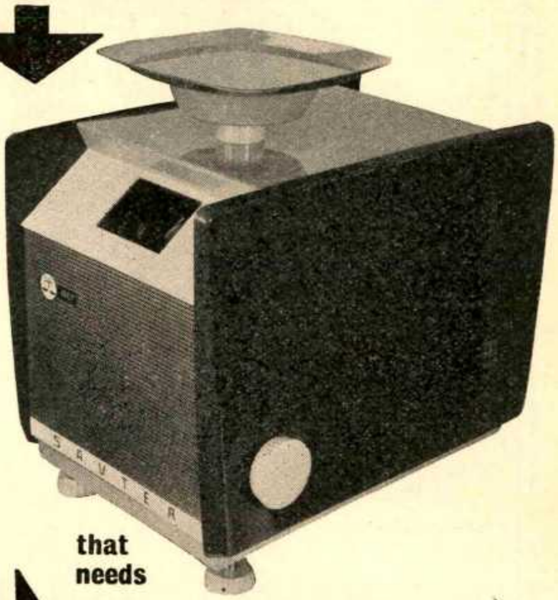
Manufacturers of high grade microscopes for more than a century

This quarterly journal of instrument engineering contains information of latest developments. It is available post free on request—ask to be put on the mailing list.

MUIRHEAD & CO. LIMITED, Beckenham, Kent, England. *Telephone: Beckenham 4888*
MUIRHEAD INSTRUMENTS LTD., Stratford, Ontario, Canada. *Telephone: 271-3880*
MUIRHEAD INSTRUMENTS INC., 441 Lexington Ave., New York 17, N.Y., U.S.A. *Telephone: Murray Hill 2-8131*

613

The Precision Balance



that needs

NO LEVELLING

with these outstanding features, too

- *TOP-MOUNTED PAN*
- *BACK AND FRONT READING SCALES*
- *NEAR-INSTANTANEOUS MAGNETIC DAMPING*
- *SUBSTITUTION PRINCIPLE*

Here's a superbly-designed substitution precision balance that offers really worthwhile new features—a **built-in levelling device** making critical levelling unnecessary; a **clear, illuminated scale both back and front,** making checking easy; **almost-instantaneous magnetic damping;** large, conveniently-mounted, **interchangeable top pan.** The Sauter TOP-PAN **is** what it **looks**—an intelligently-designed finely-engineered solution to an old problem. The TOP-PAN is available with capacities of 1 kg (0.1 g divisions), 2 kg and 4 kg (both with 1 g divisions). Models are also available with a special tare facility, but without the levelling device.

Write to-day for full details and a demonstration on your own premises.

SHANDON

SHANDON SCIENTIFIC COMPANY LTD.

6 Cromwell Place · London SW7 · Telephone: KNIghtsbridge 1131

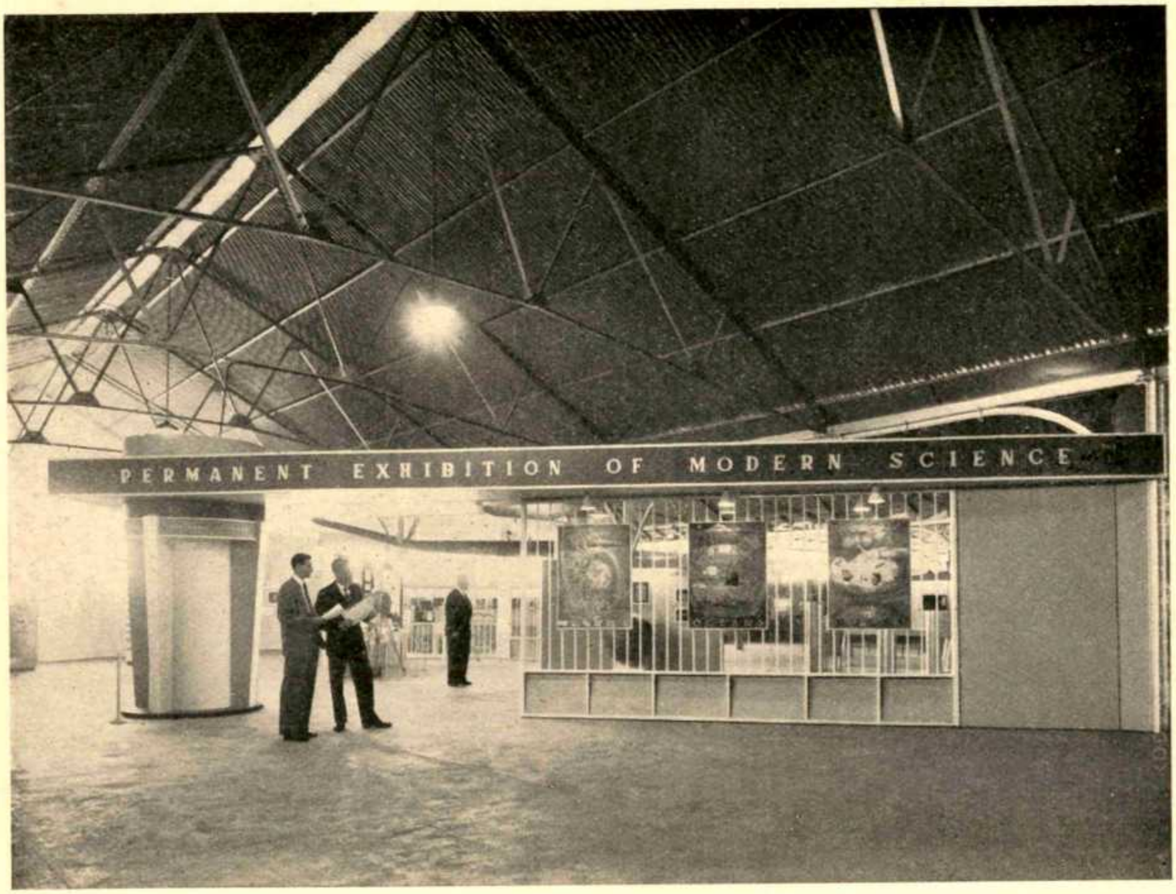


Fig. 1. Entrance to the Exhibition of Modern Science, Pretoria. The three posters seen in the foreground form part of a display symbolizing the main theme of the International Geophysical Year: space, Sun and Earth, weather and climate, oceans and poles

The inhabitants of the capital of South Africa have taken the initiative, and quite rightly so, for nowhere on the continent of Africa is scientific research and activity more concentrated than in and around Pretoria. Here are situated the headquarters of the Council for Scientific and Industrial Research with its two national research laboratories, seven national research institutes, the South African Bureau of Standards and associated activities; the headquarters of the Department of Agriculture, with its numerous research divisions including chemical services (soil fertility), botany, plant pathology, horticulture, dairying and the famous Veterinary Research Institute at Onderstepoort; the Department of Forestry, the Geological Survey and Trigonometrical Survey; the Weather Bureau and the Radcliffe Observatory; the National Zoological Gardens, National Botanic Gardens and the Transvaal Museum; the Fuel Research Institute and the developing research laboratories of the South African Iron and Steel Industrial Corporation (Iscor); the University of Pretoria, with its rapidly developing faculties of medicine and engineering, in addition to the faculty of science; and the University of South Africa, Pretoria. Here, too, are the headquarters of the various agricultural marketing boards.

As these scientists seldom meet except in their own specialized groups, it has created an opportunity for scientists from different fields of specialization to meet together and even more so to work together on matters of common interest. Furthermore, scientific organizations are obliged to participate from time to time in shows and exhibitions which involve a great deal of time, effort and expense; these exhibits are usually on display for relatively short periods, after which they have to be broken down. The result is that all the effort and expense are virtually lost.

It was against this background that on February 5, 1960, Mr. Kingwill, head of the Information and Special Services Department of the Council for Scientific and Industrial Research, proposed the establishment of a Permanent Exhibition of Modern Science to a meeting of Pretoria members of the South African Association for the Advancement of Science. The proposal was accepted and an organizing committee appointed. The committee found immediate support from the Pretoria City Council, the Northern Transvaal Chamber of Industries, the Transvaal Museum, the Council for Scientific and Industrial Research and South African Iron and Steel Industrial Corporation. It was decided to launch the Permanent Exhibition of Modern Science in conjunction with the Pretoria Industrial Show, in one of the halls with an area of 30,000 sq. ft., which the Pretoria City Council agreed to make available for an indefinite period.

The success of the exhibition depended entirely on the support of the main research organizations in Pretoria in order to be able to present a reasonably complete picture of research in science and engineering in relation to the development of the material and human resources of South Africa. Despite the short

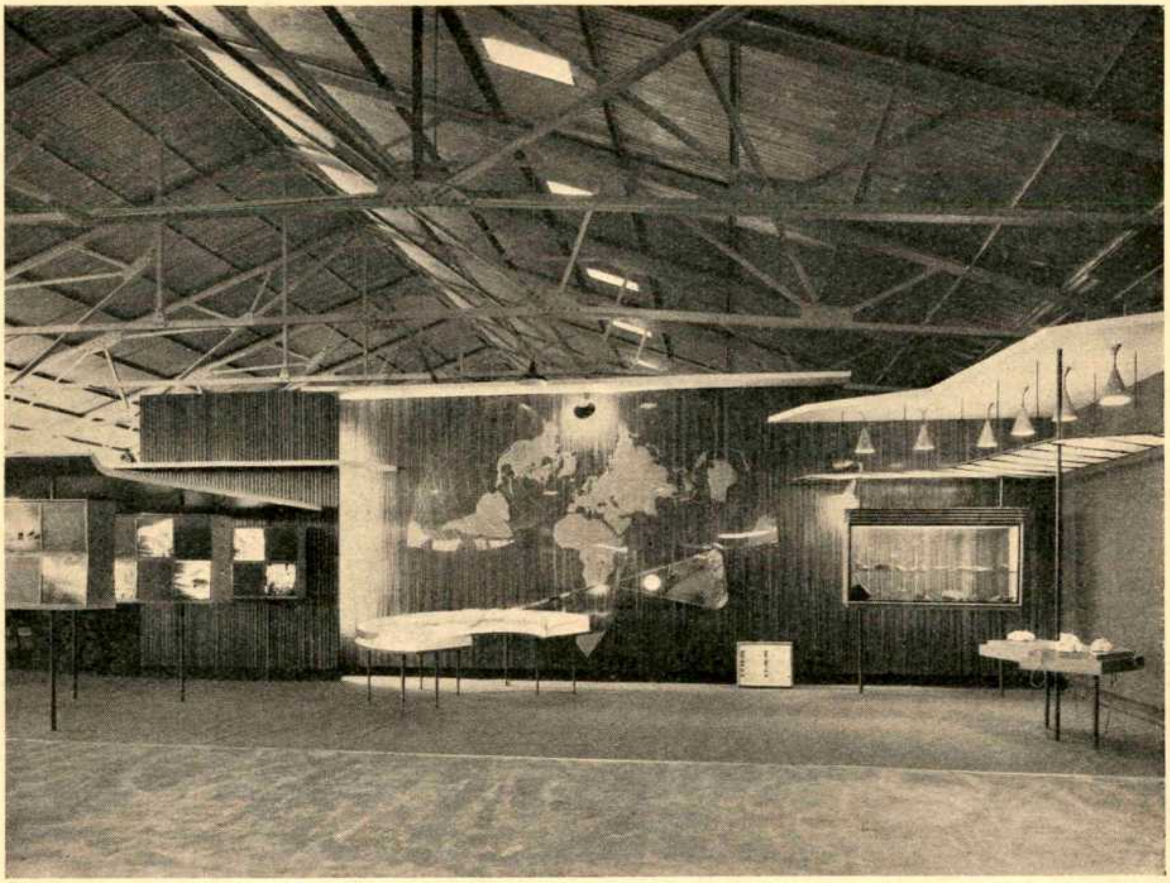


Fig. 2. An exhibit first shown at the Atoms for Peace Conference in Geneva, illustrating the occurrence and mining of uranium in South Africa

notice this support was forthcoming. In addition, exhibits on sugar production were provided by the South African Sugar Association, paper production by the South African Pulp and Paper Industries, on power generation by the Electricity Supply Commission for South Africa, English Electric and John Thompson, Ltd., petroleum by the Shell Co. of South Africa, Ltd., aviation by British Overseas Airways Corporation, gas turbines by the South African Air Force, and the recovery and uses of diamonds by the Diamond Research Laboratory. Major contributions were received from the Information Offices of the United Kingdom and the United States—the former on radioactivity and nuclear power, the latter on space research.

As a result of this wonderful co-operation and support, it was possible to stage a very comprehensive exhibition of modern science in an unbelievably short time. The exhibits have been so arranged that they lead the visitor from research on outer space and its relationship to the measurement of the Earth, to the measurement of the ionosphere, on which we are dependent for long-distance radio communication, to meteorological observations and weather forecasting; and from there to research on hail, air-pollution and radioactive fall-out. Descending to Earth the structure and interrelationships of rock formations are explained. These are followed by exhibits illustrating the mining, recovery, preparation, transformation and utilization of coal in South Africa. From here the exhibition leads off in three directions: the use of coal in the generation of electric power; the occurrence of uranium in association with gold in South Africa, the processing and use of uranium in the generation of electric power, radioisotopes and the cultivation of the surface of the Earth, that is, agriculture.

The main aspects of agricultural research, such as soil and vegetation, crops and pastures and animal husbandry are demonstrated in comprehensive displays. In the exhibit on forestry the following aspects are dealt with in detail: afforestation, genetics and tree breeding, plantation operations, saw milling, seasoning, grading, uses and wood products. With this exhibit is associated an exhibit on the production of pulp and paper.

The theme then switches to the basic sciences of chemistry and physics. Exhibits dealing with X-ray crystallography, spectrochemical analysis and the three techniques of chemical separation, chromatography, ion exchange and distillation, illustrate that the separation and identification of substances is an important aspect of science. These displays are followed by an exhibit on the history, meaning, purpose and application of standardization. Parallel to them are exhibits on engineering: gas turbines and aviation, stress analysis, slag cement, steel production and the exploration, recovery, transportation and uses of petroleum.

This leads to research on public services: road construction and maintenance, traffic engineering and road safety; the treatment and purification of water supplies and sewage effluents; vitamins, trace-elements and nutrition; Bantu housing and, finally, aptitudes and abilities of the people of South Africa and their adjustment to changing conditions.

The present housing of the exhibition in the Pretoria Show grounds is a temporary arrangement, and eventually it is intended to provide permanent accommodation which will be more conveniently situated. A film auditorium is included in the exhibition hall, and on Wednesday evenings a programme of lectures and films is arranged to amplify the exhibits in the hall and other items of general scientific interest.

The establishment of the Permanent Exhibition of Modern Science has been made possible through the voluntary efforts of members of the organizing committee and the support and co-operation of numerous sponsors. It is a long-term project, and success will depend on continued support and sponsorship of industrial, commercial and public undertakings; on the active participation in its activities by scientists working in these organizations; on the interest and participation of school teachers and scholars; and on the Association for the Advancement of Knowledge and Culture.

OBITUARIES

Prof. Camille Guérin

PROF. CAMILLE GUÉRIN died on June 9 in Paris in the hospital of the Pasteur Institute after a long illness.

Probably not all who are aware of, or are responsible for organizing, vaccination with BCG, finally but only very recently accepted in Britain as safe and effective and having an important ancillary part to play in the control of tuberculosis in man, recognize that the initials perpetuate so eloquently the close and fruitful collaboration of two of the most illustrious of Pasteur's disciples which, more than forty years ago, resulted in the development of this antituberculosis vaccine—Bacille Calmette-Guérin.

Guérin was born in Poitiers in La Vienne on December 22, 1872. He pursued his veterinary studies in the Ecole d'Alfort near Paris. It was fortunate that Nocard, one of the professors at this school, realizing his interest in research in the bacteriological field, initiated Guérin's technical training and encouraged his vocation. Nocard was an able master, for he was associated with Pasteur in the early studies on rabies in the rue d'Ulm and later, in addition to continuing his teaching at Alfort, he brought his clinical science and experience in experiments on animals to a collaboration with his friend Roux at the Pasteur Institute in studies on tuberculin, mallein and on the development and improvement of bacteriological techniques, just as Guérin did later to his collaboration with Calmette. It was Nocard who brought Calmette and Guérin together.

In 1896, the year following Pasteur's death, Calmette was appointed director of the newly founded Pasteur Institute in Lille on his return from a mission to Saigon, starting in 1891, to establish a centre for smallpox vaccination and for the diagnosis of and vaccination against rabies with the Pasteur vaccine. This latter became one of the three Pasteur Institutes in the great French colony of Indo-China.

In 1897 Guérin was appointed assistant to Calmette in Lille. Their collaboration lasted for thirty-six years and was only terminated by Calmette's death in 1933.

As Guérin once waggishly commented, he could not be accused of having tackled too many problems without devoting sufficient time to any one of them. Only two were the subjects of his researches, vaccinia and tuberculosis. Experiments on Jenner's vaccine were indeed the first subject of his researches, and the regeneration of the virus by passage on the shaved skins of rabbits led to an original method of titrating the virus which was later adopted in 1927 by the League of Nations for the standardization of the vaccine for smallpox.

However, it was to the experimental study of the bacillary infection and the immunity against tuberculosis that in collaboration with Calmette he consecrated the major part of his long scientific career, extending over forty-six years. They had come to the conclusion that only a living vaccine would be likely to prove efficacious, and their long, patient efforts from 1908 over a period of thirteen years, during which a bovine strain of tuberculosis was passaged 230 times in a medium to which they had added bile which they had found in early work had an attenuating effect on the bacilli, finally led to a culture which proved to be non-infective for guinea pigs and cattle while retaining immunizing properties for these species. Investigations were made on the stability of this attenuated culture in respect of lack of tendency to reversion of pathogenicity when subcultured in media without bile or evidence of similar reversion in vaccinated animals. At this stage consideration was given to the possibility that the vaccine would be safe and effective for the immunization of man. The first vaccinations of children were carried out by Weill-Hallé in 1921 with successful results. BCG vaccination became increasingly popular so that in 1928 the number of vaccinated children in France increased to more than 100,000. During this period the activities of the Lille Institute had been interrupted by the 1914–18 War. In 1919 Calmette went to the Pasteur Institute in Paris, where Guérin soon joined him to continue the work. Their culture is the parent of all the cultures used for BCG vaccination, and the number of persons vaccinated with BCG throughout the world is now estimated to be about 200 million. There is no evidence of reversion to virulence in the body of vaccinated persons. Moreover, following trials organized by the Medical Research Council (Rep. 1956, 1958 and 1959), which indicated safety, substantial protection for at least 6½ years and satisfactory conversion-rates to tuberculin sensitivity following vaccination with liquid or freeze-dried BCG, it has at long last come to be recommended in Britain for the vaccination of infants born to tubercular parents, children of 13–14, doctors, nurses and medical students coming in contact with tubercular patients and those who have been in contact with cases of open tuberculosis, provided that all these categories show negative results in tuberculin tests before vaccination. The important part that BCG can play in economically developed countries, as it is pointed out, must be considered as ancillary to all the instruments available for the control of tuberculosis. These have been listed in a recent review by an eminent professor and expert in tuberculosis, J. W. Crofton, as BCG and the general improvement in the standard of living to raise community resistance, miniature radiography, chemotherapy, appropriate isolation, improved ventilation and the reduction of overcrowding in home, work, play and transport.

Reference is also made to control of bovine tuberculosis; with regard to this it is appropriate to mention the recent announcement by the Ministry of Agriculture that October 1, 1960, marked the end of a concentrated effort to reduce the incidence of bovine tuberculosis in Britain to a degree that it is no longer a national problem in that it has been virtually eradicated by attestation of the herds of the whole country. It is stressed that the present position with regard to human tuberculosis does not justify complacency. However, it is considered that a stage has been reached when it is possible to see how the main weapons against tuberculosis can be applied, and that provided there is enthusiasm and public backing to the end, there is a prospect of foreseeing the elimination of tuberculosis in the not too distant future. The problem in the undeveloped countries is more difficult. It is commented that BCG has an important part to play in economically developed countries, and it might be even more useful in the others provided that the efficacy and appropriate use of the vaccine is kept under constant review in the laboratory and in the field.

In this connexion it is interesting that what is considered as one of the most convincing studies of the protective power of BCG vaccination was first reported by Aronson in 1948. North American Indian children and young adults who did not react to tuberculin were divided into groups of 1,550 vaccinated with BCG and 1,457 unvaccinated. There was segregation of the first group for two months after vaccination. Tuberculin testing and radiographic examination was carried out each year for seven years and at the end of this period 4–5 times more unvaccinated had developed tuberculosis than the vaccinated and the mortality-rate from tuberculosis was seven times higher in the unvaccinated. It is interesting to note, in view of the early demonstration by Calmette and Guérin of the immunizing value of BCG in cattle, that it is now considered that control of tuberculosis should, whenever possible, be by an eradication scheme based on attestation of herds by tuberculin testing. Since BCG vaccinates are sensitized to tuberculin, diagnostic procedures are difficult and thus BCG can play no part in such a scheme. Nevertheless, although the BCG vaccine has become established as safe and effective, it had a very stormy history and one of the roughest passages of any vaccine before final acceptance. Undoubtedly, one of the major setbacks was the unfortunate incident in 1930 in Lubeck in Germany when 78 out of 251 infants believed to have been vaccinated with BCG, but who as was later established by official inquiry had been inoculated in error with fully virulent human culture of the tuberculosis bacillus, died. Until the result of the official inquiry had confirmed that BCG was not responsible, Calmette and Guérin suffered adverse criticism and vituperation from all quarters, including that from those who had warned that reversion to virulence was possible and here was the proof. Although BCG was exonerated, the incident had unfortunate repercussions, and it was a very long time before confidence in the safety and efficiency of the vaccine was established. Calmette especially was greatly shaken by the incident. He did not have the resilence of Pasteur in dealing with his detractors. The worry, strain and the vilification by the critics together were too much for him. He died in 1933, a disappointed man broken in spirit and health.

Guérin continued the fight against tuberculosis as chief of the BCG service in the Pasteur Institute for another ten years, consolidating the victory, opposing the doubts and critics with his lucid, well-considered judgments. Even after his retirement, he gave encouragement and advice to those who took over from him. Guérin was a member and president or vice-president of many learned societies and, in addition to receiving many foreign decorations, his contribution to the control of tuberculosis was recognized with gratitude in 1952 by the award of the Grande Médaille de Vermeil de la Ville de Paris, in 1955 by a prize of one million francs by the Academy of Sciences and appointment as Grand Officier de la Légion d'Honneur in 1958.

Those who knew Guérin recognized his charming modesty and great affability, and what gave him the greatest satisfaction was presiding at the First International Congress of BCG in 1948 and the opportunity of surviving long enough to witness the ultimate success of BCG, the universal recognition of the safety and efficacy of the vaccine which he and Calmette had struggled to develop nearly half a century ago.

IAN A. GALLOWAY

Prof. R. M. Gordon, O.B.E.

RUPERT MONTGOMERY GORDON, professor emeritus of parasitology and entomology in the School of Tropical Medicine, University of Liverpool, died on July 26, 1961, aged sixty-seven. During his youth he lived in Phoenix Park, Dublin, and there he developed early an interest in biology. He graduated in medicine in Trinity College, Dublin, in 1916, and, joining the Royal Army Medical Corps, he spent the war years in the Middle East. He was demobilized in 1919 and joined the staff of the Liverpool School of Tropical Medicine. He was soon sent to work for some two years in the School's laboratory at Manaos in Brazil, and on his return he became house physician and clinical pathologist to the Tropical Ward in Liverpool. In 1924 he was transferred to the Sir Alfred Lewis Jones Research Laboratory in Sierra Leone, where he became director in 1929 and professor of tropical diseases of Africa. In 1937 he returned to Liverpool to the chair of entomology, and in 1941 he became the first occupant of the Dutton and Walter Myers chair of entomology and parasitology.

He was president of the Royal Society of Tropical Medicine and Hygiene during 1955–57, and he was awarded the Chalmers Memorial Gold Medal in 1937. On his retirement he received the Mary Kingsley Medal of the Liverpool School of Tropical Medicine. He was an active member of many scientific committees and an editor of the *Annals of Tropical Medicine and Parasitology* for 28 years. In collaboration with Dr. M. M. J. Lavoipierre he completed a text-book on medical entomology shortly before his death.

Prof. Gordon was author of more than one hundred scientific papers which covered a wide range of subjects. In addition to many minor studies he made major contributions to medical parasitology in connexion with the epidemiology of malaria; the vectors of schistosomiasis and the development of the worms in the final host; metazoan immunity; the reaction of the human host to the bites of insects; the epidemiology of filariasis, especially loaiasis; and the deposition and development of trypanosomes deposited in the mammalian host by tsetse flies. One of his most notable achievements was his investigation of the behaviour of the mouthparts of blood-sucking arthropods during the act of feeding. He and his colleagues devised a method by which the mouthparts could be photographed on cine colour film in the host's tissues while feeding, and several cine films were made.

As a man, Prof. Gordon earned the affection of his junior staff and colleagues. He was deeply loyal to the School and to his friends, and he took a personal interest in encouraging the development of his young research workers. He was a tireless worker, a very fine teacher and an amusing companion. His wide experience and broad vision enabled him to throw fresh light on any problem he investigated, and his stimulating approach and constructive criticism will be sadly missed by workers in the field of medical parasitology. T. H. DAVEY

NEWS and VIEWS

Fluid Mechanics at Glasgow :
Prof. T. R. F. Nonweiler

DR. TERENCE NONWEILER has been appointed to the Mechan chair of aeronautics and fluid mechanics at the University of Glasgow in succession to the late Prof. W. J. Duncan. For the past four years Dr. Nonweiler has been a senior lecturer in aeronautical engineering at the Queen's University of Belfast, having previously held the same office at the College of Aeronautics. For some years he has acted as consultant to the Admiralty. He is an Associate Fellow of the Royal Aeronautical Society and also of the Institute of Aero Space Sciences and a Fellow of the British Interplanetary Society. Dr. Nonweiler is well known for his many contributions to theoretical aerodynamics, to space technology and to the problem of the stability of submarines. In addition, he has been a moving spirit in the recent campaign for the achievement of man-powered flight.

The appointment of Dr. Nonweiler to a chair with responsibilities for the education of both aeronautical and mechanical engineers is particularly felicitous in that his interests and experience cover the whole range of fluid mechanics. Moreover, several fields in which he has long been interested are likely to become increasingly prominent in the immediate future.

Progress on the Advanced Gas-cooled Reactor at Windscale

THE 135-ft. diameter containment building which encloses the advanced gas-cooled reactor, now nearing completion at the Windscale Factory of the Atomic Energy Authority, Cumberland, was successfully tested over the August Bank Holiday week-end. The holiday period was chosen so as to cause minimum interference with other work on the site and with the Factory. Very satisfactory results were obtained from sub-atmospheric strength and leak tests. The pneumatic strength test at 12·5 lb./sq. in. (gauge) necessitated the diversion of traffic from the adjacent public road as a precautionary measure. The advanced gas-cooled reactor represents the next phase of development of the graphite-moderated gas-cooled reactor beyond the basic Calder Hall design adopted for the first round of British nuclear power stations now being built. The main objective of the reactor is to reduce the capital cost of reactor installation, and this will be done by raising the steam temperature and increasing thereby the amount of heat generated from a given size of reactor. Higher temperatures will be made possible by the use of uranium oxide fuel, which will be put into the reactor in stainless steel and beryllium 'cans'.

Industrial Water and Effluents Group

A NEW group of the Society of Chemical Industry, to be known as the Industrial Water and Effluents Group has been formed under the chairmanship of Dr. B. A. Southgate, director of the Water Pollution Research Laboratory of the Department of Scientific and Industrial Research. The new Group will be concerned with the broad subject of industrial water, from supply, through treatment and use, to ultimate disposal. Among the subjects which will receive special attention by the Group are: availability of water for the future needs of industry; quality requirements for water for industrial use; treatment of water for process, steam raising and cooling purposes; conservation and re-use of industrial water; methods of treatment and disposal of water-borne wastes; industrial location and development, as governed by water supply and waste water disposal facilities; effects of industrial effluents on natural waters. The first meeting of the Group will take place at the Royal Institution, Albemarle Street, London, W.1, on November 1. Applications for membership and further information can be obtained from the General Secretary, Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

Atomic Research and International Co-operation

A REPORT, *New Trends in Atomic Research and their International Significance*, prepared by Dr. L. Kowarski, scientific adviser to the European Nuclear Energy Agency, and submitted to the Agency's Steering Committee, surveys the scope for international action in nuclear research and the opportunities of co-operation and makes several suggestions for such action (Pp. 36. Paris: Organization for European Economic Co-operation, European Nuclear Energy Agency, 1961). These are to be examined by a meeting of experts from member and associated member countries which is expected to take place during October. Dr. Kowarski assumes that fundamental research in this field is likely to be carried out in a clearly autonomous division of a large centre or in a separate centre, and that the need for large-scale planning, especially in semi-applied research, will encourage international co-operation. He believes that, although international co-operation is compatible with secrecy regulations only under exceptional conditions, in normal times the temptation to secrecy can be kept within reasonable bounds and channels of information kept open. Of the opportunities for co-operation, he lists first studies on high-flux reactors, followed by the application of electronic computation to reactor technology. Reactor physics also offers considerable possibilities, particularly through the pooling of material resources, as does the conversion of fission-generated heat into electric power. Besides the advanced types of power reactors, research on materials and components, research on miscellaneous applications of nuclear energy whether in medical or biological research, the sterilizing irradiation of food or other utilization of by-products, and problems of the disposal of waste materials offer important opportunities. The concrete suggestions for co-operation made in this report are, first, the creation of an 'Advanced Proposals Committee', to scrutinize new trends and ideas in this field, together with study groups on the direct conversion of nuclear energy to electric power and on data processing. Authoritative discussions could also be initiated into possibilities of co-operation on fluid-fuel and heavy-water reactors, making full use of the potentialities which already exist at Oak Ridge, particularly in the molten-salts reactor.

The Frederick Soddy Trust

THE report of the Frederick Soddy Trust (dated September 1958. Pp. 16. (Hove, Sussex: The Frederick Soddy Trust, 9 The Drive)), gives some indication of the principles to be employed in selecting projects for support, and these are further emphasized by the list of rejected applications by August 1957 and the record of grants made up to September 1958 which are included in the report. One further application, a large-scale and ambitious project for studies in a Central American desert, was rejected because it required support on a much larger scale than the Trust was able to give. On the principles themselves, the report emphasizes that team work rather than individual work is to be supported; other things being equal, preference is to be given to original work leading to some published report, and the emphasis throughout must be on actual study and research in the field. Grants will normally only be made to persons reasonably qualified to carry out the work and, if possible, where the study is being carried out under competent and qualified leadership. Special value is to be attached to organized co-operation with local workers when field studies in a foreign country are undertaken. Some details are included of the programme of crofting field studies, 1957; the proposed study of Corby and the proposed survey of the parish of Ardington, Berkshire, by the University of Reading; and of a proposed expedition to the Pyrenees.

Australian Rock Art

ONCE again students of primitive native art have to thank Mr. F. D. McCarthy, who has recently published two articles in the *Records of the Australian Museum*. One deals with the rock-shelter paintings of the Conjola District in New South Wales (24, No. 13; March 9, 1959: "Cave Art of the Conjola District, New South Wales". Pp. 191–202+plate 22. Sydney: The Australian Museum); the other with certain rock engravings from the Sydney–Hawkesbury District (24, No. 14; March 9, 1959: "Rock Engravings of the Sydney–Hawkesbury District". Sydney: The Australian Museum). In both cases, conventionalized figures of animals and humans occur, as well as signs. Representations of footprints are especially noticeable among the engravings. The paintings occur in rock-shelters, the engravings on open rock surfaces. Among the illustrations given is a number of excellent photographic reproductions. So much has been done recently to render available to students this interesting native Australian art that one wonders when the various investigators, headed by Mr. McCarthy, will get together to produce a really worthy, well-illustrated volume on the subject. Museum articles and the like are all very well, but not everyone outside Australia can easily put their hands on them. It is time a more complete account appeared.

The Whaley Skull

AN interesting excavation was undertaken in Derbyshire by the late Leslie Armstrong, in company with the late Dr. Arthur Court, at the Whaley rock-shelter, not many miles from the more classic palæolithic sites at Creswell Crags. The work started so long ago as 1938, but the well-known skull was only unearthed in 1947. A number of archæological levels was identified, ranging from two Mousterian layers at the bottom to Aurignacian and proto-Solutrean levels above. Between the two series there was a sterile layer. The Whaley skull appeared to be a deliberate burial in the proto-Solutrean layer. Associated with it in the level were antler, bone and flint tools typical of the culture. The skull was buried with the base downwards and the frontal parts facing the wall of the shelter. Dr. Oakley has examined the chemical composition of the skull and the results obtained are in no way inconsistent with it being of Upper Palæolithic date, though from the point of view of the measurements of the skull itself it could almost equally be of a female of Neolithic date. In an article in *Man* of July 1961, D. R. Brothwell, of the Duckworth Laboratory of Physical Anthropology at Cambridge, describes the find in detail with comparative data from other sites. It seems likely that a proto-Solutrean family living in this rock-shelter carefully buried the skull of a woman who was fully adult, though the original suggestion that she was about fifty years of age now seems doubtful; but why she was carefully buried in the 'home' will never be known.

Natural Rubber Producers' Research Association

THE annual report of the Natural Rubber Producers' Research Association once more directs attention to the steady development of the natural rubber industry in the face of ever-growing competition from a variety of synthetics. Latest among these is the *cis*-1 : 4-polyisoprene rubber, which, for the first time, actually reproduces the essential structure of the natural rubber molecule, and which, with the closely allied *cis*-1 : 4-polybutadiene, is likely to prove a formidable challenge. This challenge is being met by concentration on improved efficiency in production and marketing, and by the continuous application of fundamental research. Among the more striking of the fundamental researches referred to is the discovery that the immediate precursor of the rubber hydrocarbon in the rubber plant is isopentenyl pyrophosphate. This substance has been shown to be converted rapidly into rubber in fresh latex. Another topic which is now becoming much clearer is the mechanism of rupture and fatigue failure. The well-known zig-zag type of tearing so characteristic of a good rubber is shown by photoelastic investigations to be a consequence of the classical stress-distribution pattern in the material ahead of a growing crack. The work on the chemistry of vulcanization, and the converse effect of degradation, has continued, and is enabling a consistent picture of the relation between the molecular structure and the physical properties of rubbers to be built up.

Railways in Rural Areas

PERHAPS inadvertently, the Newsletter of the Friends of the Lake District for June 1961 brings out clearly the dilemma which must confront railway authorities in determining their policy of supplying transport facilities in rural areas (Pp. 14. Ulverston, Lancashire: Friends of the Lake District. 1961). The association is keen that certain branch lines which provide passenger services during holiday periods should be preserved. To do this is quite uneconomical and involves considerable financial loss to British Railways. The dilemma is one which cannot be resolved by the interested parties alone and, if this desirable service is to be maintained, should be decided for all important branch lines by the Government as a means of preserving the nation's amenities.

Calouste Gulbenkian Foundation

THE chairman's report of the Calouste Gulbenkian Foundation, covering the period July 20, 1955—December 31, 1959 (Lisbon: Pp. xvii+201+17 plates), describes briefly the founder and the Foundation before outlining the investment and distribution policy which has been pursued. These have mainly comprised charitable activities and the arts in Portugal, with some work in education such as provision of scholarships (of which 122 were for study abroad, and 41 for study in Portugal; of these 45 were in the physical sciences, 80 in the medical and biological sciences and 34 in social sciences), and travelling libraries. Some limited assistance to scientific research in Portugal has been given through the provision of study, work and travel scholarships, grants to scientific institutions and for research projects and the establishment of a study centre for agricultural economics. A number of grants has been made for various purposes in the United Kingdom and elsewhere in the British Commonwealth. These include £10,000 to establish a Gulbenkian Fund at King's College, University of London; £22,000 for lecture rooms in the New Law Library, University of Oxford; £50,000 for three fellowships at Churchill College, Cambridge; £5,500 to the Pepys Library, Magdalene College, Cambridge, for preparation and publication of a catalogue; £12,000 to the Outward Bound Trust and £5,000 to the University College of Rhodesia and Nyasaland for an archaeological laboratory.

Theoretical and Applied Mechanics at the University of Roorkee

THE fifth annual congress on theoretical and applied mechanics was held during December 23–26, 1959, at the University of Roorkee, India, under the presidency of Dr. A. N. Khosla, the then vice-chancellor of the University. On the previous two days, December 21–22, a symposium on "Non-linear Physical Problems" was held at the University. The symposium was sponsored jointly by the Indian Society of Theoretical and Applied Mechanics and Unesco, and was presided over by Prof. F. K. G. Odqvist. Fifty-eight papers were presented at the congress and thirty papers at the symposium, and 132 delegates attended. Many of the papers have been published in scientific periodicals, but eighteen of the papers, including the presidential address by Dr. A. N. Khosla entitled "Shell Roof for 325 feet Auditorium Span at the University of Roorkee", presented to the congress, and fourteen to the symposium have been collected together and recently published as the *Proceedings of the Fifth Congress on Theoretical and Applied Mechanics; and the Symposium on Non-Linear Physical Problems* (Pp. xi+342. Kharagpur: Indian Society of Theoretical and Applied Mechanics, Indian Institute of Technology, 1960). The contribution by Prof. Odqvist is entitled "Engineering Theories of Creep", and an account is given of solutions of the problems of plasticity and creep as connected with bending of plates and stretching of membranes. Recent solutions obtained with Swedish digital and/or analogue computers are discussed.

Management of Pine Stands for Pulp-wood

TREES belonging to various species of *Pinus* are now widely used in afforestation operations because it is a feature of most species of that genus to be hardy pioneers able to adapt themselves to very exacting and exposed conditions. Because of the unfortunate habit of over-generalization and lack of discrimination—which has led in one case to the appearance of a text-book entitled *Conifers*, but which is solely concerned with certain species of pines—there has recently been an unwise tendency to accept certain revolutionary theories on the treatment of conifers, which might conceivably be applicable to some species of pine under certain special conditions, as worthy of general application in forestry. It was only a matter of time before the pendulum would swing back again, and it is perhaps quite significant and appropriate that this swing should receive an impulse from South Africa. Arising from the rediscovery that young or juvenile wood from trees 6–10 years old produces chemical pulp of inferior quality, J. J. Kotzé, writing on the management of pine stands for pulpwood, makes some very salutary comments (*Journal of the South African Forestry Association*, No. 35, 3; 1960). He says, among other things, that "diameter-size in the shortest possible time is of far less importance than quality and quantity. Closer initial espacement is thus indicated". By delaying thinning, lateral branch development is kept under much greater control than in the case of wider espacement and earlier thinning, while taper is reduced and the trees tend to become more cylindrical in form. There is also greater opportunity to select the best stems for further development, while the rotation can also be lengthened. These points, which have long been accepted by foresters, deserve to be emphatically re-stated. More care must be taken in drawing general conclusions for application in practice until adequate tests have been made over an adequate number of years—and in forestry that means very many years.

Mental Health Statistics

THE supplement to the Registrar General's statistical review on mental health for 1957 and 1958 continues the series dealing with statistics of patients admitted to and discharged from 'mental and mental deficiency hospitals' which began in 1949 (Pp. ix+102. London: H.M. Stationery Office, 1961. 7*s.* 6*d.* net). Previous supplements on mental health in this series have dealt with patients in designated hospitals who were under the provisions of either the Lunacy and Mental Treatment Acts or the Mental Deficiency Acts. By 1958 the modern attitude to mental sickness was finding expression in the de-designation of hospital beds for mental patients. In order to preserve the continuity of the statistics, an attempt has been made to maintain the same bed coverage in 1958 as in previous years, irrespective of whether the patients were statutory or informal. Consequently, the totals of admissions, departures and discharges, deaths and resident populations include all statutory patients for the years up to and including 1957, and for 1958 all statutory patients plus those informal patients about whom it was possible to obtain statistical information. Two trends are apparent in the departures and discharges: a general movement towards shorter durations of stay and an increase in the numbers of long-term patients leaving hospital.

Harwell Radiation Protection Course

THE fifth advanced course in "The Principles of Radiation Protection" will be held in the Harwell Reactor School during October 25, 1961–January 30, 1962. The course, which comprises lectures, practical work, demonstration and visits, is intended for graduates or persons of graduate standard either entering or already working in the field of radiological health and safety. The course will cover a wide field of fundamental background knowledge and will deal with principles rather than with particular techniques. Some of the lectures will be given by the staff of the school, but the majority will be delivered by experts from Harwell and other establishments of the U.K. Atomic Energy Authority. It is also hoped to have lectures from experts from the Medical Research Council, the Agricultural Research Council, the Central Electricity Generating Board, the Institute of Cancer Research, the Radiological Protection Service and the Ministry of Housing and Local Government. The fee for the course is £250, exclusive of accommodation, and application forms and further details can be obtained from the Manager, Reactor School, Atomic Energy Research Establishment, Harwell, Berkshire.

University News: Cambridge

THE following elections to posts in Churchill College have been announced. *Fellowship:* Dr. A. Hewish, of Gonville and Caius College, on appointment as College lecturer in physics. *Junior Research Fellowships:* J. A. Miller, of Selwyn College, for research in geophysics; R. M. Loynes, of Trinity College (Central Electricity Generating Board Fellowship) for research in mathematics. *Research Studentships:* N. W. Ashcroft, of the Victoria University of Wellington, New Zealand, in physics; S. J. Bernau (Canterbury, New Zealand), of St. John's College (Gulbenkian Studentship), in mathematics; G. R. G. Lewison, of Trinity Hall (Central Electricity Generating Board Studentship), in engineering; D. McCormack Smyth (Toronto), of Churchill College (Ford Studentship), in education; W. K. Stell, of Swathmore College, Penn. (Ford Studentship), in physiology. *Postgraduate Studentships:* S. Chatterjee, of University College of Science, Calcutta, for a course in statistics; E. A. Kohll, of the University of the Witwatersrand, for a course in chemical engineering.

Techne (Cambridge), Ltd.: 'Tempunit' Temperature Controller

THE 'Tempunit' temperature controller manufactured by Techne (Cambridge), Ltd., was erroneously priced at £15 in an advertisement in *Nature* of July 15. We understand from the manufacturers that the correct price is £25.

Announcements

SIR BERNARD LOVELL, director of the Nuffield Radio Astronomy Laboratories of the University of Manchester, has been chosen by the International Academy of Astronautics as the first winner of the Daniel and Florence Guggenheim International Astronautics Award. This Award, which carries with it a prize of one thousand dollars, is to be given each year to an individual who has made outstanding contributions to the progress of astronautics during the preceding five years.

MR. D. B. MACKAY, assistant chief officer for the past two years of the Official Seed Testing Station, National Institute of Agricultural Botany, has been appointed chief officer of the Station in succession to Dr. P. S. Wellington.

DR. DAVID M. GATES has been appointed editor of Section D, Radio Propagation, of the *Journal of Research* of the U.S. National Bureau of Standards. He succeeds Dr. James R. Wait, who has been editor of Radio Propagation for the past two years. Dr. Gates has been serving as assistant chief of the Upper Atmosphere and Space Physics Division in the Central Radio Propagation Laboratory at Boulder Laboratories. Previously he had been the scientific director of the London Branch of the U.S. Office of Naval Research.

THE third international conference on "Neurosecretion" will be held at the University of Bristol during September 8–13. Further information can be obtained from Prof. H. Heller, Department of Pharmacology, University of Bristol, Bristol 8.

THE Society for Water Treatment and Examination is holding its autumn meeting at Great Yarmouth during September 20–22. Further information can be obtained from the honorary secretary, A. W. H. McCanlis, 41 Carshalton Road, Sutton, Surrey.

THE Nutrition Society (Scottish Group) is holding a symposium at the large lecture hall, College of Agriculture, Edinburgh, on September 30. The subject of the symposium will be "Nutrition and Poultry". Further information can be obtained from the honorary secretary of the Scottish Group of the Nutrition Society, Dr. D. G. Armstrong, Hannah Dairy Research Institute, Ayr, Scotland.

A PROVINCIAL meeting of the Association of Applied Biologists will be held at the School of Agriculture, University College of North Wales, Bangor, during September 17–19. The programme will include material of interest to agricultural botanists, foresters (forest zoology and botany) and veterinary research workers. Further information can be obtained from J. Hobart, Department of Agriculture and Forest Zoology, School of Agriculture, Deiniol Road, Bangor.

RECENT SCIENTIFIC INQUIRIES IN PARLIAMENT

THE Parliamentary Secretary for Science, Mr. D. Freeth, dealt with a further 25 questions relating to science in the House of Commons on July 18, answers to which occupy 20 columns of the Official Report. Replying to a question about non-ferrous mineral resources in Cornwall, Mr. Freeth said that the results of a comprehensive study have been published in a Geological Survey memoir on the Metalliferous Mining Region of South-West England in 1956. Airborne geophysical surveys were undertaken in 1957, 1958 and 1959, and at present airborne magnetic surveys were being extended to the off-shore areas.

On international co-operation, Mr. Freeth said that high cost is an important but not the only factor in deciding whether an international project is desirable. The United Kingdom is participating in the European Organization for Nuclear Research, in the *Dragon* and *Halden* reactor experiments of the European Nuclear Energy Agency, in the Anglo-United States *Scout* satellite programme and a project for European space flights is under consideration.

Membership of the Machine Tool Industry Research Association incorporated in November 1960, includes 64 machine tool manufacturers representing about half the output of the industry, and 20 manufacturing firms in allied industries.

Repeated investigations by the Medical Research Council have shown no evidence of a higher incidence of lung cancer or respiratory disease among persons exposed to increased amounts of Diesel exhaust fumes. No figures are at present available to indicate the effect of Diesel smoke on road safety, but the Department of Scientific and Industrial Research is collaborating with the Minister of Transport in efforts to ensure the correct maintenance and operation of vehicles which will eliminate satisfactorily the nuisance from this source. Mr. Freeth also said that the Warren Spring Laboratory of the Department of Scientific and Industrial Research has examined methods for removing smoke from the exhausts of Diesel-propelled road-vehicles, but no device yet tested has proved satisfactory. The British Internal Combustion Engine Research Association is undertaking fundamental work on the combustion process in Diesel cylinders which it is hoped may lead to remedial measures. Mr. Freeth said it is a fact that correct setting of the injectors and their proper maintenance and operation provide a simple and effective remedy.

In answer to a series of questions on cancer, Mr. Freeth said that work on geographical variation in cancer incidence is already being supported by the Medical Research Council and no promising lead on cancer or similar diseases will be held up through lack of Government funds. The Medical Research Council's expenditure from public funds on cancer research was £327,000 in 1955–56 ; £400,000 in 1956–57 ; £486,000 in 1957–58 ; £499,000 in 1958–59 ; £583,000 in 1959–60 ; £650,000 in 1960–61 ; and would be about £783,000 in 1961–62. No reliable estimate can be made of expenditure on research relevant to cancer in the universities and medical schools in Britain from funds provided by the University Grants Committee, or in the National Health Service during the treatment of patients.

Other answers were as follows: The annual output of qualified scientists would be about 6,900 in the present academic year, compared with less than 5,000 in 1956, and the annual output of qualified technologists has grown from 6,200 to more than 9,600 in the same period. The Research Councils, for which the Minister for Science is responsible, are providing about £250,000 in the current year for expenditure on printing, publications and other forms of publicity. The Department of Scientific and Industrial Research at present operates 13 research grants with six different universities or colleges, and 6 further grants are contemplated. It is also intended to extend the use of scientific methods by the Geological Survey. A Committee of the Royal Society, at the request of the Advisory Council on Scientific Policy, is considering existing arrangements for supporting fundamental research in biology.

ATOMIC ENERGY RESEARCH AND DEVELOPMENT IN BRITAIN

THE seventh annual report of the Atomic Energy Authority covers the period April 1, 1960–March 31, 1961*, in which the Authority had in hand a special survey of its future requirements, both of man-power and finance, to ensure that its demands on Britain's resources do not exceed the minimum required for the efficient discharge of its responsibilities. This survey is additional to the periodic reviews made to ensure that the choice of reactor systems for major development and the priorities between them are firmly based. Although the review is still proceeding, its existence offers some re-assurance that developments on the part of the Central Electricity Generating Board will not lead to inappropriate duplication of effort. An increase in staff from 38,500 to 40,840 during the year is reported, industrial employees rising from 19,572 to 20,606.

On the civil side, the development of nuclear reactors to generate electricity on an economic basis remains the Authority's principal objective, and work for the nuclear power stations under construction, particularly the development and manufacture of magnox fuel elements, occupied a substantial part of the Authority's scientific and technical resources. Work on possible reactor systems for nuclear ship propulsion, production of radioisotopes (sales of which rose by 10 per cent to total nearly £21 million during the year) and development work on their applications, basic nuclear science, work on health and safety problems, and fulfilment of international obligations account for most of the Authority's remaining scientific work. Production of fissile material was up to programme, and unit cost was reduced by improvements in efficiency. The Calder and Chapelcross reactors, designed primarily to produce plutonium, are supplying more than 2,000 million units of electricity to the grid annually, and the stage has now been reached when the reactors are capable of generating more steam than can be used by the installed turbine capacity; turbo-alternators are being modified accordingly. In an experiment in a Calder reactor some of the standard fuel elements reached an exposure far in excess of their designed endurance. The production of a much more advanced type of fuel for the prototype advanced gas-cooled reactor at Windscale began during the year, and the main chemical extraction plant at Windscale successfully processed all the irradiated fuel from the eight Calder and Chapelcross reactors. A new separation plant is being constructed to process the irradiated fuel which will arise from the nuclear power stations operated by the electricity boards. The efficiency of the diffusion plant at Capenhurst was improved by about 4 per cent, and in addition to the highly enriched material taken from the top end of the plant, uranium hexafluoride was drawn off from the cascade at low enrichment to meet requirements for the prototype advanced gas-cooled reactor and for other research and development projects. In view of the great progress made since 1951, when work on the centrifuge method of isotope separation was abandoned, in fields such as vacuum engineering, the handling of uranium hexafluoride, and the design of gas bearings, a small development programme has been initiated to examine possible effects on this method of separating isotopes and the bearing of the new technologies on technical aspects of disarmament.

---

* United Kingdom Atomic Energy Authority. Seventh Annual Report for the period 1st April, 1960–31st March, 1961. Pp. viii + 80 + 4 plates. (London: H.M.S.O., 1961.) 5*s.* net.

Research and development undertaken in connexion with specific reactor systems are described in a separate chapter dealing with the reactor development programme. Research and development carried out with wider objectives, for example, the broad attack on scientific nuclear power problems, such as the effects of radiation on materials, the nuclear properties of materials and their effect on reactor performance, the chemical behaviour of materials in a reactor and heat-transfer problems, fundamental research in branches of science of particular importance to the Authority, and the research on isotopes or large sources of radiation, now centred on Wantage, are described in a further chapter. During the year, 517 technical reports have been published as well as 335 research articles by the Authority's staff in scientific periodicals, and lists of these publications are available on request. A further chapter of the report is devoted to controlled thermonuclear research.

Obviously it is only possible to select a few points for emphasis, and indeed even the Authority's report does not claim that its review of research and development is other than selective. A comparative economic study of various marine reactor designs made during the year indicated that, because of the comparatively small size of such reactors and the stringent safety precautions necessary if present reactor designs were installed in a ship, none of the designs at present in an advanced state of development is feasible, and the Authority is now considering the use of more advanced systems which are in a much earlier state of development. Much attention was paid to the reaction between graphite and irradiated carbon dioxide under the operating conditions of the Windscale advanced gas-cooled reactor, and the design for a large civil advanced gas-cooled reactor was carried forward in more detail. The Dounreay fast reactor is already fulfilling its purpose of developing the technology of fast reactors generally, and the next stage is intended to be a plant from which commercial fast reactors will be designed; work on this during the year was concerned mainly with reactor physics, engineering design study and the development of the fuel element, while work was started on the fabrication and irradiating-testing of possible fast reactor fuel elements. Much of the effort in support of the high-temperature gas-cooled reactor concept has been directed towards the 20 MW. (H) *Dragon* reactor experiment being built at Winfrith under the auspices of the Organization for European Economic Co-operation, but possible applications of this system to marine propulsion units and to large, land-based power stations are also being investigated. The way in which both gaseous and non-gaseous fission products diffuse out of graphite crystals and also through the pores of a polycrystalline mass of graphite is being studied. In preliminary work on the steam-generating heavy-water reactor system, a series of reactor physics experiments was performed in the *Dimple* and *Lido* research reactors.

In general research, investigations of the effect of irradiation on graphite, using the high-flux research reactors *Dido* and *Pluto*, suggested that changes produced by irradiation are essentially due to groups of interstitial atoms which congregate if the graphite is heated during or after radiation. The effect of neutron irradiation on the physical and mechanical properties of beryllium is being studied using electron microscope 'thin-film' techniques. In the increasing research on ceramic fuels a novel technique has been developed for the preparation of uranium carbide, with low open-pore space, by the 'reaction sintering' of uranium and carbon powders. Experiments on magnesium oxide have shown that its brittleness is due to the presence of surface cracks, less than one thousandth of an inch in length, which grow during deformation and lead to fracture. Investigations at Harwell directed towards studying the gas- and solid-flow patterns in fluidized material have shown that the roof of a bubble is robust, that a pocket of gas does not rise through a rain of solid particles, and that streams of gas adjacent to bubbles enter through the sides and bottom of the bubbles and rise inside them, suggesting that bubbles may help to promote gas mixing. A new method of automatic monitoring for beryllium has been developed in which a stream of the air to be examined is passed between platinum metal electrodes. A spark discharge between the electrodes excites the emission of light with wavelengths characteristic of any materials suspended in the air, and the light characteristic of beryllium can then be isolated and detected photoelectrically within a minute. A promising technique is being investigated in which wet steam is used as the cooling agent; if water is present as a film or spray, very high efficiencies for removal of heat can be obtained. In controlled thermonuclear research, work continued on the containment properties of the toroidal pinched discharge in *Zeta*, the ring-shaped apparatus in which deuterium gas is heated to high temperatures by an electric discharge. Investigation of plasma containment by magnetic fields oscillating at radio-frequencies have also been made, and in theoretical work attempts are being made to develop basic theoretical plasma physics, especially stability theory, to give predictions a secure foundation and to analyse individual experiments in detail, using available theory, to supplement and interpret the diagnostic measurements, suggest new experiments, and check the theory itself.

In written answers in the House of Commons on July 19, the Parliamentary Secretary for Science, Mr. D. Freeth, said that the volume of design work on reactor systems and other plant associated with the civil nuclear power programme carried out by industry on behalf of the Atomic Energy Authority is at the same level for 1961–62 as for 1960–61. The Authority received £2 million from the sale of fuel-elements in 1960–61, in addition to £6·5 million advance payments received in 1959–60, and expected to receive about £13 million from such sales in 1961–62. The three completed fuel canning lines at Springfields are fully employed, and all five lines are expected to be in production by May 1962, when the factory should be capable of meeting all demands now foreseen for natural uranium fuel-elements canned in magnox. It will be necessary in due course to plan new facilities for the fabrication of uranium oxide fuel elements if it is decided to include power stations using such elements in the nuclear power programme.

The Atomic Energy Authority now employs 350 professional staff on design studies and research and development for the prototype fast reactor, compared with 200 a year ago; but when full design and construction of a prototype commercial reactor can begin will depend on progress in developing a suitable fuel. Of the Authority's professional staff engaged on civil research and development, the proportions working on the main reactor systems in 1961 were: magnox reactors, 15; advanced gas-cooled reactors, 20; fast reactors, 14; high-temperature gas-cooled reactors, 8. In 1960 the corresponding figures were 20, 20, 9 and 8, respectively.

The Authority has in hand an intensive programme on the development of plutonium fuel elements for the prototype fast reactor. The fuels include mixed plutonium and uranium dioxides in a steel matrix and mixed plutonium and uranium carbides. The programme involves the preparation, irradiation testing and examination of specimen fuel materials, investigation of methods of making complete fuel elements and their testing under irradiation, and investigation of the behaviour of the fuel elements under conditions simulating those expected to be experienced in a power reactor. The number of professional staff engaged on the development of fast reactor fuels has approximately doubled since last year. It is not at present planned to run the Dounreay fast reactor completely on plutonium fuel, but shortage of plutonium is not holding up the development of fuel elements in the fast reactor. Ten engineers from the industrial consortia are attached to experimental reactor projects compared with 26 a year ago, and the Authority maintained a close association with the industrial consortia through a Nuclear Power Collaboration Committee and its supporting technical committees, including a joint committee which advises on the design and development of the Authority's experimental reactor projects and on advanced reactor systems. All information derived by the Authority on reactors is available to the industrial consortia through the Authority's reports, and the technology and progress have been discussed jointly under arrangements made by the Nuclear Power Collaboration Committee.

THE BRITISH GELATINE AND GLUE RESEARCH ASSOCIATION

THE twenty-first meeting of the Research Panel of the British Gelatine and Glue Research Association was held on May 17, under the chairmanship of Mr. S. G. Hudson (Richard Hodgson and Sons, Ltd.). The chair was taken in the afternoon by Mr. C. F. C. Simeons (British Gelatine Works, Ltd.).

Mr. W. G. Cobbett (British Gelatine and Glue Research Association) gave the first paper entitled, "Rupture Properties of Gelatine Gels". An apparatus has been built for the extension of dumb-bell shaped strips. The gels are cast from 10 per cent gelatine solutions in 'Perspex' moulds and are matured at 10° C. Extension is then performed at chosen constant rates, the gel strips being floated on mercury at 10° C. Rupture properties are more closely related to jelly strength (rigidity) than to the 40° C. viscosity of the gelatine solution. Thus gelatines with similar viscosities break at loads which follow the order of their jelly strengths while gelatines with similar jelly strengths are not greatly influenced in their breaking loads by differences in viscosity. The extension at rupture is greater for gelatines of higher jelly strength when the viscosities are equal, but is also higher for gelatines of higher viscosity where the jelly strengths are similar. The slope of the linear (lower) portion of the load-extension graph is directly related to the jelly strength as measured on the Bloom gelometer.

Discussion on the paper was opened by Prof. A. G. Ward, who had initiated the project in 1957. He recalled that the work was begun to provide information on the mechanical properties of gels in relation to food uses. The field was largely unexplored and it would be desirable to examine a series of fractions and a selection of total samples exhibiting extreme values in other physical properties. The lively discussion which followed showed that there was considerable interest in exploring the effects of additional variables on the extension properties of gelatine gels, for example, gel concentration and *p*H, chemical modification, including cross-linking, and dilution with additives such as water-soluble block polymers.

Dr. A. Courts presented his recent work on "Structural Re-orientation of Gelatine in Citrate". He has investigated the rise of viscosity with time which aqueous solutions of gelatines undergo over a period of days at moderate temperatures ($< 25°$ C.). The effect is enhanced several-fold in the presence of certain buffers, and most work has been done with approximately 0·3 per cent solutions in 0·15 *M* citrate at 20° C.; the rise is rapid during the first day, is slower but linear over the next 3–4 days and thereafter falls off. The rise is maximal near *p*H 7 and is unrelated in magnitude to the viscosity (log. viscosity number) of the sample and hence to its weight average molecular weight. It is dependent on the rigidity modulus of the gelatine and a quantitative relationship exists between the linear portion of the rate of viscosity increase and the rigidity. The increase in viscosity is explained in terms of partial reversion to a highly ordered state probably resembling that of the original collagen. This concept is supported by other properties of the matured solutions, for example, their ability to give fibres with precipitants and their resistance to complete digestion by trypsin. Dr. K. Little (Nuffield Orthopædic Centre, Oxford) showed some electron micrographs which she had obtained on the precipitated materials and which exhibited undoubted fibrous forms.

The afternoon paper, "Glueing in the Furniture Industry", was contributed by Mr. M. J. Merrick (Furniture Research Association). Attention was directed to the increasing mechanization of the modern furniture industry with the concomitant features of high production-rates and low labour content. Three main adhesives are in use, namely, animal glue, urea-formaldehyde and polyvinyl acetate. The more obvious differences between the properties of these, such as strength or resistance to water, have but a minor influence on the manufacturer's choice which is, instead, governed by the necessity for speed and reproducibility in the glueing of wood joints and veneers. Mr. Merrick then described the detailed operations involved in glueing in the furniture industry. A further point which emerged in the discussion was that synthetic glue manufacturers have built up an enviable technical service to ensure that the best use is made of the properties and behaviour of their products.

D. A. Sutton

RESEARCH BY SIXTH-FORM SCHOOLBOYS

By J. HEYWOOD

Department of Telecommunications and Electronics, Norwood Technical College, London, S.E.27

The editorial in *Nature* (**187**, 883; 1960) directed attention to the need for educational research and mentioned some specific topics. My work was cited as one of the examples in that editorial[1], and I have been concerned with several projects of this nature. The first has been concerned with project work for sixth formers, a second continuing the study previously mentioned[1] and a third into the opinions of technical college students on liberal studies. In this article the investigations made on project work will be discussed and some results presented.

This work has already been mentioned in *Nature*[2] since it has been carried out through the auspices of the Radio-Electronics Section of the British Astronomical Association. Four sixth formers have undertaken the same form of project and two of them have worked as a pair. The object was to provide a system of work which would have a liberalizing influence on the students' scientific studies and at the same time provide insight into the basic techniques of research inquiry. As an enticement the scheme aimed at providing some useful data for the Radio-Electronics Section.

Two of the students already possessed an interest in astronomy before the project was envisaged and were members of the Section. The other two were in attendance at the Salesian College, Battersea, and had no previous interest in astronomy or radio. Part of their project which is of considerable interest is at the moment in the press[3], and that of the third student will appear in the same publication[4].

Each project is in three parts, the first two parallel and the third being the item of use to the Section. The students were informed that their results should be written up in a form suitable for presentation as a 'letter' to *Nature*—there was of course no guarantee of publication anywhere but the concept of a letter in *Nature* gives the student an idea of how much has to be done to produce a small item.

An important feature of this work is the system of 'least instruction'. The students are encouraged to get on with the jobs themselves: at the same time they are equally encouraged to seek advice from their tutors but it is left to them to make the appointments. O'Neil and Borlengi[3] visited me at my home on four occasions during a three-month period and then submitted the article noted. This article was seen once previously and modified. In the case of Murdin[4], who already had an interest in radio astronomy and had worked with me previously, only two discussions were necessary. Murdin did, however, withdraw his article and make some additions before it was finally sent to press. These two articles summarize the scope and intentions of the first part of the project, while Sabbagh is concerned with the third part.

The fundamental units of radio astronomy provide an interesting introduction to the M.K.S. system and the relation between physical and radio units of brightness, brightness distribution and magnitude. A further comparison can be made with the unit of field strength as used in radio communications. In the first part of the project the student is given a number of references[5] which vary in level and told to investigate the problems of radio astronomy with particular reference to its units; he is asked to note how they differ from similar units used in optics and radio communications. His reactions to the various fields of observational radio astronomy are noted, and it is expected that a written account of this work in the form of the summary will be presented when complete. It is interesting to note that all the approaches to this same task have differed widely.

Normally the second part of the test is the construction of a simple piece of radio equipment with the view of its being used for observations. A typical project might be the construction of a sudden enhancement of atmospherics (associated with solar flares) recorder similar to that described by Ellison[6], or the construction of a simple radiometer[7] after Sander[8] or Osborne[9]. The third part would then consist of observing solar radiations or sudden enhancement of atmospherics (associated with solar flares) over a period of about two months. There is a number of possibilities open to simple investigation involving the analysis of such records.

At the time of writing all the students had completed the first part of the project. Sabbagh[10] had, however, undertaken a statistical analysis of some records made by Hyde and Castelvecchi[2] as a combined part two and three.

Two items arise from this research. First, it has been said that there is a large number of sixth formers with middle of the road Advanced level passes who would make good university students but who are nevertheless not able to obtain places. Secondly, it has also been said that there is far too much specialization in the sixth form arising from the need to cram. The shortage of university places accounts for the first and the shortage of time available to investigate candidates thoroughly undoubtedly assists this shortage. It would seem that projects such as those described would not only offset specialization, but also, if run on the lines of a second degree whereby an external oral examination was given on the candidates dissertation, provide the universities with a powerful means of selection. An alternative would be to offer a general paper on science subjects in which a candidate is required to write two fairly lengthy dissertations on any two of a variety of subjects announced during the two years preceding the examination. The former method seems to be the better since it offers the student an introduction not only to the wide aspects of scientific inquiry but to a knowledge of its tools.

[1] Heywood, J., *Nature*, **186**, 189 (1960).
[2] Heywood, J., *Nature*, **188**, 900 (1960).
[3] O'Neil, C., and Borlengi, M., *Mem. Brit. Astro. Assoc.* on "The Design of Radio Telescopes" (in the press).
[4] Murdin, P. G., *Mem. Brit. Astro. Assoc.* (in the press).
[5] Pawsey, J., and Bracewell, R. N., *Radio Astronomy* (Oxford, 1960). Lovell, A. C. B., and Hanbury Brown, R., *The Exploration of Space by Radio* (Chapman and Hall, 1957). Smith, F. G., *Radio Astronomy* (Pelican, 1961). *The Services Handbook of Electronics*, vol. on "Transmission and Propagation" (H.M.S.O.).
[6] Ellison, M. A., *J. Brit. Astro. Assoc.*, **69**, 127 (1959).
[7] Pettifer, J., *Mem. Brit. Astro. Assoc.* (in the press).
[8] Sander, K. F., *Proc. Inst. Elect. Eng.*, Part 3*A*, **93**, 1487 (1946).
[9] Osborne, J. M., *School Sci. Rev.*, No. 142, 478 (1959).
[10] Sabbagh, J., communicated to *J. Brit. Astro. Assoc.* (1961).

UNIVERSITIES COUNCIL FOR ADULT EDUCATION

DURING 1959–60, as in 1958–59, there was a small increase in the total number of adult education courses over the figure for the previous year, from 4,741 to 5,051, and *prima facie* a striking increase in residential courses, from 351 to 575*. Apart from residential courses, the most notable increase was in the number of sessional courses. At 1,647 they reached a record maximum. This upward trend has been steady over the past few years, the average rate of increase since 1955–56 being about 5 per cent per annum. Alongside this increase the downward trend of tutorial classes has continued although the fall has been at a reduced rate, from 851 to 839, a reduction of only 12 compared with 46 and 53 in the two previous years. At Manchester, where the decline in the number of tutorial classes caused concern to the Joint Committee, a leaflet outlining the advantages of tutorial class study was printed and given to every Workers' Educational Association student who was not a member of a tutorial class. From Hull comes a reminder that "in general, tutorial courses offer more obviously and more fully, opportunities for the development of classes into strenuous seminar groups". Southampton, though regretting the reduction of this type of work, wonders whether the development of tutorial work has been pushed, in recent years, beyond reasonable limits. The report of King's College, University of Durham, contains this thought-provoking paragraph:

"Part of the answer to the decline in tutorial classes is that adult education is ceasing to be the 'late night' substitute it once was. There are fewer gaps in the educational system through which the potentially highly educable boy and girl can slip out of the main stream. While it is by no means infallible, and cannot be so long as the sill of the catchment area remains at the 'eleven plus', comparatively few pupils of good standard fail to make the grade if they try hard enough. The most grievous failure of the present system is that it so easily misses those who, at the vital age, are unaware of the need to make the effort and who lack parental pressure to encourage them to do so. Alongside the elimination of much of the earlier material from which adult students were made has gone some of the incentive to seek a higher education. The social scene of the early decades of the twentieth century has changed. The Welfare State needs a smaller quota of pioneers, dedicated to the attainment of social justice, and a much larger one of trained social workers, with qualifications, for the administration of its machinery. Moreover, progress in any occupation is now achieved through certificate, diploma or degree to a much greater extent than ever before, and the provision for those ends becomes steadily more extensive. So, the tutorial class, which in theory offered an opportunity for study for its own sake, but which in fact offered all kinds of other inducements, is less an attraction because it is less needed.

"The character of adult education is undergoing change no less than other factors of twentieth-century life and, although the tutorial class should continue to attract those who recognize its essential quality, universities may soon need to consider the introduction of incentives to it other than study 'for its own sake'."

One respect in which the character of adult education is undergoing change is the continued development of residential courses. Several departments comment on this as forming perhaps the most satisfactory side of their work. The residential course is a reflexion of changed social and economic conditions. "People are, on the whole, rather better off and at the same time they have many competing claims on their time. They prefer, wherever possible, to take their adult education in small and intensive doses, a limited commitment in a limited time, instead of spreading it over a long period of time, as in the past." Manchester is to be congratulated on the success which has deservedly crowned its persistent effort to demonstrate the importance of an extra-mural residential college.

Because of changes of classification, a strict comparison of the 1959–60 figures of subjects of study with those for previous years is not possible, but there seems not to have been any significant shift of interest. Indeed, the consistency of students' interests year after year, as revealed by the table of subjects studied, suggests either that there exists a peculiarly proper balance of subjects in extra-mural work and that it has been attained, or that students are notably conservative in their concerns, or that the pattern is largely shaped by the availability of tutors. The largest number of extra-mural classes in Wales was in religion, and clergy and ministers of religion formed the second highest category in the analysis of tutors.

---

* Universities Council for Adult Education. Report on the year 1959–1960. Pp. 28. (Bristol: W. E. Salt, Honorary Secretary and Treasurer, The University, 1961.)

The number of full-time tutors showed a slight increase over the previous years, and was almost the same as in 1956–57. Nevertheless, the increase in full-time tutors did not match the increase in courses, and consequently a larger number of part-time tutors was engaged, 3,838 compared with 3,512 in the previous year. This was a continuation of the trend noticeable during the past few years. The proportion of part-time tutors who are members of a university staff has at the same time declined slightly, but steadily, from 48 per cent in 1956–57 to 45 per cent in 1959–60.

Since the War, extra-mural work has expanded into fields such as archæology, music and the visual arts in which, though it is accepted that they are properly subjects of university study, a considerable proportion of the practitioners and exponents are to be found outside the universities. Moreover, dons become busier and more heavily committed. Belfast refers to the "growing reluctance of university teachers to make weekly journeys", Newcastle comments that "the prevailing apparent disinclination on the part of internal university lecturers to commit themselves to the longer course, especially if travelling is involved", and both Liverpool and London regret the transport difficulties which make it hard, or even impossible, for members of the internal university staff to travel to the remoter parts of their extra-mural areas. At a time when students have become more mobile, dons have become less so. This is a minor, less-prominent, feature of the mid-twentieth century social revolution; it creates new problems in maintaining the university complexion of extra-mural work.

Specialized courses which relate to a professional or vocational interest are now widespread. Manchester has a one-year training scheme for hospital welfare workers, a one-year public administration course for overseas Government servants, and a course on background to management for business executives which received the commendation of the Human Sciences Committee of the Department of Scientific and Industrial Research. At Liverpool there were several courses in the natural sciences at the postgraduate level, two of them, in applied mathematics and experimental botany, leading to a certificate. London has approved the introduction of a three-year course of study leading to a certificate in transport studies, and 1959–60 saw the beginning of the course for the diploma in the history of music. At Leeds, courses in such subjects as textiles, engineering, mining and fuel were arranged in association with internal departments of the University, and other courses were arranged for members of the staffs of the Home Office, the Prison Commission, the National Association for Mental Health, the National Coal Board, and the National Union of Mineworkers.

Courses of this kind are carrying universities into new fields where extra-mural departments are having to institute their own research. Leeds has received a grant from the Nuffield Provincial Hospitals Trust for work on the staffing of the hospital service, Liverpool appointed a research worker to study the impact of social change on adult education, and Oxford has embarked on a three-year scheme of research and experimental work in the field of trade union education.

These kinds of courses appeal rather to individual students than to pre-existing social groups, although it is always hoped that every class will grow into a cohesive social group. People move about much more often than they used to, and groups are less permanent. Continuity and progression of study needs to be thought of in terms of the individual more than of the group. The Leicester report, in its survey of the thirty years 1929–1959/60, contains a statement which would command at least partial acceptance generally:

> "There has also been a decided change in the ethos of the department's classes. Formerly, it was markedly social, now it is markedly individual. Classes and courses still grow into social unities, but this is now an incidental growth and it has not the educational significance which it once had. Rarely now do classes and courses become schools of citizenship as well as vehicles for teaching and learning in specific disciplines."

Another respect in which the change is noticeable is the educational background of students. The percentage of weekly wage-earners and of those with only an elementary or secondary modern education has fallen more than 40 to less than 10, and the percentage of professional workers and of those with a college or university education has risen from less than 10 to more than 40. Of the thousand students attending grant-aided extension classes at Leeds, more than 40 per cent had been to a university, teacher training college or technical college, and another 30 per cent had attended school at least until the age of sixteen. Almost half of them were under the age of thirty-six.

There are numerous examples of extra-mural classes undertaking field-work which has added to knowledge in botany, zoology, archæology and history. Under the tutelage of the Durham Extra-Mural Board, for example, a group of 15 students has investigated the site of a medieval village at West Whelpington, and their findings make a distinct contribution to the comparatively new venture of British archæology into the later Middle Ages.

MUSEUMS AND GALLERIES

THE recent report of the Standing Commission on Museums and Galleries stresses the responsibility of the British Government with regard to these institutions, and urges some immediate action*. Contrary to past practice, this sixth report covers only two years (1959 and 1960) instead of the usual quinquennial period. In emphasizing the need for more frequent reports, the Commission feels that this is a period of active rehabilitation in museums after a time of severe financial stringency. There is an increased public interest in them and higher standards are now expected.

---

* Standing Commission on Museums and Galleries. Sixth Report, 1959–1960. Pp. iv+64. (London: H.M.S.O., 1961.) 4s. net.

Although dealing chiefly with the national museums, the provincial institutions will welcome the recommendation of the Commission that the grant (now at £25,000) administered by the Victoria and Albert Museum for purchases should be increased in the next financial year.

With regard to buildings, there are at least thirteen projects which are to be regarded as of particular urgency. These include reconstruction of the war-damaged galleries at the British Museum, planning and construction of the final quarter of the Tate Gallery, erection of a new herbarium and library wing at Kew, construction of north-east and north-west wings of the Royal Scottish Museum, work on a west wing of the National Museum of Wales and extension of the Museum block of the Welsh Folk Museum.

The Commission urges museums to aim at a steady and rapid improvement in all their services and recommends that staff should be appointed to carry this out. It considers that these services should including cataloguing existing collections, the extension of late opening, provision of automatic guide lectures, and restaurants.

The Commission reiterates its recommendations in the previous report concerning estate duty, and income tax and surtax. It is pointed out that as it is not the policy of museums to finance themselves by entrance fees, apart from minor sources, only the taxpayer and private benefactors remain. It is thus felt that all bequests of money or money's worth should be exempted from aggregation and payment of estate duty, and that this should hold even if the donor dies within a year from the making of the gift. It is also considered that tax reliefs should be made in respect of gifts of objects or money, as the present British system of taxation offers no incentive to a benefactor to give to museums and art galleries during his life-time.

Those associated with provincial museums—and there are about 800 in the British Isles—will welcome the information that the Commission hopes to complete the survey and report, with recommendations, during the summer of 1962, possibly issuing an interim factual report some time in 1961. This will be awaited with great interest.

The report also includes an impressive survey of the individual institutions comprising the national museums in London, Edinburgh and Cardiff.

F. S. WALLIS

POPULATION MOVEMENTS AND MALARIA ERADICATION

IN the early months of 1960, a World Health Organization consultant, Mr. R. Mansell Prothero, visited the Sudan, Uganda, Kenya, Somaliland, Tanganyika, Zanzibar, Southern Rhodesia, Nigeria and Ghana to study the effect of population movements on malaria eradication programmes. In his report (*W.H.O. Chron.*, 15, No. 5–6; 1961) he observes that, although no method of measuring the extent of population instability has yet been devised, there can be no doubt of the high degree of such instability in Africa.

In all countries and territories visited by Prothero, there are considerable population movements from permanent villages to farms and back again. These usually involve the erection of temporary shelters for migrants on the farms. In the Sudan, for example, people move from one area to another during the cotton-picking season; in Nigeria, during the dry season, people go the valleys where there is still water to irrigate the land. Gambia receives many temporary immigrants from neighbouring countries for groundnut cultivation. Cultivation, planting and weeding coincide with the early months of the rainy season when malaria vectors are on the increase, and when temporary shelters provide suitable resting places for them.

Although the harvesting of such products as groundnuts, cocoa, and cotton attracts a considerable amount of labour, in many parts of Africa it occurs after the peak of malaria transmission. The same cannot be said of movements of labour to mining, industrial and commercial undertakings, since these occur all the year round.

Nomadic movements of pastoral tribes, which are on a larger scale than other types of population movement, occur only in certain parts of Africa, such as northern Sudan and Somalia. Fixed settlements are foreign to the way of life of these tribes; moreover, the environment in which they live compels many of them to wander with their flocks and herds. The only religious movement of any large size are those of the West African Mohammedans, north-east towards Mecca; these cause some convergance on the Sudan, but do not involve so many people as the internal migrations of the population of the country itself.

Apart from those connected with farming, most of these population movements occur without respect for international or political boundaries. Their effect on the stability of populations is considerable, and in fact, all malarial eradication pilot projects now in operation in Africa are hampered by them. It has so far proved impossible to start a malaria eradication programme for a whole country in Africa, because there is not one that contains population movements within its own boundaries. To keep migrants out of an area by using some kind of *cordon sanitaire* would not be practicable, because there are so many ways in which an African can get through natural or man-made boundaries if he wishes to do so. International co-operation in malaria eradication would only be a partial solution. These population movements are a fundamental part of the 'way of life' of the people. Some, such as pastoral nomadism, are traditional; others, such as migrant labour, are of more recent development. In either case, they cannot within a short time be stopped or easily controlled.

The situation must be accepted, and if it raises problems in malaria eradication, it will be necessary to find ways of adjusting to it. Malariologists in Africa must try to circumvent the problems that population movements raise for them in their work. But how, for example, is it possible to deal with the nomad pastoralist whose movements do not appear to follow any fixed plan, and whose huts, being mostly composed of grass and leaves, are not susceptible to effective insecticide treatment ? It is clear that there is no generally applicable solution to problems of this kind, and each of them must be considered individually on the same level as the malariological and entomological investigations that are now regarded as an essential part of malaria eradication programmes. Population distribution, settlement patterns, types of housing, farming practices, communications, water supplies and other relevant factors vary so much from one area to another that generalization is impossible. Geographical reconnaissance, as it now exists in eradication programmes, does not cover every aspect of population movement. Where necessary, it will have to be expanded to collect much wider information on the factors described, in order to understand the whole complex of physical environment, malaria parasites, vectors and people. It may then be possible to evolve the correct technique applicable to each individual local situation. The inclusion of professional geographers in some malaria teams for full-time work at the outset of a programme would be valuable; when they had collected the required information, it would not be necessary to retain their services, since the geographical data could afterwards be kept up-to-date by a specially trained sanitarian. There is also a need for detailed individual study of every migration problem arising in the course of malaria eradication in tropical Africa.

POLLUTION FROM REFUSE TIPS

TWO problems associated with modern living conditions are the disposal of house refuse and the reclamation of land from which chalk, gravel or other material has been removed. The possibility of solving these two problems at one blow by tipping the refuse into worked-out pits is attractive, provided that this can be done safely and without nuisance.

One of the dangers of this procedure, which is widely practised, is that water supplies may be contaminated by drainage from the refuse. To study this problem the Minister of Housing and Local Government, in 1953, appointed a technical committee to supervise experiments on the tipping of refuse, with special reference to the pollution of underground water. These experiments were carried out in the Laboratory of the Government Chemist, and the report on the results has recently been published, together with the conclusions and recommendations of the Committee*.

Two series of experiments were made: one on refuse tipped dry and the other on refuse tipped into water. The volume and composition of the liquid draining from the pits were determined, and the purification effected by passing this liquid through sand and gravel was studied.

The Committee points out that the matter is complex, and is careful to avoid over-simplified conclusions in its report. It appears, however, that the results of the experiments are reasonably encouraging and that, provided certain precautions are taken, the practice of tipping refuse may safely be continued and even extended. Although a highly polluting liquid is produced by percolation of rainwater through refuse, the amount of organic pollution falls off relatively rapidly with time. In addition, when refuse is tipped dry, a considerable amount of rain has to fall on it before any drainage water is produced. Thus if the amount of rainfall reaching the refuse can be reduced by covering the material with an impermeable layer as soon as possible after tipping, the total load of pollution discharged from the pit will be decreased. This is one of the reasons why the Committee considers that, under comparable conditions, dry tipping is less likely than wet tipping to affect water supplies adversely. It does not, however, rule out the use of wet sites, for the experiments showed that considerable purification of the liquid can be effected by percolation of the water from the pit through the surrounding ground. One suggestion of the Committee is that refuse might be tipped first into a dry pit, where initial decomposition would occur, and that after about two years it should be transferred to a wet tip, leaving the first site to be used again.

It is not claimed that the experiments described were exhaustive, and several lines of investigation remain to be followed. NORA H. JOHNSON

---

* Ministry of Housing and Local Government. *Pollution of Water by Tipped Refuse.* Report of the Technical Committee on the Experimental Disposal of House Refuse in Wet and Dry Pits. (H.M.S.O., London, 1961.) 7*s*.

TOXICOLOGY OF *PENICILLIUM ISLANDICUM*

TOXICOLOGICAL research into the methanol extract of the fungus *P. islandicum* has been carried out since the spring of 1954, with collaboration in the fields of mycology, chemistry and medicine. In five biological testings, methanol extract prepared from the fungus mat of *Ub*, *Uc* or *Ea* strains was administered in the form of oil solution or water suspension to 150 mice and 10 rats, orally or subcutaneously, to observe pathological changes; in chemical testings, chemical fractionations of the methanol extract were carried out.

Biological data observed a few days after a single or several doses of the methanol extract had been administered have been summarized by K. Uraguchi, T. Tatsuno, M. Tsukioka, Y. Sakai, F. Sakai, Y. Kobayahi, M. Saito, M. Enomoto and M. Miyake of the Departments of Pharmacology and Pathology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan (*Jap. J. Exp. Med.*, 31, No. 1; 1961), as follows: Acute symptoms of poisoning are developed in mice and rats, but their life-time is prolonged with reduced doses. Poison damages show that abnormal function of the liver is detectable by the bromsulphthalein retention test, and autopsy shows, in most of the liver, centrolobular necrosis, fatty metamorphosis of liver cells and interconnexion between the necrotic areas with subsequent collapse; in part of the liver there is vacuolation of liver cells on peripheral parts of the lobules and hæmorrhage among liver cell trabeculæ.

From these results the investigators came to the conclusion that the extract must contain certain toxic agents injurious to the liver and acutely fatal to animals.

Before and after the twenty-fourth hour the symptoms differ from each other in a marked fashion. Pathological changes observed in the liver are of two kinds. It would appear that the biological response to the methanol extract must be a manifestation of two actions essentially different in properties but each injurious to the liver. Convincing evidence of this is obtained from the data concerning the solvents used for the methanol extract; rapid action seems to be detectable when the extract is suspended in water while delayed action is detectable when this extract is dissolved in oil.

With regard to the toxic metabolites of *P. islandicum*, the investigators concluded that, in the methanol extract of the fungus mat, two liver-injurious components co-exist which act fatally within a short time; one is a hydrophilic compound relatively rapid in action and the other a lipophilic compound which is much slower.

Chemical fractionation of the constituents in the methanol extract of the fungus mat indicated that lipophilic fractions, sterols and several pigments of the polyoxyanthraquinone group are obtained; among the latter is found a yellow pigment 'luteoskyrin', which has been unknown hitherto.

ICSU REVIEW

THE *ICSU Review* was initiated in 1958 as a new enterprise of the International Council of Scientific Unions, with the strong interest of the past president of Union, Dr. Lloyd Berkner. The intention was to have a publication especially devoted to the affairs of the Council with its thirteen federated scientific unions and its special committees. The *Review* would bring the progress now being made in the co-operation of working scientists from all over the world to the notice of specialists and a wider public which is not well informed on these activities. The new *Review* was edited by Sir Harold Spencer Jones.

After the death of Sir Harold, the position of the *Review* was considered and certain changes were thought to be desirable. The scope of the *Review* could be expanded so that it could cover even more effectively the full activities of the Union's constituent organizations. This objective could be reached only if the *ICSU Review* presents theories, projects or discoveries of world-wide importance, and is directed towards international science. It should bring members of the scientific community a knowledge of movements and trends in other sciences in a more comprehensive way than is done in the present scientific journals. To this end, the publication will in future include short articles from different branches of science, written in a style suitable for the non-specialist and the generally educated public. This will give a better presentation of the work of the scientific unions in its relation to the progress of knowledge and to world affairs; thus international co-operation will be presented in a living perspective.

The new issue (3, No. 1; 1961) is a first step towards realizing this goal and contains articles on *AGK*3—a co-operative programme in astronomy by F. P. Scott; international research programmes in oceanography by Dr. G. F. Humphrey; the world land use survey by Prof. L. Dudley Stamp; changing views on the Earth's crust by Prof. J. Tuzo Wilson; the mixed commission on the ionosphere by Prof. W. J. G. Beynon; biophysics by Prof. A. Engstrom; fluorine and African plants by Sir Rudolph Peters; a European project for a large observatory in the southern hemisphere by Dr. J. H. Oort; the international latitude service by C. A. Murray; and geophysical and space-tests of gravitational theories by Prof. R. H. Dicke. Dr. Donald Fraser has been appointed editor.

SYNTHESIS AND X-RAY ANALYSIS OF DIAMOND

By H. Ll. D. PUGH and J. LEES
Plasticity Division, National Engineering Laboratory, East Kilbride, Glasgow
AND
J. A. BLAND
Research Department, Plastics Division, Imperial Chemical Industries, Ltd., Welwyn Garden City, Hertfordshire

THE recent development of relatively simple ultra-high pressure equipment has stimulated research effort in this field. To the engineer the field is of importance because of its potential in producing a range of new materials, probably characterized by hardness and good refractory properties. A small team in the National Engineering Laboratory, East Kilbride, is investigating the synthesis of such new materials, and has constructed an ultra-high pressure apparatus capable of subjecting specimens to pressures of approximately 75,000 atm., and temperatures of approximately 2,000° K. In the proving of this apparatus, synthetic diamond crystals were produced. A detailed X-ray analysis was made of one of these, by the Plastics Division, Imperial Chemical Industries, Ltd.

The National Engineering Laboratory (U.K.) apparatus is based on the National Bureau of Standards (U.S.A.)[1] modification of the tetrahedral anvil apparatus[2]. In this apparatus the high-pressure cell

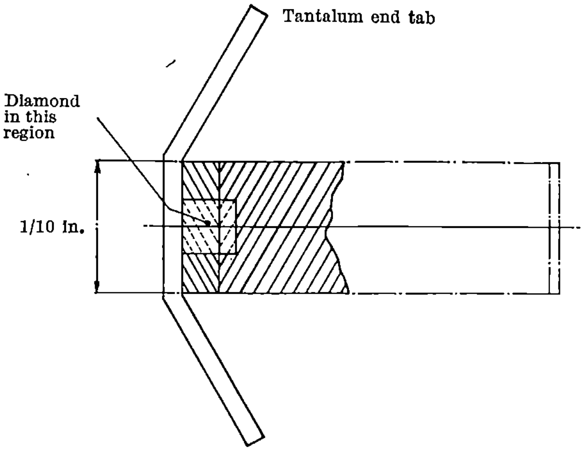


Fig. 1. Part of high-pressure cell containing nickel and graphite showing the region where diamond crystals were formed. Nickel, shading left down to right; graphite, vice versa

is a tetrahedron of pyrophyllite of ⅞ in. side, in the middle of which, extending between the centres of two opposite edges, is the test specimen 0·1 in. diam. × 0·3 in. long. The four faces of the tetrahedron are forced together by anvils acting on each face. Each anvil of tungsten carbide (6 per cent cobalt), force-fitted into *EN*26 binding rings, has a triangular face of ¾ in. side and is bevelled and shaped to fit with its neighbours so as to surround the tetrahedron completely except for small gaps. The excess pyrophyllite is initially extruded into these gaps, where it forms an efficient compressible seal allowing the bulk to transmit the pressure to the specimen approximately hydrostatically. The three lower anvils butt accurately on to the inner conical surface of a steel ring so that their thrusts are provided by wedging reactions when the top anvil is pressed downward. Contact tabs[2] enable the specimen to be heated by a large current, either through the specimen or through a graphite or metal tube surrounding it.

The apparatus was calibrated for pressure measurement in terms of the known resistance transitions of such metals as bismuth at 25,400 atm., and thallium at 36,700 atm. All pressures given here are in terms of the Kennedy and La Mori[3] recalibration, which shows Bridgman's resistance-transition values to be up to 30 per cent high. Temperatures were estimated by relating the heating power to that required to melt metal specimens, for example, nickel, for which data at high pressures are available[4].

The diamonds were synthesized using the arrangement of graphite and nickel shown in Fig. 1. With a power dissipation of 500 W., the specimen was heated to approximately 2,000° K. at a pressure of about 60,000 atm. for 30 min., followed by a 10-min. cooling cycle. On subsequent examination, the nickel disk was found to have almost completely alloyed with the carbon and a cluster of relatively large diamonds was obtained in the position shown. In the diamond-growing region, the temperature was probably somewhat below 2,000° K., and a considerable temperature gradient existed due to thermal contact with the cold anvils. Bovenkerk *et al.*[4], describing the diamond synthesis by General Electric, mention the necessity for a catalyst metal such as nickel, and also refer to an accelerating effect of temperature gradient on diamond growth.

The diamonds obtained were black in colour. One crystal 0·3 × 0·3 × 0·4 mm. (Fig. 2), the largest synthesized in Great Britain, was examined by X-ray methods using Laue, rotation and Weissenberg diffraction photographs obtained with copper radiation. This diamond shows several well-developed crystal faces, mostly hexagonal and square. It sank slowly in cadmium borotungstate at room temperature, showing its density to be somewhat greater than 3·3 gm. cm.$^{-3}$ compared with 3·514 gm. cm.$^{-3}$ for natural diamond.

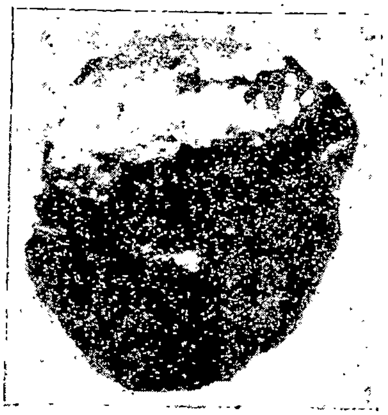


Fig. 2. Photograph of synthetic diamond showing crystal faces (× 135)

The following results show that the diamond is similar to the synthetic crystals from the General Electric Co., Schenectady, New York, which were examined by Grenville-Wells and Lonsdale[5], and by Lonsdale, Milledge and Nave[6]. A complete rotation picture taken in a 6-cm. diameter camera with filtered radiation (Fig. 3) was interpreted in terms of three separate patterns:

(*a*) The dominant pattern consists of spots corresponding in position and intensity to those from a single crystal of natural diamond rotating about $[0\bar{1}1]$ with the 'forbidden reflexions' 200 and 222 absent and weak respectively. Determinations of the *d*-spacings of the spots on the zero layer are given in Table 1 (*a*). All the spots are sharp with small spikes along constant θ arcs; the 222 spot is sharper than the others and does not have spikes showing that it may be caused by simultaneous 'Renninger reflexions'[7] from planes $\bar{1}\bar{1}\bar{1}$ and $\bar{1}33$. The main

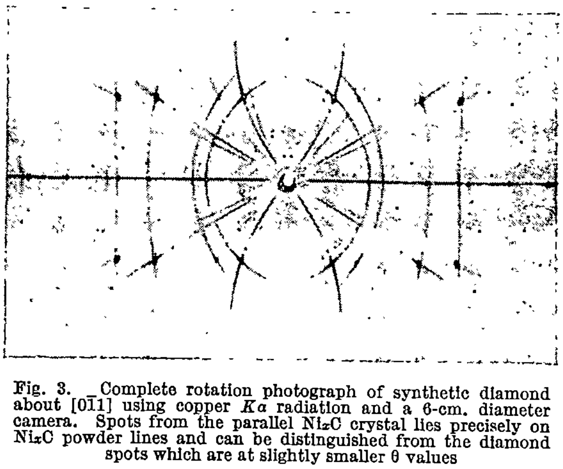


Fig. 3. Complete rotation photograph of synthetic diamond about $[0\bar{1}1]$ using copper $K\alpha$ radiation and a 6-cm. diameter camera. Spots from the parallel Ni$_3$C crystal lies precisely on Ni$_3$C powder lines and can be distinguished from the diamond spots which are at slightly smaller θ values

layer lines arise from a lattice repeat of length $a/\sqrt{2}$ where $a = 3{\cdot}565$ Å.; but there are weak subsidiary layer lines corresponding to another repeat of length $3a/\sqrt{2}$. These extra layer lines come from a small diamond crystal related to the main component by a reflexion twin across $(\bar{1}\bar{1}1)$ which brings the $[41\bar{1}]$ zone axis, with a repeat length of $3a/\sqrt{2}$, into coincidence with $[0\bar{1}1]$ (ref. 6).

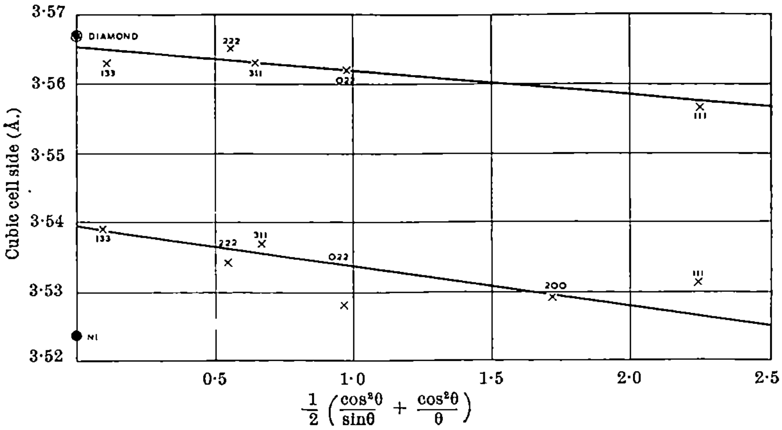


Fig. 4. Extrapolation curves giving accurate lattice parameters of diamond and Ni_xC structures. ⊙, Published lattice parameters for diamond and pure nickel; ×, experimental points with indices of reflecting planes

(*b*) There is another pattern from a cubic single crystal, also rotating about $[0\bar{1}1]$ which has a face-centred lattice exactly parallel to that of the main diamond crystal. The lattice parameter is 3·539 Å., intermediate in value between diamond (3·567 Å.) and face-centred nickel (3·524 Å.). Precise measurements show that the strong 200 and 222 spots on the photograph belong to this pattern; some of the intensity of the strong 222 spot may also arise from a double Renninger reflexion. There is no indication that this crystal is twinned across $(\bar{1}\bar{1}1)$ as in the diamond.

(*c*) A partially oriented powder pattern of phase (*b*) is present with intensities in qualitative agreement with those from nickel (Table 1 (*b*)). Careful measurements of reflexion positions were made on a random rotation photograph from an 11·46-cm. diameter powder camera, and the cell constant given by each reflexion is plotted against the usual extrapolation function in Fig. 4. It is clear that there are two separate sets of powder lines and spots confirming the presence of diamond with $a = 3{\cdot}565 \pm 0{\cdot}003$ Å., and the second cubic phase (*b*) with $a = 3{\cdot}539 \pm 0{\cdot}003$ Å. The interpretation of the smooth powder rings in the centre of the photograph is hindered by the pattern from the shellac used to mount the crystal. In a photograph taken by Dr. H. J. Milledge of another diamond from the same source, one of these smooth rings occurs alone and is almost certainly the 0002 reflexion of graphite. The occurrence of graphite with synthetic diamond is unusual.

In their study of the General Electric diamonds, Lonsdale, Milledge and Nave[6] also report the presence of a second cubic phase with $a = 3{\cdot}539$ Å., but with a different distribution of observed intensities (Table 1 (*b*)). The most important difference is in the relative strengths of the 111 and 200 reflexions. The new set of intensities is consistent with the nickel–carbon compound Ni_xC($x > 4$) (ref. 8), which has a lattice parameter of 3·536 Å. Presumably the carbon atoms are randomly arranged on octahedral sites in the face-centred nickel structure, but it is not necessary to have a random arrangement of nickel atoms as required by the previous intensity observations. It is assumed that this compound is also cubic because no deviations from cubic symmetry could be detected on Laue photographs. The compound is analogous to martensite, γ-Fe, which is a solid solution of carbon in a face-centred iron structure.

Table 1

(*a*) Spacings of diamond spots on the complete rotation photograph, compared with A.S.T.M.* Card 6–0675.

hkl	Present work *d* (Å.)	A.S.T.M.* Card, diamond *d* (Å.)
111	2·054	2·060
022	1·259	1·261
311	1·074	1·0754
222	1·0292	—
400	0·891 approximately	0·8916
133	0·8174	0·8182

(*b*) Spacings and intensities of powder rings of the second cubic phase Ni_xC ($x > 4$) compared with A.S.T.M.* Card 4–0854, 4–0850 for pure nickel.

hkl	Present work *d* (Å.)	I	Lonsdale, Milledge, Nave I	A.S.T.M.* Card, Ni *d* (Å.)	I
111	2·039	*s*	53·8	2·034	100
200	1·765	*s/m*	100	1·762	42
022	1·259	*m*	36·0	1·246	21
311	1·067	*m*	25·4	1·0624	20
222	1·0201	*m/w*	15·5	1·0172	7
400	0·883 (approx.)	*w*	9·6	0·881	4
133	0·8119	*m*	8·8	0·8084	14

* X-ray Powder Data File (1960), American Society for Testing Materials, Philadelphia.

X-ray reflexions on the zero and first layers corresponding to rotation about the $[0\bar{1}1]$ axis were recorded with a Weissenberg camera. The pictures confirmed the results from the rotation photograph and showed the presence of two additional diamond components obtained by reflexion across the twin-planes (111) and $(\bar{1}11)$. The patterns from the twin components have no detectable satellite spots for these exposures, so if any Ni_xC compound is associated with the subsidiary twins, the amount is much smaller than that with the main diamond.

We wish to thank Dr. C. W. Bunn and Mr. W. R. D. Manning for their interest and Dame Kathleen Lonsdale and Dr. H. J. Milledge for valuable advice on and discussion of the interpretation of the X-ray data. Thanks for discussion are also due to Mr. E. A. Cole, Mr. R. P. Palmer and Miss A. Turner-Jones; and for assistance to Mr. J. Andrew, Mr. M. R. E. Ball and Mr. S. F. Bossom of Imperial Chemical Industries, Ltd. We wish to acknowledge the assistance of Mr. M. P. Littleson of the National Engineering Laboratory with the experimental details of the diamond synthesis, and of the National Engineering Laboratory Workshop for the construction of the tetrahedral-anvil apparatus.

This article is published by permission of the Director of the National Engineering Laboratory.

[1] Lloyd, E. C., Hutton, U. O., and Johnson, D. P., *J. Res. Nat. Bur. Stand.*, **63**, *C*, 59 (1959).

[2] Hall, H. T., *Rev. Sci. Instrum.*, **29**, 267 (1958).

[3] Kennedy, G. C., and La Mori, P. N., *Lake George Conf. Very High Pressure Res.* (John Wiley and Sons, 1960).

[4] Bovenkerk, H. P., Bundy, F. P., Hall, H. T., Strong, H. M., and Wentorf, R. H., *Nature*, **184**, 1094 (1955).

[5] Grenville-Wells, H. J., and Lonsdale, K., *Nature*, **181**, 758 (1958).

[6] Lonsdale, K., Milledge, H. J., and Nave, E., *Min. Mag.*, 32, 185 (1959).

[7] Renninger, M., *Acta Cryst.*, **8**, 606 (1955).

[8] Bernier, R., *Ann. Chim.*, **6**, 104 (1951).

GEL FILTRATION OF SEA-WATER: SEPARATION OF FREE AND BOUND FORMS OF VITAMIN B_{12}

By Dr. K. W. DAISLEY*

National Institute for Research in Dairying, Shinfield, nr. Reading

When Porath and Flodin[1] published details of the separation of serum proteins from ammonium sulphate by their new technique of gel filtration, it was evident that this same method might be useful in oceanographic research, where work on the organic substances in sea-water is often hindered by the salts present.

The most sensitive microbiological assay methods for vitamin B_{12} will not function at sea-water salinity. Often, however, the sea-water can be diluted before use[2], but some oceanic waters contain amounts of vitamin B_{12} so small that dilution takes them below the measurable range. Extraction and concentration methods can be applied[3], but they need large volumes of sea-water and take much time and labour.

It was therefore hoped that filtration through 'Sephadex *G*-25' gel (Pharmacia, Uppsala) would separate vitamin B_{12} from salt, but a filter column 72 cm. × 3·6 cm. did not achieve this separation; the vitamin was delayed by the filter to about the same extent as was the salt (Fig. 1).

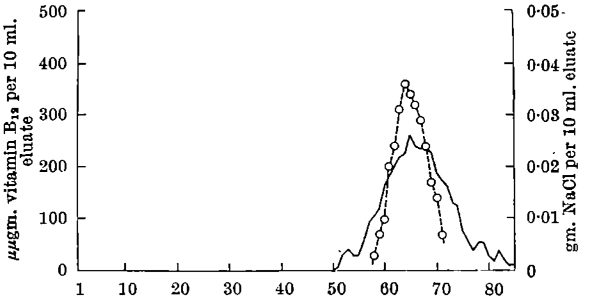


Fig. 1. Gel filtration of 10 ml. 3·5 per cent sodium chloride solution containing 500 μμgm. vitamin B_{12}/ml. Elution by distilled water. Continuous line, vitamin B_{12}; broken line, sodium chloride

There is reason for wanting to obtain separate estimates of the free and the bound forms of vitamin B_{12} in natural waters[4]. To see whether gel filtration would facilitate such separation, a binding agent prepared from pig pyloric mucosa ('Bendogen', G.E.A., Copenhagen) was added to a vitamin B_{12}–sodium chloride solution in an amount sufficient to bind half the vitamin present, and this solution was then passed through the 72 cm. × 3·6 cm. gel filter column. Free and bound forms of the vitamin were clearly separated (cf. Fig. 2 with Fig. 1), the bound form coming through first.

Gel filtration therefore does provide a simple means of separating free and bound forms of vitamin B_{12} before assay, and it can be used in obtaining separate estimates of free and bound vitamin B_{12} in fresh-water samples, and in sea-waters where the vitamin concentration is sufficiently high.

It is when the vitamin B_{12} concentration in sea-water is very low that it becomes essential to separate the vitamin from the salt, so as to permit concentration of the vitamin before assay. The result of the experiment in which a binding agent was added to the vitamin B_{12}-saline solution (Fig. 2) leads to a method of overcoming the failure of the filter to separate free vitamin B_{12} from salt. An excess of binding agent is added to the vitamin B_{12}–saline solution or to the sample of sea-water; the vitamin B_{12} present is thus converted to the bound form, which is then separated from the salt by passage through the gel filter column.

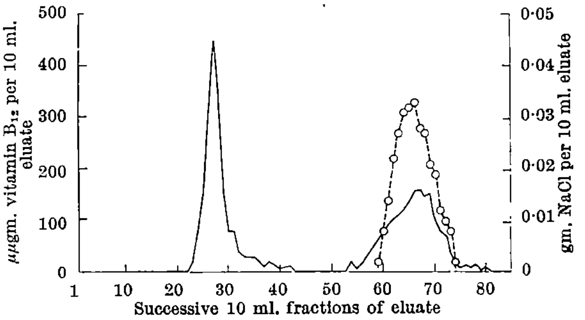


Fig. 2. Gel filtration of 10 ml. 3·5 per cent sodium chloride solution containing 500 μμgm. vitamin B_{12}/ml. and 1 μgm. 'Bendogen'/ml. Elution by distilled water. Continuous line, vitamin B_{12}; broken line, sodium chloride

After this separation, the bound vitamin, in solution in the distilled water which is used for washing it through the column, can be concentrated to the extent necessary to bring the vitamin within the range of the microbiological assay methods. For such concentration, evaporation under reduced pressure can be employed; alternatively, dry 'Sephadex' powder can be stirred into the solution. It takes up more than twice its weight of water in forming the gel, but excludes the bound vitamin B_{12}, which therefore remains in the supernatant liquid and can be poured off after centrifugation. The degree of concentration achieved by addition of the 'Sephadex' powder can be readily observed by dissolving a coloured protein (hæmoglobin) in the solution before the 'Sephadex' is added. This protein is also excluded by the 'Sephadex', and so the increase in optical density of the supernatant liquid after addition of the 'Sephadex' provides a direct measure of the increase in concentration of the coloured protein and therefore also of the vitamin B_{12}. With the degree of concentration thus known, it is not necessary to attempt the difficult complete recovery of the supernatant liquid for the purpose of the assay; measurements on a portion of the supernatant liquid can be recalculated for the whole original sample. If the dry 'Sephadex' is added by known weight, one experiment with the hæmoglobin can serve as a calibration for the whole

---

* Unit for Biochemical Research bearing on Fisheries' Problems (grant-aided by the Development Fund).

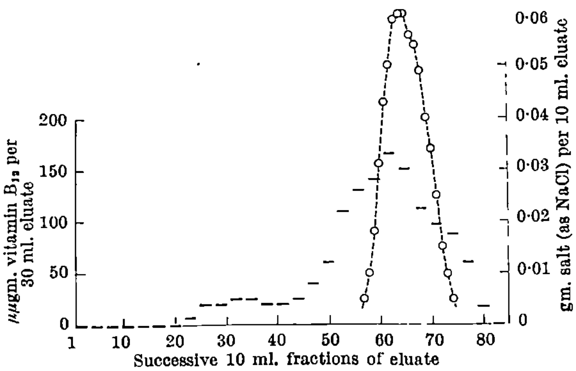


Fig. 3. Gel filtration of 30 ml. sea-water, after storage frozen since collection. Elution by distilled water. Horizontal bars, values for vitamin B_{12} content of eluate fractions combined in threes; broken line, salt (expressed as sodium chloride)

batch of 'Sephadex', and further use of hæmoglobin is unnecessary.

Figs. 3 and 4 illustrate the gel filtration technique, applied to a sea-water sample from Loch Fyne collected on February 15, 1961, by Dr. L. R. Fisher of this laboratory (the Director and Staff of Millport Marine Station are thanked for provision of collecting facilities). In this sample, the vitamin B_{12} was measurable when the eluate fractions were combined in threes; with a less-rich water, the binding agent is added and the gel filtration carried out, but then the fractions predicted to contain the vitamin (20th to 45th with this particular column) are all added together and concentrated before assay. Filtration experiments with different portions of the same water sample, with and without the addition of binding agent, provided evidence on how much of the vitamin B_{12} was originally bound.

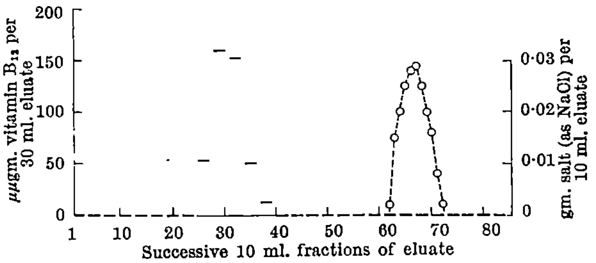


Fig. 4. Gel filtration of 10 ml. sea-water (from the same sample as in Fig. 3) + 20 ml. solution of 'Bendogen' 1 mgm./ml. Elution by distilled water. Horizontal bars, values for vitamin B_{12} content of eluate fractions combined in threes; broken line, salt (expressed as sodium chloride)

In the experiments described, vitamin B_{12} was measured by an appropriately simplified version of the *Euglena* assay previously described[2], and sodium chloride by titration against standard silver nitrate solution.

This work is being extended to other sea-waters and fresh-waters, and to microbiological culture liquors; further results will be published elsewhere.

[1] Porath, J., and Flodin, P., *Nature*, **183**, 1657 (1959).
[2] Daisley, K. W., *J. Mar. Biol. Assoc. U.K.*, **37**, 673 (1958).
[3] Cowey, C. B., *J. Mar. Biol. Assoc. U.K.*, **35**, 609 (1956).
[4] Daisley, K. W., *Nature*, **180**, 1042 (1957).

INFLUENCE OF MOISTURE CHANGES ON DEFORMATION OF WOOD UNDER STRESS

By L. D. ARMSTRONG and G. N. CHRISTENSEN

Division of Forest Products, Commonwealth Scientific and Industrial Research Organization, Melbourne

RECENTLY it was reported[1] that creep in wooden beams was markedly increased when their moisture content changed while they were under load. In addition, it was found (Gottstein, J. W., Moglia, P. J., and Stashevski, A., private communication) that if veneers were bent to a predetermined extent and held there under conditions of either constant or decreasing moisture content, the residual deflexion after releasing the veneers was much greater in the latter case, and nearly proportional to the moisture content change over the range studied. It was considered possible that these effects resulted from interaction between the applied stress and stresses accompanying the differential shrinkage arising from moisture gradients. An alternative explanation is that the rheological properties of the wood itself were affected transiently during moisture content changes.

A distinction between these two possibilities may be made by studying very small specimens, as it has been shown[2,3] that, in specimens less than approximately 1 mm. in thickness, the rate of sorption in the absence of air no longer appears to be controlled by diffusion of moisture through the wood. Consequently, moisture changes may occur without development to the same extent of moisture gradients and stresses due to differential shrinkage of the type found in beams of larger size. (Some gradients must, nevertheless, occur within the wood cell wall as moisture movement takes place.)

The influence of changes of moisture-content on the deformation of wood under bending load has therefore been studied further using very small specimens as well as larger beams. In the present tests, the larger beams were of *Eucalyptus gigantea* of similar dimensions to those used previously (90 × 2 × 2 cm.) and were tested in an air-conditioned cabinet. The small specimens were of klinki pine (*Araucaria klinkii*, 6 × 0·1 × 0·1 cm.) and were set up in an evacuable apparatus. As well as reducing drying stresses, the use of small specimens permitted much faster, and more readily controlled, changes in moisture content. In a series of experiments, cyclic changes in moisture content were effected with the specimens loaded or unloaded as desired. Bending loads of less than 25 per cent of the short-term breaking load were used. From experiments using small beams several phenomena have been observed, and

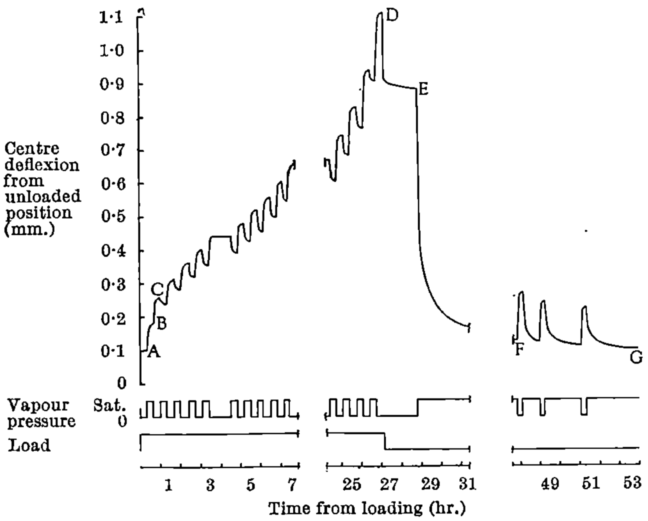


Fig. 1. Effect of changes in moisture content on bending of wood under load

these are described here. Qualitatively similar behaviour was displayed by the 2-cm. beams where-ever comparable tests were made. Some of the phenomena are illustrated by the results from one of the 1-mm. specimens in Fig. 1.

(1) When the specimen was loaded in the completely dry state, very little creep occurred within a short time of loading (Fig. 1, *0A*).

(2) If, after loading the specimen in the dry state, water vapour at saturation pressure was admitted (Fig. 1, *AB*), the deflexion increased rapidly to almost twice the initial deflexion; the rate of deflexion decreased rapidly, usually falling to a very low value within 2–3 hr. From sorption kinetics[2], it would be expected that sorption of water would have been virtually completed within this time. In the larger beams, where the sorption process was slower, similar behaviour occurred over a period of about 50 hr.

(3) When, during the first adsorption of water vapour by a loaded sample, the rate of admission of vapour to the sample was reduced (taking several hours to reach saturation vapour pressure), it was found that the final deflexion of the sample after saturation had been reached was not affected by the slower sorption-rate. In the case of the 2-cm. beams, observation of the effect of rate of change of moisture content was confined to different rates of drying from the green state, no difference being found in the final deformation reached on drying to the same moisture content over one and two weeks respectively.

(4) On re-drying the loaded specimen, the deflexion increased further (Fig. 1, *BC*), but generally by a smaller amount than during the first adsorption.

(5) During subsequent moisture content cycles, the loaded specimen showed partial recovery during each adsorption followed by increased deflexion during each desorption (Fig. 1, *CD*). Deflexions several times the instantaneous elastic deflexion were thus ultimately reached. Such large deflexions could not generally be obtained under short-term loads at constant moisture content without breaking the specimen. Similar phenomena were demonstrated by the 2-cm. beams when the moisture content was cycled between 12 and 22 per cent of dry weight.

(6) If, during moisture content cycling under load, the range of change of vapour pressure was made narrower, the changes in deflexion during each complete cycle were much less. Nevertheless, a decrease in deflexion for each absorption and an increase for each desorption were still found for each cycle after the first.

(7) If the deformed specimen was unloaded in the dry condition, little recovery other than the initial elastic response occurred within an hour (Fig. 1, *DE*). However, on admitting water vapour at saturation pressure after unloading in the dry state (Fig. 1, *EF*), a large recovery occurred.

With the 2-cm. beams, nearly half the deflexion was recovered within a month of unloading in the dry state. The greater part of the remaining deformation was recovered on soaking the beams in water for five days.

(8) When further moisture content cycling occurred after unloading, the recovery was enhanced slightly (Fig. 1, *FG*), but complete recovery did not appear to be possible by vapour adsorption and drying alone. It was noted that, during this cycling, marked changes in deflexion of the specimen occurred, whereas there were no such changes in a specimen which had not previously been loaded.

The result of these investigations is to confirm that when wood is subjected to a sustained bending load, a change in moisture content affects the rate and magnitude of deformation. Most of the change in deformation occurs within the period during which moisture content change takes place. Its magnitude depends mainly on the extent rather than the rate of moisture content change. It is not known whether these phenomena result from an acceleration of normal creep behaviour (at constant moisture content) or whether different mechanisms are involved. However, as similar effects are observed with beams of 1 mm. and 2 cm. size, stresses arising from non-uniform shrinkage are not likely to be responsible. It is possible that large transient stresses[2] are introduced into the molecular network as the moisture content changes. Under the bias of an external force, deformation may thus occur more readily for as long as the moisture change continues.

In the cycling experiments (as in Fig. 1), the decrease in deformation that accompanies all adsorptions after the first may arise from an additional process. This might be a relative change in the longitudinal components of swelling of the tension and compression portions of the beam as its deformation increases. The persistence of the effect after the deformed beam has been unloaded supports such an explanation. The large recovery obtained during the first increase of moisture content after unloading suggests that the deformed beam contains a proportion of strained but stable elements of structure sufficient to provide a restoring bias when the external force is removed.

The magnitude of the effect of the moisture content changes on deformation points strongly to the necessity for avoiding such changes not only during long-term rheological testing of wood but also during short-term mechanical testing.

[1] Armstrong, L. D., and Kingston, R. S. T., *Nature*, **185**, 862 (1960).

[2] Christensen, G. N., and Kelsey, Kathleen E., *Holz als Roh- u. Werkstoff*, **17**, 178 (1959).

[3] Christensen, G. N., *Austral. J. App. Sci.*, **11**, 295 (1960).

DISTRIBUTION OF ISOTOPIC NITROGEN IN NITROGEN GAS DURING DENITRIFICATION

By R. D. HAUCK and D. R. BOULDIN

Division of Agricultural Relations, Tennessee Valley Authority, Wilson Dam, Alabama

NITROGEN gas, formed as a result of denitrification in soil to which isotopically enriched nitrate–nitrogen had been added, was shown[1] to retain its identity although it was evolved into a confined atmosphere already containing nitrogen gas. The atoms of nitrogen-14 and nitrogen-15 in the original atmosphere were randomly distributed among the molecules of nitrogen gas. Presumably, the atoms of nitrogen-14 and nitrogen-15 in the nitrogen gas which was formed as a result of denitrification also were randomly distributed among the molecules of this enriched gas.

However, the data presented in refs. 1 and 2 indicate that when two sources of nitrogen gas with different contents of nitrogen-15 are mixed under the conditions of ordinary biological experimentation, the nitrogen-14 and nitrogen-15 atoms in the whole mixture are no longer distributed randomly among the nitrogen molecules, because isotopic exchange among the various species of molecules does not proceed at an appreciable rate. The fact of this non-equilibrium condition was used to calculate the amount of nitrogen evolved from the substrate, as well as to allow certain inferences about the mechanism of gas formation.

Equations were derived[1] which expressed the fraction of nitrogen gas in the atmosphere which was derived from the added, enriched nitrate–nitrogen as a function of the mass spectrometric ion currents at masses 28, 29 and 30, and the atom fractions of nitrogen-15 in both the original atmospheric gas and in the enriched nitrate source. Implicit in the equations was the assumption that all the nitrogen evolved from the added nitrate–nitrogen had the same atom fraction of nitrogen-15 as the original source material. Any nitrogen from other sources which pooled with the added nitrogen and was denitrified afterwards, could not be considered accurately, once the experiment was begun.

The equations presented here permit calculation of the mole-fraction of nitrogen gas which is evolved from soil into an atmosphere containing nitrogen even though the amount of nitrogen-15 in the source material changes as a result of mixture with other soil nitrogen. The basis of these equations has already been discussed[1]. A brief restatement of some of the definitions and equations contained in that article will be appropriate here.

The mass spectrometer determines the ratios r and r', which are defined as follows: r, corrected ratio of ion currents at mass 29 to ion currents at mass 28; r', corrected ratio of ion currents at mass 30 to sum of ion currents at masses 28 and 29.

The mole fractions of the various molecular species of nitrogen are given by:

$$a + 2b + c = 1$$

where a = mole fraction of $^{14}\mathrm{N}^{14}\mathrm{N}$; $2b$ = mole fraction of $^{14}\mathrm{N}^{15}\mathrm{N}$; c = mole fraction of $^{15}\mathrm{N}^{15}\mathrm{N}$. hence:

$$r = 2b/a \tag{1}$$

$$r' = \frac{c}{a + 2b} \tag{2}$$

The nitrogen isotope fractions are represented by the following symbols: X^{14}_{N}, atom fraction of nitrogen-14 in the material undergoing denitrification; X^{15}_{N}, $(1 - X^{14}_{\mathrm{N}})$ = atom fraction of nitrogen-15 in the material undergoing denitrification; X^{14}_{A}, atom fraction of nitrogen-14 in the original atmosphere; X^{15}_{A}, $(1 - X^{14}_{A})$ = atom fraction of nitrogen-15 in the original atmosphere.

Let d be the fraction of the nitrogen gas (in the atmosphere at the time of sampling) which is derived from the isotopically enriched substrate, and let $(1 - d)$ be the fraction of the nitrogen gas (in the atmosphere at the time of sampling) which was present in the atmosphere originally.

When two sources of nitrogen gas, each with different atom fractions of nitrogen-15 randomly distributed among the molecules of nitrogen, are mixed, the resulting mole fractions are given by equations (3), (4) and (5) as functions of the atom fraction of nitrogen-15 in the two sources (X^{15}_{N} and X^{15}_{A}) and the mole fractions (d and $(1 - d)$) of the resulting mixture derived from the two sources:

$$a = (1 - d)(X^{14}_{A})^2 + d(X^{14}_{\mathrm{N}})^2 \tag{3*}$$

$$2b = 2(1 - d)(X^{14}_{A}X^{15}_{A}) + 2d(X^{14}_{\mathrm{N}}X^{15}_{\mathrm{N}}) \tag{4*}$$

$$c = (1 - d)(X^{15}_{A})^2 + d(X^{15}_{\mathrm{N}})^2 \tag{5*}$$

Equations (3), (4) and (5) can be combined and rewritten in terms only of r, r', X^{14}_{N}, X^{14}_{A} and d, as follows:

$$r = \frac{2(1 - d)(X^{14}_{A})(1 - X^{14}_{A}) + 2d(X^{14}_{\mathrm{N}})(1 - X^{14}_{\mathrm{N}})}{(1 - d)(X^{14}_{A})^2 + d(X^{14}_{\mathrm{N}})^2} \tag{6}$$

$$r' = \frac{(1 - d)(1 - X^{14}_{A})^2 + d(1 - X^{14}_{\mathrm{N}})^2}{(1 - d)(X^{14}_{A})^2 + d(X^{14}_{\mathrm{N}})^2 + 2(1 - d)(X^{14}_{A})\,(1 - X^{14}_{A}) + 2d(X^{14}_{\mathrm{N}})(1 - X^{14}_{\mathrm{N}})} \tag{7}$$

Equations (6) and (7) contain two unknowns, X^{14}_{N} and d. The value of X^{14}_{A} is obtained by direct mass spectrometric determinations of atmosphere nitrogen samples; r and r' are primary analytical data. No assumptions have been made about the source material characterized by X^{14}_{N} (or X^{15}_{N}). The symbol d specifically refers to the mole fraction of total atmospheric nitrogen which was derived from the source X^{14}_{N}.

The value for X^{14}_{N} can be obtained from the quadratic relation:

---

* Corresponding to equations (6), (7) and (8), respectively, in ref. 1.

$$X^{14}{}_{\mathrm{N}} = \frac{-B \pm \sqrt{B^2 - 4AP}}{2A} \tag{8}$$

where $A = P(r' + 1) + R(r + 2)$, $B = -2[P(r' + 1) + R]$ and $P = 2(X^{14}{}_A)(1 - X^{14}{}_A) - r(X^{14}{}_A)^2$, $R = (1 - X^{14}{}_A)^2 - r'[2(X^{14}{}_A)(1 - X^{14}{}_A) + (X^{14}{}_A)^2]$.

Using this value for $X^{14}{}_{\mathrm{N}}$, d may be calculated from the expression:

$$d = \frac{P}{(r + 2)(X^{14}{}_{\mathrm{N}})^2 - 2X^{14}{}_{\mathrm{N}} + P} \tag{9}$$

A value for d can be calculated from equations (3), (4) or (5), as well as from equation (9). The difference between the two methods of calculation lies in the fact that with equations (3), (4) or (5), as previously used[1], the value for $X^{14}{}_{\mathrm{N}}$ (or $X^{15}{}_{\mathrm{N}}$) must be measured independently at the beginning of the experiment (or from sub-samples of soil during the experiment), and may not change with time, while the $X^{14}{}_{\mathrm{N}}$ used in equation (9) is an average value calculated from mass spectrometric analysis of an atmosphere into which an admixture of gaseous components was made. If no dilution of the original and supposed source material occurs, then either equations (3), (4) or (5) or equation (9) will accurately calculate the mole fraction of nitrogen gas evolved from the substrate $X^{14}{}_{\mathrm{N}}$. However, should dilution occur, then this change in isotope content of the source material will be quantitatively reflected in the value for $X^{14}{}_{\mathrm{N}}$ as obtained (equation (8)) from the measured ratios r and r'.

Table 1. CALCULATION OF X^{15}N AND MOLE FRACTION (d) OF TOTAL NITROGEN THAT WAS EVOLVED FROM ADDED NITRATE ASSUMING EITHER DILUTION OF ADDED NITRATE OR NO DILUTION

Exp. No.	Incubation period (days)	Initial X^{15}N	Calculated* X^{15}N	d_1†	d_2‡
8*a*	19	0·3470	0·3398	0·0183	0·0190
8*a*	19	0·3470	0·3397	0·0074	0·0076
12*a*	4	0·3470	0·347–	—	—
12*a*	14	0·3470	0·3439	0·0152	0·0154
12*a*	22	0·3470	0·2735	0·0364	0·0579
13	22	0·3470	0·2889	0·0114	0·0147
14	19	0·2395	0·2164	0·0514	0·0569

* Atom fraction of ^{15}N in enriched nitrate calculated from equation (8).

† Mole fraction of nitrogen evolved from source, assuming no dilution.

‡ Mole fraction of nitrogen evolved, calculated from equation (9).

Some data selected from a previous paper[3] are re-interpreted using equations (8) and (9) and presented in Table 1, to illustrate the effect of dilution of source material on the value of d obtained. Values for $X^{15}{}_{\mathrm{N}}$, that is $(1 - X^{14}{}_{\mathrm{N}})$ are also given.

The results from experiment 12*a* (Table 1) illustrate the progressive dilution of nitrate source material (characterized by the calculated value for $X^{15}{}_{\mathrm{N}}$) with time. Although it is not the object of this article to draw conclusions concerning the interplay of mineralization and denitrification reactions in soil, the observation nevertheless can be made that little dilution of the nitrate substrate was observed within the first week of incubation, whereas under the conditions of the experiments from which the data were taken, appreciable dilution occurred about two to three weeks after the beginning of incubation. The calculated value for $X^{14}{}_{\mathrm{N}}$ (or $X^{15}{}_{\mathrm{N}}$) represents the average isotope content of the material which is undergoing denitrification over a given period of time. In this respect, it approximates the composition of a composite source material integrated with time. The calculated value for $X^{14}{}_{\mathrm{N}}$ will become increasingly more important in the estimation of d as mineralized nitrogen pools with the added, enriched material. The accuracy with which the value of $X^{14}{}_{\mathrm{N}}$ characterizes the source of the evolved nitrogen gas increases as both rate of dilution and denitrification approach constancy.

In summary, the equations presented above will permit calculation not only of the amount of nitrogen gas evolved into a nitrogen-containing atmosphere as a results of denitrification, but will give as well a quantitative estimate of the relative contributions to the evolved gas by both the original source and by other nitrogen-containing material in soil from a consideration only of mass spectrometric data obtained from the atmosphere above the soil.

[1] Hauck, R. D., Melsted, S. W., and Yankwich, P. E., *Soil Sci.*, **86**, 287 (1958).

[2] Rittenberg, D., *Symp. Preparation and Measurement of Isotopic Tracers*, 31 (J. W. Edwards, Ann Arbor, Michigan, 1946).

[3] Hauck, R. D., and Melsted, S. W., *Soil Sci. Soc. Amer. Proc.*, **20**, 361 (1956).

CALCIUM METABOLISM IN MAN WITH CALCIUM-45: MALABSORPTION SYNDROME AND EXUDATIVE ENTEROPATHY

By DR. G. MILHAUD and DR. P. VESIN

Laboratoire des Isotopes, Institut Pasteur and Service de Médecine,
Hôpital Saint Antoine, Paris

SEVERAL diseases of the small intestine are accompanied by such manifestation of impaired calcium metabolism as hypocalcæmia, hypocalciuria, tetany and osteoporosis. We have investigated changes in calcium metabolism in subjects with malabsorption syndrome and exudative enteropathy. The malabsorption syndrome (non-tropical sprue) is characterized by fatty diarrhœa and impaired absorption of several substances, including fats, carbohydrates, lipo-soluble vitamins and iron. In exudative enteropathy, described by Gordon[1] and Schwartz[2], one observes diarrhœa of variable severity, intestinal leakage of serum proteins, hypoproteinæmia and œdema; impairment of intestinal absorption is not constant.

The syndromes can be differentiated by studying the results of the intravenous injection of serum albumin-^{131}I and of polyvinylpyrrolidone-^{131}I (PVP-^{131}I). Fœcal radioactivity is marked in exudative enteropathy and negligible in sprue.

We have studied four patients with either the malabsorption syndrome or exudative enteropathy.

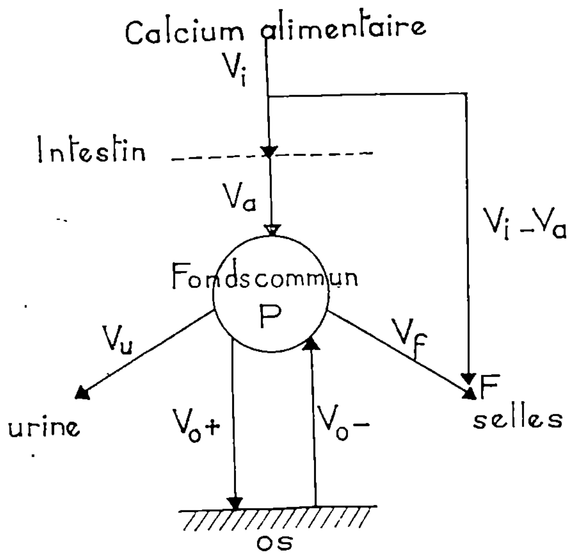


Fig. 1. Scheme of calcium metabolism

In all, radiological examination of the small intestine was typical: dilatation or hypertony of loops and dilution or scattering of barium. Two patients showed disorders of calcium metabolism.

Examination of calcium metabolism was carried out with calcium-45, using a method described previously[3,4]. Steatorrhœa was demonstrated by the triolein-^{131}I test and protein loss into the intestine was determined indirectly by means of the PVP-^{131}I test.

Case 1 (No. 110)[5]: a 31-yr.-old man, weighing 71 kgm. with exudative enteropathy. Had observed lower limb œdema and ascites for 9 yr. Total serum protein was 4·5 gm./100 ml. Steatorrhœa was marked and the PVP-^{131}I test was strongly positive. The patient complained of muscular spasms, paræsthesias and headaches. Serum calcium was 4·5 mgm. per cent, and there was marked hypocalciuria. A roentgenogram showed a calcification of the pericardium.

Case 2 (No. 121)[6]: a 14-yr.-old girl, who weighed 43 kgm., suffered from exudative enteropathy. For 6 months lower limb œdema and ascites have been present; total serum proteins were 2·7 gm./100 ml. Steatorrhœa and a very positive PVP-^{131}I test were found. The patient complained of paræsthesias and tetany; serum calcium was 6·8 mgm. per cent and there was marked hypocalciuria.

Case 3 (No. 128): a 40-yr.-old man weighing 51 kgm. had malabsorption syndrome and mild œdema. Serum protein was 5·5 gm. per cent. Marked steatorrhœa and a PVP-^{131}I test typical of exudative enteropathy were found. The oral glucose tolerance curve was flat. Serum and urinary calcium were normal and there were no clinical or radiological signs of disturbed calcium metabolism.

Case 4 (No. 117): a 70-yr.-old man, weighing 52 kgm., had experienced the onset of the malabsorption syndrome 7 months before admission. Steatorrhœa was marked and the oral glucose tolerance curve was flat. The induced hypersideræmia curve was low. The PVP-^{131}I test was normal. Serum proteins was 7 gm. per cent. There was neither œdema nor clinical or radiological signs of disturbed calcium metabolism. Serum and urinary calcium were normal.

Calcium metabolism can be represented by the scheme shown in Fig. 1. The various symbols have the following meaning: V_i, calcium ingested per time unit; V_a, calcium absorbed by the intestine; V_u, calcium excreted in the urine; V_f, calcium excreted in the fæces; V_{0+}, calcium deposited in bone; V_{0-}, calcium released from bone; Δ balance.

In addition, the exchangeable calcium pool P can be considered to include four compartments of different sizes. One corresponds to serum calcium, M, which exchanges with the calcium of the three other compartments at different rates. These three compartments can be identified respectively in first approximation as extracellular fluid calcium, M_1, exchangeable soft tissue calcium, M_2, and with exchangeable bone calcium, M_3 (Fig. 2).

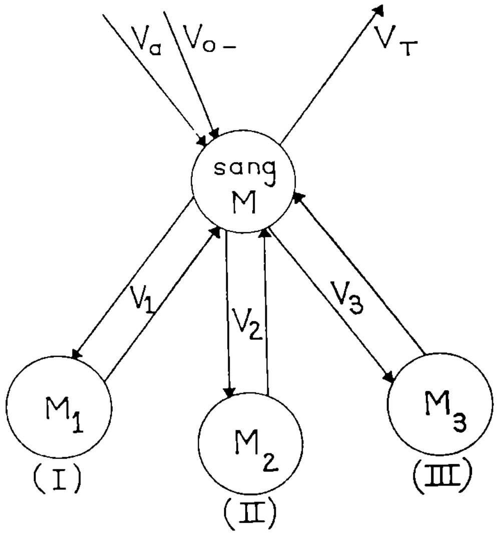


Fig. 2. The four compartments of the exchangeable calcium pool

Table 1 shows principal values of calcium metabolism for the four patients. In the first two cases, the disturbances of calcium metabolism are marked; in addition to hypocalcæmia and hypocalciuria, the values of the exchangeable calcium pool and of the rate of bone fixation represent, respectively, 50 and 60 to 70 per cent of the normal. On the other hand, the rate of osteolysis is somewhat increased, and the balance is strongly negative (– 0·544 gm./day and – 0·253 gm./day).

In cases 128 and 117, serum and urinary calcium are normal and the patients are in the balance. However, the exchangeable calcium pool and the rates of bone formation and of bone destruction are decreased.

The problems presented by the first two patients made it necessary to ascertain the role of the intestinal disease in the negative calcium balance. Intestinal malfunction may result in: (1) diminished absorption of calcium; (2) increased secretion of calcium; (3) a combination of both.

In case No. 110, the first factor applied, since no absorption of calcium could be demonstrated. However, this factor was not sufficient to account for the abnormalities found, because when the patient was studied again, following one month of weekly intramuscular injections of 15 mgm. of vitamin D, absorption became normal, but the excretion of calcium, though slightly lowered, was still abnormally

Table 1. CALCIUM METABOLISM IN FOUR PATIENTS WITH EXUDATIVE ENTEROPATHY OR MALABSORPTION SYNDROME

Number of case report	No. 110	No. 110 after vitamin D and calcium	No. 121	No. 128	No. 117
Age (yr.)	31	31	14	40	70
Height (m.)	1·76	1·76	1·61	1·72	1·67
Body-weight (kgm.)	71	81	43	51	52
Blood vol. (l.)	6·84	6·05	3·32	5·06	4·7
Plasmatic vol. (l.)	3·58	3·05	1·63	2·83	3·24
Calcæmia (mgm. per cent)	4·5	7·1	6·8	9·4	10·8
Exchangeable calcium pool (gm.)					
M	0·161	0·216	0·110	0·266	0·350
M_1	0·340	0·356	0·380	0·182	0·375
M_2	0·528	1·778	0·810	0·995	0·998
M_3	2·856	5·702	1·790	2·781	1·573
P	3·885	8·052	3·090	4·224	3·296
Intest. abs. (gm./day)					
V_i	1·062	0·814	0·633	0·908	0·829
F	1·616	0·659	0·876	0·685	0·714
V_a	0	0·507	0·237	0·358	0·282
Balance	−0·554	+0·145	−0·253	+0·002	+0·015
Abs. rate (per cent)	0	62	37	39	34
Excretion (gm./day)					
V_u	0·010	0·010	0·010	0·221	0·100
V_f	0·544	0·352	0·480	0·135	0·167
Osteoblastosis and osteolysis (gm./day)					
V_{0+}	0·540	1·788	0·630	0·510	0·713
V_{0-}	1·094	1·643	0·883	0·508	0·698

high. In case No. 121, the second factor was involved because the intestinal absorption of calcium was found to be in the lower range of normal values.

Case No. 128 also had a pathological PVP-^{131}I test, but differed from the two preceding in that the œdema and hypoproteinæmia were recent. However, his intestinal excretion of calcium was normal, a finding consistent wih the normal serum calcium and the absence of clinical or radiological signs of disturbed calcium metabolism. In case No. 117. fæcal calcium excretion was increased as compared with the normal; intestinal absorption was normal. These results are represented in Fig. 3, which shows the relation between the amount of calcium absorbed, V_a, and that excreted, V_f, by the intestine in the four patients with small intestine diseases.

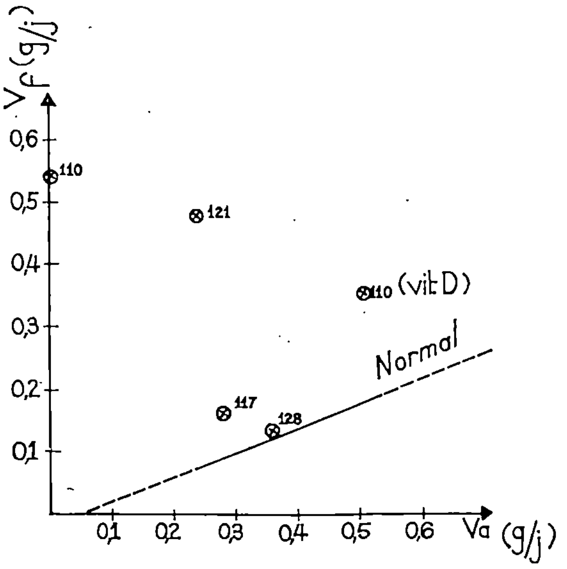


Fig. 3. Relation between the intestinal excretion of calcium, V_f, and the absorption, V_a, in the malabsorption syndrome and exudative enteropathy

It is apparent in cases No. 110 and 121 that the ratio of excreted calcium to absorbed calcium is abnormal. The case of malabsorption, No. 117, had a normal absorption and a slight disturbance of fæcal calcium excretion, observations which are consistent with the absence of clinical or radiological signs of disturbance of calcium metabolism. Table 2 summarizes the studies of absorption and excretion in the four patients.

Table 2. DISTURBANCES OF DIGESTIVE ABSORPTION AND INTESTINAL EXCRETION IN EXUDATIVE ENTEROPATHY AND MALABSORPTION SYNDROME

Case report	Absorption		Fæcal excretion of PVP-^{131}I (per cent injected radioactivity)	Absorption of calcium (gm. day)	Excretion of calcium (gm. day)	Balance
	Fat (trioleine-^{131}I) (per cent)	Carbohydrates (oral glucose tolerance test)				
110	82·5	normal	10·7	0	0·544	negative
121	51·2	normal	14·6	0·237	0·480	negative
128	50·0	impaired	5·5	0·358	0·135	balanced
117	69·5	impaired	1·2	0·192	0·167	balanced
Normal control	> 95	normal	< 1·5	0·25 to 0·50	0·100 to 0·117	balanced

The case of non-tropical sprue which Laszlo *et al.* studied using a different method showed hypocalcæmia and hypocalciuria. The fæcal excretion of calcium was very high and the ratio V_f/V_a similar to that found in our cases of exudative enteropathy. It is possible that this case, known as non-tropical sprue, was actually either a case of exudative enteropathy as the hypoproteinæmia and the high fæcal nitrogen excretion suggested, or a transition form similar to case 128, as suggested by the disturbed glucose absorption.

It should be noted that the injection of vitamin D and the oral administration of calcium profoundly changed the metabolism of calcium in case 110: the exchangeable calcium pool doubled, the rate of bone formation trebled, and the balance became positive. At the same time the paræsthesias, cramps and headaches disappeared.

We are indebted to Dr. J. P. Aubert for the mathematical analysis of the data and to Prof. Renault for the PVP-^{131}I and trioleine-^{131}I test.

[1] Gordon, R., *Lancet*, i, 325 (1959).
[2] Schwartz, M., and Jarnum, S., *Lancet*, i, 327 (1959).
[3] Milhaud, G., and Aubert, J. P., *La Semaine des Hôpitaux, Pathologie et Biologie*, 405 (1958).
[4] Aubert, J. P., and Milhaud, G., *Biochim. Biophys. Acta*, **39**, 122 (1960).
[5] Vesin, P., Renault, H., and Cattan, R., *Bull. Mém. Soc. Méd. Hôp. Paris*, **75**, 319 (1959).
[6] Vesin, P., Trupel, S., Acar, J., Renault, H., Desbuquois, J., and Cattan, R., *Bull. Mém. Soc. Méd. Hôp. Paris*, **76**, 261 (1960).
[7] Fink, V., and Laszlo, D. A., *Gastroenterology*, **32**, 689 (1957).
[8] Milhaud, G., Aubert, J. P., and Bourichon, J., *La Semaine des Hôpitaux Pathologie et Biologie* (in the press).

USE OF RADIO-ISOTOPES IN BIOLOGICAL WORK IN BURMA

By Dr. MAUNG MYA THAUNG

Department of Agricultural and Biological Applications, Union of Burma Atomic Energy Centre

THE post-war development of atomic energy has had a tremendous impact on the thought and plans of many nations. The majority of small under-developed countries, including Burma, have been prompted to embark wisely on programmes of development with the view of enjoying the fruits of social, economic and scientific advancement which the atom might yield for humanity. Atomic energy, dedicated entirely to peaceful purposes, seems to hold the key to progress.

Burma is a recently independent neutral country with a population of about twenty million in an area of 261,789 square miles. Since nearly 75 per cent of the whole population is undernourished and disease-ridden, it seems natural and logical that an effort be made first in the domains of health and wealth, that is, in medical and agricultural applications, for producing tangible benefits in the none too distant future.

The installation at the Department of Radiotherapy of the Rangoon General Hospital early in 1958 of a 1,000-c. 'Theratron Junior' cobalt-60 (1·3 MeV.) teletherapy unit from Canada under the Colombo Plan had undoubtedly stimulated the interest of Burmese medical men (comprising one doctor for 10,000 people) in the medical exploitation of atomic energy. A few Burmese medical practitioners have made an observation tour of the United States to become acquainted with some clinical applications of radioisotopes. Furthermore, two Burmese medical graduates were awarded International Atomic Energy Agency fellowships for training in radio-isotope techniques and radiology in the United Kingdom.

The visiting radiologists were provided by Canada under the Colombo Plan until June 1960. The hospital radiotherapist and physicist on the staff of the Department of Radiotherapy were trained in Canada on Colombo Plan fellowships. The gamma unit has been doing excellent work, especially in the treatment of numerous tumour cases, mostly cancer of the mouth, pharynx, larynx, nose, breast, and cervix. Besides, both normal X-ray and radium needle facilities are available in the Department. Neither medical diagnostic nor therapeutic work with unsealed sources of radio-isotopes is performed in Burma at present.

Some pioneering work of mine was carried out on the utilization of radiation and radioisotopes as radiomutagens and radiotracers in rice plants[1]. I investigated the effects of low levels of nuclear radiations, namely, 0 r., 500 r., 1,000 r., and 1,500 r. of gamma rays from a sealed cobalt-60 source and 0 μc., 748·36 μc., 1,517·92 μc., and 2,288·54 μc. of beta rays from phosphorus-32 in a labelled fertilizer, on the development and productivity of rice plants from two varieties of Burmese rice, *C*33-18 and *D*17-88. The results obtained suggested that low levels of gamma and beta rays influenced grain yield. The intervarietal difference in response to an equal dose of the same kind of nuclear radiation was also noted.

I used a total of 18·8 mc. of phosphorus-32 in the form of ammonium dihydrogen orthophosphate imported from the United Kingdom as a tagged fertilizer to study the effect of time on grain yield and phosphorus uptake by rice plants from soil and fertilizer at three varying levels of fertilizer applications, made separately 9, 54 and 80 days after transplanting. During the first 7–9 weeks after transplanting, plants increasingly absorbed more fertilizer phosphorus than soil phosphorus; from that point onwards, the reverse was true at all levels of fertilization studied. From the ninth to tenth week the trend of relative absorption of phosphorus by plants from both sources appeared to be almost similar in spite of differences in time of fertilization. During weeks 7–9, the phosphate-supplying capacity of the soil of transplanted plot dropped to a low point, but increased after that until, during the flower primordial initiation and floral emergence stages, it reached peak values.

The best yield, found to result from fertilization 9 days after transplanting, was apparently due to an early and efficient plant utilization of more fertilizer phosphorus and also ammoniacal nitrogen, by which growth and reproduction were improved. It was suggested that fertilization with ammonium dihydrogen orthophosphate within 9 days after or during transplanting would produce beneficial effects on yield of rice.

I also initiated a radiation plant-breeding programme by having four varieties of Burmese rice, namely, *A*28-81, *B*24-92, *C*33-18 and *D*17-88 bombarded each with thermal neutron fluxes of 6·92 × 10^8/cm.^{2}/sec. and 6·70 × 10^8/cm.^{2}/sec. in the graphite reactor of the Brookhaven National Laboratory, for 15 and 20 hr., respectively. The radioactivity thus induced in the grains was allowed to become dissipated completely before return shipment. Many and varied radiobiological effects in plants of the first generation (*N*1) were recorded and reported. Transplants of *N*1 generation raised in 1959–60 were isolated by bagging to prevent cross-pollination. Similar observations and screening for seemingly useful mutants in the second generation (*N*2) were made during 1960–61. In addition, X-rays were also used at the same time to treat seeds from five other varieties of rice. Hence, *R*1 and *R*2 generations were obtained from treated seeds.

The policy of the Union of Burma Atomic Energy Centre (UBAEC) has been to co-operate with interested parties until these have had sufficient practical experience to use isotopes; the Agricultural Research Institute (ARI) has now been asked to take over radiotracer work on their own.

The International Atomic Energy Agency specialist and his co-workers have been using autoradiographic methods to estimate qualitatively the phosphate-supplying capacity of soils from ten different areas by using rice as an indicator plant. It seems that, if a soil is relatively rich in native phosphate, rice plants will absorb more soil phosphorus than added phosphorus; the reverse will be true if the native phosphate content of the soil were low.

This is an account of the practical use of radioisotopes in biological sciences in Burma so far. Attempts are being made to introduce and encourage the uses of radioisotopes in other aspects of biological research.

---

[1] Thaung, M. M., *Nature*, **186**, 982 (1960); *Soil Sci.*, **90**, 284 (1960); *Nature*, **190**, 242 (1961); *Bur. Agric. J.*, 1 (1961) (in the press).

A COMPARISON OF COD AND BOVINE INSULINS

By Dr. S. WILSON and Dr. G. H. DIXON

Connaught Medical Research Laboratories and Department of Biochemistry, University of Toronto

THOSE mammalian insulins the structures of which have been examined are very similar and show only minor differences in amino-acid sequence[1,2], but little is known about insulins from other vertebrates.

Extracts of islet tissue from several species of cartilaginous and bony fishes have been found to possess the physiological properties characteristic of insulins prepared from mammalian sources[3]. It was therefore of interest to purify insulins from various orders of fish in order to study their properties. The work to be described yields comparisons between certain structural features of fish and mammalian insulins and provides evidence that there are large differences in the amino-acid sequences of insulins from these two classes of vertebrates.

Preparations of insulin were obtained from the principal islets of seven species of bony fish. Like mammalian insulins, these materials caused convulsions in starved mice[4] and promoted glycogen synthesis in the isolated mouse diaphragm[5]. Four of the fish insulins (from burbot, *Lota lota lacustris*; pike, *Esox lucius*; sunfish, *Lepomis gibbosus*; and sucker, *Catostomus commersoni*) were found to be immunologically similar to mammalian insulins, in that their hormonal activity was neutralized by anti-bovine-insulin sera obtained from hyperimmunized horses or guinea pigs[6]. However, the other three fish insulins (from cod, *Gadus callarias*; pollock, *Pollachius virens*; and catfish, *Ictarulus punctatus*) were very different; for example, the amount of horse anti-bovine insulin serum required to neutralize 1 unit of cod insulin was 40 times greater than that required for 1 unit of bovine, horse or whale insulins (cf. ref. 7). Because of the immunological differences between these three fish insulins and bovine insulin, it was thought likely that they might be very different chemically.

It was possible to obtain cod insulin in a highly purified state (Moloney, P. J., and Wilson, S., unpublished work) with a potency of 21 units/mgm. for the air-dried material (95 per cent confidence limits, 13–34 units/mgm.) by two mouse convulsion assays[4] using 144 animals in each test. Within the limits of the assay, this value for the hormonal activity of cod insulin is in the range, 22–26 units/mgm., reported for highly purified mammalian insulins. The fact that purified cod and bovine insulins possess similar potencies but different capacities for combining with a specific antibody to bovine insulin indicates that the groups involved in this combination need not be the same as those which determine biological activity.

When cod and bovine insulins were subjected to electrophoresis in a starch gel at *p*H 3·5 (8 *M* urea, 0·23 *M* formic acid, 0·01 *M* sodium hydroxide) the cod insulin yielded only one band which migrated faster towards the cathode than bovine insulin, as seen in Fig. 1. Paper electrophoresis of the two proteins in 5 *M* acetic acid gave a similar result. Since the cod insulin migrated more rapidly towards the cathode in both these systems, it seemed probable that it had a higher isoelectric point than bovine insulin. On partition chromatography, using the upper phase of a mixture of 2-butanol/1 per cent acetic acid as solvent, cod insulin moved only slightly from the origin ($R_F < 0{\cdot}1$) whereas bovine insulin had an R_F of 0·3. In *n*-butanol/acetic acid/water (3 : 1 : 4, upper phase) cod and bovine insulins had R_F values of 0·47 and 0·59 respectively. This is consistent with cod insulin being more polar than bovine insulin.

In order to determine whether cod insulin consisted of two polypeptide chains, such as the *A* and *B* chains of bovine insulin, linked by disulphide bonds, cod and bovine insulins were treated according to two methods which are known to split —S—S— bonds: namely, oxidation with performic acid to give the sulphonic acid form[8] and reaction with sulphite to give the S-sulpho form[9]. The products of both treatments were separated by high-voltage paper

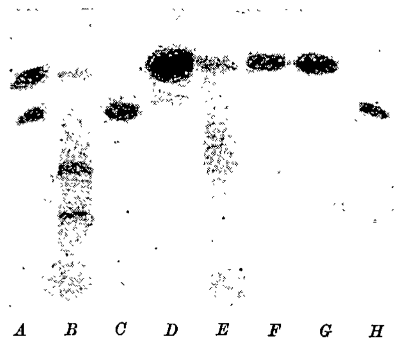


Fig. 1. Electrophoresis of various insulin preparations in starch–urea gel at *p*H 3·5 for 6 hr. at 10 V./cm. (gel stained with amido-black). *A*, artificial mixture of bonito, tunny and swordfish (BTS) and bovine insulin; *B*, pollock fish insulin (crude, 8 units/mgm.); *C* and *H*, bovine insulin (crystalline, 23 units/mgm.); *D*, BTS insulin (purified, 14 units/mgm.); *E* and *F*, cod insulin (partially purified); *G*, cod insulin (purified, 21 units/mgm.)

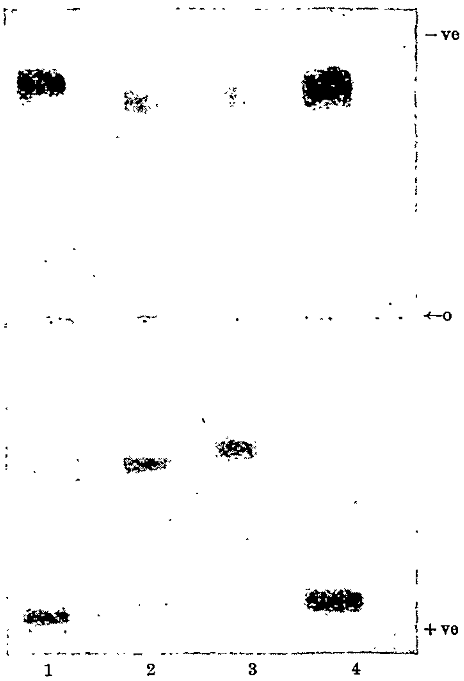


Fig. 2. Separation by high-voltage electrophoresis (75 V./cm. for 100 min.) of the *A* and *B* chains from various insulins in the sulpho- or S-sulpho-forms (ref. 7). 1, sulpho-*A* and -*B* chains from bovine insulin; 2, sulpho-*A* and -*B* chains from cod insulin; 3, sulpho-*A* and -*B* chains from BTS insulin; 4, S-sulpho-*A* and -*B* chains from bovine insulin

electrophoresis in an aqueous buffer at *p*H 4·1 containing 0·12 *M* pyridine, 1·75 *M* acetic acid and 8 *M* urea[9] or in 5 *M* acetic acid, and stained by spraying the electrophoretogram with diazotized sulphanilic acid. It was observed that cod and bovine insulins yielded two polypeptide chains in all cases, but the *A* chain from cod insulin had a much lower mobility than bovine chain as seen in Fig. 2. In addition, the spot corresponding to cod *A* chain showed the strong cherry-red colour characteristic of histidine, whereas bovine *A* chain had only the weaker orange colour due to tyrosine. The *B* chains from both insulins gave the red colour indicative of histidine, but cod *B* chain had a somewhat lower mobility towards the cathode than bovine *B* chain.

The *A* and *B* chains from cod insulin in the S-sulpho form were separated on a preparative scale by the method of Dixon and Wardlaw[9]. The solutions of the separated polypeptide chains were freed from urea and salts on a 'Sephadex' *G*–25 column (equilibrated with 0·1 *M* ammonia) and finally lyophilized. The S-sulpho *A* chains from cod and bovine insulins were hydrolysed by digestion with an aminopeptidase preparation (kindly supplied by Dr. K. S. V. Sampathkumar, Department of Biochemistry) and the resulting amino-acids identified by 2-dimensional electrophoresis/chromatography.

Histidine, arginine, phenylalanine and proline were found to be present in cod *A* chain, whereas none of these four amino-acids occurs in bovine *A* chain. Tryptophan was found to be absent from cod *A* chain, although it would have been conserved in the above procedure. For quantitative amino-acid analysis, cod insulin was oxidized with performic acid and the two chains separated by a modification of the above method[9] in which the cod *B* chain was eluted from the resin column at *p*H 3·8 with a buffer containing 2·6 *M* formic acid, 0·63 *M* pyridine and 8 *M* urea. Analyses were then carried out by Dr. D. B. Smith (National Research Council, Ottawa) on acid hydrolysates of these derivatives (5·7 *M* hydrochloric acid under vacuum for 48 hr. at 105° C.) using a Spinco amino-acid analyser and the results are summarized in Tables 1 and 2. It will be noted from Table 1 that there are considerable differences in the amino-acid composition of the *A* chains from cod and bovine insulins. One residue each of histidine, arginine, phenylalanine and proline were found in cod *A* chain, confirming the qualitative results obtained with aminopeptidase. The presence of two basic amino-acids in cod *A* chain could account for it being more basic than bovine *A* chain. Serine (2 residues) and alanine (1 residue) are present in bovine *A* chain but absent from cod *A* chain. The *A* chains also differ in the amount of glutamic acid (4 residues in bovine and 2 in cod) and aspartic acid (2 residues in bovine and 5 in cod).

The *B* chains are less unlike (Table 2), but cod *B* chain contains a residue of methionine, which is not present in any known mammalian insulin, and the threonine residue of bovine *B* chain is absent from cod *B* chain. As in the *A* chains, there is more aspartic acid in cod *B* chain (3–4 residues) than

Table 1. COMPARISON OF THE AMINO-ACID COMPOSITION OF THE ISOLATED *A* CHAINS FROM COD AND BOVINE INSULINS

Amino-acid	Cod *A*			Bovine *A*	Difference in number of residues
	No. of μmoles	No. of residues	Integral No. of residues	No. of residues (ref. 1)	
Histidine	0·077	1·0	1	0	+1
Arginine	0·084	1·0	1	0	+1
Cysteic acid	0·288	3·7	4	4	0
Aspartic acid	0·387	4·8	5	2	+3
Serine	0·007	0·1	0	2	−2
Glutamic acid	0·166	2·1	2	4	−2
Proline	0·093	1·2	1	0	+1
Glycine	0·086	1·1	1	1	0
Alanine	0·017	0·2	0	1	−1
Valine	0·066	0·8	1	2	−1
*Iso*leucine	0·134	1·7	2	2	0
Leucine	0·085	1·1	1	1	0
Tyrosine	0·077	1·0	1	2	−1
Phenylalanine	0·081	1·0	1	0	+1
		Total	21	21	0

Table 2. COMPARISON OF THE AMINO-ACID COMPOSITION OF THE ISOLATED *B* CHAINS FROM COD AND BOVINE INSULINS

Amino-acid	Cod *B*			Bovine *B*	Difference in number of residues
	No. of μmoles	No. of residues	Integral No. of residues	No. of residues (ref. 1)	
Lysine	0·207	1·2	1	1	0
Histidine	0·317	1·8	2	2	0
Arginine	0·208	1·2	1	1	0
Cysteic acid	0·311	1·8	2	2	0
Methionine sulphoxide	Traces	< 0·1	0	0	0
Aspartic acid	0·611	3·4	3	1	+2
Methionine sulphone	0·149	0·9	1	0	+1
Threonine	0·063	0·3	0	1	−1
Serine	0·194	1·1	1	1	0
Glutamic acid	0·353	2·0	2	3	−1
Proline	0·502	2·8	3	1	+1
Glycine	0·509	2·9	3	3	0
Alanine	0·398	2·2	2	2	0
Valine	0·362	2·0	2	3	−1
*Iso*leucine	0·054	0·3	0	0	0
Leucine	0·698	3·9	4	4	0
Tyrosine	0·301	1·7	2	2	0
Phenylalanine	0·326	1·8	2	3	−1
		Total	31	30	+1

bovine *B* chain (1 residue). Although the amino-acid compositions of the *A* and *B* chains from cod insulin differ greatly from those of bovine insulin, the total number of residues in each case is similar (last line of Tables 1 and 2).

Cod insulin was examined for N-terminal residues by the dinitrofluorobenzene method according to procedure 3 of Harris, Fraenkel-Conrat and Levy[10]. An uptake of 10·2 moles base per mole of cod insulin (based on molecular weight of 5,700) was observed during the reaction with dinitrofluorobenzene. This could be accounted for by the reaction of the lysyl, three histidyl and three tyrosyl groups known to be present, together with two N-terminal residues (total theoretical base requirement = 10·1 moles). Dinitrophenyl-serine and dinitrophenyl-glycine were found in equimolar amounts in an acid hydrolysate (5·7 *N* hydrochloric acid, 8 hr. at 105° C.) and there was no significant contamination with other dinitrophenyl-amino-acids. Since the only serine residue present in cod insulin is in the *B* chain, it must be the source of the dinitrophenyl-serine, and thus occupy the N-terminal position of this chain. The dinitrophenyl-glycine therefore came from cod *A* chain, and this was confirmed by the observation that the isolated *A* chain, like bovine *A* chain, reacted with ninhydrin to give a bright yellow colour characteristic of an N-terminal glycyl group.

Digestion of the two insulins with carboxypeptidase *A* (ref. 11) released alanine, asparagine and aspartic acid from bovine insulin but only asparagine and aspartic acid from cod insulin. Alanine was released from isolated bovine *B* chain whereas cod *B* chain yielded no amino-acid in significant amounts. However, treatment of cod insulin with carboxypeptidase *B* (ref. 12) released lysine, and since the only residue of lysine in the molecule is present in the *B* chain, it must be the C-terminal amino-acid of this chain. The asparagine residue removed by carboxypeptidase *A* from intact cod insulin therefore came from the C-terminal position of the *A* chain, as in the case of bovine insulin. It is significant that carboxypeptidase *A* does not release asparagine from either of the chains in the sulphonic acid form. In the case of bovine *A* chain, Sanger and Thompson[11] suggested that the cysteic acid residue in the penultimate position inhibited the release of the C-terminal asparagine. It is therefore probable that the C-terminal sequence in cod *A* chain is —$CySO_3H \cdot AspNH_2$ as in the bovine *A* chain.

In view of the large differences in amino-acid composition of the corresponding chains from cod and bovine insulin, certain portions of the polypeptide chains must have different amino-acid sequences. When peptic, tryptic or chymotryptic hydrolysates of the separated chains from the two insulins were compared by 2-dimensional electrophoresis/chromatography on paper, it was found that very few of the peptide fragments appeared to be the same. In Fig. 3*a*, chymotryptic digests of the *A* chains from cod and bovine insulin are compared, and Fig. 3*b* shows peptic digests of the *B* chains. Even when intact cod and bovine insulins were hydrolysed to small peptides by prolonged digestion with subtilisin (Nagase Co., Japan) the peptide maps were strikingly different.

Complete amino-acid sequences are available for insulins from seven mammalian species[1,2], and in all cases, except human insulin, the alterations in sequence occur in a sharply delineated region between residues 7 and 11 of the *A* chain. In human insulin there is an additional substitution of threonine for alanine at the C-terminal position of the *B* chain. However, the differences between cod and bovine insulins are much more profound, particularly in the *A* chain, and it is doubtful whether any of the peptic or chymotryptic peptides are identical. Zuckerkandl *et al.*[13] have reported a parallel situation in the case of hæmoglobins from a wide range of vertebrates. They found that the tryptic fingerprints of hæmoglobins from certain primates were almost identical, and had some peptides in common with those from other mammals such as ox and pig, whereas the fingerprints from three sub-classes of fish (bony fish, cartilaginous fish and lungfish) were very different from the mammalian hæmoglobins and from each other. The present results with insulins support the idea that the molecular structure of functionally related proteins becomes progressively different the greater the phylogenetic divergence between the species producing the proteins.

One of the difficulties in the present work was lack of sufficient cod insulin, and therefore methods for purification were first developed using another fish insulin which was available in large quantities (Shimizu Co., Japan), but only as a mixture from three fish (bonito, tunny and swordfish) referred to here as BTS insulin. This preparation had a potency of 14 units/mgm. (95 per cent confidence limits, 12–17 units/mgm.). Although it was a

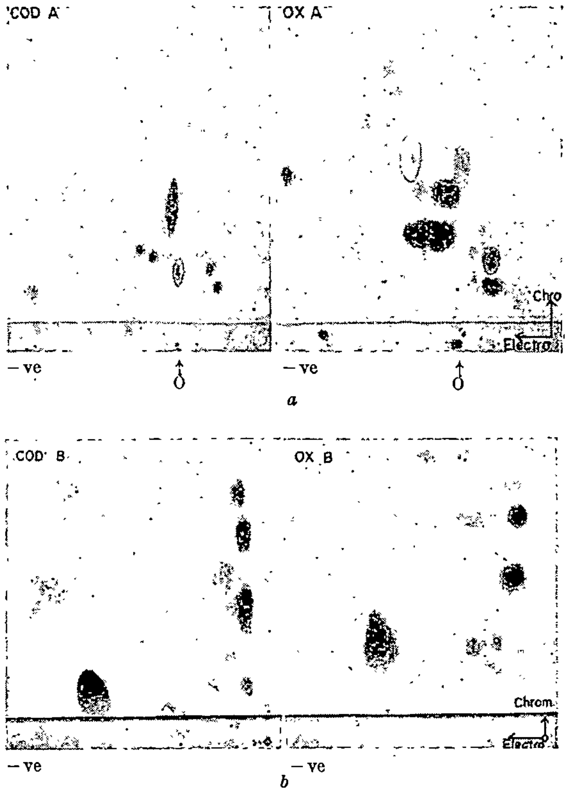


Fig. 3. Peptide maps of enzymic digests of the separated *A* and *B* chains from cod and bovine insulins. The maps were obtained by high-voltage electrophoresis on Whatman No. 3 paper in pyridine/acetic acid buffer at *p*H 3·6 followed by stitching the strip of paper bearing the peptides to another sheet for ascending chromatography in the second dimension (butanol/acetic acid/water; 3:1:1). (*a*) Chymotryptic digests of cod and bovine *A* chains in the S-sulpho form (19 hr. at 37° C. in 1 per cent ammonium carbonate solution at *p*H 8·5); (*b*) peptic digests of cod and bovine *B* chains in the sulpho form (48 hr. at 37° C. in 0·1 *M* acetic acid)

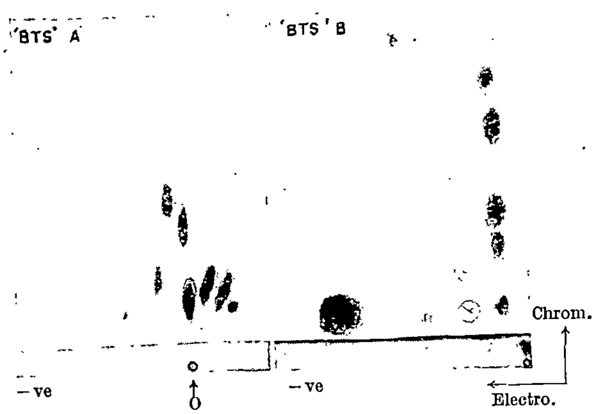


Fig. 4. Peptide maps of (left), chymotryptic digest of BTS insulin *A* chain (S-sulpho) and (right), peptic digest of BTS insulin *B* chain (sulpho-). Conditions as for Fig. 3

mixture of three insulins, the purified 'BTS' preparation behaved like cod insulin both as the intact protein (see Fig. 1) and after oxidation with performic acid (see Fig. 2). It contained two C-terminal residues, lysine and asparagine, as in cod insulin. Fingerprints of peptic and chymotryptic digests of BTS *B* chains were almost identical to those of cod *B* chain, and it is therefore likely that cod *B* chain and the three *B* chains from BTS insulin all have very similar amino-acid sequences. These findings are surprising in view of the fact that the bonito–tunny–swordfish group and cod represent two different orders of bony fish (Perciformes and Gadiformes, respectively). In contrast to the above, fingerprints of peptic and chymotryptic digests of BTS *A* chains appear to have only a few peptides in common with those of cod *A* chain, and since the total number of peptides from the hydrolysed BTS *A* chains is much greater, it is probable that the structure of the *A* chains of bonito, tunny and swordfish insulins differ among themselves as well as from cod *A* chain. Fingerprints of a chymotryptic digest of BTS *A* chain and a peptic digest of BTS *B* chain are illustrated in Fig. 4.

On treatment with dinitrofluorobenzene reagent[10], BTS insulin yielded four N-terminal residues, dinitrophenyl-glycine, dinitrophenyl-alanine, dinitrophenyl-valine and dinitrophenyl-leucine, indicating the presence of several polypeptide chains in this mixture of insulins. Yamamoto, Kotaki, Okuyama and Satake[14] have separated two insulins obtained from the bonito and showed that the N-terminal position of both *A* chains was occupied by glycine, but that one insulin had leucine at the N-terminal position of its *B* chain whereas the other had alanine.

The above comparisons of cod insulin with BTS insulin indicate that these fish insulins, representing two orders within one class of animals, have many parts of their amino-acid sequence in common but that they differ radically from insulins of another class—the mammals.

If it is assumed that the biological activity of insulin is determined by a 3-dimensional array of certain amino-acid side-chains then this specific configuration is likely to be the same for both cod and bovine insulins, since they have the same potency in a mammalian test system. Despite the similarity in potency of these insulins, it is clear that large regions of the molecule, particularly in the *A* chain, have altered in the course of evolution without changing the biological activity. Investigations of the amino-acid sequence of cod insulin are under way, and it is hoped that the establishment of those parts of the sequence which are common to this and other insulins may make it possible to suggest which regions of the molecule govern its biological action.

We wish to acknowledge the support and interest of Dr. P. J. Moloney in this work, the testing of insulin potency by Dr. A. C. Wardlaw and advice in preparation of the manuscript by Prof. C. S. Hanes. The work of one of us (G. H. D.) has been generously supported by the National Research Council of Canada.

[1] Sanger, F., and Smith, L. F., *Endeavour*, **16**, 48 (1957).
[2] Nicol, D. S. H. W., and Smith, L. F., *Nature*, **187**, 483 (1960).
[3] Macleod, J. J. R., *J. Metab. Res.*, **2**, 149 (1922).
[4] Young, D. M., and Lewis, A. H., *Science*, **105**, 368 (1947).
[5] Wardlaw, A. C., and Moloney, P. J., *Canad. J. Biochem. Physiol.*, **39**, 695 (1961).
[6] Moloney, P. J., and Goldsmith, L., *Canad. J. Biochem. Physiol.*, **35**, 79 (1957).
[7] Moloney, P. J., and Aprile, M. A., *Canad. J. Biochem. Physiol.*, **38**, 1216 (1960).
[8] Hirs, C. H. W., *J. Biol. Chem.*, **219**, 611 (1956).
[9] Dixon, G. H., and Wardlaw, A. C., *Nature*, **188**, 721 (1960).
[10] Harris, J. I., Fraenkel-Conrat, H., and Levy, A. L., *Methods of Biochem. Anal.*, **2**, 393 (1955).
[11] Sanger, F., and Thompson, E. O. P., *Biochem. J.*, **53**, 366 (1953).
[12] Folk, J. E., and Gladner, J. A., *J. Biol. Chem.*, **231**, 379 (1958).
[13] Zuckerkandl, E., Jones, R. T., and Pauling, L., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1349 (1960).
[14] Yamamoto, M., Kotaki, A., Okuyama, T., and Satake, K., *J. Biochem.* (Tokyo), **48**, 84 (1960).

INHIBITION BY OXYGEN OF BIOSYNTHESIS AND ACTIVITY OF NITRATE-REDUCTASE IN *AEROBACTER AEROGENES*

By FRANCIS PICHINOTY and LILIANE D'ORNANO

Laboratoire de Chimie Bactérienne, Centre National de la Recherche Scientifique, C.R.S.I.M., Marseille

CELL suspensions of *Aerobacter aerogenes* grown in the absence of nitrate acquire anaerobically a nitrate-reductase activity in the presence of this compound. The process of adaptation is due to a *de novo* biosynthesis of this enzyme from free amino-acids[1]. Moreover, the formation of nitrate-reductase induced by the ions NO_3^- or NO_2^- is greatly inhibited in aerobic cultures on a synthetic medium[2]. In the present article, we describe the effect of oxygen on the biosynthesis and the activity of the nitrate-reductase in non-proliferating suspensions of the same organism.

A. aerogenes (strain *L* III–I) is cultivated on a synthetic medium described previously[2]. Aerobic cultures are grown in Erlenmeyer flasks with agitation. Anaerobic cultures are grown in flasks under vacuum or in deep cylindrical bottles. After incubation at

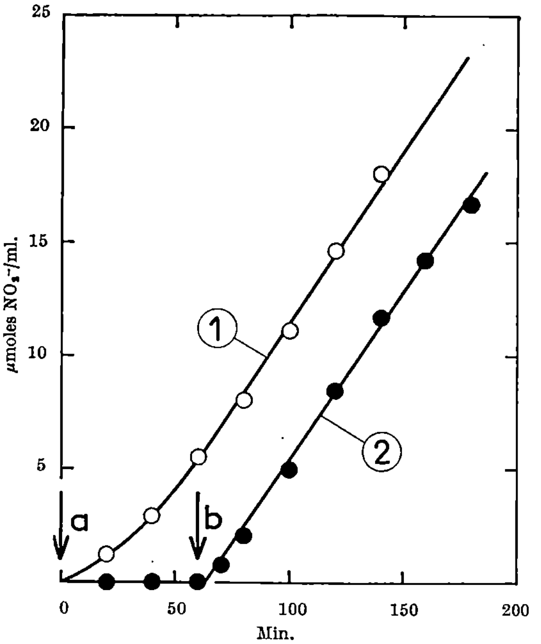


Fig. 1. Effect of aeration on the activity of nitrate-reductase. Cells: 1·5 mgm./ml. potassium nitrate is added at time 0. *a*, start of aeration; *b*, end of aeration. 1, system maintained in anaerobiosis; 2, system maintained in aerobiosis for 60 min, followed by anaerobiosis

32° C. for 22 hr., the cells are centrifuged and washed with 0·2 per cent sodium chloride. In each experiment the different systems contain the following: glucose, 50 µmoles/ml.; potassium nitrate, 25 µmoles/ml.; washed cells, 1–4 mgm. (dry weight)/ml.; phosphate buffer, *p*H 7, 0·033 *M*; temperature 37° C. Chloramphenicol is used at a concentration of 50 µgm./ml. In the adaptation experiments, 1 mgm./ml. of casamino-acids (Difco) is added. At regular time-intervals 1-ml. aliquots are taken and the nitrite assayed colorimetrically. The nitrite-reductase activity of this strain being very weak, the formation of 1 µmole NO$_2^-$ corresponds to the reduction of 1 µmole NO$_3^-$. The nitrate-reductase activity of the cells and of the extracts is determined by the rate of oxidation of a definite quantity of benzyl–viologen by an excess of nitrate, *in vacuo*, using phosphate buffer *p*H 7, 37° C., by a technique described in an earlier paper[3]. One unit of enzyme is that quantity which catalyses the reduction of 1 µmole/hr. of NO$_3^-$. The number of units contained in the cells and the extracts is reported per mgm. of protein nitrogen.

In the first part of this work, we describe the effect of oxygen on the activity of intracellular nitrate-reductase. The reduction of nitrate to nitrite in induced cells is compared under aerobic and anaerobic conditions. This experiment is carried out in the presence of chloramphenicol with a very active cell suspension derived from an anaerobic culture grown on nitrate. During aeration of the suspension, no trace of nitrite appears, but nitrite formation resumes its normal rate when aeration is stopped. Curves 1 and 2 are exactly parallel. This experiment indicates that enzyme activity is inhibited reversibly by oxygen. We have verified that aeration does not affect the stability of the enzyme. Two identical systems, one aerobic and one anaerobic, are maintained for 60 min. under conditions of the previous experiment. The cells are then centrifuged and washed. The activities of the cells and corresponding extracts measured in the presence of benzyl–viologen (Table 1) show no diminution in the course of the aeration: the intracellular enzyme retains its activity after prolonged contact with oxygen.

Table 1. EFFECT OF AERATION ON THE ACTIVITY OF NITRATE-REDUCTASE

Cell suspension	Enzyme units	
	Cells	Extracts
Anaerobiosis, 60 min.	182	75
Aerobiosis, 60 min.	187	93

In a second series of experiments, we investigated the action of oxygen on the formation of the enzyme system. The basal activity of a cell suspension of an aerobic culture grown on nitrate, measured in the presence of chloramphenicol, is practically zero (curve 2, Fig. 2). In the absence of the antibiotic, the cells acquire rapidly a high activity under anaerobic conditions (curve 1, Fig. 2). About 40 min. after the addition of the inducer, the biosynthesis of the enzyme system stops and the quantity of nitrite formed after this time becomes linear with time. Two systems identical to the preceding one are subjected to violent aeration for 60 min. After aeration, chloramphenicol is added to one. No activity is found afterwards (curve 4, Fig. 2). In contrast, in absence of chloramphenicol, adaptation begins when aeration ceases and the cells thus acquire 50 per cent of the activity of a suspension maintained constantly under anaerobic conditions (curve 3, Fig. 2). These results indicate that the adaptation of the cells is inhibited reversibly by oxygen. This phenomenon, which has likewise been demonstrated

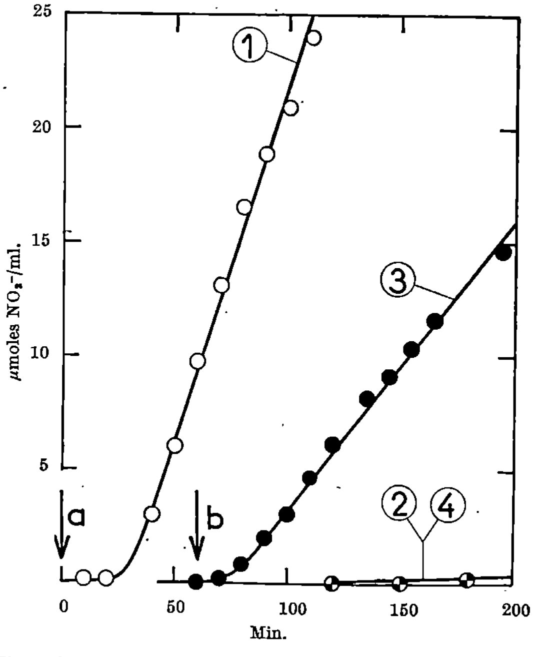


Fig. 2. Effect of aeration on the biosynthesis of nitrate-reductase. Cells: 1·5 mgm./ml. potassium nitrate added at time 0. *a*, Start of aeration; *b*, end of aeration. 1, System maintained in anaerobiosis; 2, system identical to 1 + chloramphenicol; 3, system maintained in aerobiosis for 60 min, followed by anaerobiosis; 4, system identical to 3, chloramphenicol added after 60 min. of aerobic incubation

Table 2. EFFECT OF AERATION ON THE BIOSYNTHESIS OF NITRATE-REDUCTASE

Cell suspension	Enzyme units	
	Cells	Extracts
Initial	0	0
Induced in anaerobiosis, 60 min.	287	100
Induced in aerobiosis, 60 min.	0	0
Induced in aerobiosis, 60 min., followed in anaerobiosis, 60 min.	214	95

by Pollock for *Escherichia coli*[4], can be attributed either to the inhibition of the biosynthesis of the enzymic system or to the irreversible inactivation of the system by contact with air. The experiments reported in the first part of this work definitely eliminate this second possibility. In order to confirm the previous observations, we have compared the activities of extracts of cells induced under aerobic and anaerobic conditions. This experiment is performed exactly as the one described here with cells cultivated aerobically on an ammonium chloride medium. After incubation for 60 min. in the presence of the inducer, the suspensions are cooled to 0° and chloramphenicol added to arrest protein synthesis immediately. The cells are then centrifuged and washed. The activities of the cells and extracts are measured in the presence of benzyl–viologen. The results shown in Table 2 indicate that only extracts of cells induced under anaerobic conditions have significant activity. Non-induced cells and cells induced aerobically are completely inactive. On the other hand, cells which have been incubated aerobically synthesize as much enzyme as the un-aerated suspension when anaerobic conditions are commenced. The significance of this latter experiment is clear : oxygen reversibly inhibits the biosynthesis of nitrate-reductase.

Many facultative aerobic bacteria are able to oxidize anaerobically certain organic compounds or mineral substances in the presence of nitrate and utilize the energy thus liberated for the biosynthesis of cell matter. There is reason to think that with these organisms, where nitrate replaces oxygen as the final electron acceptor in the respiratory chain, nitrate-reductase plays a part analogous to cytochrome-oxidase. It is also known that the oxidation-reduction process of denitrification is generally absent in aerobic cultures[5]. One can now anticipate that one of the causes of this important phenomenon resides in the inhibition of biosynthesis and activity of the nitrate-reductase. These investigations will be extended to true denitrifying organisms in the sense of Kluyver[5].

[1] Pichinoty, F., and D'Ornano, L., *C.R. Acad. Sci., Paris*, 252, 793 (1961).
[2] Pichinoty, F., and D'Ornano, L., *Biochim. Biophys. Acta*, 48, 218 (1961).
[3] Pichinoty, F., *Folia Microbiol.*, 5, 1 (1960).
[4] Pollock, M. R., "Induced Formation of Enzymes", in *The Enzymes* (Academic Press, Inc, 1959).
[5] Kluyver, A. J., "Some Aspects of Nitrate Reduction", in *Symposium Microbial Metabolism* (Istituto Superiore di Sanità, Rome, 1953).

COMMON CARRIER SYSTEM FOR SUGAR TRANSPORT IN HUMAN RED CELLS

By DR. L. LACKO and DR. M. BURGER

Institute of Hæmatology and Blood Transfusion and Institute of Biology, Czechoslovak Academy of Science, Prague

THE interesting findings of Heinz[1,2], who described the counterflow transport of amino-acids in Ehrlich ascites carcinoma cells, made it possible to study transport systems across the cell membrane from a new point of view. By applying this concept to the study of the mechanism of sugar influx into cells (for example, erythrocytes), transport systems common to various sugars can be determined.

We are presenting some facts to prove that, whereas one group of sugars is transported into erythrocytes by an identical carrier system, another group is not. Two experimental observations form the basis of our work. (1) It has been proved that red cells are capable of exchanging sugars such as glucose–mannose by the counterflow mechanism[3]. (2) Counterflow transfer can be distinguished from normal transport (without exchange) by incubating erythrocytes at a lower temperature (0° C.) at which, as has been shown, exchange transfer prevails. At 0° C. a marked influx of sugar into red cells only occurs if another sugar leaves the cells simultaneously[4].

Human blood was collected into an *ACD* (trisodium citrate 1·1 gm.; citric acid 0·4 gm.; glucose 3·5 gm.; water 100 ml.) solution and left at room temperature for 30 min. to allow for the entry of glucose into erythrocytes. The red cells were separated from the plasma by centrifugation; 1 ml. of the packed red

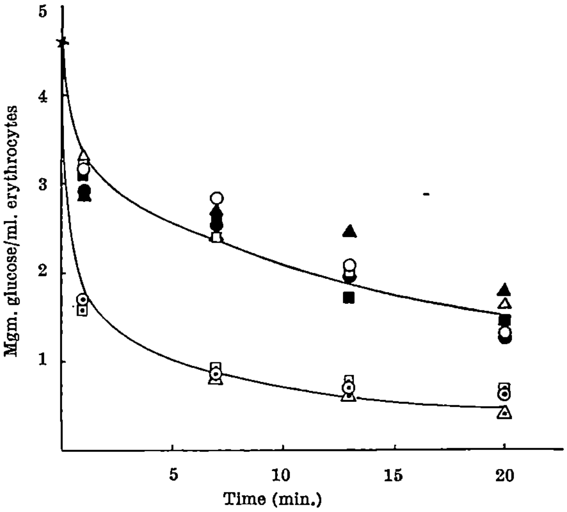


Fig. 1. Content of glucose in erythrocytes during incubation of glucose containing cells in media with different sugars.

×, level of glucose in erythrocytes at the beginning of the experiment. Content of medium: ○, 0·90 per cent sodium chloride; □, 0·98 per cent sodium chloride; △, L-sorbose; ●, D-fructose; ■, D-arabinose; ▲, D-ribose; ⊙, D-galactose; ⊡, D-mannose; ◬, D-xylose.

The sugars were present in the media in concentration 2·8 × 10^{-2} *M* dissolved in 0·90 per cent sodium chloride

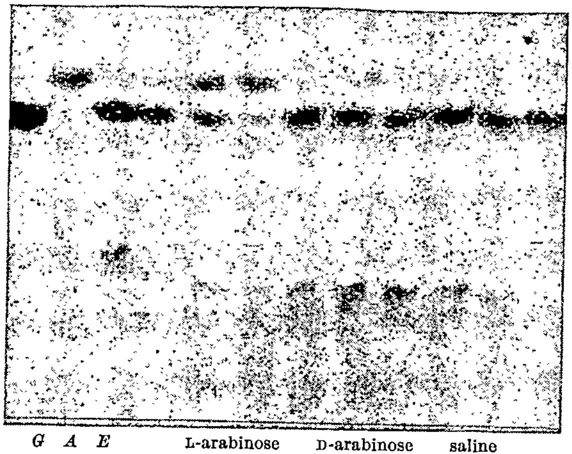


Fig. 2. Content of sugars in erythrocytes during incubation of cells containing glucose in L- and D-arabinose. The first two columns represent standards of D-glucose D- and L-arabinose; *E* represents content of glucose in erythrocytes at the beginning of the experiments. The following columns represent the amount of sugars in red cells after various time (min.) of incubation in L- and D-arabinose and saline

cells was incubated at 0° C. in 10 ml. of a 2·8 × 10^{-2} *M* solution of sugars in 0·90 per cent sodium chloride and, in a control experiment, in a sugar-free solution of 0·90 per cent and 0·98 per cent sodium chloride, respectively. At various time-intervals the sugar content of the red cells was determined by the process described in ref. 4. Fig. 1 shows that glucose leaves the red cells rapidly if D-galactose, D-mannose and D-xylose are present in the medium; however, in the presence of D-fructose, L-sorbose, D-arabinose and D-ribose the efflux of D-glucose is considerably slower, as it is into media containing sodium chloride only. By means of paper chromatography[5] we found that if the glucose efflux from the red cells was fast the sugar present in the medium appeared simultaneously in the red cells instead of glucose and that the sugar influx corresponded approximately to the glucose efflux.

The chromatogram (Fig. 2) shows an example of the substitution of glucose by another sugar (L-arabinose) in the red cells; yet another sugar (D-arabinose) does not have this effect, so that during the experiment the glucose-level in the red cells remains unaltered whether they are incubated in D-arabinose or in saline.

Similar experiments showed that there are two groups of sugars, one of which (D-galactose, D-mannose, L-arabinose, D-xylose, D-lyxose, 2-deoxy-D-glucose, 2-deoxy-D-galactose) enters the erythrocytes at 0° C., whereas another group of sugars (D-fructose, L-sorbose, D-arabinose, D-ribose, L-rhamnose, L-fucose) does not enter the red cells under these conditions.

It can be assumed that the first group of sugars shares the same carrier system when entering the red cells, and that this system is not identical with that used by the other group of sugars. We were interested to ascertain whether the second group, though not using the same carrier system as the first group, has not one carrier system in common as well. However, on using erythrocytes containing sorbose, we did not observe any exchange of this sugar by D-fructose, D-ribose and D-arabinose.

A comparison of the structure of the sugars investigated showed that aldoses using the same carrier system have one structural property in common, in that they belong to the homomorphous series of sugars with L-arabino, D-xylo and D-lyxo configuration, whereas sugars of the homomorphous series with D-arabino, D-ribo and L-lyxo configuration do not have this carrier system in common. This does not hold for ketoses, judging from the fact that D-tagatose, which belongs to the homomorphous series with the D-lyxo configuration, nevertheless did not replace glucose in our experiments. Our results are partly in keeping with LeFevre's[6] conception that the greater the affinity of sugars to the carrier system, the greater the stability of sugars in the C-1 conformation[7]. However, our results suggest that there is no gradual transition between the two groups but a sharp division, the first group having the same carrier system in common whereas the other has not. It is also important to note that glucose was substituted in our experiments by D-lyxose but not by D-ribose although, according to LeFevre, the former has a higher dissociation constant in the carrier complex than D-ribose[6].

Our thanks are due to Mrs. L. Hejmová and Mrs. H. Kopecká for technical help and to Dr. M. Černý for valuable criticism and for the gift of some sugars.

[1] Heinz, E., *J. Biol. Chem.*, **211**, 781 (1954).

[2] Heinz, E., and Walsh, P. M., *J. Biol. Chem.*, **233**, 1488 (1958).

[3] Rosenberg, T., and Wilbrandt, W., *J. Gen. Physiol.*, **41**, 289 (1957).

[4] Lacko, L., Burger, M., Hejmová, L., and Rejnková, J., *Membrane Transport and Metabolism—Symposium*, 399 (Publishing House of Czechoslovak Academia of Science, 1960).

[5] Burger, M., Hejmová, L., and Kleinzeller, A., *Biochem. J.*, **71**, 233 (1959).

[6] LeFevre, P. G., and Marshall, J. K., *Amer. J. Physiol.*, **194**, 333 (1958).

[7] Reeves, R. E., *J. Amer. Chem. Soc.*, **72**, 1499 (1950).

STEROL REQUIREMENTS OF THE SILKWORM, *BOMBYX MORI*

By Dr TOSHIO ITO

Sericultural Experiment Station, Suginami-ku, Tokyo

In a previous paper[1] I showed that soybean oil was necessary for the sub-optimal growth of the silkworm, *Bombyx mori*, and that no larva reached the second instar without oil, so far as the diet, containing 10 per cent mulberry leaf powder, 14 per cent cellulose powder, and other nutrients such as sucrose, soybean casein, and inorganic salts, was used. Subsequent experiments[2] showed that the effect of soybean oil could be attributed principally to the unsaponifiable matter, and not to fatty acids of the oil. In this connexion, the nutritive effects of sterols were tested recently and it was proved that silkworm larvæ required them for growth and development. The present communication gives a preliminary account of the results.

For testing the effect of sterols, artificial diets were used. The methods of preparing the diets and of rearing the larvæ have already been described[1,2].

The composition of the diet is presented in Table 1, which is somewhat improved on that used in an earlier work[1]. The vitamin mixture employed is shown in Table 2. The amount of sterol added was 1 mgm. to each 1-gm. dry diet. Rearing was carried out in a Petri dish, as shown previously, 20 larvæ for each dish, in duplicate. All tests were started with newly hatched larvæ, and dishes were kept at 25° C. The criteria used determining efficiency of a diet were the increase in weight and the number of larvæ which reached through various instars in 17 days.

All the tests with sterols were carried out in combination with soybean oil (4 per cent), since the presence of the latter substance enhanced feeding greatly, while the addition of some of the sterols used, and no oil addition, resulted in the reduction of the feeding. The results obtained are summarized in Table 3.

On the control diet without sterol, most of the larvæ reached the second instar, because of the presence of 4 per cent oil. The effect of the oil at varying concentrations has been presented already[2]. Those larvæ growing on such a diet deficient in sterol eventually died without reaching the third instar. However, the addition of sterol to the diet had a marked positive effect on the rate of growth. Such an effect varied with different sterols ; most noticeable with either β-sitosterol or stigmasterol, intermediate with cholesterol, and rather poor with ergosterol. When the diet contained approximately 0·1 per cent additional β-sitosterol, silkworm larvæ increased their weight most and reached the fourth instar rather sooner, and the lowest mortality occurred. Almost similar effects were obtained with one of the phytosterols, stigmasterol.

Table 1. COMPOSITION OF THE DIET

Substance	Amount used (gm.)	Dry diet (per cent)
Mulberry leaf powder	0·6	10
Starch (potato)	0·9	15
Sucrose	1·2	20
De-fatted soybean casein	1·2	20
Soybean oil (unrefined)	0·24	4
Wesson's salt mixture	0·06	1
Cellulose powder (General Biochemicals, Inc., Ohio)	1·8	30
	6·00	100
Vitamin mixture	2·37 mgm.	
Sterol, where necessary	6 mgm.	
Distilled water	9·0 ml.	

Table 2. COMPOSITION OF THE VITAMIN MIXTURE

Vitamin	Amount added to 6 gm. dry diet (μgm.)
Biotin	10
Calcium pantothenate	100
Choline chloride	1,000
Folic acid	10
Inositol	1,000
Niacin	100
Pyridoxine hydrochloride	50
Riboflavin	50
Thiamine hydrochloride	50
Total	2,370

All vitamins, except for choline chloride and inositol, were kindly supplied by Dr. G. S. Fraenkel, Department of Entomology, University of Illinois.

Table 3. EFFECT OF STEROLS ON GROWTH, DEVELOPMENT AND SURVIVAL OF THE SILKWORM

Number of larvæ reached through various instars in 17 days, out of 20 newly hatched larvæ (mean of two batches)

Sterol	Second instar	Third instar	Fourth instar
Nil	17·5	0	—
Cholesterol	18·5	13·0	3·5
Ergosterol	18·0	6·5	0
β-Sitosterol	17·5	17·0	14·5
Stigmasterol	19·0	17·5	11·0

Number and average weight of surviving larvæ, out of 20 (mean of two batches)

Sterol	After 14 days		After 17 days	
	No.	Weight (mgm.)	No.	Weight (mgm.)
Nil	1·0	6·6	0	—
Cholesterol	14·0	16·5	12·5	28·6
Ergosterol	8·0	7·7	4·5	7·7
β-Sitosterol	18·0	43·4	17·0	82·0
Stigmasterol	17·5	30·6	15·5	49·5

β-Sitosterol was purchased from Nutritional Biochemicals Corp., Ohio, and other sterols were kindly supplied by Dr. H. Lipke, Army Chemical Center, Maryland

The requirements for sterols in insects have been well established and several reviews on this subject are available[3]. So far investigations show that no insect exists which does not require a dietary source of sterol. Nevertheless, the majority of such data is based on the experiments with non-phytophagous insects, and sterol requirements in leaf-feeders have seldom been analysed. Though the testing period in this work is limited to a certain period of life of the silkworm, it is evident that the silkworm requires sterols for growth. Cholesterol has been used in common in investigations concerning nutrition of insects, but it has been shown that in some forms other sterols can be substituted for cholesterol. The effectiveness of substitution varies depending not only on the insect species under investigation but also on the kind of sterols tested. In some insects it has been reported that phytosterols must be used at a higher concentration in the diet, when substituting cholesterol for them. In the silkworm β-sitosterol and stigmasterol were more effective than cholesterol. The effectiveness of cholesterol might be reduced, at least partly, by its retarding or inhibitory effect on feeding (Ito, T., unpublished work).

One of the components of the test diet, mulberry leaf powder, contains phytosterols[4,5], which have also recently been extracted in a crystalline form by me from mulberry leaves. Furthermore, soybean oil added as one of the ingredients contains phytosterols. However, it is clear that the quantity of sterols from both sources only is not enough for further growth beyond the second instar. The formula $C_{27}H_{46}O$ was suggested for mulberry leaf sterol some thirty years ago, and another one later[5]. However, this must be re-investigated, in view of recent progress in steriod chemistry. Mulberry sterols were also of positive nutritive effect for the silkworm (Ito, T., unpublished work).

Further details will be published in *the Bulletin of the Sericultural Experiment Station.*

[1] Ito, T., *Proc. Japan Acad.*, **36**, 287 (1960).

[2] Ito, T., *Bull. Seric. Exp. Sta.*, **16**, 311 (1961).

[3] Trager, W., in *Insect Physiology*, edit. by Roeder, K. D., 350 (John Wiley and Sons, Inc., New York, 1953). Lipke, H., and Fraenkel, G., *Ann. Rev. Entomol.*, **1**, 17 (1957). Horning, M. G., in *Cholesterol*, edit. by Cook, R. P., 445 (Academic Press, Inc., New York, 1958). Fraenkel, G., *Ann. New York Acad. Sci.*, **77**, 257 (1959).

[4] Yoshida, T., *Bull. Seric. Exp. Sta.*, **8**, 35 (1930).

[5] Murao, S., *Kumamoto Med. J.*, **13**, 681 (1937).

PATTERN OF PYRETHRIN-RESISTANCE IN HOUSEFLIES

By B. C. FINE

London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1

IN 1953, Busvine[1] found that a strain of houseflies, selected for resistance to paralysis (knockdown) induced by DDT, had its tolerance to Pyrethrum raised incidentally to a level of 11·5 times the normal. Correlations between DDT- and pyrethrin-resistance have since been reported in bed bugs[2] and the tick, *Boophilus decoloratus*[3]. It appears that in some circumstances resistance to the two insecticides is related.

In order to gain a wider understanding of pyrethrin-resistance and its associations with DDT-resistance, selection experiments and toxicological work have been carried out on three strains of houseflies. One strain, the Swedish, was provided by Mr. J. Keiding in 1959. By 1956 these flies had developed resistance to many of the chlorinated hydrocarbons and parathion. Synergized pyrethrins were then used to control the flies, but in 1958 it was reported that they had become resistant to Pyrethrum and synergized Pyrethrum. The levels of resistance were respectively 11–14 times and 10·7 times that of a susceptible strain[4]. The second strain originated in Omdurman, Sudan, where it developed high levels of resistance to γ-BHC and related compounds[5]. The third strain was a normally susceptible strain.

The flies were reared according to standard techniques and toxicological measurements were made by topical application of a measured drop (0·34 μl.) to the back of the thorax of female flies[6]. The insecticides were dissolved in either Shell Risella oil or di-(2 ethylhexyl) phthalate (DOP), which are non-volatile solvents. The use of volatile solvents such as acetone is known to exaggerate the measure of resistance to DDT appreciably[7]. Preliminary tests carried out on the Swedish strain showed that resistance to Allethrin in acetone was approximately eight times the level recorded for Allethrin dissolved in Risella oil. In Table 2 it will be seen that resistance-levels for Allethrin and Pyrethrum in Risella and DOP are not the same: when compared to Risella oil, the use of DOP tends to exaggerate resistance to certain pyrethroids by a factor of approximately two. In view of the fact that toxicity varies according to the nature of the solvent, caution should be exercised in comparing the results presented here with those of other authors who employed different techniques. Susceptibility to knockdown was determined by exposing female flies to dry residues of DDT applied to glass surfaces at a rate of 0·01 mgm./cm.[2,8].

The Swedish strain was inbred and then selected for resistance to Pyrethrum over eight generations. Selection was carried out by topical application. The Omdurman strain was selected over 23 generations for resistance to knockdown by DDT. In this case the DDT-treated glass surfaces acted as the selective agent. In Table 1 the effects of these selection procedures are presented. During the last stages of selection, the toxicity of various pyrethroid and non-pyrethroid insecticides was measured by topical application against the Swedish, Omdurman and susceptible flies. The results are presented in Table 2 and illustrated in Fig. 1. The results are expressed in terms of LD_{50} (median lethal dose) or KT_{50} (median knockdown time). These values were estimated from log-dose probit lines. Insecticides dissolved in Risella or DOP are indicated in Fig. 1 and Tables 1 and 2 by the symbols (*R*) and (*D*) respectively.

Table 1. EFFECTS OF SELECTION ON THE SWEDISH AND OMDURMAN STRAINS

	Swedish		Omdurman		Increase in resistance as a result of selection*	
	Before selection	After 8 generations of selection with Pyrethrum	Before selection	After 23 generations of selection with DDT	Swedish	Omdurman
LD_{50} μgm./fly Pyrethrum (*R*)	3·6	14·3	0·54	11·9	3·9	22
LD_{50} μgm./fly DDT (*R*)	>10·2	>10·2	2·7	>10·2	No measurable loss in resistance	>3·7
KT_{50} hr. DDT	—	>24	0·8	>24	—	>30

* Represented by ratio: $\dfrac{LD_{50} \text{ or } KT_{50} \text{ after selection}}{LD_{50} \text{ or } KT_{50} \text{ before selection}}$

The results presented in Table 1 show that selection with Pyrethrum in one strain and with DDT in another each serve to raise resistance to Pyrethrum. Furthermore, the patterns of resistance to pyrethrins and related compounds as illustrated in Fig. 1 are similar, suggesting that the type of Pyrethrum-resistance in the two strains is fundamentally the same. It may be argued that the apparent correla-

Table 2. MEDIAN LETHAL DOSES IN μGM. PER FLY OF VARIOUS INSECTICIDES FOR THE SUSCEPTIBLE, SWEDISH AND OMDURMAN STRAINS

Insecticide	LD_{50} μgm./fly			Level of resistance*	
	Susceptible	Swedish	Omdurman	Swedish	Omdurman
Pyrethrum (*R*)	0·46	14·3	11·9	31·1	25·8
Allethrin (*R*)	0·40	14·6	9·5	36·5	23·7
Cyclethrin (*R*)	0·52	27·2	19·0	52·3	36·5
Barthrin (*R*)	0·88	21·7	22·4	24·6	25·4
Pyrethrum (*D*)	0·71	43·1	34·1	60·7	48·0
Pyrethrum injected (*D*)	0·37	3·7	3·1	10	8·4
Allethrin (*D*)	0·81	61·2	—	75·5	—
Furethrin (*D*)	1·4	±292	±170	208	121
DDT (*R*)	±0·27	>10·2	>10·2	>37·7	>37·7
Dieldrin (*R*)	±0·027	0·14	1·9	5·2	70·3
Lethane (*R*)	4·4	8·8	6·5	2·0	1·5
Malathion (*D*)	±1·2	5·4	9·2	4·5	7·6

* Represented by ratio: $\dfrac{LD_{50} \text{ Swedish or Omdurman strains}}{LD_{50} \text{ susceptible strain}}$

±, Log-dose probit line based on two points only.

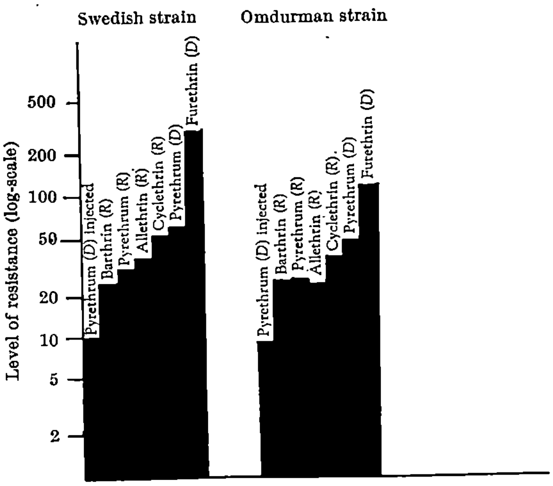


Fig. 1. Patterns of pyrethrin-resistance in two strains of houseflies

tion between DDT- and pyrethrin-resistance in the Swedish strain has resulted from prior use of both insecticides in the field. However, a definite relationship between resistance to the two insecticides is shown here for the Omdurman flies, which were susceptible to Pyrethrum and only moderately tolerant to DDT before selection was initiated (Tables 1 and 2). The nature of this relationship remains obscure. It could be dependent on a single mechanism of resistance which acts with equal facility against either insecticide. Such a mechanism is unlikely to be related to the activity of the enzyme DDT-dehydrochlorinase because DDT and pyrethrins are chemically very different. An alternative explanation for the association between DDT- and pyrethrin-resistance is that there are separate resistance mechanisms which have a correlated genetic basis in the form of pleiotropism, modifying genes or closely linked genes. However, it is doubtful whether the same complex genetic apparatus would be responsible for DDT- and pyrethrin-resistance occurring together in different species.

The two resistant strains are also more or less resistant to dieldrin and malathion. Dieldrin-resistance, being greatly different in the two strains, is probably not related to pyrethrin-resistance. Tolerance to malathion in both strains may be due to previous use of this insecticide in the field.

The results presented here also provide some preliminary information about the nature of pyrethrin-resistance. The high levels of resistance obtained for Pyrethrum and the synthetic pyrethroids are indicative of physiological resistance rather than 'vigour tolerance'. Lethane, an organic thiocyanate, is chemically unrelated to any of the other insecticides used. Resistance to Lethane can, therefore, be used as a measure of 'vigour tolerance'. Tolerance to this insecticide is of a low order in both resistant strains, and therefore the contribution of non-specific factors to pyrethrin-resistance is negligible. The high order of resistance to Pyrethrum in the Swedish and Omdurman strains is greatly reduced when measured drops of the insecticide are injected into the flies instead of being applied to the back of the thorax (Table 2). This does not necessarily indicate that the cuticle of resistant flies is less permeable to insecticides than the integument of susceptible individuals. The loss of resistance on injection may be due to a sudden flooding of the protective mechanism of resistant flies[9]. Alternatively, the protective mechanism may be concentrated in the cuticle and is by-passed on injection.

It will be observed from Fig. 1 and Table 2 that resistance to Pyrethrum extends to all the pyrethroids tested. It would appear that the same defence mechanism can cope with all these insecticides. It is also noteworthy that the highest levels of resistance are obtained for the two compounds, Cyclethrin and Furethrin, which do not have an open side chain to the keto-alcohol.

Further genetic, biochemical and toxicological work is being undertaken in order to investigate some of the problems raised in this article.

This work was supported in part by a grant from the South African Council for Scientific Research while I was holder of a Union Postgraduate Scholarship from the University of the Witwatersrand, Johannesburg. The Pyrethrum Board of Kenya and Mitchell Cotts and Co., London, supplied samples of Pyrethrum. Allethrin, Barthrin, Cyclethrin and Furethrin were supplied as samples of commercial products by McLaughlin Gormley King Co., Minnesota.

[1] Busvine, J. R., *Nature*, **171**, 118 (1953).
[2] Busvine, J. R., *Bull. World Health Org.*, **19**, 1041 (1958).
[3] Whitehead, G. B., *Nature*, **184**, 378 (1959).
[4] Davies, M., Keiding, J., and Von Hofsten, C. G., *Nature*, **182**, 1816 (1958).
[5] Busvine, J. R., *Nature*, **174**, 783 (1954).
[6] Busvine, J. R., *Ent. Exp. Appl.*, **2** 58 (1959).
[7] Busvine, J. R., *Nature*, **168**, 193 (1951).
[8] Busvine, J. R., *Ceylon J. Med. Sci.*, (*D*), **6**, Pt. 4 (1949).
[9] Oppenoorth, F. J., *Nature*, **181**, 425 (1958).

ARTHROPOD TRANSMISSION OF MICRO-ORGANISMS

By Dr. JOHN MACLEOD

Field Research Laboratory, Animal Health Division, Ministry of Agriculture, Blackford, Carlisle

IT is becoming increasingly difficult to treat as separate subjects the propagation of a micro-organism which is acting as an animal or plant pathogen and the similar propagation of one not causing disease. There is a growing need for a common terminology, but, until our knowledge of microbial biology has expanded very considerably, it is not likely that agreement about this will be reached. In the meantime we can at least so define some of the terms in present use that they can if necessary be applied without further *ad hoc* definition outside the particular field for which they were coined. The terminology of biological transmission of disease agents by arthropods has now reached a stage where precise definition and some revision are necessary. The reasons for these and the rationale of a suggested system are given here in the hope that they may help to form the basis of an agreed terminology in which a term means what it says, and can so be used irrespective of the precise context.

The usual division of transmission mechanisms is into mechanical and biological. The distinction between these has been variously interpreted as either or both multiplication of the agent within the arthropod and existence of an extraneous incubation period of the disease; different interpretations of what constitutes multiplication within the vector, and even the acceptance of simple invasion of the vector tissue prior to transfer as sufficient criterion for biological transmission, have further confused a classification at no time adequately defined, and now untenable as a simple dichotomy in the light of modern knowledge. Day[1], reviewing the mechanisms of arthropod transmission of viruses, suggested that "the cyclopropagative type best describes most examples of biological transmission of viruses by arthropods". This, however, begs the question of defining the limits of the biological type. In the following proposed scheme this time-hallowed term has been dropped as a description of a defined category.

We could usefully take the two terms 'horizontal' and 'vertical transmission', suggested by Gross[2] for transmission of a pathogenic agent, as describing the limiting conditions. 'Horizontal' means spread between contemporaries, that is, from one host to another, usually of the same generation, whether by contact, by fomites, or biological being immaterial. 'Vertical transmission', on the other hand, is from one generation to the next in the same line, that is, hereditary, though not genetically inherited. Pathogenic agents thus transmitted from host to offspring are often latent, and their presence may be unsuspected until circumstances upset the host–microbe equilibrium, or horizontal transmission occurs to another host, and disease results. Such agents may enter a spell of horizontal propagation before reverting to the vertical mode. It thus seems theoretically possible for the same organism to have a strictly hereditary (vertical) mode of transmission, and a strictly infectious (horizontal) mode, and to oscillate irregularly between, as, for example, with the phages[3].

Between these limiting types of transmission there are the types which involve two host species—the ziz-zag transmission of Andrewes[4]. It is with these I am mainly concerned here, but Gross's terms are useful not only in defining the extremes of the range of propagation methods but also in helping to clarify our appreciation of the fundamental differences between these extremes. In horizontal transmission it is not of immediate importance—to the organism—whether or not the host be killed. In vertical transmission the organism obviously must not kill its host, at least until the next generation is established; it is generally avirulent or inactive. In vertical transmission the microbes might indeed be regarded as disease agents 'looking for a disease', but because of the possibility of oscillation of their mode of transmission they cannot be ignored, even by the specialist interested only in the 'disease' reaction of the definitive host to infection.

The concept is so useful that where the terms can be adapted to the special case of arthropod transmission, without doing violence to their original definition, there is a case for using them.

Types of Transmission

Excluding the accidental type of transference arising from surface contamination, for example of the feet or proboscis of the insect, eight types of process or sequence can be recognized in significant transmission of disease to vertebrates by arthropods which actually ingest the infected tissue; they are probably equally applicable in the case of plant hosts:

(1) *Mechanical.* This may be by direct inoculation (flying needle), as in myxomatosis, or indirect, by contamination, for example by regurgitation or defæcation on food, as with poliomyelitis and flies. There need not be multiplication in the vector, and the duration of infectivity is often quite short. This type probably includes groups I and II of Watson's[5] list of plant viruses.

(2) *Simple multiplicative.* For example, plague bacilli in fleas. This may also be the type occurring in lice infection with *Rickettsia quintana* of trench fever, that is, there may even be an 'extrinsic incubation period' before transmission of the organism is possible. Nevertheless, this should be regarded as direct transference from host to host, on the grounds that the organism does not dwell in the vector tissue. Multiplication is only incidental to the sojourn in the shelter of the vector's intestinal tract, and is not essential, since theoretically the original units themselves might cause the infection of the new host. Some of Watson's group III viruses are probably transmitted in this manner, although some, and all his later, groups belong to my next main category.

The transmission in these two types is strictly horizontal in the sense that Gross uses the term, and it is suggested that, for arthropod transmission, the term be used to separate them from the second main category, in which the organism invades the tissue of the vector.

In other cases the organism may become more intimately associated with the vector. Invasion of the tissue occurs, and the association, so far as the original units are concerned, may be regarded as the end of the journey, instead of the mere sojourn it represents in horizontal transmission. Multiplication of the organism within the tissues of both hosts is probably necessary to propagation, and Andrewes's term 'zig-zag' is suggested as a comprehensive term for this class of transmission. The term is less confusing than 'developmental transmission', since this has been used also for transmission involving development of the vector from the infected instar to the next before it becomes infective.

These 'zig-zag' transmission sequences might be encompassed within one stage of the vector's life-history (intra-stadial); may involve stage-to-stage survival, or the organism may survive throughout a complete stage, for example from the infected larva of a fly through the pupa to the infective adult. The term 'trans-stadial' is probably the most useful for this last. Unfortunately, this term has also been used, wrongly, for stage-to-stage, that is, implying 'trans-moult' only. The confusion is not restricted to comparison of descriptions by different writers; in a recent American paper the term was defined early in the article as meaning passage of disease agents between active stages of a vector, and used later in the same article to refer to passage from the larva to the female tick, that is, across a complete intermediate stadium.

For the above three types the following names are suggested:

(3) *Intra-stadial transmission.* The infected stage becomes infective. For example, the products of intra-cellular multiplication of *Rickettsia prowazekii* in the epithelium of the louse gut may result in the fæces of the same instar becoming infective. Classical examples are the mosquito-malaria and tsetse-trypanosomiasis associations.

(4) *Stage-to-stage transmission.* That is, one stage becomes infected, the next infective. This accepted term is clear and convenient, and only for adverbial use would a single term be more convenient: 'ecdysial' might serve. This type is common in arthropods which have one meal per stadium, for example three-host ticks.

In this and the following types it is assumed that multiplication of the organism occurs in the vector. It may happen that among the Protozoa, and possibly the *Rickettsiae*, there is elimination of all the ingested elements except a limited number which survive either because of their stage of development or by accident of their location; these could conceivably remain in a non-multiplying form for long periods, but an ultimate phase of multiplication seems an unavoidable precursor to infectivity. With the viruses, multiplication has been demonstrated in, for example, Colorado tick fever, and it is probably a safe assumption that this must usually occur in the vector, in view of the die-away rate to be expected and, in the case of ticks, of the long periods usually involved in metamorphosis.

When stage-to-stage transmission occurs and the infective vector feeds, three things may happen: (*a*) it may lose its infection, either by 'cleansing', due to some immunity process, or through absence of opportunity for re-infection; (*b*) it may become re-infected by its own strain (type 5); (*c*) it may retain a residual infection in its tissues, so that the next stage in that generation is also infective (type 6).

In (*a*) and (*c*) it is to be supposed that the result is not affected by the fate of the infection passed to the host; the invading organism may have been killed 'at the beach-head', may have multiplied to cause an inapparent infection, without high concentration at the infection site, or may have increased rapidly in frank infection. Where the vertebrate host reacts to infection the (*b*) kind of transmission sequence—a modified stage-to-stage type—may be generated: if the vector's meal is sufficiently prolonged, as is sometimes possible with ixodid ticks, or where it can have a series of meals on the same host, as with argasids, fleas or bed-bugs, then re-infection may occur.

(5) *Re-infecting transmission.* In this the infecting stage gets back its own infective strain, possibly made more virulent or otherwise conditioned by its short homoiothermous spell. Such re-infection has also been described as perpetuation of infection. This term is obviously unsuitable where the vector is limited to one meal per life-stage, since it might have to be applied to continuation of infectivity merely through a single ecdysis—nymph to adult—in a case where infection was not hereditary.

With certain organisms, for example *Theileria parva*, this re-infection of the infecting tick is necessary, that is, trans-stadial survival apparently does not occur, but in many instances it will be difficult to determine whether the infectivity of the vector is due to trans-stadial survival or re-infection.

Where re-infection occurs regularly, and there is no hereditary barrier in the vector, then indefinite perpetuation should occur, differing from the vertical transmission of Gross only in that the organism is subjected to a regular alternation of arthropod and vertebrate host. It is tempting to use Andrewes's term 'zig-zag' to distinguish this modified form of vertical transmission sequence, but this would be an unjustifiable restriction of the original intention of the term.

(6) *Trans-stadial transmission.* This means the carrying over of infection, latent or residual, throughout a complete stage, and through two ecdyses. The intermediate stage may be active, as in the case of a three-host tick, where infection acquired by the larva would be transmissible by the female, whether or not the nymph was infective.

It is to be noted that in these definitions the term infection is applied, for convenience, to the stage which takes in the infected meal. A tick larva thus acquires infection in an uncritical sense, but the infective agent may never invade an organized larval tissue, that is, the nymph may well be the first stage to become infected in the strict sense. In trans-stadial transmission, the nymph may or may not be infective. With ticks infected as larvæ with tick-borne fever, for example, the nymphs are infective; if they feed on a non-susceptible host, the female is still infective. In the intermediate stage, however, the infection may be latent, or infectivity may be frustrated as in a pupal stage.

(7) *Transoval transmission.* This is merely a special instance of type 6—trans-stadial transmission—since the egg is the equivalent of a stadium, but it requires to be separately described for two reasons: (*a*) The mechanism of a transoval barrier to propagation of a microbe may be different to that preventing trans-stadial propagation. Trans-stadial survival is possible without hereditary survival, for example in tick-borne fever of sheep. (*b*) Transmission from the adult to the larval stage may not be through the female; infection of the egg may be due to the male. This possibility of male transmission will probably receive more attention in the future than it gets at present, for the possibility of doubling the infection-rate in the larval population is obviously going to be extremely important in those cases where there is only a very low percentage 'take' among adults exposed to infection.

The most commonly used term to-day for hereditary transmission in the vector is 'transovarial'. It is regrettable that this word has crept into general usage, for it is unsatisfactory on two counts: (*a*) it excludes transmission by the male to the zygote; (*b*) although the prefix 'trans' is permissible for an imagined passage across a complete stadium, 'trans-ovarial' merely implies passage across an adult organ. Even conceding that it could be taken to mean penetration into the ovariole, it is still inadequate as it does not necessarily imply entry into the egg or persistence into the larval stage.

There may be objection to the term 'hereditary' because of the implication of a genetic factor. 'Egg-borne', which has also been used, has an undesirable connotation with fomites. Probably the most appropriate word is congenital, but for homology with trans-stadial the term transoval is suggested.

The scrub-mite, which feeds on animals only as a larva, is an interesting case in that transmission by it of the rickettsia of mite typhus must necessarily be both trans-stadial and transoval.

(8) *Interrupted vertical transmission.* Basically from one generation to another in the same lineage of either host, the term, in relation to arthropod-transmitted disease, would imply ability of an organism to survive repetitive trans-stadial/transovval propagation in the vector, as possibly in an arthropod reservoir of a vertebrate pathogen. A break in the series of vertical transmissions might conceivably be due merely to a casual accident, such as eating of the arthropod, and here we are up against the limiting case where it is doubtful if we can justifiably regard the arthropod as vector rather than definitive host. Such vertical transmission obviously must be occasionally interrupted, for us to become aware of it. The next type in the series, unbroken vertical propagation, carries our subject beyond the limits of the field of disease transmission.

I am grateful to Dr. C. H. Andrewes and Dr. C. E. Gordon Smith for their comments on the classification, which have removed some at least of its weaknesses.

---

[1] Day, M. F., *Exp. Parasit.*, **4**, 387 (1955).

[2] Gross, L., *Blood*, **9**, 557 (1954).

[3] Darlington, C. D., *Trans. Roy. Soc. Trop. Med. Hyg.*, **54**, 90 (1960).

[4] Andrewes, C. H., *Adv. Virus Res.*, **4**, 1 (1957).

[5] Watson, M. A., *Rep. Seventh Commonwealth Ent. Conf.*, 157 (1960).

PHYSIOLOGY AND PATHOGENESIS OF THE STEIN–LEVENTHAL SYNDROME

By Dr. VIRENDRA B. MAHESH and Dr. ROBERT B. GREENBLATT
Department of Endocrinology, Medical College of Georgia, Augusta

Urinary Androsterone, Etiocholanolone, Pregnanetriol and Δ^5-Pregnenetriol of Ovarian Origin in a Patient with Stein–Leventhal Syndrome

It has been observed by several workers that patients suffering from idiopathic hirsutism and Stein–Leventhal syndrome have elevated levels of urinary androsterone (5α-androstane-3α-ol-17-one) and etiocholanolone (5β-androstane-3α-ol-17-one)[1–4], and that there is a marked decrease in the excretion of these steroids on wedge resection of the ovary[1,3,4]. Cox and Shearman[5] have found normal levels of pregnanetriol (5β-pregnane-3α,17α,20α-triol) and elevated levels of Δ^5-pregnenetriol (Δ^5-pregnene-3β,17α,20α-triol) in patients with Stein–Leventhal syndrome. The role of the adrenals and the ovaries in the secretion of these steroids has been a subject of much discussion in the past. In the present work a complete fractionation and individual estimation of urinary steroids of a typical case of Stein–Leventhal syndrome has been carried out under various medications and the results compared with those of a normal subject, in order to establish the origin of the above-mentioned compounds.

Aliquots of the 24-hr. urine (1/20 volume) were evaporated to near dryness at 45° on a flash evaporator and incubated with 0·5 ml. gluculase (50,000 units β-glucuronidase; 25,000 units phenol sulphatase) in acetic acid–sodium acetate buffer (*p*H 4·8) at 40° for 16–18 hr. After a preliminary extraction, the urine and washings were hydrolysed with hydrochloric acid at *p*H 1 at room temperature for 24 hr. and re-extracted. The extracts were combined, and the separation and estimation of steroids made using the method of Bush and Mahesh[6]. The estimation of pregnanetriol and Δ^5-pregnenetriol was carried out by eluting the appropriate zones from paper, followed by oxidation with periodic acid and assayed as etiocholanolone and dehydroepiandrosterone.

The patient with Stein–Leventhal syndrome was sent to hospital, and two 24-hr. urine samples were collected as controls (Controls 1 and 2). She was then given 25 units of adrenocorticotrophic hormone (ACTH) intravenously for two consecutive days and urines were collected (ACTH 1 and 2). This was followed by 4 days of β-methasone, 0·6 mgm. four times daily, and the urines were collected on the last two days (β-methasone 3 and 4). Finally, 0·3 mgm. of β-methasone four times daily and 5 mgm. of human follicle-stimulating hormone (FSH) once a day were administered concurrently for 6 days, and the urines collected on the last two days (FSH 5 and 6). This was followed by wedge resection of the ovary, and another 24-hr. urine specimen was collected a week after surgery and withdrawal of medications (post-

Table 1. Urinary Steroid Levels in mgm. per 24 Hr. in a Normal Patient and one with Stein–Leventhal Syndrome subjected to Stimulating and Suppressive Measures

Patient with Stein–Leventhal syndrome	Dehydro-epiandrosterone	Etiocholan-olone	Androsterone	Pregnanetriol	Δ^5-Pregnene-triol	Tetrahydro-corticosteroids	11-Oxy-17-ketosteroids
Control 1	< 0·1	3·2	3·3	1·4	< 0·1	9·1	1·1
Control 2	< 0·1	3·3	3·5	1·0	< 0·1	9·3	1·3
ACTH 1	1·0	5·5	5·9	1·8	1·5	37·7	5·2
ACTH 2	1·4	5·9	6·4	2·8	2·9	39·2	5·7
β-Methasone 3	< 0·1	1·9	2·1	0·8	< 0·1	0·7	0·3
β-Methasone 4	< 0·1	2·1	2·3	0·3	< 0·1	0·4	< 0·1
FSH 5 (+ β-methasone)	0·5	7·4	6·1	3·2	8·8	< 0·1	< 0·1
FSH 6 (+ β-methasone)	0·8	6·0	5·7	7·3	9·4	< 0·1	< 0·1
Post-operative	< 0·1	0·5	0·3	< 0·1	< 0·1	2·6	0·8
Normal subject							
Control 1	0·2	2·1	0·9	0·4	0·2	4·0	2·1
Control 2	0·1	2·2	0·7	0·3	0·2	4·2	1·8
β-Methasone 3	< 0·1	0·7	0·3	< 0·1	< 0·1	0·4	0·1
β-Methasone 4	< 0·1	0·4	0·2	< 0·1	< 0·1	0·3	< 0·1
FSH 5 (+β-methasone)	< 0·1	0·9	0·5	< 0·1	< 0·1	< 0·1	< 0·1
FSH 6 (+β-methasone)	< 0·1	0·8	0·4	< 0·1	< 0·1	< 0·1	< 0·1

operative). A similar examination was made of a normal subject with carcinoma *in situ* of the cervix, with the exception that ACTH was not administered to this case. The results of the urinary assays are presented in Table 1.

These results show that the patient with the Stein–Leventhal syndrome had elevated levels of androsterone and etiocholanolone before and after β-methasone suppression as compared with the normal subject. Human FSH brought about an average increase of 8·4 mgm. of androsterone and etiocholanolone in the patient suppressed with β-methasone, whereas the corresponding increase in the normal was 0·5 mgm. Further, there was an almost ten-fold increase in the levels of pregnanetriol and Δ^5-pregnenetriol when the patient was on human FSH. The increase on ACTH administration was only four-fold. The large amounts of androsterone, etiocholanolone, pregnanetriol, and Δ^5-pregnenetriol in the urine, when the patient was stimulated with human FSH, appear to be of ovarian origin. The human FSH administered appeared to be reasonably free of ACTH contaminants as indicated by the absence of urinary tetrahydrocorticosteroids and 11-oxygenated 17-ketosteroids. The ovarian origin of Δ^5-pregnenetriol is further confirmed by the isolation of 17α-hydroxy-Δ^5-pregnenolone in the ovaries of the patient. The ovaries, as well as the peripheral blood, also showed the presence of large amounts of dehydroepiandrosterone when the patient was given human FSH. Furthermore, incubation of ovarian slices from the patient with various steroid precursors indicated a failure of the enzyme 3β-ol dehydrogenase, thus accounting for the presence of large quantities of 17α-hydroxy-Δ^5-pregnenolone and dehydroepiandrosterone in the ovary[3]. The urinary pregnanetriol, androsterone and etiocholanolone may be metabolites of the ovarian 17α-hydroxy-Δ^5-pregnenolone (ref. 7) and dehydroepiandrosterone[8].

Role of FSH in the Pathogenesis of the Stein–Leventhal Syndrome

Hypergonadotrophism has been cited as the basis for the polycystic ovary ever since the report of Stein and Leventhal[9]. McArthur *et al.* have pointed out that luteinizing hormone (LH) assays are persistently though only moderately elevated[10]. The constancy of theca cell hyperplasia has been interpreted as synonymous with theca luteinization and excess LH has been implicated[11]. More recently, Gallagher suggested an adrenal component in the Stein–Leventhal syndrome[2]. Our investigations seem to indicate an inherent enzymic defect in the ovaries leading to hyperovarianism and resulting from persistence of FSH stimulation.

For some time we have suspected that FSH might be a factor in the causation of the large pale polycystic ovaries. Wedge resection has proved beneficial because of reduction of ovarian mass which in all probability permitted a better ratio of endogenous FSH and LH per unit of ovarian tissue. Total urinary gonadotrophins have been shown repeatedly to assay within normal limits, so that the claim of hypergonadotrophism has not been substantiated. The administration of human chorionic gonadotrophin (APL), a substance more closely allied to LTH (luteotrophin) than LH, has not been attended by any real degree of enlargement of the ovaries. In the present work, 5 mgm. of human FSH was administered daily for 6 days to a normal female and to two patients with Stein–Leventhal syndrome. The administration of human FSH induced at least a five-fold enlargement of the ovary in the two patients with Stein–Leventhal syndrome (Fig. 1), but no noteworthy increase in size of the ovary of the patient with normal ovulatory cycles. Keetal *et al.*, employing highly purified sheep FSH, noted markedly responsive ovaries in patients with the Stein–Leventhal syndrome but not in normal women[12]. Furthermore, when APL was administered following FSH to one of the Stein–Leventhal syndrome patients, multiple corpora lutea in various stages of development were induced. The ovaries were not enlarged any further over those of the FSH stimulation as to gross size except for the corpora lutea bulging the surface (Fig. 2). Histologically, FSH alone caused marked follicular growth. The follicles were lined by many layers of granulosa cells and the cells of the theca interna frequently appeared swollen and hyperplastic (Fig. 3). When APL was given following the FSH, corpora lutea in various stages of development were numerous; the granulosa cells were hypertrophied and luteinized comparable to that seen in corpus luteum formation (Fig. 4). In a measure, the effective addition of gonadotrophins to women with unresected polycystic ovaries is comparable with the effectiveness of endogenous gonadotrophins on the wedge resected ovary. In one of our experiments, simply removing one of the polycystic ovaries, leaving the contralateral ovary intact and untouched allowed the same chain of events to occur with induction of cyclic ovulatory menses[13]. Furthermore, the employ-

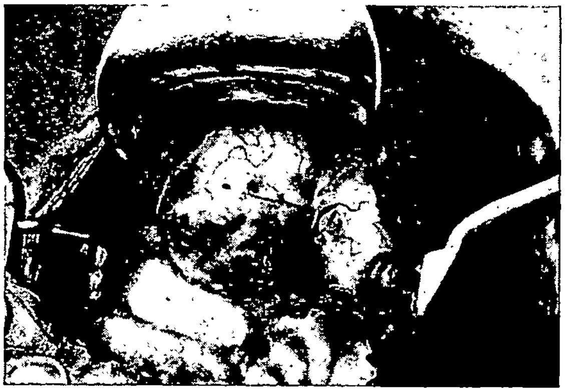


Fig. 1. Generalized enlargement of ovaries following FSH stimulation

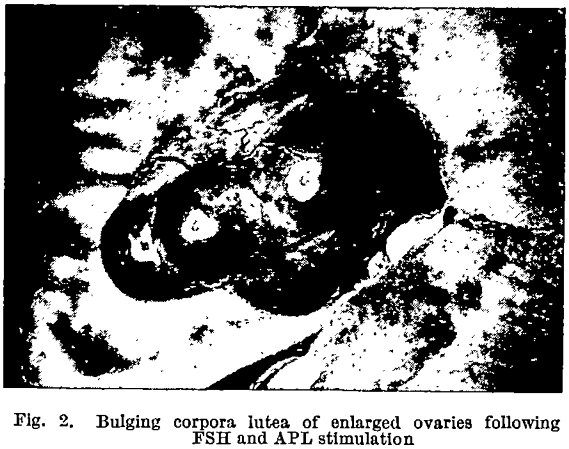


Fig. 2. Bulging corpora lutea of enlarged ovaries following FSH and APL stimulation

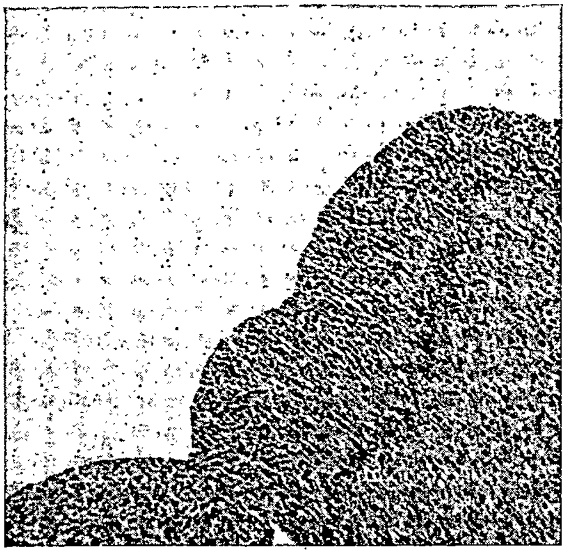


Fig. 3. Histological section from one of the many enlarged follicle cysts showing marked proliferation of granulosa cells and hypertrophy of theca interna (× 80)

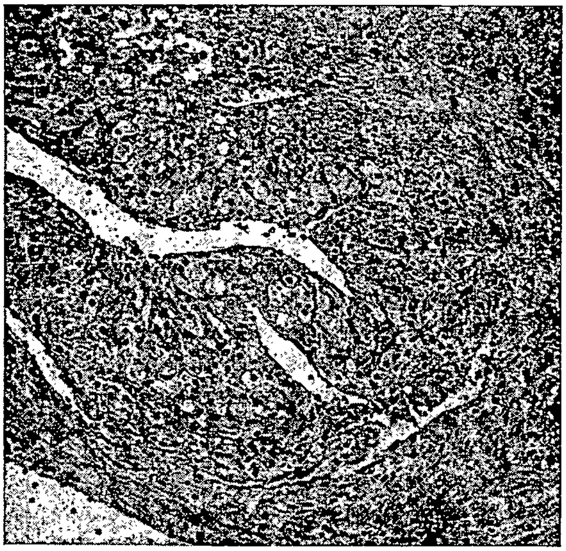


Fig. 4. Histological section of one of the many corpora lutea. This one is in an early stage of development, showing luteinization of the granulosa cell layer and theca interna (× 80)

ment of glucocorticoids in certain patients with Stein–Leventhal syndrome has frequently proved successful[4,14,15]. This finding confounded the confusion as to its etiology and seemed to support the adrenal origin for the syndrome. The similarity in response to wedge resection and corticoid therapy has been noted in several instances and this observation helped to clarify the situation. For example, one recent patient responded satisfactorily to wedge resection by cyclic ovulatory menses and conception. A few years later this patient experienced a recurrence of the syndrome. Dexamethasone therapy was instituted with return of cyclic ovulatory menses and subsequent pregnancy. It may be assumed that glucocorticoids act by permitting release of more gonadotrophins either through suppression of ACTH or some of the adrenal steroids. Sohval and Soffer have shown increased release of gonadotrophins following cortisone therapy[16]. These observations lend support to the concept that whatever improves the ratio of gonadotrophins to ovarian mass, either by exogenous administration of gonadotrophins, wedge resection, removal of one ovary, or glucocorticoid therapy, may prove corrective at least temporarily.

It may be conjectured that the Stein–Leventhal ovary is sensitive and highly responsive to endogenous FSH so that hundreds of follicles receive inadequate stimulation, producing a myriad of microfollicular cysts and a general enlargement of the ovary. The available LH is insufficient to induce ovulation not only because of its dilution per unit of ovarian tissue but also because the maturation of the prime follicles is incomplete. However, when exogenous FSH is administered certain follicles are brought to a further stage of maturation so that when APL is added these follicles are transformed to corpora lutea.

It is probable that the Stein–Leventhal ovary is the result of persistence of endogenous FSH stimulation due to an inherent enzymic defect in the ovary. This view is confirmed by incubation studies with ovarian slices from two patients with the Stein–Leventhal syndrome in whom a failure of the enzyme 3β-ol dehydrogenase was demonstrated[3]. According to the present concept of steroid biosynthesis in the ovary, cholesterol is transformed into œstrogens via Δ^5-pregnenolone, progesterone, 17α-hydroxyprogesterone and Δ^4-androstenedione. In our studies the synthesis of steroids in the Stein–Leventhal ovary did not seem to follow the normal sequence. It appeared that the ovaries, because of enzyme failure, converted Δ^5-pregnenolone to dehydroepiandrosterone via 17α-hydroxyΔ^5-pregnenolone, so that output of ovarian œstrogens is considerably diminished. The lessening of a regulatory influence of the œstrogens on the pituitary permits the persistence of FSH stimulation with the ultimate development of large pale ovaries—the polycystic ovary of the Stein–Leventhal syndrome.

A detailed report of this investigation will be presented elsewhere, for we have found several variants of the Stein–Leventhal syndrome. We are grateful to Dr. Elmer Alpert of Merck, Sharp and Dohme for the supply of human FSH, and to Drs. S. Conrad and W. Hermann for estimation of blood dehydroepiandrosterone sulphate. This work was supported by grant No. *A*–4429 from the National Institute of Arthritis and Metabolic Diseases.

[1] Hermann, W., Buckner, F., and Morris, J., *Fertility and Sterility*, **2**, 74 (1960).

[2] Gallagher, T. F., Kappas, A., Hellman, L., Lipsett, M. B., Pearson, O. H., and West, C. D., *J. Clin. Invest.*, **37**, 794 (1958).

[3] Mahesh, V. B., and Greenblatt, R. B., Forty-third Meeting of the Endocrine Society (Program), New York (June 1961).

[4] Greenblatt, R. B., *Amer. J. Obst. and Gynec.*, **66**, 700 (1953).

[5] Cox, R. I., and Shearman, R. P., *J. Clin. Endocrinol. and Metab.*, **21**, 586 (1961).

[6] Bush, I. E., and Mahesh, V. B., *J. Endocrinol.*, **18**, 1 (1959).

[7] deCourcy, C., *J. Endocrinol.*, **14**, 164 (1956).

[8] Mason, H. L., and Kepler, E. J., *J. Biol. Chem.*, **160**, 225 (1945).

[9] Stein, I. F., and Leventhal, M. L., *Amer. J. Obst. and Gynec.*, **29**, 181 (1935).

[10] McArthur, J. W., Ingersoll, F. M., and Worcester, J., *J. Clin. Endocrinol.*, **18**, 1202 (1958).

[11] Robinson, M. R., *Amer. J. Obst. and Gynec.*, **30**, 18 (1935).

[12] Keetal, W. C., Bradbury, J. T., and Stoddard, F. J., *Amer. J. Obst. and Gynec.*, **73**, 954 (1957).

[13] Greenblatt, R. B., *Maryland State Med. J.*, **10**, 120 (1961).

[14] Jones, G. E. S., Howard, J. E., and Langford, H., *Fertil. and Steril.*, **4**, 49 (1953).

[15] Greenblatt, R. B., *et al.*, *Metabolism*, **7**, 25 (1958).

[16] Sohval, A. R., and Soffer, L. J., *J. Clin. Endocrinol.*, **11**, 677 (1951).

EVALUATION OF QUANTAL NEUTRALIZATION TESTS

By Prof. S. FAZEKAS de ST. GROTH

Department of Microbiology, Australian National University, Canberra

VIRUSES are readily neutralized by specific antibody, and there are several well-tried methods for the assay of antisera. Unfortunately, the slope of dose-response curves varies when different sera are tested in the same host system, or when the same serum is tested in different hosts. Neutralizing titres can be compared, therefore, only by ignoring a basic property of the reaction; if this property, the variability of slopes, is recognized, no comparisons can be made at all. It is a pity that the practical immunologist cannot go about his job both intelligently and with a clear conscience.

The dilemma, however, is not insoluble. It will be shown that by a simple transformation, based on the theory of virus-antibody interactions[1], the quantal dose-response curve becomes linear with unit slope over the practically important range of concentrations. The new metameter defines a natural unit for the comparison of antisera, and also allows the estimation of other constants entering the neutralization process.

Definition of the metameter. The basic equilibrium between antigenic sites on the virus particle and antibody molecules is defined by:

$$K = \frac{[sV(1+n) - x][A - x]}{[x]} \qquad (1)$$

where K is the dissociation constant, s the number of antigenic sites per virus particle, V the concentration of infective and nV the concentration of non-infective particles, A the concentration of antibody molecules, and x the concentration of formed antigen-antibody complexes.

If, as is likely[2], a single molecule of antibody bound to a critical site neutralizes the infectivity of a virus particle, the fraction of survivors will be:

$$\frac{V_s}{V} = \left(1 - \frac{x}{sV(1+n)}\right)^c \qquad (2)$$

where V_s is the concentration of surviving infective particles, and c the number of critical sites per virus particle. In a quantal test V_s is unity, by definition. Here equation 2, solved for x and written now in logarithmic form, will read:

$$\log V = -c \log\left(1 - \frac{A}{sV(1+n) + A + K}\right) \qquad (3)$$

At low virus input the value of $sV(1+n)$ will be smaller than K by several orders of magnitude, and can therefore be safely neglected. This leads to:

$$\log V = c \log(1 + A/K), \text{ if } sV(1+n) \ll K \qquad (4)$$

The term $\log(1 + A/K)$ may be expanded to give:

$$\left[\frac{A}{K} - \frac{1}{2}\left(\frac{A}{K}\right)^2 + \frac{1}{3}\left(\frac{A}{K}\right)^3 - \ldots\right] \log e$$

Since in this region A/K is at least two orders of magnitude lower than unity, its higher powers are completely negligible in any biological argument. Thus we may write, with good approximation, by using the first term only of the rapidly converging series:

$$\log V = c\frac{A}{K} \log e \qquad (5)$$

for equation 4. It is evident that equation 5 can now be turned into a linear function of A, with unit slope, by taking logarithms of both sides:

$$\log\log V = \log A + \{\log c - \log K - 0{\cdot}362\} \qquad (6)$$

This, the loglog plot of quantal neutralization tests, is seen to be linear over the range defined above, with a slope of unity.

To demonstrate that the approximations incorporated in equation 6 are fully justified, a family of theoretical neutralization curves[3] will be re-plotted on the new scale.

Such theoretical curves have been obtained by programming a digital computer to solve equation 2 for a wide range of the parameters V, A, K, s, c, n. Representative sets of these curves were plotted on a ($\log A$, $\log\log V$) scale. The empirical choice of this scale is due to Dr. K. J. Lafferty; it was the then unaccountable discrepancy of $-0{\cdot}36$ which prompted closer examination of the equations and led to the present treatment. It was evident that for all values of K, s, c, and $(1 + n)$ likely to occur in practice the loglog plot is linear from $V = 1$ to at least 10^4, and usually beyond 10^6. This covers most of the doses used in routine neutralization tests.

The unit of neutralization. The effect which altered the slope of orthodox ($\log A$, $\log V$) curves—the bracketed term of equation 6—is here seen to govern the intercept of the dose-response line with the abscissa. This intercept commends itself as the natural unit of potency by which antisera may be compared. It corresponds to that concentration of antibody which allows one out of ten infective units to remain free. It should be noted that this value, which may be called the neutralizing dose, is a compound of the quantity (that is, $\log A$) and the quality (that is, $\log c - \log K$) of antibody; the numerical constant, $-0{\cdot}362$, arises from using common rather than natural logarithms, and equals $\log\log e$.

The neutralizing dose is a practical unit: it measures the potency of antisera as they function in laboratory tests and, indeed, in Nature. It is common knowledge that the infective dose by which diseases are transmitted under field conditions lies in the range of 1–10^4 ID_{50}, and it has been shown above that these values fall on the straight limb of the loglog plot. A further practical advantage is that in this range the neutralizing dose is independent of $(1 + n)$, that is, of the ratio of infective to non-infective particles. The importance of this aspect will be appreciated fully on reflecting that the $(1 + n)$ term varies widely from one host system to another, and from one virus preparation to another; its value under field conditions is not readily amenable to measurement, and usually unknown. Differences in test volume, shown to have a profound effect on both the shape of the dose-response curve and the end-points obtained[4], have a simple additive effect on the loglog plot: if the test volume is not unity (1 cm.^{3}), its actual volume will appear in the bracketed term of equation 6, giving $\{\log c - \log K - \log v - 0{\cdot}362\}$, where v stands for the test volume in cm.^{3}. It is obvious that by this method results obtained in one system should be directly comparable with results obtained in any other quantal neutralization test.

The concept of the neutralizing dose may be taken one step further. If an antiserum is found to contain one neutralizing dose when diluted $10 - d$, its potency may be expressed as the negative logarithm, d, of this dilution. So defined, the *neutralizing potency* or *pN* is a form of notation entirely analogous to *p*H. The analogy holds even to the extent that *p*H is a compound of the concentration and activity of dissociable hydrogen atoms. But while the activity there can be defined by a single equilibrium constant, in the more complex system of virus and antibody it involves the bracketed term of equation 6, even over the linear part of the loglog plot. Based on this analogy, we shall talk about neutralizing activity of a particular serum when referring to the quantity $\{\log c - \log K - 0{\cdot}362\}$. Clearly, this value can be read off the loglog plot as $(\text{loglog } V - \log A)$, provided the concentration of antibody molecules has been determined by some independent method, as shown elsewhere[5].

Calculation of *pN*, the Neutralizing Potency

Conventionally, viruses are reckoned in median infective doses (ID_{50}), and not in infective units. In a system where susceptibility does not vary from host to host, one ID_{50} equals 0·693 infective unit. As both the concentration of antibody and the number of ID_{50} are usually expressed in $\log_{10}$ units, the neutralizing potency of a serum can be calculated very simply: the logarithm of the neutralizing titre ($\log V$) minus 0·16 (that is, log 0·693) is subtracted from the final concentration of serum. The actual procedure will be made clearer by applying the method to the first assay from our paper on the neutralization of influenza viruses[4].

An antiserum, diluted in two-fold steps, was mixed with five-fold dilutions of the homologous virus in all possible combinations, using ten replicates at each level. The observed infectivity end-points, worked out by the method of cumulative totals, are given in the first row of Table 1. By subtracting these from the control (titre of virus in the absence of serum), one obtains the number of ID_{50} neutralized (row 2); taking away 0·16 leaves the number of infective units neutralized (row 3). All these operations were in $\log_{10}$ units, that is, in 'tubes' of dilution. Now the

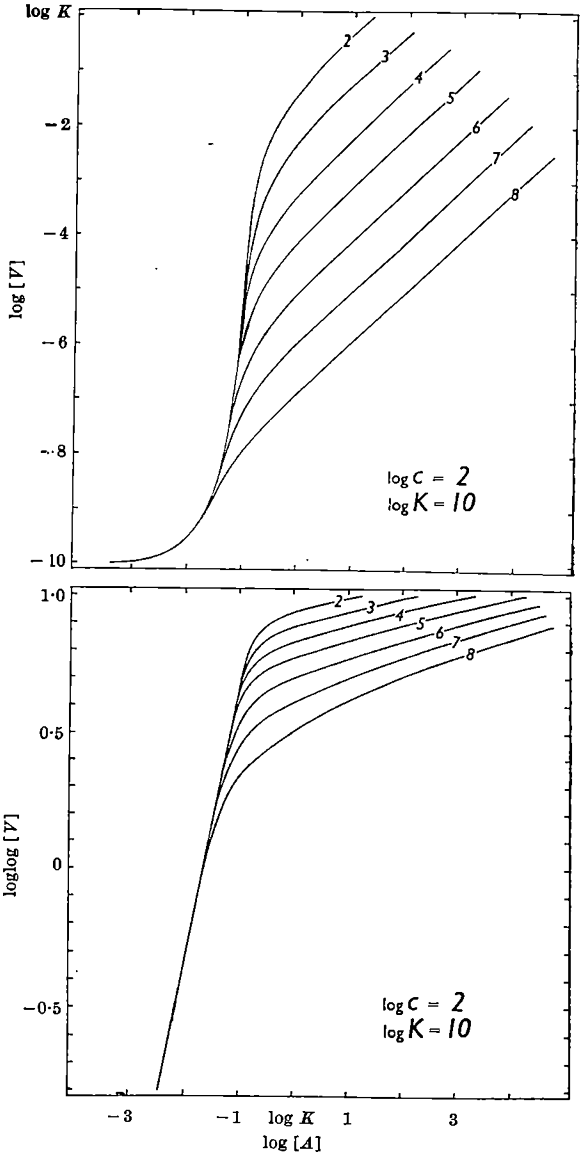


Fig. 1. A family of neutralization curves, computed from equation 2 with the values of $K = 10^{10}$, $c = 100$, and $\log s(n + 1)$ as shown on the curves

Table 1. CALCULATION OF THE NEUTRALIZING POTENCY, *pN*
(Data, on the neutralization of an influenza virus by fowl antiserum, from Fig. 2*A* of ref. 4)

Row	Operation	Computed value	log concentration of antiserum at end-point (= log *A*)													
			−2·70	−2·80	−2·87	−3·00	−3·01	−3·28	−3·23	−3·30	−3·40	−3·50	−3·60	−3·74	−3·90	Control (no serum)
1	—	log ID_{50}	0·58	0·58	1·28	1·81	1·98	2·68	3·38	3·72	4·08	4·78	4·93	5·48	5·99	7·04 7·12
2	Control ID_{50} − Test ID_{50}	Neutralizing titre	6·50	6·50	5·80	5·27	5·10	4·40	3·70	3·36	3·00	2·30	2·15	1·60	1·09	≡0·00
3	Entry 2 − 0·16	log *V*	6·34	6·34	5·64	5·11	4·94	4·24	3·54	3·20	2·84	2·14	1·99	1·44	0·93	
4	log (entry 3)	loglog *V*	0·80	0·80	0·75	0·71	0·69	0·63	0·55	0·51	0·45	0·33	0·30	0·16	−0·03	
5	log *A* − entry 4	log *A*— loglog *V*	−3·50	−3·60	−3·62	−3·71	−3·70	−3·91	−3·78	−3·81	−3·85	−3·83	−3·90	−3·90	−3·87	
6	Entry 5 − log volume	log *ND*	−4·02	−4·12	−4·14	−4·23	−4·22	−4·43	−4·30	−4·33	−4·37	−4·35	−4·42	−4·42	−4·39	

Mean neutralizing potency ± *S.E.* : *pN* = 4·345 ± 0·025

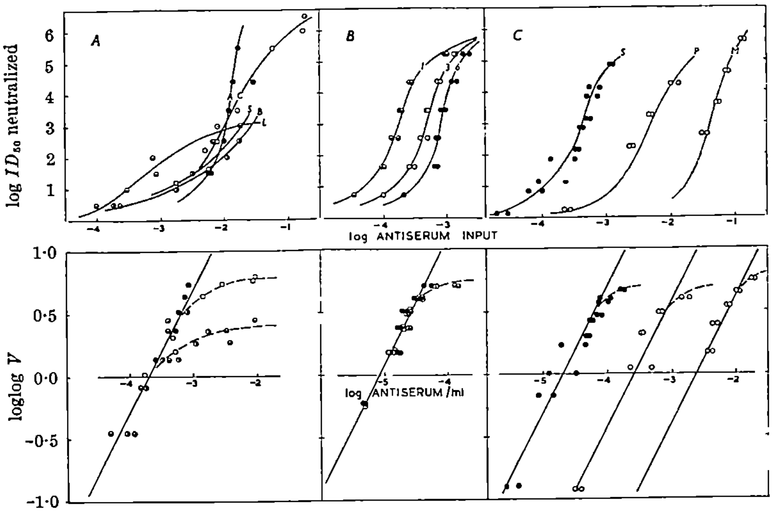


Fig. 2. Comparison of orthodox and loglog plots of data from quantal neutralization tests. *A*, tests of the same virus–antibody mixtures in five host systems (*A* = allantois; *C* = chorion; *L* = mouse lung; *B* = mouse brain; *S* = surviving chorio-allantois). Data of Tyrrell and Horsfall (ref. 6). *B*, tests on the same virus–antibody mixtures in three test volumes (0·35 ml., 1·05 ml., 2·10 ml.) (ref. 3). The ratio of the volumes is shown on the curves. *C*, tests of an antiserum against the homologous and two heterologous antigens (hyperimmune rabbit serum against *SW*-virus; *S* = *SW*-virus; *P* = *PR*8-virus; *M* = *MEL*-virus) (ref. 3)

last value is transformed to loglog *V* by taking its logarithm; a slide-rule will give the required accuracy. The figures obtained (row 4) are subtracted from the serum concentration (head of Table 1) to give row 5, and finally the log of the test volume is added (log 0·30 = – 0·52), giving the neutralizing dose, *ND*. If, as in the example, several estimates are available, we may average them and say that $10^{-4\cdot35}$ ml. of the serum contains one *ND*. The neutralizing potency of the serum will be, of course, $pN = 4\cdot35$.

The average error of such estimates has been determined under a variety of conditions previously[4]; if replicate assays are performed, the error can be determined from the internal evidence of the test. In this example it comes to ± 0·025, in terms of *pN*.

The steps leading to *ND* or *pN* of a serum are set out in detail for purposes of demonstration; they involve four simple subtractions and the looking up of a logarithm to two mantissal places. Obviously, the former can be done by mental arithmetic, and the whole computation takes less than a minute.

Finally, it may be demonstrated, on examples from the literature, that the factors which fundamentally influence the shape and position of orthodox neutralization curves still give a linear loglog plot with unit slope, and lead to a single *ND* or *pN* value for each serum.

Fig. 2*A* shows the data of Tyrrell and Horsfall[6] obtained by testing the same virus-antibody mixture in five host systems. The orthodox plots are incommensurable; the loglog plot leads to the unique *pN* value of 4·70. Figs. 2*B* and 2*C* are our results[3], the former showing the dilution effect, the latter the behaviour of an antiserum when tested against various antigens. Whereas changes of test volume do not influence the loglog plot, the difference of equilibrium constants for the homologous and heterologous systems shows up clearly in their respective *pN* values of 4·68, 3·57 and 2·62.

The aspect to be stressed is the linearity of the loglog plot over almost the whole range of virus concentrations used in practical tests. The variation due to changes in other parameters shows up only in the top part of the curves and, as will be shown later, can be used for the definition of these in the same way as the behaviour of orthodox (log *A*, log *V*) plots has already been used[3,5].

[1] Fazekas de St. Groth, S., Watson, G. S., and Reid, A. F., *J. Immunol.*, **80**, 215 (1958).

[2] Dulbecco, R., Vogt, M., and Strickland, A. G. R., *Virology*, **2**, 162 (1956).

[3] Fazekas de St. Groth, S., and Webster, R. G., *J. Immunol.* (in the press).

[4] Fazekas de St. Groth, S., Withell, J., and Lafferty, K. J., *J. Hyg., Camb.*, **56**, 415 (1958).

[5] Fazekas de St. Groth, S., and Webster, R. G., *Austral. J. Exp. Biol.* (in the press).

[6] Tyrrell, D. A. J., and Horsfall, jun., F. L., *J. Exp. Med.*, **97**, 845 (1953).

INDUCTION OF TUMOURS BY POLYOMA VIRUS: ENHANCEMENT BY CHEMICAL AGENTS

By K. E. K. ROWSON, F. J. C. ROE, J. K. BALL and Dr. M. H. SALAMAN

Cancer Research Department, London Hospital Medical College, London, E.1

It has been shown that in certain cases viruses and chemical carcinogens may interact so that tumour production is modified. For example, Rous *et al.*[1,2] found that when Shope papilloma virus was injected intravenously into rabbits the ears of which had been painted with tar for 1·5–3 months, many warts and several metastasizing epitheliomata soon arose on the tarred ears. Tarring alone gave rise to a few warts, and only one malignant tumour among ninety rabbits, while the intravenous injection of Shope virus alone was ineffective. Ahlström and Andrewes[3] injected tar or carcinogenic hydrocarbons intramuscularly or subcutaneously into rabbits, and Shope fibroma virus intradermally. In some rabbits the tumours due to the virus grew progressively, in others they slowly regressed. In controls which received the virus alone, rapid regression was the rule. Duran-Reynals[4] found that the intradermal injection of vaccinia virus into cortisone-treated mice produced much more severe local lesions than in untreated mice. These more severe lesions were followed by scar formation. If mice were given a course of paintings with methylcholanthrene on both flanks, before treatment with cortisone and vaccination, benign and malignant tumours developed at the site of the vaccination scar. No tumours were seen in control animals which received no previous treatment with methylcholanthrene or no cortisone at the time of vaccination.

In the present work the interaction of polyoma virus, injected into new-born mice, and two chemical carcinogens, 9 : 10 - dimethyl - 1,2 - benzanthracene (DMBA) and 3,4-benzpyrene (BP), and a tumour-promoting substance, croton oil, applied to the skin of the injected mice, has been examined. The experiments were designed to show, first, whether treatment with a chemical carcinogen or a tumour-promoter can modify tumour induction by polyoma virus, and secondly, whether polyoma infection modifies tumour induction by chemicals; for example, whether it can play any part in the two-stage mechanism of carcinogenesis, as tumour-initiator or promoter, or alter the incidence of malignancy. The second question has an important practical bearing, since polyoma is widespread as a latent infection in mouse stocks[5] such as are used for the testing of chemical substances for carcinogenicity. By injecting the virus into new-born animals, and choosing the dorsal skin as the site for later application of the chemical agents, it was reasonable to expect that answers to both these questions could be obtained at the same time. Chemical carcinogens applied to the skin give rise mainly to skin tumours; mammary, ovarian, and pulmonary tumours, subcutaneous sarcomas, and leukæmia may also be induced, but much less frequently. Polyoma virus, on the other hand, induces tumours of the parotid and mammary glands, renal pelvis, bone, and subcutaneous tissues, and less frequently at a variety of other distinctive sites[6,7]. No superficial tumours of the epidermis have been reported, though one strain has been found to produce enormously hypertrophied hair follicles[6]. When distribution and relative frequency are taken into account there should be little difficulty in distinguishing the characteristic effects of each type of agent.

In order to produce tumours in mice with polyoma virus it is necessary to introduce it either before birth, or during the early neonatal period, into mice from a polyoma-free stock. Mice exposed to the virus after the end of the neonatal period develop antibodies to it, but no tumours. These antibodies may pass to fœtal mice through the placenta, or to new-born mice in the milk, and so provide passive immunity[8]. The mothers of the mice used in the present experiment were all free from polyoma hæmagglutination-inhibiting antibodies. They were divided randomly into two main groups which were thereafter housed in separate rooms under otherwise identical conditions. Both groups were fed diet 41*B* supplemented with rolled oats twice weekly, and provided with water *ad libitum*. All the litters from one group were injected subcutaneously during the first 24 hr. of life with 0·02 ml. of fluid containing 10^6 infective doses of polyoma virus (determined by the antibody production test[9]). The strain of virus used, LHP_1, was originally isolated in this department from a C_3H mouse[10] and the material used for injection in the present experiment was the tissue culture fluid and homogenized cells from the fourth mouse embryo tissue culture passage. The group of mice injected with polyoma virus was designated '$P+$' and the control group 'P-free'. The latter litters were injected while less than 24 hr. old with culture fluid from the fourth passage of uninfected mouse embryo tissue. 9,10-Dimethyl-1,2-benzanthracene (DMBA) and 3,4-benzpyrene (BP) were obtained from L. Light and Co.; croton oil from Stafford Allen and Co.; and acetone ('Analar' grade) from British Drug Houses, Ltd.

In general the $P+$ mice grew less well than the P-free mice. Several of the former showed abnormalities of hair growth in the form of bald patches and a sticky appearance of the hair. However, from the tenth week onwards these symptoms disappeared and the mice grew progressively to the same size as the P-free controls. It is most unlikely that the early effects on growth and on the skin were due to the polyoma virus. We were able to isolate a virus in tissue culture from the tissues of similarly affected mice which when injected into mice 5–7 days old reproduced the condition, but was lethal to new-born mice. A similar syndrome has been described by Stanley *et al.*[11]. The inoculum used in the present experiment was found to be free of this agent, which was clearly distinguishable from polyoma virus by the type and species specificity of its cytopathogenic effect in tissue culture. It would seem that the $P+$ groups became accidentally infected with this virus, whereas the P-free groups, housed in another room, remained free of it. All the mice were weaned and sexed at four weeks of age. Ten experimental groups were then constructed, five consisting of $P+$ mice and five of P-free mice. Each group consisted of sixteen males and sixteen females, and mice from individual litters were distributed as widely as possible among the five groups for which they were eligible. By using an ear-punch system of numbering it was possible throughout the experiment to determine from which litter a mouse was derived.

The ten groups were treated with chemical agents (Table 1) and have now been observed for six months. Taking the results in the four groups treated with chemical substances (groups 2–5) together, they had a higher incidence of polyoma tumours than the control unpainted group (group 1). This difference was significant according to the χ^2 test ($0{\cdot}01 > P > 0{\cdot}001$). With regard to epidermal tumours attributed to the treatment with chemical agents, apart from one papilloma in group 9 (P-free and croton oil only), they were only seen in the groups treated with DMBA + croton oil (groups 3 and 8), or with BP twice weekly (groups 5 and 10). The injection of polyoma virus into new-born mice had no marked influence on their subsequent response to treatment with DMBA + croton oil; papillomas began to appear after the sixth week of croton oil treatment in both groups, approximately the same proportion of mice developed papillomas, and the total numbers of tumours after fifteen weeks of croton oil treatment, though on an average somewhat greater in the $P+$ than P-free mice (6·4 and 4·4 per survivor, respectively), was not regarded as significantly different. However, in the group which received weekly BP-treatment a definite difference in response was seen: tumours began to appear during the thirteenth week in the $P+$ mice (group 5) and not until the seventeenth week in the P-free (group 10). At the present time (twenty-first week of treatment with BP) there are forty-eight benign and six malignant tumours in twenty-three $P+$ survivors, but only fourteen benign and one malignant tumour in thirty P-free survivors. Treatment and observation of these two groups continue. In addition to the tumours which could without

Table 1

			Incidence of polyoma tumours before 6 months of age					Incidence of skin tumours 20 weeks after beginning of treatment with chemical agents			
Group*	Inoculated with polyoma virus	Treatment with chemical agents†	Mice with tumours	Total parotid‡ tumours	Total kidney tumours	Total tumours of other sites§	Total tumours all sites	Sur-vivors	Mice with tumours	Total benign tumours	Total malig-nant tumours
1	+	None	4	2	3	1	6	25	0	0	0
2	+	150 μgm. DMBA in 0·2 ml. acetone once	14	15	1	8	24	25	0	0	0
3	+	DMBA as group 2 followed after 3-week interval by 15 once-weekly applications of 0·25 ml. 0·1 per cent croton oil in acetone	13	11	4	5	20	21	15	135	0
4	+	15 once-weekly applications of croton oil as in group 3	12	10	4	7	21	21	0	0	0
5	+	Twice-weekly applications of 50 μgm. BP in 0·2 ml. acetone for 20 weeks	7	7	3	2	12	23	17	48	4
6	0	As group 1			None			32	0	0	0
7	0	As group 2						29	0	0	0
8	0	As group 3						30	21	131	0
9	0	As group 4						30	1	1	0
10	0	As group 5						30	7	14	1

* Each group consisted of 32 mice at start of experiment.

† Applied to dorsal skin after removal of hair. Treatment begun when mice were 7 weeks old.

‡ Parotid gland tumours were multicentric in origin. Involvement of one gland counted as one tumour, and both glands as two tumours.

§ Including bone, mammary glands, and subcutaneous tissues.

doubt be attributed to the polyoma virus, or to treatment with chemical agents, there were seen, in the groups 1–5, a number of cystic lesions in the subcutaneous tissues. Several of these appeared to be simple lymph-filled cysts arising in lymph glands, others were multilocular or filled with thin blood-stained fluid, and not obviously associated with a lymph gland. None of them had any solid elements, and it is doubtful whether any of them were neoplastic. The incidence of mice with these lesions in groups 1–5 was 4, 3, 2, 5, and 4 respectively.

At this point it is necessary to consider a factor which slightly complicated the experiment. During the ninth week of treatment with chemical agents, eight males and eight females from each of the five experimental groups of *P*-free mice (groups 6–10) were tested for hæmagglutination-inhibiting antibodies. All tests were negative. At this time there were already several benign papillomas in group 8. Nine weeks later, a second check for antibodies was made, and on this occasion several positives were found (0/32 in group 6, 1/29 in group 7, 9/30 in group 8, 10/30 in group 9, and 4/30 in group 10). Clearly the polyoma virus had found its way into the *P*-free colony and the infection had begun to spread. However, by this time, except in the case of groups 5–10, the experiment was over, and this incidence of mice with antibodies in the *P*-free group was not sufficient to invalidate conclusions which could otherwise be drawn. In point of fact there was no association between the number of tumours and the presence of antibodies in individual mice of group 8.

The number of tumours which developed in the group of mice which were infected with polyoma virus when newly born, but received no other treatment, was less than was expected from previous experience of this strain of virus. It is possible that an accidental secondary viral infection, which has been described, had an interfering action. However, since all the polyoma-injected mice suffered from this infection, the difference in polyoma-type tumour incidence between the groups which received subsequent chemical treatment and the group which did not is not invalidated. It is conceivable that the difference in the incidence of skin tumours between *P*+ and *P*-free mice treated with chemicals may have been due in part to both viruses, but very unlikely that the accidental infection both inhibited tumour-induction by polyoma virus and enhanced the effect of chemicals on it. Despite the complicating factors the following conclusions appear justified: (1) Mice inoculated with polyoma virus when newly born and later treated by application to the skin of carcinogenic or tumour-promoting agents developed significantly more polyoma-type tumours than mice which received the virus alone. (2) Skin tumours which developed in mice treated repeatedly with BP appeared sooner and in larger numbers, and more became malignant, in those which had been injected when new-born with polyoma virus than in those which had not. (3) Injection of polyoma virus into new-born mice did not initiate tumour formation, as shown by the failure of subsequent treatment with croton oil to produce skin tumours. (4) Polyoma virus injected into the newly born mouse is known to persist throughout life. The presence of this virus did not promote the formation of tumours in mice given an initiating application of DMBA at 7 weeks of age. (5) An intercurrent infection which produced symptoms in all polyoma-injected mice is described. Reasons are given for the view that these conclusions are not invalidated by this accident.

We gratefully acknowledge the technical assistance of Miss C. de Mengel. The cost of this research was in part defrayed out of a block grant from the British Empire Cancer Campaign.

[1] Rous, P., and Kidd, J. G., *J. Exp. Med.*, **67**, 399 (1938).
[2] Kidd, J. G., and Rous, P., *J. Exp. Med.*, **68**, 529 (1938).
[3] Ahlström, C. G., and Andrewes, C. H., *J. Path. Bact.*, **47**, 65 (1938).
[4] Duran-Reynals, F., *Ann. N.Y. Acad. Sci.*, **68**, 430 (1957).
[5] Rowe, W. P., *Bact. Rev.*, **25**, 18 (1961).
[6] Stanton, M. F., Stewart, S. E., Eddy, B. E., and Blackwell, R. H., *J. Nat. Cancer Inst.*, **23**, 1441 (1959).
[7] Dawe, C. J., National Cancer Institute Monograph No. 4, 67, edit. by Beard, J. W. (Pub. U.S. Dept. of Health, Education and Welfare).
[8] Law, L. W., Dawe, C. J., Rowe, W. P., and Hartley, J. W., *Nature*, **184**, 1420 (1959).
[9] Rowe, W. P., Hartley, J. W., Estes, J. D., and Huebner, R. J., *J. Exp. Med.*, **109**, 379 (1959).
[10] Salaman, M. H., and Rowson, K. E. K., *Lab. Animals Centre Collected Papers*, **9**, 61 (1960).
[11] Stanley, N. F., Dorman, D. C., and Ponsford, J., *Austral. J. Exp. Biol. Med. Sci.*, **31**, 147 (1953).

LETTERS TO THE EDITORS

SPACE SCIENCE

Analysis of Satellite Data

Using the method outlined in previous papers[1-3], I have completed an analysis of an extensive series of observations on satellite 1958 δ2 (ref. 4), and propose to outline an interesting implication of this work prior to the publication of the detailed analysis.

The method of analysis makes use of the near linearity of v^2 as a function of t, where $v = ae/a_0e_0$ in standard orbital notation. Fig. 1 shows the departure (δ) of v^2 from linearity after the removal of the important oblateness terms. The curve is composed effectively of three distinct nearly linear sections, *AB*, *BE*, *EF*, the change of type being unrelated to solar or any other obvious effects. However, the analysis is based upon the constancy of the scale height H, and for any linear section:

$$\delta = 1 - Kt$$

where[3] $\rho\sqrt{H}/f(v) \approx K$. The mean value of H is determined by the behaviour of the perigee distance[3], a least-squares fit giving the value 46·0 km. for this satellite, and the mean value of K is $1 \cdot 468 \times 10^{-3}$. If then we compare the gradient of a linear section with the mean gradient we can form an estimate of the local value of H. Since the v^2-trace does not depart from the mean trace by more than 2 per cent we can ignore the variation of $f(v)$ in the comparison. If ρ is greater than its implied mean value then the effect of ignoring the variation of ρ is to over-estimate the corresponding value of H and conversely.

An examination of Fig. 1 shows that the gradient changes very sharply at B and E, in fact almost discontinuously, the changes being unrelated to changes from day to night. If the main part of the gradient change is due to variations in H this would imply a step-like temperature distribution and consequently a considerable degree of stratification. The section *CD* is anomalous in its behaviour, showing a day–night variation in gradient more than three times as large as that of the neighbouring regions. The single oscillation apparent is fitted almost exactly by a multiple of sinψ, where ψ is the difference in longitude between the perigee and the sub-solar point. This implies a maximum of $\rho\sqrt{H}$ at midday and a minimum at midnight, the magnitude being independent of altitude in this limited region. A daytime rise in density due to the lifting of warmed air would be expected to produce a uniform variation of gradient over a limited region, hence it is reasonable to attribute the major part of the oscillation over *CD* to variation in scale height. In Fig. 2 scale heights calculated in this manner are compared with King-Hele's[5] estimates of H, modified to give true scale heights. The agreement is fair, sufficient to justify the general interpretation, though the implied temperature peak is much sharper than King-Hele's necessarily smoothed values. The excessively low values below 190 km. are probably the result of a progressive fall-off in solar activity at this time, which has not been taken into account.

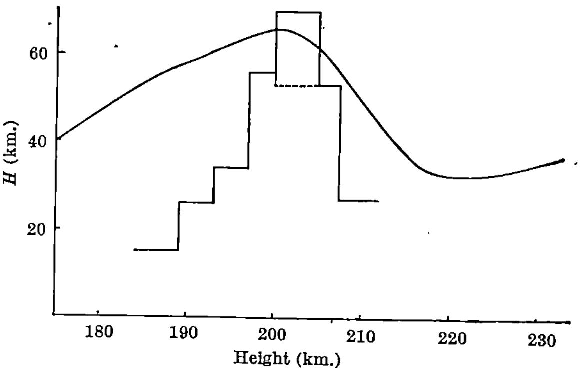


Fig. 2. Graph of scale height, comparing values deduced from K-variation with estimates from density values

The anomalous behaviour between 200 km. and 205 km., which is most probably the result of a marked day–night temperature difference, suggests strongly that this is the region of high absorption of solar energy which is the cause of the F_1-layer. Whatever interpretation is finally accepted for this anomalous behaviour, its relation to the F_1-layer is difficult to avoid. For example, if its explanation is sought in a marked variation in charge drag, one is faced with a high concentration of charged particles in a region of limited depth.

When this analysis was begun, one of my main aims was to obtain further justification for my earlier interpretation of oscillations in the v^2-trace for 1958 δ1 as a third harmonic tide. In Fig. 3 the remaining variations in v^2 after the removal of known effects are shown and compared with the calculated effects of a third harmonic tide. Solar flux variations do not account for these oscillations, which happen to coincide with a particularly quiet solar period, and furthermore, the oscillations occur at approximately the same level as those observed in the data for 1958 δ1. The disturbance is restricted

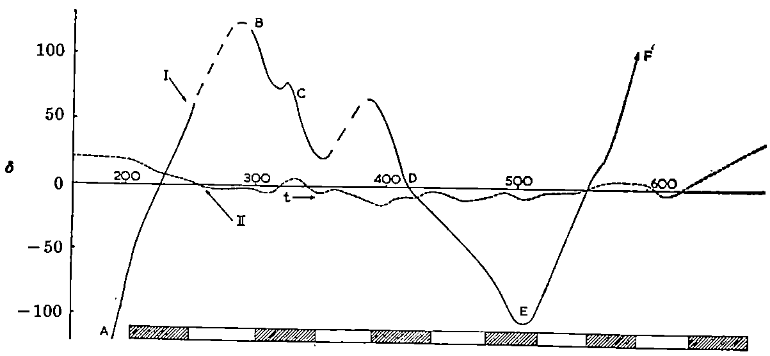


Fig. 1. Graph of δ vs. t for 1958 δ2 with the oblateness effect removed. Curve I, δ × 10⁴; Curve II, effect of variation of solar flux estimated from relative sunspot numbers. Time t in y.d. 1958. Shaded sections denote daytime (| ψ | < 90°)

to the section *BE* of the trace and is therefore limited to a depth of about 10 km. Since the earlier set of data for 1958 δ1 was of short duration, it is impossible to say whether or not the oscillations were restricted to the temperature peak, but a sharp rise in v^2 after the values given in Fig. 1 of my earlier communication[2] would suggest that the oscillations recorded there belong to the section *DE*. It is evident that a slight change of phase with height occurs, but it would be surprising if a tidal phenomenon did not show this effect. Although classical tidal theory is scarcely applicable to these regions and my earlier estimates of temperature were in error, there are strong indications that if a third harmonic tide occurs it must occur in a relatively thin shell near the 200-km. level. There has been some criticism of the suggestion of tides at this level[6–8], but in the face of this evidence the interpretation must surely be considered justified. This tidal oscillation is of small amplitude and is not particularly important in the general density pattern, but its implications for the dynamical structure of the atmosphere are worthy of consideration.

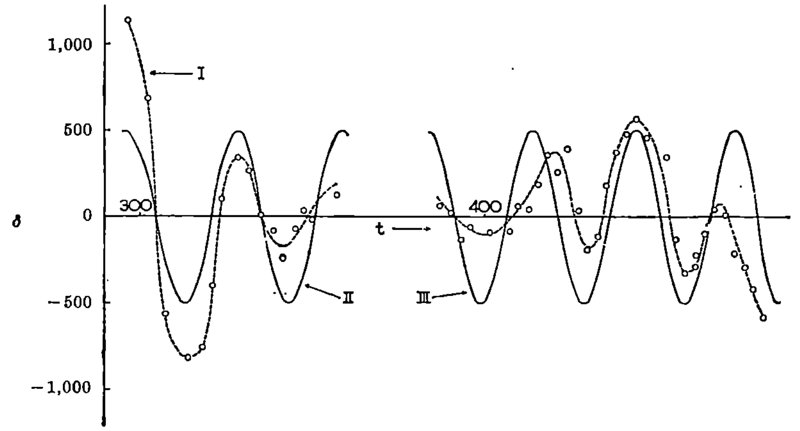


Fig. 3. Graph of δ vs. *t* for section *BE*. Curve I, $\delta \times 10^6$; Curve II, $\cos (3\psi + \pi/4)$: Curve III, $\cos 3\psi$. Time *t* in y.d. 1958.

It must be emphasized that the effects considered here are long-term, in contrast to the violent day-to-day fluctuations observed in the rate of change of periodic time. Since the v^2-analysis uses the periodic time rather than its rate of change, the effect of integrating density variations is to enhance the slower oscillations. As a result it is found that solar flux variations are scarcely noticeable in the resulting traces, although such variations are closely correlated with the daily variations of the orbital acceleration.

I wish to thank the South African Mutual Life Assurance Society for the use of its computer for the early stages of the numerical analysis.

D. G. Parkyn

Rhodes University,
South Africa.

[1] Parkyn, D. G., *Amer. J. Phys.*, **26**, 436 (1958).

[2] Parkyn, D. G., *Nature*, **183**, 1045 (1959).

[3] Parkyn, D. G., *J. Geophys. Res.*, **65**, 9 (1960).

[4] Kozai, Y., *Smithsonian Inst. Astrophys. Obs. Spec. Rep.*, No. 40, 10 (1960).

[5] King-Hele, D. G., paper at the Symp. Aeronomy, Copenhagen (1960).

[6] King-Hele, D. G., paper at the C.O.S.P.A.R. Symp., Florence (1961).

[7] Kolegov, G. A., *Iskuss. Sputniki Zemli*, **4**, 31 (1960).

[8] Priester, W., Martin, H. A., and Kramp, K., *Nature*, **188**, 200 (1960).

PHYSICS

Spatial Distribution of Free Radicals as studied by Electron Spin Resonance

The spatial distribution of free radicals produced by irradiation is of interest in radiation chemistry and radiation biology. Although there have been many theoretical treatments of this problem, direct experimental evidence has so far been lacking. Electron spin resonance spectroscopy provides a means of measuring the distribution of free radicals; and we report here a preliminary investigation by this method.

The technique depends on the phenomenon of dipolar broadening, in which the width of the electron spin resonance absorption line is increased by the perturbing effect of neighbouring free radicals. Since the perturbing effect varies inversely with the cube of the distance between two radicals, the broadening is a measure of the local concentration and not of the overall concentration, for cases where these differ owing to a non-uniform distribution.

Kittel and Abrahams[1] have calculated the broadening due to a uniform random distribution of magnetic centres in a single crystal. They showed that for moderate concentrations the line shape is approximately Lorentzian; and they gave a numerical formula for the line width. This may be written in the convenient form $B = 68\ M$ gauss, for $M \leqslant 1$, where M is the molar concentration of free radicals, and B is the increased separation, due to dipolar broadening, between the points of inflexion. The formula can be used for powders, glasses or liquids, with a different numerical value of the constant; and also for a non-uniform distribution of radicals, provided that M now represents the local concentration of radicals.

We have applied this technique to glasses of H_2O_2 in H_2O and of D_2O_2 in D_2O, irradiated and measured at 90° K.[2,3]. The spectra are complicated by *g*-value anisotropy and by hyperfine splitting; but in many cases we have been able to fit the experimental line shape to one calculated[4–6], and thus obtain the line width. As a preliminary check we used very large doses of radiation (up to 10^8 rads, giving concentrations of radicals up to 0·5 M) in order to obtain a uniform distribution of radicals. As the dose increased the line shape changed from Gaussian to Lorentzian, in accordance with the calculations of Kittel and Abrahams[1]; and the increase in separation between the points of inflexion was measured as $35 \pm 18\ M$ gauss. The line shape with no dipolar broadening was obtained by softening the glass at a temperature of about 140° K. until there was no further change in shape. This process produces a low concentration of radicals distributed uniformly. (The change in line shape as the glass softens was incorrectly attributed in an earlier communication to the production of a second radical[2].)

We then observed that different types of radiation produced spectra with different line widths, greater

Table 1. Local Concentration of Radicals

Type of radiation	Average linear energy transfer (keV./μ)	Local radical concentration	Corresponding average separation
250 keV. X-rays	0·6	(0·08 *M*)	(27 Å.)
15 MeV. deuterons	14	(0·08 *M*)	(27 Å.)
5·7 MeV. α-particles	86	0·22 *M*	20 Å.
2537 Å. ultra-violet	—	0·33 *M*	—
3100–3500 Å. ultra-violet	—	0·45 *M*	—

The values given in parentheses are near the limit of measurement, and are consequently very approximate.
The values in column 3 are the uniform random distributions which give the same line width.
The values in column 4 are obtained from the formula: average separation = $(6\cdot02 \times 10^{20} M)^{-1/3}$ cm.

than for the spectrum with no dipolar broadening. These line widths were independent of dose, provided that the dose did not exceed a certain value. Furthermore, the principal *g*-values and hyperfine splittings, when present, were the same in every case, indicating that the same radical was always produced. Hence any broadening observed must be due to the local concentration of radicals. The local concentrations obtained in this way are given in Table 1.

With ultra-violet irradiation the primary act must be the splitting of an H_2O_2 molecule into two OH radicals. The radical observed by electron spin resonance has not been positively identified, but it is unlikely to be OH. The ultra-violet observations of Table 1 show that the two radicals observed, or their precursors, diffuse a few Å. apart before being stabilized. A naïve calculation shows that the two OH radicals, when first formed, have sufficient energy to raise the temperature of a sphere of radius 20 Å. (rather more for 2537 Å., rather less for 3300 Å.) to a temperature at which the radicals have been observed to diffuse very rapidly. It is likely that local softening of the glass takes place with ionizing radiation also, so that the initial concentrations of radicals would be higher than those given in Table 1.

This work has been supported by the British Empire Cancer Campaign.

Robert C. Smith
S. J. Wyard *

Department of Physics,
Guy's Hospital Medical School,
London, S.E.1.

---

* Present address: Biophysics Laboratory, Stanford University, Stanford, California.

[1] Kittel, C., and Abrahams, E., *Phys. Rev.*, **90**, 238 (1953).
[2] Smith, R. C., and Wyard, S. J., *Nature*, **186**, 226 (1960).
[3] Smith, R. C., and Wyard, S. J., *Bull. Ampère*, 9e année, Fasc. Spécial, 224 (1960).
[4] Searl, J. W., Smith, R. C., and Wyard, S. J., *Proc. Phys. Soc.*, **74**, 491 (1959).
[5] Searl, J. W., Smith, R. C., and Wyard, S. J., *Bull. Ampère*, 9e année, Fasc. Spécial, 236 (1960).
[6] Searl, J. W., Smith, R. C., and Wyard, S. J., *Proc. Phys. Soc.* (in the press).

Magnetic Relaxation in Magnetite and in its Solid Solutions with Manganese and Nickel Ferrites

Our previous investigation of magnetic relaxation in $(Ni,Zn)_xFe_{3-x}O_{4+y}$ and $(Mn,Zn)_xFe_{3-x}O_{4+y}$ solid solutions with magnetite ($0\cdot85 \leqslant x \leqslant 1$) showed certain similarities to exist among the phenomena which occur in both systems[1]. Such similarities have since been reported by other authors[2,3].

In NiZn and MnZn ferrites the magnetic diffusion occurs owing to the presence of Fe^{2+} cations, by means of vacancy migration. A few distinct relaxations appeared in separate temperature-ranges, with activation energies still of about 1 eV. or 2 eV. (The application of the method of Rathenau[4] has given values of about 0·5 eV. and 1 eV. respectively. This method is not applicable to ferrites having a broad band of relaxation-time constants.) It was feasible to assume that the presence of magnetite or, more exactly, of the $Fe^{2+} - Fe^{3+}$ configurations is essential here.

We then began investigating relaxation in the systems $Mn_xFe_{3-x}O_{4+y}$ and $Ni_xFe_{3-x}O_{4+y}$ by means of permeability disaccommodation measurements, beginning with the particular case of magnetite, $x = 0, y \gg 0$.

The disaccommodation measurements were made on polycrystalline samples in the temperature-range between liquid hydrogen and corresponding Curie points using the Maxwell–Wien bridge. The intensity of the measuring alternating field was 10 m.oersteds, its frequency 1 kc./s. The magnetic permeability was measured as a function of time. Disaccommodation equals $\mu_0 - \mu_{30}/\mu_0$, where μ_0 denotes the value found 1 sec. after demagnetization, and μ_{30} after 30 min.

In a typical disaccommodation spectrum of magnetite $Fe_3O_{4+\gamma}$ we have observed three vacancy diffusion peaks: I, II, III (Fig. 1). The activation energies found are: $W_I = 1\cdot7 \pm 0\cdot3$ eV. (extrapolated from W_I values determined for $x > 0$, intensity of peak I being too small in samples, $x = 0$, $y \gg 0$), $W_{II} = 0\cdot85 \pm 0.10$ eV., $W_{III} = 0\cdot85 \pm 0\cdot10$ eV. In order to determine values of W, we have approximated the $\mu(t)$ curve with the after-effect function[5] $\psi(t)$.

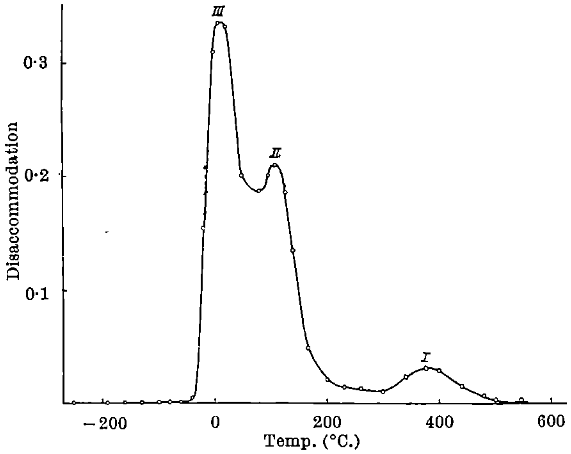


Fig. 1. A typical disaccommodation spectrum of magnetite Fe_3O_{4+y}. $y > 0$

A suitable concentration of vacancies is a necessary condition for the presence of each peak. The $y_{min.}$ value increases progressively for lower temperature effects: for III $y_{min.}$ is greater than for II, etc. For increasing values of $y > y_{min.}$ the peak I is markedly displaced to lower temperatures; the displacements of II and II are much smaller.

In both of the manganese- and nickel-ferrite systems mentioned above one finds the peaks I, II, III for almost all compositions $0 < x < 1$. As x approaches unity, the effect III is the first to disappear, then II, and finally, for the largest values of x, effect I.

The temperatures of peaks I, II, III are almost independent of x for the nickel-ferrite system. This is also true for peaks I and III in manganese ferrite – magnetite solid solutions. One observes, however, a shift of peak II from about 100° C. to approximately 200° C. for compositions $0 \cdot 5 < x < 0 \cdot 85$, having positive cubic anisotropy constant K_1 (ref. 6). Manganese ferrites show also a small peak IV at approximately – 70° C., indicating the contribution of manganese ions to the diffusion process.

For increasing values of y the displacement of peaks occurred in both systems as it did in the case of magnetite.

We therefore believe that in solid solutions of magnetite with other cubic ferrites local configurations of anisotropic space charge ($Fe^{2+} - Fe^{3+}$) are formed owing to the presence of vacancies in octahedral sites having trigonal symmetry. The configurations re-arrange themselves by migration of vacancies when the direction of magnetization changes in magnetic domains from one to the other [111] or [100] axis under the action of alternating demagnetizing fields. Relaxation I corresponds probably to the migration of a few individual vacancies or, equivalently, very limited space charge. The effects II and III might be due to the formation of more complicated arrangements, for example, pairs.

More detailed results and discussions will be published shortly.

We are indebted to Dr. Ch. Guillaud, director of this Laboratory, for his help.

A. Bragiński*
Th. Merceron

Laboratoire de Magnétisme
et de Physique du Solide,
C.N.R.S., Bellevue,
Seine-et-Oise.

* On leave of absence from 'Polfer' Research Laboratory, Warsaw

[1] Marais, A., and Merceron, Th., *C.R. Acad. Sci., Paris*, **249**, 2511 (1959); **251**, 3553 (1961). Braginski, A., Marais, A., and Merceron, Th., *ibid.*, **251** (in the press).
[2] Krupicka, S., and Vilim, F., *Czech. J. Phys.*, B, **11**, 10 (1961).
[3] Ohta, K., *J. Phys. Soc. Jap.*, **16**, 251 (1961).
[4] Rathenau, G. W., *J. App. Phys.*, **29**, 239 (1958).
[5] Becker, R., and Döring, W., *Ferromagnetismus* (Springer Verlag, Berlin, 1939).
[6] Penoyer, R. F., and Shafer, M. W., *J. App. Phys.*, **30**, 3158 (1959).

Surface Waves in Anisotropic Elastic Media

The possibility of elastic waves confined to the neighbourhood of the free plane surface of semi-infinite isotropic medium was first deduced by Lord Rayleigh in 1885. He found that the amplitudes of such waves decay exponentially with the distance from the surface. Recently there has been interest in the propagation of surface waves in anisotropic media, and Stoneley, Gold, and Deresiewicz and Mindlin have discussed the propagation of surface waves in particular directions and special planes of certain crystalline substances[1].

The general case has been considered by Synge[2]. He expressed the condition for surface wave propagation in a given plane in terms of a complex valued determinant, and deduced that surface waves are propagated only in those isolated directions for which the real and imaginary parts of this determinant both vanish. He also showed that, in general, the variation of the amplitudes of surface waves with depth is sinusoidal as well as exponentially decaying.

More recently[3] it has been shown that if the free surface is normal to the monoclinic axis of a crystal (or equivalently it is a plane of reflexional symmetry), then the determinant is real and, therefore, Rayleigh waves may be possible in any direction. Even for these special planes, however, the wave amplitudes may vary harmonically as well as decay exponentially with depth. Further, using a Fourier transform method, similar to that used in the case of body wave propagation[4], it has been shown[6] that the geometry of the surface waves can be described by 'slowness' and 'wave' curves, where the terminology is similar to that now commonly used in the three-dimensional case[5]. The wave curve represents the positions at unit time of waves which were initially at the origin; it is the polar reciprocal of the slowness curve with respect to the unit circle. Solutions of the determinantal equation give points on the slowness curve; the corresponding points on the wave curve can then be calculated. It is most important that the wave curve should be obtained as, physically, it describes the energy flow.

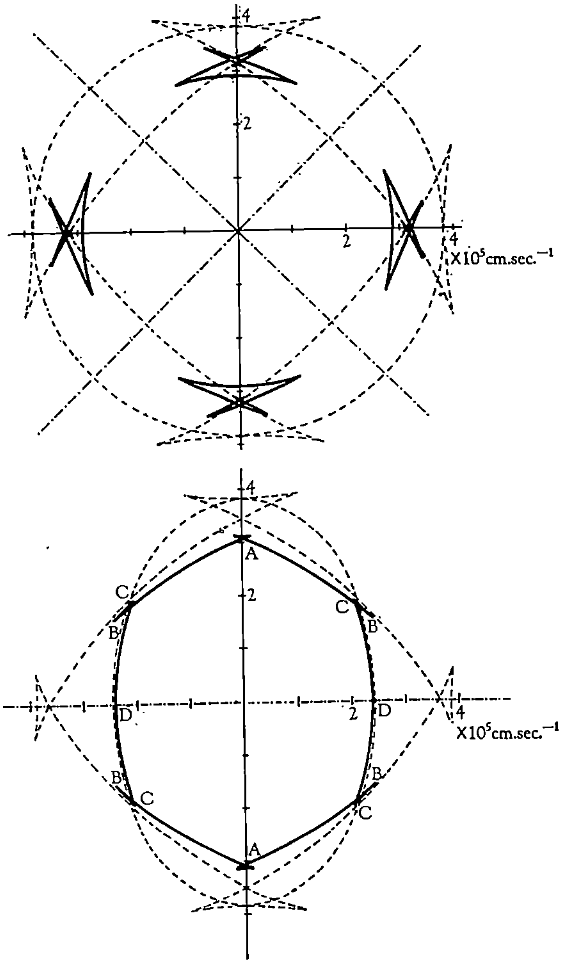


Fig. 1. Above, surface wave curves of iron in the (1,0,0) plane, and, below, similar curves for the (1,1,0) plane. In both figures the fourfold axes are represented by ——, the twofold axes by —·—·—, the surface wave curves by ——, and the curves – – – – represent the intersections of the body-wave surfaces with the appropriate plane. Below, the segments *CBABC* represent waves, where the amplitude variations with depth contain sinusoidal factors, while the waves corresponding to the remaining segments *CDC* have only purely exponential decay

The determinantal equation is too complicated for a closed solution to be practicable. Numerical solutions have been obtained for transversely isotropic materials[6], and graphs given of the slowness and wave curves for apatite, beryl, cobalt and zinc, in the case where the axis of rotational symmetry is parallel to the free surface. Since then a programme has been written for the University of Manchester *Mercury* computer, which produces the relevant information for all classes of monoclinic crystals. For example, in the two figures the full lines are the wave curves for the (100) and (110) planes of iron (the broken lines are the intersections with these planes of the 'transverse' sheets of the body wave surfaces). The stiffnesses used were $C_{11} = 23{\cdot}30$, $C_{12} = 13{\cdot}92$, $C_{44} = 11{\cdot}62$ in 10^{11} dynes/cm.^{2}, the density being 7·87 gm./cm.^{3} (ref. 7). In the (100) plane, surface waves are confined to four wedges, of angle about 45°, containing the four-fold axes. There are five surface waves in directions near these axes and, as the wedges with no surface waves are approached, it can be seen that there are regions where progressively fewer such waves exist. All the waves contain components which decay sinusoidally. In the (110) plane, surface waves are propagated in all directions. Near the four-fold axis decay is simply exponential for all components; near the two-fold axis there is also some sinusoidal variation. Between these two axes there is a small range of directions where there are two waves with sinusoidal components and one wave without. The wave curves for both planes have cusps (corresponding to points of inflexion on the slowness curves). The Fourier transform method of solution gives the additional information that the rate of damping of amplitudes with distance travelled of waves in the cusp directions is of a lower order of magnitude than the rate of attenuation of waves in ordinary directions. If the distance from the source is r, in ordinary directions the amplitudes are proportional to $r^{-1/2}$, but in the cusp directions the amplitudes decay as $r^{-1/3}$.

V. T. Buchwald

Department of Applied Mathematics,
University of Sydney.

A. Davis

Department of Mathematics,
Manchester College of Science and Technology.

[1] Hearmon, R. F. S., *An Introduction to Applied Anisotropic Elasticity*, 84 (Oxford Univ. Press, 1961).

[2] Synge, J. L., *J. Math. Phys.*, **35**, 232 (1957).

[3] Buchwald, V. T., *Quart. J. Mech. and App. Maths.* (to be published).

[4] Buchwald, V. T., *Proc. Roy. Soc.*, A, **253**, 563 (1959).

[5] Hearmon, R. F. S. (ref. 1, page 69).

[6] Buchwald, V. T., *Quart. J. Mech. and App. Maths.* (to be published).

[7] Musgrave, M. J. P., *N.P.L. Basic Phys. Div. Rep.* No. 7 (1961).

CRYSTALLOGRAPHY

Molecular Geometry of a 1 : 1 Crystalline Complex between 1,3,7,9-Tetramethyluric Acid and Pyrene

As part of a research programme on the interaction between purine and polycyclic aromatic hydrocarbons, the crystal structures of molecular complexes between 1,3,7,9-tetramethyluric acid and pyrene, 3,4-benzopyrene and coronene, first described by Weil-Malherbe[1], are being investigated in our laboratory.

In a previous communication[2], the molecular packing in hydroxymethyl purine crystals was discussed with reference to the results of an X-ray determination of the crystal structure of 1,3,7,9-tetramethyluric acid. We wish to report here the most significant aspects of the molecular arrangement in a 1:1 complex between that acid and pyrene as

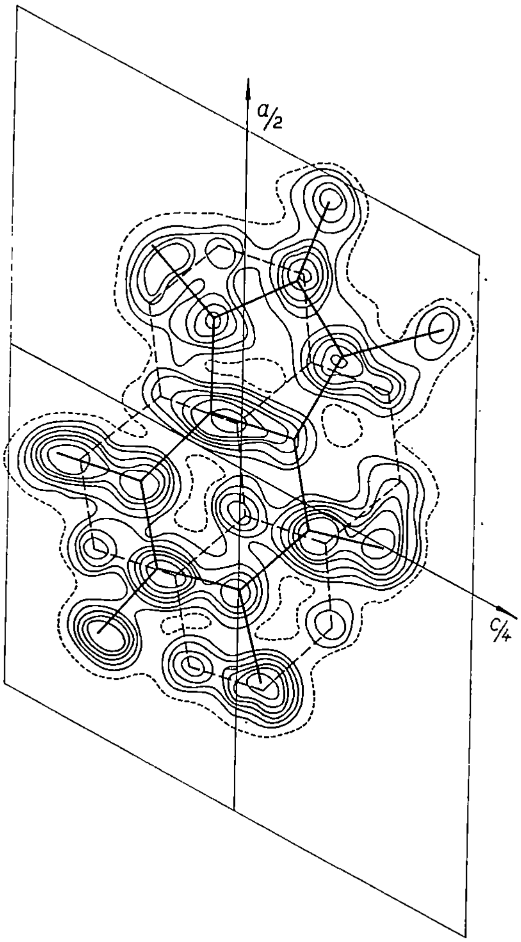


Fig. 1(*a*). Fourier projection of the electron density on (010)

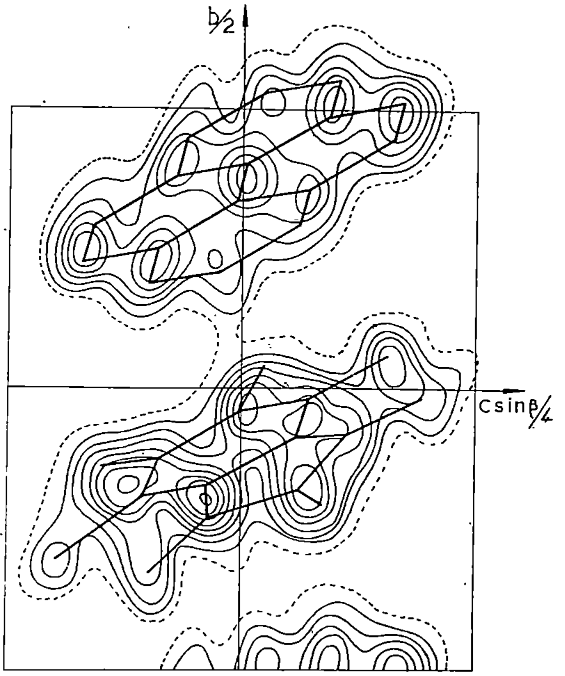


Fig. 1(*b*). Fourier projection of the electron density on (100)

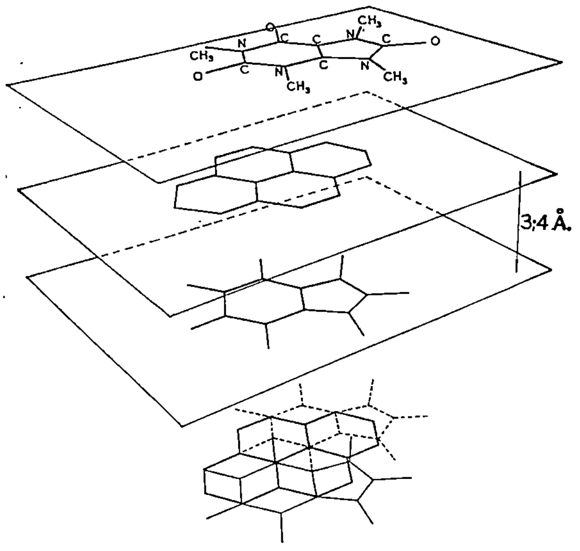


Fig. 2. Schematic drawing of the perpendicular separation of the molecules in one stack through the unit cell

revealed by an X-ray investigation of the crystal structure.

Two molecules of 1,3,7,9-tetramethyluric acid and two molecules of pyrene are contained in the unit cell of dimensions $a = 9{\cdot}71$ Å.; $b = 8{\cdot}00$ Å.; $c = 15{\cdot}04$ Å.; $\beta = 117°$. The space group is P/c—$C_s{}^2$ and the asymmetric unit contains one molecule of 1,3,7,9-tetramethyluric acid and one molecule of pyrene in general positions.

Fourier projections were calculated on (010) and (100) after the phases of a sufficiently large fraction of the observed reflexions had been established through a combination of optical Fourier transform and Patterson methods[3]. A refinement was then carried out through reiterate cycles of structure factor calculations on a 650 I.B.M. electronic computer up to a degree which was judged to be consistent with the complexity of the structure and the falling off of the intensities with the Bragg angle.

The most interesting feature of the crystal structure is a plane-to-plane alternate stacking of 1,3,7,9-tetramethyluric acid and pyrene molecules in infinite columns parallel to the b-axis. Two such columns run through the unit cell and are related to each other by a glide plane. A projection of the electron density on (010) and (100) is shown in Figs. 1(a) and 1(b).

In Fig. 2 the perpendicular separation and the relative orientation of 1,3,7,9-tetramethyluric acid and pyrene molecules in the complex is illustrated schematically. A graphite-like displacement from coincidence of the perpendicular projections of the atoms in one molecule with respect to the atoms in the next molecule may be noticed. This mode of packing is typical of molecular complexes the stability of which is commonly ascribed to 'polarization bonding'[4] between the components, the complexes between aromatic hydrocarbons and s-trinitrobenzene being a well-known example[5]. Weak charge transfer and dipole-induced dipole forces between the polar purine and the polarizable aromatic molecule, in addition to Van der Waals interactions, must therefore make the dominant contribution to the stability of the molecular complex between the acid and pyrene.

Just as in other similar cases, it is very likely that the relative orientation of the two components in the complex is the result of a compromise to produce maximum overlap between the π-orbitals involved in the electron transfer and between the polar regions of the purine and the polarizable regions of pyrene.

A correlation with other properties will be needed in order to try to assess the relative importance of the different forces contributing to the stability of the complex.

It may also be expected that the determination of the crystal structures of 2:1 complexes between 1,3,7,9-tetramethyluric acid and larger aromatic hydrocarbons in progress in our laboratory will be of considerable help in this respect, as well as in the elucidation of the mechanism of solubilization of polycyclic hydrocarbons in purine[1] and DNA water solutions (Liquori, A. M., Ascoli, F., Botrè, C., De Lerma, B., and Tresciotti, M., work to be published).

Full details of this work, which was supported by the Consiglio Nazionale delle Ricerche and by a grant from the Rockefeller Foundation, will be published shortly.

F. DE SANTIS
E. GIGLIO
A. M. LIQUORI
A. RIPAMONTI

Istituto Chimico,
Centro Studi di Chimica Fisica
del Consiglio Nazionale delle Ricerche,
Università, Naples.

[1] Weil-Malherbe, H., *Biochem. J.*, **40**, 351 (1946).
[2] De Santis, P., Giglio, E., and Liquori, A. M., *Nature*, **188**, 46 (1960).
[3] Lipson, H., and Cochran, W., *Determination of Crystal Structures* (G. Bell and Sons, Ltd., London, 1953).
[4] Wallwork, S. C., *J. Chem. Soc.*, 494 (1961).
[5] McGlynn, S. P., *Chem. Rev.*, **58**, 1113 (1958).

A New Technique for the Production of Synthetic Corundum

LARGE crystals of corundum have been grown for many years by the flame-fusion process originated by Verneuil[1]. Crystals coloured by small amounts of chromium, titanium, vanadium and other transition metal oxides are used in the gem industry and as jewel bearings and gramophone styli. The recent interest in ruby (chromium-doped corundum) as a paramagnetic material for use in masers and lasers has directed attention to the imperfections of the flame-fusion crystals. In particular, the inhomogeneity of chromium distribution in the crystals, the excessive residual strain and gross lattice imperfections detract from their usefulness in micro-wave and optical devices. Alternative methods of growth capable of giving better quality crystals are therefore of considerable interest for research in solid-state physics.

The hydrothermal growth of corundum has been accomplished (ref. 2 and Butcher, J., private communication, Hirst Research Centre of the General Electric Co., Ltd.), but considerable difficulties are involved in the production of good-quality crystals. It is necessary, for example, to work under conditions of high temperature and pressure where serious attack of conventional autoclave materials is experienced. The development of autoclaves of new design with heat-resisting alloys and inert metal liners is necessary, and the process is not yet suitable for the consistent production of large crystals.

Frémy and Feil[3] in 1877 produced small flakes of corundum from solution in lead oxide, but no further attempts to develop this method are recorded, and presumably after the success of Verneuil in 1890 the incentive to synthesize ruby was largely removed. The fluxed-melt technique for crystal growing has, in recent years, been revived for the laboratory production of comparatively large crystals of several inorganic oxide materials, for example, barium titanate, yttrium iron garnet and calcium tungstate[4].

Good-quality crystals of corundum, pure and doped with transition metal oxides, have now been produced from solution in lead fluoride. The process is carried out in a platinum crucible heated in a conventional laboratory furnace. The crystals are produced by spontaneous nucleation on slow cooling as hexagonal plates up to 2–3 cm. across. The thickness of the plates is largely dependent on the initial conditions of cooling, and in favourable circumstances they measure up to 1 cm. along the *c*-axis.

The crystals grow as truncated trigonal trapezohedra bounded by (0001) and $(10\bar{1}1)$ type faces, but $(10\bar{1}2)$ faces are occasionally observed. The surfaces of the latter appear roughened and they evidently disappear if the duration of growth is sufficiently prolonged. The $\{10\bar{1}2\}$ faces are thus more commonly observed on thick crystals, which require more growth perpendicular to the *c*-axis for these faces to grow out.

There is some tendency for the incorporation of lead fluoride in planes parallel to (0001); but many good-quality clear crystals, free from obvious faults, are obtainable.

The incorporation of high concentrations of paramagnetic ions can be readily achieved by this technique, and the products have the additional advantage of being strain-free.

E. A. D. WHITE

Central Research Laboratories,
Hirst Research Centre,
General Electric Co., Ltd.,
Wembley.

[1] Verneuil, *C.R. Acad. Sci., Paris*, **135**, 791 (1902); *Ann. Chim.* (France), **3**, 20 (1904).

[2] Laudise, R. A., and Ballman, A. A., *J. Amer. Chem. Soc.*, **80**, 2655 (1958).

[3] Frémy, E., and Feil, C., see Smith, Herbert, *Gemstones* (Methuen, London, 1958).

[4] See White, E. A. D., *Quart. Rev. Chem. Soc.*, **15**, 1 (1961).

RADIATION CHEMISTRY

Electron Spin Resonance Absorptions induced by Light in Adsorbed Layers of Cyanine Dyes on Silver Bromide Microcrystals

In connexion with the investigation of the mechanism of spectral sensitization of the photographic process, light-induced electron spin resonance absorptions in gelatinless precipitates of silver bromide dyed with cyanine dyes have been observed. No signals were detected under any conditions from the undyed silver bromide precipitates.

Electron spin resonance observations were made at 77° K. and at room temperature, utilizing the 100-kc./s. Varian *ESR* spectrometer. Samples were illuminated in the resonance cavity with a 500-W. tungsten lamp and a mercury-vapour lamp, from which light of selected wave-lengths was isolated by means of filters.

The sensitizing dyes examined constituted vinylogous series of quino-2,2′-cyanines and of thiacyanines. The dyes were (1) 1,1′-diethyl-2,2′-cyanine *p*-toluenesulphonate; (2) 1,1′-diethyl-2,2′-carbocyanine bromide; (3) 1,1′-diethyl-2,2′-dicarbocyanine bromide; (4) 3,3′-diethylthiacyanine bromide; (5) 3,3′-diethylthiacarbocyanine bromide; (6) 3,3′-diethylthiadicarbocyanine bromide; (7) 3,3′-diethylthiatricarbocyanine bromide; and (8) 3,3′-diethyl-9-methylthiacarbocyanine bromide.

Pure crystalline cyanine dyes (1), (2) and (4) did not exhibit detectable electron spin resonance absorptions under any conditions. Pure crystalline cyanine dyes (3), (5), (6), (7) and (8) exhibited absorptions at 77° K. and at room temperature, and also weak absorptions induced by light at 77° K.

Two reports[1] have appeared describing electron spin resonance at room temperature by crystalline dyes having extended systems of conjugated double bonds. The authors concluded that the observed absorptions were characteristic of long, unsaturated, conjugated chains, that they were not associated with excited states, and that they were possibly characteristic of the crystalline state of the conjugated dye molecules.

All precipitates of silver bromide containing adsorbed layers of each of these dyes (probably no more than a complete monolayer) exhibited at 77° K. narrow, structureless electron spin resonance signals at $g = 2{\cdot}005$ when exposed to light absorbed by the dye or by the silver bromide. In steady illumination, the electron spin resonance signals induced by light grew to an equilibrium intensity in about 4 min., and they decayed incompletely upon cessation of illumination if the temperature was maintained at 77° K. Warming the sample dissipated the light-induced signal completely. The cooling–illuminating–warming cycle was reversible and could be repeated many times, with no detectable change in behaviour of the samples.

The electron spin resonance response by the dyed precipitates exposed at 77° K. was greater in a vacuum than in an air atmosphere. Evacuated samples dyed with dyes (2), (6) and (8) showed weak, reversible, light-induced absorptions at room temperature; silver bromide dyed with dye (2) exhibited a weak, reversible, light-induced absorption in air at room temperature. Re-admission of air to an evacuated sample restored the sample to its original behaviour.

The action spectrum of the electron spin resonance signal induced by light and exhibited at 77° K. by an evacuated sample of silver bromide dyed with dye (8) was observed by utilizing for exposure narrow-band spectral regions isolated by means of interference filters. The action spectrum followed the long wave-length edge of the absorption band of the adsorbed dye through the maximum at about 610 mμ. The action maximum and the absorption maximum coincided within the limits of experimental error. On the short wave-length side of the maximum, however, the intensity of the electron spin resonance signal induced by light decreased with decreasing wave-length much more rapidly than the absorption of the adsorbed dye. The absorption of this dye at wave-lengths shorter than the maximum is in the form of an *H*-band. For some dyes, the sensitizing efficiency of light absorbed within their *H*-bands is known to be low[2]. Although the sensitizing efficiency of dye (8) is high in emulsions at room temperature at wave-lengths within the *H*-absorption band, it is suggested that at low temperatures the inefficiency of the *H*-band in sensitization, shown by some thiacarbocyanines, may overtake dye (8) and account for the feebleness of the electron spin resonance response to wave-lengths in the *H*-band.

The electron spin resonance signals induced by light shown by the dyed samples of silver bromide are much more intense than those exhibited by the pure crystalline cyanine dyes. There seems to be no doubt that these signals are associated with the adsorbed dye monolayer as it exists on the silver bromide crystallites. A weak signal induced by light was exhibited at 77° K. by a sample of lead bromide dyed with dye (1); this was very similar to the more intense signal induced by light and exhibited at 77° K. by silver bromide dyed with dye (1).

These observations seem to show without doubt that unpaired electrons, associated with the presence of an adsorbed layer of sensitizing dye on the surface of the silver bromide microcrystals, are produced by illumination of the sample with light absorbed by the dye. The conditions of the experiments preclude the possibility of detecting electron spin resonance absorptions by the triplet states of the dye molecules[3]. The observed electron spin resonance absorptions induced by light most probably arise from free radicals derived from dye molecules that are stabilized at low temperatures. The instability of the free radical derived from the dye molecule at room temperature, coupled with the lower sensitivity of the electron spin resonance spectrometer and the difficulties encountered in illuminating the samples, probably account for the negative results of the two earlier attempts[4] to detect electron spin resonance signals induced by light and exhibited by dyed silver bromide precipitates.

At present, the precise origin of the free radicals in these experiments cannot be stated. They may be the residue of dye molecules which have sensitized the silver bromide by transferring an electron into the conduction band of the crystal. But there are other possible modes of production of free radicals, for example, the action of bromine atoms on the adsorbed dye molecules. We hope to study such questions in the near future.

W. C. NEEDLER
R. L. GRIFFITH
W. WEST

Research Laboratories,
Eastman Kodak Co.,
Rochester 4, New York.

---

[1] Moshkovskii, Yu. Sh., *Doklady Akad. S.S.S.R.*, 130, 1277 (1960). Chernyakovskii, F. P., Kalmanson, A. E., and Blyumenfel'd, L. A.. *Optika i Spektroskopiya*, 9, 786 (1960).

[2] Carroll, B. S., and West, W., *Fundamental Mechanisms of Photographic Sensitivity*, 176, edit. by Mitchell, J. W. (Butterworths Sci. Pub., Ltd., London, 1951. Levkoev, I. I., and Lifshits, E. B., *Zhur. Nauch. i Priklad. Fot. i Kinemat.*, 3, 419 (1958).

[2] van der Waals, J. H., and de Groot, M. S., *Molec. Phys.*, 2, 333 (1959); 3, 190 (1960).

[4] West, W., *J. Chim. Phys.*, 672 (1958); experiments performed in co-operation with J. H. Chaudet, Tennessee Eastman Co., Research Laboratories, Kingsport, Tenn. Bourdon, J., Kodak-Pathé Research Laboratories, Vincennes, France (private communication).

Radiolytic Oxidation of Ferrous Solutions with Standardized Internal Sources of Polonium-210

THE *G*-value of the oxidation in the aerated 'Fricke' dosimeter solution for irradiation with the α-particles of polonium-210 does not appear to be established accurately. Values ranging from 6·3 to 5·1 have been published with a tendency towards the lower figures as time progressed. The latest values, 5·5 and 5·1, published simultaneously by Lefort and Tarrago[1] and Trumbore and Hart[2] respectively, do not agree. We have redetermined this value by radiolysis with polonium-210 dissolved in 0·8 *N* sulphuric acid containing ferrous ammonium sulphate and sodium chloride both at a concentration of 10^{-3} *M*. The energy dissipated by radioactive decay, and absorbed by the system, was determined from standardization of the specific activity by 4π-internal liquid scintillation counting and an acceptance of the value 5·305 MeV. (ref. 3) for the energy of the polonium-210 particle. No account was taken of possible effects caused by the recoiling lead-206 nucleus.

Radiochemically pure polonium-210, obtained from the Radiochemical Centre, Amersham, was converted to nitrate-free sulphuric acid solution by thorough boiling with concentrated sulphuric acid. Since the polonium was added to the dosimeter solution carrier-free, it was necessary to establish that it stayed in solution in the strong acid medium without significant adsorption on the container walls. The percentage of radioactivity lost by adsorption was found to be totally insignificant in the range of surface-to-volume ratios of the glass apparatus used in this work. Irradiations were carried out at constant temperature in spherical flasks ranging in volume between 10 ml. and 500 ml. Owing to the small α-particle range in the solutions no significant loss of energy over the boundaries was expected or observed.

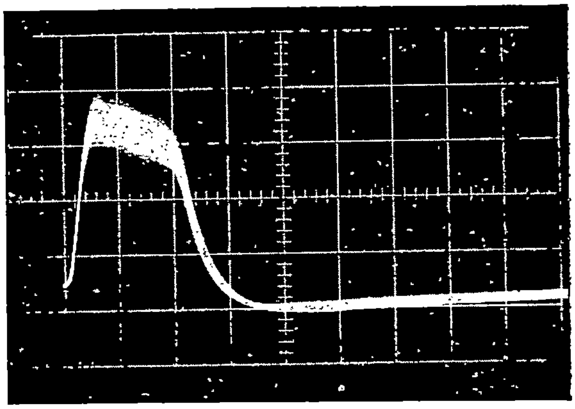


Fig. 1. Impulse spectrum, polonium-210 α-particles in the liquid scintillation counter

The specific activities of the dosimeter solutions after addition of the radioactivity varied between 9·8 μc./gm. and 25·6 μc./gm. Samples were taken at different times during the irradiations and diluted with 2 *N* hydrochloric acid to activity-levels convenient for counting. It was found that the polonium was very efficiently carried by the hydrochloric acid even after further dilution by the liquid scintillator, and no significant adsorption or precipitation was encountered during standardizations. The accuracy of the very convenient liquid scintillation method of precision α-particle counting has already been demonstrated by its use in half-life determinations[4]. Maximum error in counting measurements was well below 1 per cent.

Altogether five different irradiations were made yielding a mean *G*-value of:

$$5 \cdot 08 \pm 0 \cdot 02 \text{ ions per 100 eV.}$$

The error quoted is statistical only, calculated for a confidence limit of 68 per cent. Our result is thus in complete agreement with the value of Trumbore and Hart.

J. Steyn
D. van As

Radioactivity Division,
National Physical Research Laboratory,
South African Council for Scientific and Industrial Research,
Pretoria.

[1] Lefort, M., and Tarrago, X., *J. Phys. Chem.*, **63**, 833 (1959).

[2] Trumbore, C. N., and Hart, E. J., *J. Phys. Chem.*, **63**, 867 (1959).

[3] Allen, R. A., Millett, R. J., and Smith, D. B., *A.E.R.E.-R.* 2938 (1959).

[4] Steyn, J., and Strelow, F. W. E., *Proc. Symp. Metrology Radionuclides*, 155 (I.A.E.A., Vienna, 1960).

CHEMISTRY

Non-Uniformity of Cross-linking in Ion-Exchange Polymers

In theories of ion-exchange, the exchanger material is usually treated as a homogeneous phase, and transfer phenomena between the exchanger and aqueous solutions have always been explained on the basis of equilibria between two homogeneous phases. Recent experiments have shown that this simple picture cannot be maintained. The exclusion of co-ions by ion-exchangers in equilibrium with an aqueous solution, for example, the exclusion of cations by anion-exchangers with fixed cationic groups, or vice versa, has been well known for many years (for numerous references see the recent paper by Freeman[1]). Qualitatively this phenomenon is well understood on the basis of a Donnan equilibrium, which requires that the product of the ion activities of the electrolyte in the exchanger must be proportional to that in the solution. For a 1 : 1 electrolyte solution in contact with an anion-exchanger:

$$\underbrace{\underbrace{\{\bar{m}\}}_{\text{positive ions}} \cdot \underbrace{\{M+\bar{m}\}}_{\text{negative ions}}}_{\text{in exchanger}} = k \cdot \underbrace{\{m\}^2}_{\text{ion product in solution}} \qquad (1)$$

Hence, at low concentrations where $\bar{m} \ll M$, the uptake $\bar{m}$ of a 1 : 1 electrolyte like sodium chloride in an anion-exchanger of high fixed charge molality M should be proportional to the second power of the concentration, or more precisely, of the ion activity $(m\gamma)$ of this electrolyte in the equilibrating solution.

Verifications of this simple relation, however, have been far from perfect, largely due to the fact that it is difficult to remove quantitatively the aqueous phase from the surface of the exchangers. However, the conviction of research workers in the applicability of the Donnan law has always been sufficiently strong to introduce plausible though arbitrary correction terms, sufficient in number to restore the expected power law.

To overcome this experimental difficulty, a more refined measuring technique was introduced, in which amounts of electrolyte released from the equilibrated and wiped membrane into pure water are studied as a function of time. By extrapolating the observed curve back to zero time, according to the theoretical diffusion curve, it is then possible to eliminate the effect of the surface layer, which is released at the moment of immersion and does not affect the subsequent diffusion process. This permits an accurate analysis of the amounts of electrolyte taken up by the body of the exchanger independent of the amounts adhering to the surface. This, and the use of radiotracers, made it possible to measure the amounts of actual uptake over variations of more than 6 powers of ten. Fig. 1, curve *A*, shows the measured uptake results for sodium chloride on an A.C.I. anion exchanger membrane (made by the Asahi Chemical Industry Co., Ltd., Tokyo), with the uptake in equiv. per gm. of wet exchanger membrane plotted against the ion activity $m\gamma_{\pm}$ of the solution. This membrane is a homogeneous styrene–divinylbenzene co-polymer (approximately 8–10 per cent divinylbenzene) with quaternary ammonium groups and, when fully swollen, has a water content of 0·27 gm./gm. Instead of the expected proportionality of the uptake $\bar{m}$ with $(m\gamma)^2$, an exponent of 1·42 was observed.

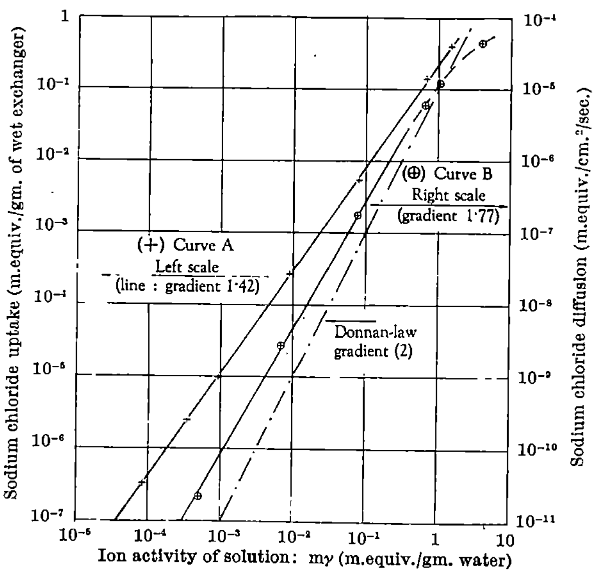


Fig. 1. Experimental determinations of electrolyte uptake and through-diffusion

The only rational explanation which will give a constant exponent over such a wide range of observations is that the exchanger is not a homogeneous phase, but that the fixed-charge density (and the cross-link density) in the exchanger varies continuously over a very wide range. Moreover, in order to give the fractional power law, the fixed-charge molality must vary in such a way that a fraction $d\varphi$ of the swollen exchanger has a fixed-charge molality between M and $M + dM$ defined by:

$$\frac{d\varphi}{dM} = K \cdot M^{-z} \qquad (2)$$

where K and z are constants, K being fixed by z and the experimental mean exchanger molality $\tilde{M}$. Then, if the appropriate Donnan relation is applied to each locality, and integration is carried out over the whole mass of the exchanger, one obtains a relation for the mean electrolyte uptake $\tilde{m}$ of the type:

$$\tilde{m} = K' \cdot (m\gamma)^{2-z} \qquad (3)$$

The results of Fig. 1 thus agree with this concept.

The observed behaviour of the electrolyte uptake is therefore a clear indication that the fixed charge molality M, and hence the distribution of cross-links in the exchanger polymer, is very far from uniform.

For the case investigated ($\tilde{M} = 4{\cdot}75$) the local fixed charge molality in the exchanger appears to vary from $M_{\text{max.}} \simeq 17$ to $M_{\text{min.}} < 10^{-4}$ equiv. per 1,000 gm. of swelling water, the distribution following equation (2) with $K = 0{\cdot}131$ and $z = 0{\cdot}58$.

The determinations of electrolyte uptake described, utilizing the diffusion release from the membrane, gave a clear indication that the apparent diffusion constant D' in the exchanger based on a Fick's law equation:

$$\frac{\partial c}{\partial t} = D' \cdot \frac{\partial^2 c}{\partial x^2} \qquad (4)$$

fell from 9×10^{-7} cm.²/sec. at $\tilde{m} = 0{\cdot}4$ m.equiv./gm. H_2O continuously to $1{\cdot}5 \times 10^{-7}$ cm.²/sec. at $\tilde{m} = 4 \times 10^{-7}$ m.equiv./gm. H_2O. As, at low uptake values, the ionic strength in an assumed homogeneous exchanger would be practically constant at $\tilde{M} = 4{\cdot}75$ m.equiv./gm. H_2O, there is no reason why in this case the true diffusion coefficient should change significantly. The obvious cause of the varying values of D' is that the exchanger is non-homogeneous. In this case the left side of equation (4) refers to the change in mean electrolyte concentration in the exchanger, while the concentration c on the right side refers to the concentration gradient in that fraction of exchanger which governs the diffusion process, namely, the more plentiful highly cross-linked fraction. Under these conditions, D', calculated according to equation (4), is not a true diffusion coefficient and it must vary in the observed direction.

On the basis of the foregoing explanation one would expect that steady-state diffusion through the membrane would be governed predominantly by the diffusion resistance of the highly cross-linked part, and this should follow a Donnan law more closely than the electrolyte uptake, which is largely influenced by the small fraction of low cross-link density. Experiments were carried out, therefore, in which the amount of diffusing electrolyte was measured, with one side of the membrane (thickness 0·019 cm.) in equilibrium with a solution of molality m, and with the other side washed with distilled water.

Fig. 1, curve B, shows the m.equiv. of sodium chloride diffusing per cm.² per second plotted against $m\gamma$ of the solution on a log-log scale. The diffusing amounts follow a power law of $(m\gamma)^{1{\cdot}77}$, thus showing a rather closer approach to the value of 2 expected for a homogeneously cross-linked system.

While the experiments (through the value of the constant z) give a clear indication of the degree of non-uniformity of the exchanger phase, they cannot decide the question of how it originated. A reasonable assumption would be that during the initial stages of the polymerization process, the divinylbenzene molecules combine preferentially with each other, leaving some very large chains of polystyrene with little cross-linking between them. Appropriate variations in the technique of manufacture combined with the analysis of non-uniformity by means of the determinations of electrolyte uptake described could throw some light on this question.

These investigations are being extended to other types of electrolytes and to other exchangers; these have so far shown a similar behaviour.

E. GLUECKAUF
R. E. WATTS

Atomic Energy Research Establishment,
Harwell.

[1] Freeman, D. H., *J. Phys. Chem.*, **64**, 1048 (1960).

Quantitative Analysis of Milk and other Emulsions by Infra-Red Absorption

A BEAM of radiation passing through an emulsion can be attenuated by both absorption and scattering effects. Quantitative absorption measurements on such systems are only reliable when the effects of scattering are made small in comparison with those of absorption ; that is, when the ratio of the refractive indices of the two phases approaches unity and when the size of particle is considerably less than the wave-length. For milk, the ratio of the fat/serum refractive indices is approximately 1·1 and since the mean fat globule diameter of homogenized milk samples is about 1μ, scattering effects will be expected to be small at wave-lengths greater than 5μ. Homogenization will therefore reduce photometric errors caused by variations in the sizes of fat globules of milk samples from cows of different breeds and in different states of lactation.

Under suitable experimental conditions, the infra-red spectra of homogenized milk samples show absorption peaks near 5·8, 6·5 and 9·6μ, these bands being due mainly to the fat, protein and lactose components respectively. Except for the effects of the fat on the 6·5μ protein band, the interaction coefficients are small and, to a first approximation, the optical densities measured at 5·8 and 9·6μ are directly proportional to the concentrations of fat and of lactose.

Analysis of a series of milk samples of 20 cows of different breeds and in different states of lactation gave standard deviations of 3 per cent or better for all three components. The concentrations of fat and of lactose were estimated from the measured optical densities of the 5·8μ and 9·6μ bands, and the protein absorption at 6·5μ was corrected for changes in fat concentration as measured by the optical density at 5·8μ. Further corrections should reduce all these standard deviations, since 'synthetic' milk samples made by mixing varying amounts of cream and separated milk from the same bulk-milk sample gave a correlation of fat percentage and optical density at 5·8μ, which approached the limit of the photometric accuracy, namely, ± 1 per cent.

Milk samples were examined in a cell of path-length approximately 50μ, a similar cell containing water being placed in the solvent position of a double-beam spectrometer fitted with filters to remove stray radiation. Since the absorption by water was so intense at all three wave-lengths, only a few per cent of the total radiation was transmitted and the peak optical densities were about 0·2 for this path-length. Little improvement was obtained by increasing the path-length, since the reduced solvent transmission necessitated the use of a larger slit-width causing a decrease in resolution and increase in stray radiation. Except for the contribution of the protein to the 9·6μ lactose band, all the interaction coefficients were negative, indicating that the non-absorbing component had an apparent negative absorption effect due to displacement of the more intensely absorbing water molecules.

Fig. 1 illustrates part of the spectra of homogenized milk and separated milk, the negative absorption effect of the lactose and protein being apparent in the separated milk spectrum at 5·8μ.

Tests showed that a change in homogenization pressure from 50 to 200 kgm./cm.², giving a change of mean diameter of fat globules of 1 to 0·5μ, made no difference to the measured absorption intensities. Thus the scattering contribution was either constant,

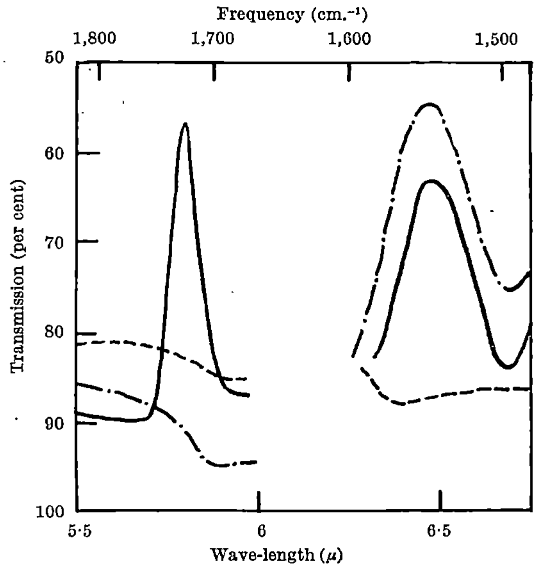


Fig. 1. Spectra of homogenized milk versus water (——), water versus water (– – –) and separated milk versus water (—·—·—). Path-lengths of approximately 50μ

or less than the limits of experimental error. Unlike previous turbidimetric methods for the analysis of milk, the infra-red absorption method is readily adapted to continuous recording of all three components, since the milk can be pumped directly from a small homogenizer into the infra-red absorption cell without dilution or addition of reagents. Further, the homogenizer is not required to operate under exactly reproducible conditions.

Analysis of hydrocarbon and other emulsions should be possible by use of the appropriate absorption bands, and samples of a few millilitres could be analysed using the small laboratory homogenizer described by Phipps[1]. Provided the sizes of the particles of the suspended phases were small enough, homogenized emulsions could be analysed directly by the infra-red absorption method.

This method is subject to U.K. Patent Application Nos. 3308/61 and 22,692/61, and full details will be published later (probably in *J. Dairy Res.*).

J. D. S. GOULDEN

National Institute for Research in Dairying,
University of Reading.

[1] Phipps, L. W., *Lab. Practice*, 9, 313 (1960).

Detection by Ionization of Atmospheric Gases during Analysis by Gas Chromatography

WHEN small quantities of atmospheric gases are added to pure helium, the electrical conductivity of the helium increases. This effect forms the basis of a sensitive method for detecting these gases in the effluent of a gas chromatography column.

Berry[1], who used a Lovelock ionization detector[2] in conjunction with helium carrier gas, reported that painstaking purification of the helium was essential to obtain this effect. The work of Landowne and Lipsky[3] and Ellis and Forrest[4] tends to confirm this. These investigators reported obtaining decreases in ionization current with the addition of atmospheric gases when they used unpurified helium under otherwise similar experimental conditions.

By modifying the design of the detector cell so as to minimize contamination of the gas by air, we have achieved the effect and order of sensitivity reported by Berry although commercial helium without further purification was used. As the result of experiments in which the helium was deliberately contaminated, we were able to estimate that the purity required is not more than that specified for commercial helium.

The design of the detector cell is shown in Fig. 1. The central hole in the poly-tetrafluoroethylene insulator was drilled 0·010 in. smaller in diameter than the diameter of the anode pushed through it. The portion of the insulator projecting into the cathode cylinder was machined to a diameter 0·008 in. greater than that of the cylinder into which it was forced. The outlet of the detector cell is 0·050 in. inner diameter tubing at least one inch long to minimize back diffusion of air. In this detector cell, the junctions of insulator and electrode surfaces were capable of withstanding at least two atmospheres of gas pressure without apparent leakage. This is the only apparent significant difference between this design and that of Lovelock[2,5].

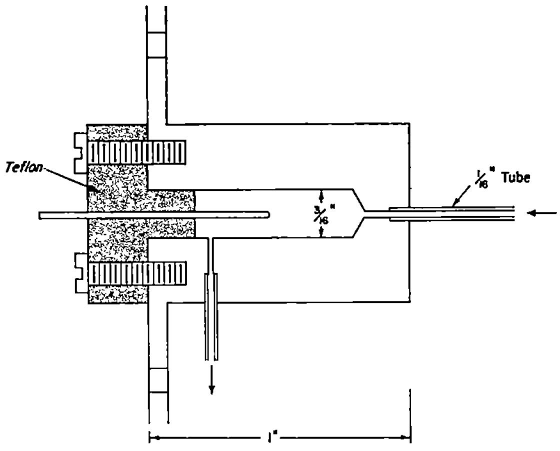


Fig. 1

We chose to operate this detector cell so that the voltage across it was recorded while 1 μamp. from a source of constant current flowed through it. Under these conditions, a decrease in voltage, rather than increase in ionization current, indicates an increase in the electrical conductivity of the gas. This mode of operation is very convenient, makes the presence or absence of a radioactive source in the detector immaterial, and extends the dynamic range of the detector when decreases in the conductivity of the gas are to be followed. Qualitatively comparable results were obtained by impressing 400–600 V. across a similar detector containing a tritium foil and monitoring the current.

Table 1

Gas	Initial sensitivity (V./volume per million volumes of helium)	Increment of concentration for max. conductivity of the mixture (volumes per million volumes of helium)	Peak change in conductivity (V.)
Oxygen	11	280	180
Nitrogen	4	520	250
Argon	3	960	270
Carbon dioxide*	5	80	184

* Determined using silica-gel column.

The response of the detector was determined for oxygen, nitrogen, and argon analysed using a column containing a Linde molecular sieve 5*A*. Carbon dioxide was analysed using columns containing silica gel. As the concentration of each of these gases in the helium increased, the electrical conductivity of the mixture increased, reached a maximum, and then decreased. The sensitivity, the concentration of each gas necessary to bring the conductivity of the binary mixture with helium to a maximum and the electrical conductivity at that maximum varied among the different gases. Values for these parameters under one set of experimental conditions are shown in Table 1. The stability of the system is such that random noise is less than 0·1 V., from which the ultimate sensitivity of the detector may be estimated.

Commercial helium is specified to be at least 99·99 per cent pure[6]. From the increases in concentration of contaminating gases required to bring helium to maximal electrical conductivity, one would infer that the purity of the helium is less significant than the experimental conditions in determining whether the conductivity will increase or decrease on the addition of permanent gases. In our experience the response was determined largely by the design and construction of the detector cell.

ARTHUR KARMEN
LAURA GIUFFRIDA
ROBERT L. BOWMAN

Laboratory of Technical Development,
National Heart Institute,
National Institutes of Health,
U.S. Public Health Service,
Bethesda 14, Maryland.

[1] Berry, R., *Nature*, 188, 579 (1960).
[2] Lovelock, J. E., *J. Chromatography*, 1, 35 (1958).
[3] Landowne, R., and Lipsky, S., *Nature*, 189, 571 (1961).
[4] Ellis, J. F., and Forrest, C. W., *Anal. Chim. Acta*, 24, 329 (1961).
[5] Lovelock, J. E., *Anal. Chem.*, 33, 162 (1961).
[6] Deaton, W. M., *Purity of Grade A Helium* (U.S. Bureau of Mines Helium Activity, Amarillo, Texas, 1959). *Matheson Gas Data Book*, 221 (The Matheson Co., Inc., East Rutherford, New Jersey, 1961).

Effect of Ozone on the Fluorescence and Ultra-violet Absorption Spectra of 3,4-Benzopyrene

PREVIOUS investigations concerning the quantitative determination of the 3,4-benzopyrene content of the air by fluorescence emission spectroscopy[1] showed that the fluorescence intensity of the samples exposed for some hours to air decreased markedly. Experiments were carried out with benzene solutions of 3,4-benzopyrene to investigate this effect. Standard concentrations of 1·2, 12, 120 and 1,200 μgm./ml. were prepared. Ozone was generated in an experimental chamber with an electric spark generator, Kocsis system, and the ozone-contaminated air of the chamber was aspirated through an impinger filled with 6 ml. solution. The ozone content was determined with 2 *N* potassium iodide by the analytical method described by Thorp[2]. The fluorescence emission spectra of the 3,4-benzopyrene solutions were taken with a fluorescence spectrophotometer specially designed for these investigations by one of us. At the same time, the ultra-violet absorption of the samples was measured with a Beckman *UD* spectrophotometer.

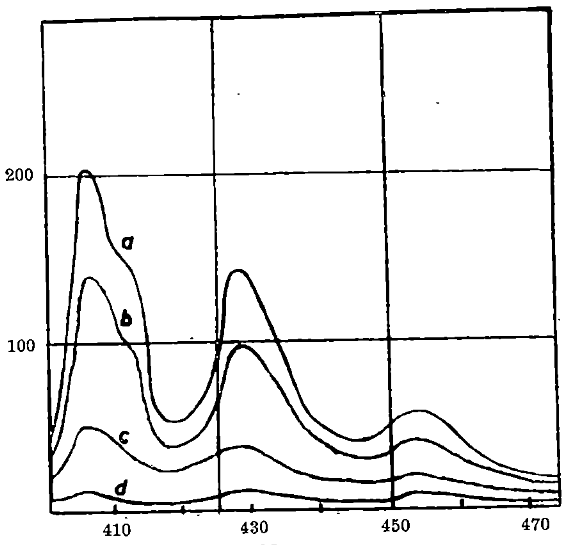


Fig. 1

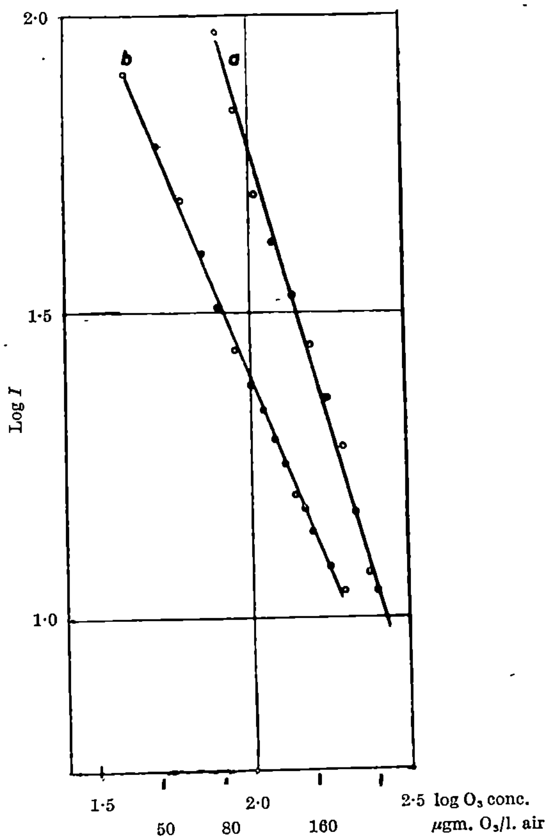


Fig. 2

Fig. 1 shows that, as a result of the ozone treatment, the fluorescence of the samples decreased with increasing time of treatment, that is, increasing amount of ozone introduced into the samples. Curve *a* of Fig. 1 refers to the fluorescence before the ozone treatment, *b* to that after 3 min. aspiration (33 μgm. ozone/l. air), *c*, after 7 min. (104 μgm. ozone/l. air), and *d*, after 10 min. treatment (255 μgm. ozone/l. air). At the same time, the absorption peaks decreased too. If the ozone treatment continued long enough, fluorescence and absorption were extinguished. The time necessary to decrease the initial fluorescence peaks to a tenth of their original value appeared to depend on concentration, and for larger concentrations on wave-length with shorter decay times of peaks at 406 mμ. The process of decreasing fluorescence intensity can be accounted for by a bimolecular law, since the logarithm of the peak intensity versus the logarithm of the ozone concentration gives straight lines (Fig. 2: *a* refers to the peak at 406 mμ; *b*, to the peak at 428 mμ).

1,200 μgm./ml. concentrations were crystallized from solution after, as a result of the treatment with ozone, the fluorescence was extinguished. Microscopical investigations showed a structural difference between the ozone treated and the original crystals. Electron diffraction patterns taken with a Hitachi electron microscope operated at 75 kV., 50 m.amp., give a marked difference too. The melting point of the ozone-treated crystals was not sharply defined (110–130° C.) whereas the melting point of 3,4-benzopyrene crystals is 176° C.

A detailed account of this work will be published elsewhere. We thank Dr. J. Mórik for valuable discussions and J. Dvorzsák for his assistance in taking the electron diffractograms.

Z. Morlin

Institute for Technical Physics
of the Hungarian Academy,

K. M. Sáringer

State Institute for Hygiene,
Budapest.

[1] Kelenffy, S., and Morlin, Z., *Egészségtudomány*, 1, 193 (1957).

[2] Thorp, C. E., *Indust. Eng. Chem.*, Anal. ed., 12, 209 (1940).

Supercontraction of Iodinated Wools

Haly and Feughelman[1] have shown that iodination of wool, when using ethyl alcohol as the solvent, causes a large reduction in the rate of second-stage supercontraction. They express the opinion that "it does seem possible that iodination of tyrosine (or other) residues could, through steric hindrance, make movement of groups, and therefore disruption, more difficult". Leveau *et al.*[2] attributed the lowering of the urea–bisulphite solubility of the iodinated wools to a strengthening of the hydrogen bonds of the phenolic hydroxyl groups by the substitution of the halogens on the tyrosine molecules. In a recent publication the explanation was offered by me[3] that the decrease in supercontraction of the second stage by iodination was due to the steric hindrance caused by the two bulky iodine atoms substituted in the 3,5 positions on the tyrosine molecule, whereby the partially hydrated lithium ion, with its comparatively large volume, is prevented from reaching the hydrogen bonds formed by the hydroxyl group. These hydrogen bonds will therefore remain intact during the supercontraction reactions and the subsequent restriction of the movement of the chains will reduce the rate of disulphide interchange taking place, thus reducing the rate of second-stage supercontraction.

While investigating the ability of different alcohols to penetrate into keratin fibres, Harrison and Speakman[4] found that fibres treated with a solution of iodine in either methyl alcohol or ethyl alcohol undergo total iodination of the tyrosine residues present in the keratin. Butanol and higher alcohols do not penetrate the fibre at all and, consequently, iodination with these higher alcohols as solvents for the iodine is effective for less than 1 per cent of the tyrosine residues present. When the iodine is, however, dissolved in *n*-propyl alcohol the reaction was shown to proceed to a point where about one-third of the tyrosine residues in the keratin could be accounted for. Haly and Feughelman[5] investigated the X-ray diffraction patterns of wools iodinated with an ethanolic and an *n*-propanolic solution of iodine respectively, and concluded that both ethanol and propanol solutions iodinate tyrosine residues in the matrix, but only the ethanol solution produces iodination of tyrosine in the microfibrils.

The series zone model for ketatin fibres[6] explains many of the physical properties of animal fibres, such as the two-stage supercontraction in concentrated solutions of lithium bromide at elevated temperatures and the stress–strain behaviour. According to this model, the matrix plays no part in the supercontraction, contraction occurring exclusively in the microfibrils. The two different kinds of zones occurring alternately along the length of the microfibrils are responsible for the two distinct contraction stages.

In view of this theory, it seemed appropriate to investigate whether selective iodination of the matrix alone would have any influence on the supercontraction of the fibres.

One end of a 1-cm. section from the root end of a 64's Merino wool fibre which had previously been washed in petroleum ether, ethanol, 1 per cent aqueous solution of 'Lissapol' (I.C.I.), water, ethanol and ether successively, was attached to a glass rod with molten polystyrene. A snippet, 0·5 mm. in length, was cut from the free end of this fibre section and supercontracted at 90° C. in an unbuffered 7·8 *M* lithium bromide solution, using the technique of Haly and Griffith[7]. The fibre section remaining on the glass was then iodinated in the way described in ref. 4. Special care was taken to ensure that the fibre section was absolutely dry and that the propyl alcohol contained no lower homologues or water. The iodination reaction was allowed to proceed for 14 days at 22° C., after which it was washed with sodium thiosulphate solution and water. After drying in a stream of cold air, a 0·5-mm. snippet was again cut off from the free end of the section and supercontracted. This procedure ruled out the variations, with regard to supercontraction, found along the length of the fibre as the two snippets were adjacent in the original fibre and with a total length of only about 1 mm.

Four determinations were carried out in this way using four different wool fibres. In none of these cases could any significant difference be detected between the supercontraction curves before and after iodination in the presence of *n*-propyl alcohol.

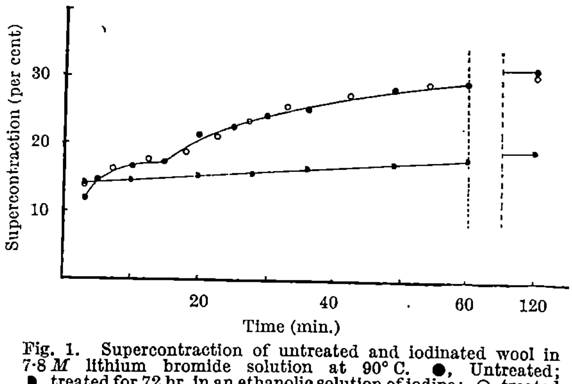


Fig. 1. Supercontraction of untreated and iodinated wool in 7·8 *M* lithium bromide solution at 90° C. ●, Untreated; ◗, treated for 72 hr. in an ethanolic solution of iodine; ○, treated for 14 days in an *n*-propanolic solution of iodine

The accompanying graph shows the kinetic supercontraction of untreated wool and wool iodinated with an *n*-propanolic solution of iodine in comparison with wool treated with a solution of iodine in ethanol for 72 hr.

The results are in agreement with the X-ray results in that the matrix alone is iodinated with the *n*-propanolic solution, with no effect on the supercontraction, while almost total iodination of matrix and microfibrils is achieved by using ethanol as the solvent, resulting in a marked depression of the second-stage supercontraction.

O. A. SWANEPOEL

South African Wool Textile Research Institute,
Rhodes University,
Grahamstown, South Africa.

[1] Haly, A. R., and Feughelman, M., *Text. Res. J.*, **30**, 365 (1960).
[2] Leveau, M., Coillet, M., and Demonmerot, N., *Bull. Inst. Text. France*, **84**, 17 (1959).
[3] Swanepoel, O. A., *Text. Res. J.* (in the press).
[4] Harrison, O., and Speakman, J. B., *Text. Res. J.*, **28**, 1005 (1958).
[5] Haly, A. R., and Feughelman, M., *Text. Res. J.*, **30**, 622 (1960).
[6] Feughelman, M., and Haly, A. R., *Kolloid Z.*, **168**, 107 (1960).
[7] Haly, A. R., and Griffith, J., *Text. Res. J.*, **28**, 32 (1958).

BIOCHEMISTRY

Identification of a Compound related to 6-Aminopenicillanic Acid, isolated from Culture Media of *Penicillium chrysogenum*

It has previously been reported[1] that when a high-yielding strain of *Penicillium chrysogenum* was grown in shake flasks in a chemically defined arginine-lactose medium[2] containing sodium sulphate labelled with sodium-35 in the absence of phenylacetic acid, a radioactive compound extractable with *n*-butyl-acetate was formed instead of benzylpenicillin. This compound differed in its chromatographic properties from any of the known penicillin degradation products.

Afterwards it was found that this compound was also formed when the mould was grown in stirred 90-l. fermenters under standard conditions, using the vortex system of aeration on a chemically defined ammonium acetate – ammonium lactate – lactose medium[3], and this made it possible to isolate the compound in larger amounts and to elucidate the structure. This work is reported in the present communication.

Isolation: The culture medium (50-l. per fermenter) was gathered after 4 days fermentation (aeration condition: vortex system, eight-bladed turbine stirrer 130 mm. diam., 700 r.p.m., air flow 50 litres per min.), filtered, cooled to 5° C., acidified to *p*H 2, extracted, first (to remove traces of penicillin) with half a volume of chloroform in which the compound is insoluble, and then three times with two volumes of *n*-butyl acetate to remove the compound. The partition coefficients of the compound in different solvents were established in preliminary experiments in which labelled sodium sulphate was added to the culture medium and the distribution in solvents followed by measuring the radioactivity. The compound was transferred from the solvent into one quarter of its volume of water by adjusting the *p*H to 7, the aqueous solutions concentrated *in vacuo* at 40° C., cooled to 5° C., acidified to *p*H 2, and percolated through a column of granular charcoal. The adsorbed compound was fractionally eluted by 70 per cent aqueous ethanol; iodine titration was used for detecting the compound in the different fractions. The fractions containing the compound were concentrated *in vacuo* for complete removal of ethanol, an excess of *N,N'*-dibenzylethylenediamine added, and, after ether extraction, the aqueous solution was concentrated to an oil. The crystalline *N,N'*-dibenzylethylenediamine salt, melting point 110–114° C., was obtained by dissolving the oil in a minimal amount of hot water, adding acetone and cooling slowly. The dried salt was hygroscopic (found: C, 59·04; H, 6·65; N, 11·14; S, 6.40; O (direct determination), 17·00 per cent; $C_9H_{12}O_5N_2S,C_{16}H_{20}N_2,1/2H_2O$; requires: C, 58·92; H, 6·52; N, 11·00; S, 6·29; O, 17·27 per cent). The yield was about 20 mgm./l. of culture medium. Altogether, about 25 gm. of *N,N'*-dibenzylethylenediamine salt were prepared.

The free acid was obtained from this salt by passing an aqueous solution cooled to 5° C. through a cation exchange column of 'Amberlite *IR* 120'(H) and lyophilizing the percolate. The amorphous powder was hygroscopic (found: C, 39·61; H, 4·81; N, 9·76; S, 11·35 per cent; $C_9H_{12}O_5N_2S,1/2H_2O$ requires: C, 40·14; H, 4·87; N, 10·40; S, 11·91 per cent; $C_9H_{12}O_5N_2S,1H_2O$ requires: C, 38·84; H, 5·07; N, 10·07; S, 11·52 per cent). The free acid could be obtained crystalline from ether. Analysis suggested the presence of one mole of ether of crystallization, which was firmly bound; it was retained even on heating at 100° C. at 15 mm. pressure, but was lost on heating to the melting point (loss of weight roughly equivalent to one mole of ether). The compound effervesced between 110 and 120° C., losing its crystalline structure, and melted at 140° C. (for two different samples, found : C, 45·85, 45·79; H, 5·98, 6·37; N, 8·81, 8·73; S, 10·29, 9·68 per cent; $C_9H_{12}O_5N_2S,(C_2H_5)_2O$ requires: C, 46·69; H, 6·63; N, 8·38; S, 9·59 per cent). A crystalline dimethyl ester was obtained by adding an ethereal solution of diazomethane to a solution of the free acid in ether-ethanol, evaporating to dryness and recrystallizing from ethanol. It had a melting point of 168–170° C. (found: C, 45·64; H, 5·61; N, 9·92; S, 10·96 per cent; $C_{11}H_{16}O_5N_2S$ requires: C, 45·82; H, 5·59; N, 9·72; S, 11·12 per cent).

Elementary composition. The analyses of the *N,N*-dibenzylethylenediamine salt, the free acid, and the dimethylester indicate the formula of this compound to be $C_9H_{12}O_5N_2S$.

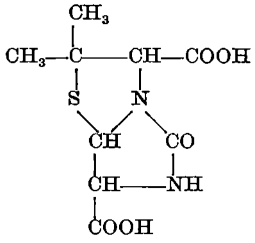
Properties. The compound is a dibasic acid, having *pK* values of 2·5 and 3·4 as determined by electrometric titration. It gives no colour reactions with hydroxylamine-ferric chloride, nitroprusside or ninhydrin. It is strongly dextrorotatory, $[\alpha]^{26}_{D} = +282°$ ($c = 1·2$, in water), as are the *N,N'*-dibenzylethylenediamine salt, $[\alpha]^{23}_{D} = +160°$ ($c = 1·0$, in water) and the dimethylester, $[\alpha]^{23}_{D} = +218°$ ($c = 1·4$, in ethanol). It has no characteristic ultra-violet absorption in the *p*H range of 1–14. With mercuric chloride it gives a precipitate which, after decomposition with hydrogen sulphide, gives a product showing a positive reaction for sulphydryl groups with ferric chloride, but a negative ninhydrin reaction. The original compound reacts with iodine, taking up six equivalents of iodine per mole, and this reaction can be used for its quantitative determination.

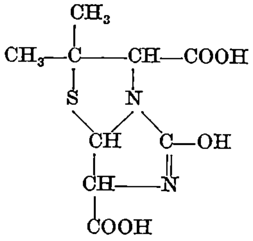
Degradation. On heating with 1 *N* hydrochloric acid for 20 min. one mole of carbon dioxide is evolved, and a compound is obtained which, after crystallization from ethyl acetate, has a melting pioint of 173–175° C. (found: C, 44·64; H, 5·73; N, 13·22; S, 14·89 per cent; $C_8H_{12}O_3N_2S$ requires: C, 44·43; H, 5·59; N, 12·95; S, 14·83 per cent). This compound is a monobasic acid ($pK = 3·4$) giving no ninhydrin or sulphydryl reaction. It precipitates with mercuric chloride and reduces iodine. Its methyl ester, prepared by treatment with diazomethane, has a melting point of 130° C. after crystallization from carbon tetrachloride-light petroleum (found: C, 47·22; H, 6·34; N, 12·31; S, 14·18 per cent; $C_9H_{14}O_3N_2S$ requires: C, 46·94; H, 6·13; N, 12·17; S, 13·92 per cent).

On oxidation with sodium periodate or hydrogen peroxide or bromine water followed by hydrolysis (6 *N* hydrochloric acid, 7 hr. at 105° C.), the compound produces two ninhydrin-positive substances which were identified by paper electrophoresis and by paper chromatography (in eight solvents) as penicillaminic acid, $(CH_3)_2C(SO_3H)CH(NH_2)COOH$, and glycine. Acid hydrolysis without previous oxidation gave a more complex mixture of degradation products.

Structure. With the isolation of penicillaminic acid, glycine and carbon dioxide, 8 of the 9 carbon atoms and 4 of the 5 oxygen atoms of the compound were accounted for, as well as their sequence; the four oxygen atoms form part of the two carboxylic groups revealed by electrometric titration.

From this as well as chemical and physical properties the most likely formula for the compound is that of the imidazolidone I (2,2-dimethyl-5-oxo-hexahydro-imidazo [5,1-*b*]-thiazolo-3,7-dicarboxylic acid), or its imidazoline tautometer II indicated below; that unaccounted-for carbon and oxygen forming the CO link between the two nitrogens.


(I)


(II)

Structure II is a penillic acid type of compound in which the penicillin side-chain residue is replaced by OH.

This structure is confirmed by the fact that the dimethylester of the compound as well as the methyl ester of the decarboxylated compound have properties identical to those reported in the literature for products previously obtained by unequivocal syntheses[4,5].

During the course of this work it became clear that the compound described here was identical with the compound VI of Tardrew and Johnson[6].

Mode of formation. Synthesis of this compound was attempted by treating phosgene with 6-amino-penicillanic acid which had previously been incubated in alkaline solution to destroy the β-lactam ring. Best results were obtained by using aqueous bicarbonate solutions, and paper electrophoresis of samples taken both before and after the addition of phosgene revealed that a substance with the same mobility as the natural compound was formed immediately on dissolving 6-amino-penicillanic acid in aqueous sodium bicarbonate. High yields of the synthetic compound were obtained by passing a stream of carbon dioxide into a solution of 6-aminopenicillanic acid in potassium phosphate buffer, *p*H 7·0. The *N,N′*-dibenzylethylenediamine salt prepared from the product of this reaction has properties identical with those of the salt of the naturally occurring compound (optical rotation, paper chromatographic mobility, melting point, infra-red spectrum).

The reaction of 6-aminopenicillanic acid in bicarbonate solution to yield a compound identical with that described in the present communication has independently been observed by Drs. Batchelor and Rolinson of Beecham Research Laboratories (personal communication to E. B. Chain; see following communication).

Thus, it appears that the compound is formed by carboxylation of the amino group of 6-amino-penicillanic acid followed by a penillic acid rearrangement. The rearrangement was hitherto only known to occur at acid *p*H.

We wish to thank Drs. R. Falini and F. Ricci and the staff of the pilot plant for their invaluable help, and Drs. Margherita Marzadro and Lavinia Carta De Angeli for analytical determinations.

The compound was isolated by Ballio, Chain, di Accadia, Mauri, Rauer and M. J. Schlesinger. The chemical investigations were carried out by Ballio, Chain, Schlesinger and S. Schlesinger.

A. BALLIO
E. B. CHAIN
F. DENTICE DI ACCADIA
M. MAURI
K. RAUER*
M. J. SCHLESINGER†
SONDRA SCHLESINGER‡

International Centre for Chemical Microbiology,
Istituto Superiore di Sanità,
Rome.

---

* Aided by a grant from the Chemische Fabrik Dr. R. Pfleger, Germany.

† Post-doctoral Fellow of the U.S. National Science Foundation.

‡ Post-doctoral Fellow of the U.S. National Foundation.

[1] Ballio, A., Brandl, E., Chain, E. B., Dentice di Accadia, F., Navazio, F., and Rossi, C., *Selected Sci. Papers Ist. Sup. Sanità*, 2 (II), 314 (1959).

[2] Brandl, E., Carilli, A., and Chain, E. B., *Selected Sci. Papers Ist. Sup. Sanità*, 2 (II), 212 (1959).

[3] Jarvis, F. G., and Johnson, M. J., *J. Amer. Chem. Soc.*, **69**, 3010 (1947).

[4] Bachmann, W. E., and Cronyn, M. W., *The Chemistry of Penicillin*, 891 (Princeton Univ. Press, 1949).

[5] Cook, A. H., and Heilbron, I. M., *The Chemistry of Penicillin*, 949, 970 (Princeton Univ. Press, 1949).

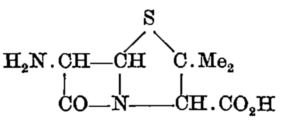
[6] Tardrew, P. L., and Johnson, M. J., *J. Biol. Chem.*, **234**, 1850 (1959).

Action of Carbon Dioxide on 6-Aminopenicillanic Acid

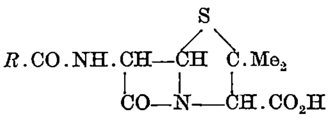
THE nucleus of the penicillin molecule, 6-amino-penicillanic acid (I)[1], is in some respects more stable than most penicillins (II), being, for example, only slowly destroyed by mineral acids. It was therefore surprising to find that carbon dioxide, which is without effect on penicillins, readily attacked 6-amino-penicillanic acid to yield a product which could no longer be converted into penicillins by acylation. The effect was first observed when 6-aminopenicillanic acid was dissolved in water by neutralization with sodium bicarbonate, but a solution of sodium 6-aminopenicillanate in aqueous sodium bicarbonate was much more stable, indicating that the reaction required free carbon dioxide. When carbon dioxide was bubbled continuously through an aqueous solution of 6-aminopenicillanic acid at 37° C. and an initial *p*H of 7·0 the 6-aminopenicillanic acid content, determined by phenylacetylation followed by biological assay, fell by 50 per cent every 3 hr. The hydroxylamine assay[2] and the leucine equivalent as determined by ninhydrin fell at the same rate, indicating that both the β-lactam ring and the amino-group were absent from the reaction product.

For preparative purposes carbon dioxide was bubbled through a 2 per cent solution of 6-aminopenicillanic acid until more than 90 per cent had been destroyed, and the resulting solution was passed through a column of 'Amberlite *IR*/120'(H) to remove sodium ions and residual starting material. Freeze-drying then gave a strongly acidic white solid (70 per cent yield) which was very soluble in water and polar solvents, but insoluble in non-polar solvents. Neither the acid nor its salts could be recrystallized; but analysis of the amorphous material, which had no definite melting point, indicated that it was derived from 6-aminopenicillanic acid by the addition of one molecule of carbon dioxide. (Found: C, 39·5; H, 4·7; N, 10·3; S, 12·0. $C_9H_{12}O_5N_2S$, $0·5H_2O$ requires C, 40·1; H, 4·9; N, 10·4; S, 11·9 per cent.)

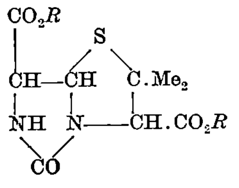
The substance appeared to contain a labile carboxyl group since one molecule of carbon dioxide was lost when the acid was: (*a*) treated at room temperature with mercuric chloride solution or (*b*) dissolved in water and boiled for 2–3 hours. The product from (*b*) was isolated in 84 per cent yield and crystallized from water in colourless rosettes, m.p. 175–176° C., of a monocarboxylic acid isomeric with 6-aminopenicillanic acid (found: C, 45·0; H, 5·9; N, 12·8; S, 14·9; $C_8H_{12}O_3N_2S$ requires C, 44·4; H, 5·6; N, 13·0; S, 14·8 per cent). These properties suggested that the original carbon dioxide adduct was a dicarboxylic acid closely related to the penillic acids[3], most probably 2,2-dimethyl-5-oxohexahydro-imidazo-[5,1-*b*]-thiazole-3,7-dicarboxylic acid (III; *R* = H). Methylation with diazomethane in the usual way confirmed this structure, giving 68 per cent of the ester (III; *R* = Me) which crystallized from water or ethyl acetate in needles, m.p. 166–167° C., $[\alpha]^{21}_{D}$ + 220° ($c = 0·5$ in EtOH) (found: C, 46·0; H, 5·8; N, 9·4; S, 11·0; calc. for $C_{11}H_{16}O_5N_2S$: C, 45·8; H, 5·6; N, 9·7; S, 11·1 per cent). The decarboxylation product, 2,2-dimethyl-5-oxohexahydro-imidazo-[5,1-*b*]-thiazole-3-carboxylic acid (IV; *R* = H) was similarly converted in 90 per cent yield to the ester (IV; *R* = Me) which crystallized from water in needles, m.p. 129–130° C. (found: C, 47·2; H, 6·4; N, 12·3; S, 13·9; calc. for $C_9H_{14}O_3N_2S$: C, 46·9; H, 6·1; N, 12·2; S, 13·9 per cent). From their physical properties, these esters would appear to be identical both structurally and stereochemically with specimens previously prepared by synthesis from D-penicillamine methyl ester[4].



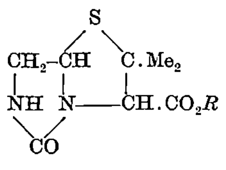
(I)



(II)



(III)



(IV)

The facile formation of (III; *R* = H) from (I) in essentially neutral solution may involve formation of the carbamic acid (II; *R* = OH; 'hydroxypenicillin') followed by penillic rearrangement[5], although the latter normally occurs only at low *p*H. Alternatively, there may be a concerted attack by the carbon dioxide molecule on both the amino group and the highly reactive cyclic amide functions.

None of the derivatives described here exhibited antibacterial activity.

We wish to thank Prof. E. B. Chain, for helpful discussions (see preceding communication).

F. R. Batchelor
D. Gazzard
J. H. C. Nayler

Beecham Research Laboratories, Ltd.,
Research Division,
Brockham Park,
Betchworth, Surrey.

[1] Batchelor, F. R., Doyle, F. P., Nayler, J. H. C., and Rolinson, G. N., *Nature*, **183**, 257 (1959).

[2] Batchelor, F. R., Hardy, T. L., Mansford, K. R. L., and Rolinson, G. N., *Proc. Roy. Soc.*, B (in the press).

[3] Clarke, H. T., Johnson, J. R., and Robinson, R. (edit.), *The Chemistry of Penicillin*, 106 (Princeton Univ. Press, 1949).

[4] Clarke, H. T., Johnson, J. R., and Robinson, R. (edit.), *The Chemistry of Penicillin*, 891, 970 (Princeton Univ. Press, 1949).

[5] Clarke, H. T., Johnson, J. R., and Robinson, R. (edit.), *The Chemistry of Penicillin*, 445 (Princeton Univ. Press, 1949).

Extraction of Lipids from the Precursor of the Stylet-Sheath Material of a Pentatomid

It has been reported[1] that the stylet-sheath material of the American milkweed bug, *Oncopeltus fasciatus* (Dall.), yields oily sudanophil droplets when warmed in chlorated nitric acid. In the three-lobed principal salivary glands, the contents of the anterior and lateral lobes have been identified as the precursors of the sheath material, and the contents of the lateral lobe show stronger sudanophilia than the contents of the other lobes[2]. On this evidence, it was suggested that lipid is present in the sheath material and is elaborated in the lateral lobe; and on the basis of the reaction of *Oncopeltus* sheath material to the Liebermann–Burchardt test for sterols, it was concluded that a steroid might be present. The following concerns the presence of lipids in the sheath material and its precursor in the salivary glands of an Australian pentatomid, *Eumecopus* sp., which feeds on eucalypts.

The sheath material of *Eumecopus* also produced oily sudanophil droplets when warmed in chlorated nitric acid. The principal salivary gland is bilobed. The contents of the anterior lobe could be distinguished from the contents of the posterior lobe and identified as the precursor of the sheath material by the immediate coagulation of the contents of the anterior lobe in water, physiological saline, and glycerol; the production of oily droplets when the contents of the anterior lobe were warmed in chlorated nitric acid; and the ability of toluidine blue to stain the contents of this lobe the same characteristic turquoise-blue colour as it did sheath material. The precursor could be collected in much larger quantities than the sheath material itself, and an attempt was made to extract lipids from the precursor with organic solvents.

The contents of the anterior lobes of several glands were first gelled by immersing the glands in glycerol. When transferred to water the glands swelled, causing the walls of the anterior lobes to separate from the coagulated masses of sheath precursor. These were removed from the glands, freed of adherent cellular material, washed thoroughly, and dried. About 5 mgm. were sealed in a capillary tube with about 15 µl. of a 4 : 1 mixture of chloroform and methanol, and incubated at 60° C. for 18 hr. When the solvent was removed and evaporated, it was found that approximately 350 µgm. of a grease-like material had been extracted. Preliminary tests on smears of the grease either on slides or filter paper showed that the extracted material was strongly fluorescent; had a strong affinity for the sudan colorants for lipids; was coloured blue by nile blue sulphate, suggesting the presence of acidic lipids; gave a positive test for amines with ninhydrin; and gave a positive result for 'Lipine' with Baker's acid hæmatin test[3] and for phosphate with a modified Hanes–Isherwood reagent[4] in an atmosphere containing hydrogen sulphide. When run in a test-tube paper-chromatogram, using *n*-butanol/acetic acid/water (4 : 1 : 5) as solvent, fluorescent material was carried to the solvent front and gave a positive reaction with ninhydrin and Hanes–Isherwood reagent, while substances giving reactions with either or both reagents were left in the path of the solvent. With the Liebermann–Burchardt test, the grease gave a purple-brown colour which developed and faded slowly; no positive reaction was obtained with other colour tests for unsaturated steroids[5].

These results suggested the presence of phospholipid and related compounds, and separation of the grease into components was attempted by treating it with different solvents. These extracts were spotted on filter paper previously soaked in a large volume of chloroform–methanol, and further separation was attempted either by allowing another solvent to run past the spot by capillary ascent or by running test-tube paper-chromatograms.

A water extract run in butanol/acetic acid/water gave a faint spot with ninhydrin about one-third the distance from the origin to the solvent front, and a blue ring with Hanes–Isherwood reagent just above the origin.

An acetone extract was further separated with benzene. The benzene-soluble fraction gave no reaction with ninhydrin or Hanes–Isherwood reagent, but took up sudan colorants and coloured blue with nile blue; it ran with the solvent front in butanol/acetic acid/water and was probably fatty acid. The benzene-insoluble fraction gave at the most a brown colour with Hanes–Isherwood reagent, and was barely coloured by nile blue or the sudan colorants, but gave a reaction with ninhydrin, even after repeated washings with water.

A benzene extract of the original grease, when run in butanol/acetic acid/water, gave a large spot with ninhydrin near the solvent front and other faint spots along the path of the solvent; a streak coinciding with ninhydrin-positive material near the solvent front gave a positive reaction with Hanes–Isherwood reagent and was coloured blue by nile blue. The material near the solvent front thus gave the reactions of a phospholipid. However, fluorescent material which remained at the origin and showed no reaction with ninhydrin or Hanes–Isherwood reagent was coloured blue by nile blue and took up the sudan colorants, suggesting that a lipid other than phospholipid was also present.

These experiments showed that lipid is present in the stylet-sheath material of *Eumecopus* and in the precursor elaborated in the anterior lobe of the salivary glands. The lipid extracted from the precursor is partly phospholipid, but there was evidence of the presence of other lipid substances. However, in contrast to the reaction of the sheath material of another plant-bug, *Oncopeltus fasciatus*, to the Liebermann–Burchardt test, the reaction of *Eumecopus* sheath material was negative; and no specific steroid reactions were obtained in the grease extracted from the precursor.

P. W. Miles

Waite Agricultural Research Institute,
University of Adelaide,
Adelaide, South Australia.

[1] Miles, P. W., *J. Insect Physiol.*, **4**, 209 (1960).

[2] Miles, P. W., *J. Insect Physiol.*, **4**, 217 (1960).

[3] Baker, J. R., *Quart. J. Micro. Sci.*, **87**, 441 (1946).

[4] Amelung, D., and Böhm, P., *Hoppe-Seyl. Z.*, **298**, 199 (1954).

[5] Fieser, L. F., *The Chemistry of Natural Products related to Phenanthrene* (Reinhold Pub. Corp., New York, 1957).

A Selective Toxin produced by *Periconia circinata*

Toxins with species and varietal specificity have often been postulated, but so far only one such substance has been isolated from a plant pathogen[1]. *Helminthosporium victoriae*, causal agent of a blight of certain oat cultivars, produces an extremely powerful exotoxin which has the same specificity for plants as does the fungus itself. Thus, the *Helminthosporium* blight is a valuable model for study of the biochemistry of disease. However, there are difficulties; most strains of the fungus give low yields of toxin *in vitro*, and the compound is very unstable[2]. Isolation of other selective toxins might overcome such difficulties and should indicate whether or not specific toxins are commonly involved in plant diseases. A second selective toxin which shows promise for further study has now been found.

The fungus *Periconia circinata* is the causal agent of the 'milo disease' of certain cultivars of *Sorghum vulgare* var. *subglabrescens* (grain sorghum or milo). Other sorghum cultivars are highly resistant. Leukel[3] first identified the causal agent and gave some evidence that a toxin affecting only susceptible plants might be involved in disease development. This interesting possibility was not investigated further.

P. circinata was isolated from a sample of Kansas soil supplied by Mr. W. D. Stegmeier of the Kansas Agricultural Experiment Station. The fungus was grown at approximately 25° C. in a modified Fries No. 3 basal medium[2], supplemented with 0·1 per cent Difco yeast extract. Filtrates were collected from cultures of various ages and tested for selective toxic action against seedlings of *Periconia*-resistant and susceptible sorghums. The seedling assay was patterned after that used for the *Helminthosporium* toxin[2]. Seeds were germinated at 24° C. for 24 hr. between sheets of moist filter paper. Serial dilutions of filtrates were made and 5 ml. of each dilution was placed in a 60 × 15 mm. Petri dish with five germinated seeds. The assay end-point was determined after 48 hr. as the maximum dilution which prevented roots from growing more than 1 cm. long. Control seedlings in water had roots 5 cm. or more in length at 48 hr. Positive assays were obtained from culture filtrates, when *Periconia*-susceptible sorghum seedlings were used as the test object. The highest assay end-point obtained was at a dilution of 1: 3,200. Culture filtrates did not damage seedlings of *Periconia*-resistant sorghum, even when diluted only 1: 10. The peak of toxin content was reached in about twenty-one days in resting culture, whether the fungus was grown in small Erlenmeyer flasks or in large Blake-type bottles, containing 500 ml. of medium. Most cultures grown on a shaker failed to produce toxin.

Filtrates were tested for toxicity against cuttings of *Periconia*-resistant and susceptible sorghum plants. The basal ends of cut shoots were placed in filtrates at dilutions from 1: 50 to 1: 800 and kept at room temperature under artificial light. In all cases the susceptible cuttings were dead and shrivelled within 48 hr., while the resistant cuttings were unaffected and were comparable to controls placed in water. Symptoms of affected cuttings were similar to those of inoculated plants in the greenhouse.

Selectivity of culture filtrates was tested further against seedlings of several sorghum cultivars and non-host plants. The following *Periconia*-resistant sorghums were tried and were not affected by the filtrate: Westland, *SA*-7078, Colby (*Periconia*-resistant selection), Feterita, Texas Blackhull Kafir, Double Dwarf Yellow Milo 38, Atlas Sorgo, and Double Dwarf White Sooner Milo. The susceptible sorghums used, which were all equally sensitive to the filtrate, were Colby, Western Blackhull *X* Day 481004, and Double Dwarf Yellow Milo. Filtrates were tested against the following non-host plants: rye, barley, wheat, oats (Clinton and Park), radish, tomato, crimson clover, carrot, cabbage (Wisconsin Ballhead and Ferry's Hollander), and the fern *Onoclea sensibilis*. There was no indication of toxicity to any of these. Various bacterial and fungal contaminants also were observed growing in toxin-containing solutions from time to time.

Toxin production apparently was correlated with pathogenicity and virulence of the fungus. A total of thirty-two *Periconia* isolates was taken from the soil sample. Eight isolates were pathogenic and produced toxin, while the others were non-pathogenic and did not produce toxin in culture. Four of the pathogenic isolates produced much less toxin in culture than did the other four. Preliminary inoculation experiments gave some indication that the low-toxin producers were less virulent as well.

Some chemical characteristics of the toxin were determined. The toxic activity of culture filtrates passed freely through dialysis membranes, indicating that the toxin has a relatively low molecular weight. Like the wildfire toxin[4] and the *Helminthosporium* toxin[2], the *Periconia* toxin was readily inactivated in alkaline solution, but was stable at acid reactions. Preliminary work indicated that the *Periconia* toxin is more stable than the *Helminthosporium* toxin, since activity was not lost in slightly alkaline solution nor after evaporation of solutions to dryness. These advantages have made it relatively simple to concentrate the material some one-hundredfold and to examine its chemical characteristics. Like the *Helminthosporium* toxin, the *Periconia* toxin appears to be polypeptide in nature. However, the chemical properties of the two toxins are distinctly different as would be expected since there is no biological cross-reactivity between them. The chemical nature and mode of action of this toxin will be investigated further.

Sorghum seeds were kindly supplied by Mr. W. D. Stegmeier of the Kansas Agricultural Experiment Station, Garden City, and by Dr. D. G. Smeltzer of the University of California, Davis.

This investigation was carried out during the tenure of a special fellowship to the senior author from the National Institute of Allergy and Infectious Diseases, United States Public Health Service.

R. P. SCHEFFER
R. B. PRINGLE

Rockefeller Institute,
New York 21.

[1] Braun, A. C., and Pringle, R. B., in *Plant Pathology: Problems and Progress 1908–1958*, edit. by Holton, C. S., 88 (University of Wisconsin Press, Madison, 1959).

[2] Pringle, R. B., and Braun, A. C., *Phytopathology*, **47**, 369 (1957).

[3] Leukel, R. W., *J. Agric. Research*, **77**, 201 (1948).

[4] Woolley, D. W., Pringle, R. B., and Braun, A. C., *J. Biol. Chem.*, **197**, 409 (1952).

Formation of Phenethyl Alcohol and Tyrosol during Fermentation of a Synthetic Medium lacking Amino-acids

THE fusel oils, or the higher alcohols produced during fermentation, play an important part in the bouquet of wine and the flavour of beer[1]. Since the classical studies of Ehrlich it has been thought that they are produced by degradation of amino-acids, and this point of view has gained considerable support from the recent work of SentheShanmuganathan[2,3]. Nevertheless, Castor and Guymon[4] have pointed out that the concentration of the appropriate amino-acids in grape must is insufficient to explain the level of fusel oils found in the wine, and when Genevois *et al.*[5] found that 1-^{14}C-sodium acetate is incorporated into 3-methylbutanol during fermentation they suggested that the bulk of fusel oil is produced from this or similar intermediates. In considering the formation of fusel oil during beer production it has been pointed out that although the amount of the various amino-acids present in wort and metabolized during fermentation is sufficient to account for the higher alcohols formed by the Ehrlich pathway, this is probably not the only route to these compounds[1].

Harold *et al.*[6] considered that phenethyl alcohol was a key compound for distinguishing between the Ehrlich pathway and that proposed by Genevois, and as they could not verify their earlier identification of this alcohol in beer they favoured the latter mechanism. The presence of phenethyl alcohol in beer, however, has now been well established[7,8] and it was again suggested that although the level of β-phenylalanine present in wort was sufficient to explain the level of this alcohol found in beer, it seemed likely that yeast could produce this alcohol from other metabolites[8]. It is now shown that during fermentation of a synthetic medium lacking amino-acids, yeast produces, in addition to 2-methylpropanol and 2- and 3-methylbutanol previously recognized, both phenethyl alcohol and the non-volatile alcohol, tyrosol, although the corresponding amino-acids, valine, leucine, β-phenylalanine and tyrosine, could not be detected in the final medium.

Eighteen litres of the synthetic medium *FRM*[9] were fermented at 25° using *Saccharomyces carlsbergensis* (N.C.T.C. 396). After 11 days, 84 per cent of the glucose but only 9 per cent of the total nitrogen had been assimilated. The majority of the unassimilated nitrogen was still in a simple form since the same amount of ammonia was found by distilling an aliquot portion with alkali directly as was found after digestion with concentrated sulphuric acid. Two litres of the fermented medium, however, were concentrated by ion-exchange chromatography on 'Amberlite *IR*-120' and the fraction eluted with ammonia was desalted, and examined by two-dimensional chromatography[10]. Only very faint spots corresponding to glycine, glutamic acid, alanine and γ-amino-butyric acid(?) were revealed with ninhydrin while no evidence for valine, leucine, phenylalanine or tyrosine could be seen.

The fermented medium, which contained 1·0 per cent v/v of ethanol and 30 mgm./l. of fusel oil as estimated colorimetrically[11], was distilled and the first 25 per cent of the distillate was collected and extracted continuously with a mixture of ether and light petroleum (b.p. 30–40°) (1:1) for seven days. The extract was dried (potassium carbonate) and the solvent was removed through a column of Dixon gauze spirals from a water-bath at 50°. The resulting extract (151 mgm.) was examined by gas chromatography on dinonyl phthalate at 78° when peaks with retention times corresponding to *n*-propanol (trace) 2-methylpropanol (16 per cent), and 2- and 3-methylbutanol (not separated on this stationary phase) (84 per cent) were observed. The figures in parenthesis correspond to the percentage of the total peak areas. Further examination of this volatile fraction by gas chromatography on 'Apiezon *M*' at 170° revealed the presence of a peak with retention time identical with that of phenethyl alcohol. By use of an internal standard it was calculated that the original concentration of this alcohol in the fermented medium was 1·5 mgm./l.

The residue from the distillation was concentrated to low bulk and extracted with ethanol (3 times). The ethanol solution was evaporated, the residue dissolved in water and the solution continuously extracted by means of ether. When the extract so obtained was examined by paper chromatography in three solvent systems: (1) butanol/acetic acid/water (4:1:1); (2) ethanol/2 *N* ammonia (9:1); (3) 10 per cent aqueous acetic acid, the major component had the same R_F value as authentic tyrosol and gave the same colorimetric reactions with diazotized sulphanilic acid, diazotized *p*-nitroaniline and α-nitroso-β-naphthol. Only in the last solvent system is tyrosol separated from tryptophanol. On these chromatograms diazotized sulphanilic acid revealed a very pale yellow spot with the same R_F as tryptophanol in the fermented medium extract, but it failed to give the characteristic colour change in presence of *p*-dimethylaminobenzaldehyde.

These observations cannot exclude the possibility that the higher alcohols secreted into the medium arise from amino-acids synthesized within the cell. This, however, seems unlikely as the corresponding keto-acids are intermediates common to amino-acid synthesis and the first stage of degradation via the Ehrlich pathway. It is suggested, therefore, that the higher alcohols are by-products of amino-acid and protein synthesis and that the two schemes of Ehrlich and Genevois are not in contradiction but the two extremes of a common mechanism. Thus it is visualized that yeast will assimilate intact amino-acids if available[12]; but if the balance is disturbed it will synthesize the required carbon skeleton, or keto-acid, and deaminate any surplus amino-acid to get the necessary nitrogen. The deaminated amino-acid and any surplus keto-acid intermediate will then be decarboxylated, reduced and secreted into the medium[13].

My thanks are due to Dr. A. H. Cook for his interest and encouragement during this work.

R. STEVENS

Brewing Industry Research Foundation,
Nutfield, Redhill,
Surrey.

[1] Stevens, R., *J. Inst. Brewing*, **66**, 453 (1960).

[2] SentheShanmuganathan, S., and Elsden, S. R., *Biochem. J.*, **69**, 210 (1958).

[3] SentheShanmuganathan, S., *Biochem. J.*, **74**, 568 (1960).

[4] Castor, J. A. B., and Guymon, J. F., *Science*, **115**, 147 (1952).

[5] Genevois, L., *Brauwissenschaft*, **14**, 52 (1961).

[6] Harold, F. V., Hildebrand, R. P., Morieson, A. S., and Murray, P. J., *J. Inst. Brewing*, **67**, 161 (1961).

[7] Äyräpää, T., *J. Inst. Brewing*, **67**, 262 (1961).

[8] Stevens, R., *J. Inst. Brewing* (in the press).

[9] Kilkenny, B. C., and Hinshelwood, C. N., *Proc. Roy. Soc.* B, **138**, 375 (1951).

[10] Hardy, T. L., Holland, D. C., and Naylor, J. H. C., *Anal. Chem.*, **27**, 971 (1955).

[11] Mathers, A. P., and Schoeneman, R. L., *J. Assoc. Off. Agric. Chem.*, **39**, 834 (1956).

[12] Thorne, R. S. W., *Wallerstein Lab. Commun.*, **13**, 319 (1950).

[13] Hough, J. S., and Stevens, R., *J. Inst. Brewing* (in the press).

Ultra-Violet Absorption Spectra of Proteins in Deuterium Oxide

TAKASHIMA[1] recently reported that when dissolved in deuterium oxide (D_2O) several proteins exhibited a new absorption band at about 265 mμ, in addition to the well-known absorption bands due to aromatic amino-acid residues[2]. The additional band was also found in a solution of clupein in deuterium oxide; this protein contains no aromatic amino-acid residues, and when pure shows no significant absorption in aqueous solution at wave-lengths longer than 250 mμ. The 265 mμ band was also found in deuterium oxide solutions of six of ten amino-acids examined.

Attempts were made here to confirm the appearance of a new electronic absorption band in deuterium oxide as solvent. Lysozyme, human serum albumin and ovalbumin were examined in 90 per cent (approx.) deuterium oxide, hæmoglobin in 95 per cent (approx.) deuterium oxide, and clupein, insulin, histidine and glycine in 99·8 per cent deuterium oxide. In some cases identical dilutions of a suitable aqueous stock solution were prepared in water and in deuterium oxide and examined side by side; in other experiments the proteins or amino-acids were dissolved directly in deuterium oxide. The solutions were examined with manual and recording spectrophotometers and the positions of the fine-structure bands were located by the moving-plate spectrographic method[3]. Difference spectra of protein solutions of equal concentration in water and in deuterium oxide were also determined on the recording spectrophotometer. In all these experiments no trace of a new absorption band at 265 mμ was observed in the solutions in deuterium oxide, and their spectra did not differ in any significant respect from those of the same solutes in aqueous solution. For hæmoglobin the absorption bands due to the hæm groups were also identical, within experimental error, in water and deuterium oxide. The sample of clupein used was slightly contaminated with nuclear material, giving a broad absorption band ($\lambda_{max.}$ *c.* 265 mμ) of moderate intensity when examined at a concentration of about 10 mgm./ml.; this band was present at the same intensity in both solvents.

The results were communicated to Dr. Takashima and he was able to show that his original findings were erroneous, because of absorbing impurities in the sample of deuterium oxide used. In subsequent observations on amino-acids with saturated side-chains dissolved in pure deuterium oxide, he found selective absorption in the 260–300 mμ region, which was later found to be due to the presence of aromatic impurities, probably tyrosine, in the amino-acid samples.

In view of the agreement which has now been demonstrated between the present observations and Dr. Takashima's subsequent findings, it may be concluded that the use of deuterium oxide as a solvent does not in fact result in the appearance of new bands in the ultra-violet absorption spectra of amino-acids and proteins.

G. H. BEAVEN
ELIZABETH BAILEY

Medical Research Council Laboratories,
Hampstead,
London, N.W.3.

---

[1] Takashima, S., *Nature*, 187, 597 (1960).

[2] Beaven, G. H., and Holiday, E. R., in *Advances in Protein Chemistry*, 7, 319 (1952).

[3] Holiday, E. R., *J. Sci. Instr.*, 14, 166 (1937).

Passive Transfer of Contact Sensitivity to Picryl Chloride in Guinea Pigs, with Subcellular Material

THE possibility of passively transferring delayed hypersensitivity in the guinea pig with viable cells from lymph node, spleen and peritoneal exudate was shown by the work of Landsteiner and Chase[1]. Since then, a number of workers have tried to identify some subcellular material responsible for the transfer. Jeter, Tremaine and Seebohm[2] described the passive transfer of contact sensitivity to 2: 4-dinitrochlorobenzene with sonically disrupted peritoneal exudate cells, but Eisen[3] observed that sonic disruption of lymphoid cells abolished their capacity to transfer contact sensitivity to 2: 4-dinitrofluorobenzene.

The following experiments describe the successful passive transfer with disrupted cells of contact sensitivity to picryl chloride in guinea pigs and the partial separation of the components responsible.

Guinea pigs of the Hartley strain were actively sensitized with picrylated guinea pig red cell stromata in Freund's adjuvant, containing tubercle bacilli, followed three weeks later by successive skin paintings with 4 per cent picryl chloride in absolute alcohol, a technique based on that described by Chase[4]. The donors were killed within two weeks of the last skin sensitization. Spleen and lymph node cell suspensions were prepared as described in ref. 5. Cells were disrupted by freezing and thawing seven times in the presence of deoxyribonuclease[7], or sonically disrupted for 5 min. at 25 kc./sec. Homogenates were prepared in a Potter–Elvehjem-type homogenizer for 3 min. at 3,000 rev./min. in chilled buffered sucrose medium[6] from chopped lymph nodes and spleen cell suspension free from red cells[5]. Cell sap and microsome fractions were prepared from tissue homogenized in the sucrose medium and centrifuged at 5,000*g*. The supernatant was centrifuged in a model *L* Spinco ultracentrifuge at 100,000*g* for 2 hr., and the pellet re-suspended in the sucrose medium.

Cell sap was fractionated by chromatography on N,N-diethylaminoethyl cellulose (DEAE cellulose), by the technique in ref. 8, but using stepwise elution. The DEAE cellulose was buffered to *p*H 7·4 and suspended in 0·01 *M* sodium chloride. The cell sap was diluted to the equivalent of 0·01 *M* sodium chloride before being put on the column. The light absorption of the effluent was examined at 260 and 280 mμ with a Unicam spectrophotometer. The effluent was collected in 10-ml. fractions and three consecutive fractions were pooled for injection into one animal.

Materials were administered intravenously except for the microsome fractions and the eluate from chromatographic fractionation of the cell sap, which were injected intraperitoneally. All recipients were skin-tested with 1 per cent picryl chloride in olive oil immediately after transfusion, and were examined 24–48 hr. later. Reactions were graded according to Chase[4]: + + + +, pink usually very slightly elevated; + + +, pink, but either somewhat pale or macular; + +, pale pink; +, faint pink; ±, many faint pink small spots.

Table 1. COMPARISON OF THE TRANSFER OF PICRYL CHLORIDE HYPERSENSITIVITY BY LIVE CELLS AND DISRUPTED CELLS

(*a*) Disrupted by freezing and thawing

No. of cells	Live cells	Disrupted cells
$1·4 \times 10^9$	+ +	+ +
$7·2 \times 10^8$	+ + +	+ + ±
$5·6 \times 10^8$	+ ±	+ +
$5·6 \times 10^8$	+ + +	+
$6·7 \times 10^8$	+ + +	+ +
	Supernatant after centrifuging at 5,000*g*	
$8·4 \times 10^8$		+ + +
$1·2 \times 10^9$		+ +
$1·2 \times 10^9$		+ +
1×10^9		+ +

(*b*) Sonically disrupted

No. of cells	Live cells	Disrupted cells
$1·5 \times 10^9$	+ +	+ ±
$9·0 \times 10^8$	+ +	+ ±
$6·5 \times 10^8$	+ + +	+ +
$5·0 \times 10^8$	+ + +	+ +
$5·2 \times 10^8$	+ + +	+
	Centrifuged at 5,000*g*	
No. of cells	**Pellet**	**Supernate**
$2·1 \times 10^9$	±	+ + ±
$2·4 \times 10^9$	+ ±	+ + +
$1·8 \times 10^9$	±	+ + +
$1·25 \times 10^9$	−	+ + ±

All reactions read 24–48 hr. after transfer.

Table 2. TRANSFER OF PICRYL CHLORIDE HYPERSENSITIVITY WITH CELLS DISRUPTED BY HOMOGENIZATION. COMPARISON OF ACTIVITY IN CELL SAP AND MICROSOME FRACTIONS

Cell sap	Microsomes
+ +	+ + ±
+ +	+ +
+ +	+ +
+ + ±	+ +
+ + +	+ + ±
+ + +	+ + +
+ + +	+ +
+ + ±	+ +

All samples from lymph nodes and spleens of two donors. All reactions read 24–48 hr. after transfer.

A comparison of the transfer of contact sensitivity by live cells and cells disrupted by freezing and thawing or sonically disrupted is shown in Table 1. There was a marked fall in the activity of the preparation on disruption. Nevertheless, considerable activity remained in the supernatant after centrifugation at 5,000*g*. In the case of sonically disrupted cells, the 5,000*g* supernatant contained almost all the activity. On fractionation of the supernatant, after homogenization in sucrose medium, by centrifugation at 100,000 *g*, activity was found in both the cell sap and microsome fractions (Table 2). The activity of the cell sap survived dialysis overnight at 4° C. Preliminary experiments have shown that the active material in the cell sap can be partially separated by chromatography, being retarded on DEAE cellulose at 0·01 *M* sodium chloride, whereas most of the protein (as judged by the absorbance ratio 260/280 mμ) is completely retained at this ionic strength.

I wish to thank Dr. H. J. Rapp for advice, and Mr. G. Kenny for technical assistance.

J. L. TURK

National Institute for Medical Research,
London, N.W.7.

[1] Landsteiner, K., and Chase, M. W., *Proc. N.Y. Soc. Exp. Biol. Med.*, **49**, 688 (1942).

[2] Jeter, W. S., Tremaine, M. M., and Seebohm, P. M., *Proc. N.Y. Soc. Exp. Biol. Med.*, **86**, 251 (1954).

[3] Eisen, H. N., in *Cellular and Humoral Aspects of the Hypersensitive State*, 89 (Paul B. Hoeber, Inc., New York, 1959).

[4] Chase, M. W., *Int. Arch. Allergy*, **3**, 163 (1954).

[5] Turk, J. L., *Int. Arch. Allergy*, **17**, 338 (1960).

[6] Askonas, B. A., *Biochem. J.*, **79**, 33 (1961).

[7] Turk, J. L., *Nature*, **189**, 889 (1961).

[8] Rapp, H. J., Sims, M. R., and Borsos, T., *Proc. N.Y. Soc. Exp. Biol. Med.*, **100**, 733 (1959).

PHYSIOLOGY

Inhibition by Physostigmine of the Increase of Oxygen Consumption induced by Potassium in Muscle

THE immersion of frog muscle in Ringer solution, with a high potassium content, is known to cause a decrease in the resting potential of the fibres accompanied by a large increase in oxygen consumption and heat production of long duration; no mechanical change of muscle is observed in these conditions. A possible cause of this effect was discussed by Hill and Howarth[1], and a hypothesis was suggested according to which the depolarization produced by potassium releases the catabolic processes normally accompanying contraction of muscle. Nevertheless, the whole mechanism is still very obscure.

The experiments to be reported here suggest that some special energy-consuming system, different from the main energy-producing metabolic pathways, is involved in the process. The experiments were carried out on sartorius muscles of the frog *Rana temporaria* during February–April. The muscles were used within 30 min. after dissection. Oxygen consumption was measured at a temperature of 22° C. in a Fenn respirometer. Fig. 1 shows that the increase of oxygen consumption in 18 m*M* potassium Ringer is practically completely inhibited by the addition of 10^{-4} *M* physostigmine. This inhibition is not caused by blocking the energy-producing metabolic system (respiration or glycolysis), because subsequent addition of 3×10^{-5} *M* 2,4-dinitrophenol to the same experimental vessel increases the oxygen consumption again to the same high level as is observed with muscles not treated with physostigmine.

Because physostigmine inhibits cholinesterases in concentrations of about 10^{-6} *M*, different lower concentrations of physostigmine were tested (Table 1). The concentration of 10^{-5} *M* was still effective, but to obtain a pronounced effect it was necessary to soak the muscle for 90 min. in 10^{-5} *M* physostigmine before adding 18 m*M* potassium Ringer solution. This we believe to be due to the slow penetration of physostigmine into the muscle cells. The concentrations inhibiting the potassium effect are distinctly higher than those required for inhibition of cholinesterase in homogenates; however, the conditions in intact cells and in homogenate need not be strictly comparable.

Fenn[2] has reported that sucrose in isotonic concentration causes an increase of oxygen consumption similar to that produced by 18 m*M* potassium[2], but we have found that, unlike the increase induced by potassium, this increase is not inhibited by physostigmine (Table 1). The results suggest that these two processes are due to different mechanisms.

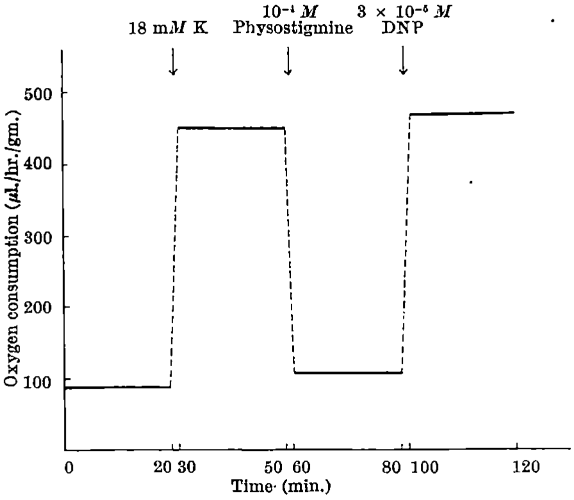


Fig. 1. Influence of 18 m*M* potassium Ringer solution, 10^{-4} *M* physostigmine and 3×10^{-5} *M* 2,4-dinitrophenol on the oxygen consumption of frog's sartorius muscle measured successively in the same experiment. The first part of the graph gives the resting value in normal Ringer

Physostigmine is known to be a potent inhibitor of both cholinesterase and pseudocholinesterase; however, with respect to the well-known role of cholinesterase in various excitation processes, it may be assumed that the inhibitory effect of physostigmine is due to its action on true cholinesterase. It is suggested that the acetylcholine cycle proposed by Nachmansohn[3] for regulation of the permeability of nerve membrane may be activated by high external concentration of potassium. The cyclic splitting and re-synthesis of acetylcholine might be the energy-consuming process which brings about the increase of metabolism.

The possibility that the high rate of metabolism, due to application of potassium, may be associated with active transport of ions was rejected by Hill and Howarth[1]. In agreement with their view, we have found that this metabolic increase is not influenced by ouabain in concentrations inhibiting active transport. The findings of Van der Kloot[4] that physostigmine influences the exchange of ions in frog muscle is not necessarily in conflict with our results, because the effects reported in his paper could be due to the change of permeability. It appears improbable that a high concentration of potassium ions acts by uncoupling the oxidative phosphorylation[5], because the muscle after inhibition by physostigmine is still normally sensitive to 2,4-dinitrophenol.

Table 1. EFFECT OF DIFFERENT SUBSTANCES ON THE OXYGEN CONSUMPTION OF THE FROG'S SARTORIUS MUSCLE. DURATION OF EVERY EXPERIMENT WAS 30 MIN.

Treatment	No. of experiments	Oxygen consumption (μl./hr./gm.)
Normal Ringer solution	6	86 ± 19
Ringer with 18 m*M* potassium	6	437 ± 82
+ 10^{-4} *M* physostigmine	6	112 ± 31
+ 10^{-5} *M* physostigmine	6	226 ± 75
+ 10^{-6} *M* physostigmine	3	441 ± 134
Ringer with 18 m*M* potassium + 10^{-4} *M* physostigmine + 3×10^{-5} *M* 2,4-dinitrophenol	7	483 ± 136
Isotonic sucrose	5	456 ± 108
Isotonic sucrose + 10^{-3} *M* physostigmine	6	427 ± 110

The possibility cannot be neglected, however, that physostigmine may act as membrane stabilizer[6], decreasing the sensitivity of the membrane to the depolarizing action of potassium.

We are indebted to Dr. V. Kubišta for helpful advice and discussion during this work.

IVAN NOVOTNÝ
FRANTIŠEK VYSKOČIL

Zoological Institute,
Charles University,
Prague 2

[1] Hill, A. V., and Howarth, J. V., *Proc. Roy. Soc.*, B, **147**, 21 (1957).
[2] Fenn, W. O., *Amer. J. Physiol.*, **97**, 635 (1931).
[3] Nachmansohn, D., *Chemical and Molecular Basis of Nerve Activity* (Academic Press, London, 1959).
[4] Van der Kloot, W. G., *J. Gen. Physiol.*, **41**, 879 (1958).
[5] Briner, G. P., Simon, S. E., Frater, R., and Tasker, P., *Biochim. Biophys. Acta*, **35**, 485 (1959).
[6] Shanes, A. M., *Pharmacol. Rev.*, **10**, 59 (1958).

Response of the Labellar Hairs of the Blowfly, *Phormia regina* Meigen, to Protein

STRANGWAYS-DIXON[1] has shown that the blowfly *Calliphora* has a cyclic preference for protein. Females ingest relatively more protein than carbohydrate during the early stages of egg growth, but relatively more carbohydrate during yolk formation. They will not reproduce without a protein diet[2].

Receptors responsive to protein have not yet been described. The work presented here was done with another blowfly, *Phormia regina* Meigen, and shows that the chemosensory hairs of the labellum can act as protein receptors. Electro-physiological studies were made on the hairs by recording from the tip in the manner of Hodgson, Lettvin and Roeder[3], and by side-wall recording[4].

There are at least three sizes of chemosensory hairs on the labellum : large hairs situated peripherally, intermediate hairs inside these and very small hairs situated centrally and between the other hairs. There may be a further size of hair slightly larger than the small hairs. Most workers appear to have studied the large peripheral hairs.

When stimulated with hæmoglobin solution (6 gm. in 100 ml. water) or brain-heart infusion (10 gm. in 100 ml. water), the large hairs give records in which two sizes of spike are present (see Fig. 1*A*). Hæmoglobin typically causes the large spike to fire at a frequency of 1–5/sec., whereas the small spike fires at 8–13/sec. Both fire somewhat irregularly. There is some indication that the large spike becomes adapted more quickly than the small spike. Most of the large hairs respond in this fashion, but some at least of the intermediate hairs give a response in which only the smaller spike fires. On the other hand, some of the small hairs give a response in which only the larger spike is firing. It cannot yet be said whether all the intermediate and small hairs give these distinctive responses.

Male *Phormia* have a largely carbohydrate diet. However, electrically their labellar hairs appear to respond identically to those of females towards protein solutions. The virtual absence of a behavioural response to protein must be due not to a difference in the peripheral mechanism but to a different central interpretation of afferent input.

Thus, two nerve fibres seem to be stimulated by protein solutions. The fibre giving the smaller spike has the characteristics—(1) longer time course and (2) greater variability of height of spike when recording from the tip—of the fibre which is now known to be responsive only to water[5]. It fires together with the fibre responsive to salts (*L* fibre) when weak solutions of salt stimulate the hair and it fires together with the fibre responsive to sugar (*S* fibre) when solutions of sugar stimulate the hair (see Fig. 1*B*). In fact, when a mixture of weak salt and sugar solutions stimulates the hair, spikes of three sizes are usually detectable in tip records. These may only become apparent on statistical analysis, as in Fig. 2. The three sizes of spike represent the *L*, *S* and *W*(ater) fibres.

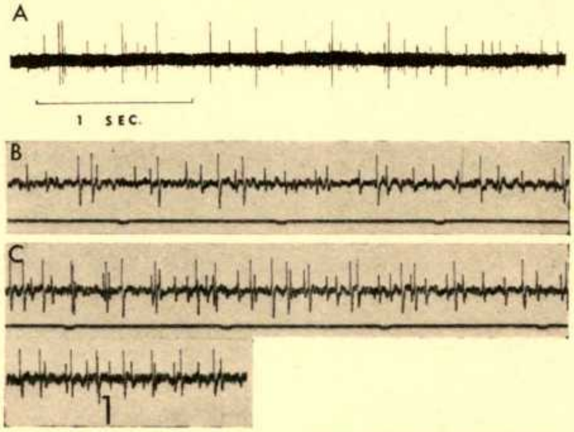


Fig. 1. *A*. Tip recording from a large labellar hair stimulated by hæmoglobin solution. A small and a large spike are present. (Tracing of original record.) *B*, Side-wall recording from a large labellar hair stimulated by 1 *M* fructose solution. Time base, 200 msec. *C*, Side-wall recording from a large labellar hair stimulated by a mixture of 50 per cent 0·1 *M* sucrose and 50 per cent hæmoglobin solution. The pointer indicates the possible firing of one large spike in the refractory period of another. Time base, 200 msec.

Experiments are in progress to determine whether the large spike firing to protein solutions is one of the four nerve fibres known to be present in the chemosensory hairs or a further one. Dethier[6] showed that two fibres, the *L* and the *S*, ascend the shaft of the hair, and a mechanoreceptor fibre terminates somewhere in the socket region. Larsen (personal communication) has since shown that a third fibre ascends the shaft and this is presumably the *W* fibre. Since protein produces an acceptance response, it is unlikely that the protein stimulates the *L* fibre which mediates behavioural rejection. Side-wall recording when stimulating with a mixture of 50 per cent hæmoglobin and 50 per cent 0·1 *M* sucrose gives records which, though difficult to interpret, may indicate that protein has a fibre specific to itself and separate from the *S* fibre. With side-wall recording, the sizes of spikes are (for the

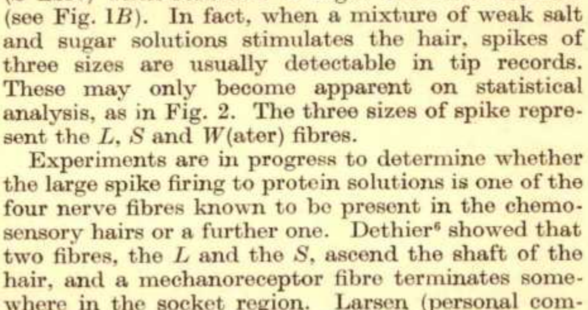


Fig. 2. Histogram showing the frequency distribution of size of spikes obtained from records where a large labellar hair was stimulated with a mixture of 50 per cent 0·1 *M* sodium chloride and 50 per cent 0·1 *M* sucrose solution. The largest spike is the *L* fibre, the smallest the *W* fibre and the middle spike the *S* fibre. (Tip recording)

L and *S* spikes) often identical. In Fig. 1*C* the occurrence of firing by one fibre in or very close to the refractory period of another indicates that three spikes may be present. One of these spikes must be the *S* fibre, another the *W* fibre, and if a third is present it must be responding specifically to the protein. This evidence is tentative, but it is significant that J. R. Larsen (personal communication) has histological evidence of some hairs with four fibres ascending the shaft. These could be the *L*, *S*, *W* and a protein fibre.

This work was supported by a *NATO* Department of Scientific and Industrial research fellowship and grant 6–6015 from the National Science Foundation.

D. I. WALLIS

Department of Zoology,
University of Pennsylvania.

[1] Strangways-Dixon, J., *Nature*, **184**, 2040 (1959).
[2] Fraenkel, G., *J. Exp. Biol.*, **17**, 18 (1940).
[3] Hodgson, E. S., Lettvin, J. Y., and Roeder, K. D., *Science*, **122**, 417 (1955).
[4] Morita, H., and Yamashita, S., *Science*, **130**, 922 (1959).
[5] Mellon, DeF., and Evans, D. R., *Amer. Zool.* (in the press).
[6] Dethier, V. G., *Quart. Rev. Biol.*, **30**, 348 (1955).

Changes in the Membrane during Recurrent Disinhibition of Spinal Motoneurons

IT has been proposed on the basis of pharmacological evidence that recurrent facilitation of spinal motoneurones in the cat is actually a removal of background inhibition, that is, a disinhibition[1]. The results of initial intracellular investigation have been consistent with this hypothesis[2]. It is the purpose of this communication to present direct evidence which lends strong support to the disinhibition hypothesis.

All experiments have been performed on cats the spinal cord of which had been transected at the atlanto-occipital membrane. The carotid arteries were tied, the vertebral arteries clamped, and the preparation was immobilized by intravenous injections of 'Flaxedil' (gallamine triethiodide, American Cyanamid Co.). The experimental arrangement has been described previously[3]. Intracellular recording was by means of glass micropipettes filled with 2·7 *M* potassium chloride or 2 *M* potassium citrate. By utilizing a bridge circuit similar to that used by Frank and Fuortes[4], it was possible to use the same micro-electrode for recording from and for passing current through the cell.

As a result of a recurrent facilitatory volley, a small depolarization (recurrent facilitatory potential) frequently develops in a conditioned motoneurone[2]. Such a recurrent facilitatory potential could be due to either of two events. First, it could be due to an excitatory process such as that which produces the usual excitatory post-synaptic potential. Second, it could be due to removal of inhibition. If background inhibitory activity is one of the factors determining the level of resting potential, which in the motoneurone is less negative than is the inhibitory equilibrium potential[5], then any decrease in the background inhibitory activity would appear as a depolarization. A clear-cut distinction between an excitatory depolarization, accompanied by an increase in conductance, and a depolarization due to removal of inhibition and accompanied by a decrease in conductance, can be drawn by changing the level of membrane potential. An excitatory post-synaptic potential (EPSP) would be decreased by depolarization, while its amplitude usually would not be changed significantly by hyperpolarization[6]. A different situation would prevail with a disinhibitory depolarization. Decreasing the membrane potential would move it further from inhibitory equilibrium, and increase both the effect of background inhibition on the membrane potential[5] and the subsequent recurrent facilitatory potential. Hyperpolarization would cause an opposite effect. Moving the membrane potential closer to the inhibitory equilibrium would decrease the effect of background inhibition on the membrane potential[5] and the recurrent facilitatory potential. If the membrane potential is shifted to the other side of inhibitory equilibrium, background inhibition will cause depolarization of the cell and release from inhibition will cause hyperpolarization; that is, the recurrent facilitatory potential will be reversed. Precisely such a reversal has been obtained in the present experiments.

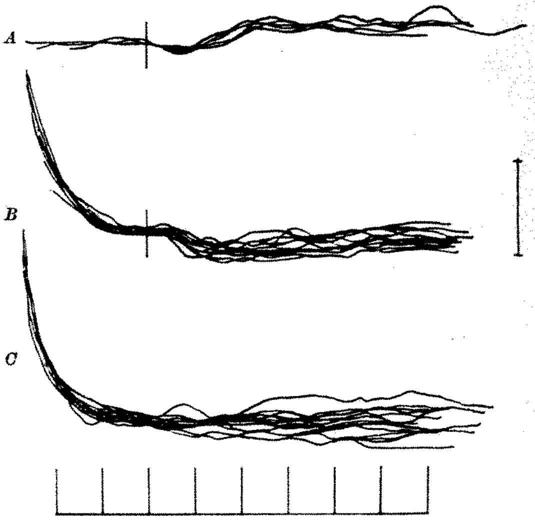


Fig. 1. Effect of hyperpolarizing pulses on recurrent facilitatory potential. The potentials shown in this figure were traced from a number of single frames. Conditioning stimulus delivered at artefact. *A*, Recurrent facilitatory potential alone; *B*, accompanied by hyperpolarizing pulse; *C*, hyperpolarizing pulse alone. Voltage calibration, 10 mV.; time, 10 msec.

Fig. 1 shows the effect of hyperpolarizing pulses on the recurrent facilitatory potential recorded with a 13-megohm potassium chloride electrode in a deep peroneal motoneurone. Superimposed tracings of control recurrent facilitatory potentials are shown in Fig. 1*A*. Such controls were interspersed among other frames, illustrated in Fig. 1*B*, which show the effect of pulses of hyperpolarizing current 100 msec. in duration and $1·2 \times 10^{-8}$ amp. in magnitude on the recurrent facilitatory potential (the latter has been delayed sufficiently so as not to fall on that part of the base-line distorted by the bridge transient). It is clear that the polarity of the recurrent facilitatory potential has been reversed. Fig 1*C* is a control which shows that aside from the bridge transient the base-line, with the current pulse on but conditioning stimulus off, is quite flat.

Similar results have been obtained in a quadriceps cell in which the recurrent facilitatory potential was reversed by a current pulse which also reversed a recurrent inhibitory post-synaptic potential (IPSP). In several other cells, both quadriceps and deep peroneal, electrode polarization negated attempts to reverse the recurrent facilitatory potential. However, even under such conditions the recurrent facilitatory potential was consistently decreased by hyperpolarizing pulses or increased by depolarizing pulses.

These results are inconsistent with the possibility that the recurrent facilitatory potential is produced by a mechanism similar to that which produces the excitatory post-synaptic potential, and completely in accord with the hypothesis that the recurrent facilitatory potential is produced by removal of inhibition. This direct evidence of the nature of the membrane changes confirms the interpretation of the previous pharmacological[1] and intracellular[2] experiments. Disinhibition, therefore, must be considered as a mechanism which contributes, under physiological conditions, to the integrative action of the central nervous system. Parenthetically, these results demonstrate that in the spinal cat both extensor and flexor motoneurones of hind limb muscles are under the influence of tonic inhibitory activity.

This investigation was supported in part by grant *B*–2619 from the Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

VICTOR J. WILSON
PAUL R. BURGESS

The Rockefeller Institute,
New York 21, New York.

[1] Wilson, V. J., Diecke, F. P. J., and Talbot, W. H., *J. Neurophysiol.*, **23**, 659 (1960).

[2] Wilson, V. J., and Burgess, P. R., *Science* (in the press).

[3] Wilson, V. J., *J. Gen. Physiol.*, **42**, 703 (1959).

[4] Frank, K., and Fuortes, M. G. F., *J. Physiol.*, **134**, 451 (1956).

[5] Eccles, J. C., *The Physiology of Nerve Cells* (Johns Hopkins Press, 1957).

[6] Coombs, J. S., Eccles, J. C., and Fatt, P., *J. Physiol.*, **130**, 374 (1955).

Stimulation of the Heart of the Spinal Dog by Phenoxybenzamine

ANTISYMPATHOMIMETIC drugs have been shown to interfere with the inactivation of the sympathetic transmitter substance. Brown and Gillespie reported that the amount of noradrenaline appearing in the splenic venous blood following sympathetic stimulation at frequencies below 30/sec. is increased by phenoxybenzamine or dibenamine, and these authors concluded that the mechanism for the destruction of noradrenaline at the nerve endings is linked to the receptor for this substance and can be inactivated with them[1]. Brown, Davies and Gillespie found that large amounts of noradrenaline appear in the venous blood from both colon and small intestine if the sympathetic nerves were stimulated following the administration of phenoxybenzamine[2]. Millar *et al.* showed that phenoxybenzamine enhances the rise in plasma noradrenaline during hæmorrhagic hypotension in adrenalectomized dogs[3]. Benfey *et al.* found that the urinary excretion of noradrenaline following ganglionic stimulation by acetylcholine is increased by phenoxybenzamine[4]. Hukovic showed that phenoxybenzamine augments the response to stimulation of the atrium of the heart isolated with its sympathetic nerves[5]. Benfey *et al.* reported that phenoxybenzamine elevates the urinary recovery of infused noradrenaline[6]. Martin *et al.* found that chlorpromazine can enhance and prolong the vasoconstrictor and chronotropic actions of adrenaline and noradrenaline[7].

It appears that antisympathomimetic drugs can also release the sympathetic transmitter substance. In the present experiments dogs in pentobarbitone anæsthesia were subjected to a cord transection between C_1 and C_2 and to a bilateral vagotomy. The contractile force of the exposed heart was recorded by a Cushny myocardiograph, the blood pressure was measured from the carotid artery, and artificial respiration was given. Fig. 1 shows that the intravenous injection of 10 mgm./kgm. of phenoxybenzamine (dibenzyline hydrochloride, Smith, Kline and French) briefly depressed and then increased the force of contraction of the heart. The increase in contractility was associated with a rise in heart-rate (from 120 to 180/min.), and there was a small hypertensive effect. 50 min. later, when the heart-rate had dropped slightly, the intravenous injection of 0·5 mgm./kgm. of reserpine ('Serpasil', Ciba) increased the heart-rate further (from 170 to 230/min.). The ganglion-blocking drug pentolinium was originally used in repeated doses of 2·5 mgm./kgm. to counteract an excessive rise in blood pressure that was observed during the manipulation of the spinal cord. In the absence of pentolinium the results were similar, but phenoxybenzamine stimulated cardiac contractility immediately and the chronotropic effect was somewhat greater.

Since it has been found before that in anæsthesia phenoxybenzamine has hypertensive and positive inotropic and chronotropic actions following ganglion-blocking drugs or atropine even in dogs injected with 20 mgm./kgm. of phenoxybenzamine the day before, but not in animals given reserpine or guanethidine 24 hr. earlier[8], it is concluded that the drug releases catecholamines in small amounts. It has also been reported earlier that phenoxybenzamine increases the plasma concentration and urinary excretion of adrenaline and noradrenaline which is associated with a fall in blood pressure[3,4]. However, no rise in the urinary output of the catecholamines was detected when ganglion-blocking drugs or vasopressin eliminated a reflex activation of sympathetic fibres by hypotension[4,6]. Chlorpromazine has recently been

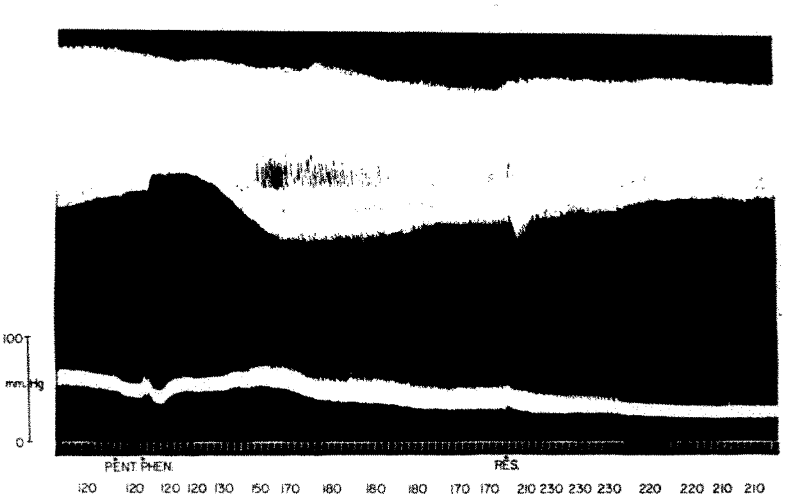


Fig. 1. Cardiac contractility, blood pressure and heart-rate of a dog (10·0 kgm.) subjected to cervical spinal cord transection and vagotomy. Time in minutes. PENT, 2·5 mgm./kgm. pentolinium; PHEN. 10 mgm./kgm. phenoxybenzamine; RES, 0·5 mgm./kgm. reserpine injected intravenously

shown to have hypertensive and positive chronotropic actions in the spinal vagotomized cat[7].

It is suggested that the antisympathomimetic effect (adrenergic blockade) involves both an impaired inactivation and a release of the sympathetic transmitter substance.

This work was supported by a grant from the National Research Council of Canada.

B. G. Benfey
D. R. Varma

Department of Pharmacology,
McGill University,
Montreal.

[1] Brown, G. L., and Gillespie, J. S., *J. Physiol.*, **138**, 81 (1957).

[2] Brown, G. L., Davies, B. N., and Gillespie, J. S., *J. Physiol.*, **143**, 41 (1958).

[3] Millar, R. A., Keener, E. B., and Benfey, B. G., *Brit. J. Pharmacol.*, **14**, 9 (1959).

[4] Benfey, B. G., Ledoux, G., and Melville, K. I., *Brit. J. Pharmacol.*, **14**, 142 (1959).

[5] Hukovic, S., *Brit. J. Pharmacol.*, **14**, 372 (1959).

[6] Benfey, B. G., Ledoux, G., and Segal, M., *Brit. J. Pharmacol.* **14**, 380 (1959).

[7] Martin, W. R., Riehl, J. L., and Unna, K. R., *J. Pharmacol. Exp. Therap.*, **130**, 37 (1960).

[8] Benfey, B. G., *Brit. J. Pharmacol.* (in the press).

HÆMATOLOGY

Large Lymphocytes and the Simonsen Phenomenon

When blood cells from an adult fowl are inoculated on the chorioallantoic membrane of the 12-day-chick embryo, easily visible opaque foci are produced[1,2]. This reaction is thought to be similar to the induction of splenomegaly following intravenous inoculation of adult fowl blood into the chick embryo[3]. The cells in blood responsible for this latter action have been found to be the lymphocytes[4]. Fowls are typical of the avian family in having a high proportion of circulating large lymphocytes, usually about 10 per cent, and, as autoradiographic investigations[5,6] have shown that large lymphocytes and not small can synthesize deoxyribonucleic acid and proliferate, an investigation was made to determine the type of lymphocyte responsible for the induction of lesions on the chorioallantoic membrane. Blood from a group of 25 random fowls was titrated on the chorioallantoic membrane, the number of small and of large lymphocytes in the inoculated blood having been determined. A highly significant correlation (0·1 per cent in Student's t test) of the number of large lymphocytes in the inoculum with the median focal count was demonstrated. The correlation of small lymphocytes with the focal count was not significant ($>$ 5 per cent). In repeated titrations of individual fowls, the focal count was also shown to depend on the number of large lymphocytes in the inoculum and to be quite independent of the number of small lymphocytes.

Quantitative determinations of the number of large lymphocytes required to produce foci were carried out. This was found to be dependent on the number of cells inoculated. Over the average range of inoculum used, about 2,000 large lymphocytes were required to produce one focus. As the inoculum was progressively diluted, more large lymphocytes seemed to be able to produce foci, and as few as 40 were shown to be capable of this action.

As the large lymphocytes were now satisfactorily identified as the competent cells in this phenomenon, the competence of large lymphocytes from other organs was studied, namely spleen and thymus. Thymic large lymphocytes were shown to be virtually incompetent in the production of foci, the small number of foci being easily accounted for by a 2 per cent contamination with blood large lymphocytes, in accordance with Terasaki's results[4]. The relative competence of the large lymphocytes of blood and spleen was assessed by: (1) direct titration on the chorioallantoic membrane; (2) the number of cells required to produce a maximal increase in spleen weight after intravenous inoculation; (3) by passaging cell suspensions of these enlarged embryonic spleens on to further chorioallantoic membranes, and establishing the number of passage cells required to produce foci. By all three criteria, the large lymphocytes from spleen were demonstrated to have about 20 per cent the competence of the large lymphocytes in blood.

This work was supported by research grants from the National Health and Medical Research Council, Canberra, and we are grateful to Sir Macfarlane Burnet for helpful discussions during the course of the investigations.

A. Szenberg
N. L. Warner

Walter and Eliza Hall Institute
of Medical Research,
Melbourne.

[1] Boyer, G. S., *Nature*, **185**, 327 (1960).

[2] Burnet, F. M., and Boyer, G. S., *J. Path. Bact.*, **81**, 141 (1961).

[3] Simonsen, M., *Acta Path. Microbiol. Scand.*, **40**, 480 (1957).

[4] Terasaki, P. I., *J. Embryol. Exp. Morph.*, **7**, 394 (1959).

[5] Everett, N. B., Reinhardt, W. O., and Yoffey, J. M., *Blood*, **15**, 82 (1960).

[6] Schooley, J. C., and Burnam, I., *Blood*, **16**, 433 (1960).

Influence of Serum Factors on the Species-specificity of Tissue Thromboplastin

It is well recognized that a difference in species may influence the time of clotting of recalcified plasma in the presence of tissue extract. Using Quick's one-stage procedure, tissue extract will give a shorter clotting-time with homologous plasma than with plasma from a different vertebrate. Mann and Hurn[1] demonstrated that the specificity of a tissue extract could be eliminated by incubating it with a small amount of homologous serum in the presence of calcium ions before adding the heterologous test plasma. At this time, factor VII (cothromboplastin, S.P.C.A., proconvertin) and factor IX (Christmas factor, P.T.C.) were the only clotting factors known to be present in serum, and as factor IX plays no part in the conversion of prothrombin to thrombin by tissue thromboplastin, Mann and Hurn concluded that the species-specificity was eliminated by the presence of homologous factor VII.

It is now recognized that normal mammalian serum contains another factor which plays a part in the activation of prothrombin, namely, factor X (Stuart Prower factor). The experiments of Mann and Hurn have been extended in the light of this knowledge in order to determine whether the so-called 'cothromboplastin effect' is due to factor VII alone, or whether factor X also influences the reaction.

The tests were carried out in plasma from the South African clawed toad, *Xenopus laevis*, using human brain thromboplastin. Toad plasma was obtained from animals anæsthetized by immersion in 2 per cent urethane solution for 10–15 min. The heart was exposed and, holding the animal in a dorso-ventral position, the ventricle was caused to protrude by pressure on the dorsal surface behind the head. The pericardium was removed and the ventricle punctured with a sharp blade, allowing the blood to drop into a graduated polythene tube containing 3·8 per cent trisodium citrate. Nine volumes of blood were collected into one volume of citrate solution. The blood from six toads was pooled to make a single specimen.

Human brain thromboplastin was prepared by the method of Hjort[2]. The dilution was adjusted to give a satisfactory one-stage thromboplastin time with normal human plasma. Human blood was collected into 3·8 per cent trisodium citrate in the proportion of nine volumes of blood to one of citrate, a siliconed syringe being used. Platelet-poor plasma was obtained from citrated blood by centrifuging for 20 min. in an International clinical centrifuge, model *CL*, with a 7 in. head. Serum free from prothrombin and thrombin was prepared by incubating clotted blood for 5 hr. at 37° C. in a glass container, and was stored after separation at $-20°$ C. for up to one week. Serum free from factor VII was obtained from a patient with a pure congenital deficiency in factor VII. Serum free from factor X was obtained from a patient with a pure deficiency in factor X. Serum from a patient under treatment with phenylindanedrone (phenindione, B.P.) was also tested; this contained 1 per cent of factor VII and 3 per cent of factor X. In all the above sera factor VII was assayed by the method of Dische[3] and factor X by the method of Bachmann *et al.*[4].

All sera were diluted 1 in 10 with normal saline. For the test 0·2 ml. brain thromboplastin was incubated at 37° C. with 0·1 ml. diluted serum and 0·2 ml. 0·025 *M* calcium chloride for 3 min. A stop-watch was started on the addition of 0·2 ml. of plasma, and the clotting-time recorded. The results are shown in Table 1.

Table 1. EFFECT OF DILUTE HOMOLOGOUS SERA ON THE REACTIVITY OF THROMBOPLASTIN AND PLASMA

Thromboplastin	Reagent added	Plasma	Clotting time (sec.)
Human	Saline	Human	12·5
		Toad	61
	Normal human serum 1 in 10	Human	12·5
		Toad	13
	Factor VII deficient human serum 1 in 10	Toad	25
	Factor X deficient human serum 1 in 10	Toad	29
	Phenindione serum 1 in 10	Toad	58

These results indicated that the species-specificity of tissue thromboplastin is dependent on the presence of both factor VII and factor X.

This report was included in a thesis accepted by the University of London for an M.Sc. degree. We wish to thank Dr. Harold Anstall for his advice.

CHRISTINE HAWKEY

Blood Bank,
Massachusetts General Hospital,
Boston.

J. L. STAFFORD

St. George's Hospital,
Hyde Park Corner, London, S.W.1.

[1] Mann, F. D., and Hurn, M. M., *Proc. Soc. Exp. Biol. and Med.*, **79**, 19 (1952).

[2] Hjort, P., *Scand. J. Clin. Lab. Invest.*, **9**, suppl. 27 (1957).

[3] Dische, F., *Brit. J. Hæmat.*, **4**, 201 (1958).

[4] Bachmann, F., Duckert, M., and Koller, F., *Thromb. et Diath. Hæm.*, **2**, 24 (1958).

PATHOLOGY

Induction of Cancer in Suckled and Unsuckled Female Rats

SPONTANEOUS mammary carcinomas are a very rare event in female Sprague–Dawley rats[1], but this type of tumour can be produced easily and rapidly by repeated administration of 3-methylcholanthrene (3-MC) given by stomach tube[2]. The neoplasias are more easily produced in young than in old rats and regress under appropriate hormonal modifications. Ovariectomy, hypophysectomy and critical amounts of œstrogens and gonadotropins are effective means of inhibiting neoplastic proliferations[2,3]. Tumours of this kind are known as hormone-dependent. The hormonal conditions during pregnancy are of great significance in growth of tumour. It has been shown that rat mammary tumours appear more rapidly when 3-MC feeding is followed by pregnancy and the incidence of mammary cancers is reduced if the carcinogen is given during pregnancy. No induction of tumours is observed by giving 3-MC during lactation[4,5]. Also, in mice the influence of pregnancy and lactation on 3-MC induction of mammary tumour has been stressed[6].

The following experiment has been aimed to determine whether a previous life-history of different events (for example, pregnancy, lactation) modifies the subsequent susceptibility of rat mammary tissue to 3-MC tumour induction.

3-MC, 10 mgm. in 1 c.c. sesame oil, was administered by gastric tube 6 days a week for 50 days to female Sprague–Dawley rats. Two groups of rats, intact and ovariectomized, received the first administration at 50 days of age. Three other groups, which underwent treatment at age 310–320 days, were (*a*) virgin females, (*b*) females which had previously reared four litters, (*c*) females which had previously been four times pregnant but never suckled. Breeding had begun at 80 days; henceforth, matings occurred two weeks after weaning of the litters at 21 days. Lactating mothers nursed a total of 20–30 young each. Non-lactating mothers rested about 35 days before being mated again. Breeding was completed before treatment with 3-MC began. Diet (18 per cent protein ration plus tap water *ad libitum*) and environmental conditions were alike for all animals during the experiments. The rats were inspected daily for palpable tumours and observed for 120 days from the last administration of 3-MC. Histological sections were stained with hæmatoxylin–eosin.

3-MC, 10 mgm., given by mouth six times weekly for 50 days to intact 50-day-old rats invariably

Table 1. INFLUENCE OF DIFFERENT HORMONAL CONDITIONS ON INDUCTION OF MAMMARY TUMOUR BY 3-MC IN YOUNG AND OLD FEMALE RATS

Category	No. of rats	Toxic deaths	Rats with tumours	Tumour appearance (days)		
				Range	Median	Mean
Young virgin females	8	—	8/8	43–72	51	52·83 ± 3·50†
Young ovariectomized*	11	4	5/7	53–96	56	70·40 ± 9·50
Old virgin females	8	3	3/5	42–79	—	—
Suckled mothers	8	2	2/6	48–71	—	—
Unsuckled mother†	9	8	0/1	—	—	—

* Ovariectomized at age 42 days † Standard error.

elicited multiple mammary tumours (Table 1). Also it produced no toxic deaths. Unsuckled mothers, subjected to the same treatment, died before tumours could be palpated. Suckled mothers tolerated the carcinogen fairly well and two of six developed tumours. Young, ovariectomized and old, virgin rats had similar mortality-rates. Three rats out of five survivors showed tumours in intact old females. While incidence of tumours was variable, the time for appearance of tumours after 3-MC treatment showed no variation. The only exception was registered for ovariectomized rats, in which induction of tumours was delayed.

Histologically, all observed tumours were recognizable as adenocarcinomas. The only variant was observed in the castrated females, the tumours of which exhibited a sclerotic and cystic appearance. Gland atrophy with occasional proliferating areas featured in the mammary tissue of old rats. The mammary glands of young intact and ovariectomized rats displayed epithelial proliferations and atrophy, respectively.

The experiment described shows that intolerance to 3-MC affects ovariectomized young and unmated old females, while surprisingly, the carcinogen was lethal to all the unsuckled mothers, except one. Despite the high mortality, the results suggest that the breast of rats which lactated and that of unbred old rats is less susceptible to 3-MC mammary cancer induction than that of young unbred or ovariectomized rats. Age or different events in the past reproductive activity of female rats have no influence on the rate of tumour appearance after 3-MC treatment. It seems that mammary epithelium (though atrophic) of old rats retains different reacting capacity to a carcinogenic stimulus (3-MC) in relation to its previous functional activity.

GIULIANO BRIZIARELLI

Vister Research Laboratories,
Casatenovo, Como, Italy.

[1] Huggins, C., Grand, L. C., and Brilliantes, F. P., *Nature*, **189**, 204 (1961).

[2] Huggins, C., Briziarelli, G., and Sutton, H., *J. Exp. Med.*, **100**, 25 (1959).

[3] Huggins, C., and Briziarelli, G., *U.S. Nat. Acad. Sci., Ann. Meeting*, 11 (1959).

[4] Dao, T. L., and Sunderland, H., *J. Nat. Cancer Inst.*, **23**, 567 (1959).

[5] Dao, T. L., Bock, F. G., and Greiner, M. J., *J. Nat. Cancer Inst.*, **25**, 991 (1960).

[6] Marchant, J., *Nature*, **183**, 629 (1959).

Amino-Acid Composition of Serum Mucoprotein in Rheumatic Fever

INTEREST in serum mucoprotein tyrosine levels as one of the most sensitive, albeit non-specific, gauges of the disease process in cases of rheumatic fever suggested the possibility that there might be differences in the amino-acid composition of the serum mucoprotein at a time when serum-levels were significantly elevated.

Mucoproteins from five cases of active rheumatic fever whose mean serum mucoprotein tyrosine level was 14·6 (11·9–16·1) mgm./100 ml. and from three normal children of comparable age and with normal serum mucoprotein tyrosine levels of 3·9 (3·2–4·3) mgm./100 ml. were separated from other serum proteins by their solubility in 1·2 *M* perchloric acid. After precipitation with 5 per cent phosphotungstic acid the mucoprotein was washed with ethanol, dissolved in a minimum of 0·1 *N* sodium hydroxide, and finally hydrolysed *in vacuo* in 6 *N* hydrochloric acid for 22 hr. at 110° C.

Analysis of the acid hydrolysate for amino-acids was carried out on a Beckman/Spinco continuous flow column chromatography apparatus using 150-cm. and 50-cm. columns with standard buffers and temperatures. The mean of the analyses for normal people and for the rheumatic fever cases are shown in Table 1. To facilitate comparison the results are expressed as moles/mole of alanine.

Table 1

	Normal (moles/mole of alanine)	Rheumatic fever (moles/mole of alanine)
Aspartic acid	1·46	1·66
Threonine	1·09	1·25
Serine	0·66	0·82
Glutamic acid	2·07	2·20
Proline	1·00	0·87
Glycine	0·91	0·94
Alanine	1·00	1·00
Valine	0·87	0·84
Methionine	0·16	0·14
*iso*Leucine	0·62	0·71
Leucine	1·23	1·34
Tyrosine	0·57	0·66
Phenylalanine	0·72	0·76
Lysine	1·19	1·15
Histidine	0·35	0·35
Ammonia	3·98	3·32
Arginine	0·57	0·63

Values for tryptophan, glucosamine, and cystine are affected by acid hydrolysis and are not given. The figures suggest no difference in amino-acid composition between serum mucoproteins in health and during acute rheumatic fever.

D. O'BRIEN
C. H. M. WALKER
D. WALKER

Department of Pediatrics,
University of Colorado Medical Center,
4200 East Ninth Avenue,
Denver 20.

Effect of Calf Thymus Histone on Rats bearing Transplantable Acute Myeloid Leukæmia

THE bactericidal activity and the cytotoxic effect of basic proteins, protamines and histones have been investigated by different workers. However, the cytostatic effect of histones on tumour cells *in vitro*[1–3] and *in vivo*[4] deserves more attention. Do histones possess a specific growth regulatory activity as was suggested by Zbarskii[4], are they gene inhibitors as Stedman has outlined in his theory[5], or is the inhibitory action of histones on tumours due only to their high toxicity in the experimented animals ?

According to recent work[1], histones and protamines as very basic proteins are incorporated rapidly into the cells *in vitro*. The presence of such positively charged proteins within the cells produces changes in their structure and metabolism, with the resulting destruction of the treated cells.

In an effort to estimate the cytostatic effect of histones, rats bearing acute myeloid leukæmia[6] were treated with calf thymus histone prepared according to the method described by Butler *et al.*[7].

The acute myeloid leukæmia was transplanted into adult 150-gm. albino rats by intravenous injection of the homogenate from leukæmic rat liver, which was heavily infiltrated with leukæmic leucocytes. Histone was administered in 5 per cent solution in physiological saline in 500 mgm./kgm. doses. The first injection of histone was intraperitoneal and the subsequent ones, if any, were placed in the dorsal subcutis. Three experimental and one control group of rats were used.

In the first group the animals were injected 1 hr. before transplantation of leukæmia. In the second group, histone was injected 3 hr. after transplantation and then on the third and fifth day. The third group was treated with histone on the second and fourth day following transplantation.

All animals in the control group were injected with 1·5 ml. of physiological saline 3 hr. after transplantation, and then on each alternate day following the transplantation.

Sections of liver and spleen of all animals which died during the experiment were examined histologically for the presence of leukæmic infiltrates using the method of direct imprints.

Table 1

Group No.	No. of injected animals	Animals died due to toxicity of histone	Animals died of leukæmia	No. of survivors
I	10	1	9	0
II	15	2	11	2
III	20	4	14	2
Controls	10	0	6	4

The toxicity of calf thymus histone in rats depends on the manner of administration. Thus the LD_{50} was 50 mgm./kgm. for intravenous, 700 mgm./kgm. for intraperitoneal and 1,500 mgm./kgm. for subcutaneous (dorsal subcutis) administration. Therefore for better tolerance all injections following the first intraperitoneal one were subcutaneous.

It follows from the results in Table 1 that the calf thymus histone has no cytostatic effect on rats bearing acute transplantable leukæmia. On the other hand, probably as a result of toxicity, the average survival of all animals treated with histone was less than 30–40 per cent of controls and of transplantation experiments[6].

LUBOMIR HNILICA*
VIKTOR HOLOUBEK†

Cancer Research Institute,
Bratislava, Czechoslovakia.

---

* Present address: Chester Beatty Research Institute, Institute for Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W.3.

† Present address: Virus Laboratory, University of California, Berkeley, California, U.S.A.

[1] Becker, F. F., and Green, H., *Exp. Cell Res.*, **19**, 361 (1960).

[2] Sandritter, W., Fischer, H., Süssenberger, K., and Schiemer, H. G., *Exp. Cell Res.*, **17**, 197 (1959).

[3] Stedman, E., Stedman, E., and Pettigrew, F., *Biochem. J.*, **38**, xxxi (1944).

[4] Zbarskii, I. B., and Perevozhchikova, K. A., *Byull. Ekseptl. Biol. i Med. (U.S.S.R.)*, **38**, 61 (1944).

[5] Stedman, E., and Stedman, E., *Nature*, **166**, 780 (1950); *Phil. Trans. Roy. Soc.*, B, **235**, 565 (1951).

[6] Hlavayova, E., *Neoplasma*, **4**, 10 (1957).

[7] Butler, J. A. V., Davison, P. F., James, D. W. F., and Shooter, K. V., *Biochim. Biophys. Acta*, **13**, 224 (1954).

IMMUNOLOGY

A Relation between the Reactivity of Human Red Cells to Antiglobulin Tests and their Surface Structure

SEVERAL authors[1] have suggested that changes in the immunological reactivity of a cell may be correlated with changes in its surface structure. We present evidence here that human red cells giving a positive antiglobulin reaction show changes in their surfaces demonstrable with the electron microscope.

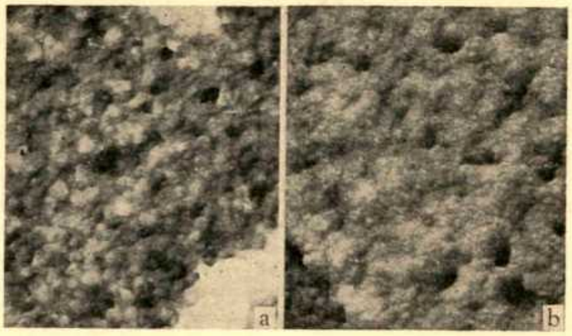


Fig. 1. Surface of (*a*) hæmolysed norma lerythrocyte; (*b*) surface of hæmolysed sensitized erythrocyte from same blood sample. (× 40,000)

Rh(D) positive human red cells of types *A*, *B* and *O* were obtained from normal persons and non-cancer patients, and from cancer patients.

Red cells from normal persons were washed three times in saline and incubated with incomplete anti-*D* serum for 1½ hr. at 37° C. Non-sensitized controls were obtained by incubating cells with serum from group *AB* persons. All cells were then re-washed three times and made up to 30 per cent suspensions in saline, and tested for sensitization by the addition of appropriate dilutions of rabbit anti-human-globulin serum.

Intact hæmoglobin-free red-cell membranes were prepared by the gradual hæmolysis method previously described[2], mounted on carbon-filmed grids, shadowed with chromium and examined with an RCA *EMU–2C* electron microscope.

Almost all the non-sensitized cells from normal persons were found to have the typical structure, with plaques of diameter 200–500 Å. (mean diameters 350–400 Å.) (Fig. 1*a*). After sensitization, the plaque diameters were much larger (500–800 Å.). Of 25 sets of cells from normal persons examined before sensitization, only 2 had a mean plaque diameter greater than 500 Å. ; after sensitization, 16 of the 25 samples had plaques of diameter 500–800 Å. (Fig. 1*b*). In 58 per cent of the specimens from cancer patients, the plaques were of similar size whereas only 30 per cent of non-cancer patients showed plaques in this range. When the results were compared with those of the antiglobulin test, they indicated, in any event in the cancer group, a strong correlation between a positive test and abnormal plaque size.

It appears, therefore, that either natural or experimental modification of the red cell surface, so that cells give a positive antiglobulin test, is associated with enlargement of the surface plaques.

We have recently found that ghosts of red cells can be sensitized to give a positive antiglobulin test ;

Table 1

Cell samples from	No. of cell samples with:			Totals
	Antiglobulin test	Average plaque diameter High	Average plaque diameter Normal	
Non-cancer patients	Weak positive	7	2	9
	Negative	6	28	34
	Total	13	30	43
Cancer patients	Weak positive	25	2	27
	Negative	6	20	26
	Total	31	22	53

such cells show the same surface changes as those sensitized before hæmolysis.

The facts that changes in the surface of red cells of more than half the cancer patients tested could be detected by electron microscopy, and that the change is readily induced by sensitization with incomplete antibody, suggest that many such cases have a similar type of antibody on their red cells. This would confirm our previous results[3] showing that anti-globulin tests on the red cells of cancer patients were significantly more often positive, albeit weakly, than in non-cancer patients and very much more so than in healthy people.

JUDITH E. DAVIS
H. N. GREEN
PAMELA W. TYMMS

Department of Experimental Pathology
and Cancer Research,
University of Leeds.

[1] Liu, C. K., and Evans, R. S., *Proc. Soc. Exp. Biol. and Med.*, **79**, 194 (1952). Rebuck, J. W., *Anat. Rec.*, **115**, 4 (1953). de Vries, A., *et al.*, *Harefuah* (*J. Med. Assoc. Israel*) (Jan. 1, 1957). Goldberg, B., and Green, N., *J. Exp. Med.*, **109** (5), 505 (1959).

[2] Hillier, J., and Hoffmann, J. F., *J. Cell. Comp. Physiol.*, **42**, 203 (1953).

[3] Green, H. N., Wakefield, S., and Littlewood, G., *Brit. Med. J.*, ii, 779 (1957).

HISTOCHEMISTRY

Pigment Bodies of the Spinal Neurones of Some Teleosts

PIGMENT bodies have been observed in the untreated living spinal neurones of the frog *Rana tigrina*[1] and some reptiles[2]. As I could demonstrate corresponding pigment bodies in the spinal neurones of some teleosts, these bodies must no doubt be considered to be of more general importance. The species investigated in this connexion were *Nannostomus beckfordi*, *Phenacogrammus interruptus*, *Corynopoma riisei*, *Otocinclus vestitus*, *Chriopeops goodei*, *Phalloceros caudimaculatus*, *Nanochromis nudiceps* and *Ctenopoma kingsleyi*.

The spinal neurones were studied in the living condition under the phase-contrast microscope and with basic dyes used *super vitam*. In this way the pigment bodies presented themselves as discrete, dirty-yellow to dark-brown bodies. The pigment, a refractile, pale yellow substance, appeared to be confined to certain duplex lipid bodies only. As in reptiles[2], this was not seen in any of the fixed preparations. The living neurones of the teleosts may reveal in addition a diffused pigment in the cytoplasm, like that of *Rana tigrina*[1], and the water snakes[2]. The general coloration of the ganglia in these teleosts and reptiles[2] was not pale yellow, in spite of the pigment in the neurones, unlike that in *Rana tigrina*[1]. As in reptiles[2], the pigment bodies in the teleostean neurones were distributed at random, although they tended occasionally to aggregate. In general, these bodies consisted of spheroids, sub-spheroids and particulates with irregular contours varying in diameter, approximately 1–4μ. Some of these bodies displayed variously distorted binary structures. The cells, which abounded in these pigment bodies, rarely contained lipid particulates.

The formaldehyde-calcium (gelatin and Helly-Zenker-Bouin) paraffin sections of the ganglia, stained with ethanolic sudan black *B*[3], revealed some pigment bodies fairly coloured, while other bodies in the same site were only partly positive. With chrome alum hæmatoxylin the pigment bodies give a strong positive reaction, with periodic acid–Schiff and methyl green/pyronin *G* a faint red coloration, while the reaction to sudan IV was almost negative. The plasmal reaction of these bodies appeared to be negative and the performic acid–Schiff reaction gave erratic results. The fat solvents, ether, chloroform, cold ethanol, cold and hot acetone and hot pyridine, appeared to be resisted by such bodies. The reaction to most of the dyes was negative. A few of them were found to segregate neutral red *super vitam* and coloured somewhat red. A negative result was obtained with Lison's test for carotenoids tried on gelatin sections of formaldehyde-calcium[3] fixed material. Moreover, the other tests for carotenoids (formic acid, hydrochloric acid, sulphuric acid, trichloroacetic acid and Carr–Price reaction), when applied to the living ganglia, reacted negatively with the pigment bodies. As in reptiles[2], on the strength of these results these pigment bodies must probably be considered as lipofuscins. Moreover, accumulations of brownish-black pigment bodies, defying all the histochemical reactions mentioned here, were observed in the surroundings of the entire ganglion.

According to Sharma[2] and correctly in my opinion, these pigment bodies are not the secretory products from the classical Golgi apparatus as claimed by Moussa and Banhawy[4], since one has not found any such apparatus or even a remotely comparable structure either in the living or in the processed young neurones. Identical views are held by Thomas[5], Baker[3], Nath[6] and Malhotra[7]. On the other hand, the pigment bodies appear to have originated as a result of total or partial oxidation of some of the lipid bodies. Cohn[8] also advocated the participation of lipids in the synthesis of pigment. The significance of this oxidation resulting in the pigment accumulation is unknown.

The pale yellow, refractile pigment, which was demonstrated in the cores of certain duplex lipid bodies (*vide supra*) originated in all probability within the interna of the lipid particulates or Golgi bodies of Hirsch in much the same way as described by Baker[3], Lacy, Hirsch and Kanwar[9]. In the neurones of *Iphita limbata* (Hemiptera) a neurosecretory product occurs, which Nayar[10] considers as lipofuscins. At this stage it is unknown whether or not the pigment bodies of reptiles[2] and teleosts could be correlated with the neuro-secretory product of *Iphita limbata*[10]. In any event it is certain that these products bear a close histochemical relationship, inasmuch as both contain lipofuscins.

A. STOLK

Histology Laboratory,
Free University,
Amsterdam.

[1] Gupta, B. L., and Sharma, S. P., *Res. Bull. Panj. Univ.*, **10**, 267 (1959).

[2] Sharma, S. P., *Res. Bull. Panj. Univ.* (N.S.), **11**, 183 (1960); *Experientia*, **17**, 125 (1961); *Res. Bull. Panj. Univ.* (in the press).

[3] Baker, J. R., *Quart. J. Micro. Sci.*, **90**, 293 (1949); *J. Roy. Micro. Soc.*, **74**, 217 (1954); *Sym. Soc. Exp. Biol.*, **10**, 1 (1957).

[4] Moussa, T. A., and Banhawy, M., *J. Roy. Micro. Soc.*, **74**, 162 (1954).

[5] Thomas, O. L., *Quart. J. Micro. Sci.*, **89**, 333 (1948); *J. Comp. Neurol.*, **95**, 73 (1951).

[6] Nath, V., *Nature*, **180**, 967 (1957).

[7] Malhotra, S. K., *Res. Bull. Panj. Univ.* (O.S.), **151**, 179 (1958); *Quart. J. Micro. Sci.*, **100**, 339 (1959).

[8] Cohn, S. A., *J. Histochem.*, **3**, 342 (1955).

[9] Lacy, D., *J. Roy. Micro. Soc.*, **73**, 179 (1953); **74**, 226 (1954). Hirsch, G. C., *Symp. Cell Secretion* (1955). Kanwar, K. C., *Res. Bull. Panj. Univ.*, **10**, 10 (1959); **10**, 99 (1959).

[10] Nayar, K. K., *Biol. Bull.*, **108**, 296 (1955).

Nucleolar Localization of Succinic Dehydrogenase in Normal Rat Hepatic Cells

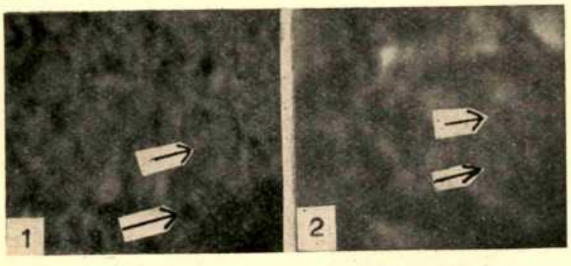
SCRUTINY of the literature[1–6] revealed that the oxidative enzyme, succinic dehydrogenase, is present in the mitochondria, occasionally in the cell membranes[2], of all mammalian cells. The absence of this enzyme in the nuclei including the nucleoli is a very characteristic feature, except in bird erythrocytes, which according to Brachet[7] may be due to the fact that bird erythrocytes have no mitochondria.

This report describes the cytochemical localization of succinic dehydrogenase in the nucleoli of normal rat hepatic cells besides their well-established cytoplasmic counterpart.

Frozen sections (8μ) of rat liver were cut, removing the endogenous substrates for succinic dehydrogenase in ice-cold 0·1 *M* phosphate buffer. Then the sections were incubated for 1 hr. at 37° C. in a medium containing 5 c.c. 0·1 *M* phosphate buffer *p*H 7·6, 5 c.c. 0·1 *M* sodium succinate, 5 c.c. distilled water and 10 mgm. triphenyl tetrazolium chloride. The common controls for confirming specificity of the reactions were applied[4,5,8]. The tetrazolium salt used in this experiment is considered to be very suitable for its high tissue penetrating power and the monochromatic nature of its formazan[9].

In the formazan-stained section, the same hepatic cell was studied with and without a phase-plate under a Zeiss phase-contrast microscope. During each of the two observations, photomicrographs were taken. Then the formazan colour was extracted from the above section by post-fixing it in Carnoy acetic alcohol and restained with pyronin methyl green for staining the nucleoli[10]. From the vernier readings of the mechanical stage recorded previously, pyronin methyl green-stained cells, in the same microscopic field as that of the formazan cells stained with formazan, were studied. A photomicrograph was taken.

It was observed that cytoplasmic formazan granules of rat-liver cells were stained in the usual manner; they showed the topography of mitochondria. The nuclei remained completely unstained and clear without any diffusion of cytoplasmic formazan inside them. In regard to shape, size and position, nucleolar-like formazan deposits of the same formazan-stained cell, with and without a phase-plate, yielded the same results as nucleoli extracted with formazan and stained by pyronin methyl green. The photomicrographs present evidence for nucleolar succinic dehydrogenase, and this unusual finding, besides the well-established cytoplasmic aspects of mitochondrial activity, is quite new.



Figs. 1 and 2. Arrows point to the nucleolar-like formazan deposits of the same formazan-stained hepatic cells without and with phase-contrast, respectively. (× 1,170)

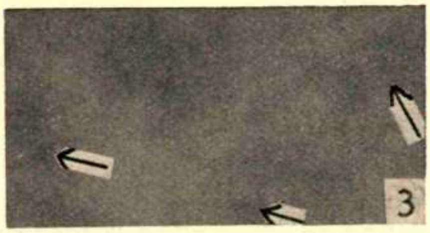


Fig. 3. Arrows point to the formazan-extracted and pyronin methyl green-stained nucleoli of hepatic cells of the same microscopic field (without phase-contrast). (× 1,340)

Thanks are due to Dr. S. K. Brahma, Dr. T. Mukherjee, Mr. S. Bose, Mr. S. Bhattacharya and Mr. N. Saha.

P. DE
R. CHATTERJEE
S. MITRA

Cell Research Department,
Chittaranjan National Cancer Research Centre,
37 S.P. Mookerjee Road, Calcutta.

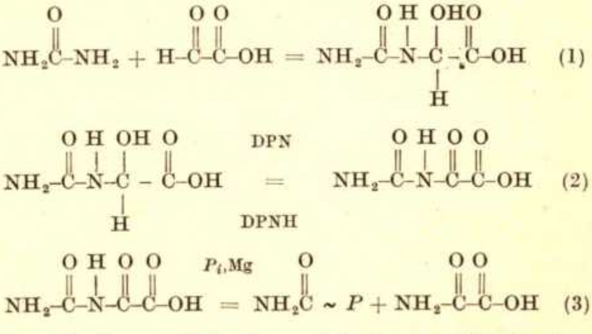
---

[1] Marcuse, P. M., *Lab. Invest.*, **6**, No. 2, 137 (1957).
[2] Monis, B., Nachlas, M. M., and Seligman, A. M., *Cancer Res.*, **12**, No. 6, 1238 (1959).
[3] Nachlas, M. M., Tsou, K. C., De Souza, E., Cheng, C. S., and Seligman, A. M., *J. Histochem. Cytochem.*, **5**, No. 4, 427 (1957).
[4] Ogawa, K., and Zimmerman, H. M., *J. Histochem. Cytochem.*, **7**, No. 5, 342 (1959).
[5] Pearson, B., and Defendi, V., *Cancer Res.*, **15**, No. 9, 593 (1955).
[6] Wachstein, M., and Meisel, E., *J. Biophys. Biochem. Cytol.*, **1**, No. 6, 484 (1955).
[7] Brachet, J., *Biochemical Cytology*, 94 (Academic Press, Inc., New York, 1957).
[8] Friede, R., *J. Histochem. Cytochem.*, **6**, No. 5, 350 (1958).
[9] Inversen, O. H., *Acta Path. et Microbiol. Scand.*, **47**, fasc. 3, 216 (1959).
[10] Gurr, R., *Methods of Analytical Histology and Histochemistry*, 157 (Leonard Hill (Books), Ltd., London, 1958).

MICROBIOLOGY

Phosphate-dependent Degradation of Urea

Jones and Lipmann[1] recently proposed a direct mechanism for the phosphorolysis of urea. We have found that urea is phosphorolytically cleaved by extracts of *Streptococcus allantoicus*. However, the urea moiety is indirectly decomposed as made evident by the requirement for glyoxylate. Co-factors for the following scheme of urea degradation in which glyoxylurea is an intermediate are diphosphopyridine nucleotide (DPN), magnesium, and phosphate or arsenate:



$$\mathrm{NH_2\overset{O}{\overset{\|}{C}}{-}NH_2 + H{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OH = NH_2{-}\overset{O}{\overset{\|}{C}}{-}\overset{H}{\overset{|}{N}}{-}\underset{H}{\underset{|}{\overset{OH}{\overset{|}{C}}}}{-}\overset{O}{\overset{\|}{C}}{-}OH} \quad (1)$$

$$\mathrm{NH_2{-}\overset{O}{\overset{\|}{C}}{-}\overset{H}{\overset{|}{N}}{-}\underset{H}{\underset{|}{\overset{OH}{\overset{|}{C}}}}-\overset{O}{\overset{\|}{C}}{-}OH \overset{DPN}{\underset{DPNH}{=}} NH_2{-}\overset{O}{\overset{\|}{C}}{-}\overset{H}{\overset{|}{N}}{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OH} \quad (2)$$

$$\mathrm{NH_2{-}\overset{O}{\overset{\|}{C}}{-}\overset{H}{\overset{|}{N}}{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OH \overset{\mathit{P_i},Mg}{=} NH_2\overset{O}{\overset{\|}{C}} \sim \mathit{P} + NH_2{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OH} \quad (3)$$

Oxamic transcarbamylase catalysing reaction (3) has been previously described by Valentine and Wolfe[2].

Procedures used for growth of *S. allantoicus* and preparation of cell-free extracts have been described previously[2,3]. Degradation of urea was measured under the following conditions: a suitable amount of an enzyme fraction[2] was incubated with 100 μmoles

Table 1. CO-FACTORS OF UREA DEGRADATION

Tube No.	Constituent omitted	Oxamate formed (μmoles)
1	Glyoxylate	0·0
2	Enzyme*	1·5
3	Urea	0·0
4	Arsenate	1·7
5	DPN	0·0
6	$MgSO_4$	4·9
7	None	14·1

* A dialysed enzyme fraction precipitated by ammonium sulphate (ref. 2) was used, 1·1 mgm. of protein being added per tube.

sodium glyoxylate (Sigma), 100 µmoles urea, 40 µmoles potassium arsenate (*p*H 7·5), 1 µmole DPN, 5 µmoles magnesium chloride, and water to a final volume of 1 ml. Reaction-time was 1 hr. at 30°. Oxamic acid, formed in the series of reactions mentioned above, was measured in the manner of acetamide in the hydroxamate method of Lipmann and Tuttle[4]. 'Dowex' chromatography of oxamic acid was carried out using 'Dowex 1–*X*8' (formate). Glyoxylic acid and glyoxylurea were estimated by the method of Young and Conway[5]; protein was determined by the method of Lowry *et al.*[6]; urea was determined according to the method of Archibald[7].

Co-factor requirements for urea degradation are shown in Table 1. The extract used in these experiments also contains enzymes which cleave glyoxylate. Products of this pathway (to be reported elsewhere) react in the assay of Lipmann and Tuttle[4] but contain no nitrogen and can be distinguished from oxamate. In the experiment described in Table 1 this blank amounted to 4 µmoles per tube and has been subtracted. 100 µmoles of methylurea, ethylurea, and thiourea, respectively, were tested as substitutes for urea but were found to be inactive, being below or near the blank value for the omission of urea. The role of arsenate and phosphate in oxamate formation from urea and glyoxylate is shown in Fig. 1. 2 µmoles of potassium arsenate catalysed the formation of 14 µmoles of oxamate in this reaction. Higher concentrations of inorganic phosphate (P_i) gave similar results. Carbamyl phosphate formed in this reaction was measured by coupling with the ornithine transcarbamylase system as reported previously[3]. Fig. 2 is an elution diagram obtained by 'Dowex' chromatography of the compound formed enzymically in the standard reaction mixture. The elution peak of the enzymically synthesized compound corresponds to that of potassium oxamate.

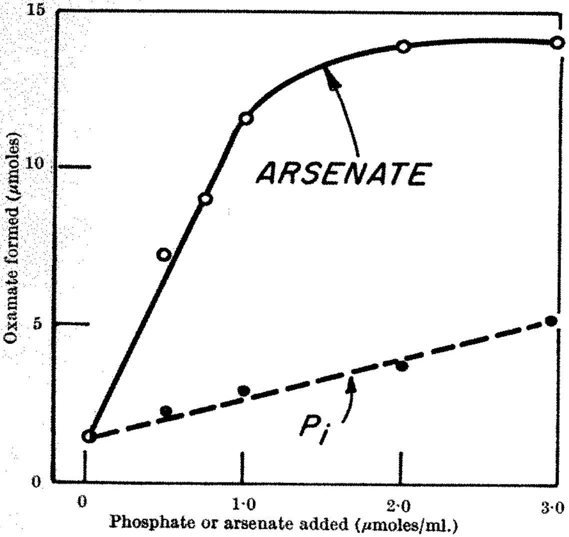


Fig. 1. Arsenate or phosphate requirement for oxamate formation from urea and glyoxylate. Standard reaction mixture

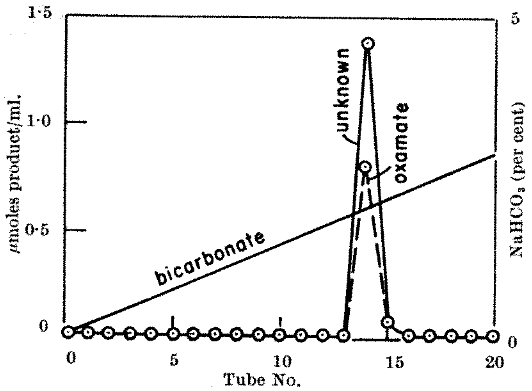


Fig. 2. 'Dowex 1-*X*8' (formate) elution diagram of potassium oxamate and the product formed enzymically from urea and glyoxylate

Energy derived from the urea moieties of allantoin is utilized by *Streptococcus allantoicus* for growth. It was of interest to see if this organism could obtain energy directly from urea. No direct phosphorolytic cleavage of urea was observed; rather, urea combined with an aldehyde moiety and was degraded indirectly via glyoxylurea and carbamyl oxamic acid to carbamyl phosphate and oxamic acid.

This investigation was sponsored by the National Science Foundation grant 6453.

R. C. VALENTINE
R. S. WOLFE

Department of Microbiology,
University of Illinois,
Urbana, Illinois.

[1] Jones, Mary Ellen, and Lipmann, Fritz, *Proc. U.S. Nat. Acad. Sci.*, **46**, 1194 (1960).

[2] Valentine, R. C., and Wolfe, R. S., *Biochem. and Biophys. Res. Comm.*, **2**, 384 (1960).

[3] Valentine, R. C., and Wolfe, R. S., *Biochim. Biophys. Acta*, **45**, 389 (1960).

[4] Lipmann, F., and Tuttle, L. C., *J. Biol. Chem.*, **159**, 21 (1945).

[5] Young, E. G., and Conway, C. F., *J. Biol. Chem.*, **142**, 839 (1942).

[6] Lowry, O. H., Rosebrough, J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

[7] Archibald, R. M., *J. Biol. Chem.*, **156**, 121 (1944).

Use of Cellulose Acetate Membranes for detecting Penicillinase-producing Organisms

DURING investigations into the gain or loss of penicillinase in cultures of staphylococci[1] there was a need for a simple method of screening these so as to be able to detect small numbers of penicillinase positive or negative organisms in mixed cultures. In addition, in the study of hospital cross-infection a quick reliable method was required for investigating many different strains of staphylococci for penicillinase production. A number of chemical and microbiological methods for detecting the ability of organisms to destroy penicillin have been described[2-7]. However, none of the methods in common use is really adapted for the study of mixed penicillinase-positive and negative populations on the surface of solid media, nor is it easy to use them so as to detect a few colonies of one type in the presence of a predominant growth of the other. One of the most useful diagnostic methods for pour plates is that described by Manson, Pollock and Tridgell[8]. We have modified their method by pouring agar containing penicillin and Andrade's indicator gently over colonies growing on the surface of agar plates, but under these conditions the method is not so sensitive nor so reliable as when deep cultures are used.

The method described here is suitable for use with surface cultures ; it can be used entirely as a chemical method or can be combined with microbiological confirmation showing that microbiologically active penicillin has disappeared. Essential features of the technique are as follows : Cultures were inoculated on to the surface of an 8-cm. cellulose acetate membrane (Oxo, Ltd.) previously laid on a nutrient agar plate. The inoculum was usually 0·1 ml. of a suitably diluted culture which was spread on the membrane with a glass spreader so as to give 100–200 colonies per membrane. Inoculation could also be made by means of Lederberg's velvet pad technique[9] from master plates containing suitably spaced colonies. In the examination of many strains of bacteria, as required in the investigation of air flora, each strain was inoculated on to one square of the grid marking of membranes which are supplied already marked. Inoculated membranes were then incubated overnight, though when the inoculum was replicated on to the membrane incubation for a few hours was adequate. After incubation the membrane was removed with forceps and placed gently on to three Whatman No. 1 filter papers which were previously moistened with 3–4 ml. of a solution of penicillin *G* containing 25,000 units per ml. with Andrade's indicator (2·0 per cent) contained in a Petri dish. After quite short periods of incubation, often as little as a few minutes, penicillinase-producing colonies developed a pink colour which slowly spread into the filter paper but did not stain the surrounding membrane to a great extent. Usually 2-hr. incubation was used in order to detect weak penicillinase producers.

Penicillinase production could also be induced by exposing cultures to low concentrations of a powerful inducer such as methicillin ('Celbenin')[10]. This could be done either by placing the membrane with its colonies on pads soaked in a solution of methicillin (0·5 μgm. per ml.) and incubating for about 2 hr. or by placing the membrane on a nutrient agar plate containing 0·1–0·5 μgm./ml. of methicillin and then inoculating it and incubating overnight before transferring it to the pad containing strong penicillin *G* solution.

The method could be combined with a microbiological method by the following variation. The membrane, instead of being placed on the surface of a nutrient agar plate, was placed on the surface of a plate containing 0·01–0·05 μgm./ml. of penicillin *G* in nutrient agar and then inoculated. After incubation the membranes were removed and tested for penicillinase as described above. The area on the plates previously occupied by the membranes could now be inoculated by means of a velvet pad loaded with a confluent growth of *Sarcina lutea* known to be highly sensitive to penicillin. If penicillin had been destroyed during the growth of the test organisms on the membrane, then the *Sarcina lutea* grew on the area below the colonies.

If a mixed culture was diluted sufficiently to give single colonies, it was possible to detect which colonies had destroyed penicillin. This, taken together with the production of penicilloic acid as indicated by the pink colour given by Andrade's indicator, made it easy to confirm in an experiment whether a group of colonies in a culture were capable of destroying penicillin and if so whether they did it by means of penicillinase or by some other mechanism.

R. KNOX
J. T. SMITH

Department of Bacteriology,
Guy's Hospital Medical School,
London Bridge, S.E.1.

[1] Knox, R., *Brit. Med. J.*, ii, 690 (1960).
[2] Abraham, E. P., and Chain, E., *Nature*, **146**, 837 (1940).
[3] Harper, G. J., *Lancet*, ii, 569 (1943).
[4] Gots, J. S., *Science*, **102**, 309 (1945).
[5] Henry, R. J., and Housewright, R. D., *J. Biol. Chem.*, **167**, 559 (1947).
[6] Boxer, G. E., and Everett, P. M., *Anal. Chem.*, **21**, 670 (1949).
[7] Penau, H., Philippe, J., and Benoist, D., *Ann. Pharm. Franc.*, **9**, 419 (1951).
[8] Manson, E. E. D., Pollock, M. R., and Tridgell, E. J., *J. Gen. Microbiol.*, **11**, 493 (1954).
[9] Lederberg, J., and Lederberg, E. M., *J. Bact.*, **63**, 399 (1952).
[10] Robinson, G. N., Stevens, S., Batchelor, R. F., Cameron-Wood, J., and Chain, E. B., *Lancet*, ii, 564 (1960).

BIOLOGY

A Hypodermic Probe using Fibre Optics

It has long been the desire of physicians, physiologists, and biophysicists alike to be able to observe, microscopically, living human tissue in its original environment. Numerous ingenious approaches to the problem have been made, and there are few organs of the experimental animal that have not been subjected to transillumination and microscopic examination in the living state, either *in situ* or externally. However, in spite of marked advances in these various techniques, none has proved suitable for the microscopic examination of human tissue *in vivo* at any depth.

If an ideal system is to be developed for this application, its resolving power should approach the average wave-length of visible light in order to permit observation of the many structures, such as striations of the muscle, which are only a little greater than one micron in size. It is also important that such a system should transmit all visible wave-lengths without selective absorption, thus permitting the use of the standard vital staining techniques at present used to improve the contrast between tissues of otherwise similar optical properties. In addition, the system should permit magnification adequate to utilize fully the advantages of such high resolution and colour reproduction.

Recent advances in the field of fibre optics[1,2] have made feasible the development of such a system, incorporated in a hypodermic probe. Theoretical and experimental work on the properties of glass fibres of very small diameter[3] has shown that it is possible to use fibre bundles, containing cores as small as 1μ in diameter, to transmit images in colour over considerable lengths. Such a fibre bundle, 0·5 mm. in diameter, would contain approximately 200,000 fibres, each capable independently of conveying light along its length, and would, under magnification, give a resolution element of about 1μ.

A distinct advantage to be gained from the use of a fibre optical, as compared to a conventional optical, system in such an application is that it obviates the necessity of introducing a lens system into the tissue under observation. In this instance it is far more satisfactory to place the entrance end of the fibre bundle in contact with the object under examination and illuminate the object either by 'trans-illumination' or 'vertical illumination' using an externally placed light source.

Fig. 1*a* illustrates diagrammatically a probe for the examination of human muscle tissue *in situ*, using a vertical illumination system. Two other methods of illumination are diagrammatically illustrated in Figs. 1*b* and 1*c*. Fig. 2 shows the prototype instrument constructed for preliminary experimentation. This instrument consists of a fused fibre bundle of 0·064 in. diameter and 1·5 in. long comprised of cores of 0·001 in. diameter contained within a standard 15-gauge hypodermic needle. To protect the fibre bundle against breakage during insertion, it is normally retracted into the hypodermic needle. In order to observe the muscle tissue, the bundle is extended beyond the needle, once inserted, and brought into contact with the tissue. This movement within the needle is controlled by a trigger. The light source and condensing system are located at right angles to the optical axis of the system, and the fibre bundle is illuminated by a perforated reflector. The image cast on the end of the fibre bundle is viewed through the perforation by a low-power microscopic system.

Fig. 3 shows a photograph taken through the instrument while in use to observe blood vessels in frog mesentery using a trans-illumination system. When vertical illumination was used with this instrument the image contrast was found to be low due to back-scattering and reflexion.

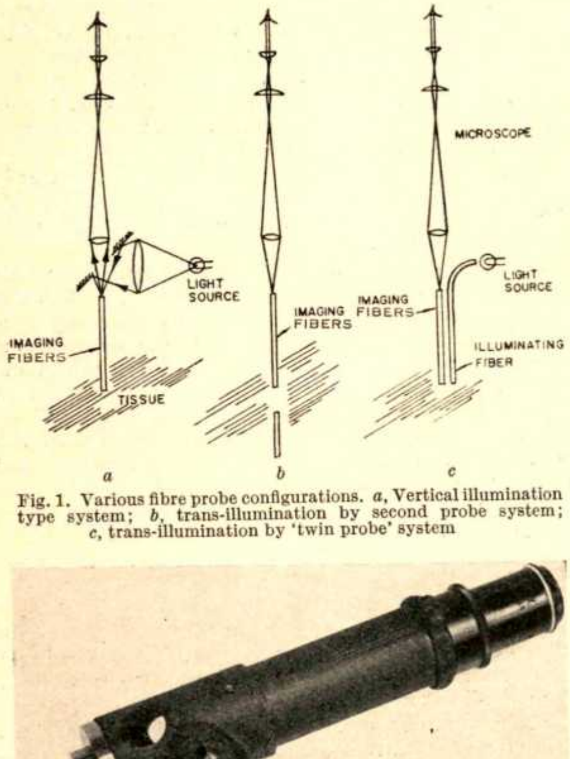


Fig. 1. Various fibre probe configurations. *a*, Vertical illumination type system; *b*, trans-illumination by second probe system; *c*, trans-illumination by 'twin probe' system

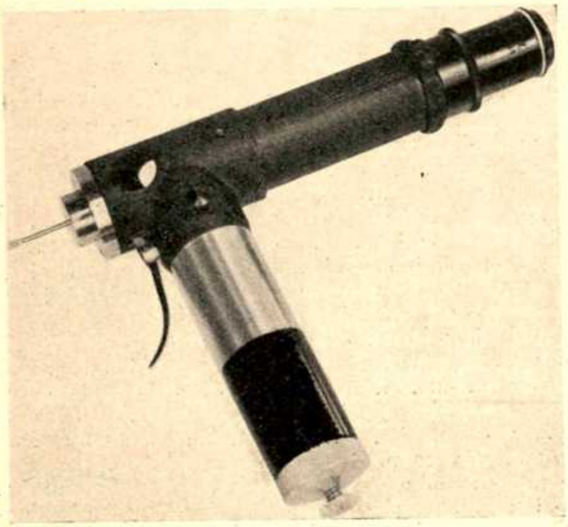


Fig. 2. Protot ype hypodermic fibre optics probe

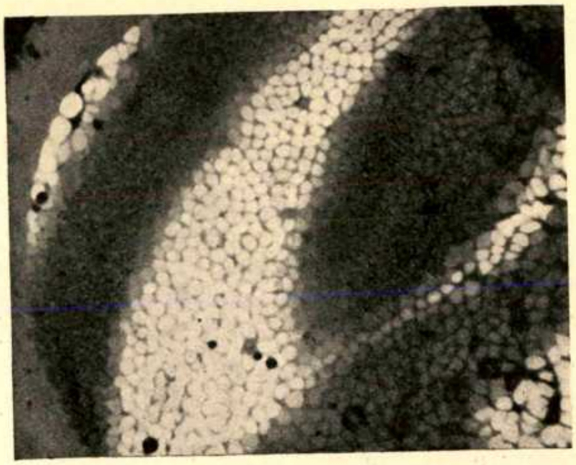


Fig. 3. A view of the arteries in the mesentery of a living frog taken by trans-illumination

It is interesting to note that, although the average size of an individual blood corpuscle in the circulatory system of the frog is the same order of magnitude as the fibre diameter, the motion past the end of the fibre probe was clearly visible due to refractive index changes varying the illumination condition of the fibres.

The results of preliminary experiments with this prototype instrument indicate numerous potential applications. Further work along these lines would allow a complete review of morphological relationships to be undertaken in the living human being under normal and pathological conditions. It would be possible, for example, to conduct biopsies on suspect nodules without incision or excision. Similarly, living bone marrow studies and liver biopsies could be performed easily. The optical system of the probe could be utilized as a carrier for micro-electrodes or micropipettes, allowing measurement of electrical activity of living cells under direct observation, for example in the brain or muscles, and permitting the injection of drugs or vital stains. Clinically, the optical probe could be used by the neurosurgeon to determine his position in the brain from the local architectonics under visualization in preparation for obliterative chemosurgery, the obliterative chemicals being injected through the same probe. Such an optical system would be compatible with near ultra-violet light transmission and could be used for the investigation of natural or induced fluorescent phenomena in the body.

Further work is in progress to produce fibre optics probes capable of 1μ resolution. These probes will be designed to operate under conditions of vertical illumination or transillumination and with visible, near ultra-violet, and polarized light.

DAVID F. CAPELLARO
NARINDER S. KAPANY

Optics Technology, Inc.,
248 Harbor Boulevard, Belmont, California.

CHARLES LONG

Highland View Hospital,
3901 Ireland Drive, Cleveland 22, Ohio.

[1] Kapany, N. S., Appendix *N*, *Concepts of Classical Optics* by John Strong, 553 (W. H. Freeman and Co., San Francisco, 1958).

[2] Kapany, N. S., *Nature*, **184**, 881 (1959).

[3] Kapany, N. S., and Burke, J. J., *J. Opt. Soc. Amer.* (in the press).

Seasonal Fluctuations and Distribution of Nitrite in a Tropical West African Estuary

VERY little is known about the distribution and seasonal fluctuations of inorganic nitrogen in West African estuaries. I have given[1] some preliminary figures based on a series of spot analyses and found that in the Sierra Leone River estuary there are both horizontal and vertical gradients in the concentrations of nitrite, nitrate and ammonia, with the higher values in the upstream direction and with increasing depth. The concentration of nitrite was found to vary from 8 to 4 mgm. nitrogen/m.^{3}, nitrate from 141 to 74 mgm. nitrogen/m.^{3} and ammonia from 22 to 10 mgm. nitrogen/m.^{3}—samples taken over a period of only 3 months were, however, examined.

The present investigation was carried out in the Sierra Leone River estuary over a period of one year, using the Griess–Ilosva method for determining nitrite nitrogen as modified by Orr[2] and Wattenberg[3]. Only the nitrite content of the estuarine water was determined in this investigation, but it is hoped that later it will be possible to study the seasonal changes and distribution of both nitrate and ammonia in order to throw some light on the nitrogen cycle. It is probable that fluctuations in nitrogen and phosphorus may, with other factors such as the amount of effective light, be responsible for the phytoplankton 'bloom' which has been shown by Bainbridge[4] to occur during the period January–July. This 'bloom' was also found by me[5] and by Bainbridge[6] to coincide with an increase in the fat content of the local shad, *Ethmalosa dorsalis* (C. & V.), which is caught in the estuary in fairly large numbers.

Fig. 1 shows the average concentration of nitrite in the surface water for the central region of the estuary during the year 1960. There is a marked seasonal fluctuation, with the higher values occurring in the wet season (June–November). The maximum concentration of nitrite and minimum salinity do not, however, fall within the same month; this suggests that nitrite accumulates in the drainage area during the dry season (October–May) and is removed at the beginning of the wet season. The increment to the nitrite content of the estuary through the direct action of rainfall was not measured; but it may be appreciable since large quantities of dust of Sahara origin are removed from the atmosphere by the convectional rains in June and early July.

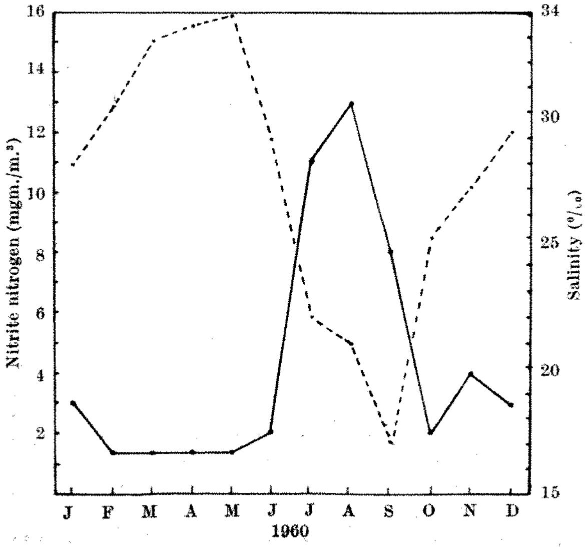


Fig. 1. Seasonal fluctuation of nitrite in the surface water for the central region of the Sierra Leone River Estuary. Nitrite, ——; salinity, - - - -

Nitrite values for the feeder streams are also high in the wet season—a fact which suggests that the high values for nitrite in the estuarine water are not entirely due to increased turbulence as a result of the larger volume of fresh-water entering the upper reaches at this time of the year. I[7], and also Longhurst[8], have shown that the bottom deposits in the central region of the estuary are largely composed of muds with a relatively high organic content, whereas in the upper reaches and mouth the deposits consist of sand or gravel. From this distribution of the organic material in the estuary, it would be expected that vertical mixing should result in higher nitrite values in the central reaches rather than at the two ends, since the suspended material in the central reaches would contain a higher proportion of organic matter brought up from the bottom. That this is actually the case may be seen from Table 1, although the amount of nitrite contributed by the suspended detritus is clearly less than that from the feeder streams.

Table 1. CONCENTRATION OF NITRITE IN THE ESTUARY WATER

Range of salinity (gm. per 1,000 gm.)	Average nitrite nitrogen (mgm./m.^{3})	No. of observations
0–15	8	15
16–20	10	13
21–25	10	26
26–30	4	73
> 30	1	27

Values for nitrite obtained during the flood and ebb at springs and neaps differed by no more than 1 mgm. nitrogen/m.^{3} during the dry season and 3 mgm. nitrogen/m.^{3} during the wet season. The horizontal gradient was most marked in the wet season; in July, the average value for nitrite at the mouth was 7 mgm. nitrogen/m.^{3} and 18 miles upstream 13 mgm. nitrogen/m.^{3}. During the greater part of the dry season, however, nitrite appears to be fairly evenly distributed in the surface water throughout the length of the estuary.

J. C. D. WATTS

Fisheries Development and
Research Unit,
Freetown.

[1] Watts, J. C. D., *Bull. IFAN*, **20**, A, 3 (1958).

[2] Orr, A. P., *J. Mar. Biol. Assoc.*, **14** (1926).

[3] Wattenberg, H., *Ann. d. Hydrogr.*, **59** (1931).

[4] Bainbridge, V., *Col. Off. Fish. Pub.*, 13 (H.M.S.O., 1960).

[5] Watts, J. C. D., *Bull. IFAN*, **19**, A, 2 (1957).

[6] Bainbridge, V., *Nature*, **179**, 874 (1955).

[7] Watts, J. C. D., *Bull. IFAN*, **19**, A, 3 (1957).

[8] Longhurst, A. R., *Col. Off. Fish. Pub.*, **11** (H.M.S.O., 1958).

Diurnal Rhythm in Cave Crayfish

NUMEROUS investigations have suggested persistent diurnal physiological rhythmicity to be the rule among organisms, inhabiting the natural day–night environment. However, the only report known to me of results of a study of an animal fully removed for innumerable generations from such daily changes in light and temperature, the case of the cave crayfish, *Orconectes pellucidus* (Tellkampf), clearly established that these possess no difference in activity between day-time and night-time when kept under controlled conditions in the laboratory[1].

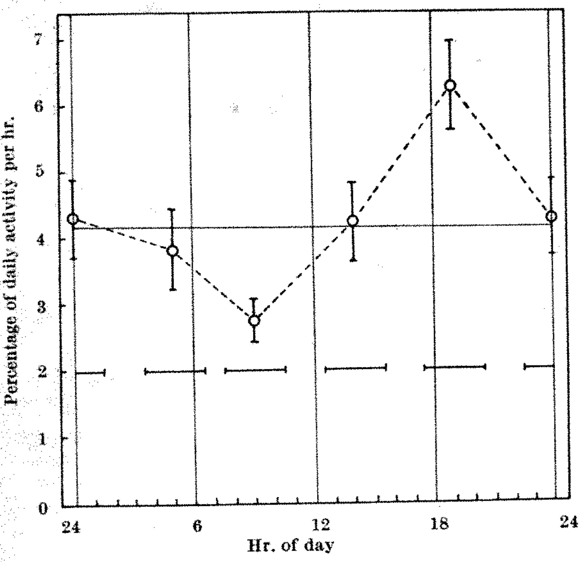


Fig. 1. Relation between the percentage of daily activity and hour of day for 3-hr. periods shown by the horizontal bars. Standard errors of means are indicated

However, a re-examination of the published data[1], in the light of recent advances in our knowledge of persistent diurnal rhythmicity in metabolism, has indicated a statistically significant ($P < 0\cdot001$), 24-hr. rhythm of activity to be present (Fig. 1) with minimum activity about 9 a.m. and maximum about 7 p.m. Activity from 8 to 10 a.m. was less than half that from 6 to 8 p.m. This relationship was found on reconsideration of all the published data for complete days in the report already mentioned for animals kept in constant darkness. There was also a statistically significant daily rhythm in standard deviation of hourly activity values.

The crayfishes involved were collected from River Styx, Mammoth Cave, Kentucky, in March 1935 and November 1936. The cave is presumed to have been formed beginning in the late Pliocene or early Pleistocene, in other words, 1–4 million years ago. The crayfishes are highly adapted to cavernicolous life. They are pigmentless and have no eyes. Young are carried on the pleopods, reportedly chiefly during winters, thus implying an annual reproductive rhythm, despite essentially no annual temperature change. Prof. Orlando Park informs me that his temperature measurements of River Styx at various times of the year during the past 25 years have varied within the range 14 ± 1° C. with no suggestion of an annual cycle.

The specific form and phase relations (times of maximum and minimum) of the diurnal fluctuation discovered resemble remarkably that of oxidative metabolism in the incubating chick egg[2], are essentially the mirror image *in toto* of the diurnal metabolic rhythm of the fiddler crab and, treating a.m. and p.m. periods independently, are related directly or as a mirror-image to the daily cycle of potato plants[3] and numerous other organisms investigated so far. These metabolic rhythms have been established to be extrinsic in character, and phase-locked to the Earth's rotation, through demonstration of day-by-day modifications, correlated with aperiodic, weather-associated fluctuations in subtle geophysical factors[4] in what had formerly been deemed conditions constant for the organisms.

This discovery of such a diurnal rhythm, especially with its particular characteristics, in an ancient cavernicolous species long shielded from any diurnal light and temperature changes gives strong additional support to the concept of the universal presence of an extrinsic 24-hr. rhythmicity timed by the Earth's rotation, available for use in turn, as the timer for specific, adaptive diurnal patterns of surface-dwelling species, phased by light or temperature cycles, or adaptively dissociated from specific, overt physiological patterns in cavernicolous species such as the cave crayfish[1].

It seems probable that the reported annual reproductive cycle reflects an extrinsic annual metabolic rhythm such as has been reported even in unvarying light and temperature[5].

Frank A. Brown

Department of Biological Sciences,
Northwestern University, Evanston, Illinois.

[1] Park, O., Roberts, T. W., and Harris, S. J., *Amer. Nat.*, **75**, 154 (1941).

[2] Barnwell, F. H., *Proc. Soc. Exp. Biol. and Med.*, **105**, 312 (1960).

[3] Brown, jun., F. A., *Science*, **130**, 1535 (1959).

[4] Brown, jun., F. A., *Cold Spring Harbor Symp.*, **25**, 57 (1960).

[5] Brown, jun., F. A., *Biol. Bull.*, **115**, 81 (1958).

Furunculosis in Lough Neagh Pollan *Coregonus pollan*, Thompson

Reports were received during the early part of August 1960 that considerable numbers of Lough Neagh pollan were dying. An inspection revealed that the north-eastern shore in the Antrim Bay area was littered with fish (approximately one for each yard of beach) and from their condition it was evident that the mortality had been occurring for some time, possibly 2–3 weeks. It has since been learned that dying and dead fish continued to be noted until the end of August at least. Although pollen was the only species noted at the time of the inspection, commercial fishermen on the lake have since stated that small numbers of bream, *Abramis bramae* L., and lake trout, *Salmo trutta* L., were also affected.

Three pollan were collected and examined macroscopically in the laboratory. Except for an apparent slight hæmorrhage at the base of the anal fin and in the surrounding area in one of the fish, nothing abnormal was noted. Despite the absence of pathological indications of bacterial invasion, cultures were prepared from the pulped kidney of each of the three pollan and these all developed colonies typical of *Bacillus salmonicida* (Emmerich and Weibel). The organism was a small, non-motile, Gram-negative staining diplobacillus fermenting glucose and mannite without gas production. There was no reaction with lactose, dulcite or saccharose; gelatine was liquefied, indole was not produced and on serum agar a profuse growth with brown pigment was produced.

The results of the confirmatory tests carried out on each of the cultures from the three pollan using gold-fish, *Carassius auratus* L., are given in Table 1. In each case the inoculum was 0·05 ml. of a saline suspension of a two-day agar slope culture, the opacity of which was approximately that of Brown No. 2 or a dilution thereof. The fish were used in groups of three.

In addition, oral infection of the gold-fish was attempted, but in none of the three cases was an infection produced.

Table 1

Dilution of inoculum	Intraperitoneal injection			Applied to scarified tissue		
	Survival (in days)	Pathological effect	*B. salmonicida* isolated	Survival (in days)	Pathological effect	*B. salmonicida* isolated
Pollan No. 1 Undiluted	1–2	General œdema and liquefaction of internal organs	+	3	Necrosis of musculature in scarified area	+
Pollan No. 2 Undiluted 1/2·5 1/5·0	3 3 3	Liquefaction of internal organs	+ + +	4 5 4	Necrosis of musculature in scarified area. Liquefaction of internal organs	+ + +
Pollan No. 3 Undiluted 1/10 1/100	2 alive alive	Liquefaction of internal organs	+	3 alive alive	Necrosis of musculature in scarified area. Liquefaction of internal organs	+

Thirty-six control gold-fish were kept during the period of the investigation and, of these, three were injected intraperitoneally with sterile saline; three others were scarified and sterile saline placed on the wounds and a further three fish were injected intraperitoneally with a culture of *B. salmonicida* which had been standardized to Brown's opacity Tube No. 2 and the suspension killed by moist heat. All the control fish together with these three groups survived.

There is therefore no doubt but that *B. salmonicida* was the epizootic responsible for the mortality of the fish in Lough Neagh, but unfortunately it is not possible to advance any plausible theories as to the reason for the outbreak. It should be noted, however, that in the Ministry's records is a report that *B. salmonicida* was responsible for the death of numbers of salmon, *Salmo salar* L., in the River Bann during the mid 1930's (Furunculosis Committee Reports: Interim, 1930; Second Interim, 1933; Final, 1935; H.M.S.O., Edinburgh).

Acknowledgment is made to Dr. A. J. Howard, the director of the Department of Industrial and Forensic Science, Ministry of Commerce, for his co-operation, and all others who so kindly assisted in the work and the correction of the manuscript.

K. U. Vickers
Fisheries Branch,
R. McClean
Department of Industrial and Forensic Science,
Ministry of Commerce,
Government of Northern Ireland.

An Example of Mechanical Co-ordination of Cilia

The membranelles (compound cilia) of the protozoan *Stentor* are arranged in a single row around the oral end of the animal. Each membranelle swings through an angle of beat of about 140° in a plane at right angles to the row, while the metachronal waves pass along the row from one end to the other. It has been established[1,2] that these membranelles are normally co-ordinated to beat in a metachronal rhythm by an internal transmission mechanism, so that the conduction of metachronal 'impulses' and the beating of the cilia are separable activities of the system, having quite different properties.

Recent observations on the cilia of lamellibranch gills[3,4] have shown that the metachronal co-ordination rate (wave velocity) can be markedly increased by an increase in the viscosity of the medium around the cilia. This would not be expected on the basis of an internal conduction theory. In work on the metachronal co-ordination of *Stentor* membranelles in media of increased viscosity, I[1,5] found that the wave velocity did not change at viscosities below about 3·5 centipoises, but that at higher viscosities the metachronal rhythm was abnormal. Further experiments on *Stentor* membranelles reported here have provided an explanation of these apparently conflicting reports.

The experimental methods used, including those of measuring the frequency of beat and the wave velocity, were the same as those described previously[1], except that in these experiments methyl cellulose was replaced by polyacrylamide as the viscous agent as recommended by Gosselin[3].

The results of these experiments are summarized in Fig. 1. The records of change of wave velocity below

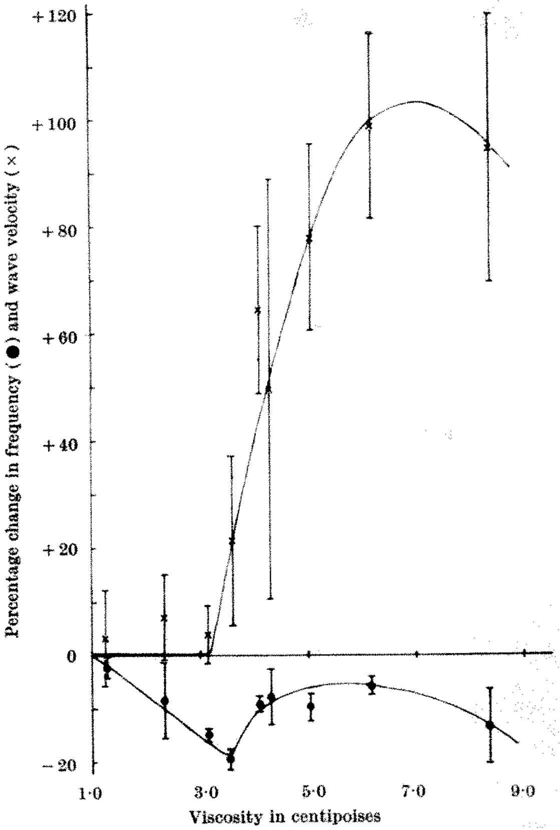


Fig. 1. Effect of polyacrylamide solutions of viscosities between 1·2 and 8·5 centipoises on frequency of beat and velocity of metachronal wave transmission of the membranelles of *Stentor*. Each value plotted is the mean of many observations, the vertical line showing 95 per cent confidence limits

3·3 centipoises were found by the *t* test ($P = 0{\cdot}05$) not to differ significantly from zero, which is in accord with previous results. At the higher viscosities there is a dramatic and significant increase in wave velocity. All the frequency records except the first differ significantly from zero, showing that at viscosities below about 3·5 centipoises the frequency steadily decreases, but it rises again at about the same viscosity as the increase in wave velocity. In solutions of high viscosity there is also an obvious decrease in the amplitude of beat.

The best explanation of these observations seems to be as follows. At the higher viscosities the tendency for the viscous medium to slow the ciliary beat falls away slightly, evidently because the movement of one cilium assists the movement of its neighbours through the increased drag communicated through the viscous medium. This same viscous-mechanical interaction between adjacent cilia allows an alternative means of communicating a metachronal rhythm which is faster than the internal co-ordination and increases in speed as the viscosity is increased. Under these conditions of high external viscosity the cilia are thought to be co-ordinated solely by the sort of mechanical means suggested by Gray[6]. The same group of cilia may thus show this second method of co-ordination under these special conditions.

The amplitude of beat decreases progressively with increase in viscosity, until eventually the angle of beat is so small that viscous interaction becomes negligible, so that the frequency falls rapidly away and the metachronal rhythm breaks down. The cilia may beat singly and sporadically at very high viscosities (above 10 centipoises) but the movements are very feeble.

M. A. SLEIGH

Zoology Department,
The University,
Exeter.

[1] Sleigh, M. A., *J. Exp. Biol.*, **33**, 15 (1956).
[2] Sleigh, M. A., *J. Exp. Biol.*, **34**, 106 (1957).
[3] Gosselin, R. E., *Fed. Proc.*, **17**, 1468 (1958).
[4] Aiello, E. L., *Physiol. Zool.*, **33**, 120 (1960).
[5] Sleigh, M. A., Ph.D. thesis, University of Bristol (1956).
[6] Gray, J., *Proc. Roy. Soc.*, B, **107**, 313 (1930).

A Method of replicating Dry or Moist Surfaces for Examination by Light Microscopy

THE use of transparent replicas is a convenient method for the microscopic study of surfaces. Perfectly dry structures can be replicated with plastic substances such as 'Collodion', 'Formvar' or methacrylate, but a serious disadvantage to the general use of these materials is that they 'mist' when allowed to harden in a moist atmosphere. Accordingly, they cannot be used satisfactorily with specimens that either become distorted when dry or, like many biological specimens, have a naturally wet surface. The following method, which overcomes this difficulty, consists essentially of taking a primary impression of the surface with a silicone rubber plastic and using this to form secondary transparent replicas.

Silicone rubber monomer ('Silflo Dental Plastic', Flexico Developments, Ltd., 25 Devonshire Place, London, W.1) is mixed with the requisite amount of catalyst and immediately poured over the material to be examined. The plastic hardens in about 5 min. and it is then gently lifted away with forceps. The primary replica is now washed in water containing detergent ('Teepol') with the aid of a small sable-hair brush and finally swilled in distilled water.

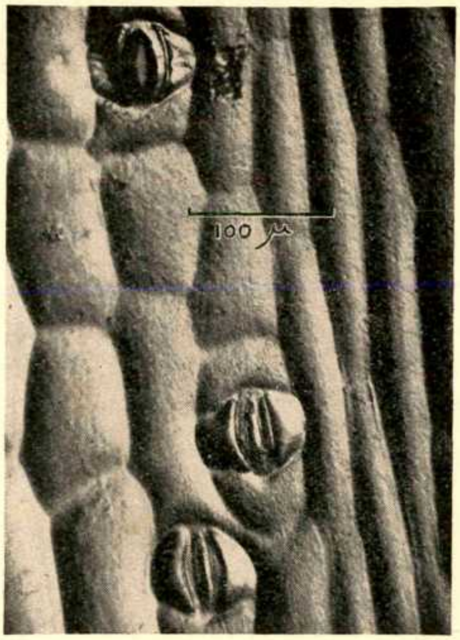


Fig. 1. Replica of abaxial surface of leaf of *Tradescantia virginiana* showing flaccid and turgid guard cells

Before proceeding to the next stage it is most important that the primary replica be thoroughly dried. The method adopted is to blot the rubber with fluff-free filter paper (Whatman No. 50) and to place it flat, impressed surface upwards, in a desiccator containing phosphorus pentoxide for 10–15 min.

A nitrocellulose preparation ('clear' nail varnish) is poured or spread over the dry, undisturbed rubber

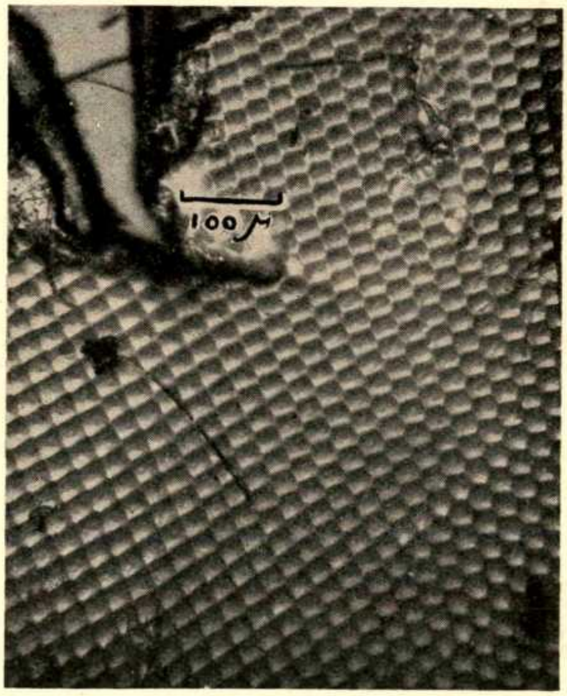
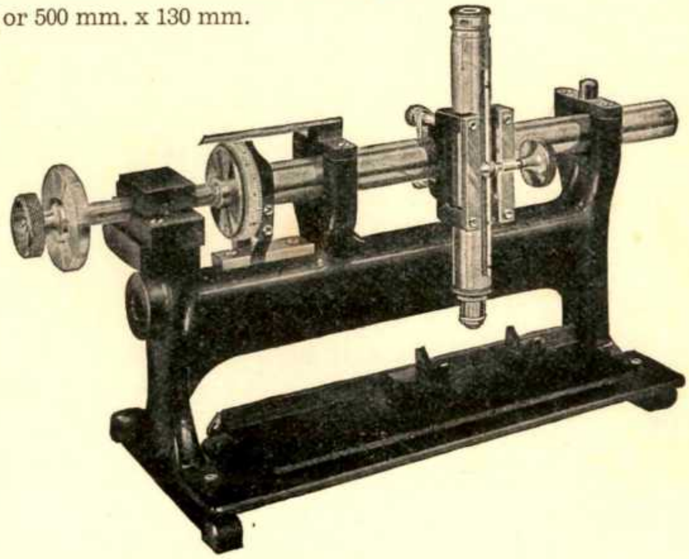


Fig. 2. Replica of surface of a wasp's eye

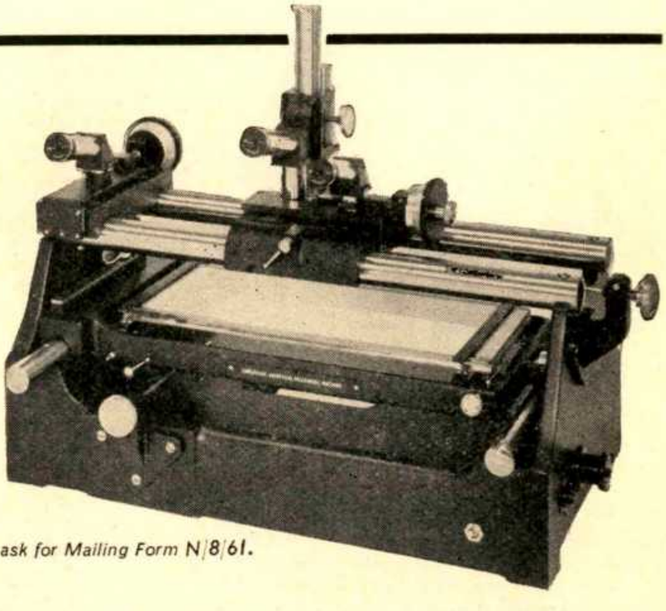
Accurate measurements
ON FLAT MATERIALS

Cambridge measuring machines and microscopes are geometrically designed and this makes possible a *lasting* degree of accuracy not easily achieved otherwise. Two basic types are made: the *Cambridge Measuring Microscope* for linear measurements up to 50 mm. and the *Cambridge Universal Measuring Machine* for two linear measurements (at right angles) either to 330 mm. x 130 mm. or 500 mm. x 130 mm.



CAMBRIDGE MEASURING MICROSCOPE

This reads directly to 0.01 mm. or by estimation to 0.001 mm. Its robust construction and easy manipulation ensure that the instrument is a useful tool both for workshop and laboratory purposes. It can be supplied with tripod and cathetometer telescope for vertical measurements.



CAMBRIDGE UNIVERSAL MEASURING MACHINE

The object is mounted on or between glass plates which have a transverse movement of 130 mm., and measurement is made by viewing through a microscope with longitudinal travel of 350 mm. (or 500 mm. in the larger model). Microscopes with micrometer adjustments read in both directions (at right angles) to 0.01 mm. or by estimation to 0.002 mm.

A convenient holder for strip film is available as an accessory.

Write for Lists 14N and 134N. For future publications, ask for Mailing Form N/8/61.

Cambridge **PRECISION MEASUREMENT**

CAMBRIDGE INSTRUMENT COMPANY LIMITED 13 GROSVENOR PLACE, LONDON, S.W.1

PUROX*

RECRYSTALLISED ALUMINA 99·7%
STABILISED ZIRCONIA 97%
PURE MAGNESIA 98%

* *Registered Trade Mark*

Purox is the name of a new range of high purity Alumina, Magnesia and Zirconia refractories. These refractories contain no glassy bond, and the Alumina is impervious. They are hard, strong, and have extremely high softening points. New techniques developed at Neston allow of the manufacture of complex shapes to close tolerances, and of single parts of considerable size. These materials open up entirely new possibilities in many fields.

MORGAN REFRACTORIES LIMITED,
NESTON, WIRRAL, CHESHIRE.
TEL: NESTON 1406
A Member of The Morgan Crucible Group

MORGAN Refractories Ltd

NE 177A

surface and the lid of the desiccator replaced immediately. When quite hard (10–15 min.) the transparent secondary replica may be separated and mounted between two thin, polished microscope slides, the edges of which are afterwards bound together with adhesive tape.

The stripping process may present some difficulty owing to the tendency of the replica to curl. The transparent layer should be lifted slowly and, as soon as it is free, transferred swiftly to a slide and sandwiched flat. When the mount is viewed by eye the back, that is, the unreplicated surface, should be clear. Unsatisfactory replicas look cloudy and the fault almost invariably lies in the failure to dry the primary replica.

On microscopic examination the replicas are seen to their best advantage if the sub-stage mirror is tilted to one side so that the incident light strikes the specimen obliquely.

This method is a simple, quick and reliable way of obtaining permanent records of a variety of surfaces suitable for examination by light microscopy (Figs. 1 and 2). It can be used for living tissues and, since the rubber appears to be non-toxic, serial impressions from the same region can be made provided care is taken not to damage the surface when removing the rubber.

I am indebted to Dr. F. A. L. Clowes for his encouragement and advice and to Mr. F. Bradley for the photographs.

JOAN SAMPSON

Botany School,
South Parks Road,
Oxford.

ENTOMOLOGY

Distribution of *Acarapis woodi* (Rennie)

A. R. Brimblecombe and C. Roff state in *Nature* of May 6, p. 561, that "Mites determined as *Acarapis woodi* (Rennie) were found recently on one of the escorts with a queen bee (*Apis mellifera* L.) from California". They also report that subsequent surveys indicated that this particular mite was found on bees from other States of the United States, and was widespread in Queensland.

The queen shipment in question was sent to Brisbane in June of 1959, and the identification was made a short time later. In subsequent letters with the British Museum and with Canberra, it was determined that no evidence of the internal mite was found in the tracheæ of the bee, but only forms on the outside of the thorax. The California State Department of Agriculture and the University of California made extensive surveys of bees in California, and other apicultural workers made surveys in other States and in different Provinces of Canada, examining thousands of bees for the internal mite, *Acarapis woodi*, with entirely negative results. No specimens of *A. woodi* have ever been found in the United States or Canada.

External acarapis mites were found widely scattered in both the United States and in Canada, and these were identified as *Acarapis dorsalis* and *A. externus*. These mites are innocuous forms that apparently have a world-wide distribution and cause no noticeable damage. They have never been found to invade the tracheæ of bees and can be distinguished from *Acarapis woodi*, the internal species, by several distinct morphological as well as ecological characteristics, which are now known to the acaralogists of the British Museum (Natural History) and to the National Museum in Washington. I personally have sent slides of the external mites to both museums and to various places in Australia, including Canberra and Adelaide.

If there is any doubt still in the minds of the acaralogists as to whether the external acarapis mites are distinct species or merely 'strains' of one mite, then for scientific as well as economic reasons they should identify the external forms as *A. woodi dorsalis*, or *A. woodi externus*, or *A. woodi vagans*, whichever they chance to be. It is certainly not by chance that the external acarapis mites have distinctly different physical characters from the internal mite, or that they have never been found to invade the tracheæ of their host.

J. E. ECKERT

Department of Entomology and Parasitology,
University of California,
Davis, California.

Brain Hormone of the Silkworm, *Bombyx mori*

It is generally accepted that the neurosecretory cells concerning the metamorphosis of insects contain a small amount of a secretory substance at any time of development[1,2], notwithstanding a marked effect which they exert when implanted or transplanted. This means that accumulation of the secretory substance released continuously from the cells in the brain is necessary to become effective. This is the reason why a great many brains were used to extract a sufficient amount of the active principle[3]. However, we have found that only several devitalized brains of *Bombyx mori* could cause adult differentiation when inserted into the non-diapausing pupæ of *Philosamia cynthia ricini* caused to diapause by prompt removal of their brains just after pupation. Devitalization was brought about by various ways such as subjecting brains to freezing in a deep-freezer at $-18°$ C. for 21 days, desiccating them over silica gel for one day, or heating them at 90° C. for 90 min. After being treated, the brains were all proved to be as effective as live ones, so far as they were tested by five for each test pupa. Needless to say that in this case the brain hormone, enough to stimulate adult differentiation of the recipient, had to be present in the five inserted brains. The possibility that the adult differentiation was caused by the prothoracic gland hormone which contaminated the inserted brains can be ruled out, since neither the same amount of fatty tissue nor blood similarly treated exerted any perceptible effect on the dormant pupæ. It seems, however, worthy of mentioning that after treatment brains of *Philosamia* were inferior to brains of *Bombyx* in their effectiveness. This presumably indicates that the situation at a given moment is different between the two species, a larger amount of the hormone being contained in the latter.

Kobayashi and Kirimura[3] reported first the preparation of the brain hormone. In their case, however, an extract from some 400 brains was necessary to let each pupa develop into an adult. By contrast, our finding stated above may suggest that the extraction from only five brains would yield sufficient quantity of the active principle, if the procedures of extraction are successfully manœuvred.

Thus we started work on the chemical properties of the brain hormone after Kobayashi and

Kirimura's method with the view of getting an active principle from the small number of brains.

The complexes of brains and subœsophageal ganglia from pupæ of *Bombyx* that had been mostly shortly before the completion of adult development were stored in a deep-freezer at – 18° C. and used as material to be extracted. Brains were counted before crushing to facilitate an easy computation of dose per test pupa. All the work was carried out at room temperature.

240 brains (fresh wt. 97 mgm.) were homogenized with 1 ml. of methanol and centrifuged. The same extraction was repeated twice more with the residue. The extracts thus obtained were combined and dried *in vacuo*. A small amount of yellow matter thus prepared was dissolved in 2 ml. of distilled water plus 5 ml. of ether, the latter being removed after thorough shaking. The water layer was washed with ether twice more and a trace of ether remaining in the water was removed *in vacuo*. The water fraction thus obtained was made up with distilled water to 2 ml., 0·1 ml. of which was injected into each of 20 test pupæ of *Philosamia*. 19 out of the 20 pupæ metamorphosed into moths 18–24 (average 19) days later at 25° C. (At this temperature normal pupæ can emerge as moths 17–19 days after pupation.) The combined ether washings were dried also *in vacuo* to yield yellow oily matter. It was dissolved in 2 ml. of sesame oil and 0·1 ml. of the solution was injected into each of 20 test pupæ with the result that not one of them indicated an appreciable differentiation toward adult. Control experiment of injecting water or sesame oil alone proved also to be ineffective. A repetition of the same experiment yielded essentially the same result.

The active principle was also prepared by extraction with 2 per cent sodium chloride solution. 350 brains were homogenized with 0·7 ml. of the saline solution and centrifuged at 9,000 r.p.m. for 30 min. The same procedure was repeated three times more with the residue, and the extracts thus obtained were about 2·8 ml. All 37 pupæ, in which 18 pupæ were injected each with 0·1 ml. of the extract and 19 with 0·05 ml., were able to metamorphose into moths 18–25 (average 20) days and 19–25 (average 21) days later respectively. Injection of 0·1 ml. and 0·05 ml. of the saline solution brought about no sign of adult differentiation.

Thus it is evident that the active extract of the brain hormone can be prepared successfully from a relatively small number of brains. Here it must be emphasized that there is an important difference between our finding and that obtained by Kobayashi and Kirimura: namely, our principle is water-soluble, while that obtained by Kobayashi and Kirimura is ether-soluble, as tested by the water–ether partition. Further purification now in progress will give further information about the chemical nature of the brain hormone.

We are indebted to Dr. S. Morohoshi, director of Gunze Silkworm and Mulberry Research Institute, and Dr. S. Sasaki, director of Ayabe Branch of Sericultural Experimental Station, who supplied the silkworm pupæ.

Mamori Ichikawa
Hironori Ishizaki

Zoological Institute, College of Science,
University of Kyoto.

---

[1] Williams, C. M., *Biol. Bull.*, **110**, 201 (1956).

[2] Karlson, P., in *Proc. Fourth Intern. Cong. Biochem.*, edit. by Levelbook, L., 37 (Pergamon Press, 1959).

[3] Kobayashi, M., and Kirimura, J., *Nature*, **181**, 1217 (1958).

GENETICS

Sterile Crosses between Mutations of *Tribolium confusum* Duv.

'McGill Red' is a wild strain of *T. confusum* maintained by me for some thirty years. 'McGill Black' is a jet black mutation which arose in the wild strain in 1950. The life-histories of both strains have been described by H. M. Slatis and me[1]. In the spring of 1960 another black mutation which may be called for convenience 'Slough Black' was received from the Pest Infestation Laboratory at Slough. An extensive programme of research has been under way to study the genetics of 'Slough Black' in relation to 'McGill Black' and 'McGill Red'.

It has been found that, when females of 'McGill Black' are mated to any male other than that of 'McGill Black', numerous eggs are produced, but these are all non-viable, no development occurring. The males of all the genotypes obtainable from crosses between the three strains have been mated with females of 'McGill Black' with similar results.

The eggs have been sectioned, but there is no trace of development, and eventually the eggs dry up and wither. Superficially they appear quite normal. There would seem to be four possible causes: (*a*) the matings are ineffective, no sperms being transferred; (*b*) there is some genetic lethal effect; (*c*) there is a cytoplasmic defect; (*d*) there is the faint possibility that rearing the insects on flour plus yeast has had an effect. Further work is being done on this problem, and some stocks are being reared on flour plus wheat-germ to check (*d*).

John Stanley

Department of Zoology,
McGill University,
Montreal, Quebec.

---

[1] Stanley, J., and Slatis, H. M., *Ecology*, **36**, 473 (1955).

5-Methyltryptophan and Darkening of the Hair in Yellow A^y/a Mice

Previous experiments have shown that powdered preparations of the skin and hair of yellow (A^y/a) mice oxidize L- (but not D-) tryptophan and also DL-5-hydroxytryptophan with the production of yellow solutions[1,2]. To obtain further evidence on the question of whether tryptophan, or a derivative, might be implicated in the production of yellow pigment in the hairs of this strain of mice, *in vivo* experiments were carried out using an antimetabolite of tryptophan[3]. Starting at 3–4 days of age, daily subcutaneous injections of DL-5-methyltryptophan were made in these mice for a period of 7–10 days. This is a period of vigorous pigment production. (Mice were obtained from the R. B. Jackson Memorial Laboratories, Bar Harbor, Maine. DL-5-methyltryptophan was a product of Sigma Chemical Co., St. Louis, Mo.)

While lower doses gave equivocal results, the injection of 1 mgm./gm. DL-5-methyltryptophan into these suckling mice daily for a week (a dosage high enough to cause measurable depression in growth-rate) resulted in animals the coats of which were markedly less yellow than those of animals given saline or L-tryptophan daily (Table 1). Instead, coats of experimental animals appeared darker in colour than those of controls. When unstained 10μ cross-

Table 1. APPEARANCE OF COATS OF *Ay/a* MICE AFTER INJECTIONS OF 5-METHYLTRYPTOPHAN OR CONTROL SOLUTIONS

Exp. No.	Age during injections	No. of animals	No. *Y*	No. *G*	No. *I*
II	3–12 (10 days)	2 Experimental	0	1	1
		2 Control (L-tryptophan)	2	0	0
III	4–10 (7 days)	3 Experimental	0	2	1
		2 Control (isotonic saline)	2	0	0
IV	4–14 (11 days)	3 Experimental	0	3	0
		2 Control (isotonic saline)	2	0	0

Y, Yellow coats; *G*, grey coats; *I*, coats intermediate in colour· 5-Methyltryptophan and L-tryptophan were injected as suspensions in distilled water (20 mgm./ml.). Judgments of colour were made by at least one naïve observer as well as the experimenter and agreed in all cases. In each experiment control and experimental animals were litter-mates.

Table 2. PERCENTAGE OF HAIR CROSS-SECTIONS CARRYING YELLOW OR BLACK PIGMENT

Exp. No.	Level at which hairs were counted		*Y*	*B*	*Y* + *B*
II	Just above the skin surface	Exp.	32	53	15
		Cont.	89	0	11
III	Just below the skin surface	Exp.	59	11	30
		Cont.	99	0	1
III	Just above the skin surface	Exp	28	14	58
		Cont.	94	0	6
IV	Just below the skin surface	Exp.	62	12	26
		Cont.	99	0	1

Y, Yellow pigment; *B*, black pigment; *Y* + *B*, yellow and black pigments both visible in the hair cross-section studied. A rectangular piece of dorsal skin and hair was fixed in formalin, embedded, cut at 10μ, and examined without any staining. At least five cross-sections of skin were examined in each case. Counts were made of a series of 100 or more consecutive cross-sections of hairs. To avoid duplication, hairs were counted at a given level parallel to the surface of the skin. Colour was estimated under the microscope with daylight illumination, and with the source of the slide unknown. This table gives no information on the relative distribution of pigments at other points along a given hair.

sections of skin and hair were examined; those from experimental animals could be readily distinguished because of increased amounts of black pigment in the follicles and hairshafts. Quantitatively, the percentage of hairs containing visible black pigment increased from 1–10 per cent in controls to 40–70 per cent in the experimental animals (Table 2). The effect depended on the continued injection of the methyltryptophan; two animals were kept after the completion of one experiment without further injections, and a typical yellow coat was visible under the dark outer coat of hair after about a week.

Preliminary trials failed to demonstrate a reversal of the darkening effect by the simultaneous administration of an equal amount of L-tryptophan daily; however, since in mice injected tryptophan is rapidly metabolized by an inducible enzyme system in the liver[4], it may take a very large amount of systemically administered tryptophan to antagonize the effect.

The above results support the idea[1] that tryptophan, or a derivative, may be utilized for pigment formation by these mice. If the methyltryptophan acts primarily by inhibiting this system, the increase in black pigment may be a secondary effect. It is well known that neural crest cells, or dendritic melanocytes of *Ay/a* mice, form black pigment rather than yellow in locations other than the hair bulb: the ear skin, the harderian gland, and the nictitans, for example[5], as well as when transplanted into the follicles of genetically black mice[6], or when explanted into the chick cœlom[7]. That the appearance of the black pigment may be a secondary effect is supported by some equivalent experiments on the effect of methyltryptophan on black (*C57Bl*/6) mice. Here, injections of the analogue resulted in a slight diminution in the intensity of black pigmentation. This result, while not quantitative, shows that methyltryptophan does not in itself have a stimulating effect on the system responsible for forming black pigment, namely, the tyrosinase system. The only other explanation would have to be that the methyltryptophan competes successfully with both the yellow and black systems to form an 'intermediate' black, which seems unlikely.

The experiments reported here are in agreement with the idea that yellow pigment in the hairs of *Ay/a* mice represents not simply a different degree of polymerization of melanin formed by the tyrosinase system, but is formed, at least in part, by a different route.

I am indebted to Dr. L. B. Flexner, of the Department of Anatomy, University of Pennsylvania, Philadelphia, and to Dr. R. K. Enders, of the Zoology Department, Swarthmore College, Swarthmore, Pa., for facilities for the initiation and completion of this work. Part of the work was carried out during tenure of a U.S. Public Health Service postdoctoral fellowship.

VIVIANNE T. NACHMIAS

Department of Anatomy,
University of Pennsylvania Medical School,
Philadelphia 4, Pa.

[1] Foster, M., *J. Exp. Zool.*, **117**, 211 (1951).
[2] Nachmias, V. T., *Soc. Exp. Biol. and Med.*, **101**, 247 (1959).
[3] Fildes, P., and Rydon, H. N., *Brit. J. Exp. Path.*, **28**, 211 (1947).
[4] Knox, W. E., in *Methods in Enzymology*, **2**, edit. by Colowick, S. P., and Kaplan, N. O., 245 (Academic Press, 1955).
[5] Markert, C., and Silvers, W. K., *Genetics*, **41**, 429 (1956).
[6] Silvers, W. K., and Russell, E. S., *J. Exp. Zool.*, **130**, 199 (1955).
[7] Silvers, W. K., *J. Exp. Zool.*, **135**, 221 (1957).

Genetics of a Difference in the Male Genitalia of East and West African Stocks of *Papilio dardanus* (Lep.)

THE famous African butterfly *Papilio dardanus* Brown is highly polymorphic in the female, while the males are monomorphic and non-mimetic[1]. The species has been divided taxonomically into a number of sub-species, mainly on the basis of quantitative variation in wing pattern of the males[2–4]. Several of these races are isolated from all others on islands and mountainous areas. However, five named races are not geographically isolated from their neighbours and the male wing pattern, since it tends to show a clinal distribution, is unsatisfactory for delimiting their exact distributions. The western race (*dardanus*) might be thought to be more distinct than the others, because of the morphology of the male genitalia[4]. In the eastern races there is a long spine on the inner surface of the valve; the western race usually has no spine (Fig. 1). In a narrow zone north of Lake Victoria where the races meet, both types are found, together with intermediates with very short spines, which in west Africa are known only as rarities.

During recent investigations into the genetics of *P. dardanus*[5], we have accumulated information on the mode of inheritance of the long spine of eastern races. The histograms (Fig. 2) show the distribution of spine-length in the western males from the region of Lake Victoria, the eastern males from South Africa, the F_1 and F_2 hybrids between the stocks,

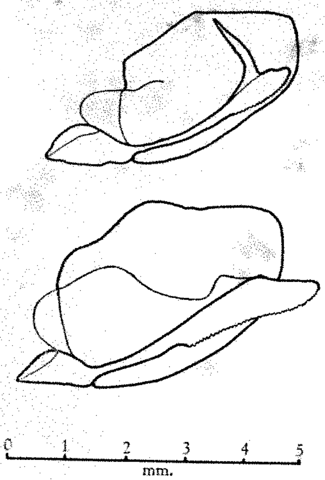


Fig. 1. The upper drawing shows a valve of the male genitalia of the eastern type; the lower one that of a male of the western race. In the latter, note the absence of the long spine present in the eastern males

and the back-cross broods to west African material. The results demonstrate that the presence of the spine is dominant to its absence, and that the character is controlled in the main by a single major gene. If we divide the F_2 individuals into two groups at the antimode (at 2·5 units) we find a ratio of 107 long-spined to 30 short- or no-spined individuals. This is a satisfactory approximation to the expected 3: 1 ratio ($P > 0·3$). The back-cross broods containing 25 long-spined to 20 short- or no-spined individuals also give a satisfactory approach to the expected 1: 1 ratio ($P > 0·3$). Variation between broods in the mean length of the spine of the dominants, coupled with the less clear-cut division into two classes in the F_2 compared with the parental populations and back-cross broods, suggest that modifiers are segregating in the hybrids.

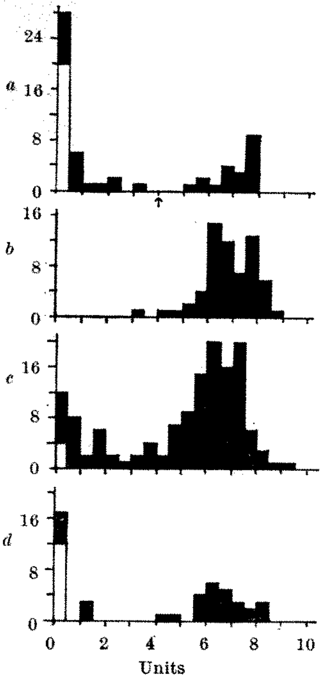


Fig. 2. The histograms show the numbers of males with different spine lengths in various stocks of *P. dardanus*, each unit being 0·156 mm. (*a*) To the left of the arrow at 4 units, all the males are of the western race, to the right of the arrow, of the eastern race; (*b*) the F_1 hybrids between the eastern and western races; (*c*) the F_2 hybrids; (*d*) the backcross of the F_1 to the western race. The white area of the histogram between 0 and 0·5 unit shows the number of individuals with no spine

The morphology of the male genitalia has been used in Lepidoptera and some other insects as an important or often the only taxonomic character. These results show that one such character, used to separate a sub-species, is mainly controlled monogenically.

The similarity between the histogram of the F_2 broods and histograms compiled for such human characters as the threshold for the ability to taste PTC solutions, or the ability to metabolize isoniazid[6], suggests that a more detailed picture of the genetics of spine-length in *P. dardanus*, where breeding can be controlled, may serve as a useful model in detailed investigations of the genetics of human characters having a bimodal distribution.

J. R. G. Turner
8 Dunbabin Road,
Liverpool, 15.
C. A. Clarke
Department of Medicine,
P. M. Sheppard
Sub-department of Genetics,
University of Liverpool.

[1] Ford, E. B., *Trans. Roy. Ent. Soc.*, **85**, 435 (1936).

[2] Carpenter, G. D. H., *Proc. Roy. Ent. Soc.*, B, **16**, 55 (1947)

[3] Carpenter, G. D. H., *Proc. Roy. Ent. Soc.*, B, **17**, 11 (1948).

[4] Jordan, K., *Z. wiss. Zool.*, **83**, 151 (1905).

[5] Clarke, C. A., and Sheppard, P. M., *Genetics*, **45**, 439 (1960).

[6] Evans, D. A. P., Manley, K. A., and McKusick, V. A., *Brit. Med. J.*, i, 485 (1960).

AGRICULTURE

Plant Growth in Irradiated Soil

One of the difficulties in any investigation of the action of micro-organisms on plants growing in soil is that sterilization of the soil by heat or by chemical means prior to planting causes complex physical and chemical changes, some of which are phytotoxic[1]. The reports that urease activity continued in soil sterilized by an electron beam[2] and that γ-irradiation of humus resulted in little physical and chemical change[3] indicated that irradiation sterilization may overcome the disadvantages of soil sterilized by more conventional methods. The present report is concerned with a comparison of growth of subterranean clover (*Trifolium subterraneum* L.) in two soils (Urrbrae red-brown earth and Mount Compass podzolized sand) sterilized by heat, by propylene oxide and by irradiation.

Soil was added to 20·5 cm. × 3·2 cm. test-tubes to a depth of 5 cm. and moistened to field capacity with distilled water. These were sterilized either by steam at 120° C. for 1 hr. on each of three successive days, or by propylene oxide introduced into an evacuated sterilization chamber at the rate of 0·6 gm./litre capacity and held at 25° C. for 72 hr. Other samples of the moistened soils were sealed in polythene bags placed in sealed tins and sterilized by γ-irradiation with a dose of 2·5 megarads. The bags were afterwards opened and placed in sterile test-tubes. All sterilization was carried out fourteen days before planting with surface-sterilized subterranean clover seed pre-germinated on agar[4]. Three sterile germinating seeds were transferred to each of eight tubes in each treatment and grown for six weeks in a glasshouse with supplementary heating and cooling.

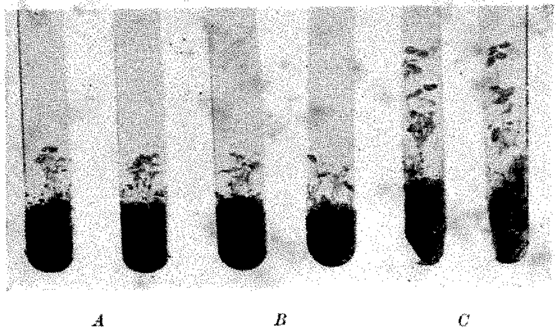


Fig. 1. Growth of subterranean clover in Urrbrae red-brown earth at 3 weeks. *A*, Heat-sterilized; *B*, propylene oxide treatment; *C*, irradiation-sterilized

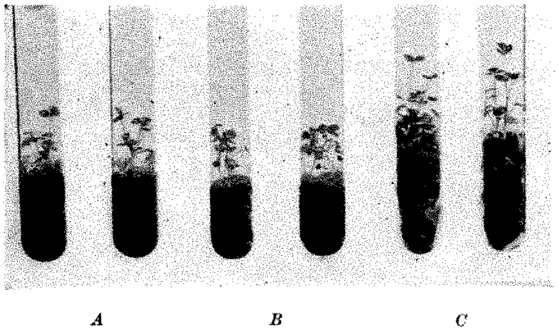


Fig. 2. Growth of subterranean clover in Mount Compass sand at 3 weeks. *A*, Heat-sterilized; *B*, propylene oxide treatment; *C*, irradiation-sterilized

Plating of soil at both planting and harvesting showed that whereas heat- and irradiation-treated soils were sterile, the soils treated with propylene oxide were not. In this treatment there were some surviving bacteria which proliferated in all tubes during the experiment.

In both Urrbrae red-brown earth and Mount Compass sand, plant growth and development in soils sterilized by heat or propylene oxide was retarded, plants in the latter case being particularly small, with rosetting and yellowing. Several of these plants died during the six-week experimental period. By contrast, plant growth in irradiated soil was good (Figs. 1 and 2, Table 1) and only slightly less than that of plants growing in unsterilized soil.

In irradiated Mount Compass sand, roots and root hairs were well developed, but in heat-sterilized sand, root growth was significantly less, light brown lesions occurring on some roots, and root hairs being few, short, and irregularly distributed. In heat-sterilized Urrbrae red-brown earth, severe stunting of primary and the many secondary roots occurred, with dark brown lesions frequently present; root hairs were absent. Subsequent experiments have shown that the phytotoxic effects produced by heat sterilization of soil can be overcome by the introduction of specific fungi and bacteria. While no obvious stunting of roots occurred in irradiated Urrbrae red-brown earth, root hairs were short and sparse compared with those on plants in unsterilized soil.

Table 1. Growth of Subterranean Clover in Heat- and Irradiation-Sterilized Soils after 6 Weeks

Soil	Mean total root length/tube		Mean total top weight/tube	
	Heat (cm.)	Irradiated (cm.)	Heat (mgm.)	Irradiated (mgm.)
Mount Compass sand	46·1	86·1**	23·4	29·0
Urrbrae red-brown earth	16·9	86·5***	33·4	40·5***

** Differences between heat- and irradiation-sterilization significant at 1 per cent level.
*** Differences between heat- and irradiation-sterilization significant at 0·1 per cent level.

The results suggest that this method may be the best at present for work on plant growth in sterilized soils. However, the suppression of root-hair formation and growth (especially in Urrbrae red-brown earth) indicates that irradiation is not without some deleterious effect. In fact, recent experiments with irradiated Urrbrae red-brown earth stored at room temperature for 10 weeks prior to planting indicate that phytotoxicity increases in such soils with storage. This result parallels the observation[5] that colour and odour changes occur in grass following irradiation sterilization, due possibly to the continued enzyme activity[2].

We wish to thank Dr. J. G. Clouston, of the Australian Atomic Energy Commission, Lucas Heights, New South Wales, for irradiation of the soils.

G. D. Bowen
A. D. Rovira

Commonwealth Scientific and
Industrial Research Organization,
Division of Soils,
Adelaide,
Australia.

[1] Warcup, J. H., *Soils and Fert.*, **20**, 1 (1957).
[2] McLaren, A. D., Resketko, Lola, and Huber, W., *Soil Sci.*, **83**, 497 (1957).
[3] Groenewound, van H., *Proc. Canad. Phytopath. Soc.*, **26**, 12 (1959).
[4] Rovira, A. D., *Plant and Soil*, **11**, 53 (1959).
[5] Grossbard, Erna, Minson, D. J., and Raymond, W. F., *Exps. in Progress*, **11** (*Grasslands Res. Inst., Ann. Rep.*, 1957–58, 80, 1959).

Assessing the Damage caused by *Heterodera rostochiensis* Woll.

Previously there have been no published accounts of the effect of any species of *Heterodera* on the yield of the host crop under field conditions. Jones[1] attempted to obtain conditions approaching those found in the field by the use of small, artificially constructed plots or microplots. However, these were too small to give a realistic estimate of yield per acre. For insect pests differential population density-levels have been established by the use of insecticides. This is impractical, for eelworms as nematicides, in addition to affecting the eelworm population, also often exert an effect on plant growth. I have therefore investigated the effect on the yield of potatoes of differences in initial density of eelworm population. This method, possibly with some modification, should prove suitable for other *Heterodera* species or for any species of nematode for which reliable population estimates can be made.

Six experiments all on various fen peat soils were performed in 1960 on fields chosen for uniformity of soil depth, texture and previous cropping. Twenty small plots (each 4 rows × 5 yd.) per site were distributed on a grid pattern covering a total area of 44 rows × 80 yd. These were marked out and soil sampled (25 cores per plot) shortly after planting and the eelworm population on each plot was estimated by the usual technique[2]. The crop yield was obtained by hand digging the whole of each plot and riddling over a $1\frac{1}{2}$ in. riddle.

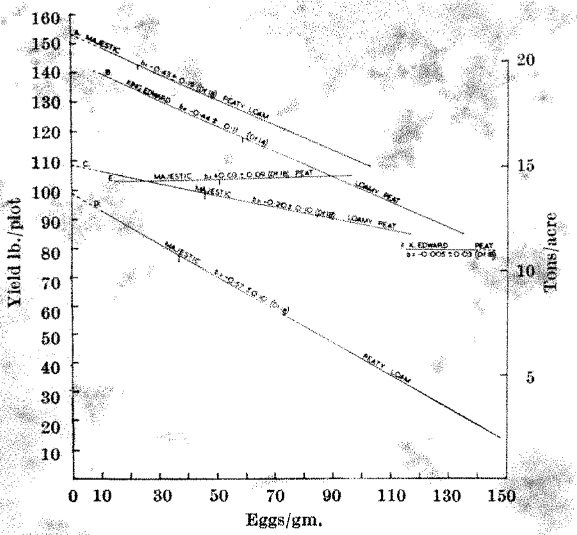


Fig. 1. The regressions of yield on eggs/gm. (The *F*-line should continue to 456 eggs/gm.)

Regression analyses were computed of yield on 'full' cysts per 100 gm. (square root transformation) and also on eggs per gm. (log transformation). A full cyst is defined as one containing more than 50 eggs.

Table 1. REGRESSION COEFFICIENTS

Site	$\sqrt{\text{Full cysts/100 gm.}}$	Log eggs/gm.
A	− 9·08 ± 2·74†	− 26·40 ± 6·70‡
B	− 10·34 ± 2·15‡	− 49·40 ± 11·20‡
C	− 5·21 ± 1·81†	− 14·37 ± 6·69*
D	− 12·25 ± 1·64‡	− 33·30 ± 5·40‡
E	+ 2·16 ± 2·05	+ 5·27 ± 9·30
F	− 0·91 ± 1·80	− 4·64 ± 17·49

* Significant at P = 0·05.
† Significant at P = 0·01.
‡ Significant at P = 0·001.

Significant regressions were obtained at sites *A–D*. At sites *E* and *F*, on adjoining farms and extremely acid peat soils, yield appeared to be independent of eelworm population and no explanation can be advanced at the moment.

The eelworm population estimates were transformed because the error increases with the size of the count. For ease of interpretation and because it seemed that yield and initial eelworm population were linearly related over the range studied, regression coefficients for sites *A–D* were also calculated from untransformed data. The regressions of yield on eggs per gm. are shown in Fig. 1 and the results for full cysts and eggs summarized in Table 2. The range of full cyst counts was approximately 0–55 per 100 gm. for each of the sites *A–D*.

Table 2

Site	Full cysts/100 gm.		Eggs/gm.	
	Regression coefficients	Yield loss/ 10 full cysts/ 100 gm. (tons/acre)	Regression coefficients	Yield loss/ 20 eggs/gm. (tons/acre)
A	−0·93 ± 0·44	1·29	−0·43 ± 0·19	1·20
B	−1·10 ± 0·24	1·53	−0·44 ± 0·11	1·22
C	−0·64 ± 0·25	0·89	−0·20 ± 0·10	0·56
D	−1·59 ± 0·26	2·21	−0·57 ± 0·10	1·58
	Means	1·48		1·14

The mean figures for yield reduction on these four sites are about 1½ tons per acre for every 10 full cysts per 100 gm. and a little more than 1 ton per acre for every 20 eggs/gm. Although efforts were made to pick uniform sites, estimates of loss of yield due to eelworm will be to some extent biased, if there are unknown soil factors which vary within these fields and have an effect on yield and on eelworm population. These preliminary trials need to be repeated in different seasons on the same and on different soils.

In advisory work it has been necessary to decide on a 'critical value' for eelworm population above which advice has been given that a satisfactory yield of potatoes is unlikely. For example, Johnson and Thompson[3] gave a figure for Yorkshire of 50 cysts with contents per 100 gm. However, damage is a continuous process commencing at low levels of eelworm and approaching a maximum when visible symptoms appear in the crop. (It is uncertain what happens at very low levels at which there might be no effect or even a slight stimulation to growth[4-6].) On all sites except *D*, where some patchiness occurred, no obvious symptoms of eelworm attack were seen throughout the growing season. Also, what constitutes an acceptable potato yield will depend on how much the crop has cost to grow, and on the cropping potential of the field, and the 'critical value' will therefore vary accordingly. This is portrayed in Fig. 1 where the lines although nearly parallel are widely separated. It therefore seems more logical to express losses due to eelworm in terms of x tons per acre for every y eelworms per z gm. of soil before planting.

I wish to thank F. G. W. Jones and B. M. Church, of Rothamsted Experimental Station, for their helpful advice on the presentation of these results.

E. B. BROWN

National Agricultural Advisory Service,
(Block 'C'),
Brooklands Ave.,
Cambridge.

---

[1] Jones, F. G. W., *Ann. App. Biol.*, **44**, 25 (1956).
[2] Goodey, J. B., *Tech. Bull.*, No. 2 (H.M.S.O., 1957).
[3] Johnson L. R., and Thompson, H. W., *Agriculture*, **52**, 266 (1945).
[4] Jones, F. G. W., *Nematologica*, **2**, 257 (1957).
[5] Lownsbery, B. F., and Peters, B. G., *Phytopathol.*, **45**, 163 (1955).
[6] Peters, B. G., *Rep. Rothamsted Exp. Sta.*, *1954*, 96 (1955).

SOIL SCIENCE

Role of Interparticle Forces in Development of Upper Plastic Limit of Clays

THE standard laboratory test method in soil mechanics for determination of the liquid limit of clays (upper plastic limit) depends on the facility of movement of particles relative to one another under conditions created by the test procedure. An interpretation of the upper plastic limit of clays has recently been suggested[1] in terms of distance between particles or structural units of particles wherein forces of interaction become sufficiently weak to allow slipping to occur.

Since the standard liquid limit test requires the samples to be remoulded in the performance of the test itself, initial structure or orientation of particles in the undisturbed or natural state is not of prime importance. The degree of particle interaction arising from interparticle forces of attraction and repulsion will determine to a large extent the facility for movement of particles. The distance between particles or structural units of particles following the remoulding of soil sample for test may be better characterized in terms of degree of particle interaction arising from influence of interparticle forces. In many natural soils, where the presence of interparticle forces may not be felt except within very close limits, particle interaction dependent on effective specific surface area may be the criterion for establishment of the liquid limit. In tests on selected clay soils using distilled water and ethylene glycol ($C_2H_4(OH)_2$), the influence of interparticle forces and effective specific surface area on the moisture content at the liquid limit is demonstrated. Correction for the specific gravity of ethylene glycol was made in the calculation of moisture content expressed in terms of the ratio of weight of fluid to the oven-dry weight of solids. The clay soils selected for test were natural bentonite, kaolinite, fire clay (primarily illite) and ball clay (approximately 75 per cent kaolinite with remainder illite, montmorillonite, feldspar and quartz). Table 1 summarizes the results of the tests.

Table 1. LIQUID LIMIT OF SELECTED CLAY SOILS WITH DISTILLED WATER AND ETHYLENE GLYCOL

Soil type	Moisture content at liquid limit (per cent)	
	Distilled water	Ethylene glycol
Natural bentonite	437·0	114·6
Kaolin	70·2	97·4
Ball clay	62·6	105·3
Fire clay	39·0	42·7

As would be predicted, the liquid limit of bentonite is reduced considerably when ethylene glycol is used in place of water. This is in accordance with the concept of interaction volume, and with results presented by previous investigators on the influence of electrolyte concentration on liquid limit[2,3]. The elimination or reduction of interparticle forces of repulsion reduces the interaction volume and thereby decreases the liquid limit.

The results for kaolin and ball clay show an increase in the liquid limit with the use of ethylene glycol in place of water. This would suggest that the resultant structure or orientation of the particles is of a dispersed nature. Although remoulding in water would tend to cause the soil to have a dispersed structure, the slightly acidic nature of the soil and the edge charges associated with kaolinite would cause the particles to reassume an edge-to-face particle arrangement. The resultant interaction volume, in a situation where edge-to-surface particle orientation exists, is small compared with that of a dispersed structure. For purposes of consideration in terms of a mechanism, it may be expedient to visualize slipping of particles or flocs of particles at the critical liquid limit point being dependent upon the external surface area. In this instance, in the absence of interparticle forces of any significant magnitude, the dominant factor is the external specific surface area which would require a sufficient coating of fluid only for lubrication purposes. Particle interaction would be in terms of facility of movement of particles relative to one another.

The absence of strong interparticle forces in the fire clay accounts for the very slight increase in liquid limit in the substitution of glycol for water. The initial structure of the clay soil mixed with water is dispersed, and subsequent replacement of water by glycol yields the same structure or orientation of particles.

Complete data and test results on other properties of the soils described will be published elsewhere.

Raymond Yong
E. T. Selig

Armour Research Foundation
of Illinois Institute of Technology,
Chicago, Illinois.

---

[1] Warkentin, B. P., *Nature*, **190**, 287 (1961).

[2] Mielenz, R. C., and King, M. E., *Proc. First Nat. Conf. Clays and Clay Tech.*, California Bureau of Mines, *Bull.* 169, 218 (1955).

[3] Warkentin, B. P., *et al.*, *Proc. Soil Sci. Soc. Amer.*, **21**, 495 (1957).

Soil Temperatures in Iceland

INFORMATION on soil temperatures in Iceland is scant except for interrupted and now discontinued observations made by the Meteorological Bureau of Iceland at two and earlier three different locations in the country[1]. These observations had mostly been restricted to the 1- and 2-m. depths.

A study of the influence of soil temperature on the soil–plant relationships has therefore been undertaken, especially with the more effective use of fertilizers in mind.

Preliminary measurements at three agriculturally important depths, namely, 5, 10 and 20 cm. under permanent grass and in an exposed soil, were carried out at the experimental ground of this Institute during the summer 1960. The soil at the particular site was a coarse sandy loam with 10–15 per cent loss on ignition.

All measurements were carried out with permanently installed mercury-in-glass soil thermometers with a collar of non-hardening roof-compound around the soil-air joints to exclude so far as possible free air temperature gradients caused by the shrinking of soil around the inserted parts of the thermometers. Readings had to be restricted to the normal working hours of each day and were made at 9 a.m. and 5 p.m., local summer time (= G.M.T.), thus usually giving six morning observations and five in the afternoon of each week.

Table 1 shows a few typical weekly mean values of the soil temperatures thus obtained.

The highest soil temperatures noted under the grass cover during the above period occurred in mid-July as follows: at 9 a.m., 13·6, 14·0 and 14·0° C.,

Table 1. TYPICAL WEEKLY MEAN VALUES OF RECORDED SOIL TEMPERATURES AT 5, 10 AND 20 CM. DEPTHS UNDER PERMANENT GRASS AND IN AN EXPOSED SOIL AT VARMA, ICELAND

Week	Under grass						Exposed soil					
	9 a.m.			5 p.m.			9 a.m.			5 p.m.		
	5 cm.	10 cm.	20 cm.	5 cm.	10 cm.	20 cm.	5 cm.	10 cm.	20 cm.	5 cm.	10 cm.	20 cm.
1/6– 4/6	9·5	9·8	9·5	10·9	10·1	9·4	9·8	9·0	9·1	13·1	11·9	9·2
7/6–11/6	11·2	11·6	11·2	13·4	12·0	11·3	10·4	10·2	10·6	14·8	13·5	10·9
11/7–16/7	13·1	13·6	14·0	15·8	14·4	14·0	12·6	12·5	13·1	20·9	17·1	14·1
8/8–13/8	12·2	12·8	13·3	13·9	13·5	13·4	11·9	11·7	12·9	19·9	16·9	13·7
5/9–10/9	10·5	11·0	11·5	11·0	11·2	11·4	9·0	9·6	10·6	11·7	11·1	10·7
26/9– 1/10	8·3	8·6	8·8	9·2	9·0	8·8	7·3	7·8	8·1	9·7	9·1	8·7

respectively at the 5, 10 and 20 cm. depths, while corresponding values at 5 p.m. were 16·8, 14·8 and 14·1° C., respectively. In the bare soil the highest temperatures observed at 9 a.m. were 14·2, 14·0 and 15·0° C. at the above depths, while corresponding values at 5 p.m. were 22·6, 19·0 and 15·5° C., respectively. It should be mentioned that the highest temperatures noted at each soil depth do not necessarily coincide, but may occur at different dates.

The highest air temperatures observed at these two hours of observations were 15·8 and 19·2° C., respectively.

Ground frost as recorded 5 cm. above ground occurred only twice over the bare soil, but 8 times above the grass during the period of observations. The lowest grass-minimum temperatures recorded were in June −1·1° C., in July −0·2° C., in August −1·3° C. and in September −2·2° C.

This limited material does not allow for much discussion, but it gives some valuable indications for future work on the processes of soil formation in Iceland. It also provides additional paths for studies of ecological differences in the country's natural vegetation, but fuller information is needed before definite conclusions can be reached. Therefore further work is in progress.

BJARNI HELGASON

Department of Agriculture,
University Research Institute,
Reykjavík, Iceland.

[1] *Veðráttan* (*Bull. Meteorol. Bur. Iceland*), **1–22**; **25** (1924–45; 1948).

PSYCHOLOGY

Perceptual Defence and Filter Theory

It has been suggested by Broadbent[1] that his general theory of selective processes in the human nervous system could give an account of the phenomenon of perceptual defence. His theory assumes that information entering the central nervous system can be thought of as arriving along certain 'channels' and that a 'filter' can select one (or occasionally more) channels and suppress the input along others. He has said: "It is economical for a series of stimuli to be analysed first for simple physical properties conveying little information . . . (if) classes of words may behave in the same way as sensory channels do . . . the filter might detect that a word belongs to the general class of sexual words without distinguishing which of those words it is and pass the word to the later perceptual mechanisms for more detailed analysis if the filter is set to pass sexual words. . . . The main factual point at which evidence seems to be needed is the question whether a class of words can act in the same way as a sensory channel".

On this last point one may comment that a class of words, where class is defined by meaning only, has no physical characteristics common to all its members. Hence if the filter works on physical characteristics, as stated above, it would not be able to select such a class without selecting the individual members in it, which Broadbent was trying to avoid.

To accept his suggestion leads to the following prediction. The threshold for detecting what class a word belongs to should be lower than that for identifying individual members of that class. The present communication presents an experiment to test this.

Two lists of monosyllabic words were prepared on magnetic tape, seven words in each list. These were played to subjects at different signal-to-noise ratios. Two groups of undergraduates were used as subjects. Group A ($n = 14$) were presented with a list of colour names, and asked to make a forced choice at each signal-to-noise level as to whether the class of words was colours or animals. They were also given the second list and asked to identify the individual words on it, without being told anything about the context. The list was actually the names of clothes. This design biases the experiment in favour of the prediction, due to the effect of context on threshold[2]. Group B used the lists the other way round (clothes for class, and colours for individual, recognition). The results are shown in Figs. 1 and 2.

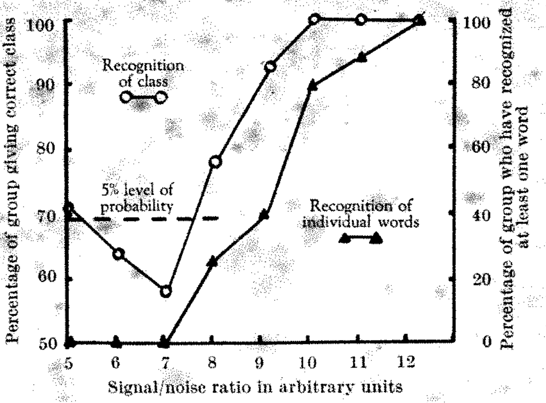


Fig. 1

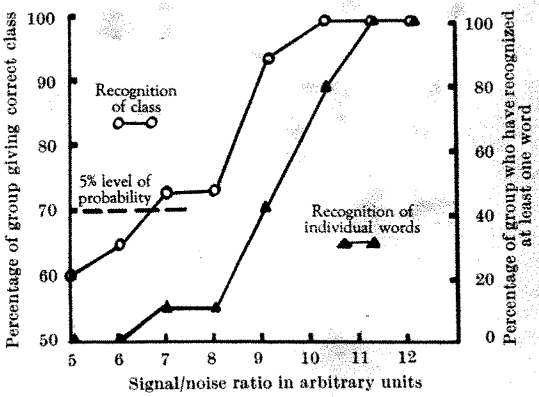


Fig. 2

For both lists the point at which the class recognition curve rises above the 5 per cent level of confidence (by the binomial test[3]) is also the point at which the first words are recognized correctly. Since contextual help was given for the class-recognition thresholds but not for the individual word-recognition thresholds, the curve for the latter is, if anything, displaced farther than it should be to the right. This finding agreed with the subjects' introspections.

Thus the prediction from Broadbent's theory is not fulfilled, and we must conclude that classes of words cannot function like a sensory channel, at least in this type of situation.

NEVILLE MORAY

Department of Psychology,
University of Sheffield.

[1] Broadbent, D., *Perception and Communication* (Pergamon Press, London, 1958).

[2] Bruce, D., *Language and Speech*, **1**, 79 (1958).

[3] Seigel, S., *Nonparametric Statistics* (McGraw-Hill, New York, 1956).

FORTHCOMING EVENTS

Wednesday, August 30—Wednesday, September 6

British Association for the Advancement of Science (at Norwich)—123rd Annual Meeting.

Wednesday, August 30

At 8 p.m.—Sir Wilfrid Le Gros Clark, F.R.S.: "The Humanity of Man" (Presidential Address).

Thursday, August 31

At 10 a.m.—Prof. D. J. E. Ingram: "From Radar to Spectroscopy—New Regions of the Spectrum" (Kelvin Lecture).

At 10 a.m.—Prof. R. G. W. Norrish, F.R.S.: "Chemistry and Spectroscopy" (Presidential Address, Section B).

At 10 a.m.—Prof. C. M. Yonge, F.R.S.: "Life and Environment on the Bed of the Sea" (Presidential Address, Section D).

At 10 a.m.—Sir Gordon Radley: "World Communications" (Presidential Address, Section G).

At 10 a.m.—Dr. H. E. Holttum: "Plant Taxonomy as a Scientific Discipline" (Presidential Address, Section K, K*).

At 10 a.m.—Sir William Slater, F.R.S.: "The Revolution in Agriculture" (Presidential Address, Section M).

At 11.15 a.m.—Dr. L. Chalk: "Wood Anatomy" (Chairman's Address, Section K*).

At 11.30 a.m.—Prof. Max Gluckman: "African Jurisprudence" (Presidential Address, Section N).

At 8 p.m.—Prof. C. D. Darlington, F.R.S.: "Why People are Different" (*Eidophor* programme).

Friday, September 1

At 10 a.m.—Dr. R. Stoneley, F.R.S.: "The Interior of the Earth" (Presidential Address, Section A).

At 10 a.m.—Prof. K. C. Dunham, F.R.S.: "Black Shale, Oil, and Sulphide Ore" (Presidential Address, Section C).

At 10 a.m.—Prof. S. H. Beaver: "Technology and Geography" (Presidential Address, Section E).

At 10 a.m.—Prof. Sir Bryan Matthews, F.R.S.: "Senses and the Sciences" (Presidential Address, Section I).

At 10 a.m.—Dr. B. E. Lawrence: "Education in Uganda" (Presidential Address, Section L).

At 11.15 a.m.—The Earl of Cranbrook: "Science and Social Service" (Presidential Address, Section X).

At 11.30 a.m.—Prof. George Seth: "From Galton to Crowther" (Presidential Address, Section J).

At 8 p.m.—Field Marshal Viscount Alanbrooke: "Some Birds of Spain" (Evening Discourse).

Saturday, September 2

At 8 p.m.—Prof. S. Tolansky, F.R.S.: "Seeing the Very Small" (*Eidophor* programme).

Sunday, September 3

At 11 a.m.—Service in Norwich Cathedral. Preacher: The Lord Bishop of Norwich, the Right Reverend Dr. Launcelot Fleming.

Monday, September 4

At 10 a.m.—Prof. C. F. Carter: "The Economic Use of Brains" (Presidential Address, Section F).

At 10 a.m.—Dr. K. P. Oakley: "Dating Man's Emergence" (Presidential Address, Section H).

At 11.30 a.m.—Dr. Richard G. Lipsey: "Can there be a Valid Theory of Wages?—A Study of Fact and Fiction in Economics" (Lister Lecture).

At 2.30 p.m.—Dr. Judith Milledge: "Form and Colour in Nature" (Darwin Lecture).

At 8 p.m.—Dr. B. V. Bowden: "The Impact of Automation" (Evening Discourse).

Tuesday, September 5

At 8 p.m.—Mr. Kenneth Adams: "Seeing in the Round" (*Eidophor* programme).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Research Chemist or Biochemist (graduate or postdoctoral), to pursue studies in the chemical dynamics of bones and teeth—Dr. S. A. Leach, Turner Dental School, The University, Manchester 13 (September 1).

Research Assistants (2) in Theoretical Physics at St. Salvator's College, for D.S.I.R. financed posts in support of research on difference equations, asymptotic expansions and perturbation theory, and on associated problems in theoretical solid state physics—particularly metals and semiconductors—The Secretary of the University, College Gate, St. Andrews (September 2).

Senior Library Assistants (2) (graduates with library experience), for duties in the Cataloguing Department—The Librarian, The Main Library, The University, Edgbaston, Birmingham 15 (September 8).

Assistant (graduate in botany or agriculture, or equivalent qualifications) to the Mycologist, for work involving testing the susceptibility to disease of crop varieties, present work is mainly concerned with the cereals—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (September 15).

Lecturer (graduate in medicine, science or veterinary science preferably with a master's degree and research experience) in Physiology at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (September 15).

Senior Lecturers (2) in Chemistry at the University of Nigeria, Nsukka—The London Representative, University of Nigeria, 33 Craven Terrace, London, W.2 (September 15).

Chemical Engineer, Physical Chemist or Physicist (honours graduate with at least two years research experience) in the Unit Operations Group, Chemical Engineering Section, C.S.I.R.O., Melbourne, to be engaged on either of the following projects: (*a*) a study of the dynamic characteristics and controllability of closed circuit grinding, and other solid-handling processes, using modern techniques including frequency response; or (*b*) investigations within the general field of water desalination—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 608/14 (September 16).

Senior Research Assistants (2) (preferably with qualifications in medicine, psychology or sociology), for a research project on the prescribing of drugs by doctors and the distribution of products by drug houses—The Registrar, The University, Liverpool, quoting Ref. CV/TES (September 16).

Senior Research Fellow or Research Fellow (with a Ph.D. degree in organic chemistry, with additional experience in general synthetic organic chemistry) in the Biological Inorganic Chemistry Unit, John Curtin School of Medical Research, Institute of Advanced Studies, Australian National University, to work on the synthesis of multidentate organic ligands, as part of the programme of the Unit on the effects and utilization of metal complexes in biological systems—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (September 18).

Chair of Sociology in the University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (September 25).

Chair of Philosophy in the University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (September 25).

Lecturer in Solid State Physics in the School of General Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 29).

Chair of Veterinary Science in the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

Experimental Officer or Senior Experimental Officer (preferably with a degree or diploma in physics or electrical engineering; previous experience of high voltage engineering, electronics and nuclear physics would be an advantage), to take charge of maintenance and operation of a 6-MeV. electrostatic generator—The Registrar, The University, Manchester 13 (September 30).

Lecturer or Assistant Lecturer (with an honours degree in psychology and preferably research interests in experimental or social psychology) in Psychology at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, September 30).

Professor of Animal Husbandry at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (London Branch), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

Professor of Microbiology at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

Lecturer in Philosophy at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 6).

Lecturer in Physiology at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 7).

Lecturer (with a degree in science or agricultural science, with experience in research and teaching in plant pathology) in Botany at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (October 31).

Chair of Psychiatry in the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (November 1).

Assistant Director (with a good knowledge of helminthology in its application to agriculture) of the Commonwealth Bureau of Helminthology, St. Albans, for duties which include abstracting, editorial work and general administration—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (November 15).

Biochemist (science graduate), to carry out biochemical tests in the area laboratory—The Group Pathologist, Group Laboratory, c/o St. Thomas's Hospital, Hydestile, Godalming, Surrey).

Director (scientist with experience of research in fibre technology) of the Jute Research Institute, Dacca, Pakistan, for duties including advice to the Government of Pakistan on research on jute and other fibres; initiating and conducting research, and lecturing—The Department of Technical Co-operation, Almack House, King Street, London, S.W.1, quoting PAK/163.

Postdoctoral Research Fellow (preferably with experience in the use of gas chromatography and/or mass spectrometry), for research in the gas phase reactors of methylene—Dr. H. M. Frey, Chemistry Department, The University, Southampton.

Postgraduate Research Assistants (with a degree in biochemistry, chemistry, physiology, physics or zoology) in the Department

OF PHYSIOLOGY AND BIOCHEMISTRY—The Secretary and Registrar, The University, Southampton.

RESEARCH ASSISTANT (with a good degree in zoology, and with an interest in the physical and chemical properties of animal tissues) **IN THE DEPARTMENT OF BIOMOLECULAR STRUCTURE**, for the study of molecular structure of biological systems—The Registrar, The University, Leeds 2.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 7: Results of Potato Variety Trials. Pp. 13. Leaflet No. 24: Liver Fluke Disease. Pp. 4. Leaflet No. 35: The Feeding of Bacon Pigs. Pp. 8. Leaflet No. 37: Turkey Production. Pp. 13. Leaflet No. 48: Beef Production. Pp. 7. Leaflet No. 49: The Cultivation of Gooseberries. Pp. 10. Leaflet No. 57: Have Eggs of First Quality. Pp. 7+4 plates. Leaflet No. 64: Warble Flies of Cattle. Pp. 4. Leaflet No. 68: The Cultivation of Strawberries. Pp. 12. Leaflet No. 73: Potatoes. Pp. 27. Leaflet No. 74: Flax Variety Trials. Pp. 4. Leaflet No. 81: The Construction of Farm Dairying Buildings. Pp. 32. Leaflet No. 88: Tomato Growing in Glasshouses. Pp. 17. Leaflet No. 19: Parasitic Worm Diseases of Farm Livestock. Pp. 4. Leaflet No. 94: The Growing and Harvesting of Flax. Pp. 6. Leaflet No. 97: Growing Field Beans. Pp. 4. (Belfast: Ministry of Agriculture, 1961.) [57

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 101: The Making of Silage. Pp. 15. Leaflet No. 108: Bee-Keeping. Pp. 14. Leaflet No. 111: Nutritional Leg Weakness in Chickens. Pp. 5. Leaflet No. 124: Weed Control in Agriculture. Pp. 20. Leaflet No. 130: The Cultivation of Lettuce. Pp. 11. Leaflet No. 138: Separated Milk and Milk Powder for Stock Feeding. Pp. 4. (Belfast: Ministry of Agriculture, 1961.) [57

Lawes Agricultural Trust. Report of the Rothamsted Experimental Station for 1960. Pp. 329. (Harpenden: Rothamsted Experimental Station, 1961.) 10*s*. [57

The British Mycological Society. Transactions—Ten Year Index, (1947–1957; Volumes 31–40.) Compiled by J. Webster. Pp. iii+119. (Winchester: Warren and Sons, Ltd., 1961.) 20*s*. [57

Bulletin of the British Museum (Natural History). Zoology. Vol. 7, No. 8: A Young *Macristium* and the Ctenothrissid Fishes. By N. B. Marshall. Pp. 353–370. (London: British Museum (Natural History), 1961.) 6*s*. [57

The Aluminium Development Association. Directory of Members, with Index of Products. Pp. vi+63. (London: The Aluminium Development Association, 1961.) [57

The Royal Society of Arts Industrial Art Bursaries Competition. Particulars for 1961. Pp. 11. (London: The Royal Society of Arts, 1961.) [57

The Institution of Electronics: Northern Division. Catalogue of the Sixteenth Annual Electronics, Instruments and Components Exhibition and Convention 1961, held in the College of Science and Technology, Sackville Street, Manchester, July 6–12, 1961. Pp. xx+150+A34. (Rochdale: Institution of Electronics, 78 Shaw Road, 1961.) [57

Department of Scientific and Industrial Research. Building Research 1960: The Report of the Building Research Board with the Report of the Director of Building Research. Pp. iv+91+16 plates. (London: H.M. Stationery Office, 1961.) 7*s*. net. [57

Department of Scientific and Industrial Research. Torry Research Station Annual Report 1960 on the Handling and Preservation of Fish and Fish Products. Report of the Torry Research Station Steering Committee and Report of the Director of the Torry Research Station. Pp. iv+44. (Edinburgh and London: H.M. Stationery Office, 1961.) [57

Hopkin and Williams, Ltd. Organic Chemical Reagents. Monograph No. 42: Curcumin—Reagent for Boron. By Eileen A. Johnson and W. Jablonski. Pp. 4. (Chadwell Heath, Essex: Hopkin and Williams, Ltd., 1961.) [57

Gowland Hopkins: a Memoir on the Discoverer of Vitamins. By Prof. Ernest Baldwin. Pp. iii+26. (London: Van den Berghs, Ltd., 1961.) [57

Department of Scientific and Industrial Research. Research for Industry 1960: A Report on work done by Industrial Research Associations in the Government Scheme. Pp. iv+148+12 plates. (London: H.M. Stationery Office, 1961.) 8*s*. net. [57

Colonial Office. Commonwealth Research Publications, No. 25: A Study of the Thomson's Gazelle (*Gazella thomsonii* Gunther) in Tanganyika. By A. C. Brooks. Pp. vi+147. (London: H.M. Stationery Office, 1961.) 13*s*. 6*d*. net. [57

Jackson's Millboard and Fibre Co., Ltd. Directors' Report and Accounts, 1961. Pp. 11. (Bourne End, Bucks: Jackson's Millboard and Fibre Co., Ltd., 1961.) [57

Memoirs of the British Astronomical Association. Vol. 39, No. 1: Apparitions of Jupiter, 1956 to 1958. Pp. 32+12 plates. (Hounslow West, Mddx.: British Astronomical Association, 1960.) [57

The Rowett Research Institute. Collected Papers: Summary and Subject Reviews, Vol. 17. Pp. iv+63. (Bucksburn, Aberdeen: The Rowett Research Institute, 1961.) [57

Royal Observatory Bulletins. No. 25: Three-Colour Photoelectric Photometry with the Cape Refractors. By A. W. J. Cousins, O. J. Eggen and R. H. Stoy. Pp. ii+E39–E57. 4*s*. net. No. 26: Photoheliographic Results, 1957. Pp. i+C89–C184. 9*s*. 6*d*. net. No. 28: Mechanization of Spherical Harmonic Analysis. By B. R. Leaton and S. R. C. Malin. Pp. i+D319–D328. 2*s*. 6*d*. net. No. 29: Three-Colour Photometry of Red Variables. By O. J. Eggen. Pp. i+E73–E90. 4*s*. net. No. 30: The Double-Lined Spectroscopic Binary HD 133822, with Notes on Methods of Investigation. By D. S. Evans. Pp. E91–98. 2*s*. 6*d*. net. No. 31: The Period-Colour Relation for Contact Binaries. By O. J. Eggen. Pp. E99–E118. 4*s*. net. No. 32: Time Service 1960, April–June. Pp. B21–B40. 4*s*. net. (London: H.M. Stationery Office 1961.) [57

Other Countries

Commonwealth of Australia: Department of External Affairs. Australian National Antarctic Research Expedition. A.N.A.R.E. Reports, Series D, Vol. 10: Meteorology—Mawson, Davis, Taylor and Macquarie Island, 1957. Pp. 277. (Melbourne: Antarctic Division, Department of External Affairs, 1961.) [226

Indian Forest Bulletin (New Series). Entomology. No. 231: Insect Borers of Newly Felled Timbers and their Control. Part 4: The Sillari Investigation of 1953–1955. By M. L. Roonwal, P. N. Chatterjee and R. S. Thapa. Pp. 7. (Delhi: Manager of Publications, 1961.) Rs. 0.75nP.; 1*s*. [226

Forschungsberichte des Landes Nordrhein-Westfalen. Nr. 975: Über die Herstellung von Kernspuremulsionen. Von Prof. Dr. A. Nareth. Pp. 36. (Köln und Opladen: Westdeutscher Verlag, 1961.) 11.50 D.M. [226

Institut pour la Recherche Scientifique en Afrique Centrale, Bruxelles. Douzième Rapport Annuel, 1959. Pp. 253. (Bruxelles: Institut pour la Recherche Scientifique en Afrique Centrale, 1961.) [57

Annals of the New York Academy of Sciences. Vol. 91, Article 4: Automatic Process Monitoring. By Sidney Siggia and 10 other authors. Pp. 819–935. (New York: New York Academy of Sciences, 1961.) [57

Smithsonian Miscellaneous Collections. Vol. 142, No. 3: Some Osteological Features of Modern Lower Teleostean Fishes. By Prof. William A. Gosline. Pp. ii+42. (Publication 4458.) (Washington, D.C.: Smithsonian Institution, 1961.) [57

Calouste Gulbenkian Foundation. Chairman's Report, 20th July 1955–31st December 1959. Pp. xvii+201+17 plates. (Lisbon: Calouste Gulbenkian Foundation, 1961.) [57

Ontario Research Foundation. Annual Report, 1960. Pp. 46. (Toronto: Ontario Research Foundation, 1961.) [57

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 63: Cretaceous Rocks in the Region of Liard and Mackenzie Rivers, Northwest Territories. By D. F. Stott. Pp. ix+36 (4 plates). 75 cents. Bulletin 69: Design of a Curie Point Meter. By A. Larochelle. Pp. vii+18. 50 cents. Paper 60–32: Buries Valleys in Central and Southern Alberta. By A. MacS. Stalker, Pp. ii+13. 50 cents. Paper 61–6: The Sequence of Marine Triassic Faunas in Western Canada. By E. T. Tozer. Pp. ii+20. 25 cents. (Ottawa: Queen's Printer, 1961.) [57

A Collection of Papers of the Rational Theory, Book X2. By F. Harrison Stamper. Pp. 12. (Los Angeles F. Harrison Stamper, 1990 Barnett Road, 1961.) [57

Forschungsberichte des Landes Nordrhein-Westfalen. Nr. 904: Untersuchung über die Vorgänge in Feststoffbeladenen Gasströmen. Von Dr.-Ing. Otto Adam. Pp. 165. (Köln und Opladen: Westdeutscher Verlag, 1960.) [57

Fiskeridirektoratets Skrifter. Serie Havundersøkelser. (Reports on Norwegian Fishery and Marine Investigations.) Vol. 12, No. 10: Phytoplankton Distribution on the Norwegian Sea in June 1952 and 1953. By Einar Ramsfjell. Pp. 112. Vol. 12, No. 11: Phytoplankton Distribution in the Norwegian Sea in June 1954, related to Hydrography and Compared with Primary Production Data. By E. Paasche. Pp. 77. (Bergen: A. S. John Griegs Boktrykkeri, 1960.) [57

British Territories in Borneo: Geological Survey Department. Memoir 11: The Geology and Mineral Resources of the Lower Rajang Valley and Adjoining Areas, Sarawak. By Dr. E. B. Wolfenden. Pp. xiii+167+39 plates. (Kuching: Government Printer, 1960.) 6 Malayan dollars; 14*s*. [57

United States Department of the Interior: Geological Survey. Topographic Instructions of the United States Geological Survey. Photogrammetric Rectification: Instructions for Preparing Equivalent Vertical Prints from Tilted Aerial Negatives—a Discussion of the Theory of Rectification. Book 3—Mapping Procedures; Part 3C—Photogrammetric Mapping; Chapter 3C3—Photogrammetric Rectification. Pp. viii+42. (Washington, D.C.: Government Printing Office, 1961.) 40 cents. [57

Annals of the New York Academy of Sciences. Vol. 91, Article 3: Genetic Perspectives in Disease Resistance and Susceptibility. By Henry A. Wallace and 15 other authors. Pp. 595–818. (New York: New York Academy of Sciences, 1961.) [57

Mellon Institute Pittsburgh. Annual Report of the Institute for the fiscal year ended February 28, 1961. Pp. 43. (Pittsburgh: Mellon Institute, 1961.) [57

Chicago Natural History Museum. Fieldiana: Zoology. Vol. 35, No. 8: Notes on Nepal Birds. By Robert L. Fleming and Melvin A. Traylor. Pp. 441–487. (Chicago: Chicago Natural History Museum, 1961.) 1.25 dollars. [57

Kungl. Skogshögskolans Skrifter. (Bulletin of the Royal School of Forestry, Stockholm.) Nr. 35: Kronhjortens Skadegörelse på Granskog: Examensarbete vid Skogshögskolan i ämnet Skogszoologi med Viltvård Utfört ar 1959. Av Åke Sjöström. Pp. 36. 5 kr. Nr. 37: Some Chemical Properties of the Humus Layer in Swedish Natural Soils. By Hugo Sjörs. Pp. 51. 7 kr. Nr. 38: Accumulation and Distribution of Phosphorus in Pine Seedlings (*Pinus silvestris* L.) By Göran Lundeberg. Pp. 26. 4 kr. (Stockholm: Skogsbiblioteket, Skogshögskolan, 1961.) [57

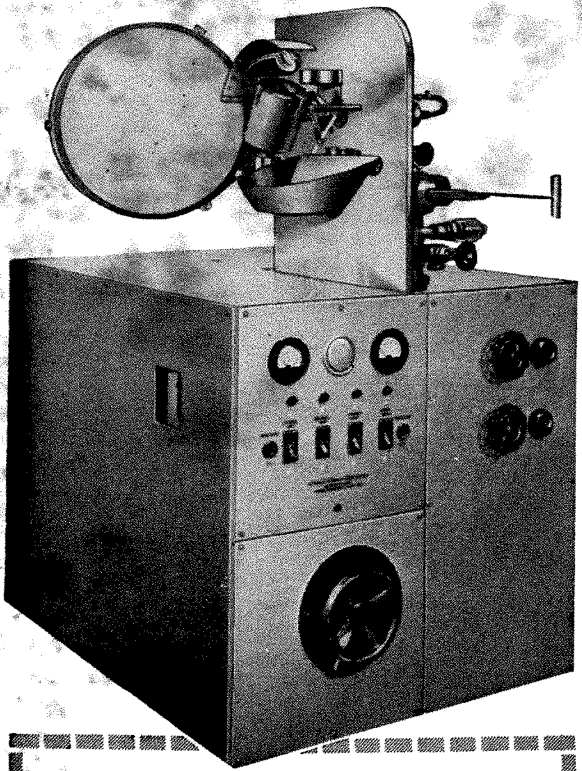
Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office

WILD BARFIELD NRC*

Laboratory Vacuum Furnace



Model 2904

2 lb. to 8 lb. capacity

(FOR OPERATION UP TO 2,000°C)

Made in Britain to the designs of the National Research Corporation of Mass., U.S.A. who have built more high vacuum furnaces now in successful operation than any other firm in the world. The Model 2904 Vacuum Furnace is a self-contained unit for high-temperature operation at high vacuum or under controlled atmosphere. It is heated by a tungsten resistance heating element with molybdenum heat shields in a water-cooled stainless steel sleeve.

This permits:

- Close control of temperature
- Good heat distribution
- Long life
- No outgassing or contamination from refractories

FOR MELTING • POURING • SINTERING
ALLOYING • HEAT-TREATING • ANNEALING

Vacuum Arc and Vacuum Induction Furnaces
Vacuum Analysis Equipment • Vacuum Diffusion Pumps

STANDARD EQUIPMENT

- High vacuum pumping system with Wild-Barfield-NRC oil diffusion pump and Gas Ballast mechanical pump.
- Metal cabinet base, overall dimensions 35″ x 43″ x 34″ high.
- Pyrex bell jar, 18″ diameter x 12″ long, protected with a wire mesh guard, or optionally a stainless steel bell jar.
- Water-cooled basin to shield the bell jar and to catch spilled metal.
- Feed-through for thermocouple wires or heater leads for special work.
- 15 kW. power supply, controlled with a variable auto-transformer. Power is supplied to the split tungsten sleeve through water-cooled coaxial leads.

* NRC *is the trade-mark of the National Research Corporation, registered in the United States Patent Office.*

WILD BARFIELD

ELECTRIC FURNACES
FOR *ALL* HEAT-TREATMENT PURPOSES

WILD-BARFIELD ELECTRIC FURNACES LIMITED

ELECFURN WORKS, OTTERSPOOL WAY, WATFORD BY-PASS, WATFORD, HERTS. TELEPHONE: WATFORD 26091 (8 LINES)

NRC8

APPOINTMENTS VACANT

UNIVERSITY OF ST. ANDREWS

Applications are invited for a Lectureship or Assistantship in Psychology in the Department of Psychology, Queen's College, Dundee, in the University of St. Andrews, commencing October 1, 1961, or as soon as possible thereafter. Salary scales: Lectureship, £1,050 by £50 to £1,400 (efficiency bar) by £75 to £1,850 (initial appointment in lower part of scale); Assistantship, £800 by £50 to £950. F.S.S.U., family allowance and grant towards furniture removal expenses.

Applications (six copies, naming three referees), to be lodged not later than September 7, 1961, with the undernoted, from whom further particulars may be obtained.

SECRETARY OF THE UNIVERSITY.
c/o Queen's College, Dundee.

R.R.E. COLLEGE OF ELECTRONICS

MINISTRY OF AVIATION
ROYAL RADAR ESTABLISHMENT
MALVERN

Lecturer required, owing to expansion of courses, for January 1, 1962, to teach Physics and Mathematics for Higher National Diploma Courses. Applicants should have a degree or equivalent qualification, and have teaching or other suitable experience. Burnham salary scale £1,370 by £35 to £1,550 for Lecturers; subject to the Teachers' Superannuation Acts.

Further details and application forms obtainable from the Principal for return within two weeks of the appearance of this advertisement.

AUSTRALIAN NATIONAL UNIVERSITY

CANBERRA
INSTITUTE OF ADVANCED STUDIES
DEPARTMENT OF ASTRONOMY

The University proposes to make three appointments in the Department of Astronomy (Head of Department: Professor B. J. Bok, Mount Stromlo Observatory). Each appointment will be made at one of several levels between those of Senior Fellow and Research Fellow, dependent on the qualifications and experience of the appointee. The salary ranges are: Senior Fellow, £A.3,000 to £A.3,550; Fellow, £A.2,120 to £A.3,000; Senior Research Fellow, £A.2,620 to £A.3,160; Research Fellow, £A.1,625 to £A.2,450. Superannuation is on the F.S.S.U. pattern. Reasonable travel expenses are paid and assistance with housing is provided.

A summary of conditions of appointment and descriptive papers setting out the research programme and facilities of Mount Stromlo Observatory and the Department of Astronomy, A.N.U., are available from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications will be considered immediately after June 1 and October 1, 1961.

UNIVERSITY OF MELBOURNE

OLYMPIC CONSOLIDATED INDUSTRIES LTD.
POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for the above fellowship in the Chemistry Department of the University of Melbourne. The research will be on physico-chemical investigations of the surface chemistry of organic polymers of interest to the tyre industry. The salary is in the range £A.1,250 to £A.1,500. Tenure one, two or three years. Tourist sea fare to Melbourne will be paid to an overseas fellow.

Entries should be lodged with the Dean of Graduate Studies, University of Melbourne, Parkville, N.2, Victoria, Australia, by November 30, 1961, and the Fellow is expected to assume duty early in 1962. Further particulars may be obtained from the Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

COMMONWEALTH BUREAU OF PASTURES and Field Crops, Hurley, Berkshire, requires Indexer (female). Duties: indexing literature on agricultural science; proof reading; compilation of bibliographies. Qualifications: knowledge of scientific terminology; must be conscientious, methodical, and accurate. Salary in scale £443 to £989. Superannuation scheme. Age, qualifications and experience considered when fixing starting salary.—For further details and application form apply to Director. Closing date September 4, 1961.

UNIVERSITY OF MANCHESTER

TURNER DENTAL SCHOOL

Applications are invited for a research Chemist or Biochemist, graduate or postdoctoral, to pursue studies in the chemical dynamics of bones and teeth. The work in progress involves various aspects of the mechanisms of calcification and demineralization and there will be ample opportunity for an individual approach to these problems. This is a Medical Research Council Staff Appointment and the salary and conditions are similar to those of University staff of similar qualifications and experience.

Further details may be obtained from Dr. S. A. Leach, Turner Dental School, the University, Manchester, 13, to whom applications should be addressed not later than September 1, 1961.

NEW ZEALAND

SENIOR RESEARCH FELLOWSHIPS

Applications are invited for Senior Research Fellowships to be taken up in New Zealand in 1962. Applicants should possess a Ph.D. degree, or an equivalent qualification, or whilst not having such qualification have a distinguished record of scientific research. There is no age limit and no restriction regarding nationality, but successful applicants must meet New Zealand immigration requirements. A good working knowledge of English is required.

The Fellowships will be awarded for research at any of the branches of the New Zealand Department of Scientific and Industrial Research. They will be tenable for periods of between six months and one year initially, but may be extended to a total period of two years. The value of each Fellowship will range between £1,400 and £1,900 a year, with an additional £250 a year for a married man. These amounts are taxable. (£N.Z.1 approximately equals £1 Sterling.) A grant for return fares by surface transport to New Zealand is payable to each Fellow, and an allowance of two-thirds of this grant may be paid where a Fellow is accompanied by his wife. While in New Zealand reasonable travelling expenses incurred in connection with the Fellow's research work will be paid.

Application forms and further particulars are obtainable from:

The New Zealand Scientific Liaison Office,
B.C.S.O.,
Africa House,
Kingsway,
London, W.C.2, England.

The New Zealand Embassy,
19 Observatory Circle,
Washington 6, D.C.,
U.S.A.

The Office of the High Commissioner for New Zealand,
Suite 804,
Commonwealth Buildings,
77 Metcalfe Street,
Ottawa, Ontario, Canada.

The Department of Scientific and Industrial Research,
P.O. Box 8018,
Wellington
New Zealand.

Applications should reach New Zealand by November 30, 1961.

UNIVERSITY OF MANCHESTER

Applications are invited for appointment of an Experimental Officer or Senior Experimental Officer to take charge of maintenance and operation of the Manchester University 6 MeV. electrostatic generator. Previous experience of high voltage engineering, electronics, and nuclear physics would be advantageous, and a degree or diploma in physics or electrical engineering is desirable, particularly for the senior appointment. Salary scales attached to this post are: £700 by £30 to £790 by £40 to £1,000 per annum (Experimental Officer); and £1,000 by £50 to £1,250 (merit bar-line) by £50 to £1,500 (Senior Experimental Officer). The initial salary will be according to qualifications and experience. Contributory superannuation scheme.

Applications should be sent not later than September 30, 1961, to the Registrar, the University, Manchester 13, from whom further particulars and forms of application may be obtained.

METALLURGIST OR PHYSICIST WITH postgraduate research experience and preferably some knowledge of ceramics, required for fundamental research work on problems associated with welding. Excellent working conditions and facilities, attractive salary according to age and experience.—Apply in writing to Research Manager, Murex Welding Processes Limited, Hertford Road, Waltham Cross, Herts.

UNIVERSITY OF MANCHESTER

Applications are invited from suitably qualified graduates for two posts in the Department of Electrical Engineering of the Faculty of Science as Lecturer and Assistant Lecturer. Candidates should have interest in any of the branches of Electrical Engineering. The salary scales are as follows: Lecturer, £1,050 to £1,850 per annum; Assistant Lecturer, £800 to £950 per annum; initial salary and status according to qualifications and experience. Membership of F.S.S.U. and children's allowance scheme.

Applications should be sent not later than September 2, 1961, to the Registrar, the University, Manchester, 13, from whom further particulars and forms of application may be obtained. Overseas applicants should send letters of application (no forms), as soon as possible, giving details of qualifications and experience, and submit the names of at least three persons to whom reference may be made.

ASSISTANT PROFESSOR OF PHYSIOLOGY

The University of Alberta invites applications for the position of Assistant Professor of Physiology, to become effective after January 1, 1962, at a salary, depending on qualifications and experience, within the range of $6,500 to $9,200 per annum with annual increments. Duties include a limited amount of undergraduate and graduate teaching, while the new staff member will be encouraged to pursue his own programme of research. The department is interested in obtaining a staff member with some training in the field of cellular physiology but this should not exclude applicants with other special fields of interest. Inquiries for further information should be addressed to Dr. J. W. Pearce, Department of Physiology.

Applications for the above position, including a curriculum vitae, the titles of publications, a recent photograph and the names of three referees should be sent to the Dean of Medicine, University of Alberta, Edmonton, Alberta, Canada.

UNIVERSITY OF EDINBURGH

AND
SOUTH-EASTERN REGIONAL HOSPITAL BOARD, SCOTLAND
MEDICAL PHYSICS UNIT

A first- or second-class honours graduate Physicist is required to apply physics to a wide range of medical problems, initially in the radioactive isotope field. A new department at present under construction will be occupied shortly. Collaboration in joint research projects will be expected and there are ample opportunities for individual research. The salary will be in the range £730 to £1,150 per annum.

Either recent or experienced graduates are invited to submit applications, with the names of two referees, to the Secretary, S.E. Regional Hospital Board, 11 Drumsheugh Gardens, Edinburgh, 3.

BRITISH CERAMIC RESEARCH ASSOCIATION

SPECIAL CERAMICS

There are several vacancies in this newly formed and expanding Division of the British Ceramic Research Association for Senior Scientific Officers, Scientific Officers and Senior Technical Officers. A good background in chemistry and/or physics is preferred, but experience in ceramics is not essential. The work covers the development of new materials, e.g., non-oxide ceramics, inorganic polymers, refractory coatings, etc., the assessment of their properties and their potentiality in electronic components, rocketry, nuclear power and general engineering. Excellent facilities are available for this work and publication is encouraged. The posts are permanent and progressive and carry superannuation provision under the Federated Superannuation System for Universities. Salary will depend on age, qualifications and experience but will be in the range of: Senior Technical Officer, £850 to £1,150. Scientific Officer, £730 to £1,150. Senior Scientific Officer, £1,200 to £1,650.

Applications, giving details of age, qualifications and experience, should be sent to the Director, Dr. N. F. Astbury, The British Ceramic Research Association, Queens Road, Penkhull, Stoke-on-Trent.

UNIVERSITY OF LEEDS, PHARMACOLOGY Department, requires a Technician. Chemical or biological experience desirable. Salary according to age, qualifications and experience, on a scale from £490 (normally at age 21) to £675.—Apply by September 2 to Professor D. R. Wood, Department of Pharmacology, School of Medicine, Leeds, 2.

THE URGENCY OF INTERNATIONAL CO-OPERATION

IN his presidential address to the British Association, printed elsewhere in this issue (p. 977), Sir Wilfrid Le Gros Clark touches on a recurrent theme in presidential addresses to the Association since, at its centenary meeting, Smuts referred to the way in which the scientific advance of mankind had outstripped ethical development and the need to close this gap. Perhaps even more noteworthy, however, are the similarities between Sir Wilfrid's address and that on the moral un-neutrality of science which Sir Charles Snow delivered at the meeting of the American Association for the Advancement of Science early this year. There can be no such thing as the ethical neutrality of science, for by the very nature of scientific activity, the scientist, with his over-riding respect for the truth, has a built-in moral impulse that imposed on him a constant moral discipline. This, and not the æsthetic values which the scientist shared with other creative workers is what really marked off scientific activity from other intellectual activities.

Moreover, this is not the only moral component for the international character of the scientific community was evident even as late as thirty years ago. Laying some stress on the personal links which then still existed within the international work of science, Sir Charles suggested that the exchanges which took place then were, and have remained, more valuable than all the diplomatic exchanges ever invented. While this position was shattered in nuclear physics with the development of the atom bomb, much of the international community of science remains in other fields such as biology, and Sir Charles considers that the moral and intellectual leadership of science may well pass to biologists and that among them we are likely to find the Rutherfords, Bohrs and Francks of the next generation.

It is at this point that Sir Charles links up the morality of the scientists with the problems of an organized society. Only a very bold man, when a member of an organized society, has the power to say no; but, recognizing this, Sir Charles nevertheless believes that scientists have a moral imperative to say what they know. The spring of knowledge itself provides a spring of moral action in scientific activity which is at least as strong as the search for truth; and, as Sir Charles sees it, this moral imperative could supply the driving force which would enable scientists to meet their plain duty in the present world situation and explain clearly the implications of the challenge which now confronts mankind.

Sir Wilfrid Le Gros Clark arrives at essentially the same position by a very different route. Believing that the dangerous uncertainties looming ahead make it essential to take stock of the species *Homo sapiens* whose evolution has culminated in mankind to-day, he believes also that it is our duty as scientists to insist that this is a matter of quite extraordinary urgency—and to make it apparent as widely as possible. The frightening question is now beginning to present itself, whether the civilization which mankind has slowly and laboriously built up over a period of many thousands of years can avoid disastrous dissolution as the result of uncontrollable or uncontrolled struggles for political power or economic superiority; and, indeed, whether the human species can avoid at least partial extinction by the misapplication of its own ingenuity.

Sir Wilfrid begins with a rapid review of how we came to be and how we are constituted in our physical and psychological adaptability to cope with our changing environment to-day. He notes that human evolution has by no means been a record of uninterrupted, uni-directional progress, and that probably the development of the distinctively human type of social organization depended in the first instance on the use of tools and weapons in co-operative activities for foraging and hunting for food and for the protection of family groups. This demanded an accelerated development of those parts of the brain whereby emotional and instinctive impulses can be more effectively subordinated to the good of the community as a whole. It is his thesis that the emergence of the biological species *Homo sapiens* has been the resultant of selective pressures directly dependent on the invention and development of increasingly complex cultural organizations. Stressing the importance of the period of immaturity during which the growing child remains dependent on the adult members of the community, thus making possible the full post-natal development of the large and complicated brain and also providing the opportunity for educating the individual to adapt himself to the intricate inter-relationships of human society, Sir Wilfrid remarks that if man (in the broadest sense) invented culture, it was culture which invented *Homo sapiens*.

Considering now man as he is to-day, Sir Wilfrid points out that an outstanding feature of our species is a broad and plastic adaptability rather than a narrow adaptedness to some particular set of conditions. He emphasizes that our biological adaptability needs to be constantly supplemented by deliberate and conscious efforts to adapt ourselves to local circumstances as they arise. There is some reason for satisfaction, so far as physical and biological environments are concerned, in the success achieved in utilizing the resources of the physical and biological sciences and the derived technological developments, by developing more effective methods of control of pests and protection against disease and by intensive studies of the physiological and psychological factors underlying the adaptational potentialities of the human body; but the related field of ergonomics, or the study in the widest context of man in relation to his working environment, has as yet not been developed to anything like the same extent.

It could well be argued that here is to be found one important factor responsible for some of our economic difficulties to-day. Sir Wilfrid recognizes that the relevance of ergonomics to industry, so far at least as the rational design of machinery and the control of the immediate climatic working environment are concerned, is widely admitted; but the factor of adaptability enters no less into such matters as redundancy, arising out of technological change through developments such as automation and the application of computers and other electronic devices. It is a matter of the adaptability of both trade unions and of management, and is clearly a more formidable task than is presented on the material plane, where, as Sir Wilfrid observes, we can feel some assurance of ultimate success, provided adequate funds and facilities are made available for investigation by scientific methods.

Quoting John Stuart Mill's observation "There is not a more accurate test of the progress of civilization than the progress of the power of co-operation", he remarks of this question of mankind's adaptation to his sociological environment that, while there has been demonstrably a broad historical trend towards a progressive unification of mankind, this has come about as a result of conflict between opposing groups, ending in the emergence of the vanquished into the social and economic structure of the victor, as well as by mutual agreement for the furtherance of common interests. This process of unification has been to the ultimate benefit of the whole community by achieving a higher level of co-operation, permitting, for example, a more centralized organization of all means of production, more efficient control over local fluctuations in food supply, more comprehensive planning of large-scale projects of irrigation and transport and more economical division of labour. It can thus be regarded as a major factor determining sociological progress; but in these larger groups of human society, Sir Wilfrid fears that there is the basic danger that, with increasing pressure of population, the altruistic sentiments which in the course of a long evolutionary history have become ingrained in the mentality of the individual are too apt to be suppressed by the narrow interests of each particular group.

There have been some unhappy demonstrations in recent years of the way in which in Britain sectionalism works against the interests of the community as a whole; and it is undoubtedly true also that in the larger groups the attitudes and opinions of the individual come to be directed for him by all the subtleties of propaganda developed in a complex society, so that we have to face the seemingly paradoxical fact that the larger the units of co-operation in social communities the greater the tendency for their segregation into populations opposed to each other by conflicting economic and ideological interests. This has, as Sir Wilfrid observes, tended to slow up the historical process of unification and threatens to halt it; but before we question whether it is even feasible to hope for a final unification of the whole of mankind, it should be remembered that some of the difficulties at the international level arise precisely because not all members of the international community have reached the same level of culture or civilization. We cannot assume the existence everywhere of the altruistic sentiments to which Sir Wilfrid refers, and this is undoubtedly one factor that threatens world order to-day.

Nevertheless, Sir Wilfrid rightly maintains that an essential prerequisite for any attempt at further unification for the good of mankind as a whole is the ability to give full expression to the impulse for co-operation which is so characteristic a feature of the humanity of man, and that this can only be made possible by opportunities for the free interchange of ideas among the population of the world. At this point Sir Wilfrid expresses his conviction that science has a vitally important part to play. Regarding science and technology as culminating expressions of the co-operativeness on which depended the genesis of the form of human society in the past, he suggests that science is in a particularly favourable position to effect the closest contacts between peoples who may be divided by political and other interests. Thus he points to the recognition by scientists of all countries of the need to retain and develop harmonious relations, because the very advance of science depends on free communication. Secretiveness is a brake on such advance, because it prevents the unrestricted interchange and interpenetration of ideas among those engaged on common problems.

Sir Wilfrid thus recognizes this pattern of unity and co-operation among scientists even in the face of circumstances which often appear frustrating, and he asserts indeed that the quintessence of the scientific mentality is to be found in a willingness to communicate and discuss new advances in knowledge with completely open-minded freedom, uninhibited by any attempt at secrecy for partisan or sectional interests of any kind. Moreover, he finds that the universality in the logic of the scientific method permits an intimacy of understanding scarcely possible in fields of learning where there are fundamental conflicts of opinion derived from preconceived convictions and beliefs, based on unverifiable evidence and not amenable to scientific methods of inquiry by the rigorous test of repeated observation and experiment. How far this profound respect for objective truth, which may be regarded as intrinsically a self-regulating process, imposed on the scientist by the very nature of science, contributes to the promotion of a sense of toleration and intellectual humility may be a matter of opinion and is not discussed either by Sir Wilfrid Le Gros Clark or Sir Charles Snow; but there can be no gainsaying the points they make about co-operativeness being an essential element in the pursuit of science, and the responsibility of the scientist to explain the implications of advances in scientific knowledge. There may be reservations as to the extent to which international scientific associations can and do influence mankind generally, particularly in the face of national conflicts, but there can be no question as to the value of associations which are wholly international in achieving full international unity. Furthermore, even where regional research institutions and other regional institutions supplement national institutions in the study of problems of common interest, membership should, as Sir Wilfrid insists, be open unconditionally to competent scientists of any community whose welfare depends on the results of the institution's research projects.

The process of dissipating tendencies towards national rivalry and engendering an atmosphere of concord may be slow—much slower than Sir Wilfrid suggests—though he rightly points out that time is getting very short. That is not, however, to belittle the importance of scientists seeking to inculcate as widely as possible a spirit of freedom in scientific inquiry which gives full expression to the deep-rooted altruism; such altruism is an essential attribute of the humanity of man, and can possibly do much to preserve and intensify the feeling of harmony among all peoples of the world. It is rather that it strikes a fresh note of urgency in the task, particularly where it involves also fostering similar altruism among some of the less-developed and emergent nations. A concrete objective is, in fact, set before the British Association, and also associations of professional scientists and technologists, namely, to make plain, in season and out, to the individual citizens and the communities to which they belong, the probable impact on everyday life of new scientific and technological discoveries. This involves demonstration of the principles and methods of scientific inquiry by which discoveries have been made, with the inferential corollary that the same principles and method can also be applied, with some assurance of success, to other outstanding problems of a similar nature. Whether or not there is still time to complete the task, it is essential that it should be undertaken wholeheartedly and urgently. Full co-operation by all scientists of all races is essential; they should address themselves to the study of those specific problems in ergonomics and other fields which bear on co-operation in industry and in society, and the way men and women live together in communities in an era of rapid and often bewildering technological change.

VERTEBRATE PHYLOGENY

Théories de l'Évolution des Vertébrés

Reconsidérées à la Lumière des Récentes Découvertes sur les Vertébrés Inférieurs. Par Erik Jarvik. Préface et traduction du Prof. J. P. Lehman. (Monographies Scientifiques.) Pp. 104. (Paris : Masson et Cie, 1960.) 20 N.F.

IN the University of Stockholm at about the turn of the century, when the Department of Zoology acquired new premises they renamed it Zootomiska Institutet. This was to emphasize that the zoology studied there was not 'mere systematics', but the science of the detailed and comparative structure and development of animals; and perhaps nowhere has research been so faithful to this ideal. What has especially appealed to the Stockholm school is the development in ontogeny and phylogeny of the rhythmic plan of vertebrate segmentation and encephalization. While Holmgren, Pehrson and others made their discoveries on the living species, Stensiö and his school have been able to test the conclusions based on comparative anatomy and embryology by direct anatomical studies of fossil vertebrates.

The technique invented by Sollas—grinding and photographing, grinding again and photographing, until the specimen is destroyed, leaving a record of its structure from which wax models can be made—has been used to such effect by these dedicated workers that we know more about the detailed structure of some of the early chordates than about many a living species.

Stensiö's first work of this kind, on *Cephalaspis* (1927), was a great event. I remember his lectures on the subject at University College, London, when in deliberate, matter-of-fact tones he said of this palæozoic animal: "And so, you see, we are forced to the conclusion that this animal possessed a functional pronephros".

Jarvik, himself a distinguished contributor to this work, has written a little book summarizing such results of these many studies as have had most influence on our interpretation of the relationships of the classes and subclasses of lower vertebrates, and on the course of evolution within the series.

We have come to accept that the cleft between lampreys and hagfishes, based largely on the naso-hypophyseal relationships, represents the palæozoic cleft between Cephalaspidomorphs and Pteraspidomorphs, as Stensiö concluded in 1927. The discoveries on the Placoderms have not yet been incorporated into our text-books. Parker and Haswell in their *Text-book of Zoology* (Forster-Cooper's edition) too hastily accepted the conclusion of Watson that the Acanthodii possessed a ventral hyoidean gill-slit. Anyone acquainted with a range of Teleostean forms would know that the presence of a single row of gill-rakers on the ceratohyal was no argument for this, and Holmgren pointed this out in 1941 with additional evidence against Watson's theory. Stensiö has found both Arthrodires and Antiarchi to have a great deal in common with the Selachii, so that he unites them broadly as 'Elasmobranchiomorphi'. No one has yet used the Sollas technique on an Acanthodian, so this group is dismissed by Jarvik as *mal connu*.

Jarvik lays much emphasis on the results of his own work on Crossopterygii, which, he concludes, were already divided into two groups ancestral to Anura and Urodeles respectively. This conclusion is based mainly on the structure of the snout, analysed in detail in his work of 1941–42. It is an example of the way in which the new work has pushed divisions back further, so that the dotted lines of the phylogenetic tree, which once joined to form a main trunk, now vanish back into the unknown. On the other hand, Jarvik has allowed himself a theoretical tree in which, with the help of a few hypothetical groups, there are no dotted lines at all.

Uniformity of basic plan is re-emphasized, and one of the most interesting and elegant results is the inclusion of the dermal bones of the base of the skull in the segmental pattern of the visceral arches. *Latimeria* does not receive extended mention partly because this book is a kind of supplement to the corresponding parts of the *Traité de Zoologie*, where this Cœlacanth receives adequate attention; but perhaps partly too because Millot and Anthony wrote their first fine volume on its anatomy without disclosing the condition and course of the profundus nerve, a great disappointment to the Swedes, since it has an important bearing on the interpretation of the homologies of the pre-mandibular somite.

The concept of de-lamination (why not supra-lamination ?) and the lepidomorial theory, the use of the course of the lateral-line canals rather than the pineal to determine the homologies of the roofing bones of the skull and the abandoning of the theory of a wide osteogenic influence of the neuromasts, are acceptable generalizations which seem overdue. The origin of the paired fins by concentrations of originally broad-based segments of the fin folds receives new support from Stensiö's work on the Arthrodires in which (*mirabile dictu*), as in the Cephalaspids, the course of nerves, arteries and veins was preserved as hard encasing tubules. But the opposing theory quoted (to be demolished) by Jarvik, that the two paired fins were formed by the growth behind two of the spines of the ventro-lateral series in *Acanthodes* of a web, rays, muscles, etc., is cruder than any which I have ever come across.

The Swedish original of this timely and challenging book is one of a score of articles in *Svensk Naturventenskap 1959*, year-book of the Swedish national council for research in the natural sciences, and the translation was published without giving Dr. Jarvik an opportunity to equip it for its new purpose. Necessarily condensed and somewhat arbitrary, it drives the reader for evidence to the original papers, yet lacks a bibliography. The absence of an index is partly compensated by a good list of contents. There are few misprints (a full stop where there should be a colon in the middle of p. 31 is an awkward one). The many illustrations, including photographs and colour-diagrams, probably account for the expensiveness of this handsome paper-back, which should nevertheless be studied by every serious student of vertebrate morphology and phylogeny.

To most of us the French version is very welcome, but anyone with a reading knowledge of Swedish would have a bargain in the original, well bound, with two additional colour-plates of Jarvik's models and a number of articles on diverse subjects, all for the much lower price of 18 Swedish crowns.

E. TREWAVAS

SOCIAL HISTORY OF ENGINEERING

A Social History of Engineering

By W. H. G. Armytage. Pp. 378. (Faber and Faber, 1961.) 42*s*.

PROF. ARMYTAGE'S volume is the first of a series which, we are told, is designed primarily for candidates for the diploma in technology, but also for the professional scientist and engineer. The dust-jacket also tells us that the series "has been planned to help the specialist take account of the economic, social and political implications and consequences of his work and influence in society at large". Books written to this recipe are of course badly needed. The history of technology, edited by Dr. C. Singer, aroused great hopes which the published volumes have not wholly fulfilled. Their bulk and cost alone would have made them unsuitable for use by the large numbers of young men interested in the subject, or compelled to study it.

For the success of this new series the present volume augurs neither badly nor well. Books like this must serve two distinct objects—to provide a factual record of technological development in the past and to relate the development to changes in society. The two tasks require from the author a combination of aptitudes and of equipment not commonly to be found together; and it would not therefore have been surprising or unforgivable had Prof. Armytage succeeded in one of his tasks better than in the other. But this is not what Prof. Armytage has achieved. He has come within sight of both his objectives, yet the distance by which he has failed to reach either would be sufficient to damp the enthusiasm of the most indulgent of reviewers.

As a historical record the book suffers from an excess of that very quality against which Prof. Armytage warns us in his preface, that is, packaged history. Technical facts, scientific and engineering terms follow each other at the double quick. There are about 1,200 proper names in the index, and this contains only some of the names which pepper the pages. Moreover, it is precisely because he tries to tell so much that he often fails to tell us enough. Lack of space has prevented him from explaining the nature of many of the inventions and innovations and processes which he enumerates, with the result that some of the pages would be largely incomprehensible to an average student, be he even a student of technology. Now and again, Prof. Armytage is compelled to compress his facts to the point of distortion. His story of the improved harnessing of animals in the Dark Ages is told as if it concerned the replacement of oxen with horses, whereas its main achievement was greater efficiency in the use of oxen. The introduction of the open-field system in the same period is described as if it originated in the eighth century and was all organized on a three-field system from the outset. To pass to the other end of the spectrum, Prof. Armytage refers to *Zeta* as if it were a technical success, and credits Prof. Jewkes with a proposition concerning the contribution of large firms to inventions, which is exactly the opposite of the views which Prof. Jewkes is commonly believed to hold.

For the same reasons, Prof. Armytage is sometimes compelled to gloss over the technical trends which, if brought out, might have introduced order and coherence into his story of individual inventions. Thus, the technological progress in European mining is described as if it began with certain vague improvements in the 1450's. The reciprocal connexions between technical innovations in the textile industries of the eighteenth and the early nineteenth centuries receive no more than a passing reference; the linked developments in agricultural engineering are scarcely mentioned. The story of aircraft between the Wars leaves the reader unaware of the process whereby in the early thirties there emerged the first monoplanes with stressed skins, retractable undercarriages and monocoque structures. The story of radar glosses over the scientific and technical developments which proceeded and followed Watson-Watt's initiative, and there is scarcely a word in the book about Appleton, Oliphant or Roe. In the story of engineering proper nothing is said about progressive changes in working speeds and tolerances and the consequent evolution of machine tools, or about cigarette-making machinery, scientific instruments or watches and the part they played in the development of precision and automatic tools.

Much of the book's space is of course given over to non-technological matter brought in to supply the social background. Some of these data are highly relevant and valuable. Prof. Armytage's account of technical education, his story of the higher engineering schools abroad, and above all, his account of the connexions between individual engineers, who worked with whom, who established the nurseries of engineering talent, how and when the engineers began to collaborate and the professional institutions were established—all this had never been brought together so briefly and so conveniently as Prof. Armytage has done in his book. This alone would justify his effort and commend it to the general body of interested readers.

Unfortunately, the same cannot be said about many of the other non-technological facts in the book. A history of technology linking it with economic conditions, social changes and intellectual movements is something which, in the present state of the literature on the subject, no single person could be expected to conjure up; and Prof. Armytage is certainly no conjuror. The only social correlation he is able to establish at all convincingly is that of war. He has no difficulty in showing how successive wars stimulated progress in applied sciences, and above all in engineering. But many of his other correlations appear to be questionable or irrelevant. What, for example, has the destruction of drainage at Passchendaele got to do with technological changes ? How does Mr. Hollerith's tea-time conversation with Dr. Billing, who had some twenty years previously collected statistics on the Army of the Potomac, establish the connexion between the American Civil War of 1861 and the invention of the Hollerith system in 1890 ? What has the enforced sales of British securities in America in the two World Wars ("led to increasing dependence on the American stock market") got to do with technological change in the same period ? Modern architecture may or may not be part of the recent history of engineering, but if it is, its history does not begin with Gropius; and even if it owed as much to Gropius as Dr. Armstrong believes, why insist on Gropius's fleeting connexions with A. E. G. and yet fail to give an adequate account of the Bauhaus. I do not believe that a writer as pressed for space as Prof. Armytage need have bothered to bring in the Indian Mutiny, but if he did, he might have taken care not to make the Moslems venerate the cow and the Hindus abhor the pig.

These are mere examples culled at random, but they should be sufficient to explain why the reviewer's verdict is bound to remain open. The book contains some valuable information, conveniently summarized for the discriminating reader, but it cannot be recommended as a sole guide for young men about to be examined in the social history of engineering.

M. Postan

UNIVERSITY SELECTION

The Chosen Few

An Examination of some Aspects of University Selection in Britain. By W. D. Furneaux. Pp. xxvii + 245. (London: Oxford University Press, 1961. Published for the Nuffield Foundation.) 25s.

DISCUSSION on educational topics seems to go in cycles. After a spate of discussion, both informed and uninformed on the selection of children for secondary education and a number of published researches on the reliability, validity and sociological consequences of such selection methods, it is now the turn of the universities' selection procedures to be closely scrutinized. These provide a more formidable task for the investigator; the fewer university bodies with their particular faculty requirements are often less co-operative than the 146 local education authorities with their eleven-plus selection procedures. As the author of the present book points out, this lack of co-operation is often not deliberately so, but is due to the lack of availability of records and information about students who are accepted or rejected for a university course.

This research began in 1948 under the auspices of the Nuffield Foundation and was carried out by the Nuffield Student Selection Unit of the University of London Institute of Psychiatry. Access to relevant records was not granted by Oxford and Cambridge, and the main research was centred on the University of Sheffield. There is no evidence to show that this restriction provided a satisfactory sample of university entrants. One point does emerge clearly; unless each university endeavours to build into its selection scheme some follow-up measure of the trustworthiness and validity of its selection methods, the continued wastage of time and talent will continue. Interviewing candidates as a selection instrument is still as unreliable as it was found to be by Hartog and Rhodes in their report in 1935. With increased number of university applicants and the correspondingly greatly increased wastage of time by university staff in considering applications, alternative psychological methods will have to be considered and tried out. As Furneaux says of the academic staff responsible for selection: "In general they set about their work conscientiously and with devotion, but are guided by a mixture of empiricism, tradition, and personal experience, which cannot always be expected to produce the most valid results".

According to the author, 'A' level results constitute "the only reasonable and available objective evidence", but these results do not reach the selection panels early enough and even then, because of the difficulties of assessing the value of 'essay-type' questions, are not very efficient predictors. It is pointed out that the main function of 'A' level results is to confirm "that the minimum statutory entrance requirements have been complied with"; however, the recent report by J. A. Petch does show that the General Certificate of Education examination has some predictive value. One rather gathers that preliminary acceptance of a student by a university owes a great deal to the skill shown by the headmaster in the writing of his report, nevertheless, Furneaux believes that more use might be made of these headmasters' reports, if the universities would use a more precise method such as a questionnaire for eliciting relevant information.

This report underlines all the known difficulties of student selection and criticizes, at least by implication, the waste of time and effort on students' multiple university applications; it points out that there seems to be no clear evidence that the standards achieved by students admitted to a university and those applicants who were rejected by this university and admitted to another, differ in any marked way.

Points raised in Part 1 of this study relate to multiple applications, sociological background of students as a determinant of selection and wastage of talent. Part 2 deals more specifically with the Nuffield investigation. This is a useful source book, though finding one's way through the various facts and arguments, often repeated and expanded in various sections of the book, is not too easy even with the help of the index; this may be due to the ten-year time-lag between the initiation of this study and its final publication. However, few will disagree with the main points of the author's thesis, and his plea for some form of clearing-house system is timely.

Psychologists and sociologists will look forward to the second volume, and now that Mr. Furneaux has dealt with the background of selection he will be free to deal with the ways in which psychological tests and procedures can be of use in university selection.

W. H. KING

THE PHYSIOLOGY, PHARMACOLOGY AND PATHOLOGY OF MUSCLE

The Structure and Function of Muscle

Edited by G. H. Bourne. Vol. 2: Biochemistry and Physiology. Pp. xiv + 593. 118*s*. Vol. 3: Pharmacology and Disease. Pp. xiv + 489. 107*s*. 6*d*. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.)

THIS treatise on muscle is completed by the present volumes. In Volume 2, the first two chapters, on the chemistry of myofibrillar protein and on the biochemistry of muscular action, set a high standard. Chapter 3 gives an account of the biochemistry of muscle mitochondria, and is followed, in Chapter 4, by an essay on endocrinology with reference to muscle. Chapter 5, entitled, "The Neuromuscular Junction: Role of the Acetylcholine System", is a discursive review of some aspects of conduction, neuromuscular and synaptic transmission. Chapters 6 and 7 give a concise account of the biophysics of muscle, and are followed, in Chapter 8, by an excellent survey of the comparative physiology of the activation of muscle. The next two chapters are essays on human muscular physiology, with emphasis on factors limiting performance and on fatigue. Chapter 11 is a full and balanced review of the central nervous control of skeletal muscle, which covers the substantial advances made in the past two decades. This volume is concluded by a stimulating chapter on certain aspects of the physiology of cardiac muscle.

It is regrettable that much space has been given, in Volume 2, to marginal topics, such as endocrinology and the biochemistry of sarcosomes, apparently at the expense of an adequate treatment of the physiology of smooth and cardiac muscle. A disappointing feature of the book is the section on neuromuscular transmission, which unduly emphasizes the personal views of the author. Fortunately, these faults are offset by the uniformly high standard of most contributions to this volume, which can be recommended to the advanced student and teacher as a useful account of many of the recent advances in the biochemistry and physiology of muscle.

In Volume 3 two chapters are devoted to pharmacology: Chapter 1 is a review of the actions of drugs on smooth and skeletal muscle; in Chapter 2 some aspects of the effects of drugs on the myocardium are discussed. Virus infections involving muscle are described in Chapter 3, which is followed by a chapter on the parasitic infections of muscle, with emphasis on the widespread prevalence of sarcocystic infestation. An illustrated account of the degeneration and regeneration of skeletal muscle in Chapter 5 is succeeded by chapters on the effects of nutritional deficiencies on muscle, and on the histological changes in muscle due to ageing. Chapter 8 is an authoritative review of recent advances in the study of post-mortem changes in muscle, with especial reference to rigor mortis. The histochemistry of muscle is dealt with in Chapter 9. The pathology of the intrinsic diseases of muscle is described in Chapter 10, which is followed by a chapter on the clinical features of these diseases. Chapter 12 is an interesting discussion of the genetic aspects of muscular disease. A brief but stimulating essay on some fundamental problems of muscle biochemistry concludes the work.

The article on the pharmacology of skeletal and smooth muscle is outstanding, in Volume 3, in its conciseness, and will be of great value to the general reader. It seems remarkable that, in the treatment of pathology, parasitic and virus infections are considered in some detail, but no mention is made of Clostridial infections. The quality of the histological illustrations is not uniformly satisfactory: many are too small and suffer from poor definition. Nevertheless, this volume may particularly interest clinicians, and should focus attention on a field which has hitherto been rather neglected. D. H. SPROULL

ECTO- AND ENDO-PARASITES OF SOUTH-EAST ASIA

Malaysian Parasites

XXXV–XLIX. Edited by W. W. Macdonald. (Studies from the Institute for Medical Research, Federation of Malaya, No. 29.) Pp. vii + 251. (Kuala Lumpur: Institute for Medical Research, 1960.) 9 Malayan dollars; 21*s*.; 3 U.S. dollars.

THIS is the third volume of a series of collected papers on the ecto- and endo-parasites (including biting insects and arachnids) occurring in south-east Asia. It contains two long papers, both by C. B. Philip and W. W. Macdonald, on Tabanidae and Culicinae respectively, a review of the host distribution of Malayan ticks by J. R. Audy and others, and several shorter papers on mites, together with one on the rearing of ixodid ticks and another on nematode parasites of domestic rats.

Like its predecessors it is well bound, clearly and attractively printed and illustrated, and remarkable value for money. Most of the papers are for the expert, but Macdonald's paper on the ecology of mosquitoes in lowland dipterocarp forest should interest the general reader. The same author's paper on *Armigeres* sub-genus *Leicesteria* also contains much of ecological interest, with particular reference to the fauna of bamboo internodes.

It is sad to think that most of those who worked in the Institute during the publication of this series have now dispersed. Their achievements bear witness to the unique potentialities of a team of this kind. The present staff can be trusted to carry on the tradition within the limits of their resources. For the rest it is to be hoped that visiting experts will use the Institute as a centre for exploring the marvellously rich fauna of south-east Asia and the many intricate and still little understood problems of human ecology which the area presents.

P. F. MATTINGLY

ART AND THE ENGINEER*

By PROF. MISHA BLACK, O.B.E.

School of Industrial Design (Engineering), Royal College of Art,
London, S.W.7

NEGLECTING the massive contrary evidence, many engineers continue to console themselves for a lack of creative ability by assuming, and constantly reiterating, that form inexorably follows function and thus that a solely scientific or technological approach to engineering produces forms which are visually so satisfactory as to eliminate the need for conscious æsthetic endeavour. The protagonists for this view point with pride to high-speed passenger aircraft as proof of their theory, and extend the range of their evidence by mustering the hulls of ships, the control rooms of power stations, electronic equipment and the mechanism of intricate machinery to their support.

One must reluctantly counter these admirable bids with the lamp standards which still destroy the visual amenities of a thousand streets, most rail and road bridges built since 1900 and the myriad consumer and capital products which have gone far towards destroying the pleasures of city life and have eroded the rural scene.

This balancing of the good against the unsuccessful would be an amusing game if the stakes were less than our total environment, which they in fact represent.

Thus I am producing these over-simplified arguments which before now should have been transferred to a more serious level of discussion. Those who might be expected to represent the opposing views have long past composed their differences. Sir Herbert Read (whose loyalty to the arts is unquestioned) wrote in 1934[1]: "One false theory assumes that if the object in question performs its function in the most efficient way possible, it will *ipso facto* possess the necessary æsthetic qualities. To this argument we must reply that an object which functions perfectly may, and probably will, possess æsthetic qualities, *but the connexion is not a necessary one*".

The late Oscar Faber (whose engineering experience is beyond dispute) said in 1944 (in a paper delivered at the Institution of Civil Engineers)[2]: "The fallacy, which is very widely held, is that if a structure is honestly designed to satisfy all its scientific or engineering requirements it will of necessity be beautiful . . . all I can say is that I wish it were true, because most engineers can design a structure scientifically with a constant factor of safety in all its component parts, and if that were a guarantee that the result would be beautiful the problem would indeed be a simple one. Unfortunately, all that one can say about it is that such a structure may by chance be beautiful, but it will only be by chance". Yet at every conference on civic or industrial design the ancient ritual is repeated, and at least one engineer can be relied on to chant the now venerable refrain that form automatically follows function, that mechanical efficiency produces its own art form and that association with the arts is advisable only outside the drawing office and workshop.

This dogmatism on the part of some engineers has sustained the equally emotional opposition of humanitarians. They, from William Morris onwards, imagining their values threatened by developing technology, have retreated (as the forces of science are too virile and powerful for open battle) historically into the peaceful backwaters of arts and crafts and to-day into art forms which express their own despair and have meaning only for those who understand their obsessive pessimism.

The continuing vigour of the argument results from there being right on both sides. There is no doubt that the engineers of the twentieth century have produced the most memorable symbols of our time which, when examined in the perspective of future centuries, may well prove to be more important manifestations of our then past than all but a few of the more self-conscious works of our architects, sculptors and painters. But it is equally true that the majority of the works of our civil and mechanical engineers are characterized by a mediocrity only equalled by the mass of our architecture. The engrossing mystery is the unevenness of quality which can be found in the work of even a single homogeneous group of engineers, the circumstances which make it possible for the designers of a superb flying machine to produce cabin interiors redolent of a shoddy suburban hotel and for the designers of an efficient and economic street lighting system to mount the fitting on poles whose only excuse is that others are yet more disagreeable.

At the Frontiers of Knowledge

The answer to this inconsistency lies in the extent to which the engineer is working at the frontiers of knowledge. When the problem set is more technically exacting than any attempted before in that field, then the discipline of the problem, the need to probe beyond existing boundaries of knowledge, produces formal results which exactly mirror the period of history which made that technical development possible. They thus have a quality of exact correctness which is classically satisfactory. Because it was produced at the advanced headland of a civilization, it could only be materialized by men who were, for that moment, at the head of their time and through them their society expressed one aspect of its greatness. Such work crystallizes the essence of a period and, on a subsidiary plane, is comparable with the fine arts which similarly express a society annealed through the fire of a single personality.

Because such work is done at the frontiers of experience its result may not always be generally comprehensible, especially in periods of revolutionary transformation, but during the post-industrial revolution period, which is my concern here, the gap of time between achievement and public acceptance has progressively decreased. Thus to-day the creative jump forward which produced the *Caravelle* jet aircraft required no period of gestation before it was accepted as a proud symbol of the first year of this decade.

---

* Substance of a lecture delivered at Hatfield Technical College on June 23.

An appreciation of this view is obscured by the problem of early steps ahead into undeveloped technological ground when the creative probes produce unresolved intermediate solutions which appear primitive and immature in comparison with the then fully developed earlier forms which they are destined later to supersede. For example, one may remember the primitive forms of the early steamships as poor cousins to the then still extant majestic sailing vessels, the tottering early aircraft and the first motor-cars a parody of their future.

But though at times the early forms of a new technology may seem beautiful only to the enthusiast, the basic fact I think remains. Form follows function when the form is that of objects or mechanisms which are at the height of their time when the need to resolve scientific and technological problems exclude all other considerations and when the consciousness of the engineers is completely absorbed within the superhuman task of reaching ahead into the then unknown.

The purity of design which is possible in such circumstances is, however, a rare occurrence. It is hindered by the necessity for its expression at a stage of technological development which allows for mature solutions, it requires, in mechanical engineering, the opportunity for development of a completely new product instead of the more usual modification to part of an existing object or mechanism, and it postulates a problem being attempted in which the needs of human usage are of secondary importance. To this last point I shall return, but first may I complete this part of my argument by re-stating that engineer-designed objects of the type I have been describing possess an intense emotional content. If all the artefacts of our society were produced in this way, the problems we are considering and the need for this article would not exist. But they are not. The vast majority of engineering projects and products are conceived well within the boundaries of scientific knowledge and technological experience; the developments in a new project are commonly marginal—a minor step forward. In such engineering undertakings the possible variations multiply and usually reach infinity. Here conscious judgment, choice and constant decision face the engineer, and in these decisions æsthetic choice plays, as I hope to demonstrate, a major part.

The Clue to Confusion

Here is the clue to present confusion. The engineer rightly sees that the major 'break-through' works possess great beauty, and he is sometimes arrogant enough to believe that the qualities of great achievement must automatically be reflected in his more pedestrian daily tasks. He does not, in this context, accept the common knowledge that quantity affects quality, and that what operates satisfactorily at one scale does not necessarily do so when the scale is greatly increased or diminished. The beam which properly bridges a 20-ft. span should not simply increase in size when the span increases to 200 ft.—and the reverse is equally true. The engineer developing a motor-car which is only a stage in advance of last year's model is not operating at the frontiers of knowledge; the electronic engineer reducing the depth of a television tube is similarly working at a lesser pitch; the designer of a gas cooker or a Diesel locomotive is also operating at a level where application is more appropriate than vision. At this, the normal daily working level, the differences in design of objects produced to the same general performance specification demonstrate the freedom of choice which exists, within, of course, controlling technical conditions.

This is, I believe, indisputable. It is a truism which only the strange lack of its appreciation justifies repetition. I do not under-estimate the importance of the controlling factors of materials, manufacturing processes and cost; but these provide only the matrix within which other motives also determine the engineering decision. In domestic appliances the plethora of refrigerators and washing machines, for example, indicates how wide is the choice which faces the engineer even when the technical requirements have been established with reasonable exactness. The varieties of kettles, automobiles, vacuum cleaners and Diesel locomotives reveal major differences in ease of operation and of appearance which cannot be explained only by variations in specification or production facilities.

The engineer is a decision-making animal, and it would therefore be useful to examine the motivators of his decisions.

Motivators for Decisions

I believe that the majority of engineering decisions, within the boundaries of technical facility, are made from emotional and seemingly inconsequential premises. The radio engineer arranges the controls of a television set in relation to the screen mainly because he thinks 'it looks better that way': the draughtsman selects a particular form for a domestic radiator because he prefers that form to any of the thousand alternatives that exist within the specification of his task. The final appearance of a modern machine tool is as much the outcome of these proliferating individual æsthetic decisions as was the form of a nineteenth-century beam-engine elaborated with the Grecian columns and the cast-iron filigree which the engineer of that time similarly considered the appropriate (if then less inhibited) æsthetic decision.

This concern of the engineer with æsthetics, with the appearance of things, is now firmly established. It manifests itself in a desire for neatness, for invisible fastenings, for the compact and orderly disposition of units. It works towards the attainment of an elegant engineering solution in which the most simple economic solution is commonly jettisoned for one which has required difficult development to effect the elegant solution as efficiently and as economically as the more immediately obvious brash commonplace one.

During the past fifty years, however, a disturbing element has been introduced into this technological development towards what Whitehead[3] has called "... the most austere of all mental qualities ... the sense of style". The serious searching for the most fitting solution is now commonly influenced by a real or supposed knowledge of market requirements, and engineers have been known to reject both the commonplace and the elegant solution for one which is neither in order to satisfy their sales staff, who are convinced that only falsification and bogus elaboration will be acceptable to their markets.

If it is accepted that the engineer commonly makes æsthetic decisions, it is reasonable to ask whether he is fitted to do so. The answer must certainly be that he is only sometimes so fitted. I have already excluded the pioneer design projects from this consideration as almost unconsciously determining their own form, but for the rest the ugliness which excretes over our streets and homes, offices and workshops is a disquieting reminder that all is not yet well. For this the engineer is not to be blamed. He has been educated, as have people as a whole, with almost complete disregard for the cultivation of any visual sense—he has had little experience in producing or appreciating the visual arts. He has been encouraged to develop his eyes into acquisitive organs concerned only with selecting for conscious recognition those aspects of the world which are of material benefit to him. He has learned to see a lamp-post to avoid bumping into it, but not to see the lamp-post so that he knows and takes pleasure or offence from its shape.

Perceptive vision has been succinctly described by Suzanne Langer[4]: "What we see in this way becomes simply a thing of vision—a form, an image. It detaches itself from its actual setting and acquires a different context". The engineer has been given little, if any, training in the technique of looking and seeing, of the minute examination of objects, or in the sense of touch and the co-relation between visual and tactile sensations.

Slowly that situation is changing as the importance of art in general education is gaining recognition, but the average graduate engineer is still visually untrained, still partially blind and then finds himself, in that twilight of cataract, faced with the need constantly to make æsthetic, formal, visual, three-dimensional decisions. In this situation the surprise is not that so much is second rate, but that so much achieves satisfactory visual standards.

A Cure for Blindness

A cure to this problem could theoretically be found in an adjustment to the curricula of engineering students which corrected the faults of their primary education and imbued their time at university or college of advanced technology with the humanities. These human studies, it has been argued, could be biased towards the visual arts so that the graduate engineer became able to make more expertly those æsthetic decisions which must later constantly confront him.

This could satisfactorily deal with the problem if it were not for two at present insurmountable difficulties. First, the knowledge required for a primary engineering degree can only be mastered by application to essential studies which leave no time for a still further extended curriculum. There is already insufficient time after basic theory has been taught to allow for the development even of primary engineering design and for application to the technique of making decisions based even on purely factual information and specification. Secondly, the present too early division of boys and girls into scientific and humanistic specialization tends to dissuade from the scientific stream those very students who might be able to assimilate a degree course in which the arts are co-ordinated with the sciences.

The immediate answer must be found, I believe, in the training of a special type of engineer who, from the commencement of his technological education, is encouraged to concentrate on that aspect of engineering which is now commonly absent from the curriculum. He must be what in our civilization is considered to be an odd character, part engineer, part artist. He must have a feeling for form analogous to that of the sculptor or architect, he must be aware of the emotive effect of colour, he must be sensitive to the tactile qualities of surface, and, at the same time, he must be able to draw and calculate, to talk to mechanical and production engineers in their own terms and on their own ground. He must be sensitive to the society he serves, conscious of the movements in public taste, excited by problems of ergonomics and anthropometrics.

If he were all these things in full measure he would be a superman, the impossible Leonardo da Vinci of the twentieth century. But I am not, of course, suggesting a course of training for supra-engineers. My aim is more modest. Those undergraduates whose training is emphasized towards what the Americans call 'human engineering' are only partial Leonardos. Alone they are useless and even dangerous in their superficiality, but teamed with those whose training and experience have been differently directed towards technological specialization, they can provide the link between technology and humanity, between those who design the machines and those who use them.

A start has been made. A few art schools and technical institutes in Great Britain are now experimenting with training of this kind. This training is still in the elementary stages in which the instructors are groping for the teaching techniques which will achieve their known objectives, which will produce graduate engineers who can complete the production team.

The Industrial Designer

A title must be found for this comparatively new specialization, which has so far reached a degree of maturity only in the United States. There they are called 'industrial designers', and that name, with all its obvious limitations, must serve as an internationally accepted nomenclature. It is more fitting, by its very ambiguity, than the German '*Formgeber*' or the French '*estheticien industriel*'.

If we accept the need for such specialized technicians, the 'missing technician' as John Gloag called him in 1945[5], it would be useful to consider how he can best function in the industrial complex. To this, as to every question in which technology directly affects human needs, there is no simple positive answer. The answer shifts its position as circumstances change.

In the design of capital goods, machine tools, special duty machinery, locomotives and industrial electrical equipment, the industrial designer's function should be a minor one. He should be a watchdog for the operator, a worrier about detail, an expert in anthropometrics and ergonomics; he should be concerned with problems of calibration, communication and psychological well-being. He should be concerned also with finish and maintenance, with the form of the total machine so that its appearance reflects the excellence of its mechanical operation. In such cases the industrial designer is no more than the guide dog of a gifted blind man. His function is essential, but it is subservient to the needs of his master, the engineer.

In consumer products the emphasis shifts. In the design of a typewriter the human needs of the typist are as important as the mechanical efficiency of the machine. The assembly of the keyboard, the shape of the shift key, and ease of operation, become vital factors in the total design. The appearance of the machine becomes a vital element in its acceptance, and the work of the industrial designer begins to equal in importance that of the designer of the typewriter as a printing machine.

In domestic appliances the emphasis shifts still further into the territory of the industrial designer; into the strange world of consumer acceptance and product death-dates, where what the housewife will or will not like in her kitchen, what the marketing division believe they can and cannot sell, reach heights of monumental irrationality. This is a hinterland of personal preferences and peculiar dislikes, of motivation which often has but little to do with the real value or efficiency of an appliance. It is a publicity-aggravated dreamland in which the industrial designer is able to function, sustained by training and experience which have steeled him to its vagaries, and from which he can produce a reality which can materialize the highest standards society will accept instead of, in despair, looking for the lowest common denominators.

Style, Design, Obsolescence

The reservations of an engineer when asked to accept an industrial designer as a colleague are understandable. They arise first from the human weakness which suspects that this new appointment is a criticism of his own ability. This is a comparatively simple problem of human relationships which time will help to resolve. An aeronautical engineer does not question the advisability of working with a stress engineer, or with experts in hydraulics, electrics and other branches of intense specialization. Few architects doubt the wisdom of collaboration with civil engineers; few surgeons would be prepared to operate without an anæsthetist.

The other cause for lack of enthusiasm for collaboration is more serious. It arises from the engineer's deep distrust of the whole operation of industrial design. He suspects that the designer is concerned purely with superficial style, with fashionable frippery which is completely separated from, and sometimes detrimental to, the function of a mechanism.

At its most extravagant this design concept is expressed in some automobile design in which the body-work is disagreeable and in which spear-pointed exuberances turn an innocent vehicle into a lethal pedestrian-hating weapon. It is expressed in kitchen equipment which is so loaded with gadgets as to require the housewife to turn herself into a skilled mechanic (or to disregard the elaborations which she has expensively bought), and which is a pleasure only to the service engineer who is kept busy repairing this unnecessary complexity.

But having properly disparaged such baroque styling, the need for style remains.

To reduce the problem to its essential crudity: 'things are made for people'. Human needs, desires and ambitions must be the arbiters of all industrial production. There is no gain in manufacturing an excellent piece of equipment which no one needs or wants and is therefore not prepared to buy. This is a truism for any society as soon as the earnings of any large section of the community exceed their minimum requirements for shelter and food. It is already manifest in the U.S.S.R., where the increasing supply of 'non-essential' consumer products such as television sets is creating the problems of consumer demand or resistance which have for long been a characteristic of Western marketing. This sensitivity to human needs and the relation of the form and operation of engineering products to the style which mirrors each facet of a society at each moment of its complex and uneven growth is an attribute of the skilled industrial designer; it is one of his specialized functions.

The reasons for the disrepute of this quite proper function, about which the industrial designer is as sensitive as the engineer is scornful, is that too often the body-work of industrial products has been 'styled' by self-styled designers whose talents reside only in their ability to produce competent perspective sketches which satisfy the uncritical judgment of industrial management and, often, disregard all production and servicing desiderata. This process has become self-propagating as the engineering staff have always managed to find a method of producing the stylist's extravagances; a sufficiently large market has been satisfied by these monstrous objects which has become status symbols and, in the competitive battle for markets, the gadgetry of one manufacturer has been capped by another's still wilder bid for applause and profit.

The situation remains with us; but the tide shows signs of turning. The industrial designers are becoming serious and expert. They have largely renounced the extravagant showmanship which was their defence during the period when their battle for recognition was more bitter than it now remains. They are being trained to collaborate with their colleagues in the factory production team and are no longer expected by industry to produce magical rabbits at the drop of a commission. They are as concerned with production problems as are the production engineers themselves; they have willingly discarded their artist's freedom for the discipline of the factory, hopeful that serving a social purpose will compensate for the compromise this almost inevitably imposes.

Scientific Romanticism

I want, lastly, to consider an aspect of this problem which inhibits an appreciation of it in what I believe to be a proper perspective. Those designers who have accepted their essential liaison with science and industry have sometimes transformed what should be understanding and sympathetic friendship into too passionate a love affair. To quote from a recent issue of the Italian publication *Zodiac*[6]: "The important art of the twentieth century is going to be produced by the men who understand our age and who create artistic objects which are useful to-day. Those men who create these objects are the true artists of our time". In his fundamental book, *Art and Industry*, Sir Herbert Read wrote: "Such beauty as we admire in Greek vases, and in the undecorated forms of Mediterranean art generally, can undoubtedly be produced by machinery".

I wish that I could accept these adulations of the industrial designer. It would be pleasant to believe that a stainless steel coffee pot made by the hundred thousand in a Midlands factory could have the same quality as one of Isaac Dighton's silver pots of the early eighteenth century. But the mass-produced pot, however appropriate and satisfying its proportions may be, however well it may be made, however efficiently it may operate, however good the quality of its metal and finish, lacks the one quality essential to a work of art. It lacks uniqueness. That is a quality which every natural organic object possesses: it is the quality which every hand-made artefact possesses, it is the quality which in great works of art gives them a distinction which justifies man as a creator, as a god on earth.

That quality exists in the original model which the engineer and the industrial designer may together produce, but it must be ironed out and tooled away so that the machine may produce its thousand copies without the stigmata of individuality which would make a mockery of the very process of industrial production.

In almost completely obliterating uniqueness the machine obliterates humanity in its production. The formal mathematical qualities of form and proportion remain, the sense of pleasure in using an efficient object remains, positive satisfaction in handling a well-made thing exists, but these are not, in themselves alone, the qualities of a work of art.

For compensation, we have the multiplication of machine production. If the æsthetic quality of a motor-car cannot equal that of even a minor sculpture by Henry Moore, the faint æsthetic pulse in the dehumanized forms of the automobile is multiplied by the number of cars made from the same mirror-polished tools. In quantity the mild æsthetic pleasure each car evokes adds up to an important factor in our daily lives. Multiplied by the million objects of our environment, their effect on our lives begins to equate with the impact of a single work of art.

All the works of all the designers for mass production cannot equal one Rembrandt self-portrait or a Cézanne still-life, but that need not deny us a modest satisfaction in producing, if we eventually do, an environment in which, against our machine-created background of neutral and agreeable objects, the painters and the sculptors and the architects and the great engineers can produce their masterpieces.

[1] *Art and Industry* (Faber and Faber, London, 1934).

[2] "The Aesthetic Aspect of Civil Engineering Design," Inst. Civil Eng., London.

[3] Presidential Address to the Mathematical Association, 1916.

[4] Langer, Suzanne K., *Feeling and Form* (Routledge and Kegan Paul, London).

[5] Gloag, J., *The Missing Technician* (George Allen and Unwin, London).

[6] Schoener, Allan T., "Art without Pedestals," *Zodiac*, 7 (Milan).

COAL IN OXFORDSHIRE

By Dr. C. J. STUBBLEFIELD, F.R.S., and Dr. F. M. TROTTER

Geological Survey of Great Britain

THE Geological Survey of Great Britain[1] has implemented a programme of boring during each year since 1948. The programme is undertaken independently of the investigations carried out by national boards, industrial concerns and private bodies; and the purpose of the boreholes is to obtain a fuller knowledge of the geology of Great Britain. Should raw materials of commercial value come to light from this work, the information concerning them is made available to the public so that other organizations, if they so desire, may explore further and exploit them.

In order to preserve a balance between short- and long-term research, in some years it has been the Geological Survey's policy to drill shallow boreholes so that that knowledge of the sub-surface geology may be increased in areas covered by 1-in. maps which at the time are being officially surveyed geologically. In other years boreholes are planned for use in long-term investigation of the underground geological composition and structure in extensive areas of Britain covered by Mesozoic rocks and to check the results of geophysical researches; in general these are deeper boreholes.

Among the several regional studies now in hand, one concerns the nature of the sub-Mesozoic rocks in the area between the Severn basin and Oxford. In this area two exploratory boreholes—both were less than 2,000 ft. in depth—had been made in earlier years by industrial concerns; the first of these boreholes was started in 1872 and finished in 1875 at Signet, Burford (National Grid Reference 42/251101); strata classified as Coal Measures were entered at 1,174 ft. and boring continued in them to a depth of 1,409 ft. (ref. 2). The borehole was stated to have encountered a coal seam at 1,350 ft., but its thickness, presumably less than a foot, was not recorded and no samples of strata were kept. The second borehole, 15 miles farther north at Batsford, Lower Lemington (Nat. Grid Ref. 42/215346), was started in 1901 and completed in 1904; beneath Triassic rocks at 1,021 ft., it passed through Coal Measures for 525 ft. and then traversed Silurian mudstones to 1,700 ft., at which depth boring stopped[3].

The first Geological Survey borehole in this area was started in 1949 at Stowell Park (Nat. Grid Ref. 42/084118) about ten miles west-north-west of Signet, Burford[4,5]. This borehole confirmed the existence of a deep basin of Mesozoic rocks underlying the Cotswolds and the Vale of Gloucester, and drilling stopped in 1951 at a depth of 4,503 ft. without the base of the Mesozoic (Triassic) rocks sequence being reached.

The Geological Survey then turned its attention nearer to, and slightly north-west of, the site of the 1872–75 Signet borehole. Boring commenced in 1952 at Upton, Burford (Nat. Grid Ref. 42/232131). The Upper Coal Measures were reached beneath the Triassic rocks at a depth of 1,107 ft. (733 ft. below Ordnance Datum) and were proved to a thickness of 2,660 ft.; a few thin coal seams were found, but owing to technical difficulties the bore stopped at a depth of 3,767 ft. in July 1953[6,7].

For some years the area between the Severn and Oxford had been the subject of gravity, magnetic, and limited seismic surveys by the British Petroleum

Co., Ltd.[8], and although the Geological Survey[9,10] announced its intention in 1955 that boring might be continued at a site or sites progressively eastwards from the Cotswolds, its borehole activity there was deferred for some years pending the results becoming known of future boreholes by the oil company in that area. The first of these, a borehole drilled in 1955 at Faringdon, Berkshire (Nat. Grid Ref. 41/323940)[11,12], revealed Lower or Middle Old Red Sandstone (Devonian) rocks beneath Triassic at a depth of 2,150 ft. (1,852 ft. below O.D.) and another borehole drilled in 1958 for the Gas Council at Noke Hill (Nat. Grid Ref. 42/539129)[12] five miles north-north-west of Oxford found Upper Old Red Sandstone underlying Mesozoic rocks at about 380 ft. (66 ft. below O.D.).

When these results became known and no further boring by the British Petroleum Co. seemed likely in the early future, the Geological Survey started a borehole in August 1960 at Apley Barn, north-north-east of Witney Park (Nat. Grid Ref. 42/344107)[13].

The surface-level is 280·1 ft. above Ordnance Datum and the borehole commenced in the Great Oolite White Limestone. Various subdivisions of the Jurassic were proved, including the Marlstone Rock-bed (the top of the Middle Lias), which was 1 ft. 9 in. thick with the base of the bed at 171 ft. 9 in. In passing it may be noted that the Marlstone Rock-bed worked for ironstone north-west of Banbury at a thickness of about 20 ft. has thinned at Witney, as it does farther north towards a north–south line through the Vale of Moreton and the Vale of Bourton. The base of the Jurassic was found at 564 ft. and that of the Rhætic at 592 ft. 8 in.

The base of the Keuper Marl was penetrated at 760 ft. 6 in. and the sandstones below have been assigned to the Keuper Sandstone. Reddened Upper Coal Measures underlie the Keuper Sandstone without visible unconformity, thereby rendering difficult the fixing of the demarcation line; provisionally the base of the Keuper Sandstone is taken at 820 ft. 10 in. Reddening of the Upper Coal Measures persists to a depth of 927 ft. The first seatearth type of mudstone was encountered at 907 ft. and the first coal seam, 4 in. in thickness, at 1,005 ft. 9 in. Between 1,000 ft. and 1,948 ft. twenty coal seams were proved, and in this account these measures, which are principally mudstones, may conveniently be termed the Uppermost Coal Group; the total thickness of coal found in this Group was 21 ft., including one 3-ft. seam of coal with its base at 1,661 ft. 6 in. From 1,948 ft. to 2,602 ft. dominantly argillaceous measures included mudstones with ironstones, seatearths and four thin coal seams; in these strata, red, brown, purple, yellow, green and variegated measures alternated at about 20–50 ft. intervals with strata that were essentially grey, and these measures are called for convenience the Coloured Measures Group. These coloured relatively barren measures were underlain by 270 ft. of grey mudstones with subordinate sandstone bands to 2,872 ft. and they contained eleven coal seams and associated seatearths; these measures are here referred to as the Second Coal Group. The total thickness of coal in the Second Coal Group is 20 ft. 9½ in., and worthy of mention are a 2 ft. 7 in. seam at 2,644 ft., a 3 ft. 2 in. coal (including a 1-in. parting) at 2,689 ft. 8½ in., a 3-ft. coal at 2,749 ft. 3 in. and a 2 ft. 9 in. coal at 2,818 ft. 9 in.

The Second Coal Group is underlain by arenaceous strata of Pennant sandstone aspect which nevertheless contains eight coal seams; these measures are here called the Arenaceous Coal-bearing Group and the total thickness of coal in this Group is 14 ft. 2½ in. Particularly noteworthy are a 6 ft. 1 in. seam at 3,633 ft., of which the bottom 4 ft. 11 in. are coal, and a 6 ft. 4½ in. seam at 3,786 ft., of which the bottom 4 ft. 9 in. are coal. The base of the Arenaceous Coal-bearing Group is taken at 3,968 ft. 7 in. Fossil plants and non-marine lamellibranchs have been collected at intervals between 841 ft. and 3,773 ft., and a fish tooth at 3,943 ft., all indicating Upper Coal Measures age. A pulmonate gastropod was found at 877 ft. and in British Coal Measures such forms have been previously found only in the Keele Beds, which are the uppermost Coal Measures in North Staffordshire, and in the Coal Measures of Upton, Burford. The sequence between 2,872 ft. and 3,968 ft. contains sandstones of a type known in the Pennant Measures of Bristol and Somerset, and South Wales.

No evidence of older Carboniferous rocks was found in the borehole, for underlying the Arenaceous Coal Group is a Conglomerate Group comprising multicoloured sandstones, breccias and conglomerates with subordinate purplish and green mudstones; between 3,971 and 4,596 ft. Devonian fossils were found, including a coral, crinoid stem columnals, brachiopods and fish scales; between 4,511 and 4,562 ft. the flora indicates Middle or Lower Devonian age. Boring ceased at 4,942 ft.

The stratal gap between the Keuper and the Upper Coal Measures and that between the latter formation and the Devonian are not associated with discordance of dip. The highest dip recorded in the Devonian is 4°.

It has now been shown that coal occurs in seams of workable thickness beneath Apley Barn, and the quality of these seams is being studied by officers of the National Coal Board. Thus the name Oxfordshire Coalfield gains the significance which it lacked when E. N. Arber was criticized for coining the name in 1916 (ref. 14), on the evidence of the Signet Burford borehole, where no coal of workable thickness had been found.

The economic potentialities of the coalfield are as yet unproved. The completion of its exploration would be a lengthy procedure which is not the responsibility of the Geological Survey of Great Britain, though that organization would give geological advice if requested. A series of further boreholes would be required, and if these proved good-quality coal seams at a workable depth over a sufficiently wide area, then only might the need arise for the siting of a colliery shaft. Such a site would most probably be chosen near a railway line and Oxfordshire, like Kent, would have its concealed coalfield.

---

[1] Trueman, A. E., *Rep. Geol. Surv. Board*, 1945, 9 (1947).

[2] De Rance, C. E., *Trans. Manchester Geol. Soc.*, **14**, 437 (1878).

[3] Strahan, A., *Sum. Progr. Geol. Surv.*, 1912, 90 (1913).

[4] Dines, H. G., *Sum. Progr. Geol. Surv.*, 1951, 20 (1953).

[5] Green, G. W., and Melville, R. V., *Bull. Geol. Surv., Great Britain*, No. 11 (1956).

[6] Pugh, W. J., *Sum. Progr. Geol. Surv.*, 1953, 10 (1954).

[7] Edmunds, F. H., *Sum. Progr. Geol. Surv.*, 1953, 28 (1954).

[8] Falcon, N. L., and Tarrant, L. H., *Quart. J. Geol. Soc.*, **106**, 141 (1951).

[9] Drummond, W. J., *Sum. Progr. Geol. Surv.*, 1954, 6 (1955).

[10] Pugh, W. J., *Sum. Progr. Geol. Surv.*, 1954, 10 (1955).

[11] Falcon, N. L., *Proc. Geol. Soc.*, **1524**, 93 (1955).

[12] Falcon, N. L., and Kent, P. E., *Mem. Geol. Soc. Lond.*, **2**, 16 and 46 (1960).

[13] Wilson, V., *Sum. Progr. Geol. Surv.*, 1960, 34 (1961).

[14] Arber, E. A. N., *Trans. Inst. Min. Eng.*, **50**, 373 (1916).

Applied Anisotropic Elasticity

R. F. S. HEARMON

The scope of the book is indicated by the chapter headings : Fundamentals; Elastic Constants and Symmetry; Elastic Constants of Single Crystals, Sheet Materials and Polycrystalline Aggregates; Some Simple Stress Systems; Stress and Strain Distribution; Wave Propagation; Anisotropic Plates. Its aim is to collect hitherto very scattered information, including Soviet contributions, as an introduction for students, research workers, designers, and others interested in the field. *Half-tone plates* 35*s net*

Chemical Crystallography

AN INTRODUCTION TO OPTICAL AND X-RAY METHODS

C. W. BUNN

No previous knowledge of crystallography on the part of the reader is assumed here, and the book deals with the use of the principal crystallographic methods—morphological, optical, and X-ray—for the identification of crystalline substances and the determination of the precise arrangements of atoms in crystal structures. For this edition it has been expanded to cover important recent developments in the various methods of structure determination. *Half-tone plates and text-figures* *Second edition* 60*s net*

INTERNATIONAL SERIES OF MONOGRAPHS ON PHYSICS

The Principles of Nuclear Magnetism

A. ABRAGAM

The purpose of this book is to give a consistent description, on a unified basis, of the various aspects of nuclear magnetism. Emphasis throughout is on clarity and rigour rather than on the abundance of factual and bibliographical data. The outlook is mainly theoretical, but a large amount of experimental evidence is included. *Illustrated* 84*s net*

Hydrodynamics

A STUDY IN LOGIC, FACT AND SIMILITUDE

GARRETT BIRKHOFF

This is a complete revision of the first edition of ten years ago. The author has brought up to date the results of further activity in the field of hydrodynamics, added a chapter on turbulence, and expanded the work on paradoxes and modelling. *Frontispiece and figures* *Second edition* 42*s net*

PRINCETON UNIVERSITY PRESS

Tools of the Astronomer

G. R. MICZAIKA and WILLIAM M. SINTON

This volume gives a guide to the instruments used in astronomical research today. Visual refractors, bifilar micrometers and meridian circles, and the new equipment of the modern solar observatory and radio astronomy are described. 62*s net*

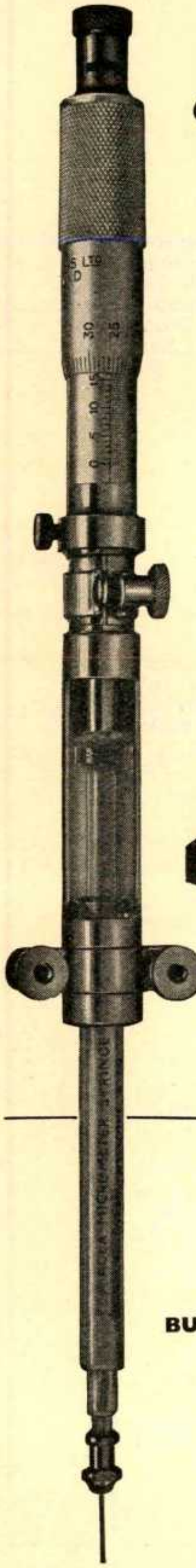
THE HARVARD BOOKS ON ASTRONOMY: HARVARD UNIVERSITY PRESS

Ripple Tank Studies of Wave Motion

W. LLOWARCH

The author describes improvements that he has made in ripple tank technique, and deals with all aspects of the properties of wave motion which are likely to be encountered at school level, and in the earlier years of a degree course, in pure or applied physics. He also illustrates and discusses the basic properties of waves in general and their use to interpret the wave-like behaviour of sound, light, and other forms of radiation. *Text-figures* 15*s net*

Oxford
UNIVERSITY PRESS

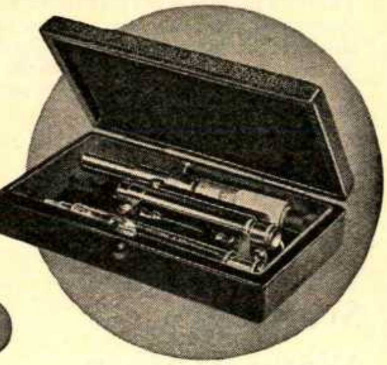


'AGLA' BRAND

MICROMETER

SYRINGE

A precision instrument capable of measuring very small volumes of fluid with an accuracy of ± 0·00005 mil.

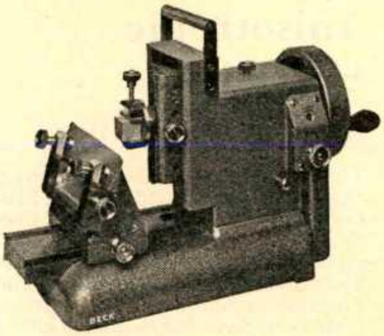


BURROUGHS WELLCOME & CO.

(THE WELLCOME FOUNDATION LTD.)

LONDON

AUTOMATIC ROTARY MICROTOME



A robust and efficient machine at very moderate cost. Re-designed with up-to-date features, for rapid production of sections from 1µ upwards.

Please write for our new general catalogue.

R. & J. BECK, LTD.

69-71 MORTIMER STREET, LONDON, W.1

FOUNDED HAWKSLEY LONDON IN 1869

CRISTALITE

B.S. 748 HAEMACYTOMETER

The only metallized haemacytometer made to British Standard 748 — the most rigid standard in the world.

Details on request.

HAWKSLEY & SONS LIMITED

17 NEW CAVENDISH ST. LONDON W1

WELBECK 8557

THE TRAINING OF BIOCHEMISTS

UNTIL comparatively recently most biochemists in Great Britain had received their early training in other disciplines such as chemistry, physiology or medicine. However, the rapid progress of biochemistry during the post-war period has been paralleled by an increase in the number of biochemistry departments within British universities, and it is likely that most of our future research biochemists will graduate from these departments, having followed courses leading to the honours B.Sc. degree.

It is probably true to say that no one is yet certain, to the point of dogmatism, as to the best way of training such biochemists, and this no doubt reflects the position of biochemistry as a 'meeting-point' of so many other disciplines. In order to create discussion and thought on this topic the Biochemical Society held, during the afternoon of July 13, a colloquium on "The Training of Biochemists". The colloquium, which formed part of the four-hundred and eighth meeting of the Society, was held in Oxford under the chairmanship of Dr. K. S. Dodgson and was attended by more than 250 members and guests.

One of the principal controversies of the proceedings centred around the contributions of the first two speakers. Prof. S. V. Perry outlined the growth and development of biochemistry in Britain and stressed the value of the three- or four-year B.Sc. honours course in the training of the biochemist. Such courses were now firmly established in their own right and provided not only an intellectual challenge but also a sound basis for education in its broader sense. In contrast, Prof. B. Spencer suggested that biochemistry was more fitted to be a postgraduate subject, and illustrated his point with detailed reference to the systems in use in most American universities. Some lively discussion followed these contributions and although the weight of opinion was perhaps loaded in favour of the undergraduate course it was clear that most people, recognizing the important links between biochemistry and other disciplines, felt that other pathways by which biochemists emerged should remain open. Prof. R. A. Morton reiterated from the floor that the educational aspects of a university course should not be neglected in favour of 'training'. Biochemistry as an undergraduate subject was intellectually stimulating, and courses should always include aspects hard enough to stretch the mind of the student. What matters is the spirit of the course rather than the factual content.

The views of industry regarding the training of biochemists were outlined by Dr. W. F. J. Cuthbertson, who pointed out that industry as a whole was still not fully aware of the potential value of the trained biochemist. He suggested that a group should be formed, composed of representatives from academic life, industry and the various industrial research associations, to determine the contribution that biochemical graduates could make in industry, their present level of employment and the possible future requirements for them. In noting the differences between research in industry and in universities or research institutes, Dr. Cuthbertson suggested that industrial research was in some ways more exciting and variable. The freedom of academic research was sometimes illusory, and the academic research worker soon realizes that certain projects and methods of approach are more likely than others to attract financial aid to research. Most large industrial concerns exhibit a liberal attitude towards research, and the industrial biochemist is usually free to work on anything he choses providing that he can persuade his authorities to supply the necessary facilities. The training that was appropriate to the industrial biochemist was not markedly different from that followed by most university departments, but additional attention might be given to special facets such as microbiology and the use of statistics. The ability to present an account of his work in unambiguous simple English was perhaps even more important for the industrial biochemist than for his academic friends, because many of his findings may have to be assessed by people with little or no training in his own discipline or, for that matter, in any other scientific subject.

After some preliminary sparring with questioners, Dr. Cuthbertson was challenged outright as to whether industry was satisfied with its biochemical recruits. In reply he said that, generally speaking, expanding university departments and research institutes were skimming the cream of the graduates and a more encouraging attitude to industry from within university departments would be welcomed. Several speakers suggested that industry was perhaps responsible for any unfavourable attitude which might exist and, in particular, the use of graduates as 'superior technicians' by industry was deprecated. This unwelcome development certainly appears to be on the increase, and reflects the present shortage of trained technical assistants—a situation which technical colleges in Britain should be given every encouragement to ameliorate.

University and departmental entrance requirements were discussed by Dr. Margaret Kerly, and the meeting was greatly indebted to her for the work she had done in collating so much diverse and scattered information. In biochemistry, perhaps more than any other subject, opinions as to the school background appropriate to students vary considerably. Dr. Kerly pointed out that the number of sixth-form students from any one school who were interested in a biochemical career varied from none to 2 or 3 only, and it was therefore unrealistic to expect schools to modify time-tables in order to cater for potential biochemists. Time-table problems usually resulted in sixth-formers taking chemistry, physics and mathematics for advanced level General Certificate of Education, in which case they were weak on the biological side, or alternatively, chemistry, physics or mathematics and a biological subject, in which case they tended to be weak in either physics or mathematics. A possible solution might be the introduction of a new advanced level subject which combined those sections of the present physics and mathematics courses which are most necessary for all biologists.

In spite of the Biochemical Society's recent pamphlet, *Careers in Biochemistry*, inquiries in schools had convinced Dr. Kerly that many science masters and sixth-form students were only vaguely aware of the potentialities of a career in biochemistry. More propaganda will be needed in schools if more good candidates for biochemistry are to be forthcoming. Further support for this contention came during discussion, and it was suggested that individual departments could and should do more in this respect, particularly in the light of the shortage of biochemists which Britain is now experiencing.

In describing the recently revised four-year biochemistry course at Oxford, Prof. D. D. Woods stressed the importance of adequate background teaching, particularly in chemistry, but pointed out the need to introduce biochemistry at an early stage in the course in order to stimulate student interest. Experimentation in biochemical research is steadily becoming more sophisticated, and recent trends have emphasized the more biological aspects of the subject. The early introduction of such topics as cell physiology and genetics is therefore an important feature of the course at Oxford, and the Biochemistry Department was particularly fortunate in having the co-operation of other departments in the teaching of such topics.

Much interest was expressed in the course at Oxford, particularly from the point of view of its breadth of outlook, although it was clear that Oxford with its resources was in a particularly favourable position to overcome time-table problems which so beset smaller departments.

In the final paper, Dr. E. M. Crook discussed the M.Sc. course in biochemistry which has been in existence for some years at University College, London. Dr. Crook suggested that a good deal can be said for not attempting to teach biochemistry as an undergraduate subject, but a major disadvantage in adopting this attitude, so far as the course at University College, London, had been concerned, was that few M.Sc. candidates have learned any biochemistry, even of the most elementary kind, before entering the course. Moreover, a first-class honours graduate in, for example, chemistry has to be exceptionally strong-minded to proceed to an M.Sc. course in another subject in the face of the psychological impulse to go on at once to prove himself as a research worker. The danger, therefore, is that the M.Sc. course may tend to attract the less-able student who may look on the course as a means of 'upgrading' a relatively poor initial degree. On the whole, however, it seems likely that postgraduate teaching must increase. Various departments could, between them, provide a formidable series of postgraduate courses of different specialist nature, and this could prove of great value in strengthening the subject in Britain.

In summarizing the proceedings, Prof. F. C. Happold confessed himself to be out of sympathy with the title of the colloquium. We were not concerned with the training of biochemists as one trains circus animals but with their education. The latter embraces the former but it is also more difficult to achieve. In any event, those departments which already had honours courses in biochemistry were certainly not proposing to abandon them. Such courses were an excellent education in themselves providing that sufficient time was available to do the job properly. In his own opinion the four-year course appeared to be particularly satisfactory from this point of view.

Judging from the conversations which followed the closing of the meeting, the original aims of the colloquium in stimulating thought and discussion had been successfully achieved. The Biochemical Society hopes to publish the proceedings of the meeting, in monograph form, at an early date.

K. S. DODGSON

NEWS and VIEWS

New President of the British Association : Sir John Cockcroft, O.M., K.C.B., C.B.E., F.R.S.

SIR JOHN COCKCROFT, Master of Churchill College, Cambridge, and part-time member of the U.K. Atomic Energy Authority, has been elected president for 1962 of the British Association for the Advancement of Science. That year, the annual meeting will be held in Manchester.

As is well known, Sir John has achieved world-wide fame as an atomic physicist. He was Jacksonian professor of natural philosophy in the University of Cambridge during 1939–46. His first researches, beginning in 1924 at Cambridge, were concerned with the heating of transformer coils, and later, at the Cavendish Laboratory, he carried out work on the deposition of surface films by atomic beams. Later, with Kapitza, he designed much of the equipment used in the production of intense magnetic fields. In 1932, with Walton, he had devised the arrangement by which nuclear disintegration by artificially accelerated particles was first observed. He was a prime mover in the development of equipment and the erection of buildings for which the physicists at Cambridge must now be grateful. During most of the Second World War period he was chief superintendent of air defence, Research and Development Establishment, Ministry of Supply, and then became director of the Atomic Energy Division, National Research Council of Canada, during 1944–46. He was then appointed director of the Atomic Energy Research Establishment at Harwell.

Such is the high esteem in which his work is held that Sir John has been or is a member of many scientific and advisory organizations and he has been the recipient of numerous State, academic and other honours. Among the most recent are the 1961 Atoms for Peace Award, which was established as a memorial to Henry Ford and his son Edsel, consisting of a gold medal and 75,000 dollars, and the chancellorship of the Australian National University, Canberra.

Norwich and its Region

VISITORS to Norwich will find *Norwich and its Region* a very pleasing work. (Pp. 224. Norwich: Local Executive Committee of the British Association for the Advancement of Science, 1961. 15*s*.). It provides a most informative survey of the region of Norfolk and north Suffolk. The survey opens with a concise account of the historical events which have moulded East Anglia. To-day it is in the midst of a second industrial and agricultural revolution. Though to some it may appear a quiet backwater, residents are conscious of the mounting pace at which it is being drawn relentlessly into the main stream. The second section deals with geology, physiographical features, climate, and the nature of the soil. Results of recent research are emphasized. In fact, advances in knowledge of the Pleistocene deposits have been so rapid during the past few years that the authors feel obliged to point to the interim character of the account they give. The biological background of the area provides the theme for another section. Despite the extent of agriculture, much natural vegetation is still preserved. The county is actively concerned with conservation of Nature. About three-quarters of the British flora abound in Norfolk, due to the climatic contrast coupled with differences in soils. The county is rich in non-marine Mollusca; the coast, however, provides little shelter for marine animals.

The fourth theme deals with the influence of man on the environment. Physical anthropology, archæology and social, political and religious history are discussed. East Anglians are proud to be individualists, ever loath to follow the accepted pattern. From very early times, social services were instituted for the care of the sick and poor, and opportunity for schooling has not lagged behind that in the rest of England. Norfolk has a record of independence and enterprise in education no less than in farming. The final section gives a detailed informative account of the economy of the area to-day. Primary industry, like agriculture, forestry and fishing, is discussed; so too is the growth of manufacturing industry and commercial and professional services. The authors of these surveys are to be congratulated, since their natural emotional involvement with the various topics is never allowed to distort their scientific objectivity. The photographs are numerous and excellent. There is a very good map of the region. The photograph on the back cover is exquisite—a golden splendour of barley. That the book has been sent to members in advance of the meeting is to be welcomed.

Anatomy at Oxford :
Sir Wilfrid Le Gros Clark, F.R.S.

Sir Wilfrid Le Gros Clark retires from the chair of anatomy in the University of Oxford at the end of the next academic year. He is probably the last in the line of the great medical men who have been able to combine a career in anthropology, in which he is among the most distinguished of its leaders, with that of an equally distinguished career in neuroanatomy. His classical work on the nuclei and connexions of the primate thalamus, his work on the visual and olfactory systems and many other parts of the brain have shown that he is equally as good at noting the morphological changes which follow controlled experimental procedures as in descriptive anatomy. His book, *The Tissues of the Body*, is a masterpiece of lucid writing and probably the best introduction to human biology which has yet been written. In this book he shows that anatomy is the science concerned with the organization of the living body and how this is achieved during embryonic, post-natal and adult life. It must embrace the whole range of organic structure, the organization of the elementary components of living matter into cells, cells into tissues, tissues into organs, organs into systems, and systems into integrated creatures. He, like Woollard before him, urged that the teaching and study of minutiæ of topographical anatomy are an unjustifiable burden to those training to become doctors, for nowadays no potential physician, surgeon or clinical scientist can afford the time to master more than a few of the details offered by any one of the basic medical sciences, least of all those of no intellectual or even practical value. This is not to deny, however, that anatomical details are of great importance to surgical specialists, but it is by them that they should, and can most easily, be taught.

Prof. G. W. Harris, F.R.S.

Sir Wilfrid's successor will be Prof. G. W. Harris, the distinguished neuro-endocrinologist, who is at present Fitzmary professor of physiology in the Institute of Psychiatry in the University of London. Prof. Harris started his professional career in anatomy at Cambridge, where he taught for eight years and where he laid the foundations of his endocrinological research work. The electors, in choosing Prof. Harris, have shown boldness and imagination. Although, at present, he is by title a professor of physiology, he should make a most worthy successor to Sir Wilfrid, for he, like Sir Wilfrid, is interested in structural organization. For example, he has demonstrated in a series of elegant experiments that the pituitary gland (adeno-hypophysis) only operates normally when it lies in the pituitary fossa. It is not sufficient that the gland, when transplanted elsewhere in the body, remains structurally intact. Only when it is returned to the site in which it originally developed (that is, lying close to and having direct vascular relationships with the hypothalamus) does it again function normally. This work is a perfect demonstration of the importance of structural organization in relation to functional requirements, and alone suffices to indicate that Prof. Harris is well suited to develop the science of anatomy at Oxford, the foundations of which have been so well laid by Sir Wilfrid. An appointment of this kind underlines the current feeling in medical educational circles that instruction in any form of detailed topographical anatomy will have to pass into the hands of surgical specialists, as indeed it already has in some medical schools.

Visit of Russian Scientists to Harwell

As a result of the agreement recently signed between the U.K. Atomic Energy Authority and the Russian State Committee for Utilization of Atomic Energy for Peaceful Purposes, a party of five Russian scientists visited England during July 20–30 to discuss fundamental solid state physics. In order to allow the Russians to see a good cross-section of British work, the Atomic Energy Authority invited a number of university scientists to attend discussions at Harwell on radiation damage, electronic and magnetic properties of solids, and on neutron diffraction. The party also visited physics and metallurgical laboratories of the Universities of Reading, Oxford and Cambridge. The visit afforded an opportunity of learning a little about the nature and scope of work carried out at some of the Russian research institutes. It is clear that substantial results in the fields of inelastic neutron diffraction and electron microscopy of thin films may be expected in the next few years, following a marked expansion in Russian efforts in these topics which started in 1959. The Russian party was led by Prof. V. E. Ivanov, of the Physico-Technical Institute of the Ukrainian Academy of Sciences, whose main interest lies in the field of purification of metals, and included Prof. N. F. Pravdyuk, of the Kurchatov Atomic Energy Institute, Moscow, who spoke on radiation damage; Mr. V. I. Bykov, of the Obninsk Physico-Energetics Institute, who spoke on neutron diffraction; Mr. B. B. Baturov, of the State Committee for Utilization of Atomic Energy, whose main interest is in nuclear reactors; and Mr. A. D.

Sokolov, of the State Committee for Utilization of Atomic Energy, whose main interest is in particle detectors and instrumentation for physics experiments.

U.S. Nuclear Power Projects

THE third edition of *Fact Sheets on U.S. Nuclear Power Projects* is a complete revision of the sheets first published in December 1958 and revised in June 1960 (Pp. iii+51. New York: Bozell and Jacobs, Inc., 230 Park Avenue, 1961). The reactors listed are classified by coolant. The water-cooled reactors include five pressurized and eight boiling-water reactors. The sodium-cooled reactors comprise two moderated and two fast-breeder reactors, and one organic-cooled and three gas-cooled reactors are also listed. The introductory article discusses the approach to nuclear power development in the United States which has not been, and is not, aimed primarily at producing kilowatts, but is designed to develop technical information for the construction of commercial plants by the electrical utility industry. At least 133 investor-owned electricity companies in the United States of America are actively engaged in civilian nuclear power projects, including plants in operation; under construction, or under contract, or planned; or in study, research, and development projects; a complete alphabetical reference list of the projects is given at the end of the *Fact Sheets*.

New Hope for African Wildlife

GREAT concern has been felt in recent years about the accelerating rate of destruction of wild fauna, flora and habitat in Africa, without regard to their value as a continuing economic, scientific and cultural resource. It is feared that by 1970 several of the largest land mammals in Africa will not survive in a wild state. The smaller wild creatures are already under constant threat as the New Africa expands and demands on its natural resources grow. At the 1960 Warsaw Congress of the International Union for the Conservation of Nature it was recognized that the crux of the problem is to persuade the people of the New Africa to appreciate the value of their wild life not only in relation to the growth and prosperity of their country but also for the sake of future generations. It was at this Congress that the African Special Project was planned in three stages: (1) survey of problems; (2) conference to discuss possible solutions; (3) operational work to implement the solutions. Stage 1, which has already been completed, consisted of a survey carried out in sixteen African countries. Stage 2 has been planned and prepared in detail. The conference on "The Conservation of Nature and Natural Resources in Modern African States" will be held in Arusha, Tanganyika, during September 5–12. It is organized jointly by the International Union with the Commission for Technical Co-operation in Africa. For practical reasons the number of delegates had to be limited, but will include representatives of 25 governments. A number of 'fellowships' has been provided so that selected Africans of the younger generation can participate. Further information can be obtained from the Press Officer to the Arusha Conference, Ian S. MacPhail, 20 Belgrave Square, London, S.W.1.

Recent Map Publications by the Ordnance Survey of Great Britain

ON August 1, the last six sheets were published by the Ordnance Survey of Great Britain, completing the Seventh Series of the One Inch to One Mile Maps, and for the first time the whole of Great Britain is covered by a single series of maps at this scale, in which all sheets are similar in projection, size and style. The six sheets are: Sheet 1, *Shetland Islands* (*Yell and Unst*); Sheet 2, *Shetland Islands* (*North Mainland*); Sheet 3, *Shetland Islands* (*Lerwick*); Sheet 4: *Shetland Islands* (*South Mainland*); Sheet 188, *Torquay*; Sheet 190, *Truro and Falmouth*. At the same time, six publications of the new post-war series of One Inch Tourist Sheets have been published: *North York Moors ; Peak District ; Wye Valley and Lower Severn ; Lake District ; Lorn and Lochaber ; Loch Lomond and the Trossachs*. With the exception of the sheet *Wye Valley and Lower Severn*, where relief is depicted by contours and layer tints, the sheets in this series show relief by various combinations of layer tints and hill shading, which give an immediate impression of ground shapes even to the inexperienced map reader. In other respects, the style remains generally the same as that used for the One Inch Seventh Series. Copies of all sheets are available in three styles from all Ordnance Survey agents and H.M. Stationery Office bookshops (Tourist Sheets: paper flat, 5*s*. ; paper folded in covers, 7*s*. ; mounted on linen in covers, 9*s*. Seventh Series: paper flat, 4*s*. 6*d*. ; paper folded in covers, 5*s*. 6*d*. ; mounted on linen in covers, 7*s*. 6*d*.).

Problems of Psychology

A NEW quarterly journal (*Problems of Psychology*, Nos. 1 and 2. Pergamon Press, 1960) aims at publishing a selection of papers in translation from *Voprosy Psikhologii*, the Russian psychological journal founded in 1955. Dr. N. O'Connor, who is acting as translation editor, states in his foreword that he hopes to choose papers which will be both representative of Soviet psychology and at the same time of special interest to English readers. The present issue contains papers on a wide variety of topics, ranging from the role of the cerebral cortex in the perception of flicker to the psychology of a man with prodigious powers of memory. Of particular interest to English psychologists is Dr. E. N. Sokolov's extremely ingenious probability model of tactile perception. Although rather uneven, this new psychological journal will be widely welcomed.

The Royal Scottish Museum

THE annual report of the Royal Scottish Museum (Pp. 29. Edinburgh: Royal Scottish Museum, 1961), Edinburgh, for 1960 will be the last to appear under the control of the present director, Dr. D. A. Allan, for he retires shortly (see *Nature*, August 12, p. 648). The report indicates progress in all directions especially as regards the carefully planned building operations. The lecture-theatre block approaches completion, fluorescent lighting has been installed in the store rooms, and a further series of exhibition galleries have been redecorated in what is now regarded as the standard colour scheme. Considerable progress has also been made on paper with the replanning of the north-east and north-west wings facing Chambers Street. The introduction of automatic stoking and thermostatic control has led to difficulties in securing adequate humidity, but further experiments to remedy this are being carried out. The consequent diminution in work, dust and dirt in the basement storage is marked and welcomed. As usual, temporary exhibitions have been a valuable feature in the activities of this Museum, and a total annual attendance of nearly 357,000 testifies to the place of this institution in the life of the community and with visitors to the City. The director and his staff continue their valuable connexions with other cultural bodies, not only nationally but also internationally, and Dr. Allan has reason to be well satisfied with the present state of the Museum which he has directed with such conspicuous success.

Aids to Agriculture and Horticulture in Northern Ireland

The Ministry of Agriculture in the Government of Northern Ireland has produced a series of leaflets giving advice to those concerned with the raising of crops and animals. The leaflets are practical, clearly written and would be of assistance to nurserymen, gardeners and farmers managing large or small farms. One hundred and thirty-eight leaflets have already been issued dealing with topics as varied as "Liver Fluke Disease", "Results of Potato Variety Trials", "The Feeding of Bacon Pigs", "Turkey Production", "The Cultivation of Gooseberries" and the "Construction of Farm Dairying Buildings". The leaflets may be obtained free of charge from the Ministry of Agriculture, Stormont, Belfast.

The Plastics Institute

Sir Laurence Merriam, chairman of British Xylonite since 1959, has been elected president of the Plastics Institute for the session 1961–62. The following have been elected vice-presidents of the Institute for 1961–62: P. C. Chaumeton, Sir John Dean, P. A. Delafield, G. Dring, S. Ellice-Clark, G. W. Hodds, J. Lesser, W. F. Mitchell, A. E. Skan, Sir Miles Thomas, W. C. Waghorne, G. J. Wevell. The new ordinary members of Council for 1961–62 are: Dr. F. P. Hiron, M. Jones, M. Kaufman, A. A. Tomkins.

Fleming Memorial Fund

The Trustees of the Fleming Memorial Fund for Medical Research announce that the first grants from the Fund will be made before the end of 1961. The Fund exists to commemorate Sir Alexander Fleming, and his discovery of penicillin, by raising money to provide assistance for basic medical research anywhere in the world. This assistance may be given by grants to universities, research institutions and others engaged in basic medical research, and by way of national and international fellowships tenable at universities and research institutions. Applications should be sent to the Secretary to the Trustees, Fleming Memorial Fund for Medical Research, 26 Upper Brook Street, London, W.1, on or after October 1, 1961.

Announcements

Mr. F. Thistlethwaite, lecturer in the Department of Economics and Politics, University of Cambridge, has been appointed the first vice-chancellor of the University of East Anglia, Norwich.

Three meetings on "Nutrition in Latin America", organized by the Food and Agriculture Organization, will be held during the autumn: *September 27–October 7*, nutrition seminar for the Caribbean (Puerto Rico); *October 11–21*, seminar on nutrition education for Mexico and Central America (Guanajuato); *October 23–November 15*, joint symposium with the World Health Organization (Guatemala City). Further information can be obtained from the Food and Agriculture Organization, Viale delle Terme di Caracalla, Rome.

The eleventh annual instrument symposium and research equipment exhibition, sponsored by the Washington Sections of the American Association of Clinical Chemists, American Chemical Society, Instrument Society of America, Society of American Bacteriologists, Society of Applied Spectroscopy, Society for Experimental Biology and Medicine, will be held at the National Institutes of Health in Bethesda, Maryland, during October 9–13. Further information can be obtained from J. B. Davis, National Institutes of Health, Bethesda 14, Maryland.

The twenty-second national conference of the Council for the Preservation of Rural England is to be held at Southport during October 12–14. The subject of the conference, which is open to all, will be "The Impact of Industrial Development on the Countryside". Further information can be obtained from the general secretary, Sir Herbert Griffin, 4 Hobart Place, London, S.W.1.

The first European symposium on "Food Technology" is now to be held in Frankfurt (Main) during October 26–27, instead of October 19–20 as previously announced. Further information can be obtained from Prof. W. Diemair, Georg-Voigt-Strasse 16, Frankfurt (Main).

An international meeting on "Psychophysiology, Neuropharmacology and Biochemistry of Audiogenic Seizure" is to be held by the French National Centre for Scientific Research during November 6–9. Further information can be obtained from Prof. R. G. Busnel, Laboratoire de Physiologie Acoustique, C.N.R.Z., Jouy-en-Josas (Seine et Oise), France.

The International Atomic Energy Agency, the Food and Agriculture Organization and the World Health Organization are jointly organizing a conference on "The Use of Radioisotopes in Animal Biology and the Medical Sciences" in Mexico City during November 21–December 1. Further information can be obtained from the Joint Secretariat, Conference on the Use of Radioisotopes in Animal Biology and Medical Sciences, International Atomic Energy Agency, Kaerntnerring 11, Vienna.

List No. 29, "Forthcoming International Scientific and Technical Conferences", issued by the Department of Scientific and Industrial Research, amends and amplifies the information contained in List No. 28, which is superseded, from July 1961 to December 1962. As is the usual practice, conferences have been given chronologically and an index added to facilitate reference. The list is obtainable from the Department of Scientific and Industrial Research, Charles House, 5–11 Regent Street, London, S.W.1.

The fourth edition, revised to November 1960, of *A Bibliography of African Bibliographies, covering Territories South of the Sahara*, issued by the South African Public Library, Cape Town, includes some 1,450 entries, more than 350 of which are new, arranged according to the Universal Decimal Classification, with somewhat more intensive geographical sub-division (Grey Bibliographies, No. 7. Pp. iv + 79. (Cape Town: South African Public Library, 1961.) R. 2.00; 20*s*.). There is also an author–subject index combined.

SPACE-FLIGHT AND RE-ENTRY TRAJECTORIES

A SYMPOSIUM on "Space-Flight and Re-entry Trajectories", organized by the International Academy of Astronautics, was held at Louveciennes, near Paris, during June 19–21, 1961. The conference was opened by Prof. Theodore von Kármán, who has chosen to follow up his long and distinguished career in aeronautics by becoming the first director of the Academy. This symposium constituted the first meeting of the Academy, and the subject of trajectories was chosen, Prof. von Kármán said, because of its fundamental importance in the successful development of space flight. Though the Academy was now only beginning its activities, Prof. von Kármán was confident that in perhaps 100 years it would be as 'respectable' as the Academy of Sciences or the Royal Society. The present situation of astronautics reminded him of that of aeronautics when he first came to France in 1908. Then, aeronauts were regarded almost as madmen, and no one truly foresaw the later commercial and military applications of aircraft. Even the pioneer Lanchester was worried by the cost of upkeep of aerodromes, and thought they would have to be divided into two parts, to be grazed by cows in alternate years. With so many false aeronautical prophecies among his memories, Prof. von Kármán was confident of the future of astronautics.

The first scientific session, on "Trajectories for Lunar and Interplanetary Missions", began with a paper by J. M. J. Kooy (Breda, Netherlands) on "Astrodynamics and Planetary Research", which investigated the motion and uses of space-vehicles placed in orbit about a planet such as Venus. Then H. Hiller (Royal Aircraft Establishment, England) described the results of a generalized study of two-dimensional trajectories in the region of the Earth and Moon, and W. E. Moeckel (U.S. National Aeronautics and Space Administration) discussed interplanetary trajectories for electrically propelled space vehicles.

The second session, on "Orbital Transfer and Rendezvous", comprised three papers on the optimization of interplanetary trajectories, by D. F. Lawden (Canterbury, New Zealand), P. Contensou (Office National d'Etudes et de Recherches Aéronautiques, France) and B. Fraeijs de Veubeke (Liège, Belgium), and a general study of the techniques of orbital rendezvous by J. C. Houbolt (U.S. National Aeronautics and Space Administration). These subjects promise to expand greatly in the future, since both are essential to the economical performance of space missions and both pose severe mathematical problems.

In the third session, on "Near Earth Satellites", J. Kovalevsky (Bureau des Longitudes, Paris) made a general analytical review of orbital perturbations, D. G. King-Hele (Royal Aircraft Establishment, England) discussed the effect of atmospheric oblateness on satellite orbits, L. Sehnal (Ondrejov, Czechoslovakia) described luni-solar perturbations on a 24-hr. satellite, and K. Schütte (Munich) commented on the changes in the orbit of *Echo* 1.

The final session, on "Dynamics of Terminal Re-entry", was, ironically, delayed because the coach conveying the participants pursued a determinedly non-optimum terrestrial path from Paris to Louveciennes and took nearly 2 hr. to complete a journey nominally of 10 miles. In this session, A. Ferri and T. Ling (Brooklyn, U.S.A.) described the effect of varying lift on re-entry trajectories, W. F. Hilton (Hawker Siddeley, England) considered re-entry paths which avoid the zones of radiation, and errors in re-entry guidance were discussed by T. R. F. Nonweiler (Queen's University, Belfast) and L. Broglio (Rome). Finally, J. V. Becker, D. L. Baradell and E. B. Pritchard (U.S. National Aeronautics and Space Administration) investigated the methods of control for re-entry from escape speeds.

Although some of the papers seemed to be slightly out of touch with the present realities of space research, this was perhaps appropriate, or even inevitable, in a forward-looking academic symposium. Certainly the symposium as a whole can be counted a success, and the International Academy of Astronautics was wise to limit the numbers of those attending to about 80. Representatives from the U.S.S.R. were invited, but were unable to come. The papers presented at the symposium are to be published in *Astronautica Acta* later this year.

D. G. KING-HELE

BIOLOGY IN BRITAIN

By DR. H. V. WYATT

Department of Bacteriology, University of Leeds

SINCE the War, more and more exciting discoveries have been made in biology, but the classical biology of pre-war years has changed little. New techniques and brilliant men have opened new and fundamental subjects of inquiry, which cannot be wholly contained by the separated disciplines of botany and zoology but have found equal application in both subjects. Perhaps for the first time there is now a subject that can be called 'biology'.

A British biologist visiting the United States has at least two shocks in store for him; the first is the breadth of interest of his American colleagues and the second is the scope of the American undergraduate programme. In the United States there are departments in biology where the staff may include an ecologist, a physiologist, a biochemist, a microbial geneticist, a plant pathologist and perhaps an embryologist. An undergraduate may study zoology, botany, biochemistry, microbiology and genetics as well as chemistry. Afterwards, if he undertakes postgraduate studies, he will attend courses in an even greater variety of subjects. We may be doubtful and scoff 'superficial' and 'multiple-choice', and there are certainly faults in the American educational system, but the American biology graduate and Ph.D. is better equipped than the British counterpart, better equipped to teach other subjects, better equipped to read the journals, better equipped to do and to direct research. The advances that are being made are the results of the application of the techniques of one subject in others. Embryology is now studied by using techniques of biochemistry and immunology ; major advances have been made in genetics by the use of micro-organisms; micro-organisms have contributed to our understanding of biochemistry and now the biochemistry of deoxyribonucleic acid, the chemical basis of heredity, is being studied by using the viruses that infect bacteria. The artificial barriers are down: only the complete biologist can hope to map the new territories, although all the time the discovery of new landmarks makes it easier to find the way.

The emphasis in teaching is on the vertical division: animals and plants are catalogued in an ascending scale of morphological complexity corresponding to the natural evolution of the groups. But British practice in research is based increasingly on a horizontal structure; people who work on the active transport of inorganic ions and organic molecules use yeast, bacteria, plant roots, parasitic worms, mammalian red blood cells and so on. The fundamental properties of active transport are probably the same in all cells and can be studied in any convenient system; methods of studying it are peculiar to active transport and do not depend on the type of cell. From these studies we may discover whether cells of different origin behave in the same way or whether those differences we find have an evolutionary or an ecological significance. Already we have a pragmatic subject, microbiology, in which unity derives from common techniques and not from an evolutionary concept of a common ancestor. Research emphasizes the unity of modern biology; our teaching must do the same, for only by teaching biology as a unified discipline can we hope to train biologists adequately. It is essential that while we teach the latest findings of biochemistry and biophysics, we also give our students a love and understanding of living plants and animals. If the biologist is taught little biochemistry, the biochemist lacks contact with the living.

Many subjects are taught in isolation when their integration would make plain their coherent pattern. Recent experiments have shown that diet and behaviour may influence the interaction of parasite and host; behaviour is affected by the hormone balance of the animal, and this in turn is influenced by the animal's environment. We must frame our courses so that the student is made aware of such complex interactions. To do this we must persuade the staffs of our universities from the isolation of their departments. A degree of trade union demarcation has begun in our subjects; we now specialize so early that there is a tendency for the student with a first degree in microbiology, for example, to be restricted to that subject; although his techniques could be used there to advantage, he is rarely encouraged to apply for a job in a zoology department.

If the new biology is experimental and not in the first place descriptive, on what shall we model our course ? At least one course that we could copy is already in existence. For the Diploma of Technology in applied biology at the colleges of technology the emphasis after the first year is on physiology, biochemistry, pharmacology and microbiology. Elementary mathematics, statistics and organic and physical chemistry are also compulsory subjects. It is significant that these courses were planned by departments of biology in colleges where there had been no fragmentation into minor subjects. Although these courses have been planned with the view of the needs of industry, they are more suited for academic biology and research than are the courses offered at the universities. Sixth-form students may well think so too, as they read the topics and experience required in the "Situations Vacant" columns. Already our students may be learning more about modern biology from the *New Scientist*, the Penguin Science Survey and television than they gain from the isolated subjects studied at a university.

It is important that, in presenting the mosaic to show both the pattern and the basic units, we do not leave the student either bewildered or complacent. Bewildered if, faced with two thousand references about a single animal, he cannot imagine what more can be discovered or where to start; complacent, if he is presented with knowledge as a set of facts and theories and techniques as unquestionable. Both might be avoided by a detailed observation that would give competence with several techniques, an appreciation of the complexity of an organism and, most of all, an awareness that science is not dormant but militant. One evident way to attempt this would be to give a class several strains of a bacterium. Each week the class would study their own cultured strains in a progressive series of experiments. These could include growth, nutritional requirements, antibiotic sensitivity, genetics and phage-susceptibility—each of which contributes to the understanding of the others. If we base our undergraduate courses on modern experimental techniques with wide application, we can shed some of the crafts which now occupy so much time in school and university. As we do so, we may attract students who now take the physical sciences.

As soon as possible we must bring all the biological sciences together in one building. This is necessary not only so that the staff may feel that they are all biologists but in order that courses can be integrated. Staff using the same or similar techniques will be encouraged to collaborate and form new loyalties. Apparatus can be shared for both teaching and research. Physical and financial separation now makes this difficult. With the present departmental system, expensive apparatus is bought by one department, used, and afterwards lies idle. In another department, similar equipment is bought from another source. While we lack in the universities much modern equipment necessary for research, we continue to buy two pieces of apparatus where one would suffice.

A director of the National Science Foundation, which is revolutionizing the teaching of science in the United States, has written of the need for more experimental biology in Europe[1]. In Britain recently there has been argument for the creation of more chairs of biochemistry and molecular biology or biophysics. The need for such chairs is urgent: Prof. Irene Manton, in a recent broadcast, suggested that more chairs be created within existing departments; and, I may add, we need fusion of the biological sciences for teaching and research. Within a unified department of biology, it will be easier to add new disciplines than it is, nowadays, to create new departments.

[1] Consolazio, W. V., *Science*, 133, 1892 (1961).

RESEARCH WORK OF THE NATURE CONSERVANCY

An adjournment debate in the House of Commons on July 21, at which was discussed the research work of the Nature Conservancy, was opened by Mr. M. Kimball with an attack on the work of the Conservancy which showed a complete misunderstanding of the purposes and nature of the Conservancy. Nevertheless, the debate as a whole should prove a useful piece of publicity for the work of the Conservancy, which still lacks the resources necessary for an adequate publicity policy to remove misunderstandings such as those exemplified by Mr. Kimball's speech. On the matter of finance, it may be noted that, although several speakers commented on the inadequacy of the Conservancy's budget of no more than £500,000 a year, the Parliamentary Secretary for Science, Mr. D. Freeth, made no comment beyond saying that it is a matter for individual judgment whether the Conservancy's resources are too small.

Mr. Freeth, recalling the Director-General's description of the research programme as "a comprehensive attack on a range of ecological problems which are regarded as of fundamental significance for the advance of conservation", maintained that besides increasing scientific knowledge, the Conservancy's work is in the long run of great benefit to the nation. Of a staff of 250, 100 are scientists, and the Conservancy also maintains 40 postgraduate students working for doctorates in ecology and related subjects. It is, he insisted, a research council, and although it does its best to encourage interest in Nature and its conservation, this is not a primary purpose, and one has to recognize that many of the reserves are established to be sanctuaries for wild life, a purpose inconsistent with free access. The Conservancy welcomes at all its reserves scientists and university workers who have research to do which is likely to be of value. Mr. Freeth does not believe that there is overlapping with the work of other Government research organizations. There is considerable interlocking of personnel at a very high level, as well as on *ad hoc* committees, and the Select Committee on Estimates which explored the question of relations with the Forestry Commission in 1958 found no evidence to justify a recommendation to transfer to the Commission some of the fundamental research now undertaken by the Conservancy. Much of the Conservancy's research is basic in character and not suitable for dissemination through the National Agricultural Advisory Service.

The Conservancy, Mr. Freeth pointed out, controls about 8,000 acres of woodland, and research here is essential: first, to provide representative examples of the more important ecological types in their earlier stages of development; secondly, to provide adequate areas of managed woodland which would otherwise be threatened by systems of management more concerned with profit-making; and thirdly, to provide specialized facilities for undisturbed research and long-term field-trials. Mr. Freeth specifically denied the suggestion that the Nature Conservancy has ever established the breeding of coypu. For publicity or educational campaigns, or the issue and enforcement of regulations, the Conservancy has to look, for example, to the Ministry of Agriculture or of Transport.

As regards liaison and co-operation with county naturalist trusts and other bodies of naturalists, Mr. Freeth pointed out that the Select Committee on Estimates was impressed with the argument that unique opportunities for research would be lost if the Conservancy did not acquire as soon as possible the whole of the 250,000 acres envisaged. At present, 88 reserves represent 177,000 acres, and Mr. Freeth does not think it reasonable to delay proceedings merely because in a particular area a county organization has not been formed. He welcomed Mr. Kimball's praise of the Nature Conservation Corps, but to what was perhaps Mr. Kimball's most important point—that for only half the reserves has a five-year management plan been established—he made no reply. If this is due simply to financial stringency, it is time that the Minister for Science—and Parliament—acted.

SALMON AND FRESHWATER FISHING IN GREAT BRITAIN

Recently the Committee on Salmon and Freshwater Fisheries produced a stimulating report in which are made several recommendations which may lead to some very necessary changes in freshwater fishery legislation.*

Of major importance are the recommendations that the powers of the River Boards be extended. The report rightly appreciates that fishery problems are often localized and suggests that River Boards should be allowed to make by-laws to suit local conditions. Equally important are the recommendations that River Boards should have the power to demand accurate returns of catches from all licence holders. Clearly, adequate salmonid fishery conservation is greatly hampered by inaccurate returns of catches. As conservation to a large degree centres around re-stocking, it is obvious that the recommendation that no person be permitted to stock his waters without the consent of the River Board is pertinent: there is much more to stocking than the mere planting of fish.

Some valuable recommendations are made on the control by River Boards of the obstructions to migratory fish, and of particular importance in this context is the suggestion that a River Board should have the power to prevent any diversion of water unless sufficient water is passed down the original channel to avoid damage to fisheries. Equally sound is the point made that old dams, sluices, or leats in poor condition should be repaired by the owners, or alternatively demolished. Overcrowding in pools below unsurmountable weirs often results in dead fish during periods of high temperature and low water.

---

* Report of the Committee on Salmon and Freshwater Fisheries. (Cmnd. 1350.) Pp. viii+151+13 plates. (London: H.M.S.O., 1961.) 8*s*. net.

Pollution is dealt with admirably; and the suggestion that the Armer Committee's recommendations relating to the control of existing discharges should be extended to estuarine waters, should, if adopted, be highly beneficial to salmonid fisheries. I would like to see this control extended to all inshore waters.

The report stresses the need for more freshwater fishery research, and I am certain that the far too few freshwater-fishery biologists that are active in Britain will applaud the proposals that electric fishing and poisoning should be permitted for the purpose of scientific fishery research.

A report of this nature, which is of great interest not only to the 2½ million anglers but also to many industrialists, water undertakings and public bodies in Britain, will inevitably suffer much criticism. To a fishery biologist it suggests a beneficent revolution, but I find a few minor points of disagreement with the Committee. First, because only 2,000 netsmen are dependent, or partly dependent, on salmon fishing for their livelihood, because salmon anglers outnumber netsmen by about fifty to one and because anglers are of greater economic importance to River Boards, riparian owners, the tackle and tourist industry, I would recommend that netting be discontinued until salmon are much more plentiful. The netsmen should be adequately compensated. Secondly, I would recommend strongly that kelts should be preserved. As the report states, previously spawned fish form 5 per cent or more of salmon catches in the United Kingdom and thus represent at least 15 tons of fish; it would need several expensive hatcheries to produce this weight of fish. Thirdly, I would prohibit angling for salmon after the first week of September; in most cases this would protect the gravid fish and dispense to a large extent with the need for an accurate definition of unclean fish as discussed in the report. Fourthly, I would advocate a close season for coarse fish and that this be suited to local conditions. In addition, I would make it illegal for fishermen to return coarse fish above the minimum size, as many coarse fisheries are vastly overstocked and consequently the fishes are stunted. Lastly, I feel that the report should have stressed more strongly the need for research on coarse fish and I would suggest that this should be carried out in a national laboratory. I am certain that the River Boards and the River Boards Association would find such an institution invaluable.

Despite these objections the report is of major importance. It rightly emphasizes the remarkable achievements of the River Boards and its suggestions for the extension of their powers or for extended use of existing powers are expert, moderate and reasonable, as are its recommendations for compensation or non-compensation of affected interests. Everyone who has at heart the interests not only of the fisheries but also of rivers and lakes and the country as a whole will watch impending legislation closely and pray that the recommendations of the report become law with a minimum of mutilation and, above all, a minimum of delay. J. W. JONES

ELECTRIC CURRENTS ASSOCIATED WITH GEOMAGNETIC DISTURBANCES IN THE AURORAL ZONE

By DR. F. J. SCRASE

Meteorological Office, London

THE electric current systems in the high atmosphere in high latitudes, especially along the auroral zones, are complex and variable. The normal networks of geomagnetic observatories suffice only to provide inferences of the broader features of these systems. With the view of their more detailed study, the Special Committee of the International Geophysical Year, at its Rome meeting in 1954, recommended that some observatories in auroral regions should be supplemented by two subsidiary stations, in directions at right angles and about 20 km. from the main station, for the measurement of the horizontal space gradients of the magnetic elements.

In an article amplifying these recommendations, S. Chapman[1] explained how knowledge of a disturbance in horizontal magnetic force produced by a horizontal current flowing directly overhead, together with the vertical gradient of the disturbance, will indicate the strength and height of the current separately. Since the divergence and curl of the magnetic field are both zero, the vertical gradients can be derived from the horizontal gradients.

In accordance with the recommendation of the Comité Spécial de l'Année Geophysique Internationale, two subsidiary stations equipped with Askania variographs were set up near the Meteorological Office Geophysical Observatory at Lerwick (60° 08′ N., 01° 11′ W.) and were operated for part of the International Geophysical Year and its successor the International Geophysical Co-operation, 1957–59. One of the stations was at Aipleton, 26·7 km. south-south-west of the main station and the other at Bridge of Walls, 22·3 km. west-north-west. This article describes preliminary results of attempts to estimate the magnitude and position of the electric currents during some magnetically disturbed periods.

Method of Analysis

The disturbances selected for measurement were mostly those of the bay type of a few hours duration, but a few were sharp peaks of shorter duration. For each station the components of the disturbance vector were derived by measuring the maximum deviation with respect to an undisturbed part of the record shortly before the disturbance, or to the interpolated undisturbed value at the time of the maximum. It soon became evident that values of the horizontal space gradients derived from differences between records from the pairs of stations did not conform with the theoretical relationships between

Table 1

	Date	G.M.T.	*Rn* (km.)	*Re* (km.)	*Rz* (km.)	*C* $\left(\frac{\text{amp.}}{1{,}000}\right)$	*A* (deg.)	*I* (deg.)
1959	Oct. 26	15.25	+64	−3	53	+88	78	+11
1959	June 28	15.30	+105	−27	268	+368	91	−5
1957	June 4	16.40	0	−16	167	+86	95	−6
1959	June 27	17.05	+92	−67	371	+598	81	−9
1959	Oct. 3	17.05	+230	−230	627	+1,180	89	−19
1959	Apr. 27	17.10	+90	+11	156	+111	113	−9
1959	Sept. 1	17.20	+177	−64	248	+353	95	−18
1959	May 15	18.00	+50	−97	576	+750	91	−10
1959	Apr. 26	18.10	+109	+3	338	+235	99	−3
1959	May 11	18.20	+113	−60	352	+232	91	−10
1957	June 3	18.25	+87	−54	246	+465	83	−10
1957	June 21	19.45	+15	+34	66	+43	146	−4
1959	May 24	19.50	+173	+12	124	+157	90	+5
		Mean:	+100	−43	276	+359	95	−7
1959	Oct. 1	21.40	+34	0	48	−149	261	−6
1959	Apr. 26	22.07	+96	+15	74	−110	288	+12
1959	Apr. 27	22.31	+8	+25	55	−23	323	−7
1957	June 5	23.30	+53	+11	96	−203	294	+7
1959	June 4	23.43	+70	−39	77	−142	261	+2
1959	Aug. 22	00.30	+87	−14	224	−300	277	+6
1957	June 6	02.15	+36	−9	65	−98	279	+12
1957	June 18	02·30	+130	−39	82	−104	262	+14
1957	June 25	03.00	+70	−10	51	−75	248	−18
		Mean:	+65	−7	86	−134	277	+2

Key to headings of columns: *Rn*, *Re*, *Rz* are distances north, east and vertically from Lerwick of the intersection of the current with the perpendicular from Lerwick; *C* is the current strength, positive sign indicating west to east; *A* is the horizontal direction of the current with respect to geomagnetic north; *I* is the vertical inclination of the current, positive directed downwards.

the field components associated with a horizontal line current. Thus, the gradients of the north components along the easterly direction were found not to be equal to the east component gradients in the northerly direction. There may be several reasons for this. For example, the differences over finite distances, about 25 km., may not be representative of the true gradients at a point; or the assumption of a simple line current may not be valid; or the effects of induced currents or of local anomalies may be significant. A similar difficulty with regard to the gradients has been reported by Cain[2] in a preliminary analysis of differential measurements made during the International Geophysical Year in Alaska.

However, it is not necessary to know the gradients in order to compute the magnitude and location of the current that produces the measured field disturbances at two or more stations. The line current should, in fact, coincide with the intersection of the planes through the stations normal to the directions of the total disturbance forces. The location and direction of such a current, relative to the stations, are thus obtainable, by co-ordinate geometry, from the measurements at any pair of stations. With three stations three sets of results are obtained, thus affording some check on the accuracy of the determinations. Knowing the perpendicular distance R of the line current from a station, the strength C of the current is obtained from $C = f.R/2$, where f is the total disturbance force at the station. To avoid introducing a questionable factor, the effect of induced currents has not been taken into account in these computations, but the magnitude of the effect is considered later.

For the preliminary investigation, 22 records of disturbances were selected for measurement; these showed station differences in one component or another sufficiently marked to lead to reasonably consistent estimates of the currents. The disturbances (in total force) ranged from about 100 to 400γ, with station differences of a few tens of gamma. In some cases measurements from only one pair of stations were available.

The main results are presented in Table 1, in which the occasions are arranged in the order of the time of day at which the disturbance (maximum) occurred. No attempt was made to select disturbances according to the time of day, however, and their distribution may be taken to indicate that measurable disturbances occurred more rarely between 04 hr. and 14 hr.; this conforms with the almost complete absence of radar echoes from aurora in the morning daylight hours 06–12 hr. The fact that the results are confined to the period between April and October, however, does not indicate that disturbances rarely occurred in winter.

The computed locations of the currents relative to the positions of the stations are plotted in Fig. 1. In each case the relative magnitude and the azimuth of the current are indicated by the length and direction of the line, the mid-point of which represents the intersection with the perpendicular (3-dimensional) from Lerwick. It will be seen that most of the currents are located to the north-north-west of Lerwick between geomagnetic latitudes 62·5° and 63·5° N. Observations at the Balfour Stewart Auroral Laboratory from the area from the Western Atlantic to Central Europe indicated that auroral displays were reported on most of the days of the magnetic disturbances and that the bases of the auroral arcs were overhead in lat. 62°–67° and rays overhead in lat. 59°–64°.

Fig. 2 shows how the main features of the currents vary with the time of day. The most marked change is in the direction of the currents, which is from west to east between 15 hr. and 20 hr., and east to west from 22 hr. to 03 hr. This is in agreement with deductions made by Goldie[3] from magnetic storm disturbance vectors and also with the visual, photographic and radar echo observations of auroral move-

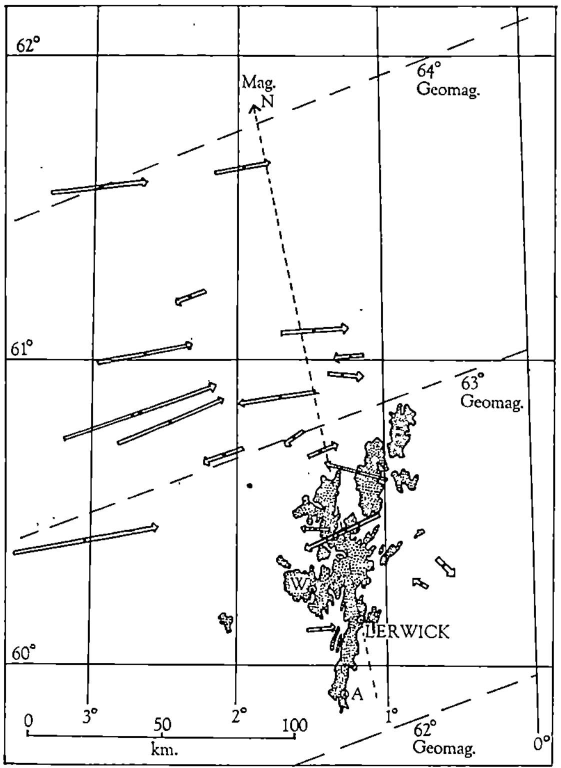


Fig. 1. Location of auroral zone currents

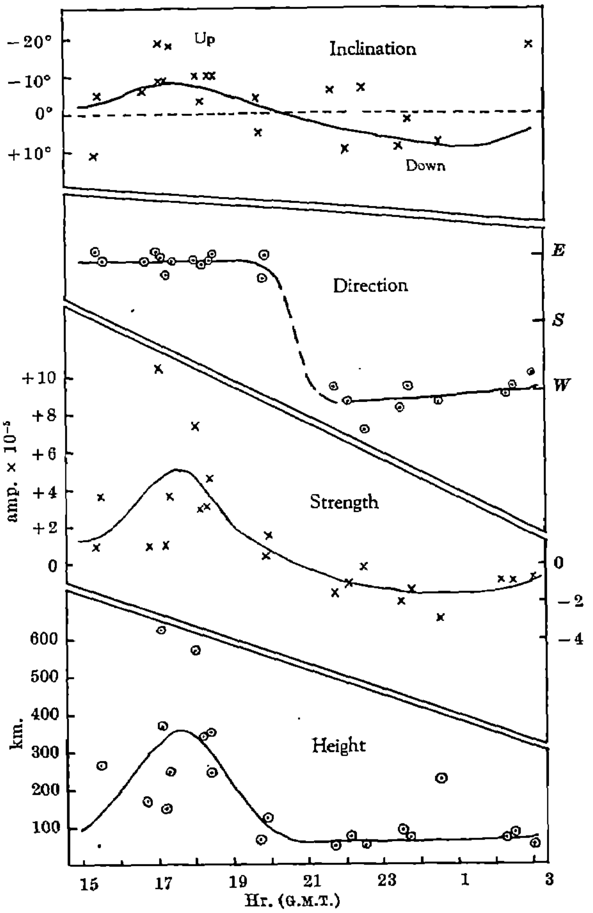


Fig. 2. Auroral zone currents derived from magnetic records

ments reported by various observers. The mean directions of the currents are more nearly parallel to the direction along local geomagnetic east and west than to the lines of geomagnetic latitude, the differences being about 5° and 15° respectively. This result is in conformity with observations of auroral rays (McInnes and Robertson[4]) and with recent auroral radio echo measurements (Watkins[5]).

The current strengths reach a maximum, of about 10^6 amp. in the west to east direction, between 17 hr. and 18 hr., fall to zero at about 21 hr. and then increase to a smaller maximum of about 3×10^5 amp. at about 0 hr. These current strengths are of the same order of magnitude as those determined by Goldie[3] for large magnetic storms.

The calculated values of the inclination of the currents to the horizontal (at Lerwick) show considerable scatter, but there does appear to be a tendency for the west–east currents to be inclined upwards and the east–west downwards by about 5°. This departure from the horizontal is too large to be accounted for by the Earth's curvature; at the extreme distance west of Lerwick of the currents shown in Fig. 1, the curvature should not cause an apparent departure of more than about 2°.

Probably the most interesting feature shown in Fig. 2 is the variation in the height of the currents. As in the case of the strengths, there is a maximum at about 17.30 hr. Between 15 hr. and 19 hr., most of the currents were at heights between 150 km. and 350 km., that is, at about the levels of the $F1$ and $F2$ layers of the ionosphere, but two appeared to be near 600 km. After 20 hr. and until 3 hr., the heights of the currents were centred at about 80 km., that is, near the E layer of the ionosphere and at about the average lower limit of auroral visual forms and of maximum auroral light. Most of the radio observations of aurora indicate that echoes originate from that layer, but radar reflexions have been detected from levels as high as 300 km. and observations of the scintillation of radio stars have indicated disturbances in ionization at these levels. From Goldie's determinations from magnetic storms of outstanding magnitude, heights of currents of between 230 km. and 390 km. at all hours of the day were deduced, but in storms of a more ordinary category the heights fell to about 100 km. between 0 hr. and 4 hr.

Effect of Induced Currents

It has already been mentioned that, in deriving the results described here, no account has been taken of the possible effects of induced currents in the Earth. That they are not negligible is shown by comparing the elevations of the vectors of disturbances recorded simultaneously at Lerwick and at Eskdalemuir about 500 km. south of Lerwick. Although the total disturbance forces recorded at Eskdalemuir are roughly of the magnitude expected at the distances from the currents computed from the Lerwick network observations, the elevations of the forces are all much greater than expected. This is also evident in the Eskdalemuir and Lerwick data used by Goldie[3] already mentioned; but fortunately he realized that the elevations at Eskdalemuir were more liable to be in error and used the total forces in his computations. The discrepancies in the elevations can, however, be largely accounted for if we assume the existence of an induced current, equal and opposite to the overhead current, at a depth of the order of 800 km. below the Earth's surface. Estimates of the depth in individual cases varied between 550 km. and 1,100 km. It is known that the electrical conductivity of the Earth, which is very small down to a depth of 600 km., where it has a value of 10^{-3} ohm.$^{-1}$ cm.$^{-1}$, rises rapidly to reach a value of at least 10^{-2} ohm^{-1} cm.$^{-1}$ at a depth of 900 km. (Lahiri and Price[6]).

In the method adopted for computing the overhead currents, the nearer the observing station to the seat of the currents the smaller are the errors caused by neglecting the induction current. If the effect of the latter at the depth of 800 km. is allowed for in the computations for the Lerwick network, the calculated heights and strengths of the overhead currents would be reduced by roughly 30 per cent, but the general trend of the daily variation shown in Fig. 2 would not be changed significantly.

Density of Ionization

If, as is now generally accepted, the currents of the auroral zone result from the movement of the ionization that is responsible for the aurora, it should be possible to relate the current strength C with the velocity of auroral motion V. At about 18 hr., when the currents reach their maximum value of about 10^6 amp. in the easterly direction, auroral speeds are of the order of 1 km. sec.$^{-1}$ in the westerly direction (Ratcliffe[7]). If, then, these currents are produced by the westerly drift of negative ionization at this speed and if the area of cross-section of the stream of ionization is A, then the electron density can be estimated from $n = C/e.V.A$, where e is the electronic charge ($1\cdot6 \times 10^{-19}$ coulomb). Recent radar echo observations of aurora (Eastwood[8]) suggest that the region of ionization in which the reflexions take place is about 20 km. thick, and so an appropriate value of A would be 400 km.^{2}. These figures lead to an estimate of the electron density of:

$$n = 10^6/(1\cdot6 \times 10^{-19} \times 10^5 \times 400 \times 10^{10}) = 1\cdot5 \times 10^7 \text{ cm.}^{-3}$$

This is a reasonable value for disturbed conditions in the ionosphere.

This communication is published with the permission of the Director-General of the Meteorological Office. It is hoped to publish a more detailed account of the work in due course.

---

[1] Chapman, S., *I.U.G.G. News Letter*, No. 9, 117 (1955).

[2] Cain, J. C., *Quart. Prog. Rep.* No. 1, I.G.Y. Contract No. 50.2, Geophys. Inst., Univ. of Alaska (1958).

[3] Goldie, A. H. R., *Trans. Roy. Soc. Edin.*, 57, Pt. 1, 143 (1931).

[4] McInnes, B., and Robertson, K. A., *J. Brit. Astron. Assoc.*, 69, 167 (1959).

[5] Watkins, C. D., *J. Atmo. Terr. Phys.*, 19, 1 (1960).

[6] Lahiri, B. N., and Price, A. J., *Phil. Trans. Roy. Soc.*, A, 237, 509 (1939).

[7] Ratcliffe, J. A., *Physics of the Upper Atmosphere* (Academic Press, London, 1960).

[8] Eastwood, E., *et al.*, *Nature*, 188, 115 (1961).

GRAVITY SURVEY ON THE AIRA CALDERA, KYUSYU, JAPAN

By IZUMI YOKOYAMA

Geophysical Institute, Hokkaido University, Sapporo

THE Aira caldera, in the southern part of Kyusyu, Japan, is one of the biggest in the world, measuring about 20 km. in diameter. It occupies the innermost part of Kagosima Bay, and an active volcano, Sakurazima (1,118 m. high), stands on its southern circumference. This volcano came into existence after the formation of the Aira caldera. The bottom of the caldera is the rather shallow (about 150 m. deep) and flat floor of Kagosima Bay and the steep cliffs occurring at several sea-shores have been supposed to be parts of the encircling caldera rim. A tremendous volume of volcanic ejecta, ignimbrite and pumice flows, which covers a wide area around Kagosima Bay, has been proved to have been ejected when the caldera was formed.

As reported before, my colleague and I[1] found a conspicuously low Bouguer anomaly on the Kuttyaro caldera lake in Hokkaido, which amounts to a maximum of 46 mgal. extending over 20 km. Since I considered such a low gravity anomaly as one of the characteristics common to the calderas of so-called Krakatoa type, the formation of which was accompanied by the ejection of vast quantities of matter, I tried to verify the similar gravity distribution on the Aira caldera. A gravity survey by means of a North-American gravimeter was carried out there in August 1960 in co-operation with the Geographical Survey Institute. Almost all the observation points are the bench-marks for precise levels. The Bouguer anomalies observed around the caldera and along the coast-line of the Sakurazima Peninsula are shown in Fig. 1, where topographical corrections are smaller than 1 mgal. for all observation points and can safely be ignored. As shown in Fig. 1, a relatively low anomaly is very conspicuous around the Aira caldera, the lines of equal anomaly being exactly concentric with the supposed caldera rim. At the northern boundary where there is no characteristic topography of the caldera rim, it is not easy to discriminate exactly only the effect of the Aira caldera on the gravity field, in other words, some residual anomalies due to the caldera, but one may easily extrapolate the tendency of decrease in the Bouguer anomaly observed on land to the centre of the caldera, though no observation in the Bay is available. At the north of Sakurazima, perhaps the minimum value of the Bouguer anomaly may reach about − 25 mgal. and hence the relative anomaly to the surroundings may amount to about − 45 mgal. Further, the centre of this anomaly coincides with one of the topographical depressions at the time of the great 1914 eruption of Sakurazima. Along the coast of Sakurazima itself, the anomalies are negative at the northern side and positive at the southern side, while the axis of inversion of the sign is almost the same as that of the crustal tilt which accompanied the recent activities of the volcano. The active volcano Sakurazima has scarcely any effects on the general distribution of the Bouguer anomaly on the caldera: this means that the size of the structure of a recent volcano cannot be compared with that of a gigantic caldera so far as density contrast is concerned. The anomaly suggested by the present survey, about

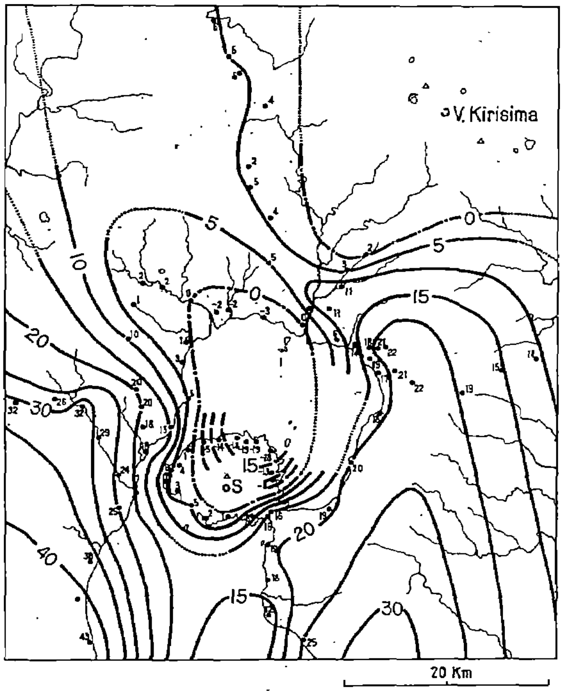


Fig. 1. Distribution of the Bouguer anomalies on the Aira caldera. Unit is the mgal. *S*, crater of Volcano Sakurazima

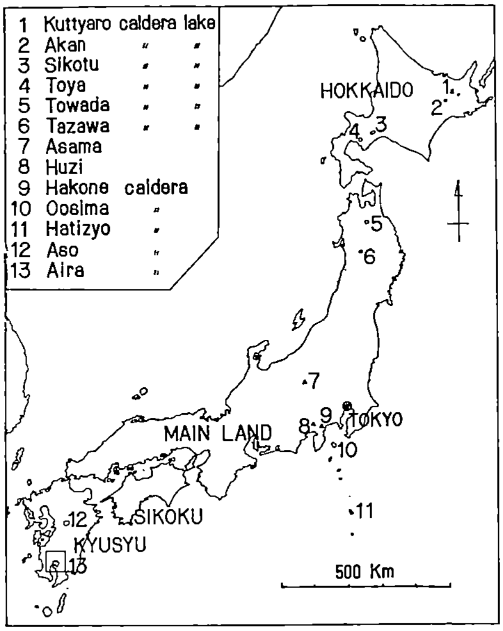


Fig. 2. Calderas in Japan

45 mgal. at its maximum extending over 20 km. in diameter, is very conspicuous and incidentally of just the same magnitude as the Kuttyaro caldera. Therefore, it is concluded that coarse material with a density about 0·3–0·5 gm./cm.^{3} smaller than that of its surroundings has accumulated at a depth of 3–4 km. beneath the caldera. The distribution of the Bouguer anomalies gives the total mass defect, which is responsible for the low anomalies, as 1·6 × 10^{11} metric tons. In fact, the material ejected from the caldera during its formation was deposited as ignimbrite and pumice flows around the caldera; its amount is estimated at 1·6 × 10^{11} m.^{3} by B. Koto[2].

Table 1

Caldera	Diam. (km.)	Age	Deposit	Bouguer anomaly (mgal.)	Observer
Kuttyaro	20–26	Pleistocene	Ignimbrite	−46	Refs. 1, 3
Akan	13–24	Pleistocene	Ignimbrite	−25	Ref. 3
Oosima	3–4·5	Holocene	Subsidence of basaltic lavas	+15	Ref. 4
Aso	17–25	Pliocene	Ignimbrite	−20	Ref. 5
Aira	23–24	Pleistocene	Ignimbrite and pumice flows	−45	
Volcano	**Height (m.)**	**Age**	**Rock**	**Bouguer anomaly**	**Observer**
Asama	2,542	Holocene	Andesite	Normal	Ref. 6
Huzi (Fuji)	3,776	Holocene	Basalt and andesite	Normal	Ref. 7

In Japan there are some ten calderas as shown in Fig. 2, some of which have been studied gravimetrically. The results of the observations on the calderas and volcanoes are summarized in Table 1.

From these data, though examples are not abundant, it can be seen that the Bouguer anomalies on the calderas can be classified in two types: (1) low anomaly, that is, Kuttyaro type; (2) high anomaly, that is, Oosima type. It can further be seen that they are almost normal on the large stratified volcanoes without caldera, such as Huzi (Fuji) and Asama, after consideration of topographical corrections. The two types of Bouguer anomalies correspond geologically to two types of calderas, the Krakatoa and the Glencoe types of H. Williams[8], according to the cause of their formations as discussed in ref. 1.

On the other hand, L. C. Pakiser, *et al.*[9] carried out a geophysical investigation in the Mono Basin, California, which is a volcano-tectonic depression surrounded by a large amount of pumice; they found a residual gravity relief of about − 50 mgal. extending over 25 km. It is very noticeable that the magnitude of this anomaly is of the same order as the Japanese large calderas. E. Medi and C. Morelli[10] made a gravity survey in Sicily and found no anomaly on Etna (3,274 m. high) as was also the case on Huzi and Asama. In effect, the contrasts of the Bouguer anomaly between large calderas and stratified volcanoes are very remarkable with respect to subterranean structure or depth of origin, shallow for the former and deep for the latter.

I wish to thank the authorities of the Geographical Survey Institute for their kind co-operation in this work.

---

[1] Yokoyama, I., and Tajima, H., *Nature*, **183**, 739 (1959).

[2] Koto, B., *J. Coll. Sci., Imp. Univ. Tokyo*, **38**, 12 (1916).

[3] Yokoyama, I., *J. Phys. Earth*, **6**, 75 (1958).

[4] Yokoyama, I., and Tajima, H., *Bull. Earthq. Res. Inst.*, **35**, 23 (1957).

[5] Tsuboi, C., Jitsukawa, A., and Tajima, H., *Bull. Earthq. Res. Inst.*, Supp., **4**, Part 8, 476 (1956).

[6] Tsuboi, C., Jitsukawa, A., and Tajima, H., *Bull. Earthq. Res. Inst.*, Supp., **4**, Part 5, 199 (1955).

[7] Yokoyama, I., and Tajima, H., *Geofisica Pura e Applicata*, **45**, 1 (1960).

[8] Williams, H., *Univ. Calif. Pub. Bull. Dept. Geol. Sci.*, **25**, 239 (1941).

[9] Pakiser, L. C., Press, F., and Kane, M. F., *Bull. Geol. Soc. Amer.*, **71**, 415 (1960).

[10] Medi, E., and Morelli, C., *Annali di Geofisica*, **5**, 209 (1952).

ETHYLENE EVOLUTION AND SUB-CELLULAR PARTICLES

By Prof. STANLEY P. BURG and ELLEN A. BURG

Department of Physiology, University of Miami School of Medicine, Florida

DURING the past ten years there have been at least five separate reports of the preparation of cell-free systems capable of evolving ethylene. The earlier claims relating to soluble systems, acetone powders and breis[1–3] have been criticized on many counts and in particular the specificity of several of the assay methods is questionable[4]. In fact, it is doubtful whether certain of the plant sources which were used actually produce measurable quantities of ethylene, for example, etiolated peas and oats[2]. More recently, the report by Spencer[5] of the preparation of a particulate ethylene-producing system from tomatoes has been disputed by Meigh *et al.*[6], who were unable to confirm the results using either tomatoes or apples. We also have had no success in preparing a sub-cellular system which evolves ethylene, using apples, tomatoes and the fungus *Penicillium digitatum* as sources of enzyme and the techniques of Hall[1,2], Dimond and Waggoner[3], Spencer[5] and other conventional procedures. The most recent report of ethylene production by a particulate preparation is that of Lieberman and Craft[7], who used an acidic medium to isolate active fractions from both tomatoes and apples. It is the purpose of the present article to indicate that the volatile substance emanating from these particles is not ethylene, and hence that ethylene production by a sub-cellular system remains to be demonstrated unequivocally.

A single tomato in an early pink stage was homogenized at 4° C. with an equal volume of *p*H 6 buffer (0·05 *M* phosphate, 0·5 *M* sucrose), using a Waring blender for 1 min. at slow speed. The homogenate was passed through four layers of cheesecloth, after which 500*g* (15 min.) and 17,000*g* (30 min.) fractions were obtained by differential centrifugation. Each fraction was washed by re-sedimentation and the pellets were suspended in 5 ml. of *p*H 6·2 sucrose–phosphate buffer. Into a 5-ml. syringe were drawn successively 1 ml. of particles, the co-factors (listed below) contained in 0·6 ml. buffer, and 3 c.c. of air. The needles were sealed by insertion into rubber stoppers, syringes were incubated at 30° C., and finally the entire gaseous contents were analysed by pressing out all the fluid and gas chromatographing the remaining air on an aluminium oxide column. A flame ionization detector similar in type and sensitivity to that used in refs. 6 and 7 was used, so that the major difference between the present experimental technique and that described in their original communication in *Nature* is the substitution of an aluminium oxide adsorption column for the silicone oil partition column. However, it should be noted that details of the procedure by which 'acidic' particles[7] were prepared from tomatoes and apples have not yet been published, so that it is impossible to state with absolute surety that our sub-cellular preparation is comparable in all respects.

It was found that a gas similar to ethylene was evolved by both washed and unwashed 500*g* and 17,000*g* particles when adenosine triphosphate, coenzyme A, magnesium chloride, phosphate and thiomalic acid were included in the incubation mixture (see Table 1: 'ethylene-like' substance). The rates per ml. are closely comparable with those reported by Lieberman and Craft, although an exact comparison is not possible since the nitrogen content of our preparation was not determined. As shown in Table 1, the particles behaved in every respect in the manner which has previously been reported[7]: a requirement for thiomalate and thioglycolate is only very slightly replaced by cysteine and glutathione, the reaction is essentially inhibited by boiling the enzyme preparation, the evolution is strongly inhibited by 10^{-3} *M* ethylenediaminetetraacetic acid (EDTA), and both 500*g* and 17,000*g* fractions are active. In addition, the rate in the presence of thiomalic acid was found to be linear for almost 60 min. at 30° C. However, the gas which the particles produce does not co-chromatograph with added ethylene, as shown in Fig. 1, its peak being distinctly separated from that of authentic ethylene. Moreover, as shown by gas chromatography, neither bromine water nor mercuric perchlorate solution removed the substance from air, whereas these reagents were found consistently to eliminate comparable quantities of ethylene from synthetic air–ethylene mixtures. Thus it is established that the gas produced by these particles definitely is not ethylene. Since the substance is observed to chromatograph between ethane and ethylene, it may be a two-carbon compound, and it is most likely organic as it produces a response with the flame ionization detector.

Table 1. Production of an 'Ethylene-like' Substance by Cytoplasmic Particles from Tomatoes in the Presence of Thiomalic and Thioglycolic Acids

Syringe contents*	μl./c.c. particles/hr.	
	'Ethylene-like' substance†	Ethylene
Particles + co-factors	0·0013	0·0003
Particles + co-factors + thiomalate	0·0343	0·0006
Particles + co-factors + thioglycolate	0·0314	0·0005
Particles + co-factors + glutathione	0·0038	0·0002
Particles + co-factors + cysteine	0·0041	0·0004
Boiled particles + co-factors + thiomalate	0·0023	0·0007
Particles + co-factors + thiomalate + EDTA	0·0035	0·0005

* The syringe contents consisted of the following: 50 μ*M* thiomalate, glutathione, cysteine or thioglycolate; 3 μ*M* adenosine triphosphate, 60 μ*M* phosphate; 0·08 μ*M* coenzyme A, 400 μ*M* sucrose, 25 μ*M* $MgCl_2$, 1·6 μ*M* EDTA. The *p*H of the reaction mixture was 6·2 and the syringes contained 1·6 ml. of liquid and 3 c.c. of air. The syringes were incubated at 30° C. for 60 min.

† These values are derived on the basis of a calibration of the flame detector for ethylene, but since the sensitivity of the instrument depends on the number of carbon atoms in the compound, the rates will be in error if the 'ethylene-like' substance does not possess two carbon atoms in its structure.

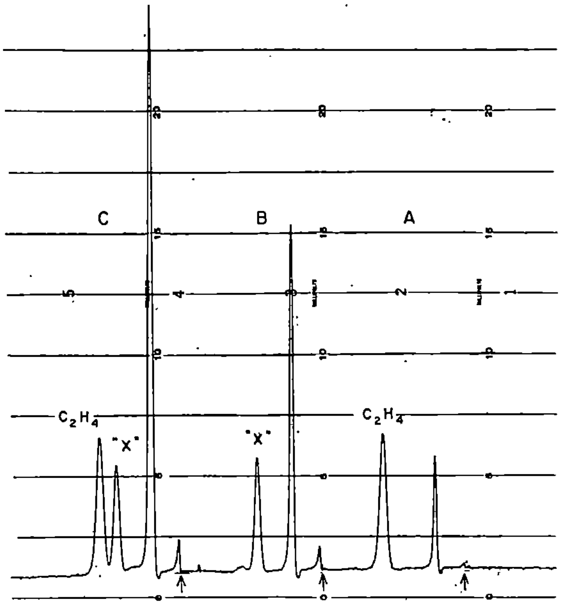


Fig. 1. Gas chromatogram of (*A*) an authentic sample of ethylene, (*B*) volatiles produced by tomato particles, and (*C*) a co-chromatograph of *A* and *B*. The graph reads from right to left and one vertical division corresponds to 30 sec. Samples were inserted at the times indicated with arrows. A 24-in. activated alumina column (Burrell) was operated at 23° C. with a nitrogen carrier gas flow-rate of 10 c.c./min. and a hydrogen flow-rate of 15 c.c./min. The first peak in each case represents a mixture of all unretarded components of the sample, and the 'ethylene-like' substance is labelled '*X*' on the graph

A close examination of Fig. 1*B* reveals that there is a minute trace of a volatile material, amounting to a few per cent of the major unknown component, which has the same retention time as authentic ethylene. Its identity as ethylene was confirmed by the observation that treating the sample with either bromine water or mercuric perchlorate reagent caused it to be absent from a subsequent chromatograph. It is important to note that the production of ethylene, within the limits of accuracy which can be obtained with such small quantities of material, is shown in Table 1 to be independent of the co-factors and substrates included, and the boiled preparation is at least as active as any other. Within a 25-min. incubation time, which was the briefest collection period investigated, the formation of ethylene had ceased in all cases. Therefore, the trace of ethylene is perhaps not due to a metabolic reaction, but rather to a contamination of unknown origin which is present, or formed by chemical reaction, at the start of the incubation.

Undoubtedly one of the stimuli which has prompted the search for a particulate preparation able to evolve ethylene is the observation that intact tissue responds to treatment with solutions of varying tonicity as though the ethylene-producing system were located in a particle having a semi-permeable membrane[8,9]. This evidence is indirect, and the interpretation can only be validated by the actual demonstration of ethylene evolution by a particulate fraction. However, the behaviour of mitochondria, which may display no endogenous uptake of oxygen although able to consume oxygen on the addition of appropriate substrates, makes it unlikely that particles will be shown to evolve ethylene unless a specific substrate is added. This situation is made even more difficult by the complication that cutting tissue into very small pieces seems to hinder ethylene production markedly[6,10].

In all properties investigated, the particles described in this article appear to be similar to the sub-cellular fractions prepared by Lieberman and Craft provided that our 'ethylene-like' substance is equated with their ethylene. If this is valid, then it must be concluded that none of the claims of ethylene production by cell-free systems can be supported, and that of Lieberman and Craft is no exception. It will be necessary to know more about the substrate from which ethylene is derived before such experiments are likely to be successful.

A grant (*RG* 8124) from the National Institutes of Health provided support for this investigation. We thank Prof. K. V. Thimann for his criticism and advice.

[1] Hall, W. C., *Bot. Gaz.*, **113**, 55 (1951).
[2] Hall, W. C., *Plant Physiol. Proc.*, **32**, vii (1957).
[3] Dimond, A. E., and Waggoner, P. E., *Phytopathology*, **43**, 663 (1953).
[4] Biale, J. B., Young, R. E., and Olmstead, A. J., *Plant Physiol.*, **29**, 168 (1954).
[5] Spencer, M. S., *Nature*, **184**, 1231 (1959).
[6] Meigh, D. F., Norris, K. H., Craft, C. C., and Lieberman, M., *Nature*, **186**, 902 (1960).
[7] Lieberman, M., and Craft, C. C., *Nature*, **189**, 243 (1961).
[8] Burg, S. P., and Thimann, K. V., *Plant Physiol.*, **35**, 24 (1960).
[9] Craft, C. C., *Plant Physiol. Proc.*, **35**, vii (1960).
[10] Burg, S. P., and Thimann, K. V., *Proc. U.S. Nat. Acad. Sci.*, **45**, 335 (1959).

OXYGEN ELECTRODE FOR MEASUREMENT OF TISSUE SLICE RESPIRATION

By E. H. STRICKLAND*, F. D. ZIEGLER and PROF. A. ANTHONY

Biophysics Laboratories, Pennsylvania State University, University Park, Pennsylvania

SUBSTANTIAL progress has been made in using polarographic methods to measure oxygen concentrations in a number of biological systems, such as blood[1], mitochondria[2] and bacteria[3]. Improved reproducibility and simplicity of operation have been achieved by refinements in the oxygen electrode, for example, the Clark electrode[4]. The present article describes a continuously recording tissue slice respirometer which uses a Clark oxygen electrode covered with a ½ mil 'Teflon' membrane. This system allows convenient measurement of rapid changes in respiratory-rates.

The tissue slice respirometer constructed in this laboratory is shown in Fig. 1. The reaction medium is kept uniformly mixed by magnetic stirring, so that addition of reagents does not alter the steady-state diffusion conditions for more than a few seconds. A sealed electrode vessel prevents oxygen transfer from the atmosphere. Thus, any oxygen consumed by the slice must come from the medium.

The uses of the tissue slice respirometer can be illustrated best by outlining the detailed procedure followed in a typical experiment. The results obtained in one such experiment are shown in Fig. 2. The first step was to fill the vessel with reaction medium and turn on the magnetic stirrer. The current-level corresponding to an air-saturated reaction medium (240 μM) was then measured. Next, the magnetic stirrer was turned off to reduce bubble formation to a minimum when introducing a slice of tissue. This accounts for the large decrease in electrode current evident in Fig. 2. A slice of heart ventricle from a rat was added to the vessel, which was then sealed with a vaccine bottle stopper containing an outlet syringe needle. About ½ ml. of reaction medium was injected into the vessel to force any trapped air bubbles through the outlet syringe. Finally, the stirrer was turned on again, causing the electrode current to increase rapidly. When the new diffusion conditions reached a steady state (about 20 sec.), the electrode recorded the endogenous respiration (0·25 μmole oxygen/sec.), which is the slope of the curve at this

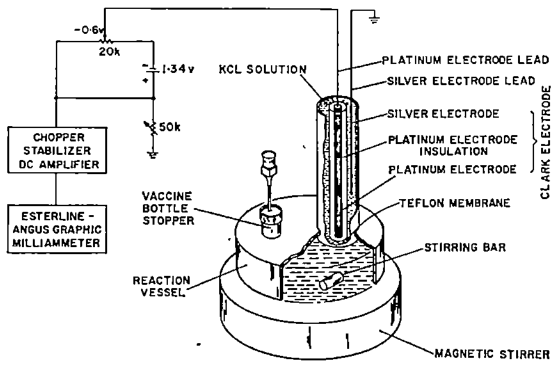


Fig. 1. Oxygen electrode system used for measurement of tissue slice respiration. The reaction medium is stirred rapidly enough so that the electrode current is independent of the rate of stirring. The electrode current is linearly dependent on the oxygen concentration (± 7 per cent) as determined by the measurement of the respiration of baker's yeast

---

* Present address: National Institutes of Health, Bethesda, Maryland.

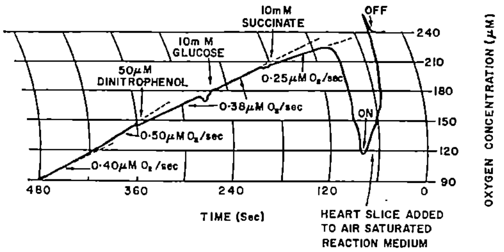


Fig. 2. Record of respiration of a slice of heart ventricle in several different respiratory states. Reaction medium containing 90 m*M* sodium chloride, 5 m*M* potassium chloride, 1 m*M* magnesium chloride and 40 m*M* phosphate buffer, *p*H 7·3. Temperature, 24° C. On–off refers to the magnetic stirrer

point. Injection of succinate into the vessel caused respiration to be stimulated by 50 per cent (to 0·38 μmole oxygen/sec.). Subsequent addition of glucose failed to stimulate respiration further. Following that, an injection of 2,4-dinitrophenol produced an additional 30 per cent increase in oxygen consumption (to 0·50 μmole oxygen/sec.).

This experiment illustrates how the oxygen electrode can be used to investigate the respiration of the same tissue slice in several different metabolic states. For example, the stimulation of respiration by dinitrophenol indicates that a low concentration of adenosine diphosphate in the tissue is limiting the rate of respiration in the presence of succinate. This agrees with recent findings of other workers, who used different methods for investigating muscle respiration[5,6]. The failure of glucose to stimulate respiration in the presence of succinate suggests that the penetration of glucose into the tissue slice is not rapid. If the glycose had penetrated into the slice, it would have been rapidly phosphorylated, thereby increasing the concentration of adenosine diphosphate. Any internally generated adenosine diphosphate would have stimulated respiration. No such respiratory stimulation was observed with the addition of glucose in the present experiment.

At the lower left corner of Fig. 2 the respiratory rate began to decline. This decreased respiration occurs at low oxygen pressures due to a decreased intracellular oxygen concentration. In practice, measurements are not made at such low oxygen pressures. By using an oxygen-saturated medium instead of an air-saturated medium, the respiratory rate of tissue slices is independent of the oxygen pressure over a much wider range of pressures.

It should be apparent from this that the oxygen electrode respirometer has many advantages over the manometric technique for work on tissue slices. The polarographic technique is especially useful for investigating the factors regulating respiration in tissue slices. Studies similar to that shown in Fig. 2 should facilitate greatly investigation of the action of chemical agents, such as drugs or hormones, on respiration. A relatively simple experiment can be used to determine whether such agents have any effect on the endogenous level of adenosine diphosphate, the substrate concentration, or the respiratory enzymes. Such information can be obtained by measuring respiratory rates in different metabolic states[6,7]. To obtain such information by manometric techniques would be much more time-consuming and less accurate. One can conclude from our work that a wider future application of the oxygen electrode for measurements of tissue slice respiration should prove extremely fruitful.

This investigation was aided in part by grant *RG*-5112 (*R*2), U.S. National Institutes of Health.

[1] Kreuzer, F., Harris, E. D., and Nessler, C. G., *J. App. Physiol.*, **15**, 77 (1960).

[2] Chance, B., and Williams, G. R., *J. Biol. Chem.*, **217**, 383 (1955).

[3] Todt, F., Teske, G., Windisch, F., Heumann, W., and Goslich, C., *Biochem. Z.*, **323**, 192 (1952).

[4] Clark, L. C., *Trans. Amer. Soc. Art. Int. Org.*, **2**, 41 (1956).

[5] Chance, B., and Connelly, C. M., *Nature*, **179**, 1235 (1957).

[6] Krebs, H., in *Ciba Found. Symp. Cell Metabolism*, edit. by Wolstenholme, G. E. W., and O'Connor, C. M. (J. and A. Churchill, Ltd., London, 1959).

[7] Chance, B., and Hess, B., *Science*, **129**, 700 (1959).

PERSISTENCE IN ERYTHROCYTE GHOSTS OF MEDIATED SUGAR TRANSPORT

By PAUL G. LEFEVRE*

Medical Research Center, Brookhaven National Laboratory, Upton, New York

THE special mechanism by which the common monosaccharides traverse the surface of the mammalian red blood cell has been the subject of considerable investigation in recent years[1–6]. Diverse experimental procedures, concerned primarily with analysis of the kinetics of sugar movements both into and out of the cells, have led to the conclusions that: (*a*) the permeation of the sugars through the cell surface occurs by way of a transient stoichiometric association with a limited number of reactive sites which are accessible at the cell surface to the two aqueous compartments (inside and outside the cell); (*b*) the translocation of the associated sugar-complex between the inner and outer interfaces of the surface layer results either from simple diffusion or from some kinetically indistinguishable process requiring no investment of metabolic energy. This mechanism, generally prominent in fœtal erythrocytes[2], does not persist at a comparable level of activity in the adult except in the human and certain other primate species. Typically, such cells are virtually impermeable to structurally related polyhydric alcohols, and even to certain sugars, which, though isomeric to the transported kinds, are of unsuitable steric arrangement[4]. Moreover, application of a variety of blocking agents[1] may completely stop the penetration of the transported sugars without radically altering permeability to small unrelated solutes.

Thus the specific reactive sites are generally held to be located at the semi-permeable barrier classically identified with the plasma membrane, which would apparently restrain all monosaccharides from entering the cell, were it not for this special mechanism. This raises the question whether the red cell ghost, retaining many of the surface characteristics of the original cell would also display the specialized sugartranslocating behaviour which the barrier shows in the intact cell. It is known from Teorell's observations that ghosts may continue to take in glucose while still excluding sucrose[7]; but Teorell did not examine whether this could be attributed to a free diffusion or to a surviving mediated-transfer activity. This question was therefore specifically examined by quantitative comparison of red-cell suspensions and derived ghost suspensions in respect to D-glucose movements. These experiments indicate that the membrane transport continues to function at full capacity after removal of the bulk of the original intracellular material. Thus, densitometric records of ghost volume changes with time (Ørskov's method[8]) continue to follow satisfactorily the predicted relation:

---

* Present address: Department of Pharmacology, School of Medicine, University of Louisville, Louisville 2, Kentucky.

$$kKt = (C_s + 1)(C_s + K)(C_s + K + 1)(V_0 - V) + (C_s + 1)(C_s + K)^2 \ln \frac{1 - V_0}{1 - V} \quad (1)$$

where k is a maximal flux constant, compounded from the diffusivity of the complex in the membrane and the fixed density of reactive sites; K is the dissociation constant of the complex and C_s is the external sugar concentration, both expressed in physiologically isosmotic units (isotones); and V_0 is the value at zero time of V, the intracellular (intraghost) water volume expressed as a fraction or multiple of its value at isotonicity. (This relation derives directly from integration of equation (1*b*) of ref. 6, for the case where the sugarless medium is isotonic and much larger in volume than the cells, as in Ørskov's procedure; though differently expressed, it is identical with equation (12) of ref. 3.)

Human blood was collected into standard ethylenediaminetetraacetic acid platelet packs ('Fenwal') from polycythæmic patients undergoing therapeutic phlebotomy, and stored at 2–4° C. when not used the same day. (In several years of related work on both normal and polycythæmic bloods, no indication has appeared of any relevance of this disease state to the sugar-transfer system.) Just prior to preparation of the ghosts, cells separated by centrifugation were taken through at least three successive washes in relatively large volumes of normal saline (Baxter) and finally two or three washings in a buffered balanced-salt medium[6]; in the course of this procedure, the major part of the white cells was discarded along with the uppermost red cell layers. Cells packed to about 75 vol. per cent in this medium were lysed by abrupt addition of (usually) 4 or 5 volumes of distilled water, and the hæmolysis reversed after 10–60 min. by addition of hypertonic sodium chloride, potassium chloride, or balanced salt mixture, sufficient to restore or slightly exceed isotonicity; finally, the ghosts were reconstituted by centrifugal washings in the isotonic medium and stabilized therein by 30–60 min. incubation at 37° C. before beginning experimentation. Cell and ghost volumes, counts and hæmoglobin contents were estimated by standard methods[6]; water contents by wet and dry weighings and sodium and potassium contents by flame photometry, through the courtesy of Dr. L. K. Dahl.

The ghosts prepared as described above were comparable with Ponder's preparations in retaining, typically, about a quarter of the original cell hæmoglobin and slightly less than half the original cell volume. In Ørskov's apparatus, they showed approximately the same transmission as intact cells at the same volume concentrations (that is, ghosts at about twice the cell count); this contrasts with the experience of Stein[10], who reported a marked increase in transmission at a given suspension concentration, with ghosts even less depleted than these. Densitometric comparison of these ghosts with intact cells in regard to glucose entry or exit at 37° C. showed essentially identical resistances to permeation: in response to a given applied sugar gradient, the ghost equilibration-rate was about double that of the cells, in appropriate proportion to the depletion of the original cell water and total solute. Compliance with this relation was further tested by varying widely the relative volumes of water used for lysis of several aliquots of ghosts, providing, after reconstitution, a series of ghosts of differing average volume. As shown in Table 1, the acceleration of equilibration progressed in pace with the depletion of the water space, implying an essentially unchanged membrane-transfer capacity even in the most shrunken ghosts.

Table 1. ACCELERATION OF GLUCOSE EQUILIBRATION WITH PROGRESSIVE DEPLETION OF GHOST CONTENTS

Half-times estimated from Ørskov's recordings. Vols. refer to average single cell or ghost. $C_s = 0{\cdot}285$; glucose initially all in medium. k given on basis that $K = 0{\cdot}02$ isotone

Preparation	Total vol. (μ^3)	Water vol. (μ^3)	$t_{1/2}$ (min.)	k (isotones per min.)
Intact cells	95	68	0·86	1·51
Ghosts, from lysis at ratio				
1:2·5	62	45	0·65	2·0
1:4·2	43	32	0·41	3·2
1:8·3	33	25	0·30	4·3
1:16·7	24	19	0·23	5·7
1:33·3	17	13	0·18	7·2

This finding, that glucose was equilibrated in the ghosts at about the absolute rate expected from continued operation of the transport system of intact cells, made it highly unlikely that any other mode of transfer of sugar had come significantly into play. (Moreover, all these preparations retained the characteristic of intact cells of total impermeability to *m*-inositol, a cyclic hexahydric molecule physically similar to glucose.) The conclusion was further examined by study of the kinetics; Table 2 shows the similarity in the degree of slowing of glucose exit, in ghosts and cells, brought about by increasing the sugar concentration in the critical range. Had this equilibration occurred by way of uncomplicated diffusion, $t_{1/2}$ would be nearly invariant at all sugar concentrations, and K could be said to be very high (indefinitely greater than 10). But Table 2 shows the much lower K's at which the $t_{1/2}$ data give minimal dispersion in graphical fitting to equation (1); the K value for the ghosts is within the range generally reported for intact cells. It appears to be somewhat higher than that of the paired cell-suspension in the parallel experiment; but since these K's are approaching the lower limit for meaningful resolution by this technique and are subject to an uncertainty of perhaps one-third, no serious evaluation can, at present, be given to this possible minor shift in the affinity constant.

Table 2. GLUCOSE EXIT FROM CELLS OR GHOSTS AS FUNCTION OF C_s
Prior equilibration $C_s = 0{\cdot}5$. Average ghost vol. = 46 per cent of average cell vol.

C_s (isotones)	Cells, $t_{1/2}$ (min.)	Ghosts, $t_{1/2}$ (min.)
0·009	0·24	0·10
0·022	0·36	0·16
0·034	0·59	0·20
0·051	0·74	
0·073	1·03	0·33
K (isotones)	0·014	0·024
k (isotones/min.)	1·8	3·5

The adequacy of these extensions of the indirect Ørskov technique was supported by direct chemical studies. Serial analyses of ghosts and cells in thick suspensions, during equilibration at 37° C. with glucose[6], gave a ghost k of 2·61 isotones/min. (again taking K as 0·02 isotone), while that for the intact cells was 1·65; in this instance the ghosts had sustained an average loss of 71 per cent of the original hæmoglobin, 46 per cent of the water, 49 per cent of the cell volume, and 95 per cent of the K^+, of which 25 per cent had been replaced by Na^+. The persistence of the specific transport activity after such radical alteration of the internal aqueous compartment argues strongly against the notion that the selective permeation is determined by the organization of a protoplasmic–water 'matrix', and points to the surface membrane as the locus of the specific reactive sites.

Membrane transports previously reported as surviving in the erythrocyte ghost under appropriate conditions include K^+ accumulation[11] and glycerol-mediated transport[10]. The former depends on supply at the time of lysis of a combination of metabolites serving later to energize the cation 'pump'; so that it is difficult to establish a fair basis for comparing its capacity in the ghost with that in the intact cell. Glycerol transfer, however, is not thought to involve such complications, and would be expected to show the same reciprocal relation of equilibration-rate to residual ghost volume as noted here for glucose transfer. Stein's finding[10] of a rather small increase in $t_{1/2}$ for entry of glycerol into ghosts, as compared with cells, appears to run counter to this expectation; however, the residual volume of these ghosts was not specified.

This work was supported by the U.S. Atomic Energy Commission.

[1] Wilbrandt, W., *Symp. Soc. Exp. Biol.*, **8**, 136 (1954). LeFevre, P. G., *ibid.*, **8**, 118 (1954). Bowyer, F., *Intern. Rev. Cytol.*, **6**, 469 (1957).

[2] Widdas, W. F., *J. Physiol.* (London), **127**, 318 (1955).

[3] Widdas, W. F., *J. Physiol.* (London). **125**, 163 (1954).

[4] LeFevre, P. G., and Marshall, J. K., *Amer. J. Physiol.*, **194**, 333 (1958).

[5] Hillman, R. S., Landau, B. R., and Ashmore, J., *Amer. J. Physiol.*, **196**, 1277 (1959).

[6] LeFevre, P. G., and McGinniss, G. F., *J. Gen. Physiol.*, **44**, 87 (1960).

[7] Teorell, T., *J. Gen. Physiol.*, **35**, 669 (1952).

[8] Ørskov, S. L., *Biochem. Z.*, **279**, 241 (1935).

[9] Ponder, E., *J. Exp. Biol.*, **28**, 567 (1951).

[10] Stein, W. D., *Exp. Cell Res.*, **11**, 232 (1956).

[11] Hoffman, J. F., and Tosteson, D. C., Twentieth Intern. Physiol. Cong., *Abstracts*, 429 (Brussels, 1956).

SEPARATION AND *IN VITRO* CULTURE OF CELLS FROM LIVER TISSUE

By DR. JUSTINE S. GARVEY

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California

A CELL-CULTURE examination was initiated to investigate more directly the role of the liver in the immunological response of rabbits to injection of antigen[1,2]. This involved the development of certain cell-culture techniques for the liver.

Most *in vitro* methods[3–9] for liver tissue have not involved separation of the reticulo–endothelial from the parenchymal cells although there have been a few such reports[10–12]. It is of distinct advantage to have separate cultures of the two types of cells, and in order to accomplish this, selection was made both in the initial isolation of cells and in the use of two different growth media. Sterility was observed throughout the following procedures, and solutions were warmed to 37° C. before use, unless indicated otherwise. A normal adult rabbit was bled from the heart, then killed by an injection of air. The abdominal area, which had been previously shaved, was swabbed with tincture of iodine, followed by ether. An incision was made and the liver removed. One lobe was perfused immediately (for parenchymal cell preparation), whereas the remaining tissue (for reticulo–endothelial cell preparation) was placed in 0·3 per cent trypsin prepared in GKN solution (8·0 gm. sodium chloride, 0·4 gm. potassium chloride and 1·0 gm. glucose in 1 litre distilled water).

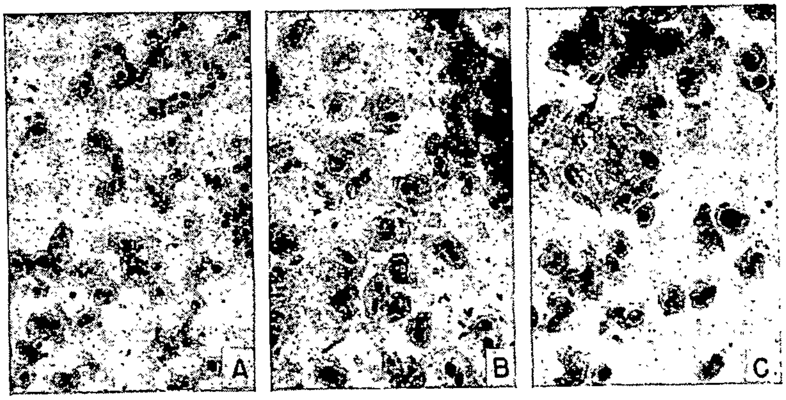


Fig. 1. Parenchymal cell culture from normal adult rabbit. *A*, 5 days after inoculation; *B* and *C*, both 10 days after inoculation. For all three fields, high-power object. × 43, oculars × 10, and photographic enlargement ~ × 10. Total magnification of *c.* 1,800 with emphasis of the fact that *A*, *B* and *C* are entirely comparable magnification

Preparation and Study of Parenchymal Cells

The blood appeared to be entirely displaced by allowing 500 ml. of GKN solution to circulate through one separated lobe, the perfusion fluid being injected directly into the blood vessels through a hypodermic syringe needle.

As a final perfusion, the lobe of liver was injected with about 10 ml. of the culture medium ; this was 6 per cent 'Hyprotigen', an enzymatic hydrolysate of casein in aqueous solution (pH 6·7), containing 0·05 M sodium chloride plus 0·20 M potassium chloride. ('Hyprotigen' is the trade mark of Don

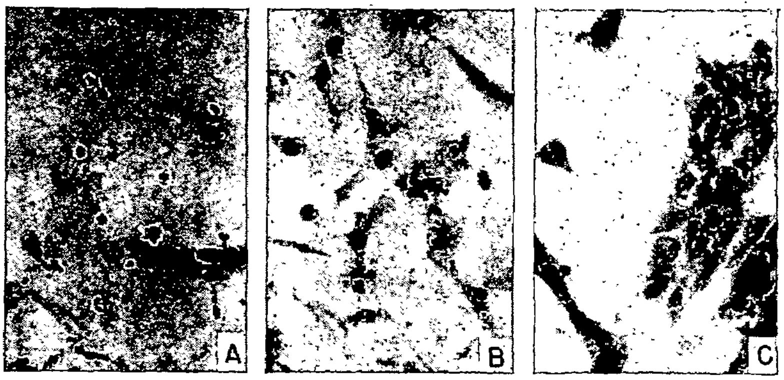


Fig. 2. Reticulo-endothelial cell culture from normal adult rabbit liver. *A*, 1 day; *B*, 3 days; *C*, 5 days after inoculation. Comparable total magnification of *c*. 1,800 for all three fields

Baxter, Inc., Glendale, California; it is supplied also by some international licensees.) This lobe was allowed to incubate for 5–6 hr. in a beaker containing the same medium, placed in a 36–37° C. humidified incubator with a 5 per cent carbon dioxide in air atmosphere. At the end of the incubation the lobe was transferred to a large Petri dish (150 mm. diameter × 20 mm. height) containing about 40 ml. of the medium. For use in dispersing cells, stainless steel cloth (30 mesh, type 304) was fabricated into two rectangular pieces, each 10 × 4 cm.; three horizontal rows of wire were removed from one 4-cm. side and the other 4-cm. side was bent under as a 2-cm. flap for easier handling. The ends from which the wire was removed were placed into the tissue, one to grip the tissue and the other to be used as a rake to release the cells into the medium. A small area was 'raked' to give almost complete dispersion of the cells, then other adjacent areas of tissue were processed similarly until only connective tissue and blood vessels remained in an otherwise homogeneous suspension of cells. The former were discarded as debris and the cell suspension was placed in an Erlenmeyer flask from which equivalent aliquots were pipetted into 15-ml. conical, graduated centrifuge tubes.

These tubes were centrifuged for 4 min. at 650 r.p.m. (50*g*) in a clinical centrifuge with trunnion head. The supernatant was discarded and the pellet was used to prepare replicate cultures of 1/3 ml. packed cells per 5 ml. medium in Petri dishes of 60 mm. diameter × 15 mm. height. These dishes contained a glass coverslip. A zero-time smear of the inoculum was examined with Wright stain, whereas a May–Grünwald Giemsa stain was used for cultures obtained at various times after incubation in the humidified 5 per cent carbon dioxide in air atmosphere where temperature was regulated at 36–37° C. The stained smear showed that the inoculum consisted of parenchymal cells with no other cell type being obvious; the majority of these cells were binucleated. After 3–5 days the cultures differed from those incubated for a shorter time both in number of cells and in the pattern of growth, that is, instead of a few isolated cells, there were fairly large groups of cells, and the cytoplasm was spreading, frequently as strands between the cells (Fig. 1, *A*). A determination of nitrogen in washed cells showed a slight increase after 5 days in culture, and at 10 days the nitrogen value had doubled compared with that for the inoculum. At 10 days there occurred amitotic activity in some areas of all cultures. The nuclear volume increased conspicuously, and the shape of the nuclei varied greatly (Fig. 1, *B* and *C*). These observations differ from those reported previously for liver tissue in culture but confirm quite precisely a discussion of amitotic activity as observed in human adult tissue just following autopsy[13].

Preparation and Study of Reticulo-Endothelial Cells

As soon as the perfused lobe was incubated, as noted above, the tissue which had been placed in trypsin was cut into pieces about 2–3 mm.[3]. With a pipette as much solution was removed as possible, and the tissue was transferred into an Erlenmeyer flask containing 100 ml. 0·01 per cent collagenase[14,15] prepared in GKN and cooled to 10° C. The suspension of tissue was stirred magnetically during refrigeration for 3 hr. The supernatant was poured from the tissue and 50 ml. trypsin was added. The pieces of tissue, suspended in trypsin, were stirred for 20 min. with an air-driven magnetic stirrer in the 36–37° C. incubator. It was very apparent that the enzyme treatment had reduced the tissue to a turbid suspension and connective tissue. The connective tissue was removed and the suspension of cells was centrifuged at 1,650 r.p.m. (330*g*) for 4 min. The supernatant was discarded and the pellet was washed twice in serumless medium (Table 1) for 4-min. periods at 1,650 r.p.m. (330*g*). About 1/3 ml. of pellet was inoculated per 5 ml. medium (Table 1)

Table 1. TISSUE CULTURE MEDIUM FOR RETICULO-ENDOTHELIAL CELLS

The quantities of amino-acids were derived as relative ratios from an analysis of the composition of rabbit antibodies (ref. 16), the remaining formulation developed from various experiments with splenic cultures, which do not differ significantly from reticulo-endothelial liver cell cultures

Amino-acids	Mgm./l. complete medium	Vitamins	Mgm./l. complete medium
l-Arginine (HCl)*	12·20	Choline chloride	4·0
l-Cysteine (HCl)*	8·14	Folic acid†	4·0
l-Cystine†	3·13	Nicotinamid	4·0
l-Histidine (HCl)*	3·79	Calcium pantothenate	4·0
*dl-Iso*leucine	17·56	Pyridoxal (HCl)*	4·0
l-Leucine	15·80	Thiamine (HCl)*	4·0
l-Lysine (HCl)*	16·15	Riboflavin	0·4
l-Methionine	2·80		
l-Phenylalanine	10·98		
l-Threonine	26·44		
l-Tryptophan	5·40		
l-Tyrosine‡	13·24	Salts and carbohydrate	Mgm./l. complete medium
dl-Valine	40·88		
l-Thyroxine		NaCl	6,400
(sodium salt)	0·025	KCl	400
l-Glutamic acid	23·60	$NaH_2PO_4 \cdot H_2O$	125
dl-Alanine	22·72	$CaCl_2$	200
l-Aspartic acid	19·34	$MgSO_4 \cdot 7H_2O$	200
Glycine	11·44	$Fe(NO_3)_3 \cdot 9H_2O$	0·1
l-Proline	17·12	$NaHCO_3$§	3,700
l-Serine	21·84		
Inositol	7·00	Dextrose	4,500

* Crystallized in hydrochloric acid.
† Dissolved in 1 ml. 1 *N* sodium hydroxide.
‡ Dissolved in boiling water.
§ Dissolved separately, then added to other salts.

The complete medium is prepared by combining in a 1-litre volumetric flask the solutions which are prepared separately, namely, the amino-acids in about 200 ml., vitamins in about 100 ml., salts and dextrose in about 500 ml. Then 1·5 ml. 1 per cent phenol red is added. The volume is diluted to 1 litre, flushed with carbon dioxide until colour becomes orange. The solution is sterilized in '02 Selas', dispensed in aliquots and refrigerated for use over a period of 2–3 months. Just prior to use in cultures, the following are added to the final concentration indicated:

Rabbit serum (optional)	150 ml./l. = 15 per cent
Insulin	0·5 mgm./l., that is, 11 units
l-Glutamine	292 mgm./l.
Penicillin	10^5 units/l.

which contained 15 per cent normal rabbit serum. The maximal contamination with parenchymal cells was estimated to be 1 per cent of the inoculum. After 1 day (Fig. 2, *A*) considerable differentiation of cells was observed in stained cover-slip cultures, and conspicuous morphological change and growth had occurred after 3 and 5 days (Fig. 2, *B* and *C*). After 10 days, there was a sheaf of cells of the type that predominated in the culture at 5 days, and these showed rather frequent mitotic figures.

Reproducibility with all the above techniques was easily achieved. The commercially available medium 'Hyprotigen', containing mostly peptides and some amino-acids, was chosen for growing parenchymal cells as a result of screening various media. With regard to the medium described in Table 1, although this was less satisfactory for parenchymal cells than was the 'Hyprotigen', the omission of serum and addition of salmine protamine sulphate improved somewhat the cultural characteristics. It should be emphasized that a parenchymal cell culture does not require serum and, in fact, is inhibited by the presence of unmodified serum. Whereas peptides and amino-acids suffice for growth of parenchymal cells, the reticulo–endothelial cell culture does have an apparent requirement for serum. The rather general failure in the past with cultures of liver tissue may be largely attributed to the fact that it is extremely difficult to attain optimal conditions for a mixed cell population, and the more slowly growing parenchymal cell is rather rapidly suppressed by the reticulo-endothelial cell which differentiates and assumes fibroblastic characteristics.

These investigations are being continued with particular emphasis given to the parenchymal cell culture which slowly metabolizes, with secretion of protein into the medium, while demonstrating a characteristic amitotic activity.

Special acknowledgment is given to Prof. Dan H. Campbell for critical discussions and encouragement during the investigation. The financial support of the U.S. National Institutes of Health is appreciated.

[1] Campbell, D. H., and Garvey, J. S., *Intern. Arch. Allergy App. Immunol.*, **12**, 70 (1958).
[2] Campbell, D. H., and Garvey, J. S., *J. Infect. Dis.*, **107**, 15 (1960).
[3] Anderson, N. G., *Science*, **117**, 627 (1953).
[4] Harris, T. N., and Harris, S., *Science*, **105**, 75 (1947).
[5] Evans, V. J., Earle, W. R., Wilson, E. P., Waltz, H. J., and Mackey, C. J., *J. Nat. Cancer Inst.*, **12**, 1245 (1952).
[6] Laws, J. O., and Stickland, L. H., *Nature*, **178**, 309 (1956).
[7] Longmuir, I. S., and apRees, W., *Nature*, **177**, 997 (1956).
[8] Kaltenbach, J. P., *Exp. Cell. Res.*, **7**, 568 (1954).
[9] Paul, J., and Pearson, E. S., *Biochem. J.*, **63**, 16 P (1956).
[10] Doljanski, L., *C.R. Soc. Biol.*, **101**, 754; **102**, 629 (1929).
[11] St. George, S., Friedman, M., and Byers, S. O., *Science*, **120**, 463 (1954).
[12] St. George, S., *Reticulo-endothelial Structure and Function*, edit. by Heller, J. H., chap. 32, 449 (The Ronald Press Co., New York, 1960).
[13] Ashworth, C. T., and Reid, H. C., *Amer. J. Path.*, **23**, 269 (1947).
[14] Rinaldini, L. M. J., *Intern. Rev. Cytol.*, **7**, 587 (1958).
[15] Hinz, R. W., and Syverton, J. T., *Proc. Soc. Exp. Biol. Med.*, **101**, 19 (1959).
[16] Smith, E. L., McFadden, M. L., Stockell, A., and Buettner-Janusch, V., *J. Biol. Chem.*, **214**. 197 (1955).

PHOTOPERIODIC EFFECTS ON STOMATAL BEHAVIOUR IN *XANTHIUM PENNSYLVANICUM*

By DR. T. A. MANSFIELD and PROF. O. V. S. HEATH, F.R.S.

University of Reading, Horticultural Research Laboratories, Shinfield Grange, Shinfield

DIFFERENCES in stomatal behaviour under different photoperiodic treatments were reported by Schwabe[1] for *Chrysanthemum* and *Kalanchoe blossfeldiana*. He found that with short days (8 hr. daylight + 16 hr. darkness) opening of the stomata occurred towards the end of the night, but with long days (8 hr. daylight + 8½ hr. low light + 7½ hr. darkness) no night opening occurred.

We have investigated the relationship between photoperiodic treatment and stomatal behaviour using the short-day plant *Xanthium pennsylvanicum*. In some preliminary experiments effects similar to those reported by Schwabe were found: under short-day conditions (16 hr. nights) the stomata were closed for the first 11–12 hr. in the dark, but then began to open slightly. This opening was relatively small compared with that in high-intensity light (20,000 lux) during the day. In long-day conditions, in which the 16-hr. night was replaced by 8 hr. of low-intensity illumination (1,500 lux) followed by 8 hr. darkness, night opening did not occur. Light of 1,500 lux given after high-intensity light was not sufficient to maintain any appreciable opening, and consequently, under the conditions of these experiments, the stomata were closed for a larger proportion of the 24-hr. cycle in long days than they were in short days.

Considerable differences were observed in the rate of stomatal opening in light in the morning under different photoperiodic treatments. A detailed investigation has revealed a close relationship between the rate of opening in light and the length of the preceding night. In Fig. 1 it is shown that stomatal opening was much more rapid after 9-hr. than 7-hr. nights. The day-time illumination was 20,000 lux; the 9-hr. and 7-hr. treatments were, respectively: (*a*) 7 hr. of low light (1,500 lux) at the end of the day, followed by 9 hr. of darkness; (*b*) 9 hr. of 1,500 lux at the end of the day, followed by 7 hr. of darkness. It is interesting that 8½–9 hr. is the critical long night for flowering in *X. pennsylvanicum*. In a factorial experiment designed to study the interaction of length of night and the intensity of the light used to shorten the night, similar results to the above were obtained using either 160 or 40 lux, and even 10 lux had a significant effect in reducing subsequent rate of stomatal opening if given for 15 hr. followed by 1 hr. darkness. In another experiment, rate of opening after nights ranging in length from 1 to 23 hr. was examined; the different lengths of night were achieved by providing different periods of light of 1,500 lux at the end of the day, shortening a 24-hr. dark period. An almost complete cycle of 'opening ability' was found (Fig. 2).

In conjunction with these measurements, observations were made on the occurrence of night opening of the stomata. During a long period of darkness the stomata were first of all closed completely, but after several hours they opened slightly, remained open for about 12 hr., and then closed again. The most important factor controlling the timing of this cycle of events was the time of termination of the low-intensity light. This is illustrated in Fig. 3, in which are recorded the times of the beginning of night opening under different illumination treatments. The plants had received high-intensity illumination (20,000 lux) until 4 p.m., and then various periods of low light terminating at the times shown. Prolonging the illumination delayed the occurrence of night opening, as indicated by the significant regression (continuous line). The broken line is the relation that would be expected if prolonging the low light by a given period had displaced the time of opening by the same period. The observed relation was significantly different from this ($P < 0{\cdot}001$), showing that the time from the termination of light to the commencement of opening was somewhat shorter after a longer period of low light. This indicated that the dark processes could go on in light of 1,500 lux, though at a reduced rate. The foregoing experiments were carried out at 27° C. In an experiment on the interaction of light and temperature it was found that in darkness night opening began sooner the lower the temperature in the range 15°–36° C.; in light of 10 lux it did not occur at all within 20 hr. of the change from 20,000 lux at 36° or 29° C., was delayed 1½ hr. at 22° C. and began at the same time as in darkness (after 9½ hr.) at 15° C.

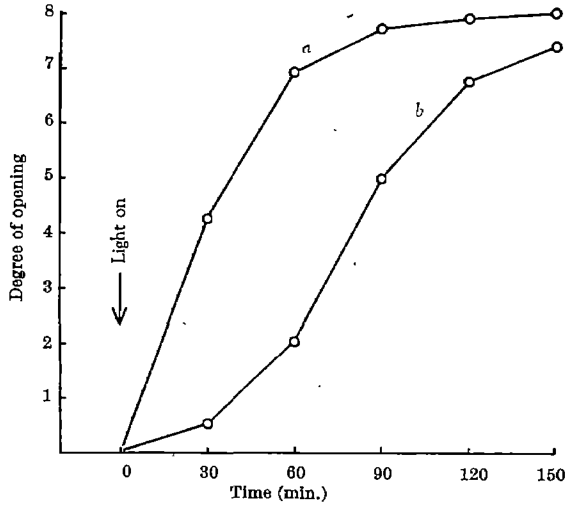


Fig. 1. Stomatal opening in light of 20,000 lux: *a*, after 9-hr. night; *b*, after 7-hr. night. Each curve is the mean from observations on six separate leaves. Ordinate scale, arbitrary units of total pressure minus pressure across the leaf, in a resistance porometer, that is, increasing values indicate opening

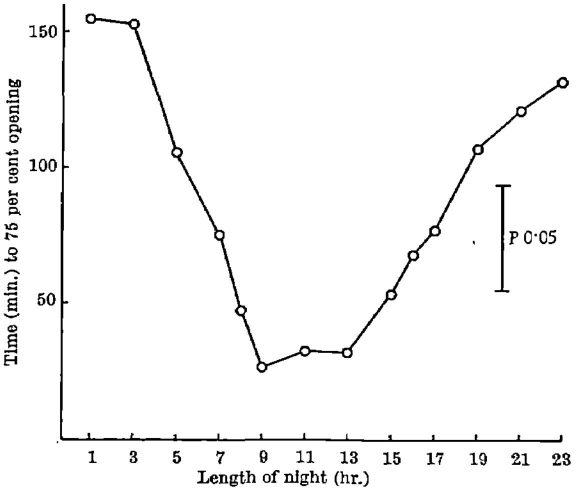


Fig. 2. Stomatal opening in 20,000 lux after different lengths of night. The time (min.) from the start of illumination to the attainment of 75 per cent of full aperture is shown. Each value is the mean of six observations on separate leaves. The least significant difference for comparison of two means at P 0·05 is indicated

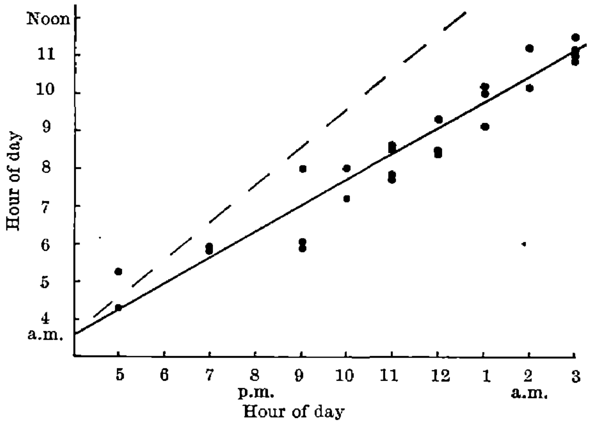


Fig. 3. Control of night opening of the stomata in *X. pennsylvanicum* by the time of termination of illumination. Ordinate, time of first detectable night opening. Abscissa, time of termination of light of 1,500 lux

We have been able to conclude that the differences in rate of opening in light after different lengths of night are related to the tendency for the stomata to open slightly for a time in continuous darkness. When they are already in the night-opening condition, however slight this opening may be, the rate of opening in light is very greatly increased.

The most satisfactory explanation of these observations would seem to be that an endogenous rhythm of 'opening ability' occurs in the stomatal cells in the dark, and that the phase of this rhythm is set mainly by the time of transition from light to dark, in much the same way as it was, for example, in the rhythm of carbon dioxide output in *Bryophyllum fedtschenkoi* reported by Wilkins[2]. We have not yet, however, shown that there is a true rhythm in which the cycle is repeated, though there has been some evidence of this in other species[3,4].

A full description of these and other findings will be published in due course, and an attempt will be made to relate them to theories of the stomatal mechanism. The results suggest that dark fixation of carbon dioxide might be concerned; this is known to occur in *X. pennsylvanicum*[5]. This aspect is being investigated.

The data were collected for attached leaves on potted plants using a new recording resistance porometer which automatically detaches and replaces *seriatim* the lower portions of the porometer cups on four leaves; the leaf surfaces within the cup areas were thus freely exposed to ambient air of normal carbon dioxide content except during the actual readings. This apparatus will be described elsewhere.

This investigation was carried out during the tenure by one of us (T. A. M.) of an Agricultural Research Council postgraduate studentship.

[1] Schwabe, W. W., *Nature*, **169**, 1053 (1952).
[2] Wilkins, M. B., *Nature*, **187**, 523 (1960).
[3] Dale, J. E., *Ann. Bot.*, N.S., **25**, 39 (1961).
[4] Sayre, J. D., *Ohio. J. Sci.*, **26**, 233 (1926).
[5] Langston, R., and Leopold, A. C., *Plant Physiol.*, **29**, 436 (1954).

INFLUENCE OF ELECTRICAL FIELD ON PUPATION AND OVIPOSITION IN *NEPYTIA PHANTASMARIA* STKR. (LEPIDOPTERA: GEOMETRIDAE)

By DR. D. K. EDWARDS
Forest Biology Laboratory, Federal Building, Victoria, British Columbia

IT is known that weather often can affect insect behaviour and development profoundly. Electrical factors associated with weather may be partly responsible for these biological reactions[1], but very little experimental work has been done to determine if an electrical field can influence insect behaviour and development. Avio and Tarozzi[2] studied *Drosophila* oviposition in an electrical field (125 V./m.) but found no significant effects. More recently, Levengood and Shinkle[3] reported higher yields of *Drosophila* progeny reared in an electrical field. They did not mention, however, whether there was an observable effect of the field on time of development. Edwards[4] has reported some observations on the behaviour of adult *Calliphora* and *Drosophila* in an electrical field, with *Drosophila* responding to establishment of the field by temporarily reducing its activity.

Electrical field effects on the development of Lepidoptera, and on the location of eggs deposited by insects, have not been reported previously. Accordingly, some tests were made to determine whether an electrical field could influence pupation-time and the number and location of eggs deposited by moths of the phantom hemlock looper, *Nepytia phantasmaria* Stkr. This moth has one generation a year, and the adults live for about five days in the laboratory.

Four wooden boxes, 22 × 25 × 34·2 cm., contained the insects. Each box was covered on one side with a plastic screen and on the opposite side with a sliding glass wall. The boxes were placed on end, so that the glass wall could be opened slightly at the bottom by sliding it upwards. Inside, on the bottom of each box, an aluminium 'field plate', 19 × 14 cm., was suspended horizontally by two glass jars 4·5 cm. high. Directly beneath the plate, a plastic tray, 16 × 7·5 cm., with walls 2 cm. high, was placed between the two jars, and its inner bottom surface was covered with aluminium foil. The foil was connected to an insulated wire running through the box wall to the earthed negative terminal of an 810-V. battery-pack. The field plates in boxes I and II were each attached to an additional insulated wire passing through the box wall. For box I, the distal end of the field-plate wire was connected directly to the positive terminal of the battery-pack, thus establishing an electrical field (180 V./cm.) between the tray and the plate. A time-switch which alternated 'on' and 'off' periods of 5-min. duration (so than an intermittent field was established) was connected to the box II field-plate wire, between the box and the battery-pack. The field plates in boxes III and IV were wired directly to the trays ; hence, there was no field between the plate and tray in these boxes. Boxes I and II were placed on top of boxes III and IV, and the unit of four boxes was illuminated continuously by a 60-W. bulb placed 8 ft. from the screened sides, so that all had approximately the same distribution of light.

Penultimate-instar larvæ were collected from Douglas fir in an outdoor cage, and allowed to pupate in the laboratory. Because all the insects did not pupate at the same time, it was necessary to add pupæ to the experiment on four different dates. Equal numbers of 1- to 2-day-old pupæ were put into each tray on August 8, 10, 12 and 15, to give a total of 98 pupæ in each box. Emergence, which began on August 22, was recorded daily by removing carefully the tray from each box through the sliding glass wall and counting the empty pupal cases.

The central portion of the *S*-shaped emergence curve was treated as a straight line, and a line was calculated for each set of emergence data by the method of least squares. The equations for the lines are : Box I : $Y = 12{\cdot}41 + 10{\cdot}53X$ ($\bar{x} = 3$; $\bar{y} = 44$). Box II : $Y = 18{\cdot}17 + 10{\cdot}75X$ ($\bar{x} = 3$; $\bar{y} = 50{\cdot}42$). Boxes III and IV : $Y = 19{\cdot}34 + 11{\cdot}10X$ ($\bar{x} = 3$; $\bar{y} = 52{\cdot}64$). The slopes of the lines did not differ significantly. An analysis of variance showed the emergence curve for insects in box I (constant electrical field), to be significantly later than the curve for the combined data from the control boxes III and IV ($P < 0{\cdot}01$). The 50 per cent emergence point for box I was slightly less than one day later than the control value. The line for box II insects (interrupted field) although shifted slightly towards a later time, does not differ significantly from the control curve. Table 1 shows that there were more males than females in all boxes, with the greatest difference in box I. However, the lag in the emergence from box I is not a result of the presence of these extra males. In fact, since males emerge somewhat before females in *Nepytia*, their predominance in box I should shift the emergence curve forward, not backward, in time. In other words, the significant shift occurred, despite the presence of more males.

Later, eggs deposited by the adults on all parts of the box, tray, and field plate were counted in each box. These data, together with pupal mortality and the numbers of each sex, are shown in Table 1. The mortality, although very low, was greatest in box I. There were also somewhat fewer eggs per female laid in boxes I and II than in the control boxes. The most definite difference, however, appeared in a comparison of eggs laid on the under-surfaces of the field plates with those on the upper surfaces of the plates. Chi-squared tests showed a significantly smaller percentage of eggs laid on the under-surfaces in boxes I and II than in boxes III and IV ($P < 0{\cdot}01$), with box I differing most from the controls. Where there was no field (boxes III and IV) the majority of the field-plate eggs were deposited on the under-

(*Continued on page 993*)

(*Continued from page 976*)

Table 1. *Nepytia* MORTALITY, NUMBERS OF INDIVIDUALS, AND NUMBERS OF EGGS DEPOSITED ON FIELD PLATE AND THE REST OF THE BOX, IN BOX I (CONSTANT FIELD), BOX II (INTERRUPTED FIELD), AND BOXES III AND IV (NO FIELD)

	Box I	Box II	Box III	Box IV
Mortality	3	1	1	0
Males	62	54	55	50
Females	36	44	43	48
Total	98	98	98	98
Total eggs	1,330	1,661	1,991	2,039
Eggs/female	36·94	37·75	46·30	42·47
Eggs on field plate	84	128	281	155
Eggs/female on field plate				
Upper surface	1·44	1·06	0·65	0·47
Under surface	0·88	1·84	5·88	2·75
Per cent on under surface	37·93	63·44	90·04	85·40

surfaces of the plates. This is probably related to the fact that *Nepytia* normally deposits most of its eggs on the under-surfaces of fir needles. In the boxes where an electrical field was present beneath the field-plate, however, and especially in box I where the field was continuously present, oviposition on the under-surfaces of the plates was conspicuously reduced. The field was very much more intense beneath the plate than above it, since the field was established between tray and plate. It was not possible to make a comparison of the numbers of eggs laid on the under-surface of the roofs of the boxes, since the numbers varied greatly between boxes.

These results show that the development of *Nepytia* pupæ, and also the number and location of eggs deposited by the adults, can be influenced by an electrical field. The magnitude of the experimental field was 180 V./cm. Fleming[5] states, "When the [thunder] cloud is within a few km., the pre-discharge field ranges from 10,000 to 30,000 volts per meter . . ." (p. 659). Thus, the intensity of the experimental field was not unreasonably high, compared with that found in Nature. However, a steadily maintained field of 180 V./cm. would probably not be found in natural atmospheric conditions, because fields associated with storms fluctuate markedly. The intermittent experimental field of 180 V./cm. in box II, which was somewhat closer to the natural situation, did not have as great an effect as the steady field. But the more intense experimental conditions were necessary in order to determine what kinds of effects to expect from application of a field, and what direction these might take.

I am indebted to W. G. Wellington and R. E. Foster of this Laboratory, for criticism of the manuscript and aid with the statistical analysis, and to H. S. Burr, Yale University School of Medicine, for his suggestions.

[1] Wellington, W. G., *Ann. Rev. Ent.*, **2**, 143 (1957).
[2] Avio, C. M., and Tarozzi, G., *Riv. di Biol.*, **49**, 419 (1957).
[3] Levengood, W. C., and Shinkle, M. P., *Science*, **132**, 34 (1960).
[4] Edwards, D. K., *Canad. J. Zool.*, **38**, 899 (1960).
[5] Fleming, J. A., *Terrestrial Magnetism and Electricity* (Dover, New York, 1949).

MELANISM IN CAPTIVE WEAVER-FINCHES (*QUELEA QUELEA*)

By DR. B. LOFTS

Department of Zoology and Comparative Anatomy, St. Bartholomew's Hospital Medical College, University of London, E.C.1

THE red-billed dioch, *Q. quelea*, is a weaver-finch which occurs in enormous numbers in Africa. Large, nomadic flocks roam the countryside in search of food and do enormous damage to crops and timber. The species has a wide distribution coinciding with the 30-in. rainfall isohyet[1]. It is sexually dimorphic: before the breeding season the male assumes a vivid nuptial plumage which temporarily distinguishes it from the permanently drab female (Fig. 1*a* and *b*). During the eclipse season, however, this cock plumage (black facial mask and light gold crown and breast) is shed and both sexes wear the same modest hen plumage, becoming morphologically indistinguishable.

During the course of a recent investigation of the breeding biology of this species[2], it was noted that some of the birds developed a black plumage. The birds, which had been flown from Africa, were housed in a large aviary under a constant photo-period of 12 hr. and a temperature of 65–75° F. Within three months of their arrival in London, some of the birds had become noticeably darker. In these specimens the entire plumage had been affected and became darker with each successive moult, until eventually the whole bird was black (Fig. 1*c*). The melanism occurred in both sexes and was common to eclipse and breeding periods.

Melanic males did not resume the typical nuptial dress during the breeding season and gave no external indication of coming into breeding condition. Autopsy revealed, however, enlarged testes of maximum size filled with bunched spermatozoa. Female *Quelea* normally demonstrate a change of beak colour during the breeding season. In the contra-nuptial period both males and females possess bright red bills, but those of the latter sex change to pale yellow when the nuptial season starts. This colour cycle was unaffected by the assumption of a melanic plumage, and during the breeding period black females were easily distinguishable from melanic males by their yellow bills (Fig. 2*a* and *b*).

During their first year of captivity, about 8 per cent of the birds (about 40 specimens) became melanic. At the end of a further two years, the proportion of affected birds had risen as high as 30–40 per cent. Mr. D. C. Lourens, chief of the Division of Entomology, Department of Agricultural Technical Services, Pretoria, South Africa, has reported (personal communication) that 75 per cent of his captive *Quelea* have undergone a similar process of melanization to that described above.

So far as I am aware, melanic *Quelea* have never been seen in the wild and only appear as a result of captivity.

In order to investigate this phenomenon and determine what artificial factor or factors might produce it, groups of *Quelea* (50 specimens) were subjected to the following conditions:

Photoperiodicity	
Group I	8 hr. day-length
„ II	12 hr. „
„ III	17 hr. „
Temperature	
Group IV	50° F. room temperature
„ V	70° F. „ „
„ VI	95° F. „ „

All the birds were fed on the same seed mixture and were kept under the above conditions for a period of four months (August–November), during which time they underwent a moult into breeding plumage. Groups I–III were all maintained at a constant room temperature of 70° F., and groups IV–VI had a uniform photo-period of 12 hr. In all groups the melanic birds were unaffected; but some of the specimens which had started in normal eclipse plumage moulted into a nuptial plumage showing signs of melanism.

In order to determine whether the quality (rather than the photoperiod) of the artificial light (fluorescent tube) may have been a contributory factor, advantage was taken of a period of mild sunny weather to expose melanic *Quelea* to sunshine. This resulted in the birds (four male specimens) reverting back to normal within the first moult. The birds were placed in an outside cage during the first week of May and all moulted to a normal eclipse condition.

One main difference in the quality of sunlight and artificial illumination is the reduction in the amount of ultra-violet in the latter. Recently, Lourens has been able to show that captive *Quelea* given a daily dose of ultra-violet light do not become melanic, whereas unexposed captive birds do. In the present investigation, 10 melanic *Quelea* were exposed to ultra-violet from an ordinary sun-ray lamp at a distance of 8 ft. from their cage. They were given two 8-min. sessions a day throughout one month while they were moulting. When feather regeneration had been completed, seven had assumed a normal nuptial dress (Fig. 3*a* and *b*), and three, though still melanic, had assumed a much lighter plumage.

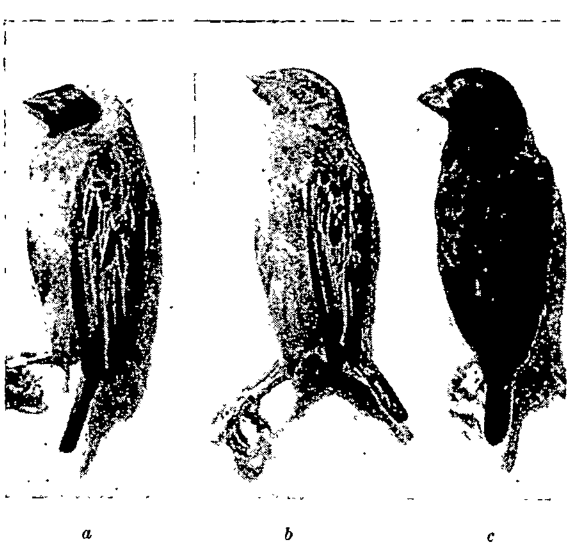


Fig. 1. (*a*) Male *Quelea* in nuptial plumage. (*b*) Female bird during breeding season. (*c*) Melanic female

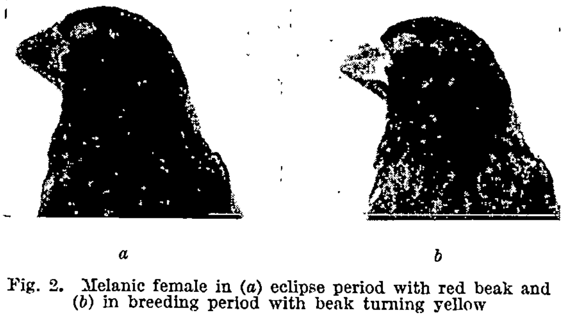


Fig. 2. Melanic female in (*a*) eclipse period with red beak and (*b*) in breeding period with beak turning yellow

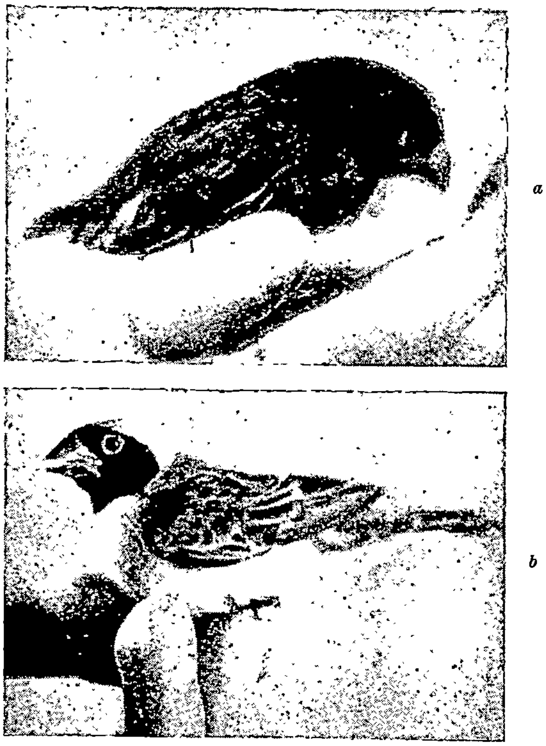


Fig. 3. Effect of exposure to ultra-violet light. (*a*) Melanic male before moult. (*b*) Same bird after exposure to ultra-violet light during moult

It is not known how the ultra-violet rays exert their influences on pigmentation in growing feathers. The cyclical assumption of a breeding plumage in this and other weaver finches is under the direct control of the pituitary[3] and, in particular, is responsive to the output of luteinizing hormone[4]. This hormone cannot be responsible for the phenomenon described in the present work, however, since all the plumage is affected, whereas luteinizing hormone will only stimulate the deposition of melanin in those regions where it is normally found in the nuptial plumage, namely, the black facial mask. It seems not impossible that the ultra-violet influence may be imposed directly on the organizing feather follicle during growth of plumage.

I thank Mr. D. C. Lourens for his kindness in allowing me to refer to some of his unpublished results. This investigation was assisted by a grant from the Nuffield Foundation.

[1] Disney, H. J. de S., and Marshall, A. J., *Proc. Zool. Soc. Lond.*, **127**, 379 (1956). Morel, G., and Bourlièr, F., *Bull. Inst. Franc. Afrique Noire*, **17**, 618 (1955).

[2] Disney, J. H. de S., Lofts, B., and Marshall, A. J., *Proc. Zool. Soc. Lond.*, **136**, 18 (1961).

[3] Witschi, E., *Wilson Bull.*, **47**, 177 (1935); *Proc. Soc. Exp. Biol.*, **35**, 484 (1936).

[4] Witschi, E., *Mem. Soc. Endocrin.*, **4**, 149 (1955). Segal, S. J., and Witschi, E., *J. Clin. Endrocrin. and Metab.*, **15**, 880 (1955).

LETTERS TO THE EDITORS

PHYSICS

Interaction of 14-MeV. Neutrons with a Silicon Semiconductor Nuclear Particle Detector

THE recent development of silicon semiconductor nuclear particle detectors has provided a convenient method for studying the charged particles emitted from the interaction of neutrons with silicon.

A silicon surface barrier detector, manufactured from 400-Ω cm. *n*-type silicon, was irradiated with neutrons of energy 14·03 ± 0·05 MeV. from the reaction $T(d,n)^4He$. Pulses from the detector, which was operated at a reverse bias of 50 V., were amplified by a charge-sensitive low-noise pre-amplifier and a modified 1430 Harwell linear amplifier and then analysed by a 400-channel *RIDL* pulse-height analyser. Fig. 1 shows a typical energy spectrum observed.

Assuming that reactions induced in silicon-28 (92 per cent abundant) are the most important then the possible charged-particle emitting reactions are $^{28}Si\ (n,p)\ ^{28}Al$, $Q = -3{\cdot}86$ MeV.; $^{28}Si\ (n,\alpha)\ ^{25}Mg$, $Q = -2{\cdot}66$ MeV., and $^{28}Si\ (n,d)\ ^{27}Al$, $Q = -9{\cdot}36$ MeV. The (n,p) and (n,α) reactions have been observed by Dearnaley and Whitehead[1] using 6·3-MeV. neutrons.

Calculation of barrier depth and comparison with range-energy values for protons and α-particles shows that unless the high-energy protons are emitted along the plane of the detector, they will escape from the sensitive volume of the detector whereas the maximum-energy α-particle range is much less than the barrier depth, and thus, apart from edge effects, all the α-particles emitted will be detected. Hence, one would expect a greater contribution to the observed spectrum from (n,α) than from (n,p).

Using the results of Hinds and Middleton[2] for the energy-levels of magnesium-25 and the energy-level scheme for aluminium-28 given by Endt and Braams[3], we calculated the expected energy of proton groups corresponding to the first seven levels in aluminium-28 and α groups corresponding to the first fifteen levels in magnesium-25. In Fig. 1, the arrows indicate the position of these groups and it will be seen that the position of the observed groups corresponds very closely with those expected from the (n,α) reaction and that there is little evidence for the (n,p) reaction under these conditions.

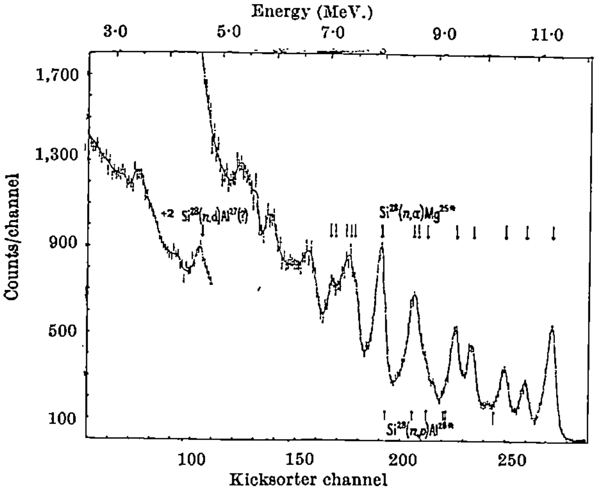


Fig. 1. Energy spectrum observed from interaction of 14·03 MeV. neutrons with a silicon surface barrier detector

The peak evident at 4·6 MeV. in Fig. 1 has been tentatively assigned on the basis of *Q*-value to the reaction $^{28}Si\ (n,d)\ ^{27}Al$.

Cross-section measurements were made for the α-groups leading to the first five levels of magnesium-25. The results are given in Table 1.

Table 1. CROSS-SECTION FOR REACTION $^{28}Si(n,\alpha)\ ^{25}Mg$

Magnesium-25 (MeV.)	$\sigma(n,\alpha)$ at 14·03 MeV.
0	1·84 ± 0·61 mb.
0·582	0·75 ± 0·25 mb.
0·976	0·98 ± 0·33 mb.
1·612	1·19 ± 0·39 mb.
1·965	1·28 ± 0·43 mb.

Further work is in progress to study the (n,p) reaction and measure the excitation function for the (n,p) and (n,α) reactions.

W. M. DEUCHARS *
G. P. LAWRENCE

Department of Nuclear Physics,
Institute of Advanced Studies,
Australian National University,
Canberra.

---

* Selby Fellow: Permanent address: Atomic Weapons Research Establishment, Aldermaston.

[1] Dearnaley and Whitehead, *A.E.R.E. Rep.* R 3662.

[2] Hinds and Middleton, *Proc. Kingston Conf. Nuclear Structure.* Res. Contrib. No. 121.

[3] Endt and Braams, *Rev. Mod. Phys.*, **29**, 683 (1957).

Magnetic Mirror Confinement of Exploding Wire Plasma

THE exploding wire phenomenon has recently been investigated by several laboratories as a high-density, high-temperature plasma source. For short durations temperatures in excess of 250,000° K. have been observed[1]. Further work has been carried out to confine the exploding wire plasma, making use of its very high current-densities and self-magnetic field[2].

It is the object of this communication to report the production and confinement of exploding wire plasmas in a magnetic mirror configuration. The high current and voltage needed for the exploding wire phenomena may also be used at the same time to produce the magnetic fields necessary for containment of the plasma. Because of the radial particle velocities released by the exploding wire[3], it is possible that a large number of ions will be trapped in an axial magnetic field. By this mechanism of producing a high-density plasma in the central region of a fast-rising, axial magnetic field, large densities of particles may possibly be achieved in the final confined state. In this experiment a capacitor bank of 1·7 μF. was

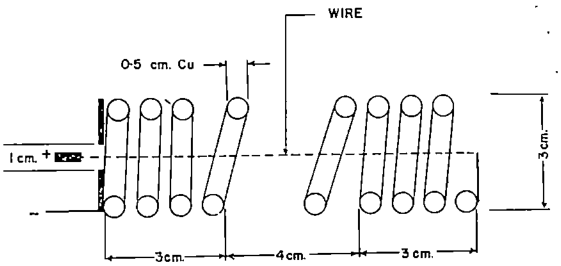


Fig. 1. Axial cross-section of magnetic mirror coil

initially charged to 22 kV. Connexions between the capacitor bank and magnetic mirror coil were made with flat sheet transmission lines, and the capacitor bank was triggered by a three-element spark gap. The capacitor bank has a rise-time of 4 μsec. after the initial triggering. The magnetic mirror coil consists of eight turns of 0·5 cm. diameter copper in the geometry as shown in Fig. 1. The wire to be exploded for producing plasma is shown in Fig. 1 to be on the axis of the coil in a series arrangement. All experiments were carried out in air at atmospheric pressure. The maximum current produced at the first current peak through the system, was estimated to be $1\cdot0 \times 10^4$ amp. This produces a maximum barrier field for the magnetic mirror coil of about 16 kilogauss, and a central field of about 7 kilogauss. A streak camera of the rotating mirror type was used for the observations of the plasma. For all photographs this camera was used at a writing speed of 13·2 μsec./cm., with the same film, at an optical stop of $f/2\cdot5$. The slit of the camera was focused on the central region of the magnetic mirror coil, perpendicular to the wire used for producing plasma. Streak photography was an effective means of recording the width of plasma with time.

Using $C = 1\cdot7$ μF., at 22 kV., and 3 mil copper wire with no external magnetic field, the exploding wire plasma shows the well-known current pause or 'dwell time'[4,5]. A streak photograph showing the 'dwell time' and 'restrike' appears in Fig. 2*a*. The beginning of the first explosion starts at $t = 0$ sec., and the 'restrike', or second current pulse, occurs at 75 μsec. During the time of the 'restrike' the light-emitting portion of the plasma is observed to increase in the radial direction to 4 cm. This pulse is then followed by an afterglow which continued beyond 210 μsec. The streak photograph in Fig. 2*b* was taken with the same conditions as Fig. 2*a* except that the plasma was produced in the central region of the axial magnetic field of the magnetic mirror coil. This streak photograph shows no 'restrike' or second current pulse. The plasma in the axial magnetic field is seen to increase in diameter to 0·75 cm. in 7 μsec., where it then remains with a relatively constant diameter during the remaining 0·01 sec. existence of the plasma. The observed confinement time is found to be increased by 50 per cent by placing a 3 cm. copper disk, with a 1-cm. hole at the centre, at the negative end of the coil as shown in Fig. 1. This disk acted as a switch that shorted the capacitor bank once the plasma had expanded to 1 cm. diameter. Light intensity decreased considerably when placed in the axial magnetic field and streak photography was only possible when high-speed film was used with a fast lens. The appearance of longitudinal striations in the plasma (Fig. 2*b*), when placed in the magnetic field, may possibly be associated with the Kruskal–Schartzschild instability. The plasma never appears to break up but continuously decreases in light intensity until photography is made impossible. The possibility that the plasma may be forming a closed current loop with its own magnetic field running axially along the plasma may explain the long time of confinement from an initially pulsed system. Although most of the work done to date has been mainly qualitative the results warrant further investigation. In the future, magnetic probe studies and other observations will be made in high vacuum.

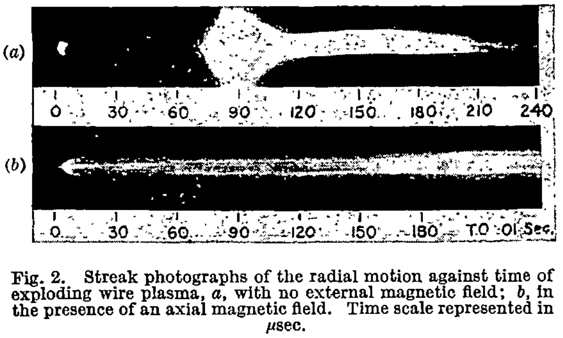


Fig. 2. Streak photographs of the radial motion against time of exploding wire plasma, *a*, with no external magnetic field; *b*, in the presence of an axial magnetic field. Time scale represented in μsec.

I thank Dr. J. D. Fox and Dr. E. A. Desloge for their helpful discussions and criticism, without which this work would have been impossible.

E. J. Seykora

Department of Physics,
Florida State University,
Tallahassee,
Florida.

[1] Scherer, V. E., *Exploding Wires*, 118, edit. by Chace, W. G., and Moore, H. K. (Plenum Press, Inc., New York, 1959).
[2] Katzenstein, J., *Bull. Amer. Phys. Soc.*, **6**, 203 (1961).
[3] Maninger, R. C., *Exploding Wires*, 156, edit. by Chace, W. G., and Moore, H. K. (Plenum Press, Inc., New York, 1959).
[4] Wrana, J., *Elektrotech. Z.*, **59**, 11 (1938).
[5] Wrana, J., *Arch. Elektrotech.*, **33**, 656 (1939).

Variation of Anisotropy Axis Dispersion with Thickness of Nickel-Iron Films

Thin films of nickel-iron evaporated in a magnetic field exhibit a uniaxial anisotropy. It has been postulated by West[1] and others that the anisotropy axes in local regions of the film vary in direction about an average axis. This angular dispersion β has been determined by West using magnetoresistance measurements and by Smith[2] with Bitter figure techniques. In the present work the variation of β with film thickness has been measured using an apparatus which has been fully described by Raffel *et al.*[3].

A field pulse H_A, greater than the anisotropy field H_K, was applied along the average difficult direction, and during the latter part of this pulse a small pulse H_D was applied along the average easy direction. The flux change parallel to the easy axis, due to the application of H_A, was measured with a single pick-up loop, and the minimum value of H_D giving full output was taken to be proportional to the maximum value of β. In practice, the approach to full output was too gradual for accurate measurement of this minimum value, and a level of H_D, H'_D say, giving 80 per cent full output, was used. An angle β' given by $\sin\beta' = H'_D/H_K$ therefore gives the maximum dispersion in anisotropy over some 80 per cent of the film. Hence, 20 per cent of the film has hard directions at angles

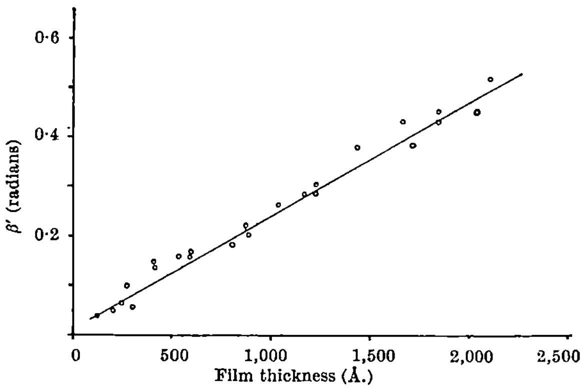


Fig. 1

to the mean greater than β', and 80 per cent at angles less than β'.

The nickel-iron films used in one series of measurements were made in a single deposition, the thickness variation being produced by partially covering the ingot with a shutter. The alloy was deposited from an ingot heated by eddy current, on 0·008-in. thick glass substrates heated to a temperature of 300° C. in an aligning field of 10 œrsteds. The pressure duringn deposition was 10^{-5} mm. mercury, the nominal ingot composition was 82–18 per cent nickel-iron and the deposition took place in some 60 sec. The film thicknesses were measured using Tolansky fringes. The anisotropy field H_K was 3·0 œrsteds and independent of film thickness, while the coercivity H_c varied with thickness in a manner similar to that reported by Methfessel *et al.*[4].

Fig. 1 is a typical graph of β' against film thickness from one such series and shows a linear increase of β' from 0·04 radian (2·3°) at 120 Å. to 0·52 radian (30°) at 2200 Å.

Series of these measurements were also carried out using nickel-iron films from different depositions. The angle β' always increased linearly with film thickness within the limits of experimental error; but the rate of increase varied from one deposition to another, showing that factors other than film thickness affect the angular dispersion. There is, for example, some evidence that β decreases with increasing deposition rate.

Bassett *et al.*[5] have observed an increase of grain size with increasing thickness for evaporated gold films, and a similar increase is to be expected with nickel-iron. Grain size, therefore, may well be the primary factor influencing β. If it is assumed[2] that the anisotropy of the films is caused by shape anisotropy of the grains or the associated anisotropic stresses, it follows that the angular dispersion of anisotropy could arise from a scatter in the orientation of these grains. The dispersion β was obtained by allowing the magnetization to rotate from a hard direction into one or other of the easy directions, forming domains of reversed magnetization. The dispersion of any small portion of film could therefore only be detected provided that its volume V were sufficiently large to form such a domain. If its volume were smaller than this, its magnetization vector would become aligned with that of the surrounding material. If the significant volume V contains grains each of volume v, one expects β to vary statistically as $\sqrt{\frac{v}{V}}$. Accordingly, β increases with grain size and hence with film thickness.

Work is now proceeding on the effect of the deposition-rate on angular dispersion and grain size.

I wish to acknowledge helpful discussions with Mr. R. S. Webley and Dr. E. P. Wohlfarth.

H. Clow

Research Laboratories,
Electric and Musical Industries, Ltd.,
Hayes,
Middlesex.

[1] West, F. G., *J. App. Phys.*, 32, 290*S* (1961).

[2] Smith, D. O., *J. App. Phys.*, **32**, 70*S* (1961).

[3] Raffel, J. I., Crowther, T. S., Anderson, A. H., and Herndon, T. O., *Proc. Int. Rad. Eng.*, **49**, 155 (1961).

[4] Methfessel, S., Middelhoek, S., and Thomas, H., *I.B.M. J.*, **4**, 96 (1960).

[5] Bassett, G. A., Menter, J. W., and Pashley, D. W., *Structure and Properties of Thin Films*, edit. by Neugebauer, C. A., Newkirk, J. B., and Vermilyen, D. A., **11** (J. Wiley and Sons, N.Y., 1960).

SEISMOLOGY

Appearance of the *T*-Phase

Robson and Barr[1] have described the recording of bursts of seismic disturbances at Roseau on the Island of Dominica in The West Indies. The ground movement begins impulsively with a 0·3-sec. period oscillation and the whole disturbance lasts only for some 4 sec. They note that the tremors can be recorded only on the sand and gravel delta of the Roseau River, but because no local source for the tremors can be found they request the comments of other seismologists who find evidence of similar seismic phases.

In Tasmania, the University has installed a radio-telemetered seismic net[2] with a base station at Hobart and satellite stations at Moorlands, Tarraleah and Savannah. The geographical distribution of these stations is shown in Fig. 1. As was pointed out by Newstead and Watt[3] when discussing the Tasmanian net, the provision for central recording at Hobart prevents the occurrence of relative time differences

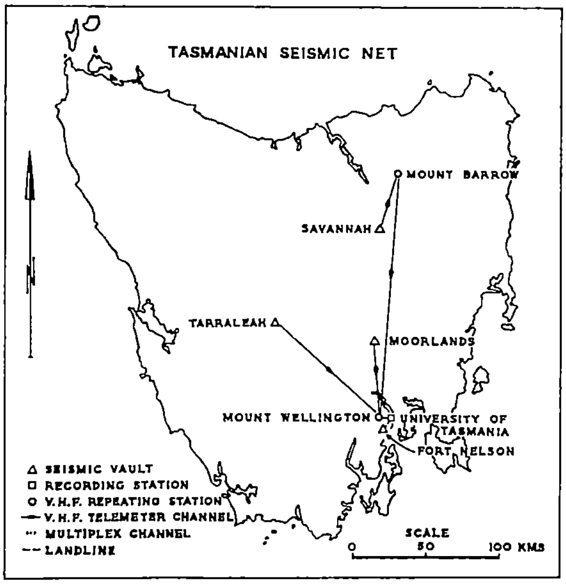


Fig. 1. Location of the stations of the University of Tasmania radio-telemetered seismic net

Table 1. DATA FOR TWO *T*-WAVES ARRIVING AT HOBART FROM MILFORD SOUND, NEW ZEALAND

Date	Epicentre	Origin time	Overall path		Water path		
			Distance (km.)	Travel time (sec.)	Distance	Travel time	Velocity
24.5.60	44·5 S. 167·5 E.	14 46 34	1,638	1,064	1,558	1050	1·48
25.5.60	44·0 S. 168·0 E.	02 37 42	1,691	1,092	1,611	1078	1·49

These seismic disturbances appear on the records as a small-amplitude, short-period tremor of limited duration. The overall epicentral distances and travel-times are given. The length of the 'Sofar' Channel which is at a water-depth of 1,500 m. is given together with the travel-time down the channel. The calculated velocity of 1·48, 1·49 is in agreement with the velocity of a compressional wave at a depth of 1,500 m.

between stations in the net. Consequently, an accurate measure of the velocity of a wave front across the continental crust of Tasmania as well as the velocity of the wave from the epicentre to Tasmania is possible.

Referring back to the appearance of the phase given by Robson and Barr[1] (see their Fig. 1), similar phases have been noted on the Tasmanian records and also on the records of other stations in eastern Australia (Doyle, H., private communication). We have identified the sources of some as due to natural earthquakes on the New Zealand side of the Tasman Sea and in other cases due to explosion of depth charges set off by naval ships. The mechanism of the transmission of the energy is as follows: From the earthquake epicentre or explosion point the energy travels as a compressional wave with a velocity of 1·47 km./sec. down the 'Sofar' Channel[4], which is a low-velocity wave-guide placed at an ocean depth of 1,500 metres. At the continental shelf conversion of energy from the channel wave phase to a *P*-phase and presumably an *S*-phase takes place. As recorded by a seismological station this *P*-phase appears to originate from a disturbance on the continental shelf.

In Table 1, the above hypothesis has been applied to two earthquakes in the Milford Sound region of New Zealand, which happens to be a prolific source of this type of wave. The channel wave can be seen to arrive 15–20 min. after the *P* wave and because of the long time-delay one may be deluded into thinking that the 'Sofar wave' or *T*-phase[5] is not connected with the initial seismic event in New Zealand. Furthermore, the wave front which forms on the continental shelf travels over the continent as a shallow *P*-wave. The late Fr. Burke-Gaffney[6] from Riverview Observatory, Sydney, examined 21 instances of this type of wave and he described them in the same terms as Robson and Barr; namely, bursts of small-amplitude high-frequency tremors. Brekhovskikh[7], in examining *T*-waves, found that the duration of the burst is dependent on the depth of water and the path-length over which the pulse travels. The constancy in appearance of the pulse as found by Robson and Barr suggests a fixed source of origin.

Byerly and Herrick[8], Dietz and Sheehy[9], and Wadate and Inouye[10] have all described the efficiency of the 'Sofar' Channel for the transmission of energy. A bathymetric map of the sea around the island of Dominica[11] shows that the 2,000-m. depth contour is very quickly reached off the coast of Roseau, thus facilitating the conversion of energy from the *T*-phase to the *P*-phase.

Shurbet[12] in 1955 examined the question of injection of energy into the 'Sofar' Channel, and he concluded that little more could be said quantitatively than to point out that the acoustical impedance across the boundary between the channel and the continent does depend on the structure of the continental shelf.

There still remains the problem of why the phase can only be positively picked up on the sediments of the delta of the Roseau River and not over the whole of Dominica Island. I would suggest that the acoustical impedance from sea water to delta sediments is very much less than from sea water to the volcanic rock of the island and as a consequence more energy is communicated to the ground in the vicinity of Roseau. Also with *P*-wave velocities as low as 0·75 km./sec. through the sediments of the delta, the amplitude of the ground movements would be enhanced, thus facilitating the recording of the phase with Willmore seismometers.

It would appear that the disturbances at Roseau are *T*-phases. These disturbances may or may not be preceded by body waves because, as pointed out by Byerly and Herrick, small earthquakes in Hawaii did not always give detectable *P* and *S* waves at California but the *T*-phase was an easily identified disturbance. If Robson and Barr could find evidence of small shocks somewhere in the Caribbean Sea[13] preceding the Roseau disturbances the problem is unambiguously resolved.

R. GREEN

University of Tasmania,
Box 252*c*, G.P.O.,
Hobart.

[1] Robson, G. R., and Barr, K. G., *Nature*, **188**, 306 (1960).
[2] Geology Dept. Pub. 84, Univ. Tasmania (1960).
[3] Newstead, G., and Watt, P. A., *Nature*, **186**, 704 (1960).
[4] Ewing, M., and Worzel, J. C., *Geol. Soc. Amer. Mem.*, **27**, iii (1948).
[5] Linehan, D., *Trans. Amer. Geophys. Union*, **19**, 229 (1940).
[6] Burke-Gaffney, T. N., *Proc. Roy. Soc. N.S.W.*, **88**, 50 (1954).
[7] Brekhovskikh, L. M., *Doklady Acad. Nauk, S.S.S.R.*, **62**, 469 (1948).
[8] Byerly, P., and Herrick, C., *Bull. Seism. Soc. Amer.*, **44**, 113 (1954).
[9] Dietz, R. S., and Sheehy, M. J., *Geol. Soc. Amer. Bull.*, **65**, 941 (1954).
[10] Wadate, K., and Inouye, W., *Proc. Japan. Acad.*, **29**, 47 (1953); *Geophys. Mag.*, **25**, 159 (1954).
[11] *The Times Atlas of the World*, **5**, 114.
[12] Shurbet, D. H., *Bull. Seism. Soc. Amer.*, **45**, 23 (1955).
[13] Coulomb, J., and Molard, P., *Ann. Geophys.*, **8**, 264 (1952).

METALLURGY

Transformation of Cathodically Impregnated and Cooled Austenite

THE stabilization of austenite in two steels by hydrogen, introduced under certain special conditions, has been recently reported by us[1]. A similar but more pronounced stabilization has also been reported, by Gulyaev and Zelenova[2], for austenite monocrystals extracted by selective anodic dissolution. The stabilization can be satisfactorily explained[3], followign Cohen *et al.*[4], on the basis of the de-activation of the strain embryos in austenite by the migration and capture of hydrogen atoms, the driving force for such migration being provided by the electrolytic or pickling potential, resulting in the generation of hydrogen at pressures estimated to reach very high values. In these investigations, impregnation as well as removal of excess hydrogen by effusion, either naturally or deliberately, have been above the M_s temperatures of the steels, when they are wholly austenitic.

The effects of continuing the impregnation to temperatures well below the M_s point have now been examined, on a low-carbon, low-chromium tool steel (carbon, 1·1 per cent; chromium, 2·83 per cent) and a high-carbon, high-chromium die steel (carbon, 2·3 per cent; chromium, 13·6 per cent). These steels, with M_s temperatures of approximately 93° C. and 170° C., respectively, have been quenched from 980° C. into a 5 per cent sulphuric acid bath held at 98° C., cooled in it to room temperature (25° C.) while being cathodically impregnated and aged in air. The Vickers hardness on ageing has been determined, using, as in the previous work, a 30-kgm. load and taking all necessary precautions, the values reported being the mean of at least four perfect impressions. As a slight preparation of the surface of the specimen, after its removal from the electrolytic bath, is necessary for obtaining satisfactory hardness impressions, the first hardness measurement could be made only about 10 min. after such removal. The hardness values, which roughly indicate the extent of transformation, are shown in Fig. 1. The hardness increases appreciably within an hour of ageing, relative to the first measured value. The rise is more rapid in the initial stages and becomes virtually negligible after about 30 min. of ageing. It was unfortunate that hardness for very short ageing periods could not be recorded. However, from the recorded values, the extrapolated hardness value for an ageing period of about 1 min. appears to be substantially lower—of the order of 300–350 Vickers pyramid numbers (V.P.N.) only for the tool steel and 550 V.P.N. for the die steel. The higher value, in the latter steel, is due to cooling through nearly 80° C. in the martensite transformation-range before it is interrupted for impregnation, resulting in the retention at this stage of not more than about 25 per cent austenite, besides the high proportion of residual carbide present. This would also account for the lower over-all increase in hardness observed in this steel.

The most satisfactory explanation for these variations in hardness appears to be on the basis of the effect of pressure on phase transformation, in accordance with the le Chatelier principle. Hydrogen is generated during electrolysis at the surface of the specimen at enormous pressures. Its rapid penetration into the rifts, supposed to exist in steel[5], would further result in a considerable pressure being exerted on the microscopic portions of the austenite within the rift network. The austenite phase may therefore be expected to be considerably stabilized and the amount of martensite formed on cooling it to a fixed reference temperature to be considerably reduced, so long as electrolytic impregnation is continued and the hydrogen pressure maintained. However, as soon as the impregnation is stopped and the steel is removed from the electrolytic bath, hydrogen commences to effuse out, with release of the pressure, and the hitherto suppressed transformation may now be expected to occur in a manner roughly corresponding to the effusion of hydrogen. This would satisfactorily account for the rapid rise of hardness in the initial stages of ageing, followed by a virtually negligible increase, as most of the hydrogen effuses out within about 30 min., at room temperature, from a cathodically soaked specimen of the size employed by us.

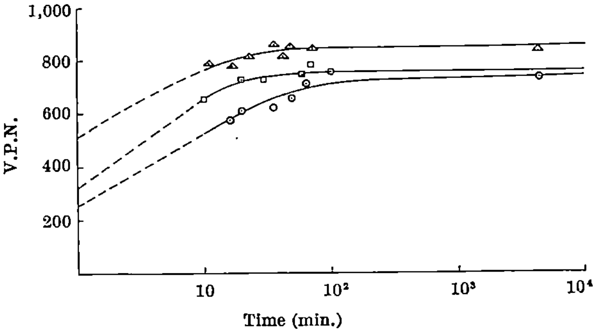


Fig. 1. Variation of hardness with time of impregnated and cooled austenite. ⊙ and □, Tool steel; △, die steel

Several authors[6–8] have examined the suppression of the austenite–martensite transformation by hydrostatic pressure. Patel and Cohen[6] estimate that the M_s point will be suppressed by about 5·7 deg. C. by a pressure of 1,000 atm. and experimentally have recorded a suppression of 8·5 deg. C. for 1,000 atm. Stregulin and Melnikov[7] have recorded a suppression of about 150 deg. C. for a pressure of about 20,000 atm. In view of the probably higher pressures prevailing during impregnation, it is quite likely that the M_s point in this investigation has been substantially depressed. Stregulin and Melnikov have also examined the subsequent isothermal transformation of austenite, the transformation of which to martensite is initially suppressed by pressure, at atmospheric as well as higher pressures, which are, however, lower than the initial suppression pressure. They indicate that in the first two or three seconds after the lowering of the pressure, the transformation proceeded very rapidly and slowed down after about ½ min. Such a very rapid initial transformation-rate may, however, be taken as improbable in our experiments, as although the effusion-rate is undoubtedly rapid, it is not so rapid as to result in the effusion of practically all the hydrogen in a few seconds. Pressure, during cathodic impregnation, is presumably exerted not only on the specimen as a whole from its exposed surface at which hydrogen is generated but also on microscopic portions of the specimen within the rift network, by hydrogen permeating into the rifts. Consequently, full release of pressure cannot be considered to follow instantaneously the removal from the electrolytic bath, unlike in the case of pressure application by classical techniques.

As the pressure of the cathodically generated hydrogen gas is dependent on the cathodic current density, it may be possible to study the austenite–martensite reaction at any value of hydrostatic pressure, merely by changing the conditions of electrolysis. The rate of release of pressure could also be probably controlled to some extent by controlling the rate of effusion of hydrogen out of the specimen—by refrigeration, for example.

Cathodic impregnation thus appears to provide a simple method of subjecting certain systems to high pressures, enabling their effects to be examined, under suitable conditions.

We thank Dr. B. R. Nijhawan, director of this Laboratory, for permission to publish this communication.

E. G. RAMACHANDRAN
C. DASARATHY

Physical Metallurgy Division,
National Metallurgical Laboratory,
Jamshedpur-7, India.

[1] Ramachandran, E. G., and Dasarathy, C., *Nature*, **187**, 684 (1960).
[2] Gulyaev, A. P., and Zelenova, V. D., *Phys. Metals and Metallog.*, **6**, No. 5, 175 (1958).
[3] Ramachandran, E. G., and Dasarathy, C., *J. Iron and Steel Inst.* (to be published).
[4] Cohen, M., Machlin, E. S., and Paranjpe, V. G., *Thermodynamics in Physical Metallurgy*, 162 (1949).
[5] Smith, D. P., *Hydrogen in Metals* (Univ. Chicago Press, 1948).
[6] Patel, J. R., and Cohen, M., *Acta Met.*, **1**, 531 (1953).
[7] Stregulin, A. I., and Melnikov, L. A., *Phys. Metals and Metallog.*, **8**, No. 3, 79 (1959).
[8] Crussard, C., *C.R. Acad. Sci.*, **237** (1953).

MINERALOGY

Tetragonal Mullite-like Phase from Co-precipitated Gels

MUCH attention has been paid to the process of formation of mullite, a refractory mineral of the composition $3Al_2O_3.2SiO_2$, through decomposition of Al–Si clay minerals and crystallization of amorphous gels. In the course of my work on allophanes and related minerals[1], a new tetragonal phase was encountered by heating the co-precipitated Al_2O_3–SiO_2 gels at the temperature-range 910°–1,100° C.

The co-precipitated gels used in the heating experiments were prepared as follows: quartz powder was fused with sodium hydroxide and dissolved in the dilute aqueous solution of sulphuric acid containing an adequate amount of alum. The concentration of SiO_2–Al_2O_3 was kept constant (1 gm./l.) for all cases. Then an excess of hexamethylenetetramine (urotropin) was added in order to attain the homogeneous precipitation of SiO_2 and Al_2O_3. The precipitate was filtered, washed with water thoroughly until either potassium, sodium or urotropin showed no trace, and then dried at room temperature.

Chemical analyses were carried out to discover what extent the ratio of Al_2O_3 to SiO_2 in the original solution would be kept in the co-precipitation, and it was found that the ratio had not been seriously affected through the co-precipitation. Eleven different gels of varying Al_2O_3–SiO_2 ratios were thus prepared (Table 1). In X-ray diffractograms the precipitate showed no diffraction lines except amorphous pattern.

Each gel was heated for 5 hr. in air at different temperatures from 800° to 1,250° C. The sample was quenched in air and examined by X-rays. The result is shown in Table 1. A mullite-like phase was detected above 910° C. in gels of composition ranging from $SiO_2 : Al_2O_3 = 10 : 90$ to $80 : 20$. This phase is converted to an ordinary mullite above 1,250° C. In the composition-range higher in Al_2O_3 than $SiO_2 : Al_2O_3 = 20 : 80$, γ-alumina was formed at about 850° C., to be converted into corundum at 1,250° C. In the composition-range higher in SiO_2 than $SiO_2 : Al_2O_3 = 70 : 30$, cristobalite was formed at 1,250° C.

Table 1. RATIO OF Al_2O_3 AND SiO_2 GELS AND PRODUCTS BY HEATING

Percentage by weight SiO_2	Al_2O_3	Molecular ratio SiO_2 : Al_2O_3	Products by heating for 5 hr. at ° C. 800	860	910	930	1,100	1,250
0	100	... : ∞	—	*A*	*A*	*A*	*Co*	*Co*
5	95	1 : 11	—	*A*	*A*	*A*	*A*	*Co, Mr*
10	90	1 : 5	—	—	*A*	*A*	*A*	*Co, Mr*
20	80	1 : 2	—	—	*Mt, A*	*Mt, A*	*Mt, A*	*Co, Mr*
28	72	1 : 1·5	—	—	*Mt*	*Mt*	*Mt*	*Mr*
37	63	1 : 1	—	—	*Mt*	*Mt*	*Mt*	*Mr*
54	46	2 : 1	—	—	*Mt*	*Mt*	*Mt*	*Mr*
70	30	5 : 1	—	—	—	*Mt*	*Mt*	*Mr, Cr*
80	20	7 : 1	—	—	—	*Mt*	*Mt*	*Mr, Cr*
90	10	15 : 1	—	—	—	—	—	*Mr, Cr*
100	0	∞ : ...	—	—	—	—	—	*Cr*

—, Gel; *A*, γ-alumina; *Mt*, mullite-like phase produced at low temperature; *Mr*, ordinary mullite; *Co*, corundum; *Cr*, cristobalite.

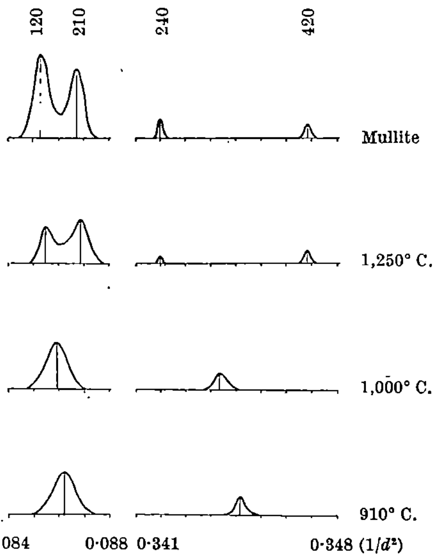


Fig. 1. X-ray diffraction pattern of products from heated Al_2O_3–SiO_2 gels and ordinary mullite

Of these eleven compositions given in Table 1, those yielding a mullite-like phase gave a sharp exothermic peak at 900°–1,000° C. in a D.T.A. curve while those yielding no mullite-like phase give no peak at the same temperature range[2]. Therefore, a close relationship between this exothermic peak in the D.T.A. curve and the appearance of the mullite-like phase might be indicated.

Further X-ray determinations were made on specimens of the composition $2SiO_2.3Al_2O_3$ heated at 910°, 1,000° and 1,250° C. with a Geiger-counter diffractometer. An arc-fusion mullite from an electrocast brick was examined for comparison. The mullite-like phase made at 910° and 1,000° C. gives generally fewer peaks than the ordinary mullite formed at 1,250° C. and arc-fusion mullite.

Table 2. LATTICE CONSTANTS AND CELL VOLUMES (v) OF PRODUCTS FROM HEATED Al_2O_3–SiO_2 GELS AND ORDINARY MULLITE

Products	a (Å.)	b (Å.)	c (Å.)	v (Å^3.)	a/b	c/b
Heated at 910° C.	7·614		2·883	167·1	1·0000	0·3786
Heated at 1,000° C.	7·625		2·882	167·6	1·0000	0·3780
Heated at 1,250° C.	7·556	7·684	2·881	167·3	0·9833	0·3749
Arc-fusion mullite	7·565	7·672	2·883	167·3	0·9861	0·3758

Especially interesting is the fact that the pairs of 120 and 210 and of 240 and 420 reflexions of ordinary mullite[3] merge each into a single peak in the mullite-like phase as shown in Fig. 1. It was found that all the peaks of the mullite-like phase can be indexed reasonably in the term of a tetragonal lattice (Table 2). When plotted in the diagram unit cell volume versus c-axis, which shows clearly the interrelations in unit-cell dimensions of various mullites and sillimanites, this tetragonal mullite-like phase falls within the range of the 'iron free synthetic mullite' designated by Agrell and Smith[4]. When plotted in the diagram *a/b* versus *c/b*, this phase falls on the linear extrapolation of the line representing the 'iron free synthetic mullite'. There is no indication that this phase has any similar evidence to sillimanite.

All the evidence appears to indicate that this new phase has a very close similarity to mullite in crystal structure.

This communication reports work from Morimoto's Laboratory of this Institute. A detailed account is now in preparation.

J. OSSAKA

Earthquake Research Institute,
University of Tokyo.

[1] Ossaka, J., *Advances in Clay Science*, Proc. Second Ann. Meeting on Clay and Clay Minerals, 337 (1960).

[2] Demeduk, T., and Cole, W. F., *Nature*, **181**, 1400 (1958).

[3] Scholze, H., *Ber. D.K.G.*, **32**, 381 (1955).

[4] Agrell, S. O., and Smith, J. V., *J. Amer. Ceramic Soc.*, **43**, 69 (1960).

GEOLOGY

Two Unexplored Records of Quaternary History

DURING the last glaciation, world sea-level is generally thought to have been about 330 ft. (100 m.) below that of to-day. The rate of recovery for the last 200 ft. has been obtained from the (radiocarbon) age of peat beds and beach deposits which were covered successively by the rising sea[1]. Search for material to date the preceding 130 ft. could be made, quite reasonably, in a region of tectonic stability such as south-west England. The existing estuaries are too shallow to carry suitable material, but there are good prospects in the ground between the south coast of Ireland and the north coast of Cornwall. Here there is a hollow, the floor of which is 420 ft. below present sea-level, cut off from water of comparable depth, some 20 miles distant, by a sill the greatest depth of which is 330 ft. below present sea-level. Mitchell[2] considers that this sill and two others at a higher level in the Irish Sea are moraines, and this is supported by what is known of the composition of the sea-floor. Material from mud to stones is common. Pleistocene erratics certainly occur on the floor of the Irish Sea[3,4], and the height of the middle sill is far less than the thickness of the uncompacted materials, revealed by a seismic refraction station nearby[5].

If the southernmost sill is of Gipping age as Mitchell suggests, then it is likely that the hollow behind it was a freshwater lake during the last glaciation, during which the ice did not reach farther south than the two presumed moraines in the Irish Sea. The deepest sill was certainly land at some time for its surface is cut by two channels.

The proving of lake deposits could show that the sea had dropped below the level of the sill during the Pleistocene. The finding of a sequence of lake deposits or of peat should reveal when the sea-level rose to − 330 ft. Similar deposits in the Irish Sea could reveal when the sea-level rose to − 270 ft.

The two hollows are the sites of contemporary deposition of mud and sand, by the tidal currents, so that older materials will have been protected from erosion, at least locally, since they were covered by the sea. Search for the older materials will give a chance to assess the amount of recent deposition and therefore the importance of the supply paths that have been proposed[6].

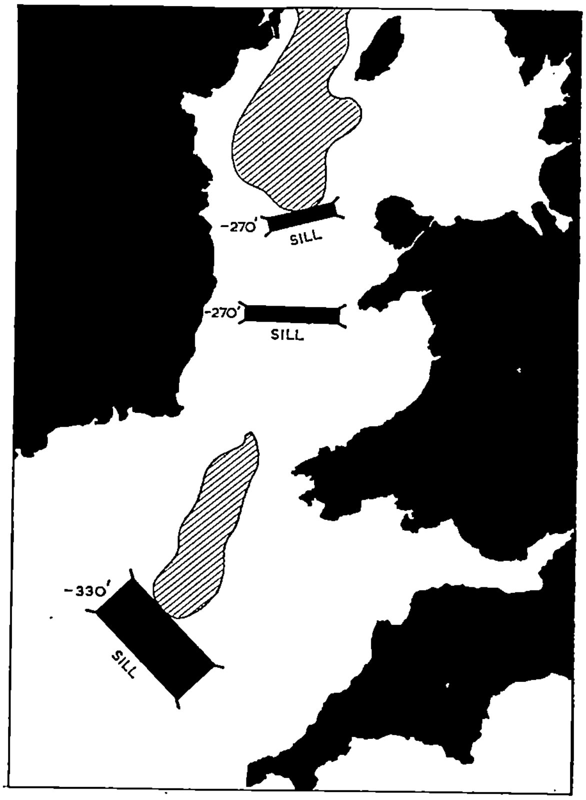


Fig. 1. The sills (? moraines) behind which freshwater lakes (diagonal shading) were probably in existence during the late Pleistocene

Examination of the northern hollow by means of the echo-sounder on R.R.S. *Discovery II* showed that there is up to 90 ft., of what experience shows can be interpreted as uncompacted material; lying partly on a rock floor. Sixty feet was proved by the same equipment in the southern hollow while a high-power, low-frequency echo-sounder, the 'Thumper', showed that the layer was underlain by other materials with a total thickness of 360 ft. (uncorrected for the velocity of sound in the material).

Further exploration is planned for the coming year, but it is anticipated that effective sampling of such sequences of deposits calls for ship-borne rotary drilling equipment of the type already used so successfully in deep water. Although such a technique is beyond the financial resources of the National Institute of Oceanography, it is hoped that the potential value of this suggested line of research will find wider support elsewhere.

A. H. STRIDE
R. BOWERS

National Institute of Oceanography,
Wormley, Godalming,
Surrey.

[1] Godwin, H., *Proc. Roy. Soc.*, B, **153**, 287 (1961).

[2] Mitchell, G. F., *Adv. Sci.*, **17**, 68, 313 (1960).

[3] Herdman, W. A., and Lomas, J., *Proc. Liverpool Geol. Soc.*, **8**, 205 (1898).

[4] Berthois, L., *Rev. Trav. Inst. Pêches marit.*, **21**, 488 (1957).

[5] Hill, M. N., *Quart. J. Geol. Soc., Lond.*, **111**, 393 (1956); and personal communication.

[6] Stride, A. H., *Dock and Harb. Author.*, **40**, 145 (1959).

CHEMISTRY

Infra-Red Spectroscopic Evidence of Four-fold Co-ordination of Titanium in Barium Orthotitanate

FOUR-FOLD co-ordination of titanium has not been reported, either in inorganic titanates or in titanium oxides. Titanium is generally present as TiO_6 octahedra, although five-fold co-ordination has been reported in a few cases[1]. However, a study of titanium-bearing garnets led me to the conclusion that titanium (Ti) replaces silicon (Si), at least in part, and thus is four-fold co-ordinated in the garnet structure[2,3].

Further spectroscopic work on synthetic compounds has now given full confirmation of this interpretation and tetrahedral co-ordination of titanium has been ascertained, not only in solid solutions of the $X_2(Si,Ti)O_4$ type, but also in a pure orthotitanate Ba_2TiO_4, in $(Ba,Sr)_2TiO_4$ solid solutions and in some glassy structures.

First, the infra-red spectra of Zn_2SiO_4, Zn_2TiO_4 and $Zn_2(Si,Ti)O_4$ compounds will be briefly discussed. Zn_2SiO_4 has been chosen as a highly discriminative case because it has tetrahedral sites only. If some silicon (1–10 mole per cent) is replaced by titanium, two possibilities only are likely to occur: (1) Titanium does enter the willemite structure and is necessarily four-fold co-ordinated. The infra-red bands related to TiO_4 tetrahedra should be characteristic both from their frequency[3] and their pattern (the infra-red pattern given by fairly dilute solid solutions has been discussed briefly in a previous paper[4]). (2) Titanium does not enter the willemite structure and thus forms a separate compound for itself, namely Zn_2TiO_4, which is an inverse spinel with titanium in six-fold co-ordination. Other possibilities are unlikely, since no intermediate ternary compound is known in the system $ZnO - SiO_2 - TiO_2$.

The observed infra-red patterns are given as block diagrams in Fig. 1. I have interpreted[5] the infra-red spectrum of Zn_2SiO_4 as follows: 1,000–850 cm.$^{-1}$, stretching modes of deformed SiO_4 tetrahedra; 460–380 cm.$^{-1}$ bending modes of deformed SiO_4 tetrahedra; 620–570 cm.$^{-1}$ stretching modes of deformed ZnO_4 tetrahedra.

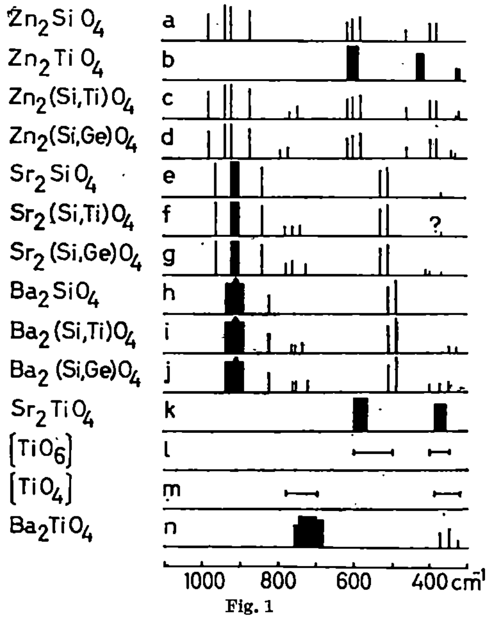


Fig. 1

No detailed interpretation is available for Zn_2TiO_4; the strong absorption centred near 590 cm.$^{-1}$ may well be due to either ZnO_4 tetrahedra or TiO_6 octahedra or both. Anyway, a detailed assignment is not needed for the present work, and the most important thing is the absence of strong bands at frequencies higher than 600 cm.$^{-1}$. This is in accordance with previous work on titanates[6], where TiO_6 octahedra have been found to absorb in the 600–500 cm.$^{-1}$ range.

The infra-red spectra of $Zn_2(Si,Ti)O_4$ samples with 1–10 moles per cent of titanium are characterized by two groups of new bands near 750 and 320 cm.$^{-1}$ (Fig. 1*c*), the intensities of which depend on the amount of titanium introduced; it is clearly impossible to attribute them to Zn_2TiO_4; moreover, their assignment to TiO_6 octahedra is inconsistent with the fairly high values 765 and 747 cm.$^{-1}$; as a matter of fact, this is within the absorption-range of GeO_4 tetrahedra as observed in orthogermanates (Tarte, T., unpublished results). On the other hand, GeO_4 tetrahedra in the $Zn_2(Si,Ge)O_4$ solid solutions gave a very similar infra-red pattern (Fig. 1*d*). This striking similarity is perhaps the most important reason for believing that titanium is also present in solid solution as TiO_4 tetrahedra.

If taken separately, each of these facts may be given several interpretations, but if considered simultaneously, they fully demonstrate that titanium has entered the willemite structure and is four-fold co-ordinated: the two groups of bands near 750 and 320 cm.$^{-1}$ are assigned to the stretching and bending modes respectively of slightly distorted TiO_4 tetrahedra.

Quite similar infra-red patterns have also been observed for other solid solutions such as $Sr_2(Si,Ge)O_4$ and $Sr_2(Si,Ti)O_4$, or $Ba_2(Si,Ge)O_4$ and $Ba_2(Si,Ti)O_4$ (Fig. 1, *e–j*), and this is again good evidence that a moderate amount of titanium may be introduced on the tetrahedral sites of these structures.

Let us now consider the case of pure Sr_2TiO_4 and Ba_2TiO_4. It turns out that these substances give quite different infra-red spectra.

In Sr_2TiO_4, titanium is six-fold co-ordinated[7] and the observed infra-red spectrum (Fig. 1*k*) agrees with the general pattern of (TiO_6) octahedra (Fig. 1*l*) as observed in a series of metatitanates[6]. In Ba_2TiO_4, the detailed structure of which is still unknown, the strongest absorption is observed near 720 cm.$^{-1}$, a rather high value if compared with Sr_2TiO_4 or other structures with titanium in six-fold co-ordination. The infra-red spectrum of Ba_2TiO_4, however, agrees nicely with the characteristic frequencies of TiO_4 tetrahedra (Fig. 1, *m* and *n*). The same pattern is observed for $(Ba,Sr)_2TiO_4$ solid solutions (containing up to 10 moles per cent of Sr), which have essentially the same structure as Ba_2TiO_4. Further proof of the occurrence of TiO_4 tetrahedra has been given by the study of the composition $Ba_2(Ti_{0\cdot95}Si_{0\cdot05})O_4$. There is full spectroscopic evidence that silicon is present in solid solution as SiO_4 tetrahedra, thus confirming the occurrence of tetrahedral sites in the Ba_2TiO_4 structure. Tetrahedral co-ordination of titanium in Ba_2TiO_4 is thus definitely established. This is the first reported example of such a co-ordination in a titanate, but this result is in accordance with the suggested analogy of structure between Ba_2SiO_4 and Ba_2TiO_4 (ref. 8).

It should be pointed out that the absorption of TiO_4 tetrahedra in the 700–800 cm.$^{-1}$-region is fairly strong and allows an easy discrimination between TiO_4 tetrahedra and TiO_6 octahedra. The method may be applied even to glassy structures, where I was able to detect spectroscopically both tetrahedral and octahedral co-ordination of titanium[9].

P. TARTE

Department of General Chemistry,
University of Liège.

[1] Andersson, S., and Wadsley, A. D., *Nature*, **187**, 499 (1960).
[2] Tarte, P., *Nature*, **186**, 234 (1960).
[3] Tarte, P., *Silicates Industriels*, 171 (1960).
[4] Tarte, P., *Bull. Acad. Royale Sci. Belg.*, 169 (1960).
[5] Tarte, P., *Fifth Symposium on Molecular Spectroscopy*, Amsterdam (1961).
[6] Last, J. T., *Phys. Rev.*, **105**, 1740 (1957).
[7] Kwestroo, W., and Paping, H. A. M., *J. Amer. Ceram. Soc.*, **42**, 292 (1959).
[8] Rooksby, H. P., *Nature*, **159**, 609 (1947).
[9] Tarte, P., *Revue Universelle des Mines* (in the press).

Selectivity of Palladium Catalysts for the Oxidation of Carbon Monoxide in the Presence of Hydrogen

IT is known that a palladium catalyst used for the removal of traces of oxygen from hydrogen at room temperature is readily poisoned by carbon monoxide[1,2].

To remove oxygen or carbon monoxide from hydrogen containing both gases it is necessary, therefore, to work at higher temperatures. Catalysts recommended for effecting a selective oxidation of a few per cent of carbon monoxide in the presence of an excess of hydrogen are the metals of the eighth group of the Periodic System, especially platinum, rhodium and ruthenium[3].

These catalysts work selectively only between about 120° and 160° C.: below 120° C. poisoning occurs, above 160° C. the selectivity becomes lost.

W. J. Hanssen[4], when determining traces of carbon monoxide in the 5–200 p.p.m. range, applied platinum gauze at somewhat higher temperatures.

We carried out experiments with hydrogen gas containing only traces of carbon monoxide (50 p.p.m.) and found that in this case selective oxidation occurs already at room temperature. The results are given in Table 1.

Table 1. SELECTIVE OXIDATION OF CARBON MONOXIDE IN THE PRESENCE OF HYDROGEN ON 0·5 PER CENT PALLADIUM ON ALUMINA CATALYST (TOTAL PRESSURE 1 ATM., 50 P.P.M. CARBON MONOXIDE AND 0·2 PER CENT OXYGEN

Temperature (° C.)	Space velocity (h^{-1})	Conversion of carbon monoxide (per cent)
20	3,000	95
20	1,000	92
30	1,000	92
50	1,000	95
75	1,000	95

Throughout the period of investigation (several days) no indication of loss of activity was noted. The carbon monoxide was shown to have been oxidized to carbon dioxide and the oxygen content had been brought down to less than 0·1 p.p.m. An increase of the carbon monoxide concentration to 200 p.p.m. resulted in total poisoning of the catalyst. The poisoning was reversible.

The reactor used worked practically isothermally. The analysis was carried out with Hanssen's method (mentioned above) adapted to our lower concentration-range.

It can be concluded that removal of traces of carbon monoxide from hydrogen (necessary in some catalytic hydrogenation reactions) can be carried out already at room temperature.

P. MARS
TH. VAN DE MOND

Central Laboratory,
Staatsmijnen,
Geleen, Netherlands.

[1] Cohn, C., *Anal. Chem.*, **19**, 832 (1947).
[2] Sergeant, G. A., and Bartlett, A. F. F., *J. App. Chem.*, 208 (1955).
[3] Brown, M. L., Green, A. W., Cohn, G., and Andersen, H. C., *Indust. Eng. Chem.*, **52**, 841 (1960).
[4] Hanssen, W. J. (private communication).

pH of Soap Solutions

THE specific conductivity and *p*H of sodium laurate solutions have been measured at several temperatures within a concentration-range of 10^{-1} *M*–3 × 10^{-5} *M*. The apparatus used was a modification of that used by Franks[1] for the chromatography of long-chain fatty acids. It enabled a concentrated (10^{-1} *M*) soap solution to be diluted continuously with rigorous exclusion of carbon dioxide at all stages, beginning with preparation of the soap.

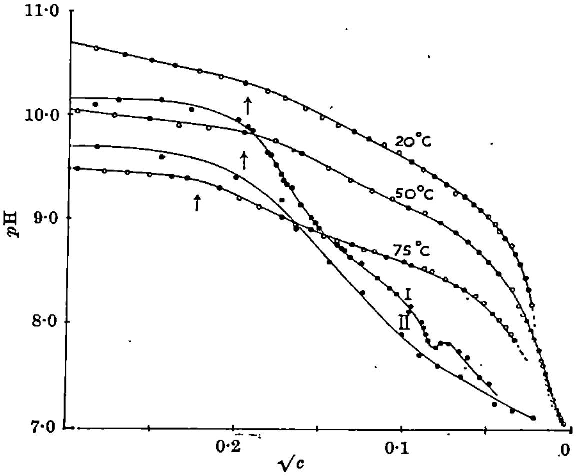


Fig. 1. *p*H of sodium laurate solutions. ○, This work; I, ref. 2 (20°); II, ref. 3 (25°)

The results are best presented graphically, and Fig. 1 shows the *p*H of sodium laurate solutions as a function of $\sqrt{c}$ at 20°, 50° and 75° C. The results of Ekwall[2] and McBain[3] are included for comparison. The present *p*H values are consistently higher than those of earlier workers and show very little scatter. The critical micelle concentration at each temperature is indicated by an arrow. Although the critical micelle concentration at 20° seems rather high compared with other published results, this could well be due to the total exclusion of carbon dioxide. Our value of 3·4 × 10^{-2} *M* is in excellent agreement with that obtained recently from e.m.f. measurements with membrane electrodes[4].

Fig. 2 shows the *p*H of a sodium laurate solution at 50° as a function of log *c* at extreme dilutions. At soap concentrations less than 10^{-4} *M* a linear relationship between *p*H and log *c* is obtained. The slope is in good agreement with the Debye–Hückel limiting law for uni-univalent electrolytes, and the soap behaves as a salt of a strong base and a weak acid. However, at concentrations greater than 10^{-4} *M*, marked deviations from ideal behaviour occur, possibly due to pre-association equilibria. The

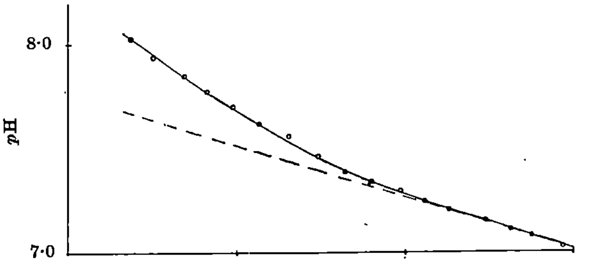


Fig. 2. pH of sodium laurate solutions at 50° C. over the low range of concentration. The dashed line represents the Debye–Hückel slope for a uni-univalent electrolyte

possibility of some form of pre-association below the critical micelle concentration has been suggested to account for the conductivity of sodium dodecyl sulphate[5], but the importance of such an effect has recently been discounted[6].

D. EAGLAND
F. FRANKS

Department of Chemical Technology,
Institute of Technology,
Bradford, 7.

[1] Franks, F., *Analyst*, **81**, 384 (1956).

[2] Ekwall, P., and Lindblad, L. G., *Kolloid Z.*, **94**, 42 (1941).

[3] McBain, J. W., Laurent, P., and John, L. M., *J. Amer. Oil Chemists Soc.*, **25**, 77 (1948).

[4] Botré, C., Cresenzi, V., and Liquori, A. M., *Proc. Third Intern. Cong. Surface Activity*, **1**, 302 (1960).

[5] Mysels, K. J., Mukerjee, P., and Dulin, C. I., *J. Phys. Chem.*, **62**, 1390 (1958).

[6] van Voorst Vader, F., *Trans. Farad. Soc.*, **57**, 110 (1961).

Theoretical Expression for Solubility Product

WHILE considering some questions on ionic interactions in a medium, I noticed a communication under this heading which includes an erroneous assumption[1].

Stating the potential energy of a 'system' of oppositely charged ions as:

$$U = \left\{ -\frac{(z_+\varepsilon)(z_-\varepsilon)}{R_0}\left(1 - \frac{1}{n}\right) - \frac{\alpha_a(z_+\varepsilon)^2 + \alpha_c(z_-\varepsilon)^2}{2R_0{}^4} \right\} \quad (1)$$

Krishnamurti derived an equation of the solubility product (equation 6) as:

$$-\log_e S_p = \left\{ \frac{\alpha_a z_+{}^2\varepsilon^2 + \alpha_c z_-{}^2\varepsilon^2}{2R_0{}^4} \right\} \Big/ RT + a \quad (2)$$

assuming that for very small values of R_0 the first term in (1), namely $\frac{-\varepsilon^2}{R_0}\left(1 - \frac{1}{n}\right)$, can be neglected in comparison with the second term representing the mutual ion-dipole interaction. (In equation 1, n is a constant which is approximately 9, α_a and α_c are polarizabilities of anion and cation respectively, z_+ and z_- are valencies of anion and cation, ε is the electronic charge and R_0 the equilibrium distance between the centres of the two ions.)

The assumption that, for very small values of R_0, the first term in (1) can be neglected in comparison with the second term is not correct. As the distance of closest approach of two ions can never be less than 10^{-8} cm. (except in the case of nuclear events), it can be seen that the first term can never be less than the second term. With α of the order of 10^{-24} cm.^{3}, $e = 4{\cdot}803 \times 10^{-10}$ E.S.U., and with minimum value of R_0 is of the order of 10^{-8} cm., and with n and 9 it is seen that the first term is always greater than the second term at least by a factor of 10 for ions of different values of Z as 1, 2, 3, etc. Hence the first term can never be neglected in comparison with the second term. The first term, representing energy due to Coulomb interaction, is of course more important than that of the attraction resulting from the polarization of each ion in the field of the other as represented by the second term.

At constant temperature and with R_0 constant for compounds of similar constitution, a plot of $\log_{10} S_p$ against $\alpha_a z_+{}^2 + \alpha_c z_-{}^2$, Fig. 1 of Krishnamurti's communication, gave a straight-line graph, which led him to think that the derivation of equation 2 above was correct. But it should be noted that with n and R_0 constant (which point is also subject to question) the first term $\frac{z_+z_-\varepsilon^2}{R_0}\left(1 - \frac{1}{n}\right)$ itself becomes constant and thus equation 7 of that communication becomes:

$$-\log_{10} S_p = K(\alpha_a z_+{}^2 + \alpha_c z_-{}^2) + \lambda$$

where λ is another constant in which the contribution of the first term is included and thus a plot of $\log_{10} S_p$ against $\alpha_a z_+{}^2 + \alpha_c z_-{}^2$ would also lead to a straight-line graph. Thus a straight-line graph for $\log_{10} S_p$ against $\alpha_a z_+{}^2 + \alpha_c z_-{}^2$ does not justify the neglect of the first term, which, as I have explained, is more important than the second term.

I also wish to point out a discrepancy in equation 6 of Krishnamurti's communication (equation 2 here). By considering the interaction energy of two ions only, as in equation 1 here and not for all ions in a mole, Krishnamurti has arrived at equation 6 assuming his equation 3; in both equations RT there should be $\varkappa T$, where $\varkappa$ is Boltzmann's constant.

B. N. BHAR

Physics Department,
College of Science,
University of Baghdad.

[1] Krishnamurti, K., *Nature*, **182**, 1227 (1958).

WHEN an ionizable compound BA is dispersed in water to form a saturated solution, equilibrium is attained with the rate at which the ions detach themselves from unit area of the surface of the solid particles (crystals) and go into solution, and is equal to the rate at which the ions recombine to form the molecules which are deposited on the same area of the surface. The rate of recombination of the oppositely charged ions $B^{\cdot}$ and A' to form BA, being deposited on unit area of the solid surface, is given by the expression: $k_1 n_{B^{\cdot}} n_{A'} . \exp(-E_1/RT)$, where $n_{B^{\cdot}}$ and $n_{A'}$ are the numbers of cations $B^{\cdot}$ and anions A' per unit volume of the solution; k_1 is a constant and E_1 is the energy per gm.-mole which the ions should possess to escape from the ionic atmosphere and from the solvation layers around the ions. This energy term was neglected in the earlier communication[1] and it should be correctly expressed as:

$$E_1 = N_0 \left\{ \frac{(z_+\ \varepsilon).(z_-\ \varepsilon)}{DR_1} + c\left(\frac{5}{9} \cdot \frac{A}{R_2^4} + \frac{7}{9} \cdot \frac{B}{R_2^2}\right) \right\} \quad (1)$$

where the first term represents the Coulomb attraction and the second[2] the energy of interaction between an ion of charge $z\varepsilon$ and c equidistant molecules of

Table 1

Compound	$N_0\left\{\frac{\alpha_a(z_+\ \varepsilon)^2 + \alpha_c(z_-\ \varepsilon)^2}{2R_3^4}\right\}\Big/RT$	$-\log_e S_p$	R_3 used in col. (2) (Å.)	R (from data in tables) (Å.)	$\gamma = \left(\frac{R_t}{R_3}\right)^4$
AgCl	22·58	22·58	2·788	2·77 (experimental)	1·47
AgBr	28·46	28·46	2·781	2·88 ,,	1·775
AgI	36·9	36·9	2·805	3·05 ,,	2·208
HgCl	40·2	40·2	2·431	2·434 (covalent)	2·351
HgBr	48·1	48·1	2·451	2·58 ,,	2·727
HgI	64·3	64·3	2·46	2·77 ,,	3·162
CuCl	13·66	13·63	3·154	2·77 (ionic)	0·595
CuBr	17·0	17·0	3·150	2·91 ,,	0·729
CuI	26·02	26·02	3·084	3·12 ,,	1·047
$HgCl_2$	33·54	33·57	3·293	3·01 ,,	0·698
$HgBr_2$	44·0	44·0	3·302	3·15 ,,	0·828
HgI_2	65·61	65·61	3·30	3·28 ,,	0·976
$PbCl_2$	9·21	9·21	4·728	3·13 ,,	0·192
$PbBr_2$	12·02	12·02	4·704	3·27 ,,	0·233
PbI_2	16·12	16·12	4·756	3·52 ,,	0·30

water at a distance R_2 from its centre ; $A = \frac{1}{2}\alpha(z\varepsilon)^2$; $B = z\varepsilon\mu$. N_0 is the Avogadro number ; z_+ and z_- denote the valencies of the positive and negative ions ; ε is the electronic charge, α the polarizability and μ the dipole moment of the water molecules; R_1 is the radius of the ionic atmosphere, and D is the dielectric constant of the medium.

The rate of re-dispersion of these ions from unit area of the surface of the solid BA in contact with the saturated solution is $k_2.n'.\exp(-E_2/RT)$, where n' is the number of pairs of ions ($B^{\cdot}$ and A') per unit area of the surface, and this re-dispersion of ions will take place when they possess energies exceeding E_2, which is the energy due to the attraction caused by Coulomb forces and the mutual polarization of ions:

$$E_2 = N_0\left\{\frac{A_0(z_+\ \varepsilon)\ (z_-\ \varepsilon)}{R_3}\left(1 - \frac{1}{n}\right) + \frac{\alpha_a(z_+\ \varepsilon)^2 + \alpha_c(z_-\ \varepsilon)^2}{2R_3^4}\right\} \quad (2)$$

where A_0 is the Madelung constant, n occurs in Born's repulsion energy term, α_c and α_a are the polarizabilities of the ions, R_3 the interionic distance in crystals. In addition to these interactions it may sometimes be necessary to consider the dispersion forces between the ions, which may be of significance at close distances of approach of the ions characterized by higher polarizabilities. At equilibrium:

$$K_1'(N_B{\cdot}N_{A'}).\exp\left[-N_0\left\{\frac{(z_+\ \varepsilon).(z_-\ \varepsilon)}{DR_1} + c\left(\frac{5}{9}\cdot\frac{A}{R_2^4} + \frac{7}{9}\cdot\frac{B}{R_2^2}\right)\right\}\right]\Big/RT$$

$$= K_2'N'.\exp\left[-N_0\left\{\frac{A_0(z_+\ \varepsilon).(z_-\ \varepsilon)}{R_3}\left(1 - \frac{1}{n}\right) + \left\{\frac{\alpha_a(z_+\ \varepsilon)^2 + \alpha_c(z_-\ \varepsilon)^2}{2R_3^4}\right\}\right]\Big/RT \quad (3)$$

It was stated in the previous communication[1] that for small values of R_3 the term $\left(\frac{(z_+\ \varepsilon)(z_-\ \varepsilon)}{R_3}\right)$ can be neglected in comparison with the second term representing the mutual ion-dipole interactions. As pointed out by Dr. B. N. Bhar, this is not correct for the commonly accepted values of inter-ionic distances. This error did not, however, invalidate the main expression (5) or the approximate expression (6) given earlier[1]. This term may be omitted from the final expression when its numerical value is of the same order of magnitude as the term:

$$\left\{\frac{(z_+\ \varepsilon)(z_-\ \varepsilon)}{DR_1} + c\left(\frac{5}{9}\cdot\frac{A}{R_2^4} + \frac{7}{9}\cdot\frac{B}{R_2^2}\right)\right\}$$

occurring on the left side of equation (3). Then we may write (3) in a simpler form:

$$-\log_e S_p = -\log_e(N_B{\cdot}N_{A'}) = N_0\left\{\frac{\alpha_a(z_+\ \varepsilon)^2 + \alpha_c(z_-\ \varepsilon)^2}{2R_3^4}\right\}\Big/RT + a \quad (4)$$

where $N_{B^{\cdot}}$,$N_{A'}$ relate to gm.ions and a is a constant. This is almost the same expression which was obtained previously[1]. Its validity is revealed by results given in Table 1. The agreement between the experimental values of $-\log_e S_p$ and those calculated from the expression (4) using the values of R_3 given in column 4 is quite good. If we use instead the inter-ionic distances (R_t) obtained from the usually accepted values of ionic radii in this expression, we have to multiply it by a factor $\gamma = \left(\frac{R_t}{R_3}\right)^4$. There is a general proportionality between γ and the extent of covalent bonding in the corresponding halides. Thus, expression (4) should be written in a more general form as follows:

$$-\log_e S_p = \beta N_0\left\{\frac{\alpha_a(z_+\ \varepsilon)^2 + \alpha_c(z_-\ \varepsilon)^2}{2R_3^4}\right\}\Big/RT + a \quad (5)$$

where β corrects for the covalent contribution to the bond between the ions in the crystal, which causes a decrease in the solubility of the salt. In fact, copper, silver and mercury show considerable tendency to form covalent compounds, and their halides (except fluorides) show very low solubilities.

K. Krishnamurti

Chemistry Department,
College of Science,
University of Baghdad.

[1] Krishnamurti, K., *Nature*, 182, 1227 (1958).

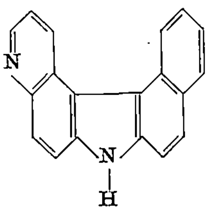
[2] Moelwyn-Hughes, E. A. *Physical Chemistry*, 859 (Pergamon Press, 1957).

BIOCHEMISTRY

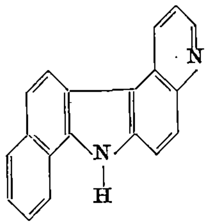
A New Family of Potent Carcinogens : Benzopyridocarbazoles

Although the introduction of heterocyclic nitrogen atoms in the molecules of polycyclic compounds sometimes decreases carcinogenic activity (for example, the inactive pyrenoline as compared with 3,4-dibenzopyrene[1]), there are many instances in which the carcinogenic potency is thereby maintained or even enhanced. Such is the case with the angular benzacridines[2], some of which are more active than the corresponding 1,2-benzanthracenes in respect of induction of skin epitheliomas and sarcomas, and with the inactive pentacene, in the molecule of which the introduction of two nuclear nitrogen atoms leads to the sarcoma-producing dinaphthazine[3]. In view of the already established carcinogenic activity of the angular dibenzocarbazoles[4], it was interesting to examine the biological properties of the recently synthesized aza derivatives[5].

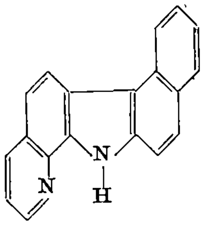
A first series of experiments concerned four such compounds: 3,4-benzopyrido(3′,2′:5,6)carbazole (I), 1,2-benzopyrido(3′,2′:5,6)carbazole (II), 5,6-benzopyrido(2′,3′:1,2)carbazole (III), and 5,6-benzopyrido(3′,2′:1,2)carbazole (IV). The first is an aza derivative of 3,4:5,6-dibenzocarbazole, and the other three are aza derivatives of 1,2:5,6-dibenzocarbazole.



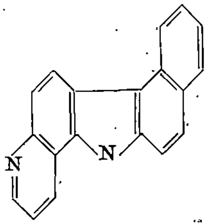
(I)



(II)



(III)



(IV)

Using male and female mice aged 4–6 months and belonging to strain XVII *nc/Z* (Radium Institute), the substances were injected subcutaneously in the flank (3 injections of 0·6 mgm. of the substance in 0·2 c.c. olive oil, with an interval of one month between injections). The results are recorded in Table 1; no hepatic lesions were observed in any of the mice.

Table 1

Substance	No. of animals	No. of sarcomas	Average latency (days)	Sarcoma index (after Iball)
I	14 7♂	1	106	13
	7♀	0	—	0
II	14 7♂	2	273	10
	7♀	1	200	7
III	16 8♂	8	157	64
	8♀	1	178	7
IV	15 7♂	7	100	100
	8♀	6	118	73

From these results, it can be seen that the group of aza derivatives includes carcinogens with various degrees of activity, some of them being extremely potent. Compound (IV), for example, ranks among the most powerful sarcomagenic substances known, with both a high proportion of tumours and a strikingly short induction period. A shift in the position of the pyridinic nitrogen atom can diminish this activity either slightly (compound III), or considerably (compound II). Hence, it is clear that, as in the benzacridine series, biological activity is to a large degree dependent on the position occupied by the pyridinic nitrogen atom; and this opens the field for a broad investigation into relationships between structure and activity in this new chemical series, an investigation which is now being pursued here.

A. Lacassagne
N. P. Buu-Hoï
F. Zajdela
F. Périn
P. Jacquignon

Institut du Radium de l'Université de Paris,
26 rue d'Ulm, Paris (Ve).

---

[1] Shear, M. J., and Leiter, J. J., *J. Nat. Cancer Inst.*, **2**, 99 (1941).

[2] Cf. Lacassagne, A., Buu-Hoï, N. P., Daudel, R., and Zajdela, F., *Adv. Cancer Res.*, **4**, 315 (1956).

[3] Hackmann, C., *Z. Krebsforsch.*, **58**, 56 (1951).

[4] Boyland, E., and Brues, A. M., *Proc. Roy. Soc.*, B, **122**, 429 (1937). Boyland, E., and Mawson, E. H., *Biochem. J.*, **32**, 1640 (1938). Lacassagne, A., Buu-Hoï, N. P., Zajdela, F., and Xuong, N. D., *Bull. Cancer*, **42**, 3 (1955).

[5] Buu-Hoï, N. P., Périn, F., and Jacquignon, P., *J. Chem. Soc.*, 4500 (1960).

Amino-Acid Activation in Tissues of Early Embryos

The work of Hoagland, Zamecnik and many others[1–4] has now clearly established that activation of the carboxyl groups of amino-acids occurs as one of the initial steps before they are incorporated into proteins during protein synthesis. The activation reaction was demonstrated by these workers, in protein-synthesizing components of a variety of mammalian tissues. Recently amino-acid activation has also been shown to occur in sea-urchin eggs; but I have seen no reports to date of any investigations on tissues of developing embryos.

The work to be reported briefly here shows that amino-acid activation can be detected in embryos of the clawed toad, *Xenopus laevis*, and it also deals with the occurrence of leucine-activation in tissues of both *Xenopus* and chick embryos. Fuller details of the work on *Xenopus* are in process of publication[6]. Amino-acid activation has been measured as an increased rate of exchange of phosphorus-32 between radioactive pyrophosphate (PP) and adenosine triphosphate (ATP) in the presence of added amino-acid, as compared with the exchange-rate without added amino-acid. The principles and details of the method have already been described[4–6]. Supernatants from tissues homogenized in ice-cold 0·05 *M* potassium chloride at *p*H 7·5 and centrifuged in the cold at 20,000*g* have been used as sources of enzyme.

Table 1 gives the results of testing supernatants from hatching-stage *Xenopus* embryos with a number of different amino-acids. It is apparent that all these show activation, that is, the PP–ATP exchange-rates are higher than when no amino-acid is added (controls). The most marked activation is shown for tryptophan, tyrosine and leucine. In later work[6] on the distribution of leucine-activation in dorsal and

Table 1. Enhancement of PP–ATP Exchange by Various Amino-acids, in Extracts of Hatching-stage *Xenopus* Embryos

Amino-acid added (2·0 μmoles)	% PP–ATP exchange*
None (controls)	0·751
Serine	3·265
Threonine	3·478
Glutamine	3·736
*Iso*leucine	4·276
Tryptophan	5·669
Tyrosine	4·341
Leucine	6·171

$$* \ \%\text{Exchange} = \frac{\text{specific activity of ATP (counts/min. } \mu\text{mole)}}{\tfrac{1}{2}\text{ specific activity of PP (counts/min. } \mu\text{mole)}} \times 100$$

Table 2. LEUCINE-ACTIVATION IN DORSAL TISSUES OF *Xenopus* EMBRYOS, AT LATE NEURULA TO EARLY TAIL-BUD STAGES
Activation expressed as percentage increases in PP–ATP exchange-rate in the presence of 2·0 μmoles of L-leucine, per 10 μgm. total nitrogen in supernatant sample. Means and standard errors of five experiment

	% increase in PP–ATP exchange
Somite tissue	34·3 ± 9·2
Nervous tissue	25·7 ± 7·8

ventral regions of embryos, it was found that dorsal halves of late neurula and tail-bud stages had a particularly high leucine-activating activity, and that when these dorsal halves were divided into somite and central nervous tissue portions, the highest leucine-activation was shown in the somite tissue (Table 2: the activation figures are here expressed as percentages and related to total nitrogen in the enzyme samples). The difference between somite and nervous tissue was highly significant ($t = 4{\cdot}9954$, $P < 0{\cdot}01$ for the paired differences between values for somite and nervous tissue in five experiments).

The same method has been used for measuring leucine-activation in somite and central nervous tissues of 48-hour chick embryos (stage 11–13 of Hamburger and Hamilton's series[7]). The only methodological difference was that the volumes of all reagents in the incubation mixture were reduced to a quarter of those used for the *Xenopus* extracts, since the total bulk of chick tissues available was very much smaller (the total nitrogen in chick supernatants was only one-tenth of that in *Xenopus* supernatants). The results with chick tissues were far more striking than those with *Xenopus*, and increases of PP–ATP exchange of more than 100 per cent were obtained (Table 3). Again, the leucine-activation in somite tissue was higher than in central nervous tissue ($t = 8{\cdot}2975$, $P < 0{\cdot}01$, for the paired differences between values for somite and nervous tissue in five experiments).

Table 3. LEUCINE ACTIVATION IN TISSUES OF 48-HR. CHICK EMBRYOS
Percentage increases in PP–ATP exchange-rate in the presence of 0·5 μmole of L-leucine per μgm. total nitrogen in supernatant sample. Means and standard errors of five experiments

	% increase in PP–ATP exchange
Somite tissue	181·89 ± 29·83
Central nervous tissue	100·64 ± 29·45

The specially high leucine-activation in somite tissue extracts of both *Xenopus* and chick embryos suggests that more leucine is being taken up into proteins of somite cells at this particular stage of development than elsewhere in the embryo. As we are dealing, in both types of embryo, with stages during which the somites are segmenting, it is of interest to note that, in chick embryos, somite segmentation can be blocked by analogues of leucine[8,9], or, conversely, speeded up by adding normal leucine to the culture medium[10]. It is not at all clear what role leucine could have in controlling the process of somite segmentation. One might, however, speculate that somitic myoblasts are prime users of this amino-acid, at least during the time that they are synthesizing myosin, for this protein contains a high proportion of leucine[11]. Holtzer, Marshall and Finck[12] have detected myosin in chick skeletal myoblasts at stages only slightly later than those used here, that is, about 56 hr. incubation (stage 16, 17). So it is reasonable to suggest that at stages 11–13 leucine could already be being taken up into myosin precursors. Autoradiographical work is needed to elucidate this point, and some has already been started by me.

Returning to the evidence (Table 1) that in late *Xenopus* embryos activation of a number of amino-acids can be demonstrated, this is of general interest because one should certainly expect embryonic tissues which are differentiating to be carrying out very active protein synthesis. It would seem that we have here a promising and wide-open field for further study.

ELIZABETH M. DEUCHAR

Department of Anatomy,
University College,
London.

[1] Hoagland, M. B., *Biochim. Biophys. Acta*, **16**, 288 (1955).

[2] Hoagland, M. B., Keller, E. B., and Zamecnik, P. C., *J. Biol. Chem.*, **218**, 345 (1956).

[3] Zamecnik, P. C., Stevenson, M. L., and Hecht, L. I., *Proc. U.S. Nat. Acad. Sci.*, **44**, 73 (1958).

[4] Novelli, G. D., and DeMoss, J. A., *J. Cell. Comp. Physiol.*, **50** (Supp. 1), 173 (1959).

[5] Scarano, E., and Maggio, R., *Exp. Cell Res.*, **12**, 403 (1957).

[6] Deuchar, E. M., *Exp. Cell Res.* (in the press).

[7] Hamburger, V., and Hamilton, H. L., *J. Morph.*, **88**, 49 (1951).

[8] Herrmann, H., *J. Embryol. Exp. Morph.*, **1**, 291 (1953).

[9] Schultz, P. W., and Herrmann, H., *J. Embryol. Exp. Morph.*, **6**, 262 (1958).

[10] Deuchar, E. M., *J. Embryol. Exp. Morph.*, **8**, 259 (1960).

[11] Block, R. J., and Weiss, K. W., *Amino-Acid Handbook* (C. C. Thomas, Illinois, 1956).

[12] Holtzer, H., Marshall, J. M., and Finck, H., *J. Biophys. Biochem. Cytol.*, **3**, 705 (1957).

Changes in the Pentose–Nucleic Acid Content of Sub-cellular Fractions of the Brain of the Rat during 'Metrazol' Convulsions

CHANGES in the content of pentose–nucleic acid in the nerve cells during increased functional activity have been reported by several workers. A depletion of pentose–nucleoprotein in the stimulated anterior horn cells has been observed by Holger Hyden[1]. Similarly, a decrease in the concentration of pentose–nucleic acid, together with an alteration of molar base ratios, has been reported in the stimulated area of the brain cortex in cats[2]. A consistent decrease in pentose–nucleic acid and protein content of the brain of the rat during 'Metrazol' convulsions has been observed and reported elsewhere[3].

In the present communication the changes in pentose–nucleic acid of various sub-cellular fractions of brain of the rat during convulsions are presented.

Convulsions were induced by injecting 'Metrazol' into albino rats[4], keeping litter-mates as controls. The animals were decapitated in the tonic phase of convulsions and the heads frozen by dropping them in liquid air. The frozen brains from two animals were pooled, quickly weighed and the nuclei separated by the method of Alfrey, Osawa and Mirsky[5]. Microsomal and *p*H 5 pentose-nucleic acid fractions were separated by the methods of Osawa, Takata and Hotta[6] and of Schweet, Lamform and Allen[7] respectively. From the sucrose nuclei, the *p*H 7·1 soluble pentose–nucleic acid fraction and the nucleolar pentose–nucleic acid fraction were separated by the method described by Alfrey and Mirsky[8]. From the fractions thus obtained pentose-nucleic acid was extracted and estimated according to the micro method of Scott, Fraccastoro and Taft[9].

Table 1 summarizes the net change in each of the sub-cellular fractions. It is apparent that the depletion in the content of pentose–nucleic acid during convulsions is primarily confined to the nuclear fractions. Ruth S. Geiger[10] has observed in histological studies that an extrusion of the nucleolar material occurred

Table 1. Pentose-Nucleic Acid (PNA) Content of Various Subcellular Fractions in Normal Rat Brain and After 'Metrazol' Convulsions

PNA fraction		Control	'Metrazol' convulsive	Percentage of change
Nuclear	Soluble	6·4 ± 0·73 (12)	4·8 ± 1·04 (12)	−25
	Nucleolar	27·4 ± 4·4 (12)	20·6 ± 4·5 (12)	−24
Cytoplasmic	Soluble	22·5 ± 3·6 (12)	21·8 ± 5·0 (12)	− 3·1
	Microsomal	52·6 ± 5·2 (10)	49·4 ± 5·3 (12)	− 6
Total	Nuclear	33·8 ± 4·4 (12)	25·5 ± 4·0 (12)	−24·5
	Cytoplasmic (excluding mitochondrial)	75·6 ± 9·6 (10)	71·3 ± 6·3 (12)	− 5·6
Total (cytoplasmic + nuclear)		107·8 ± 15·8 (10)	96·8 ± 13·6 (12)	−10·2

The figures in parenthesis indicate the number of animals used. The brains of two animals were pooled together for obtaining a single observation. Values are expressed in μgm./100 mgm. wet weight of tissue. Against each value, the mean standard error is given.

in neurons in tissue culture when subjected to electrical stimulation or 'Metrazol' action.

This fall in nuclear pentose-nucleic acid may be due to an enhanced activity of ribonuclease in the nucleus as a result of convulsive activity or it might indicate an increased passage of pentose-nucleic acid from the nucleus to the extra nuclear compartments in convulsive or electrically stimulated states. Relationship of these changes with the functional activity of the neurons is not yet defined.

G. P. Talwar
B. Sadasivudu
V. S. Chitre

All-India Institute of Medical Sciences,
New Delhi 16.

[1] Hyden, Holger, *Acta Physiol. Scand.*, **6**, Supp., 17 (1943).

[2] Geiger, A., Yamasaki, S., and Lyons, R., *Amer. J., Physiol.*, **184**, 239 (1956).

[3] Sadasivudu, B., and Talwar, G. P., *Symp. on Adv. Frontiers of Life Sci.* (National Inst. Sci. India) (in the press).

[4] Goodman, L. S., Grewal, M. S., Brown, W. B., and Surinyard, E. A., *J. Pharmacol.*, **108**, 168 (1953).

[5] Alfrey, V. G., Osawa, S., and Mirsky, A. E., *J. Gen. Physiol.*, **40**, 451 (1957).

[6] Osawa, S., Takata, K., and Hotta, Y., *Biochim. Biophys. Acta*, **28**, 271 (1958).

[7] Schweet, R., Lamform, H., and Allen, E., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1029 (1958).

[8] Alfrey, V. G., and Mirsky, A. E., *Proc. N.Y. Acad. Sci.*, **43**, 821 (1957).

[9] Scott, J. F., Fraccastoro, A. P., and Taft, E. B., *J. Histochem. Cytochem.*, **4**, 1, (1956).

[10] Geiger, R. S., *Fed. Proc.*, **15**, 71 (1956).

Rapid Infra-Red Determination of Acetone in the Blood and the Exhaled Air of Diabetic Patients

There has long been a need for a rapid method for the quantitative determination of acetone in the blood and in the exhaled breath of the patient with diabetes mellitus. Since these concentrations are a direct reflexion of the diabetic state, precise acetone measurements would provide the physician with more accurate data with which to evaluate his diabetic patient. To be clinically practical, this analytical method should be specific, sensitive and rapid. An extension of an infra-red method previously reported[1] provides the basis for this preliminary report on the analysis of actone in blood and in the expired air.

To determine the acetone in blood, a 10-ml. oxalated sample is pipetted into a 40-ml. glass centrifuge tube fitted with a ground-glass stopper. 15 ml. of carbon disulphide is added, and the tube gently shaken for 10 min. on an automatic shaker. The tube is then centrifuged in a refrigerated centrifuge for 5 min. at 1,500*g*. The solvent layer is transferred to a 15-mm. infra-red sample cell and the absorbance at 8·25μ measured. To compensate for solvent absorption a matching cell filled with carbon disulphide is placed in the reference beam of the spectrometer. The minimal amount of acetone detectable in blood by this solvent extraction technique is 1·5 mgm. per cent (15 p.p.m.). The standard deviation in the analysis of a sample of blood containing 13·7 mgm. per cent of acetone is 0·76 mgm. per cent, while that of a 61·4 mgm. per cent acetone in a blood standard is 2·2 mgm. per cent.

Expired air for acetone analysis is collected by having the diabetic patient slowly exhale into a 10-l. 'Saran' bag. This expired air is allowed to flow into an evacuated gas cell of path 40-m. long mounted on a Perkin–Elmer model 221 infra-red spectrophotometer. The absorbance at 8·25μ is measured to determine the concentration of acetone. The minimal amount of the compound detectable is 10 μgm./l. of expired air. The standard deviation in an air sample containing 600 μgm./l. is 31 μgm./l.

The infra-red analysis of blood acetone has proved to be clinically practical. It provides a precise measurement of acetone in the case of diabetic acidosis, obviating the use of the 'Acetest' method for estimating the degree of ketonæmia by serial serum dilutions. Because of the sensitivity of the method, blood acetone may be determined when present in concentrations below the sensitivity of the 'Acetest' tablet.

The determination of acetone concentration in the expired air by this method is most promising. It has been possible to detect acetone in the breath of patients with blood concentrations less than 1·5 mgm. per cent, and in the acidotic diabetic it has proved to be the most rapid means of establishing the exact degree of acidosis. As the concentration of air in the alveoli parallels the concentration in the blood, the effect of treatment of the diabetic in acidosis is easily ascertained.

Richard D. Stewart

Department of Internal Medicine,
University of Michigan Medical Center.

Edward A. Boettner
Bruce T. Stubbs

Institute of Industrial Health,
School of Public Health,
University of Michigan,
Ann Arbor, Michigan.

[1] Stewart, R. D., Erley, D. S., Torkelson, T. R., and Hake, C. L., *Nature*, **184**, 192 (1959).

Influence of Scurvy and Lathyrism (Odoratism) on Hydroxyproline Excretion

Since nearly all the hydroxyproline in the body is found in collagen, it has been suggested[1–3] that abnormal amounts of hydroxyproline may be excreted in conditions which alter the metabolism of collagen. The availability of a specific and relatively simple method for analysing hydroxyproline in urine[4] encouraged us to measure hydroxyproline excretion in scurvy and lathyrism (odoratism), two experimental conditions known to affect collagen.

To study the effect of scurvy, male guinea pigs (Hartley) were fed a scorbutogenic diet[5], and the urinary excretion of hydroxyproline was measured[4] daily for 24 days. Five test animals received the scorbutogenic diet alone, and 5 control animals received the diet and an oral supplement of 1 mgm. of ascorbic acid per day. The hydroxyproline excretion was essentially the same in both groups for the first 19 days on the diet, but from the 20th to the 24th day the animals not receiving ascorbic acid excreted significantly less hydroxyproline (0·45 ± 0·05 mgm. versus 1·12 ± 0·10 mgm. per day). On the 21st day the ascorbic acid-deficient animals showed histological evidence of scurvy[6]. In other experiments guinea pigs were placed on the scorbutogenic diet and divided into three groups : group 1 received 1 mgm. ascorbic acid per day throughout the course of the experiment, group 2 received no ascorbic acid, and group 3 received 10 mgm. of ascorbic acid on the 21st day of the experiment. Urine was collected from the 21st to the 22nd day. As shown in Table 1, urinary hydroxyproline excretions were depressed in animals on the scorbutogenic diet but were rapidly restored to normal by administration of ascorbic acid. On the 22nd day the animals in group 1 weighed 20 per cent less than those in group 2. To demonstrate that the decrease in hydroxyproline excretion was not the result of loss of weight, 15 guinea pigs were starved for 2 days. Their body-weights fell from a mean of 195 gm. to a mean of 168 gm., but their urinary hydroxyproline-levels were unchanged.

Table 1. DECREASE OF HYDROXYPROLINE EXCRETION IN SCURVY AND INCREASE OF HYDROXYPROLINE EXCRETION IN LATHYRISM

Animals	Number	Diet*	Supplement	Hydroxyproline per 24 hr. mgm. ± *S.E.*
Guinea pigs (Group 1)	10	scorbutogenic	1 mgm. ascorbic acid/day	1·13† ± 0·15
(Group 2)	31	,,	none	0·57† ± 0·08
(Group 3)	16	,,	10 mgm. ascorbic acid on day 21	1·61† ± 0·15
Weanling rats	6	low protein	none	0·63‡ ± 0·10
Weanling rats	6	low protein	160 mgm. BAPN§	2·06‡ ± 0·20
Adult rats	6	low protein	none	0·48‡ ± 0·06
Adult rats	6	low protein	160 mgm. BAPN	1·31‡ ± 0·07

* Both diets hydroxyproline-free.

† Hydroxyproline excretions for the twenty-first day of the experiment.

‡ Hydroxyproline excretions for the fifty-fifth day of the experiment.

§ BAPN, β-aminopropionitrile, supplied by Abbott Laboratories.

To study the effect of a lathyrogenic agent on hydroxyproline excretion, weanling rats and adult rats were placed on a low protein diet[7], and half the animals from each group were injected with 160 mgm. β-aminopropionitrile (BAPN) per day. No difference in urinary hydroxyproline excretion between control and injected animals was observed on the 4th and 20th days of treatment, and there was no gross evidence of lathyrism. By the 55th day, the BAPN-treated animals excreted two to three times as much hydroxyproline as controls (Table 1) and all the animals had marked scoliosis. More than 90 per cent of the hydroxyproline in the urine from both control and experimental animals was dialysable.

Several other conditions which might change hydroxyproline excretion were also investigated. Young guinea pigs (11–20 days) were found to excrete significantly more hydroxyproline than adult guinea pigs (2 years old). The young animals, weighing 100–180 gm., excreted an average of 1·84 ± 0·15 mgm. hydroxyproline per 24 hr. as compared with an average of 1·22 ± 0·20 mgm. per 24 hr. excreted by 2 kgm. adult animals. Comparable age differences have been reported previously for humans[1] and rats[8]. Preliminary experiments have confirmed the earlier report[9] that urinary hydroxyproline is not altered by parturition. In addition, our results indicate that urinary hydroxyproline-levels are not altered by parathyroidectomy or the injection of papain or carrageenan.

The results obtained here indicate that the extensive alterations induced in collagen by scurvy and experimental lathyrism, as well as by ageing, are accompanied by significant changes in the amount of hydroxyproline excreted in urine. Although most of body collagen is relatively inert[10], some fractions of collagen are both synthesized and degraded rapidly[8]. While the latter may or may not be identical with the various soluble collagens[11], several observations suggest that the amount of hydroxyproline excreted in urine reflects the amount of body collagen in these metabolically more 'active' forms. Young animals which have more soluble collagen[12] and a more rapid turnover of collagen[10] than adult animals excrete more hydroxyproline. Both the amount of soluble collagen[13] and the synthesis of collagen are decreased in scurvy[14], and the scorbutic guinea pigs excreted less hydroxyproline (Table 1). Lathyrogenic agents increase the amount of soluble collagen[15], and the increased hydroxyproline excretion in the β-aminopropionitrile-treated rats probably indicates an increased amount of metabolically active collagen in lathyrism. It is of interest that experimental lathyrism resembles Marfan's syndrome, and that some patients with Marfan's syndrome excrete increased amounts of hydroxyproline[2]. Measurements of urinary hydroxyproline may be a useful index of collagen metabolism in a number of other experimental and clinical conditions.

We are indebted to Dr. I. Zipkin for his assistance in a phase of this work.

G. R. MARTIN*
S. E. MERGENHAGEN

Laboratories of Biochemistry and
Microbiology,
National Institute of Dental Research.

D. J. PROCKOP

Experimental Therapeutics Branch,
National Heart Institute,
National Institutes of Health,
Bethesda, Maryland.

* Research Associate, American Dental Association.

[1] Ziff, M., Kibrick, A., Dresner, F., and Gribetz, H. J., *J. Clin. Invest.*, **35**, 579 (1956).

[2] Sjoerdsma, A., Davidson, J. D., and Udenfriend, S., *Lancet*, ii, 994 (1958).

[3] Prockop, D. J., and Sjoerdsma, A., *J. Clin. Invest.* (in the press).

[4] Prockop, D. J., and Udenfriend, S., *Anal. Biochem.*, **1**, 228 (1960).

[5] Woodruff, C. W., Cherrington, M. E., Stockwell, A. E., and Darby, W. J., *J. Biol. Chem.*, **178**, 866 (1949).

[6] Martin, G. R., Fullmer, H. M., and Burns, J. J., *Proc. Soc. Exp. Biol. Med.*, **106**, 157 (1961).

[7] McClure, F. J., *Pub. Health Rep.*, **65**, 1175 (1950).

[8] Lindstedt, S., and Prockop, D. J., *J. Biol. Chem.* (in the press).

[9] Woessner, J. F., *Fed. Proc.*, **18**, 461 (1959).

[10] Neuberger, A., and Slack, H. G. B., *Biochem. J.*, **53**, 47 (1953).

[11] Jackson, D. S., and Bentley, J. B., *J. Biophys. Biochem. Cytol.*, **7**, 37 (1960).

[12] Kao, K. T., and McGavack, T. H., *Proc. Soc. Exp. Biol. Med.*, **101**, 153 (1959).

[13] Gross, J., *J. Exp. Med.*, **109**, 557 (1959).

[14] Mitoma, C., and Smith, T. E., *J. Biol. Chem.*, **235**, 426 (1960).

[15] Levene, C. I., and Gross, J., *J. Exp. Med.*, **110**, 771 (1959).

PHYSIOLOGY

Analogues of Glutamic and γ-Amino-*n*-butyric acids having Potent Actions on Mammalian Neurones

NUMEROUS investigations have shown that certain amino-acids, including several which are present in the mammalian central nervous system, strongly influence the behaviour of neurones when applied extracellularly[1-4]. These amino-acids fall into two groups; neutral amino-acids related to γ-amino-*n*-butyric acid (GABA), which depress the activity of neurones, and acidic amino-acids related to glutamic acid, which excite neurones[3]. An analysis of the effects of these substances on mammalian spinal neurones indicates that whereas the mechanism of action of GABA differs from that of mammalian inhibitory transmitters[5], the action of L-glutamic acid has certain features in common with that of excitatory transmitter substances[3,6]. Recently, several substances were found to have a much greater potency than GABA and glutamic acid as depressants and excitants respectively of neurones within the isolated and sagitally hemisected spinal cord of the toad[7]. In particular, *N*-methylaspartic acid and homocysteic acid were found to be potent excitants, and 3-amino-1-propanesulphonic acid was a powerful depressant. The potencies of these and related amino-acids on mammalian spinal and cortical neurones have now been determined.

Two methods were used. In the experiments on spinal cord, the amino-acids were passed electrophoretically from solutions of appropriate *p*H into the immediate extracellular environment of interneurones located in the lumbar segments of cats anæsthetized with pentobarbital sodium[3]. Multibarrel electrodes were used so that a comparison could be made between the actions of different substances. In these experiments excitation was measured by the frequency of spike discharge evoked by a particular amino-acid and depression by the degree to which synaptically evoked spikes and negative field potentials were reduced in number and magnitude respectively[3]. Relative potencies were assessed on the basis of the electrophoretic currents required to produce a given degree of excitation or depression. The acidic amino-acids were readily passed as anions from electrode solutions adjusted to *p*H 8 with sodium hydroxide. All these compounds were in a similar ionic state at this *p*H and the concentrations of the substances obtained in the extracellular fluid can be assumed to be directly proportional to the electrophoretic currents used[8]. The relative potencies are given in Table 1, *A*.

Difficulty was experienced in passing the neutral amino-acids as anions, presumably because of electro-osmotic influences tending to pass the substances in the opposite direction when the electrodes contain solutions of high *p*H (ref. 3). These amino-acids were therefore passed as cations from solutions made to *p*H 2·8 with hydrochloric acid. Under these conditions less of the sulphonic than of the carboxylic amino-acids are in the cationic form, and the extracellular concentrations attained by the former, using a particular electrophoretic current, were probably considerably lower than those of the carboxylic acids. It is therefore probable that the true potencies of 3-amino-1-propanesulphonic acid and of taurine, relative to GABA and β-alanine, exceed the figures given in Table 1, *C*.

Table 1. RELATIVE POTENCIES OF AMINO-ACIDS

Excitants	*A*	*B*	Depressants	*C*	*D*
L-Aspartic acid	3	$> 10^{-2}$ *M*	β-Alanine	3	$7 \cdot 5 \times 10^{-3}$ *M*
D-Aspartic acid	2	$> 10^{-2}$ *M*	Taurine	2–3	$7 \cdot 5 \times 10^{-3}$ *M*
L-Glutamic acid	3	$> 10^{-2}$ *M*			
			γ-Amino-*n*-butyric acid	3	$2 \cdot 5 \times 10^{-3}$ *M*
D-Glutamic acid	2	$> 10^{-2}$ *M*			
			3-Amino-1-propane-sulphonic acid	6–15	2×10^{-5} *M*
N-Methyl-L-aspartic acid	2	$7 \cdot 5 \times 10^{-3}$ *M*			
N-Methyl-D-aspartic acid	20–30	$7 \cdot 5 \times 10^{-5}$ *M*			
L-Cysteic acid	3	$> 10^{-2}$ *M*			
D-Cysteic acid	1–3	$> 10^{-2}$ *M*			
L-Homocysteic acid	4–5	not tested			
D-Homocysteic acid	9–18	$2 \cdot 5 \times 10^{-4}$ *M*			
DL-Homocysteic acid	6	5×10^{-4} *M*			

Columns *A* and *C*, approximate relative potencies based on the responses of spinal interneurones to electrophoretically applied amino-acids. Column *B*, minimum concentrations of the amino-acids in Ringer solution required to produce spreading depression within exposed parietal cortex; topical application. Column *D*, minimum concentrations of the amino-acids in Ringer solution required to abolish negative phases of positive–negative potential evoked in parietal cortex by transcallosal stimulation; topical application.

In the cortical experiments, the amino-acids were applied as serially diluted solutions in buffered mammalian Ringer solution (*p*H 7·0–7·2) to the exposed parietal cortex of cats anæsthetized with pentobarbital sodium, and electrical records were obtained from the same cortical surface. The equivalent concentrations of depressants listed in Table 1, *D* are the average of those which just abolished the negative phase of the positive–negative potential sequence evoked by transcallosal stimulation[9]. The equivalent concentrations of excitants (Table 1, *B*) are the average lowest concentrations which produced spreading depression within two minutes of application, this phenomenon being indicated by the abolition of transcallosally evoked responses and the recording of a surface negative potential of 6–12 mV.[2,10]. A pore type of electrode permitted the recording of cortical potentials while the solutions were being applied[10].

Although many factors may limit the quantitative reliability of each of these methods, there is nevertheless a clear qualitative correlation between the two sets of results. It is apparent that 3-amino-1-propanesulphonic acid is considerably stronger than GABA, β-alanine or taurine, which were previously considered to be among the most potent of the amino-acids having depressant actions on mammalian spinal neurones[3]. 3-Amino-1-propanesulphonic acid bears the same structural relationship to homocysteic acid as GABA bears to glutamic acid, and the strong excitatory action of homocysteic acid was therefore to be expected[3]. The D-form of this compound is stronger than the L-form. A similar, though very much larger, difference is evident between the two enantiomers of *N*-methylaspartic acid, the D-isomer in this case being the most potent amino-acid excitant yet tested. The potency of *N*-methyl-D-aspartic acid is more remarkable when it is considered that alkylation of members of both the excitant and depressant series of amino-acids has usually led to lower activity[3,7]. The significance of these results in relation to the general mechanism of amino-acid excitation and depression, including the possible participation of membrane phospholipids in such processes, will be discussed elsewhere.

D. R. CURTIS
J. C. WATKINS

Department of Physiology,
Australian National University,
Canberra.

[1] Purpura, D. P., Girado, M., Smith, T. G., Callan, D. A., and Grundfest, H., *J. Neurochem.*, **3**, 238 (1959).

[2] Van Harreveld, A., *J. Neurochem.*, **3**, 300 (1959).

[3] Curtis, D. R., and Watkins, J. C., *J. Neurochem.*, **6**, 117 (1960).

[4] Honour, A. J., and McLennan, H., *J. Physiol.*, **150**, 306 (1960).

[5] Curtis, D. R., Phillis, J. W., and Watkins, J. C., *J. Physiol.*, **146**, 185 (1959).

[6] Curtis, D. R., Phillis, J. W., and Watkins, J. C., *J. Physiol.*, **150**, 656 (1960).

[7] Curtis, D. R., Phillis, J. W., and Watkins, J. C., *Brit. J. Pharm.*, **16**, 262 (1961).

[8] Curtis, D. R., Perrin, D. D., and Watkins, J. C., *J. Neurochem.*, **6**, 1 (1958).

[9] Purpura, D. P., Girado, M., and Grundfest, H., *Electroenceph. clin. Neurophysiol.*, **12**, 95 (1960).

[10] Marshall, W. H., *Physiol. Rev.*, **39**, 239 (1959).

Inhibition by Potassium of the Mechanical Responses of Frog Muscles at Low Temperature

Csapo and Wilkie[1] reported a curious effect of temperature on the twitch tension in the frog's sartorius muscles, as influenced by the action of 24 m*M* potassium chloride. The onset of the fall of tension development and the depolarization were delayed by lowering the temperature from 23° to 2° C., corresponding to a Q_{10} of 1·2–1·3. On the other hand, the rate of recovery, when normal Ringer solution was replaced, was very much reduced at lower temperature. However, if the recovering muscle were suddenly warmed again, the twitch tension recovered immediately. Grieve[2] has shown that, when recovery was delayed, it was due to delayed re-polarization. If chloride were replaced by the impermeant methyl sulphate, then there was no delay in re-polarization that could not be accounted for by diffusion in the interspaces.

I have studied the inhibition by potassium of isotonic twitching and contractions provoked in the sartorius and rectus muscles. The methods have been described[3]. The muscle was placed into a double-wall muscle chamber, cooled in its outer compartment by a fluid at −2° C.; the solution inside the chamber decreased in temperature from 20° to 0° C. within 2 min.

Both the sartorius and the rectus muscle were unable to give twitch or phasic contracture-like responses comparable in height to the control muscles, after application of potassium chloride or potassium sulphate lasting 1 min. or more, unless the time of recovery was 5–10 min. After 40 sec. of recovery, only 23 per cent of the control height of the contractions was obtained in the sartorius, when 115 m*M* potassium chloride or 95 m*M* potassium sulphate were applied, 4 per cent of the twitch response when 20 m*M* potassium chloride was applied and 15 per cent of the rectus first phase response[3] when 6–10 m*M* potassium chloride was applied. After the same solutions were allowed to act for the same intervals at low temperature, and the subsequent time of recovery of 40 sec. occurred at room temperature, the corresponding responses were several times larger, the percentage values being 63, 69 and 70. Responses of the same magnitude as the percentages mentioned were obtained if the action of potassium chloride or potassium sulphate at the lower temperature was prolonged in the sartorius about three times (contractures) and in the rectus for more than five times (Fig. 1).

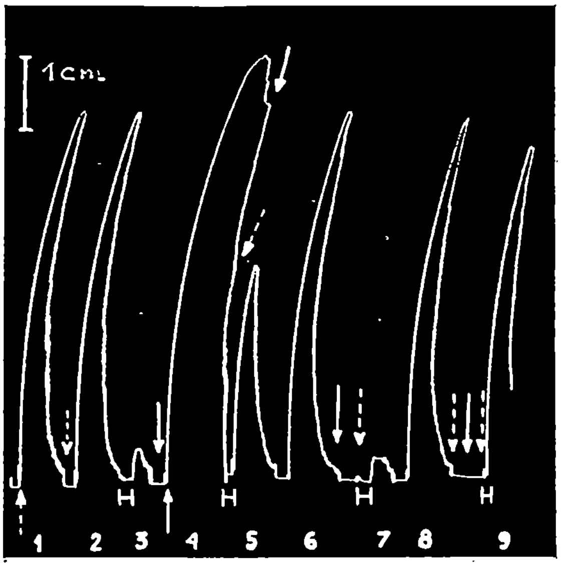


Fig. 1. Frog's rectus. Phasic response to 10 m*M* potassium chloride; 0·5 m*M* calcium in Ringer solution. Arrows upward, application of potassium; arrows downward, washing; full arrows, cold solutions (0° C.); interrupted arrows, solutions at room temperature (20–23° C.). *H*, Short interval (40 sec.); 1, 2, 3, control reactions; 4, 5, reaction at low temperature and, a short time after it, at room temperature; 6, after a reaction at room temperature, washing (15 min.) at low temperature, 40 sec. before 7, at room temperature; 8, after the reaction, first a 10-min. wash at room temperature, then at low temperature, 40 sec. before 9, again at room temperature

Low temperature, if applied a few seconds after the exposure to potassium had been terminated, prolonged the inhibition by potassium: no significant recovery could be obtained at 0° C. for 20 min. of washing. The same results were obtained with potassium chloride as well as with potassium sulphate. No rapid recovery within 40 sec. of warm washing, after prolonged washing in a cold Ringer solution, was observed.

The inhibiting action of potassium on mechanical responses at low temperature in the present experiments seems to follow a course which is slowed down relatively more than that of the decrease in twitch tension. The absence of a rapid recovery during the short period, after warming up, and the lack of differences in the rate of recovery after application of potassium chloride and potassium sulphate, prove that the two processes may indeed be different.

In a further group of experiments the response of the sartorius muscle to potassium was examined, after prolonged washing (20 min.) in a cold calcium-free solution. The effect was the same as that at room temperature, that is, the contraction was inhibited[4]. If the muscle were washed first in a calcium-free Ringer solution at room temperature, then cooled and washed in a cool, Ringer solution containing calcium, it regained the ability to respond to potassium by a contraction.

This work was supported by the Commission for Medical Research Belgrade.

H. Lorković

Institute for Medical Research,
Academy of Sciences and Arts,
Zagreb,
Yugoslavia.

[1] Csapo, A., and Wilkie, D. R., *J. Physiol.*, **134**, 497 (1956).

[2] Grieve, D. W., *J. Physiol.*, **152**, 25, *P* (1960).

[3] Lorković, H., *Amer. J. Physiol.*, **196**, 666 (1959).

[4] Lorković, H., *Pflügers Arch.*, **272**, 45 (1960).

Fatigue in a Fluid-filled Pulsating Elastic Tube

Experiments with the 'oscillator' described by me[1] have shown that fatigue affects the elastic tube, after it has been pulsating for several hours.

Aneurysmal-like bulges may occur. Alternatively, a small hole forms at the end close to the rigid outlet tube, presumably due to cavitation occurring in contact with the wall. Fluid may escape from this hole, or air be sucked in, at each pulse.

A remarkable effect is obtained by the application of cedar wood oil. Within a few minutes, the distal segment of the elastic tube, when so treated, becomes permanently stretched into a sigmoid shape and develops periodic constrictions and dilatations, affecting the system of short wave-length, that is in the range 1–2 cm.

These figures resemble the shapes of arteries in atherosclerosis.

John Erskine Malcolm

Postgraduate Medical School of London,
Ducane Road,
London, W.12.

[1] *Nature*, **190**, 88 (1961).

PHARMACOLOGY

Alleged Anticomplementary Effect of Aspirin

Van Oss *et al.*[1] recently reported that aspirin at concentrations of $2 \cdot 2 \times 10^{-3}$ *M* (40 mgm./100 ml.) inhibited the lysis of red cells in a simple complement-hæmolysis reaction, but that sodium salicylate was inactive. This was interesting in view of the therapeutic action of aspirin against diseases such as the rheumatic disorders which are thought to involve immunological phenomena[2]. Two other related compounds, salicylaldoxime and salicylaldehyde, were earlier reported[3] to inhibit red cell lysis in a similar reaction. I therefore investigated the complement-hæmolysis technique since it might usefully predict anti-rheumatic activity in other salicylate derivatives.

Sheep erythrocytes (*E*), anti-sheep erythrocyte serum prepared in the rabbit (*A*) and complement (*C′*) were standard serological reagents obtained from the Commonwealth Serum Laboratories, Melbourne. Complement was received as ampoules of freeze-dried guinea pig serum which was reconstituted with distilled water and stored at 4° C. It gave results identical with fresh guinea pig serum prepared in these laboratories. Barbital buffer, *p*H 7·4 and containing added Mg^{++} and Ca^{++}, was prepared according to Mayer *et al.*[4] and diluted 1 in 5 with distilled water on the day of use. All reagents were diluted in barbital buffer. When 0·1 ml. of a 2 per cent suspension of washed erythrocytes was sensitized with 0·1 ml. of antiserum 1/1,000, the addition of 0·5 ml. of guinea pig serum 1/200 caused complete hæmolysis of the erythrocytes within 30 min. at 37° C. The effect of added substances on the progress of the *C′* hæmolysis was determined by placing 0·25 ml. of guinea pig serum 1/100 into hæmolysis tubes and adding 0·25 ml. of a solution of the compound in barbital buffer. The latter solution was replaced by buffer alone in control tubes. The diluted antiserum and red blood cells were then added and the tubes incubated at 37° C. for 30 min. The *p*H of the solutions prior to incubation was determined with a glass electrode in duplicate tubes, which were prepared for each concentration of the compound under test.

The substances I have investigated and the concentrations that caused an approximate 50 per cent inhibition of hæmolysis in our procedure are shown in Table 1. All the acids decreased the *p*H of the final mixture proportional to their concentration, and the *p*H range at the concentrations where hæmolysis was inhibited are shown in column 2. Where applicable I have included the inhibitory concentrations reported by other workers for comparison.

Table 1. SUBSTANCES WHICH INHIBITED COMPLEMENT-HÆMOLYSIS AND THEIR EFFECT ON THE *p*H OF THE REACTION MIXTURE

Compound	Concentrations (molar) giving 50 per cent inhibition of hæmolysis in 30 min.	*p*H ranges of the reaction mixtures at the inhibitory concentrations	Concentrations (molar) elsewhere reported to inhibit complement-hæmolysis	Ref. No.
Aspirin	$2\text{–}3 \times 10^{-3}$	5·8–6·4	$2 \cdot 2 \times 10^{-3}$	1
Salicylic acid	$2\text{–}3 \times 10^{-3}$	5·8–6·4	10 per cent inhibition $3 \cdot 3 \times 10^{-3}$	
Sodium salicylate	No inhibition	7·4	No inhibition	1
Fumaric acid	$2\text{–}3 \times 10^{-3}$	5·8–6·2	—	—
Hydrochloric acid	$2\text{–}3 \times 10^{-3}$	5·8–6·4	—	—
Salicylaldoxime	8×10^{-4}	7·5	5×10^{-4}	3
Salicylaldehyde	$8 \times 10^{-4}\text{–}10^{-3}$	7·3	$2 \cdot 5 \times 10^{-4}$	3

Several important points emerge from Table 1. The capacity of the diluted Mayer solution was clearly inadequate to buffer the effects of the acidic compounds on the *p*H of the hæmolytic reaction. Furthermore, it seemed strange that while salicylic acid inhibited hæmolysis, sodium salicylate at similar concentration was inactive—yet at *p*H values above 5, both compounds would be in solution in identical form as salicylate ions. Finally all the acids tested inhibited my hæmolytic reaction at approximately the same molar concentration ($2\text{–}3 \times 10^{-3}$), hydrochloric acid being just as effective as the organic acids. At this concentration they reduced the *p*H of the reaction from 7·4 to about 6. It appeared likely therefore that inhibition of hæmolysis was due in some manner to the *p*H shift rather than a specific anticomplementary action. This explanation was further justified when the above experiments were repeated using undiluted Mayer buffer, the tonicity of which was adjusted by omitting some of the sodium chloride. Under these conditions hæmolysis was complete within 30 min. in the absence of a test compound and was inhibited by salicylaldehyde and salicylaldoxime at the same concentrations as in the dilute buffer. However, none of the other substances in Table 1 lowered the *p*H of the final mixture below 7·0 or caused any inhibition of complement-induced hæmolysis at concentrations below 5×10^{-3} *M*. Subsequent investigation with buffers of even greater capacity for hydrogen ions failed to show an anti-complementary effect of the acids in the absence of *p*H shifts at concentrations up to 10^{-2} *M*.

In conclusion, my results suggest that aspirin has no real effect *per se* on complement-induced hæmolysis of red blood cells. Since it is a weak acid, it may, if it is insufficiently buffered, lower the *p*H of a complement-hæmolysis reaction to the point where there is an unspecific inhibition of hæmolysis. The ability to do this is probably common to all acids.

It is of some interest that the anticomplementary effect of aspirin has been reported to occur also *in vivo*[1]. However, since no details were given, no comment is possible.

K. W. ANDERSON

Nicholas Institute for Medical and
Veterinary Research,
Sherbrooke, Victoria,
Australia.

[1] van Oss, C. J., Friedmann, J. C., and Fontaine, M., *Nature*, 189, 146 (1961).

[2] Anon., *Brit. Med. J.*, i, 565 (Feb. 25, 1961).

[3] Mills, S. E., and Levine, L., *Immunology*, 2, 368 (1959).

[4] Mayer, M. M., Croft, C. C., and Gray, Margaret M., *J. Exp. Med.*, 88, 427 (1948).

HISTOLOGY

Neurosecretion and Granules in Neurones of the Brain of the Leech

EXAMINATION of established neurosecretory structures with the electron microscope reveals characteristic cytoplasmic structures: the elementary neurosecretory granules. These granules are usually electron-dense and range in diameter from about 1000 Å. to about 3000 Å. Mean granule diameters differ from cell to cell in the same and in different species; but the fully formed granules are of relatively constant size for each cell type.

The elementary neurosecretory granule has been found in a variety of neurohæmal organs in representatives of many animal groups: the pars nervosa of vertebrates (mammal[1], bird[2], reptile[3], amphibian[4], and teleost[5]), the corpus cardiacum of insects[6], the sinus gland[7] and the post-commissural organ[8] of crustaceans. More recently, similar granules have been observed in the perikarya of neurosecretory cells of the caudal neurosecretory system of teleosts[9], of the preoptic nucleus of teleosts and amphibians[10,11], of the pars intercerebralis of insects[11,12], and of the brain of annelids[11,13]. The ubiquity of these elementary granules in neurosecretory systems has led to the conclusion that their presence may be virtually diagnostic of neurosecretion at the ultra-structural level.

Examination of the brain of the leech, *Theromyzon rude*, with the electron microscope reveals that every neurone contains at least some granules in the size range of elementary neurosecretory granules. (Electron microscope observations were made of the supra-œsophageal ganglion of *Theromyzon rude* fixed in Palade fixative with sucrose, embedded in methacrylate, sectioned on a Porter-Blum microtome, and observed with an R.C.A. *EMU*-3 electron microscope.) However, with the light microscope three principal types of neurones can be distinguished[14]. Two of these cell types contain cytoplasmic inclusions suggestive of intense glandular activity; cells of the third type, comprising the vast majority (95 per cent) of the neurones of the brain, show signs of minimum glandular activity only. Axons from the secretory-appearing cells pass into the dorsal commissure and appear to terminate in a neurohæmal area at its posterior surface. With the electron microscope at least some of the glandular-appearing neurones contain enormous numbers of elementary neurosecretory granules, both in the cell body and in the axon (Fig. 1). In the presumed neurohæmal area, the elementary neurosecretory granules become

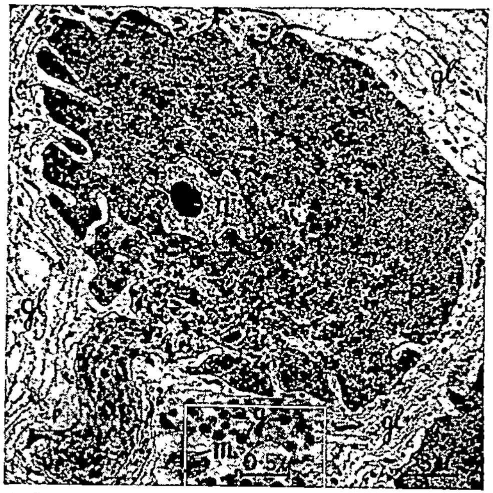


Fig. 1. Section through a neurone from the leech brain. Numerous dark neurosecretory granules are present in the cytoplasm and axone. *n*, Nucleus with a nucleolus; *a*, axon; *gl*, glia cell cytoplasm; *p*, polystyrene particle (2640 Å.). Insert. Portion of cytoplasm from a similar type of cell. *g*, Neurosecretory granules; *m*, mitochondrion

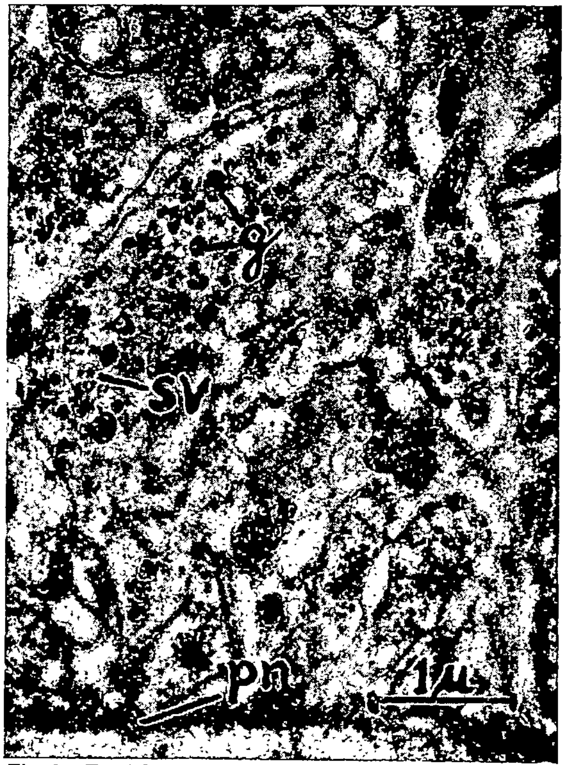


Fig. 2. Frontal section of the posterior portion of the dorsal commissure. Shown are axon endings of presumed neurohæmal structure. *g*, Neurosecretory granules; *sv*, synaptic vesicles; *pn*, perineurium

intermixed in the axones with small vesicles morphologically equivalent to synaptic vesicles (Fig. 2). Such vesicles have been reported in other neurohæmal structures[1,2,4].

On the basis of ultrastructural cytological criteria, including the consistent association of elementary granules with Golgi membranes[11], all neurones in the brain of the leech would have to be defined as neurosecretory. Since the majority of neurones in the brain must be involved in 'ordinary' nervous activities, one is hesitant to ascribe neurosecretory activity to all of them. It is possible that some of the types of granules in the leech neurones may be associated with neurotransmitter function. The usual synaptic vesicle is small (< 500 Å.)[15] and relatively electron-lucent. However, our knowledge of the range of size and appearance of presumed neurotransmitter granules is limited, particularly with respect to non-cholinergic neurones. Conceivably many of the larger, more electron-dense granules seen in leech neurones could be synaptic granules of a type associated with non-cholinergic neurohumours. Only those granule-bearing cells terminating in the neurohæmal area would be considered as properly neurosecretory.

The advice and critical reading of this communication by Prof. Howard A. Bern are gratefully acknowledged. The work was aided by grant *G*-8805 from the National Science Foundation.

IRVINE R. HAGADORN[*]
RICHARD S. NISHIOKA
Department of Zoology and its
Cancer Research Genetics Laboratory,
University of California,
Berkeley 4.

[*] National Science Foundation Predoctoral Fellow in Biology.

[1] Palay, S. L., in *Progress in Neurobiology*, edit. by Waelsch, H., 2 (Hoeber-Harper, New York, 1957).
[2] Duncan, D., *Anat. Rec.*, **125**, 457 (1956).
[3] Bargmann, W., Knoop, A., and Thiel, A., *Z. Zellforsch.*, **47**, 114 (1957).
[4] Gerschenfeld H. M., Tramezzani, J. H., and De Robertis, E., *Endocrinol.*, **66**, 741 (1960).
[5] Legait, E., and Legait, H., *C.R. Assoc. Anat.*, **45**, 514 (1959).
[6] Meyer, G., and Pflugfelder, O., *Z. Zellforsch.*, **48**, 556 (1958).
[7] Hodge, M., and Chapman, G., *J. Biophys. Biochem. Cytol.*, **4**, 571 (1958).
[8] Knowles, F., in *Zweites Internat. Symp. ü. Neurosekretion*, edit. by Bargmann, W., 105 (1958).
[9] Sano, Y., and Knoop, A., *Z. Zellforsch.*, **49**, 464 (1959).
[10] Palay, S. L., *Anat. Rec.*, **138**, 417 (1960).
[11] Bern, H., Nishioka, R., and Hagadorn, I., *J. Ultrastr. Res.* (in the press).
[12] Nishiitsutsuji-Uwo, J., *Nature*, **188**, 953 (1960).
[13] Scharrer, E., and Brown, S., *Anat. Rec.*, **139**, 271 (1961).
[14] Hagadorn, I. R., *J. Morphol.*, **102**, 55 (1958).
[15] De Robertis, E., *Int. Rev. Cytol.*, **8**, 61 (1959).

PATHOLOGY

Stimulation by Heparin of Parenchymal Liver Cell Proliferation in Normal Adult Rats

IT has been reported[1] that serum from hepatectomized rats when injected into normal rats will stimulate mitosis in the livers of the recipients. This finding could not, however, be confirmed by other workers[2]. In work reported earlier[3], we found that plasma from hepatectomized rats, which was collected using heparin to prevent clotting, stimulated both mitosis and deoxyribonucleic acid (DNA) synthesis in rat liver parenchymal cells, when injected into normal rats. On the other hand, serum or citrated plasma from hepatectomized rats had no apparent stimulating effect. For this reason, the effect of heparin itself on the proliferation of normal rat liver was examined.

Groups of five male Holtzman rats, 250–280 gm. body-weight, were used in each experiment. In a typical experiment, 100 units of heparin in 3 ml. of physiological saline or saline alone was injected intraperitoneally. If mitotic activity in the liver was to be examined, the animals were killed 24 hr. after the sample injection. Paraffin sections were cut at 6μ and stained with Harris's hæmatoxylin. Mitotic activity in parenchymal liver cells was expressed as mitotic figures in 50 high-power fields (× 400).

The effect of heparin on DNA synthesis in the liver was determined by intraperitoneal injection of orotic acid-6-^{14}C (2·5 μc./rat) 7 hr. after sample injection. The animals were killed 17 hr. later and DNA isolated essentially by the method of Hecht and Potter[4]. Isotope measurements were made using a Nuclear Chicago *D*47 gas-flow counter equipped with a 'Micromil' window. Specific activity is expressed as counts/min. per mgm. DNA.

Table 1. EFFECT OF HEPARIN AND OTHER SULPHATED POLYMERS ON DNA SYNTHESIS AND MITOTIC ACTIVITY IN NORMAL ADULT RAT LIVER

Sample injected	Mean mitotic index		Mean specific activity (c.p.m./mgm. DNA)		
	Exp. I	Exp. II	Exp. III	Exp. IV	Exp. V
Saline control	6 (1–11)[*]	6 (1–11)	15 (8–20)	18 (10–23)	9 (4–16)
Heparin (100 units)	62 (26–113)	74 (27–113)	68 (27–113)	92 (45–162)	64 (30–136)
Heparitin sulphate	—	—	—	—	115 (30–208)
Chondroitin sulphate *B*	—	—	—	—	34 (16–60)
Chondroitin sulphate *A*	—	—	—	—	19 (12–26)
Chitin sulphate	—	—	—	—	15 (9–22)
Polystyrene sulphonate	—	—	—	—	5 (4–7)

[*] Range in parentheses.

The results obtained from representative experiments using heparin are shown in Table 1. It is apparent that intraperitoneal injection of heparin resulted in marked stimulation of mitotic activity in normal rat liver parenchymal cells. The increase in mitotic activity is accompanied by increased incorporation of the radioactive precursor into DNA. Microscopic examination showed no apparent alteration in liver structure under these conditions.

To determine whether the response of the liver was specific for heparin, a number of natural and synthetic sulphated polymers were also examined for their ability to stimulate DNA synthesis in the liver (Table 1). One mgm. of each polymer in physiological saline was administered under the same conditions described here. Heparitin sulphate, which differs in structure from heparin only slightly[5], having some of the *N*-sulphate groups replaced by *N*-acetyl, stimulated liver DNA synthesis to the same extent as heparin. The higher value of the single experiment cited is not significant. Chondroitin sulphate *B* (β-heparin) also had some activity in this system, whereas, chondroitin sulphate *A*, chitin sulphate and polystyrene sulphonate had little or no stimulating effect. There was no change in this pattern on increasing the dose to the 5-mgm. level. The ability of the various polyanions to stimulate liver DNA synthesis appears to parallel the anti-coagulant and lipæmia-clearing activity of these substances.

The marked stimulation of liver-cell proliferation by heparin at doses above 20 units/rat suggests that

NORTH-HOLLAND BOOKS

recently published

***THEORY OF ELEMENTARY PARTICLES,** *Second improved and revised edition* by PAUL ROMAN.

'The book is clear, concise and complete within each topic. It seems to this reviewer to achieve the author's original purpose in a most admirable way' (AMERICAN SCIENTIST)

6 × 9″ XVI + 580 pp. Gld. 50.—; 100s.

***PROGRESS IN LOW TEMPERATURE PHYSICS, Vol. III.** Edited by C. J. GORTER.

Contributors — W. F. Vinen; G. Careri; M. J. Buckingham; W. M. Fairbank; E. R. Grilly and E. F. Hammel; K. W. Taconis; J. Bardeen and J. R. Schrieffer; M. YA. Azbel' and I. M. Lifshitz; W. J. Huiskamp and H. A. Tolhoek; N. Bloembergen; J. J. M. Beenakker; Z. Dokoupil.

6 × 9″ XII + 496 pp. Gld. 48.—; 96s.

LOW TEMPERATURE PHYSICS, Proceedings of the VIIth International Conference, held at Toronto, August 29 - September 3, 1960. Edited by G. M. GRAHAM and A. C. HOLLIS HALLETT.

6½ × 9½″ XVIII + 730 pp. Gld. 57.—; 114s.

***IMPERFECTIONS IN CRYSTALS,** *Second edition* by H. G. VAN BUEREN.

'The book is written without the need for theoretical sophistication on the part of the reader and is therefore suitable as a reference for a wide variety of scientists and engineers. It is also eminently suitable as a base for a course in a curriculum in the now suddenly popular materials sciences. Excellent illustrations and many references add to the value of the book which is heartily recommended to all inquiring minds.' (PHYSICS TODAY).

6 × 9″ XVIII + 696 pp. Gld. 55.—; 110s.

***QUANTITATIVE METHODS IN PHARMACOLOGY, Proceedings of a symposium held in Leyden on May 10-13, 1960.** Edited by H. DE JONGE.

Contents — 1. Sequential Analysis; 2. Standardization of Drugs; 3. Parametric or Non-parametric Statistical Methods; 4. Screening of Drugs; 5. Mixtures of Drugs; 6. Miscellaneous Topics.

6 × 9″ XX + 392 pp. Gld. 42.—; 84s.

***PROGRESS IN OPTICS, Vol. I.** Edited by E. WOLF.

Contributors — R. J. Pegis, K. Miyamoto, R. Barakat, D. Gabor, H. Wolter, H. Kubota, A. Fiorentini and A. C. S. van Heel.

6 × 9″ XII + 342 pp. Gld. 35.—; 70s.

***PROGRESS IN SOLID MECHANICS, Vol. II.** Edited by I. N. SNEDDON and R. HILL.

It is planned to range over the whole field in the early volumes. Here the emphasis falls on some recent outstanding advances in the two basic disciplines of elasticity and plasticity.

6 × 9″ XI + 334 pp. Gld. 35.—; 70s.

***ELEMENTARY INTRODUCTION TO MOLECULAR SPECTRA,** *Second completely revised edition* by BØRGE BAK.

Contents — 1. Introductory Survey of Practical and Theoretical Spectroscopy. 2. Derivation of some Important Equations in Spectroscopy. 3. Microwave Spectra. 4. Infrared Spectra. 5. Spectra of the Visible-Ultraviolet Region. 6. Magnetic Resonance Spectra. Table of Physical Constants. Index.

5½ × 8½″ XII + 144 pp. Gld. 15.—; 30s.

ABSTRACT SET THEORY, *Second completely revised edition* by A. A. FRAENKEL.

This book proved to be an attractive textbook. New material has been added in the text as well as in the exercises, including a modern exposition of Definition by transfinite induction.

6 × 9″ VIII + 296 pp. Gld. 30.—; $ 8.40; 60s.

***PLASMA PHYSICS,** *second revised edition* by J. G. LINHART.

'The author has the three rare qualifications of experience, mathematical facility and inventive imagination. He also possesses didactic ability. The arrangement is so clear, with full description of the experimental methods and with all the relevant theory required to explain the data, that the book can be equally recommended to the student and to the expert research worker.' (NATURE).

6 × 9″ XII + 278 pp. Gld. 25.—; 50s.

** SOLE AGENCY FOR U.S.A.: INTERSCIENCE PUBLISHERS INC. - NEW YORK*

NORTH-HOLLAND PUBLISHING COMPANY - AMSTERDAM

4 *titles worth noting*

MILESTONES IN MICROBIOLOGY

Edited by Thomas Brock

Contains a collection of the historically important papers in the field of microbiology out of which current theories have developed. Explains the various ways in which experiments are designed. Pasteur, van Leeuwenhoek, Koch, Fleming, Beijerinck, and many others are included. 30/-

EXPERIMENTAL NUCLEAR CHEMISTRY

by G. R. Choppin

Teaches the techniques and necessary basic theory for utilization of radioisotopes in scientific research and experimentation. Employs numerous experiments and techniques, using AEC license-free quantities of radioisotopes. Includes treatment of latest counting equipment, with particular reference to the simple Geiger counter for experimental use. Appendices concern isotope procurement and regulations, equipment and availability. 63/-

GAMES OF STRATEGY: Theory and Applications

by Melvin Dresher

Explores an elementary, mathematical treatment of the relatively new branch of mathematics, the theory of games of strategy. Relates rigorous theories of rational behaviour and planning within competitive environments. Problems and worked-out solutions serve to present complete theories and significant results. 42/-

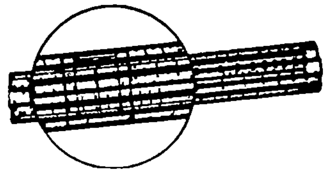
SOVIET POWER: Energy resources, Production and Potentials

by J. A. Hodgkins

Surveys geographically energy resources and solid fuel reserves, their production and potentials in the Soviet Union today. Indicates the U.S.S.R.'s technical and industrial capacity with stress upon the extensive development of power bases and energy resources. Traces Soviet growth in coal, oil, natural gases and total energy potential in relationship to expanded economic programmes. 25/-

PRENTICE-HALL INTERNATIONAL

34-36 Beech Street, London, E.C.1



SLIDE RULE EFFICIENCY *with The* **DAMIEN METAL SLIDE RULE**

Light . . . unbreakable . . . never wears out . . . indifferent to atmospheric or other conditions. Photographic printing ensures absolute accuracy, while the silver-grey finish gives figures maximum legibility. Retail Price £3 3s. 0d. each.

SPECIAL REDUCED PRICES FOR OVERSEAS

Obtainable from leading stationers and Drawing Office suppliers or direct from :

ANODISED PRODUCTS LIMITED

91 Upper Thames Street, London, E.C.4 *Tel.: MAN 7765*

BACK ISSUES OF JOURNALS IN SCIENCE & THE HUMANITIES

ALWAYS REQUIRED BY:

WM. DAWSON & SONS, LTD.,
BACK ISSUES DEPT.,
16 WEST STREET,
FARNHAM, SURREY, ENGLAND.

TEL: FARNHAM 4664 CABLES: DAWBOOKS, FARNHAM

DILLON'S UNIVERSITY BOOKSHOP Ltd

All current scientific and technical textbooks available from stock.

1 MALET ST., LONDON, W.C.1. MUSeum 2128

A Selection of Recent Books

TEXTBOOK OF PHYSIOLOGY AND BIOCHEMISTRY
Fifth Edition. By G. H. BELL, B.Sc., M.D., F.R.S.E., J. N. DAVIDSON, M.D., D.Sc., F.R.I.C., F.R.S., and H. SCARBOROUGH, M.B., Ph.D., F.R.C.P.
1,129 pages. 952 illustrations. 70s.

TEXTBOOK OF MEDICAL TREATMENT
Eighth Edition. Edited by SIR DERRICK DUNLOP, B.A., M.D., F.R.C.P., SIR STANLEY DAVIDSON, B.A., M.D., F.R.C.P., M.D.(Oslo), F.R.S.(Edin.), and STANLEY ALSTEAD, *C.B.E.*, M.D., F.R.C.P.
1,003 pages. 38 illustrations. 60s.

CEREBRAL PALSY IN CHILDHOOD AND ADOLESCENCE
Edited by JOHN L. HENDERSON, M.D., F.R.C.P.(Ed.).
411 pages. 12 illustrations. 35s.

THROMBOSIS AND ANTICOAGULANT THERAPY
Proceedings of a Symposium held in Dundee on September 29 and 30 and October 1, 1960.
Edited by W. WALKER, M.A., M.B., Ch.B., M.R.C.P.
106 pages. 86 illustrations. 17s. 6d.

PROCEEDINGS OF THE FOURTH INTERNATIONAL CONGRESS ON CLINICAL CHEMISTRY
Held in Edinburgh, August 14 to 19, 1960.
228 pages. 87 illustrations. 35s.

A SHORT HISTORY OF CLINICAL PATHOLOGY
By W. D. FOSTER, M.D.
166 pages. 32 illustrations. 27s. 6d.

E. & S. Livingstone, Ltd.

TEVIOT PLACE, EDINBURGH

the report[3] of stimulatory activity in hepatectomized rat plasma reflected the presence of heparin in the plasma.

This work was supported by the Cancer Chemotherapy National Service Centre, National Cancer Institute, under the National Institutes of Health contract No. *SA*–43–*ph*–1886.

M. ZIMMERMAN
EVEMARIE CELOZZI
Merck Institute for Therapeutic Research,
Rahway, New Jersey.

[1] Friedrich-Freksa, H., and Zaki, F. G., *Z. Naturforsch.*, **9b**, 394 (1954).
[2] Weinbren, K., *Gastroenterology*, **37**, 657 (1959).
[3] Zimmerman, M., and Celozzi, E., *Fed. Proc.*, **19**, 139 (1960).
[4] Hecht, L. I., and Potter, V. R., *Cancer Res.*, **16**, 988 (1956).
[5] Linker, A., and Sampson, P., *Biochim. Biophys. Acta*, **43**, 636 (1960).

Use of Desertomycin Medium in Routine Selective Isolation of Fungi Pathogenic to Man

THE isolation of pathogenic fungi producing superficial or systemic diseases in man from clinical materials is made difficult by different contaminating bacteria and saprophytic fungi overgrowing the culture medium and suppressing or inhibiting the growth of pathogenic fungi. In order to obtain pathogenic fungi in a pure culture, attempts were made to mix various substances in the medium in such a way that they would be liable to prevent the growth of contaminants. After a great deal of effort, the most satisfactory result was obtained by cultivation media containing actidione. Actidione (cycloheximide)[2] was the only antifungal antibiotic which, though exerting no antibacterial effect, inhibits the growth of most saprophytic fungi, yet does not inhibit the development of fungi pathogenic to man. For this very reason, it can be used with penicillin and streptomycin[2,3] or chloramphenicol[4] for the preparation of selective culture media.

The antibiotic isolated from *Streptomyces* and a completely new type called desertomycin, which is produced by us in a crystalline form, reveals a selective anti-fungal effect similar to actidione. Previously we reported on its anti-bacterial and cytostatic effect *in vitro*[5,6]. Our recent investigations, however, disclosed also its anti-fungal effect *in vitro*. Its anti-fungal effect on saprophytic fungi is much stronger than on fungi pathogenic to man. The anti-fungal concentration inhibitory to various fungi of desertomycin incorporated in Sabouraud–dextrose–agar was as follows: (*A*) *Streptomyces* strains: 1–10 μgm./ml. (*B*) Most of phytopathogenic and saprophytic filamentous fungi: 10–25 μgm./ml. (*C*) Dermatophytes: 50–100 μgm./ml. (*D*) Yeasts and yeast-like fungi (except *C. neoformans*): 250–500 μgm./ml.

By dint of its previously disclosed anti-bacterial property and the selective anti-fungal effect revealed by the findings, desertomycin promised to be a suitable agent for preparing culture media on which fungi pathogenic to man can be cultivated in pure culture.

These investigations were completed with cultivations of clinical materials liable to produce fungal contamination (such as hair, skin-scrapings, nail-clippings, sputum, etc.). The clinical materials were inoculated partly on Sabouraud-agar containing no antibiotics and partly on Sabouraud-agar containing desertomycin of 30 and 50 μgm./ml. The tubes were usually incubated for three weeks at 27° C., and the cultures were read. The results are summarized in Table 1.

Table 1

Total No. of materials	Total No. of isolations	Positive on both agars	Positive on desertomycin agar, negative on Sabouraud-agar
308	77	24	53

77 isolates were obtained from a total of 308 substances. 24 cultures of dermatophytes were obtained on both Sabouraud- and desertomycin-agars. 53 cultures of fungi pathogenic to man were isolated on desertomycin media but not on the corresponding tubes of Sabouraud-agar. These results reveal the superiority of desertomycin-containing medium to Sabouraud-agar as a routine isolation medium for fungi pathogenic to man from clinical materials.

Although desertomycin differs altogether from actidione in its chemical, physical and some biological properties, the two antibiotics closely resemble one another in their selective anti-fungal characteristics. Both can be used for the selective isolation of fungi pathogenic to man. The disadvantage of desertomycin as against actidione is that its quantities inhibitory to the growth of pathogenic and apathogenic fungi do not differ so much. On the other hand, desertomycin possesses several advantageous properties as against actidione, such as (1) stability; resistance to heat; it can be autoclaved with the culture medium without loss of activity; (2) it has also an anti-bacterial effect and so does not require the addition of any anti-bacterial antibiotic; (3) about one-tenth of the concentration is needed to attain the same effect as in actidione; (4) it does not irritate or damage the skin.

As a result of these findings, the culture medium containing desertomycin provides new possibilities for dermato-mycological laboratories. Detailed description of the experiments will be reported elsewhere.

J. URI[*]
Department of Pharmacology,
ZS. HERPAY
Dermatological Clinic,
University Medical School,
Debrecen, Hungary.

[*] Present address: Department of Biochemistry, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1.

[1] Whiffen, A. J., Bohonos, N., and Emerson, D. L., *J. Bact.*, **52**, 610 (1946).
[2] George, L. K., *Arch. Dermat. and Syph.*, **67**, 355 (1953).
[3] George, L. K., Ajello, L., and Papageorge, C., *J. Lab. and Clin. Med.*, **44**, 422 (1954).
[4] Rosenthal, S. A., and Furnari, D., *J. Invest. Dermat.*, **28**, 367 (1957).
[5] Uri, J., Bognár, R., Békési, I., and Varga, B., *Nature*, **182**, 401 (1958).
[6] Uri, J. *Nature*, **183**, 1188 (1959).

RADIOBIOLOGY

Radiation Sensitivity of *Escherichia coli* to β-Rays in Dry and Wet Condition

THE greater sensitivity of biological material to irradiation in the wet condition is due to the formation of radicals in the water surrounding the vital molecule. However, all the volume is not effective, since these radicals have a definite life-time and can diffuse only to a distance allowed by that time. This diffusion distance has variously been estimated from different types of inactivation studies in the wet and in the dry state of the molecules. Recently, the dependence of this distance on the shape of the molecule has been reported, and it has been shown that as the asymmetry of the target increases, the

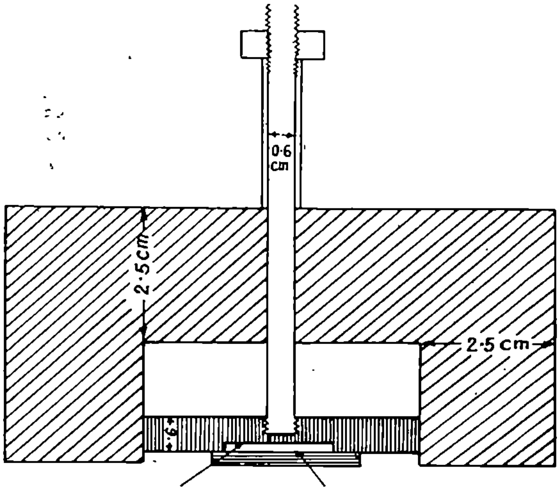


Fig. 1. Arrangement of β-irradiation of *E. coli* from strontium-90 source

diffusion distance decreases[1]. In the present report this idea has been applied to the calculation of diffusion distance for the inhibition of colony-formation by *E. coli* exposed to β-rays from strontium-90.

E. coli was grown in peptone water (peptone 1 per cent, sodium chloride 0·5 per cent in distilled water) for 4 hr. at 37° C. At the end of this period, bacteria were freed from the culture medium by repeated centrifugation and washing with Sørensen's buffer ($M/15$ KH_2PO_4 and $M/15$ Na_2HPO_4 3 : 7 v/v) at *p*H 7·00. This cell suspension, freed from broth medium, was used in different experiments.

The source of β-irradiation (obtained from the Radiochemical Centre, Amersham, England) was a strontium-90 plaque 2 cm. × 2 cm., as shown in Fig. 1. It was kept in a lead-lined 'Plexi-glas' box, the distance between the source and the sample being controlled by an adjustable screw. The total distance between the source and the sample was 2 mm.; the dose at this distance measured with an extrapolation ionization chamber was 2,460 ± 368 rads/min.

For irradiation in the wet condition, glass slides were covered with thin films of gelatin and one drop of mother suspension was taken on the slide. These glass slides containing the bacteria could be placed under the β-source in the specified position shown in Fig. 1, the centre of the drop being accurately in line with the centre of the sources. For irradiation in the dry condition, the gelatin covered glass slides containing bacteria were first dried in a vacuum desiccator for 48 hr. The preliminary tests for desiccation and the conditions of viability of the bacteria after the desiccation process have been described in detail in a previous paper[2].

Slides prepared in exactly identical ways, but not subjected to irradiation, were kept as controls. Agar platings were done by releasing bacteria both from the control and the irradiated slides separately in known quantities of phosphate buffer. Visible colonies were counted after 24 hr. of incubation at 37° C. in order to obtain the viable count in the control and irradiated samples. The ratio N/N_0 could thus be obtained for each dose of radiation, both in the wet and in the dry condition of irradiation.

Fig. 2 shows the results of irradiation of *E. coli* in dry and wet condition, with β-rays from strontium-90. 37 per cent survival doses in dry condition is 20,000 rads and that in the wet condition is 6,000 rads. If the inactivation is due to a single-hit type of process and there are n equally sensitive targets per cell the survival ratio $S = N/N_0$ after dose of D rads is given by:

$$S = \exp - [I \sigma n V] \qquad (1)$$

where $I = 5{\cdot}68 \times 10^{11} \times D$ primary ions per c.c. of unit density material, σ, the density of the material and V, the volume of the target responsible for inactivation. From the relation (1) the target volumes are found to be $6{\cdot}6 \times 10^7$ Å.^{3} and $29{\cdot}3 \times 10^7$ Å.^{3} for dry and wet conditions respectively. The density of the target material in the dry and wet condition has been assumed to be 1·33 and 1·00 respectively. Additional sensitive volume ΔV in the hydrated condition is thus $22{\cdot}7 \times 10^7$ Å.^{3}. For n equally radiation-sensitive targets per cell the additional sensitive volume is given by:

$$\Delta V = nYqv \qquad (2)$$

where Y is the number of radicals formed per primary ionization in water, q the probability of reaction between the radicals and the target and v is the effective volume of water from which the radicals can come and react with the target.

The mechanism of the inhibition of division by radiation is not clear. Very possibly it is a gene-controlled process, so that the action of radiation on any one of the sub-units of DNA molecules located in the chromosomes might be responsible for the inhibition of division[3,4]. A recent estimate of the genetic unit from the transforming principle activity is 20 Å. in diameter and 3400 Å. in length[5], giving a volume $V = 10^6$ Å.^{3}. This leads to a number $n = 60$ from the experimentally observed total volume $nV = 6{\cdot}6 \times 10^7$ Å.^{3}. For the inactivation of DNA, the value of effective radicals formed per primary ionization Yq is 1·5 (refs. 6 and 7).

The volume of available water per target v is obtained from equation (2) as $2{\cdot}5 \times 10^6$ Å.^{3}. Con-

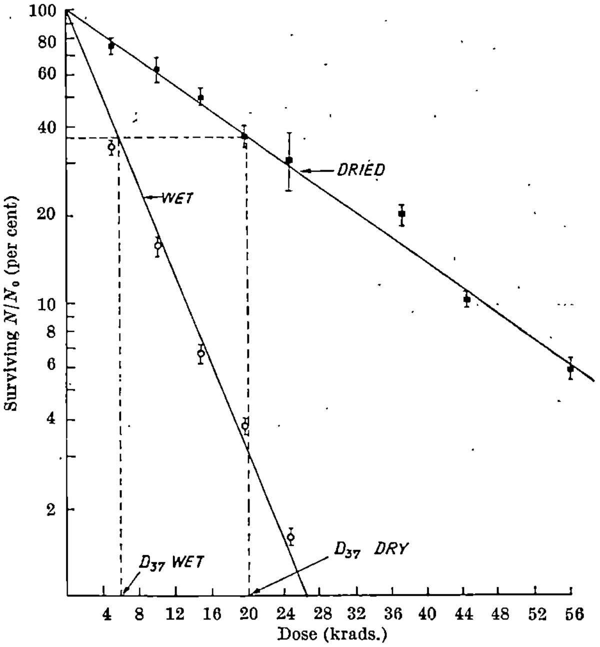


Fig. 2. Dose-survival curves of *E. coli* in dry and wet condition exposed to strontium-90 β-particles

sidering the maximum value of hydration as 40 per cent for protein, the hydration volume per target is $0{\cdot}44 \times 10^{6}$ Å.^{3}. The excess water $2{\cdot}1 \times 10^{6}$ Å.^{3} per target must be outside the target and not inside. Assuming the distribution of this water in a uniform layer around the target, the thickness of water surrounding each of the hydrated targets comes out to be 6·5 Å. So besides the hydrated target the radicals can come only from 2 or 3 molecules thickness of water.

This work was carried out under the financial sponsorship of the Council of Scientific and Industrial Research. One of us (S. B. B.) is indebted to it for a senior research fellowship.

S. B. Bhattacharjee
N. N. Das Gupta

Biophysics Division,
Saha Institute of Nuclear Physics,
Calcutta, 9.

[1] Bhattacharjee, S. B., and Das Gupta, N. N., *Ind. J. Phys.* (in the press).

[2] Bhattacharjee, S. B., *Rad. Res.*, **14**, 50 (1961).

[3] Howard-Flanders, P., *Advances in Biological and Medical Physics*, **6**, 553, edit. by Tobias, C. A., and Lawrence, J. H. (Academic Press, 1958).

[4] Hutchinson, F., *Amer. Naturalist*, **94**, 59 (1960).

[5] Litt, M., Ph.D. thesis, Harvard University (1958).

[6] DeFillipes, F. M., and Guild, W. K., *Rad. Res.*, **11**, 38 (1959).

[7] Hutchinson, F., and Arena, J., *Rad. Res.*, **13**, 137 (1960).

Gross Beta-Activity in the Thames River (Conn.)

Gross β-radioactivity has been determined in a number of marine organisms in the Thames River, Connecticut, United States. Collection areas were selected on the basis of established beds. Samples have been obtained and assayed every two weeks since April 1960. Organisms were returned to the Laboratory immediately after collection, washed free from surface contamination, weighed wet, dried under infra-red and ashed. Aliquots of the ash were prepared for radioassay and counted in an automatic β-counting system.

Radioassays were conducted on the following organisms: *Crassostrea virginica* (oyster), *Mercenaria mercenaria* (clam), *Mytilus edulus* (mussel), *Modiolus demissus* (mussel), *Laminaria agardhii*, *Fucus vesiculosus*. *Ulva lactuca* and *Chondrus crispus* (algæ). The β-activities obtained from these organisms exhibited seasonal variations, with *Laminaria* showing highest values and the oyster the lowest values.

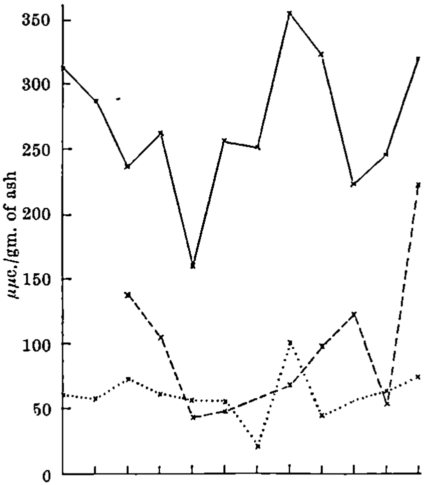


Fig. 1. Radioactivity in organisms obtained from the Thames River (Connecticut). —, *Laminaria agardhii*; · · ·, *Crassostrea virginica*; – –, *Mercenaria mercenaria*

Average monthly radioactive values for three of the above organisms are recorded in Fig. 1. Locations from which these organisms were obtained are: *Laminaria* from a point north of the Electric Boat Co., *Mercenaria* from a bed opposite the United States submarine base and *Crassostrea* from a bed north of the submarine base.

Maximum levels of radioactivity occurred in January 1961, in *Laminaria* with a total of 356 μμc./gm. of ash. Minimum values recorded during the year were in oysters in December 1960, with 23 μμc./gm of ash.

On the basis of analyses for total potassium in these organisms and calculations as to the amount of radioactivity contributed by natural potassium-40 it can be determined that most of the β-activity observed is due to potassium-40. It can be concluded, therefore, that the quantity of radioactivity present in marine organisms studied is extremely small, and does not represent a health hazard.

This work was supported by grant *RG*-6106 (*C*2), National Institutes of Health.

D. M. Skauen
T. Hatfield
D. Hanlon
J. S. Rankin, jun.

University of Connecticut,
Marine Research Laboratory,
Noank, Connecticut.

BIOLOGY

Biological Incorporation of Implant Material in the Rat

When plastic material is used as a biological implant, to improve contour or function, one of four things commonly occurs: it may cause an acute inflammatory episode and be rejected; rejection may be delayed for several months or years; it may be removed piecemeal by the body's own phagocytes; it may become encysted by fibrous tissue and remain *in situ*. In the last situation the implant has become virtually extracorporeal.

During a recent investigation of various plastic materials in rats, a completely new biological reaction, that of incorporation, was noted. The method of investigation was as follows: through a midline incision the abdomen was opened and a square 2 × 2 cm. of peritoneum and muscle was excised from the lateral abdominal wall. The defect created was covered by the material under test and sutured in place. The abdomen was then closed (Fig. 1). Naked-eye and microscopic examination of the implants were made at various times up to three months later.

In six animals a sheet of polytetrafluoroethylene, 0·3 mm. thick, was used. This material appeared to cause very little tissue reaction, and in four rats it became completely encapsulated by fibrous tissue. But in two animals normal peritoneum grew over the material. The histological picture in these two rats was quite unique; the polytetrafluoroethylene sheet had become incorporated within the tissues, with no abnormal cellular reaction. In Fig. 2, normal endothelial cells of peritoneum (*A*) are seen covering the polytetrafluoroethylene (*B*), which is permeated by normal-looking blood vessels and occasional tissue cells. The fibrous nature of polytetrafluoroethylene is evident and on superficial examination gives the appearance of collagen fibres. Deep to the implant is the normal muscle (*C*) of the abdominal wall: at this site there is no fibrous capsule nor cellular reaction to the plastic material. Examination under higher magnification (Fig. 3) confirmed the normal appearance of the blood vessels and cells within the polytetrafluoroethylene, suggesting that this was in fact a 'living implant'.

Measurements made from the histological sections indicated that the polytetrafluoroethylene sheet had increased in thickness by 30 per cent, and moreover it is stained faintly by basophilic dyes whereas before implantation it was not possible to stain this material with any of the routine histology stains. A piece of the original polytetrafluoroethylene sheet was subjected to analysis by Mr. G. W. Bowley, of Imperial Chemical Industries, Ltd., and found to be of good quality made from granular polymer, and there was no change in thickness on heating to relieve internal stresses. One is therefore driven to the conclusion that living matter is able to invade the discontinuous structure of a polytetrafluoroethylene moulding and, in so doing, open up this structure to make it readily porous to water. The effects observed in the biological alteration of the implant are thus due to physical and not chemical causes. Polytetrafluoroethylene, unlike other thermoplastics, does not have any melt state and has to be processed by methods similar to those used in powder metallurgy. Polytetrafluoroethylene fabrications are thus prone to void inclusions. No explanation, however, can be offered for the elongation of the polymer granules into fibres although elongation of particles does occur when the polymer in the dispersion form is processed into tape by extrusion and calendering: but the sheets of polytetrafluoroethylene used as implants has not been processed in this way.

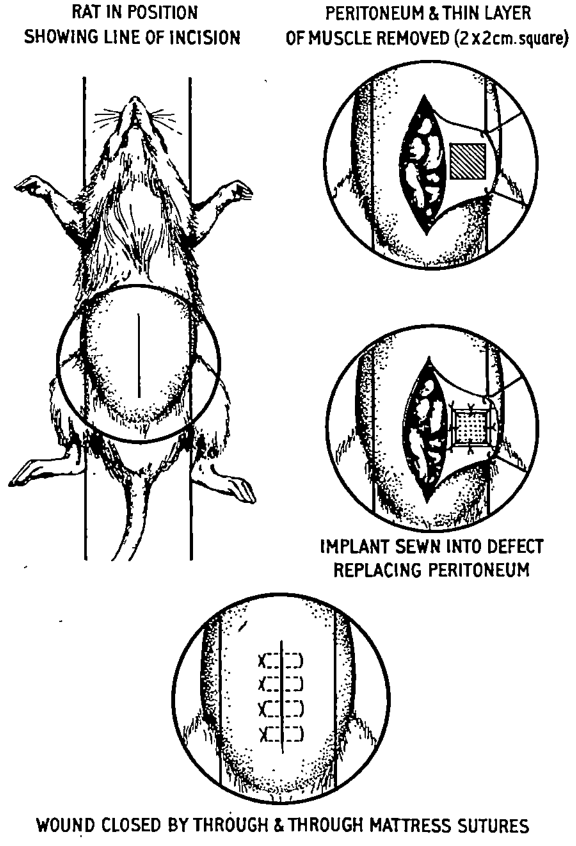


Fig. 1. Diagram of implant techniques

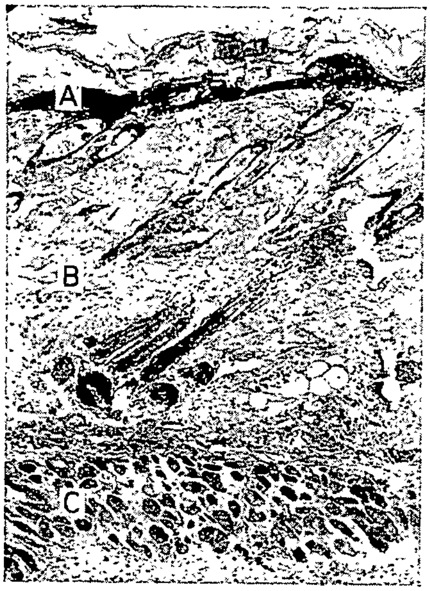


Fig. 2

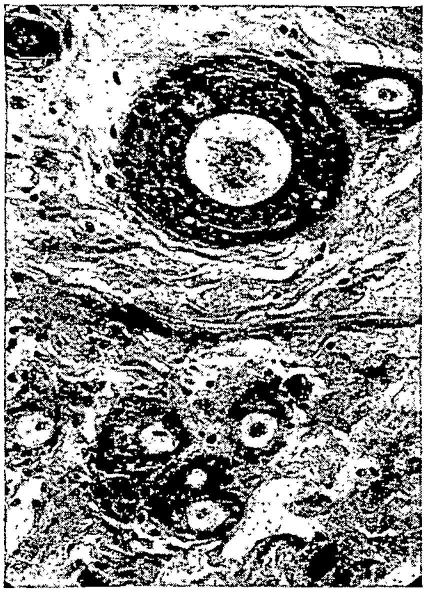


Fig. 3

These findings open up new possibilities for implant surgery.

James Calnan

Department of Experimental Surgery,
Postgraduate Medical School of London,
Ducane Road, London, W.12.

Rapid Development of Hypomagnesæmia in Lactating Cows given Artificial Rations Low in Magnesium

THE most characteristic biochemical change associated with the disorder of cattle known variously as grass staggers, lactation or nutrition tetany and Hereford disease is a fall in the blood-serum magnesium concentration from a normal value within the range 2·0–3·5 mgm./100 ml. to one of 0·7 mgm. or less. The disorder has its highest incidence in milking cows in spring, shortly after a change from winter rations to the grazing of pasture, and under such conditions frequently the fall in serum magnesium concentration is rapid; in extreme cases serum magnesium values of less than 0·7 mgm./100 ml. are observed within two days of the change of diet.

We[1,2] have previously demonstrated with milking cows that a change from winter rations to grazing is commonly associated with a decrease in the net uptake of magnesium from the gut (magnesium in the feed less the magnesium excreted in the fæces), due to a reduction in the dietary intake of magnesium and, to a lesser extent, to a decrease in its availability to the animal: hypomagnesæmia occurs in animals in which the net uptake of magnesium from the gut is reduced to such an extent that it fails to meet the magnesium requirements of the animal for milk secretion, body retention and fœtal growth, and in which, as a consequence, there is a drain on the small amount (2–3 gm.) of freely available magnesium in the blood serum and other extra-cellular fluids. The rapidity of development of hypomagnesæmia implies that the milking cow, during periods of acute magnesium deficiency, has little ability to mobilize any of the considerable amounts (100–200 gm.) of magnesium present in her soft tissues and bones.

Following the early investigations of Blaxter *et al.*[3], I have made a number of attempts to develop for milking cows artificial diets low in magnesium with the object of determining the effect of a rapid reduction in dietary magnesium on serum magnesium concentration, and of ascertaining the minimum amount of dietary magnesium, in a suitable form, on which an animal can maintain normal serum magnesium concentrations. In such work, two main problems have to be overcome: the technical difficulties of preparing the necessarily large quantities of foodstuffs sufficiently low in magnesium, and the need to ensure that such foodstuffs are acceptable to the milking cow and do not lead to digestive disturbances and subsequent loss of appetite and milk yield. It seemed preferable in the first instance, therefore, to develop rations based largely on feeding-stuffs in common use, but early experiments showed that the inclusion of large amounts of processed feeds low in magnesium, such as groats or flaked maize, effectively reduced magnesium intake but also improved markedly the assimilation of the magnesium present in the roughage part of the ration. An artificial roughage low in magnesium was therefore prepared from paper pulp[4]. Daily rations of wheat straw (2 lb.), paper pulp treated with liquid invert sugar (6 lb.), flaked maize (14 lb.), blood meal (3 lb.), supplementary minerals and distilled water *ad lib.* have now been offered for periods of 2–3 wk. to four lactating cows, yielding 1–3 gallons per day, and though the rations supplied a total of only 2–3 gm. magnesium daily, the serum magnesium concentrations of the cows were little affected and remained within the range 2·2–2·7 mgm./100 ml.

To determine the effect of a yet more extreme reduction in magnesium intake, a single animal was transferred, over three days, from the above ration to a highly artificial one, of wheat straw (2 lb.), casein (3 lb.), starch (14 lb.) and supplementary minerals, which provided only 0·8 gm. magnesium daily. The full ration was consumed for two days only, but during this period the serum magnesium fell from 2·2 to 0·8 mgm./100 ml. and an equally rapid recovery in concentration occurred with a return to the original ration.

An intermediate ration, of wheat straw (1 lb.), paper pulp treated with liquid invert sugar (6 lb.), flaked maize (3 lb.), casein (2 lb.), starch (4 lb.) and supplementary minerals, supplying 1·5 gm. magnesium daily, has, with two cows to which it has been offered, proved acceptable and yet effective in causing rapid falls in serum magnesium concentration. The results obtained with one of these cows to which, in addition to the above ration, occasional supplements of magnesium were given are shown in Fig. 1.

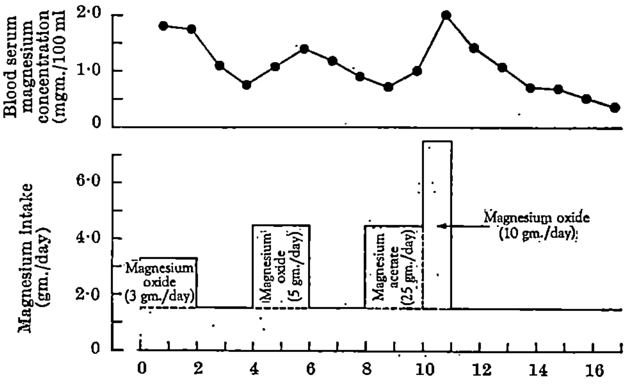


Fig. 1. Daily intakes of magnesium and the changes in the blood-serum magnesium concentration in a mature lactating cow given an artificial ration, providing 1·5 gm. magnesium daily, and occasional supplements of magnesium. (The supplement of magnesium acetate given on days 9 and 10 was not readily eaten, due to its bitter taste, and was therefore replaced by magnesium oxide. On day 17 the cow refused her food and was removed from experiment: the symptoms of tetany that can occur at a serum magnesium concentration of 0·7 mgm./100 ml. or less were not observed)

These results for a mature lactating cow show that though the net requirement for magnesium is low, the animal nevertheless depends on a continuous dietary supply for the maintenance of normal serum magnesium concentrations and at critically low levels of magnesium intake the serum magnesium concentration is rapidly affected by small changes in the intake of magnesium. They demonstrate that the freely available body reserves of magnesium must be small in relation to the requirements of a milking animal and give support to our contention[2] that hypomagnesæmia in cows at pasture is essentially nutritional in origin.

I am grateful to a number of colleagues, and in particular to Mr. V. W. Johnson, for help.

J. A. F. ROOK

Chemistry Department,
National Institute for Research in Dairying,
Shinfield, Berks.

[1] Rook, J. A. F., Balch, C. C., and Line, C., *J. Agric. Sci.*, 51, 189 (1958).

[2] Rook, J. A. F., and Balch, C. C., *J. Agric. Sci.*, 51, 199 (1958).

[3] Blaxter, K. L., and McGill, R. F., *Vet. Rev. and Annot.*, 2, 35 (1956).

[4] Rook, J. A. F., and Campling, R. C., *J. Agric. Sci.*, 53, 330 (1959).

Copper in Isopods

In crustaceans the hepatopancreas is the main organ of enzyme production. In isopods this organ consists of two types of cells which differ in size and were first distinguished by Weber[1]. This author observed in the smaller cells dense clumps of yellowish granules which became the subject of some controversy. Weber thought these granules to be 'enzymogens', a contention which was denied by Frenzel[2]. Patrick[3] held the granules to represent mucin or mucigens since they could be stained—so she claimed—only with mucin stains. Patané[4], on the other hand, found the granule in question to be untouched by mucin-specific histological techniques and he considered them as catabolic products since they gave reactions characteristic of purine bases.

So far the controversial granules have been observed in terrestrial isopods and in *Ligia oceanica*, a marine isopod which leads a semi-terrestrial life. The examination of some of these animals led me to the conclusion that the nature of the granules is quite different from that assumed by previous authors.

In an X-ray spectrophotometric investigation[5] it was found that terrestrial isopods contain enormous amounts of copper—as set out in Table 1.

Of the population with the highest copper content, *Porcellio scaber* from Cornwall, several specimens were dissected and their cuticles, intestines and hepatopancreas pooled. The analyses on these samples gave the values shown in Table 2.

Table 1

Species	Source	Copper (percentage of dry weight of whole animals) (range and mean)	No. of analyses
Porcellio scaber Ltr.	Suez, Egypt	0·022–0·039 (0·032)	5
Porcellio scaber Ltr.	Vienna, Austria	0·039–0·043 (0·041)	5
Porcellio scaber Ltr.	Cornwall	0·096–0·118 (0·106)	3
Armadillidium granulatum Br.	Spain	0·047–0·088 (0·063)	4
Armadillidium klugii Br.	Spalato, Yugoslavia	0·074–0·088	2
Armadillidium vulgare Latr.	Vienna	0·048–0·050	2
Cylisticus convexus B.L.	Vienna	0·051	1
Syspastus brevicornis Ebner	Elba	0·026–0·031 (0·028)	3

Table 2

Organ	Copper (percentage of dry weight)
Cuticle	< 0·005
Intestine + hepatopancreas	0·635
Intestine	0·076
Hepatopancreas	1·43

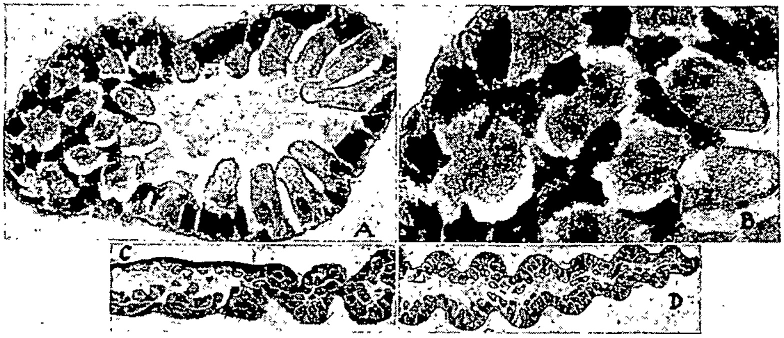


Fig. 1. Sections through one hepatopancreatic tubule of *Armadillidium granulatum* Brandt. *A,B*, transverse; *C,D*, sagittal through proximal (*C*) and distal (*D*) portion. Staining: rubeanic acid and hæm-alum. The copper appears as dense clumps of black granules

The copper content of the hepatopancreas in this species is the highest ever reported for any organ of an animal.

In sections of the hepatopancreas of *Armadillidium granulatum* stained with rubeanic acid (dithio-oximide)[6] the copper shows as dense black masses occurring intercellularly and in the small hepatopancreatic cells (Fig. 1). The large cells are free of copper which agrees with the findings of the earlier authors[1–4] that in the large cells granules do not occur. There can be scarcely any doubt that the granules observed by these authors are identical with, or contain, the copper made visible by staining with rubeanic acid.

The copper in the hepatopancreas of the animals investigated cannot be present as 'specific protein copper' since in such a state, as defined by Zuckerkandl[7], it would not react with a chelating agent like dithio-oximide. Moreover, the copper content of the hæmocyanin molecule, the cuproproteid most likely to occur in these animals, is only 0·18–0·22 per cent[8], thus even if the hepatopancreas consisted exclusively of hæmocyanin its copper content should be lower by almost one order of magnitude than it actually is. To account for its reaction with rubeanic acid the copper in the hepatopancreas of the isopods must be present in a reactive, easily dissociable state (the "cuivre naturellement réactive" of Zuckerkandl). How in this case toxicity is avoided and what the functions of these extraordinarily large amounts of copper could be remains to be investigated. Although up to now the occurrence of hæmocyanin in crustaceans has been proved only in stomatopods and decapods[9] it would not be unreasonable to expect the presence of this pigment in isopods, in which case part of the copper in the hepatopancreas might be in store for the synthesis of hæmocyanin.

All the isopods examined are reared on a diet of leaves and carrots and were kept in laboratory culture from 6 months to 6 years. Most of the isopods studied were collected and kept in culture by Prof. W. Kühnelt, whose co-operation in this investigation is gratefully acknowledged. The X-ray analyses were performed on mature, non-ovigerous, non-moulting animals in March 1961 by Dr. H. Makart at the Mineralogical Institute of the University, Vienna.

W. Wieser

2. Zoologisches Institut der Universität,
Wien.

[1] Weber, M., *Arch. Mikr. Anat.*, 17, 385 (1880).
[2] Frenzel, J., *Mitt. Zool. Station Neapel*, 5, 50 (1884).
[3] Patrick, D., *Brit. J. Exp. Biol.*, 4, 27 (1926).
[4] Patané, L., *Arch. Zool. Italiano*, 20, 303 (1934).
[5] Wieser, W., and Makart, H., *Z. Naturforschung* (in the press).
[6] Okamoto, K., and Utamura, M., *Act. Schol. Med. Univ. Imp. Kyoto*, 22, 573 (1938).
[7] Zuckerkandl, E., *Ann. Inst. Océanogr.*, 38, 1 (1960).
[8] Redfield, A., *Biol. Rev.*, 9, 176 (1934).
[9] Goodwin, T. W., in *The Physiology of Crustacea*, 1, 101 (Academic Press, Inc., New York and London, 1960).

'Quick' and 'Catch' Adductor Muscles in Brachiopods

The purpose of this communication is to direct attention to the close analogy between lamellibranch molluscs and articulate brachiopods, in the structure and function of the adductor muscles. In view of the large body of work on the physiology of the adductors in lamellibranchs, it is probable that a similar study in brachiopods would yield interesting comparisons.

Like a lamellibranch shell, the shell of an articulate brachiopod consists of two valves hinged together; but brachiopod valves are placed dorsally and ventrally, are hinged on the posterior side, and have perfect bilateral symmetry. They are closed by two pairs of adductor muscles, attached separately to the dorsal valve but uniting into a single pair of tendonous attachments on the ventral valve; and they are opened not by a ligament as in lamellibranchs but by a pair of divaricator (= diductor) muscles, of which the lines of action pass behind the hinge axis (Fig. 1). The two pairs of adductors differ macroscopically in form and lustre. Microscopically, Hancock[1] noted more than a century ago that the fibres of the posterior adductors are striated and those of the anterior adductors unstriated. This observation has been doubted more recently[2], but Atkins[3] and I have been able to confirm it in several genera. The divaricators are unstriated. It has long been known that brachiopods, like lamellibranchs, snap their valves together for protection when disturbed and at other times for the ejection of pseudofæces. It has therefore seemed probable that the analogy extends further, and that the striated and unstriated adductors in brachiopods are, respectively, 'quick' and 'catch' muscles. During a recent examination of living brachiopods in New Zealand I was able to confirm this in the terebratelloids *Terebratella* (*Waltonia*) *inconspicua* (Sowerby), *T.* (*Magasella*) *sanguinea* (Leach) and *Neothyris lenticularis* (Deshayes), and in the rhynchonelloid *Tegulorhynchia* (*Notosaria*) *nigricans* (Sowerby).

When the valves snap together spontaneously, they do not close completely, and immediately afterwards begin to re-open. If the animal is disturbed (for example, by a vibration of the vessel in which it is contained), the valves snap rapidly until their edges are nearly together, and then close slowly until their edges are tightly in contact (the shell then remains closed for a variable period before re-opening). Thus both quick and slow closing movements can be distinguished in the undissected animal. If the tendonous portions of the anterior adductors are cut, leaving the posterior adductors undamaged, the valves snap together repeatedly but are unable to close tightly. If a posterior adductor is placed between a pair of electrodes connected to a stimulator, a single pulse will induce a single rapid twitch in the muscle. Thus the striated posterior adductors are clearly the muscles responsible for the rapid snapping of the valves. When an anterior part of the valve and mantle tissue is removed (making the muscles visible through the very thin anterior body-wall) the injury causes a permanent contraction of the anterior adductors, indicating that these are the muscles responsible for slow, tight and prolonged closure of the valves. The relatively slow reopening of the valves after a closure is due presumably to the contraction of the unstriated divaricators when the antagonistic contraction of the adductors has been relaxed.

The functional role of these muscular mechanisms will be described elsewhere, but it is hoped that the physiological properties of these muscles will be investigated more thoroughly by those with access to supplies of living brachiopods. This work was done at Portobello Marine Biological Station during the tenure of a Royal Society and Nuffield Foundation Commonwealth Bursary.

M. J. S. Rudwick

Department of Geology,
University of Cambridge.

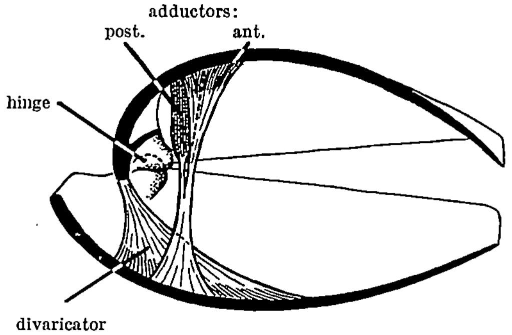


Fig. 1. *Terebratella inconspicua*, with valves gaping, cut along median plane of symmetry to show chief muscles (all other organs omitted); dorsal valve above. (× 3)

[1] Hancock, A., *Phil. Trans. Roy. Soc.*, **148**, 805 (1859).

[2] Hyman, L. H., *The Invertebrates*, **5**, 546 (New York, 1959).

[3] Atkins, D., *Proc. Zool. Soc. Lond.*, **131**, 564 (1958).

Paper Electrophoresis of Proteins while preserving the Pattern of their Localization in Tissues

The composition of protein mixtures extracted from different sources can be found by the usual methods of paper electrophoresis, but the localization of protein fractions in tissues can only be determined with preliminary preparative breakdown of tissues and organs.

I have now succeeded in combining these two functions by using special multilayer columns for electrophoresis made by folding and compressing one or two large sheets of paper and inserting imprints on filter paper of leaves or thin slices of animal tissues or organs. Fig. 1 shows examples of leaf-imprints obtained by a method described in refs. 1 and 2.

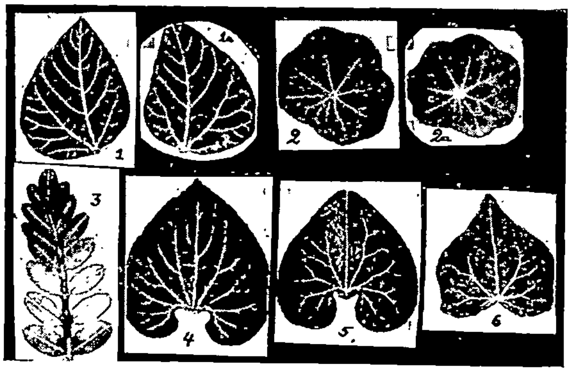


Fig. 1. Examples of leaf imprints on filter paper

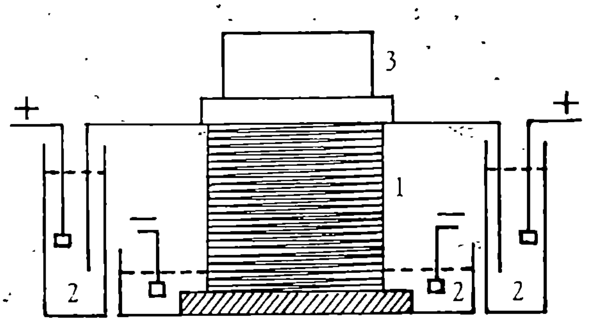


Fig. 2. Schematic diagram of electrophoresis on folded paper column. 1, Folded paper column; 2, buffer; 3, weight 1–2 kgm.

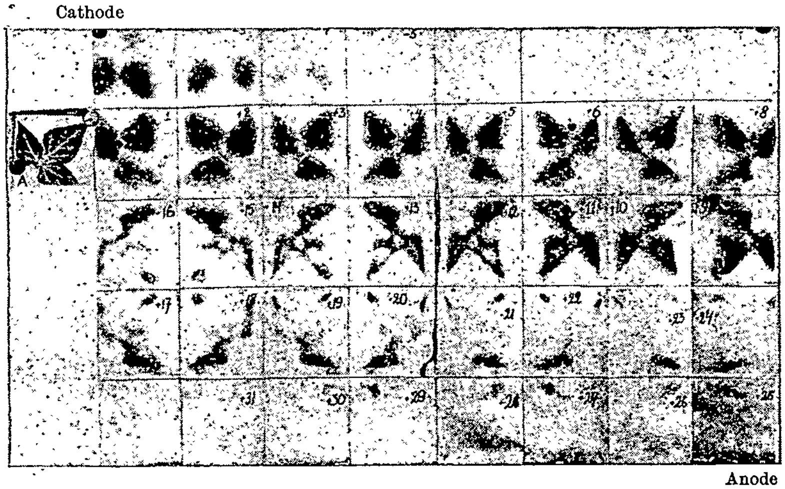


Fig. 3. An unfolded electrophoretogram of a bean leaf. Proteins are stained with bromophenol blue. Duration of electrophoresis was 2 hr. at 20 V./cm. The numbers 1, 2, 3, 4, *et seq.* indicate the sequence of layers in the folded column. *A*, the original leaf-imprint after electrophoresis. Chlorophylls and carotenoids did not migrate

Such imprints are inserted while still fresh and wet into a column of folded paper or a pile of paper disks, saturated with buffer and subjected to electrophoresis. The form of the leaf is preserved during migration of the proteins to the anode or cathode ; the localization of protein fractions may be determined by the usual methods of fixing and staining the unfolded paper electrophoretograms.

A schematic diagram of the electrophoresis is shown in Fig. 2. It is emphasized that better electrophoretograms are obtained by compressing the folded paper columns at 150–200 atm. before applying the buffer. Fig. 3 shows an electrophoretogram of a bean leaf, at the periphery of which is localized a rapidly migrating protein fraction.

This technique can probably be applied to histochemistry if materials with a finer microstructure than the usual filter paper can be used for impressing thin slices of tissues and organs. Folded, multilayer columns can of course also be used for electrophoresis of extracted mixtures of proteins.

ZH. A. MEDVEDEV

Department of Agrochemistry and Biochemistry,
Timiriazev Agricultural Academy,
Moscow, A–8,
U.S.S.R.

[1] Medvedev, Zh. A., *Proc. of the First (UNESCO) Internat. Confer.* "Radioisotopes in Sci. Res.", 3, 648 (1958).

[2] Medvedev, Zh. A., *Reports of Timiriazev Agric. Academy*, No. 3, 186 (1957) (in Russian).

CYTOLOGY

Fixation of Yeast Protoplasts for Electron Microscopy

In an attempt to study the plasma membrane of cells by correlation of physico-chemical experiments and electron microscopical observations, yeast protoplasts were considered as a suitable experimental material. These protoplasts are easily obtained in quantities sufficient for chemical experiments by treatment of a yeast cell suspension with gut juice of *Helix pomatia*[1–3]. In the present work *Saccharomyces carlsbergensis* strain 74 of the National Collection of Yeast Cultures, Britain, has been used.

The protoplasts are osmotically fragile, but suspended in a solution containing 10 per cent mannitol and 0·01 *M* sodium acetate–acetic acid buffer they can be kept intact at 4° C. for some days. By phase-contrast microscopy they are seen as globular bodies with a diameter of 6–7μ containing a large vacuole of about 4μ diameter and some granular inclusions (Fig. 1). Addition of 1 per cent osmium tetroxide to such a suspension for fixation causes the sudden disappearance of the vacuole and a swelling of the protoplasts to complete disintegration. This is remarkable in view of the fact that other single cells, for example, *Limnaea* eggs, ascites tumour cells and blood cells are perfectly fixed by the addition of osmium tetroxide to their suspensions. A similar phenomenon, however, was observed by Thorsson and Weibull[4] and by Lee[5] with the fixation of bacterial spheroplasts.

It was assumed that the plasma membrane of the normal yeast protoplasts is permeable to water, but not, or with difficulty, to small molecules such as mannitol and to ions. Then it was supposed that the action of osmium tetroxide on the plasma membrane renders this membrane permeable to small molecules and ions but not to macromolecules. The same suppositions were made for the vacuole wall.

Of the substances contained in the cytoplasm the most interesting in the present case are the nucleic acids and the salts.

From the dimensions of protoplast and vacuole, combined with results obtained by Eddy[6] it can be calculated that the cytoplasm contains about 4·3 per cent ribonucleic acid and deoxyribonucleic acid together, that is, about 0·12 equiv. per l. A potassium content of the same order may be derived from a table given by Cook[7] for yeast cells, whereas the sodium and chloride content is much lower, namely, about 0·007 equiv. per l. The nucleic acids will be almost fully ionized at *p*H 6, the *p*H of the cytoplasm.

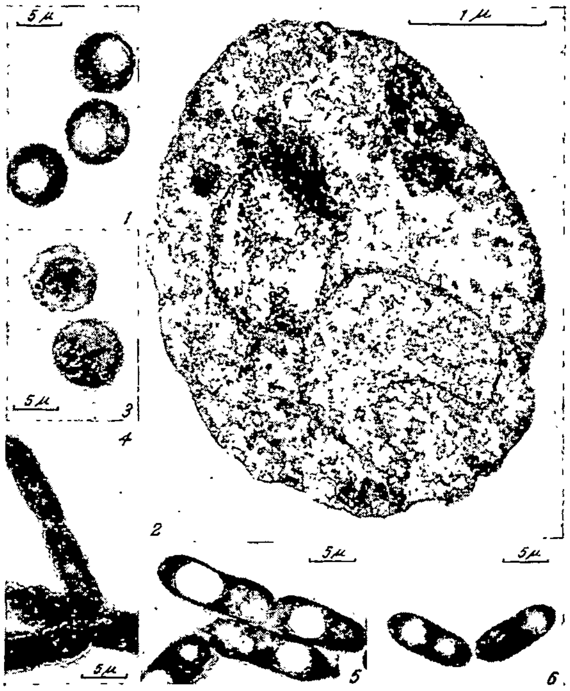
From the Donnan equilibrium, set up under the conditions which are thought to prevail after fixation, the resulting swelling pressure of the cytoplasm can be derived. This pressure is represented by the difference between the concentrations of the mobile ions inside and outside the cytoplasm. In a 10 per cent cell suspension this difference will amount to about 0·1 molar. The action of this swelling pressure leads to disintegration of the protoplast.

It proved to be impossible to counteract this swelling pressure, which makes its appearance only at fixation, by the extra addition of uncharged molecules, for example, mannitol or sucrose, to the fixation medium in order to raise its osmotic pressure.

This is in agreement with the picture given here. The effect of the swelling pressure on the protoplast will be prevented in a mechanical way by the toughness of a cell wall around the cytoplasm. In fact this is the case with normal yeast cells, which can be fixed by osmium tetroxide without disruption of the cell. The protoplasts, without such a wall, may be protected at fixation by an inversely directed Donnan effect, resulting from the addition of highly charged, non-permeating, macromolecules to the fixation fluid.

It has been found that carrageen, a sulphate colloid of equivalent weight 294, yields good results in this respect.

Fig. 2 shows an electron micrograph of a yeast protoplast fixed by osmium tetroxide at pH 5 in a solution containing 0·6 per cent calcium carragenate.



Figs. 1–6

As regards the fate of the vacuole the same line of explanation may be followed. The vacuole wall will be rendered permeable to small molecules and ions by the action of osmium tetroxide. The vacuole then collapses as a result of the swelling tendency of the cytoplasm and it does so in protoplasts as well as in normal yeast cells (Figs. 3 and 4). Fig. 5 shows unfixed normal yeast cells. As it is obviously impossible to prevent the uptake of solute by the cytoplasm out of the vacuole by the addition of highly charged macromolecules to the vacuole content, there is no issue unless the cause of the Donnan effect itself is abolished. This might be done by lowering the pH of the fixation medium to the isoelectric point of nucleic acid, assuming that the same pH will be reached in the cytoplasm.

Indeed, with fixation at pH 1·45 the vacuole of normal yeast cells does not collapse (Fig. 6).

With yeast protoplasts the picture is less clear. There is some shrinkage of the vacuole, but this may be due to an insufficient difference in diffusion-rate between osmium tetroxide and hydrochloric acid. Evidently the osmium tetroxide should arrive at the vacuole wall after the acidification of the cytoplasm, for a vacuole once collapsed will not swell again. It is feasible that the presence of a cell wall may retard the diffusion of osmium tetroxide sufficiently to this end.

The bearing of the present results on the quality of fixation of other macromolecular polyelectrolyte containing cells or isolated cell constituents seems worthy to be investigated. A more detailed report of the experiments will be submitted for publication elsewhere.

I wish to thank Dr. G. J. H. Th. Tonio for the generous supply of yeast protoplasts and Prof. J. Th. G. Overbeek for a discussion of the results.

P. F. Elbers

Centrum voor Submicroscopisch
Onderzoek van Biologische Objecten,
Rijksuniversiteit Utrecht,
Janskerkhof 3, Netherlands.

[1] Giaja, J., *C.R. Soc. Biol.*, 82, 719 (1919).

[2] Eddy, A. A., and Williamson, D. H., *Nature*, **179**, 1252 (1957).

[3] Eddy, A. A., and Williamson, D. H., *Nature*, **183**, 1101 (1959).

[4] Thorsson, K. G., and Weibull, C., *J. Ultrastr. Res.*, **1**, 412 (1958).

[5] Lee, S., *Exp. Cell Res.*, **21**, 252 (1960).

[6] Eddy, A. A., *Exp. Cell Res.*, **17**, 447 (1959).

[7] Cook, A. H., *The Chemistry and Biology of Yeast* (Academic Press, New York, 1958).

VIROLOGY

Influence of *p*-Fluorophenylalanine on the Production of Viral Ribonucleic Acid and on the Utilizability of Viral Protein during Multiplication of Fowl Plague Virus

Experiments by Zimmermann and Schäfer[1] showed that p-fluorophenylalanine (FPA), given immediately after infection of chick embryo cells by fowl plague virus, inhibits the formation of any virus material detectable by commonly used biological methods. If FPA is given 2 hr. post-infection, the s-antigen appears normally, while no hæmagglutinin activity and no infectious particles can be detected.

With the aid of isotopic techniques two questions were explored. (1) How can the synthesis of viral ribonucleic acid (RNA) be influenced by FPA ? (2) Does the s-antigen, which is synthesized in the presence of FPA, fit into the viral particle ?

In a recent paper a method was described by which virus-specific ribonucleic acid (RNA) can be demonstrated within tissue culture cells infected with fowl plague virus[2]. The following technique was used. Phosphorus-32 was added to the culture medium. In this way only the newly synthesized RNA was labelled so that the bulk of cell-RNA could be excluded. To compare the RNA of the infected cultures with that of the non-infected ones, the RNA's were digested with RNase and the specific nucleotides and oligonucleotides were determined quantitatively. The ratios of cytidine monophosphate to oligonucleotides are characteristic for each species of RNA[3]. By the shift of the oligonucleotide pattern of the infected cells towards the pattern of purified viral RNA, one can estimate the amount of virus-RNA in the mixture with the newly built cell RNA.

In preliminary experiments it was found that the overall incorporation of phosphorus-32 into the cell-

Table 1. EFFECT OF FPA ON THE SYNTHESIS OF VIRAL RNA

Exp.	C/X	FPA 0		FPA 2		no FPA		Virus
		contr.	vir.	contr.	vir.	contr.	vir.	RNS
1	U	1·25	1·22	1·24	1·10	1·23	1·08	0·86
	AC	2·97	2·99	3·01	2·62	2·96	2·68	2·60
	GC	1·83	1·85	1·83	1·96	1·84	2·04	2·08
2	U	1·44	1·44	1·43	1·28	1·48	1·28	0·86
	AC	3·29	3·31	3·47	2·98	3·34	2·66	2·60
	GC	1·81	1·89	1·87	1·99	1·84	1·87	2·08
3	U	1·18	1·17					
	AC	2·90	2·92					
	GC	1·80	1·92					
4	U			1·66	1·51	1·70	1·50	0·86
	AC			3·33	2·96	3·75	2·88	2·60
	GC			1·82	1·90	1·89	1·82	2·08

For each sample five cultures of 24-hr.-old chick embryo cells were taken. To one group immediately after infection 270 γ/ml. FPA were added (FPA 0 vir.), to another group FPA was added 2 hr. post-infection (FPA 2 vir.). The third group received no FPA (no FPA vir.). All control cells (contr.) were not infected. In experiment 1 the cultures received 0·15 mc. phosphorus-32/culture 2 hr. post infection and were collected at 5 hr. post infection. In experiments 2 and 4, the cultures received the isotope at 4 hr. post infection, in experiment 3 at 1 hr. post infection. They were collected 2 hr. later, respectively. Only four nucleotides were determined. The ratios o cytidinemonophosphate (C) to nucleotide (X) are shown.

RNA was not affected by FPA. Particular interest was directed to the problem of whether viral RNA can be synthesized under conditions where *s*-antigen production has been stopped by FPA. If FPA is given immediately after infection, there is no difference between the RNA's of infected and non-infected cells (Table 1). Under these conditions *s*-antigen production is completely suppressed. If, however, FPA is given 2 hr. post-infection, the oligonucleotide pattern of the RNA of infected cells shifts towards that of the purified virus (compared with non-infected cells), just as in experiments in which no FPA was given.

Since FPA inhibits the synthesis of viral RNA only at early stages after infection, one may assume the formation of some kind of an 'early protein', resembling that of phages[4-6] necessary for the start of specific nucleic acid synthesis. If this hypothetical 'early protein', which is apparently not identical with the *s*-antigen, incorporates FPA instead of phenylalanine it will not be functional and synthesis of viral RNA cannot start. Once it has been synthesized normally, FPA no longer interferes with the production of either viral RNA or the material behaving serologically like *s*-antigen. Since fowl plague RNA does not contain an abnormal nucleotide[7], the 'early protein' may not be a particular enzyme, because all enzymes necessary for RNA-synthesis are already present in the non-infected cell. Its function may be to stabilize somehow the genetic information of the virus.

The assumption of a faulty 'early protein' raises the question of whether the *s*-antigen formed in the presence of FPA is identical in all respects with that produced without FPA during multiplication.

In preliminary experiments it was found that FPA had no marked influence on the incorporation of ^{14}C-leucine into normal cell proteins.

The treatment of tissue cultures with FPA was the same as that in the experiments with phosphorus-32. All cultures received ^{14}C-leucine during 2–5 hr. post infection. The incorporation of isotope was stopped by addition of large amounts of ^{12}C-leucine and virus production proceeded for another 17 hr. after removal of the FPA-block 5 hr. post-infection by phenylalanine. The virus contained in the medium was purified after addition of non-radioactive-carrier virus by adsorption on and elution from erythrocytes and was then split by ether[8]. After centrifuging to remove unsplit virus the hæmagglutinin was taken off by erythrocytes. All purification steps were followed by the determination of the biological activities in order to assure that no losses in any of the samples occurred. In each experiment all samples were treated identically.

Control cultures were grown on a ^{14}C-leucine medium prior to infection and after removal of the isotope the cells were infected. This virus yield was treated in the same way as, and compared with, virus grown at the same time in the presence of ^{14}C-leucine. The *s*-antigen of the control virus contained only 10–15 per cent of the radioactivity of that grown in the presence of ^{14}C-leucine. This radioactivity probably represents normal cell components, which cannot be separated from the virus under these conditions.

Table 2. EFFECT OF FPA ON THE INCORPORATION OF *s*-ANTIGEN INTO INTACT VIRUS

Exp.	Sample	C.p.m. in *s*-antigen	Virus yield in culture fluid HA-titre
1	FPA 0	753	128
	FPA 2	750	128
	Virus	10,000	256
2	FPA 0	211	32
	FPA 2	208	32
	Virus	2,315	64
3	FPA 2	429	128
	Virus	5,500	256

For each sample 10 cultures of chick embryo cells were infected synchronously by fowl plague virus. 270 γ/ml. FPA were added immediately after infection (FPA 0) or 2 hr. post-infection (FPA 2). The virus samples received no FPA. At 2 hr. post-infection 0·5 μM ^{14}C-leucine/culture (specific activity 6·24 mc./mM) were added to all cultures. The medium was withdrawn 5 hr. post-infection and medium containing 600 γ/ml. phenylalanine and ^{12}C-leucine 300 times in excess was added. After 17 hr. the virus was isolated from the medium by adsorption on and elution from red cells, and split by ether (ref. 8). The radioactivity was determined in the isolated *s*-antigen fraction.

The experiments shown in Table 2 demonstrate that *s*-antigen, synthesized in the presence of FPA (FPA 2) and labelled by ^{14}C-leucine, is not incorporated into the intact virus in an appreciable amount. The radioactivity in this fraction does not surpass 15 per cent of that of virus grown without FPA. The same percentage of radioactivity was found in the above-mentioned controls and in experiments in which FPA was given immediately after infection (FPA 0). Under these conditions no serologically detectable *s*-antigen is produced in the presence of ^{14}C-leucine.

It is known that FPA is incorporated into proteins instead of phenylalanine[9]. From our results we conclude that the faulty *s*-antigen, which incorporated FPA, is still serologically demonstrable but does not fit into the intact virus and therefore is excluded from incorporation into it as a viral sub-unit.

We wish to thank Prof. W. Schäfer for his interest in these experiments. This work was supported by the Deutsche Forschungsgemeinschaft.

CHRISTOPH SCHOLTISSEK
RUDOLF ROTT

Max-Planck-Institut für Virusforschung,
Tübingen.

[1] Zimmermann, T., and Schäfer, W., *Virology*, **11**, 676 (1960).

[2] Scholtissek, C., and Rott, R., *Z. Naturforsch.*, **16**, b, 109 (1961).

[3] Scholtissek, C., *Biochem. Z.*, **331**, 138, 365 (1959); **332**, 458 (1960).

[4] Tomizawa, J., and Sunakawa, S., *J. Gen. Physiol.*, **39**, 553 (1956).

[5] Stent, G. S., in *Advances in Virus Research*, **5**, 95, edit. by Smith, K. M., and Lauffer, M. A. (Academic Press, New York, 1958).

[6] Crawford, L. V., *Biochim. Biophys. Acta*, **28**, 208 (1958).

[7] Zillig, W., Schäfer, W., and Ullmann, S., *Z. Naturforsch.*, **10**, b, 199 (1955).

[8] Schäfer, W., and Zillig, W., *Z. Naturforsch.*, **9**, b, 779 (1954).

[9] Munier, R., and Cohn, G. N., *Biochim. Biophys. Acta*, **31**, 378 (1959).

FORTHCOMING EVENTS

Sunday, September 3—Wednesday, September 6

British Association for the Advancement of Science (at Norwich)—Continuation of 123rd Annual Meeting.

Sunday, September 3

At 11 a.m.—Service in Norwich Cathedral. Preacher: The Lord Bishop of Norwich, the Right Reverend Dr. Launcelot Fleming.

Monday, September 4

At 10 a.m.—Prof. C. F. Carter: "The Economic Use of Brains" (Presidential Address, Section F).

At 10 a.m.—Dr. K. P. Oakley: "Dating Man's Emergence" (Presidential Address, Section H).

At 11.30 a.m.—Dr. Richard G. Lipsey: "Can there be a Valid Theory of Wages?—A Study of Fact and Fiction in Economics" (Lister Lecture).

At 2.30 p.m.—Dr. Judith Milledge: "Form and Colour in Nature" (Darwin Lecture).

At 8 p.m.—Dr. B. V. Bowden: "The Impact of Automation" (Evening Discourse).

Tuesday, September 5

At 8 p.m.—Mr. Kenneth Adams: "Seeing in the Round" (*Eidophor* programme).

Sunday, September 3—Thursday, September 14

Royal Aeronautical Society; Institute of Aerospace Sciences, United States; and the Canadian Aeronautical Institute (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1)—Eighth Anglo-American Aeronautical Conference.

Wednesday, September 6—Saturday, September 16

International Federation for Documentation (in the Caxton Hall, Westminster, London, S.W.1)—27th Conference.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer or Assistant Lecturer in Psychology at Queen's College, Dundee—Secretary of the University, University of St. Andrews, c/o Queen's College, Dundee (September 7).

Research Assistant (graduate in science, preferably physics, with a knowledge of electronics) in the Department of Agriculture, for investigations into the measurement of radioactivity in livestock—The Registrar, The University, Leeds 2 (September 7).

Lecturer (with a degree or equivalent qualification, and teaching or other suitable experience) to teach Physics and Mathematics for Higher National Diploma Courses—The Principal, Royal Radar Establishment College of Electronics, Malvern (September 9).

Assistant Lecturer in Occupational Psychology—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (September 11).

Senior Lecturer (graduate in bacteriology or dairying with considerable experience of agricultural and/or dairy bacteriology) in Bacteriology—The Secretary, West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2 (September 11).

Scientific Officers or Senior Scientific Officers (with a first- or second-class honours degree, preferably in science or engineering, with a wide and intelligent interest in scientific matters and aptitude for administrative work in a scientific field) in the D.S.I.R. Headquarters, London, for scientific administrative work—Establishment Office, Department of Scientific and Industrial Research, 5–11 Regent Street, London, S.W.1, quoting HQ.4 (September 16).

Technical Officer (preferably with experience in a teaching laboratory) in the Biochemistry Department, University of Melbourne, Australia, for the organization at technical level of undergraduate practical classes—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (September 16).

Lecturer or Assistant Lecturer in Pure Mathematics—The Secretary, The University, Exeter (September 18).

Curator (with a B.Sc. degree with particular training in mineralogy and palæontology) of Fossils and Minerals in the Natural History Museum, Adelaide, South Australia—The Agent-General for South Australia, 50 Strand, London, W.C.2 (September 22).

Economists (2) (with a first- or second-class honours degree in economics and at least three years postgraduate experience in industry or research in industrial economics; preferably with a lively interest in technological development) in the D.S.I.R. Headquarters, Economic Section, London, to take part in planning and executing studies of the research and development needs of industries or sectors of the national economy—The Manager (PE.3324), Ministry of Labour, Professional and Executive Register, Atlantic House, Farringdon Street, London, E.C.4 (September 25).

Scientific Assistant (preferably with experience in plant nematology) to undertake the identification of helminthological collections—The Director, Commonwealth Bureau of Helminthology, The White House, 103 St. Peter's Street, St. Albans, Herts (September 30).

Chemist (with a degree in chemistry from an approved university, together with at least two years postgraduate experience, and a working knowledge of experimental statistics), to be responsible for a routine soil testing laboratory and to conduct research on tea soils and on the nutrition of the tea bush—The Director, Tea Research Institute of East Africa, P.O. Box 91, Kericho, Kenya Colony (October 1).

Lecturer (preferably with special qualifications in physiology or genetics) in Botany; and a Research Fellow (with a degree, preferably some research experience, and between the ages of 23 and 35 years) in Botany at the University of Tasmania, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 2).

Lecturers in the Department of Medical Microbiology, Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 4).

Chair of Chemical Pathology—The Secretary, The University, Aberdeen (October 31).

Postdoctoral Research Fellow in the Chemistry Department, for physico-chemical investigations of the surface chemistry of organic polymers of interest to the tyre industry—The Dean of Graduate Studies, University of Melbourne, Parkville, N.2, Victoria, Australia (November 30).

Assistant Biochemist, Basic grade (preferably holding the A.R.I.C., the Grad.Mem.R.I.C., or B.Sc.(Hons.) in chemistry or biochemistry), in the Group Laboratory at Crumpsall Hospital—The Hospital Administrator, Crumpsall Hospital, Manchester 8.

Assistant Lecturer, Grade B (with a good honours degree in mathematics and preferably some teaching or research experience) in Mathematics—The Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex.

Assistant Professor of Biology—Head, Department of Biology, University of New Brunswick, Fredericton, N.B., Canada.

Assistant Professor of Physiology—The Dean of Medicine, University of Alberta, Edmonton, Alberta, Canada.

Biochemist (with a good honours degree and preferably postgraduate training or research experience, and interested in the use of advanced techniques, for example, high voltage electrophoresis, and in the invention of new techniques for the differentiation of bacteria), for duties which will include the supervision of a survey now in progress—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref. 61/26.

Biometrician (with a first- or upper second-class honours degree, together with an interest in biological research, and preferably experience in agricultural experimentation) in the Department of Biometrics—The Secretary, Grassland Research Institute, Hurley, Maidenhead, Berks.

D.S.I.R. Research Assistant (graduate) in Zoology, to study the responses to environmental stress of the reticulo-endothelial system of the spleen and liver in rodents, using histochemical and enzymological techniques—Dr. L. L. de Kock, Department of Zoology, The University, Aberdeen.

Geologist (under 35, with a first- or good second-class honours degree in geology, and preferably some background of economic geology and/or a knowledge of mapping, photogeological interpretation and surveying with theodolite and aneroid barometers) in the British Solomon Islands, for geological field surveys, studies of mineral occurrences, and petrological or palæontological studies—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting C1/RC218/18/01.

Graduate Chemist in the Soil Science Laboratory, University of Oxford, to assist research into the use of new physico-chemical techniques for assessing the availability to plants of potassium in the soil—Dr. P. H. T. Beckett, Department of Agriculture, Oxford.

Laboratory Technologist (A.I.M.L.T., and considerable experience, preferably as Chief Technician) with the Government of Tanganyika—The Crown Agents, 4 Millbank, London, S.W.1, quoting Ref. M3C/53605/NF.

Lecturer or Assistant Lecturer in Agricultural Economics; and a Lecturer or Assistant Lecturer in Farm Management in the Faculty of Agriculture (Imperial College of Tropical Agriculture), University College of The West Indies, Trinidad, West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

Lecturer (with a good honours degree and capable of teaching physiology and pharmacology up to and including the external honours B.Pharm. degree of the University of London) in Physiology and Pharmacology in the Department of Biology—The Registrar, Leicester College of Technology and Commerce, Leicester.

Lecturer (under 45, with either A.M.I.E.E. or Higher National Diploma in Electrical Engineering, and at least three years teaching experience or relevant industrial experience), for an electrical technician's course at Kampala Technical Institute, Uganda—The Director of Recruitment, Department of Technical Co-operation, Carlton House, Terrace, London, S.W.1, quoting BCD.114/9/032/T.

Librarian (woman zoologist, age 25–35, British nationality, with a degree in zoology or biology) in an ecological library, for duties which include subject-indexing—The Director, Bureau of Animal Population, The University, Botanic Garden, Oxford.

Physicist (with a first- or second-class honours degree) in the Medical Physics Unit, to apply physics to a wide range of medical problems, initially in the radioactive isotope field—The Secretary, S.E. Regional Hospital Board, 11 Drumsheugh Gardens, Edinburgh 3.

Research Assistant (honours graduate) in Physics—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18.

Research Assistant (with a good degree in zoology and an interest in the physical and chemical properties of animal tissues) in the Study of Molecular Structure of Biological Systems in the Department of Bimolecular Structure—The Registrar, The University, Leeds 2.

Scientific Officer (with a first- or second-class honours degree in chemistry or Associate or Fellow of the Royal Institute of Chemistry, and preferably postgraduate experience in analytical work in food chemistry and insecticide analysis) in the Ministry of Commerce and Industry, Nigeria, for chemical work in connexion with the deterioration and protection of raw foodstuffs during storage in Nigeria—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. E.10/2.

SENIOR LABORATORY TECHNICIAN or LABORATORY TECHNICIAN (experienced in bacteriology)—The Director, Laboratory Animals Centre, M.R.C. Laboratories, Woodmansterne Road, Carshalton, Surrey.

SENIOR LECTURERS or LECTURERS (with a first- or second-class honours degree or equivalent and preferably with teaching and/or research experience) IN MATHEMATICS, ELECTRONICS AND ENGINEERING PHYSICS—The Registrar, Royal Military College of Science, Shrivenham, Swindon, Wilts, quoting Ref. HQ.77/17.

SENIOR RESEARCH ASSISTANT (Ph.D. standard) IN PHYSICS, for a programme of work on the magnetic properties of thin films—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey.

SENIOR RESEARCH FELLOW (with a university degree in science or engineering and experience in research) IN THE DEPARTMENT OF AIRCRAFT DESIGN, to engage in research in the fields of aircraft structure theory and/or solid mechanics—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

STUDENT TECHNICIAN IN THE DEPARTMENT OF PATHOLOGY, Connaught Hospital, Walthamstow, London, E.17—Secretary, Forest Group H.M.C., Langthorne Road, London, E.11.

STUDENT TECHNICIAN or TECHNICIAN (preferably with mathematics and physics at advanced level), for research work in a pulmonary physiology laboratory—The Secretary, University College Hospital Medical School, London, W.C.1.

TECHNICIAN (male or female) IN THE MICROBIOLOGICAL TEACHING LABORATORY—Mr. Edwards, The School of Pharmacy, 29–39 Brunswick Square, London, W.C.1.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Tuberculosis in Animals: Proceedings of a Symposium held at the Zoological Society of London, May 24th, 1960. Edited by J. N. Ritchie and W. D. Macrae. (Symposia of the Zoological Society of London, No. 4.) Pp. v+94. (London: Zoological Society of London, 1961.) 22*s*. 6*d*. [146

Negretti and Zambra, Ltd. Publication S109/1: Sales and Service Overseas. Pp. 12. (London: Negretti and Zambra, Ltd., 1961.) [57

First Annual Report of the Council of the Institute of Physics and the Physical Society, 1960. Pp. 23. (London: The Institute of Physics and The Physical Society, 1961.) [57

The British Steel Castings Research Association. Eighth Annual Report, 1961. Pp. ii+37. (Sheffield: The British Steel Castings Research Association, 1961.) [57

Fourth Report of the West African Timber Borer Research Unit. Pp. 67+5 plates. (London: Crown Agents for Oversea Governments and Administrations, 1961. Published for and on behalf of the West African Timber Borer Research Unit.) 5*s*. [57

Friends of the Lake District. Report and News Letter, June 1961. Pp. 14. (Ulverston, Lancs.: Friends of the Lake District, 1961.) [57

Association of British Manufacturers of Agricultural Chemicals. Directory, 1961. Pp. 50+xxiv. (London: Association of British Manufacturers of Agricultural Chemicals, 1961.) [57

Surface Activity. By R. J. Taylor. (A Unilever Educational Booklet–Advanced Series.) Pp. 21. (London: Unilever, Limited, 1961.) [57

Ciba (A.R.L.), Limited. Technical Notes, No. 222: Araldite for Floors. Pp. 8. (Duxford: Ciba (A.R.L.), Limited, 1961.) [57

General Register Office. Studies on Medical and Population Subjects. No. 17: Mass Miniature Radiography: a Report on Three Years Examinations in Endland and Wales, 1955–1957. By M. A. Heasman. Pp. x+124. (London: H.M. Stationery Office, 1961.) 15*s*. net. [57

Courtaulds, Limited. Report and Accounts, 1960–1961. Pp. 26. (London: Courtaulds, Limited, 1961.) [57

Department of Scientific and Industrial Research. Quality Measurement in the Chemical Factory. By A. J. Clark and C. W. Munday. Pp. ii+22. (London: Department of Scientific and Industrial Research, 1961.) [57

The British Steel Castings Research Association. British and Foreign Specifications for Steel Castings, Part 2: Summaries, Comparisons and other relevant Information. Pp. xiii+29. (Sheffield: The British Steel Castings Research Association, 1961.) 40*s*.; 7.50 dollars. [57

Soil Mechanics, Limited. Geotechnical Pamphlet No. 10: Rock Mechanics—The Measurement of the Properties of Rocks in the Field and in the Laboratory. Pp. xxii. (London: Soil Mechanics, Limited, 1961.) [57

British Broadcasting Corporation. Facts and Figures About Viewing and Listening in Twelve Charts with a Commentary. Pp. 31. (London: British Broadcasting Corporation, 1961.) 5*s*. [57

Air Ministry: Meteorological Office. Geophysical Memoirs No. 105: Upper Winds Over the World, Part 3: Standard Vector Deviation of Wind up to the 100-Millibar Level over the World. By Dr. G. B. Tucker. Pp. 101. (M.O. 631e). (London: H.M. Stationery Office, 1961.) 25*s*. net. [57

Soviet Plastics, No. 7 (July, 1960.) Cover-to-cover translation of *Plasticheskie Massy*, journal of the Soviet plastics industry. Translated from the Russian by the Rubber and Plastics Research Association of Great Britain. Pp. 72. Published monthly. Annual subscription rates: U.K. £14; Canada and U.S.A. 60 dollars. (London: Rubber and Technical Press, Ltd., 1961.) [57

Transactions of the Royal Society of Edinburgh. Vol. 64, No. 14, (1960/61): The Muscular and Reproductive Systems of *Atomaria ruficornis* (Marsham) (Coleoptera, Cryptophagidae). By M. E. G. Evans. Pp. 297–399. (Edinburgh: Royal Society of Edinburgh, 1961.) 37*s*. 6*d*. (57

Fumifugium: or The Inconvenience of the Aer and Smoake of London Dissipated. By John Evelyn. Pp. 41. First published in 1661 and reprinted by the National Society for Clean Air. (London: National Society for Clean Air, 1961.) Cloth 5*s*.; Paper 2*s*. 6*d*. [67

Other Countries

National Academy of Sciences—National Research Council. Nuclear Science Series. NASNS 3030: The Radiochemistry of Selenium. By G. W. Leddicotte. Pp. v+38. 50 cents. NAS–NS 3031: The Radiochemistry of the Transcurium Elements. By G. H. Higgins. Pp. v+35. 50 cents. NAS–NS 3032: The Radiochemistry of Aluminium and Gallium. By John E. Lewis. Pp. v+48. 50 cents. NAS–NS 3033: The Radiochemistry of Antimony. By William J. Maeck. Pp. vi+54. 50 cents. NAS–NS 3034: The Radiochemistry of Titanium. By Chong Kuk Kim. Pp. v+23. 50 cents. NAS–NS 3035: The Radiochemistry of Cesium. By H. L. Finston and M. T. Kinsley. Pp. vi+68. 75 cents. (Washington, D.C.: Office of Technical Services, Department of Commerce, 1960 and 1961.) [57

Fondation Universitaire, Bruxelles. Quarantième Rapport Annuel, 1959–1960. Pp. 138. (Bruxelles: Fondation Universitaire, 1961.) [57

New Zealand Marine Department. Fisheries Technical Report No. 2: Scallop Investigation, Tasman Bay, 1959–60. By J. H. Choat. Pp. 51. (Wellington: New Zealand Marine Department, 1960.) [57

Zwanzigster Jahresbericht der Schweizerischen Gesellschaft für Vererbungsforschung, Société Suisse de Génétique (S.S.G.), 1960, mit Unterstutzung der Julius Klaus-Stiftung für Vererbungsforschung, Sozialanthropologie und Rassenhygiene in Zürich. (Separatabdruk aus Archiv der Julius- Stiftung für Vererbungsforschung, Sozialanthropologie und Rassenhygiene, Band XXXV-1960-Heft 3/4.) Pp. 313–479. (Zürich: Art. Institut Orell Füssli, AG., 1960.) [57

Northern Rhodesia: Ministry of Land and Natural Resources. Annual Report of the Department of Water Affairs for the year 1960. Pp. 8. (Lusaka: Government Printer, 1961.) 2*s*. [57

South African Public Library, Cape Town. Grey Bibliographies, No. 7: A Bibliography of African Bibliographies covering Territories South of the Sahara. Fourth edition. Pp. iv+79. (Cape Town: South African Public Library, 1961.) R.2.00; 20*s*. [57

Council of Scientific and Industrial Research, New Delhi. Annual Technical Report, 1959–60. Pp. iv+440. (New Delhi: Council of Scientific and Industrial Research, 1961.) [57

Canada: Department of Forestry. Forest Entomology and Pathology Branch. Annual Report of the Forest Insect and Disease Survey, 1960. Pp. 121. (Ottawa: Queen's Printer, 1961.) [57

Indian Central Oilseeds Committee. Castorseed and Its Products: Proceedings of the Seminar held 11–12 August 1960, at the Regional Research Laboratory, Hyderabad. Pp. viii+87. (Hyderabad: Indian Central Oilseeds Committee, 1961.) [57

Bulletin of the American Museum of Natural History. Vol. 122, Article 2: Cranial Morphology of the Hadrosaurian Dinosaurs of North America. By Prof. John H. Ostrom. Pp. 33–186+plates 1–6. (New York: American Museum of Natural History, 1961.) 2.50 dollars. [57

United States Department of the Interior: Geological Survey. Bulletin 1081–G: Stratigraphy of the Wichita Group in part of the Brazos River Valley, North Texas. By Philip T. Stafford. Pp. iv+261–280+plates 11 and 12. Bulletin 1082–J: Geology and Mineral Deposits of the Carlile Quadrangle, Crook County, Wyoming. By M. H. Bergendahl, R. E. Davis and G. A. Izett. Pp. v+706+plates 34–39. Bulletin 1098–B: Geochemical Prospecting Abstracts, January 1955–June 1957. By Ellen L. Markward. Pp. iii+57–160. 35 cents. Water-Supply Paper 1369–B: Energy Losses Associated with Abrupt Enlargements in Pipes, with special reference to the Influence of Boundary Roughness. By Carl E. Kindsvater. Pp. iv+53–75+plate 3. 25 cents. Water-Supply Paper 1535–D: Calcium Carbonate Saturation in Ground Water, from Routine Analyses. By William Baci. Pp. iii+14+1 plate. 45 cents. Water-Supply Paper 1602: Effect of Reforestation on Streamflow in Central New York. By William J. Schneider and Gordon R. Ayer. Pp. v+61. Water-Supply Paper 1639: Surface Water Supply of Hawaii, 1958–59. Prepared under the direction of J. V. B. Wells. Pp. vii+149. 55 cents. Water-Supply Paper 1640: Quantity and Quality of Surface Waters of Alaska, 1959. Prepared under the direction of J. V. B. Wells and S. K. Love. Pp. vi+114. 50 cents. (Washington, D.C.: Government Printing Office, 1961.) [57

United States Department of the Interior: Geological Survey. Professional Paper 311: Geology of Northeasternmost Tennessee. By Philip B. King and Herman W. Ferguson. With a section on the Description of the Basement Rocks by Warren Hamilton. Pp. v+136+19 plates. Professional Paper 366: Ash-Flow Tuffs: Their Origin, Geologic Relations and Identification. By Clarence S. Ross and Robert L. Smith. Pp. vi+81. (Washington, D.C.: Government Printing Office, 1960 and 1961.) [57

Annals of the New York Academy of Sciences. Vol. 92, Article 2: *In Vitro* and *In Vivo* Effects of Amine Buffers. By Gabriel G. Nahas, Emmanuel M. Papper and 104 other authors. Pp. 333–812. (New York: New York Academy of Sciences, 1961.) [67

Bulletin of the American Museum of Natural History. Vol. 122, Article 3: A Review of the Bats of Trinidad and Tobago—Descriptions, Rabies Infection, and Ecology. By George G. Goodwin and Arthur M. Greenhall. Pp. 187–302+plates 7–46. (New York: American Museum of Natural History, 1961.) 4 dollars. [67

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaperat the General Post Office

UNIVERSITIES IN BRITAIN

THE vital importance of the task confronting the Robbins Committee on the educational system of Britain is being emphasized on all sides. The recent decision of the Minister of Education regarding the status of the colleges of advanced technology is admittedly only a temporary decision pending the report of that Committee, when more fundamental decisions will need to be taken regarding not simply the government and autonomy of such institutions but also their staffing, their size and their relation to the universities on one hand and to the technical colleges and technical schools on the other. The implications of their location do not appear to have been examined as they should have been, and there are also questions of staff and their status. Again, it is obvious that the expansion of the universities requires consideration both in regard to the balance, or rather the relations, between research and teaching and to the place of academic research, quantitatively as well as qualitatively, in Britain's organization for research. This is apparent from the recent report of the Council for Scientific and Industrial Research and also from the comment which has been made on the installation of the radio telescope at the Royal Radar Establishment rather than at a university. Nor can proposals for university expansion leave out of account particular problems, like that of mathematical education, to which much attention has been given recently. Even when such problems demand immediate remedial attention, any reorganization of higher education should be concerned to see that such crises do not recur.

For such reasons, Prof. H. C. Dent's little book, *Universities in Transition**, and the broadcast talks in the Third Programme by Sir Eric Ashby, "Universities To-day and To-morrow" (*Listener*, June 1), and of Mr. Norman Fisher, "The Future of Our Universities" (*Listener*, June 29), are timely. They are addressed to the layman, to the ordinary public, rather than to those who are engaged in academic work, and they should help to build up an intelligent public opinion on the issues involved in the expansion of the universities of Britain, and the implication of such expansion both for the national well-being and for the educational system as a whole. No less important, they help to place in a true perspective the numerous special issues arising out of such questions as apprenticeship and technical training generally, university selection and the content of curricula, even when they are presented in books of the quality of *The Complete Scientist* and Mr. W. D. Furneaux's Nuffield Foundation Study, *The Chosen Few*. Prof. Dent, moreover, provides an admirable select bibliography which offers the reader a reasonable conspectus of current discussion; as, in fact, in his limited field, does Mr. Furneaux.

Prof. Dent divides his space fairly evenly between the past, the present and the future. In the first section, he describes briefly what he regards as the most significant features in the development of universities in Britain up to 1939, stressing more particularly the rise of the modern universities. In the second, he outlines in some detail the expansion of universities between 1945 and 1960 and the wartime planning which made that expansion possible. Naturally, these two sections are largely recapitulation to anyone familiar with the subject, but even so, Prof. Dent contrives here and there to emphasize points that are often overlooked and bring out their relevance in the present situation, and he indicates weaknesses which we should be at pains to avoid in any future planning.

It is in his third section, when he examines critically some of the more important problems thrown up or made more acute by the present expansion, that Prof. Dent's own judgment finds free expression, and the order of priorities which he indicates deserves careful consideration. First, however, he suggests that the difficulties confronting British universities to-day can be divided, broadly, into two groups: those concerning internal administration and the conduct of university activities, and those posed by the ethos, intentions and organization of society. Most of the problems in the first group can, in any event to some extent, be solved by the action of the universities alone. None of those in the second group can.

This, of course, is a fundamental reason why it is so important that there should be a really informed public opinion as to what the universities are doing and what is involved in their expansion, although this is frequently unrecognized even by those who are foremost in pointing out that universities are social institutions. The fundamental problem, however, underlying and determining the character of all the others, Prof. Dent rightly maintains, is what kind of institution the university of to-morrow should be, or in other words: What is its primary purpose ? He agrees with Sir Eric Ashby that the social purpose of the European university has, within broad limits, remained constant throughout the ages, namely, to be society's principal means for conserving, disseminating and advancing academic knowledge, and, consequently, to be its principal source of supply of persons highly trained in academic disciplines; but he believes that in Britain the nature and scope of that purpose need reconsideration and some of its elements realignment.

Prof. Dent points out, for example, that there are now many institutions devoted solely to research, and the university is no longer the sole institution which accepts the advancement of knowledge as a corporate responsibility. Quite apart from the desirability of the colleges of technology extending their research activities, there are the independent research institutions, and it is significant that, in passing, Prof. Dent stresses the need for more co-operation and co-ordination between various institutions engaged in research. In this context, indeed, he suggests that broad fields of learning might be allocated as a special responsibility to particular institutions.

---

* *Universities in Transition*. By H. C. Dent. Pp. 176. (London: Cohen and West, Ltd., 1961.) 15s. net.

There are obvious difficulties in such an arrangement, but it may well be worth exploring, and it is interesting to note how far Prof. Dent's brief discussion is in line in a British context with the findings of a recent report of President Kennedy's Science Advisory Committee. Moreover, he accepts the impracticability of divorcing university research from teaching, and his discussion of research is designed primarily to elucidate what it is the university can offer that is unique. Even teaching at the highest level is likely, for example, to be in the future increasingly shared between the universities and other types of institution, including the colleges of technology.

Prof. Dent suggests that the answer may be found in the idea of community; not, indeed, in terms of the medieval idea, but in terms of an environment which affords, or rather compels, a wide range of academic and social experience of a particular kind. In consequence, a university must possess coherence, and the fragmentation and departmentalism which are sometimes encountered must not be ignored but removed. This fundamental question thus leads him to consider what conditions are necessary to facilitate a sense of community and the prospects that such conditions will, in fact, be accepted and satisfied.

It is not surprising to find that Prof. Dent believes that there should be a limit to the expansion of particular universities, and that he agrees with the Association of University Teachers that there should be a deliberate policy of preserving the pattern of university life found in the smaller and medium-sized universities. In consequence, he advocates the creation of new universities and university colleges, the diversion of considerable numbers of students who would otherwise enter universities into colleges of technology or technical colleges—it is not quite clear which he means—and the shedding by universities of some subjects now in their programmes. He questions outright whether purely professional subjects should any longer figure in university programmes, and suggests that at least all purely professional studies and activities should be transferred to other institutes, possibly associated with the universities after the fashion of the teaching hospitals or the teacher-training colleges, leaving the universities with the sole task of teaching the academic disciplines which are the bases of professional activities.

That is a proposal which Prof. Dent recognizes is likely to be unpopular; but it should at least stimulate some of the constructive thinking that is needed outside as well as inside the universities. If it should leave the universities with a task that is manageable in respect both of teaching and research, the considerable readjustments that professional institutions would be required to make might be well worth while. Furthermore, it would seem likely to meet some of the criticism that has been levelled at the education of the chemist and physiologist by Prof. T. L. Cottrell and Prof. J. A. B. Gray, and might offer the universities more room for adjustments in the content of studies to meet the needs of to-day and encourage breadth as well as depth in the education of scientists and technologists. At least it should give the universities the opportunity to impart something of the ideal of public service which is as essential a part of a professional code as technical competence.

Prof. Dent's next emphasis is on the quality of a student's private life. This, he rightly observes, can make or mar a student's university career, and he sees no adequate substitute for the community life of a college or hall of residence. He does not forget that there are sometimes disadvantages and defects which initiative and imagination could remove, but he challenges outright the belief that halls of residence are in general too expensive.

On the selection of students, Prof. Dent is much less detailed than Mr. Furneaux, but he is no less emphatic as to the need for some sort of clearing-house, as has now been recommended by the *ad hoc* Committee on Procedure for Admission of Students of the Committee of Vice-Chancellors and Principals, and the need for better use of school-leaving examinations and revision of procedure. He emphasizes, moreover, the importance of proper provision for the needs of overseas students, and thinks that attention to some of the factors which affect, often powerfully, the quality of a student's life might reduce the wastage and proportion of failures among those who secure admission.

This major criticism is, however, rather of the Government than of the universities. The universities cannot hope to offer their students all that a university education ought to imply without adequate financial resources. To do so, Prof. Dent observes, means something like doubling the present annual grants for recurrent expenditure, with *pro rata* increases as the number of students increases; and, no less essential, a sufficient increase in non-recurrent grants to provide adequate residential accommodation, equipment and buildings, including library as well as laboratory facilities and lecture rooms and provision for social and recreative activities. In this connexion it may be noted that Prof. P. M. S. Blackett has since expressed the concern held by many as to whether in submitting their applications to the University Grants Committee for 1962–67 the universities have made anything like sufficient provision for the cost of the scientific research which is their responsibility, the total cost of which could be about £14 million a year.

Two problems specifically mentioned by Prof. Dent in this connexion are those of specialization and breadth of education, which, as already noted, concentration on academic rather than professional disciplines might do something to ease, and the distractions from academic studies caused by sundry matters, including vacation employment. He suggests indeed that an inquiry into the entire range of university learning and teaching, including the content of syllabuses and the length of undergraduate courses, is overdue; and he points to the desirability of giving those appointed to a university academic staff who have no previous experience of teaching, particularly if young, some instruction in the arts of lecturing and handling seminar and tutorial groups, etc., in the psychology of learning and of later adolescence, the general structure of the English educational system and the rudiments of knowledge of one or two other national systems.

This is essentially an administrative matter. A second point, the relative place of research and teaching in a university teacher's life, is not, though in the first place Prof. Dent suggests that the relative load of teaching and research and the staff/student ratio should be the subject of authoritative inquiry. It is urgently necessary to-day to use the services of every university teacher to the very best advantage, and this is not possible so long as the claims of research and teaching are regarded as conflicting. Prof. Dent believes, indeed, that every member of the academic staff of a university should be constantly engaged in research, but not necessarily, nor perhaps even usually, with the idea of immediate publication; though he equally believes that the primary duty of most university teachers to-day is to teach as well as possible, and for this they must continually enrich their knowledge and understanding of their subject.

The wise distribution of a university's resources depends, of course, on the quality of its administration, but though Prof. Dent, like Sir Eric Ashby, is critical of some procedures, he does not think that any major alteration in the structure of the existing types of government would be timely. He urges some delegation of responsibility from the vice-chancellor, some relief of professors from administrative responsibilities and greater participation in academic government by members of the non-professorial staff; he also insists that it is essential to distinguish clearly between political independence and academic freedom, between the university as an institution in and of society and the methods by which that institution performs its particular and unique task. Political independence of the State the British universities no longer possess, if they ever had, but Prof. Dent does not believe that academic freedom should be limited to full responsibility for its own appointments, the organization of its courses, and the character and standards of the instruction given within a university. He is convinced that the university should also have absolute control over the admission, examination and expulsion of students; the final voice in the distribution of public funds received for recurrent expenditure; and the absolute right, subject to freely negotiated agreements, to determine what forms of research and what research projects it will undertake or approve.

All these rights Prof. Dent holds to be necessary if the university is to undertake freely, imaginatively, and flexibly its unique task which, with Sir Charles Grant Robertson, he sees as the starting point of a new renaissance, for which it creates spiritual and moral power. That belief is at the heart of his plea for ensuring that the university remains a community the members of which wrestle with the fundamental problems of life as well as pursue their intellectual tasks together, and, by and large, it seems to be accepted by Mr. Norman Fisher in his broadcast talk, when he expresses the hope that British universities of the future will live up to what was said of medieval universities by Hastings Rashdall: "Their greatest service to mankind was simply this, that they placed the administration of human affairs . . . in the hands of educated men".

Apart from this, in their broadcast talks, Sir Eric Ashby and Mr. Norman Fisher have little to say about the purpose of a university. Sir Eric is concerned chiefly with the problems which staff and students present in the proposed expansion to 170,000 places: the matter of buildings he dismisses as not an educational problem, rather more perfunctorily than Mr. Fisher approves; though it is plain enough that Sir Eric requires the existence and provision of satisfactory buildings, including halls of residence, libraries, etc., just as much as Prof. Dent or Mr. Fisher. The provision of staff of high quality he recognizes as the key problem: in his view the supply of students of high enough quality offers no difficulty, nor does he suggest that plans for university expansion in Britain will or should offer every young person capable of obtaining a first degree at a university the chance to do so. On the contrary, he points to the alternative avenues of higher education, and suggests that pressure on university places might well be relieved if teacher-training colleges and the colleges of technology were allowed to award a degree instead of a diploma.

This appears to be in line with Prof. Dent's views, as also are Sir Eric Ashby's views on research and teaching, a matter which he points out bears vitally on the problem of attracting staff of high quality. Some changes in the criteria for academic success are suggested for that reason alone. Size and location are dismissed as smaller matters, provided a university has space to be a coherent community with staff and students living, preferably, within walking distance; this again is not seriously contested by Mr. Fisher, beyond the comment that there are weighty arguments for limited size, or in any event, in big universities, a system of devolution. The question of academic government is regarded by Sir Eric as of critical importance, though he does not admit that it is necessarily a matter of size. He challenges, indeed, the existing pattern of academic organization of knowledge and suggests that some of the new universities should eschew altogether the words 'faculty' and 'department', and invent some new system even if it is too much to expect abolition of internal frontiers in the older universities. Like Prof. Dent, however, he insists that a university is a society, not a hierarchy, and that it is essential to associate the main body of university teachers with the making of academic decisions, and not leave it in the hands of some 1,200 professors. Nor does he believe that the revision of university problems is a difficult problem to solve.

Mr. Fisher has rather more to say about the purpose of a university, emphasizing the importance of a university giving its students something much more than professional skill; his suggestion for the development of graduate schools is intended to assist the university to perform its function of the transmission of knowledge as well as the extension of its frontiers, and to bridge the gaps between one specialized field of knowledge and another. He, too, sees the need for radical changes in the organization of existing universities, in recruitment of staff and in the system of promotion. By and large, Sir Eric Ashby and Mr. Fisher in their broadcast talks and Prof. Dent in his short volume provide the Robbins Committee with plenty of critical comment on important matters.

The chief task of the Robbins Committee can be described shortly as to recommend what new educational institutions are needed, what they should do and where they should be situated. The sound academic arguments for not pushing individual universities above the size of 4,000–5,000 students are apparent in these papers, and the new universities already specifically contemplated will provide some 15,000 places. A similar number may well be available in the colleges of technology, particularly if such colleges are enabled to offer a degree instead of a diploma in technology, and this might well be preferable to increasing the places in university departments of technology. Similarly, the suggestion regarding the teacher-training colleges, taken in conjunction with Prof. Dent's suggestion as to professional studies, might help. Meanwhile, however, the universities themselves should look at such matters as their degree structure in relation to catering for more general studies, the adjustment of the balance between research and teaching to the advantage of teaching, and the ways and means of meeting the residential and tutorial needs of the increasing numbers of students drawn from homes where there is no tradition of culture or reading. Whatever recommendations the Robbins Committee may make —and they should cover many institutions like technical colleges, professional institutes, and the schools themselves, as well as colleges of technology and the universities—so far as they affect the universities, it will be for the universities to decide on the methods of implementation, even if it is for the Government to will the means. The shape of at least some of the recommendations can already be forecast with confidence, and the universities should push ahead with their plans and proposals for reform and development.

HOPES OF TO-MORROW

It is disquieting that, despite all their technical skills, scientists so often have become the dupes of opportunist politicians. How adroitly Adolf Hitler manipulated them to his service. He is not the last of his species. In both halves of our polarized world scientists and technologists are to be found harnessed to the band-wagon of some capricious potentate who has succeeded in capturing the machinery of government. The Prime Minister, in his address to the Royal Society of London at the Tercentenary Banquet, claimed that politicians were the only amateurs left. Perhaps it is not too mischievous to suggest that this amateurish pursuit has incredible professional consequences. In general, scientists tend to leave their fate much to the whims of politicians. They are unconcerned with the world outside their research establishments; indeed, it is often a logical consequence of their training. To claim, as some do, that such unawareness is a virtue in itself is rather absurd.

Recently the Minister for Science raised the spectre of a languishing land, if Britain did not resort to automation more avidly*. Countries with a lower standard of living are mastering technical skills more and more. Lord Hailsham stated that, without automation, Britain will not command competitive power, but be priced out of the market. To avert this, the country must seize the lead in technology of production and be determined to remain one jump ahead of emergent countries in technical skill. He asserted that vast changes in the whole system of education would have to be made. For example, he pointed out that there are "vital questions in the field of human sciences" which are but weakly dealt with at universities and there is a dearth of management science.

It cannot be denied that new ideas in technology are necessary to prevent a mature economy from stagnating. That automation is essential for improving technology is certainly valid too. However, to suggest that national prosperity hinges on stressing automation and that education, particularly at schools and universities, be perverted to satisfy apparent technological demands of the moment is a sorry exaggeration. What may seem of paramount urgency to-day will to-morrow perhaps not seem so important.

Economic patterns are complex. A feature of a highly industrial, capitalistic economy is its sensitivity to trade-cycle fluctuations. This tendency appears to be related to the 'roundaboutness' of production; the greater the number of sequential steps between raw material and finished product, the keener is the sensitivity. Ultimately, the most powerful determinant of economic climate is what industrial and commercial management feel about the future. It is related to the temperament and emotional patterns of individuals.

To design education, particularly university education, so that scientists will acquire the skill to conjure up automatic devices to meet the expediency of the moment could, if overdone, have dire results. For example, it is possible to place too much emphasis on the type of mathematics used for obtaining solutions by electronic computation. Numerical methods on a computer usually will provide an answer, when a function is not easily integrable by analysis or say, a troublesome differential equation has to be solved. With inadequate training in analytical mathematics, the scientist will shirk an attempt at explicit solution; he will resort to a solution with a computer. The result is found speedily, but lacks the sophistication of an explicit form. The functional form of solution might have suggested startling insight into the physical significance bearing on the problem. Use of a computer tends to block such discovery.

---

* Conference on Automation and Computation, Harrogate, June 27, 1961.

Lord Hailsham's exhortations concerning problems of living, the vital questions, need to be examined. It might be well if these could be treated by using 'human science', as he suggests. But unhappily (or happily) human emotions do not lend themselves readily to rational formation. They are frankly, but fascinatingly, inconsistent. "Nature is not bound by reasonableness", as D. H. Wilkinson (professor of experimental physics at Oxford) has said. It is interesting to recall Lord Hailsham's presidential address to the Classical Association in Birmingham on April 13. There he stated that a solution to our modern perplexities, our aberrations in politics and morals, in æsthetics and philosophy probably lay in establishing again "the real validity of our moral and æsthetic value judgments symbolized in the classical culture in which we were steeped in our youth". This seems a better approach than 'human science'.

As we plan to change curricula to satisfy the technological demands of our age, let us consider likely hazards to the nation. Without knowledge of non-technological aspects of living, the adults of to-morrow will be vulnerable. They will be manipulated and beguiled. It was Stendhal who, in an article to the *Paris Monthly Review**, June 1822, wrote rather provocatively, that a really useful 'science' a man can learn is the science of knowing the motives of men's actions. When you know these, you can try to cultivate in people other motives which will lead them to act in such a way as to bring about your own happiness. It does seem that men often lie when they speak of their motives. One must try to penetrate and undermine such untruths.

The art of knowing life is acquired by involvement with the full range of human passions. One must live. Always love and hatred will move men. Personal experience sometimes teaches. There are other sources. Our literature is rich in expression, "from the whispering of affection to the raging of madness" (Nietzsche). Not from the classics alone but from the genius of those with heightened sensibility in any age is the noble art of understanding human nature to be gleaned. It is likely to be withheld from those who persistently proffer their punched programmes to our latter-day Oracle of Delphi, the computer. We need men of science who command *savoir-vivre* and who have a degree of empathy. How encouraging it is to read in *The Times* of June 28 that in the French baccalauréat examination, the entrée to universities there, respect is still shown for the irrational element. Even those candidates who plan to read science at the university might in their examinations be required to produce a dissertation on subjects such as: "What is the nature of æsthetic pleasure ?" or "Are we responsible for our passions?"

So let us not be carried away by excessive enthusiasm for the expediency of to-day, but be prepared for the demands of to-morrow. Then even while:

"Things fall apart ; the centre cannot hold,
Mere anarchy is loosed upon the World.
The Blood-dimmed tide is loosed, and everywhere
The ceremony of innocence is drowned," (W. B. Yeats)

we may have an abiding confidence to judge and judge wisely. For "a fool sees not the same tree as a wise man sees" (W. Blake).

---

* *Selected Journalism from the English Reviews by Stendhal, with Translations of other Critical Writings*, edit. by Strickland, G. (John Calder, London, 1959).

RESPIRATORY METABOLISM IN PLANTS

Respiratory Metabolism in Plants
By Prof. Harry Beevers. (Row-Peterson Biological Monographs.) Pp. xi+232. Evanston, Ill.: Row, Peterson and Company, 1961.) 3.85 dollars.

THE title chosen for this book is meant to indicate that it does not attempt to cover the whole study of plant respiration, but is concerned only with the biochemistry of its reaction sequences. This is a rapidly developing field subject to annual inspection in various reviewing journals, but this is by no means to say that the present volume is supererogatory. It is not easy for the young student to pick the (more or less) permanent wheat from the ephemeral chaff in annual reviews. A considered statement of work from the past couple of decades is a sounder foundation, and nobody is better qualified to provide such a foundation at the present time than Prof. Beevers, who has himself made notable contributions to this work.

He has aimed at and succeeded in providing a factual statement of present knowledge with a minimum of controversy and 'interpretation' of dubious data. He covers the sequences and cycles of reactions during the production of pyruvate from glucose; the utilization of pyruvate in its various aspects, and oxidation systems and their constituent electron-transfers. These are treated at some length, and then there are briefer accounts of the energetics of respiration and the intracellular localization of respiratory enzymes.

The seventh chapter, on the regulation of respiratory-rates, seems to step a little outside the author's declared intention and to involve him rather more deeply in controversy. The remaining chapters deal with the relation of respiration to other physiological processes such as salt uptake, nitrogen metabolism, etc., and make interesting reading.

The book has been well produced in paper-back form at a price which should not put it out of the reach of its most important customers and beneficiaries—biology students. W. O. JAMES

CIVIL ENGINEERING

Civil Engineering Reference Book

Edited by J. Comrie. Second edition. Vol. 1 : Pp. xiii+518. Vol. 2 : Pp. xiii+527. Vol. 3 : Pp. xiii+599. Vol. 4 : Pp. xiii+392+lxii. (London : Butterworth and Co. (Publishers), Ltd., 1961.) £17 17s. 0d. the four volumes.

A REFERENCE book dealing with so vast a subject as the science and practice of civil engineering would assume enormous proportions if the objective was to provide a self-contained treatment of every aspect of the subject for the engineer. Its proportions would, however, be more modest if it contained a brief review of each aspect of the subject with adequate references to specialist publications. This work in four volumes, by some fifty-six contributors, appears to fall somewhere between these possible forms, though its aim (as stated in the introduction to the first edition) is to give the civil engineer a concise presentation of the fundamentals of the theory and practice of all branches of the subject.

There is a tendency, in those chapters involving mathematical analysis, to deal with matters of detail rather too briefly for the practising engineer. This is remedied in some instances by numerous references to specialist works as, for example, in the chapter on hydraulic power plants. In other instances, however, as in the chapter on strength of materials, the references given are not always adequate for the sophisticated mode of treatment adopted. (The confusion of the mathematical expressions for the strain energy and complementary energy of linear systems in this chapter is noteworthy.)

Chapters on mathematics, statistics, physics and chemistry, mechanics, mechanics of fluids, strength of materials, thin slabs and shells, surveying, building materials, cements, measuring appliances and general tables are contained in Volume 1. Volume 2 deals with soil mechanics, site investigation, foundations, hydraulic power plants (for example, dams, conduits and turbines), overhead transmission lines, tunnels and tunnelling practice, land drainage, hydraulics of rivers and canals, coastal engineering and explosives. Structural theory and practice relating to metal (including welding and welded construction), concrete, timber and brick and masonry construction, including buildings and bridges, is the subject of Volume 3. The fourth volume is concerned with waterways, highways, railways, airports, harbours and docks, water supplies, sewerage, civil engineering in town planning, works construction, specifications and quantities, fire protection of buildings, legal notes and patents (British). Extensive revision of the contents of the previous edition has been carried out, and the chapters on hydraulic power plants, overhead transmission lines, structural concrete, prestressed concrete, masonry and brickwork and aesthetics of bridge design are new.

It is surprising to find that such an important topic as the plastic theory of the ultimate load-carrying capacity of ductile metal structures is confined to a few non-mathematical paragraphs in the chapter on steel building structures, in view of the detailed treatment of many other aspects. (The chapter on the theory of structures is devoted entirely to an attempt at a comprehensive treatment of the various so-called methods of analysing frameworks in the elastic condition, and is thoroughly conventional.).

With the exception of the chapters on surveying, materials and measuring appliances and the tabulated data, the value of Volume 1 seems questionable. Almost any attempt to deal with subjects such as mathematics and mechanics adequately and in directly useful form for the engineer, within the compass of a single chapter as in this volume, would meet with very limited success. Moreover, there is no difficulty in obtaining good text-books in English on basic aspects of the engineering sciences. Works on engineering design and practice are much less common, however, and it would appear that the value of this book as a whole lies more in its presentation of this kind of matter than in the abbreviated accounts of theory which it contains. Even though a book of this kind requires fairly frequent revision (at least every ten years) to take account of progress, the appearance of a second edition is some indication of its appeal to the practising engineers for whom it is written.

T. M. CHARLTON

THE BIOLOGY OF MICRO-ORGANISMS

An Introduction to the Biology of Micro-organisms

By Lilian E. Hawker, A. H. Linton, B. F. Folkes and M. J. Carlile. Pp. 452+50 plates+48 figures. (London: Edward Arnold (Publishers), Ltd., 1960.) 35*s*. net.

THE study of micro-organisms is wide-ranging and rapidly developing. This is partly because of the increasing recognition of their economic importance as agents in the manufacture of useful products, in soil fertility, and as agents of disease and spoilage; and partly because the study of microbial physiology has much to contribute to cell physiology in general. The rapid rates of reproduction of many micro-organisms make them extremely valuable tools for the study of many phenomena of general biological importance.

There are very few books which attempt to give between one pair of covers a conspectus of the morphology, physiology and ecology of the major groups of micro-organisms. Therefore this book serves a very useful purpose as a general introduction to this wide subject, without attempting to be a complete text-book of microbiology (if such be possible). It is divided into three sections: (1) morphology and life-cycles of micro-organisms, with chapters on bacteria, fungi, protozoa and slime-moulds, algae, viruses (148 pp.); (2) physiology and metabolism (118 pp.); (3) ecology of micro-organisms, including the microbiology of soil, air and water, animal and plant pathogens, the microbiology of food and other commercial products, and industrial fermentations (140 pp.). There are an appendix (20 pp.) on some techniques used in the study of micro-organisms, and an index (26 pp.).

The book is well illustrated with many photographs and diagrams. Each chapter ends with a well-chosen list of books and articles for further reading. The book should be very useful to undergraduate students of microbiology and related fields; and to postgraduates in other sciences who wish to have in convenient form a concise survey of microbiology—of the biological forms concerned, their physiology, and the numerous fields of microbial activity. The book is well printed and well produced, and is extremely good value, by modern standards, for its modest price.

B. C. J. G. KNIGHT

WORLD-WIDE CIRCULATION OF AIR WITHIN THE STRATOSPHERE

By P. GOLDSMITH
Atomic Energy Research Establishment, Harwell
AND
F. BROWN
Atomic Weapons Research Establishment, Aldermaston

Introduction

IN 1949, Brewer[1] postulated a meridional circulation in the stratosphere to account for the low humidity measured in the lower stratosphere over southern England. At that time, the humidity measurements had been made up to heights of 42,000 ft.[2,3] using *Mosquito* aircraft of the British Meteorological Research Flight fitted with the Dobson–Brewer frost-point hygrometer[2]. These results showed that the humidity mixing ratio invariably fell rapidly with increasing height just above the tropopause. There was also some evidence, in the case of low tropopause heights, that the mixing ratio levelled off at a value of about 0·002 gm./kgm. at heights well within the stratosphere. The humidity measurements were later extended to heights of 50,000 ft. using *Canberra* aircraft[4,5]. These further data confirmed the earlier work and showed that the humidity mixing ratio steadied off at a value of about 0·002 gm./kgm., corresponding to a frost-point of about – 80° C. at 50,000 ft. This gave further support to Brewer's hypothesis that air rises through the equatorial tropopause, which acts as a cold trap since the air temperature there is about – 80° C. This dry air then moves horizontally polewards and finally sinks in the middle and high latitudes.

Dobson[6] extended this circulation model to account for seasonal variations in the total ozone over temperate and polar regions. He suggested that the cold pool over the winter pole stores ozone-enriched air which arrives by the meridional circulation from ozone-producing regions. This air subsides into the lower stratosphere of temperate latitudes in the late winter or spring and so accounts for the spring peak in the total ozone observed there.

The spring maximum of fission product fall-out from nuclear weapon tests was first attributed to the 'Dobson–Brewer' circulation by Stewart *et al.*[7]. The peaks in the fall-out distribution at temperate and high latitudes were also explained by this meridional circulation feeding the radioactive debris into the lower temperate and polar stratosphere where it becomes available for transfer into the troposphere.

However, some recent observations of the humidity and the tritium concentrations at heights of about 90,000 ft. over England[8] show that the 'Dobson–Brewer' circulation does not reach these heights. Also, Feely and Spar[9] have found that the world-wide distribution of the unique tracer tungsten-185 in the stratosphere up to 70,000 ft. is best explained by lateral turbulent mixing, rather than by large-scale meridional flow.

It is the purpose of this article to discuss these recent data and to describe a modified 'Dobson–Brewer' stratospheric model which is not only consistent with these new results but is also in accordance with other data on the distribution of radioactive debris, water vapour and ozone within the stratosphere.

Humidity Data

Measurements of the humidity of the air, the tritium content of the water vapour, the carbon-14 abundance of the carbon dioxide, together with the deuterium/hydrogen ratio of the hydrogen of the water vapour and the carbon-13/carbon-12 and oxygen-18/oxygen-16 ratios of the carbon dioxide have been made in recent years[8] at altitudes of 80,000–100,000 ft. over southern England. These measurements were made by collecting samples of the carbon dioxide and water vapour of the stratospheric air by means of a cooled vapour-trap, which was carried to these heights by balloon. From the relative amounts of water vapour and carbon dioxide collected, the humidity mixing ratio of the stratospheric air was deduced.

Table 1. HUMIDITY RESULTS ON FLIGHTS OVER SOUTHERN ENGLAND

Date	Height (ft.)	Humidity mixing ratio (gm./kgm.)
2.5.58	89,000	0·044
13.5.59	91,000	0·045
5.6.59	80,000	0·009
15.6.59	93,000	0·049
18.6.59	90,000	0·077
7.7.60	89,000	0·034
11.9.60	99,000	0·035

On the flight of 18.6.59, the balloon ascended through a thin layer of cloud, which may have resulted in water being deposited directly on to the sampling apparatus. This may account for the comparatively high humidity found on that flight.

The one measurement at 80,000 ft. on 5.6.59 is the driest (0·009 gm./kgm.) and may mean that a transition from dry air below to moist air above occurs at about this height.

It is not proposed to compare these humidity data with other measurements in great detail since this has already been done for the 1958 result[10]. However, since that publication, not only have the subsequent flights confirmed the presence of moist air above 80,000 ft., but also much independent evidence in broad agreement with these results has been accumulating. Measurements[11,12] on the infra-red spectrum of the Sun using spectrometers carried to altitudes of about 90,000 ft. by balloon have been interpreted to confirm the presence of moist air above a comparatively dry lower stratosphere. Similar measurements[13], using infra-red spectrometers borne by aircraft at heights up to 65,000 ft., lead to the same interpretation. Automatic frost-point hygrometer balloon soundings[14] up to 100,000 ft. show a similar humidity profile.

These humidity data are inconsistent with the thesis that the stratosphere above 80,000 ft. over middle latitudes receives air via a 'Dobson–Brewer' type of circulation.

Tritium Concentration

The tritium concentration of the air found over England between 1956 and 1960 using the vapour-trap technique[8] is reproduced in Fig. 1. This tritium

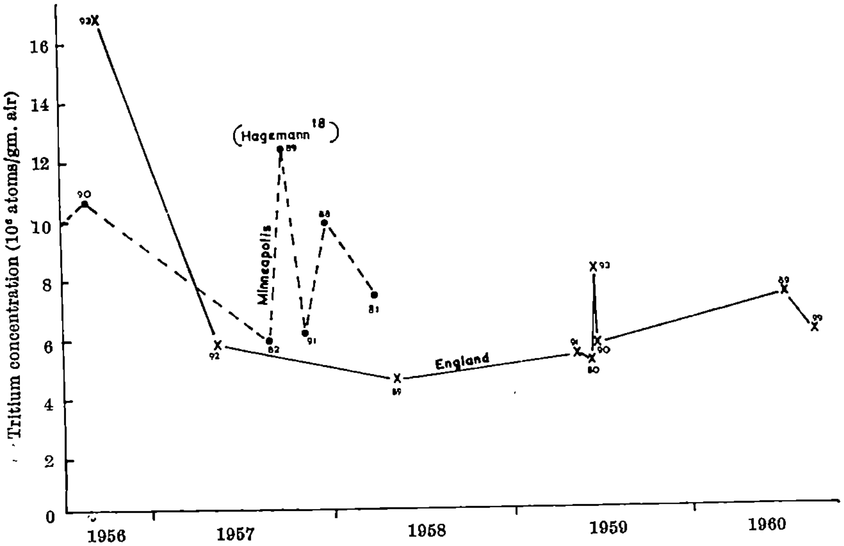


Fig. 1. Variation in the concentration of tritium above 80,000 ft.

was all contained in the hydrogen of the collected water vapour. The figures by each point give the height of the sample in thousands of feet. Also plotted are the only other comparable measurements[18] made at similar latitudes (over Minneapolis, United States) using a somewhat different technique. The bulk of this tritium has been produced during thermonuclear explosions.

Fig. 1 shows that the concentration of tritium over England since the termination of weapon testing in the autumn of 1958 has been remarkably steady and any possible large variation must have been of short duration between sampling times. The fact that the tritium concentration at 90,000 ft. has not fallen during this period suggests that the air at these heights exchanges only slowly with that at lower altitudes. This is consistent with a 'Dobson–Brewer' meridional circulation only if circulation reaching these heights is very slow. The associated humidity data, however, show that the dry circulating air does not reach 90,000 ft.

Tungsten-185

Evidence that the 'Dobson–Brewer' model is not fully compatible with the world-wide distribution in the stratosphere of late 1959 of the unique tracer tungsten-185, has recently been published by Feely and Spar[9]. The tungsten-185 was injected into the lower equatorial stratosphere at a latitude of about 12° N. during mid-1958. The maximum tungsten-185 concentration has remained at the latitude of injection near the equator above 70,000 ft., while the transfer into the lower stratosphere of higher latitudes has been quite rapid. Within one or two months of injection, tungsten-185 was found in the stratospheric debris falling out at temperate regions[15,16]. The height of maximum tungsten-185 concentration decreased progressively with increasing latitude, while the concentration compared with that at the injection latitude similarly decreased. Feely and Spar[9] suggest that lateral turbulent mixing with the possibility of some gravitational settling can best explain this tungsten-185 distribution. However, this explanation does not account fully for the observed ozone distribution, or the occurrence and maintenance of high humidities at 90,000 ft. compared with 50,000 ft.

The Requisites of a Stratospheric Model

An acceptable model of the stratosphere must be consistent with the following information pertaining to the stratosphere: (*a*) the reported world-wide distributions within the stratosphere of the following tracers: (i) tungsten-185, (ii) strontium-90, (iii) carbon-14, (iv) ozone; (*b*) the high humidities at 90,000 ft. as compared with those at 50,000 ft. in the temperate stratosphere; (*c*) the lack of a decline since 1958 in the tritium concentration in the middle stratosphere over England; (*d*) the latitudinal distribution of the radioactive fall-out of stratospheric origin reaching the ground; (*e*) the spring peak of fall-out and total ozone in temperate and polar zones; (*f*) the rapid transfer of material from the lower equatorial stratosphere to that of temperate zones, and the much greater times required for the rhodium-102 debris from rocket detonations above 100,000 ft. in the equatorial stratosphere to reach the lower extra-tropical stratosphere; (*g*) the various residence times

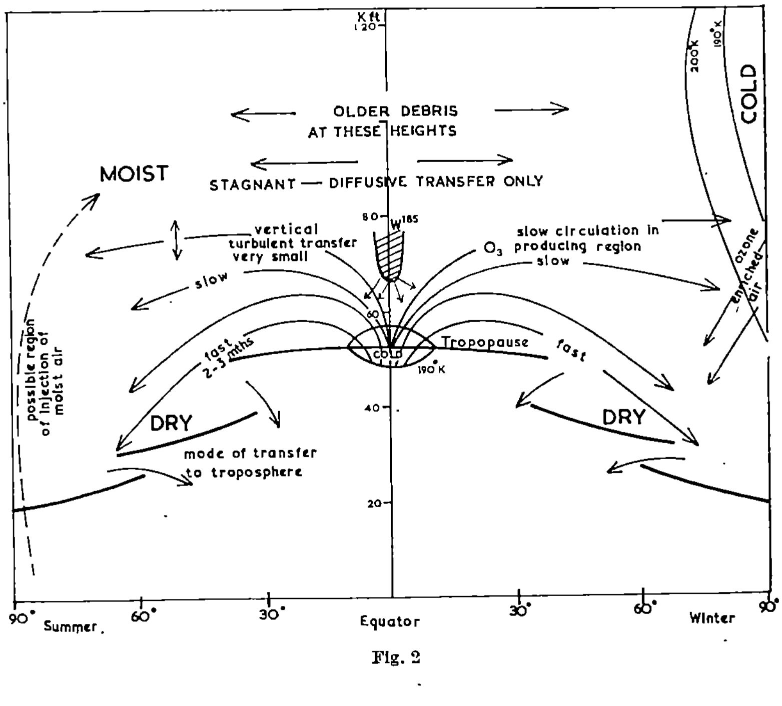


Fig. 2

of stratospheric nuclear debris depending on the latitude and height of the source.

The Proposed Model

In Fig. 2 is sketched the essentials of a model, basically the 'Dobson–Brewer' circulation with a limitation in height, which meets all the above requirements. The rising air at the equator does not reach great heights, but turns polewards almost immediately. The higher branches of this circulation reach heights of only about 70,000–80,000 ft. It is postulated that the meridional circulation is quite fast just above the tropopause, this air taking not more than two months to reach temperate zones, while the upper flow is very much slower. This slow-flowing air, therefore, stays in the ozone-producing layers for periods of about a year and slowly feeds air enriched with ozone into the polar night, where it becomes available for transfer into the lower temperate stratosphere by the 'Dobson' mechanism.

The stratosphere above about 80,000 ft. is considered to be stagnant as regards large-scale meridional and vertical air movements, and a region where small-scale turbulent diffusion may be the important means of meridional transfer. This stagnant region is moist and since the humidity gradient is probably steep in the region of the transition to the circulating air, turbulent vertical mixing must be very small, otherwise higher humidities would be observed at lower altitudes.

It is evident that some water must be lost from this stagnant region by the limited vertical mixing and during the subsidence of air from the cold pool over the winter pole. It is tentatively suggested[6] that the summer polar regions may be the sources of replacement for this water. The possibility that thermonuclear explosions themselves are the main cause of the high humidity of the middle stratosphere is considered to be unlikely. It would mean that something in the order of 4×10^9 tons of water, depending on the lateral extent of the moist layer, were lifted to these heights during the explosions. An expenditure in energy of 2,000 megatons trinitrotoluene equivalent is required to evaporate this quantity of water, that is, more than an order of magnitude greater than the published[17] energy release of all nuclear bursts over water (64 megatons equivalent).

Note added in proof. The suggestion that rising moist air over the summer polar regions may be the source of the high humidities in the middle stratosphere now appears to be doubtful. Measurements made this summer by the Meteorological Research Flight (W. T. Roach, private communication) of the frost point of the air at about 45,000 ft. at lat. 80° N., showed the air there to be dry. In view of the dry air found at all latitudes at heights of about 50,000 ft., it is difficult to see where, on the Earth's surface, the moist air of the middle stratosphere can originate. The possibility of an extra-terrestrial source, such as a high flux of low-energy protons from the Sun followed by oxidization must be given serious consideration (C. M. de Turville, *Nature*, 190, 156 (1961)).

Discussion

This model is now shown to account satisfactorily for all the broad features concerning stratospheric circulation listed above.

(*a*) (i) *Tungsten*-185: The tungsten-185 data[3] are quite consistent with the circulation pattern outlined in this article. The bulk of the tungsten-185 is in the stagnant regions, the maximum concentration therefore remaining at the injection latitude. The lower fast circulation has been provided with tungsten-185 by possible gravitational settling of the larger particles or by some turbulent mixing from above and by that part of the injection put directly into the lowest part of the equatorial stratosphere.

(ii) *Strontium*-90: The vertical distribution of strontium-90, which showed a maximum concentration above 70,000 ft. at all latitudes in 1959[9], is explained because this distribution is the result of numerous injections, since 1952, made at several northern latitudes. That part of the debris placed in the stagnant stratosphere has been spread laterally by the slow diffusion processes. The strontium-90 debris injected between the tropopause and the lower limit of the stagnant stratosphere has been carried to high latitudes by the circulation and so was in a position to transfer to the troposphere comparatively quickly.

(iii) *Carbon*-14 : The world-wide distribution in 1955 of artificially produced carbon-14 (ref. 18) similarly showed a maximum concentration above 80,000 ft. at all latitudes, with small concentrations in the lower equatorial stratosphere. This again is in full agreement with the model shown in Fig. 2.

(iv) *Ozone*: Ozone is produced naturally by photochemical action, mainly in the middle stratosphere. Sinking air in the lower stratosphere is therefore generally rich in ozone while rising air is deficient. The low ozone concentrations in the lower equatorial stratosphere[19,20] indicate rising air there. Similarly, the high concentrations in the lower temperate stratosphere during the late winter or spring[19,20] are indicative of subsiding air. The limited circulation model, like that of Dobson and Brewer, is in full agreement with these general conclusions.

(*b*) Following the subsidence of moist air from high over the winter pole during the late winter and spring, higher humidities would be expected in the lower temperate stratosphere, together with the higher ozone concentrations which account for the spring peak in the total ozone. The Meteorological Research Flight humidity measurements at 48,000 ft. over southern England do not appear, at first sight, to be in agreement with this requirement. However, it must be remembered that the aircraft frost-point hygrometer is probably not accurate to better than 2° C. when measuring frost-points of about – 80° C. There is also some evidence that it may read systematically low[21]. A study of the actual measured frost-points reported at 48,000 ft. over England[4,5] show that the humidity mixing ratio was generally 0·0015 gm./kgm., while on more than 10 per cent of occasions it was more than 0·003 gm./kgm. If a value of 0·0015 gm./kgm. were taken as the humidity mixing ratio of the air reaching the lower temperate stratosphere via the lower fast direct flow, and if 0·04 gm./kgm. as found on the balloon flights[8] were taken for that arriving from altitudes above 80,000 ft. via the polar regions, then for the actual humidity in the temperate lower stratosphere to remain within the range of uncertainty of the aircraft frost-point hygrometer, at a value of 0·003 gm./kgm., say, only about one-twentieth of the air in the lower temperate stratosphere can have come from high altitudes.

A similar calculation for the ozone concentration using the mean data on the vertical distribution given by Ramanathan and Kulkarni[19] is in good agreement with this conclusion. A value of $4{\cdot}5 \times 10^{-7}$ gm./gm.

is taken for the ozone concentration of the low direct flow, 9×10^{-6} gm./gm. is taken for that pertaining to the air at 90,000 ft., while that at 50,000 ft. over middle latitudes during the spring peak is 9×10^{-7} gm./gm. These values lead to a conclusion that one-eighteenth of the air in the latter region can have originated at 90,000 ft.

(*c*) The tritium concentrations within the middle stratosphere over England[8] show that the transfer of air from these heights to lower altitudes is slow. The replacement of tritium by lateral mixing has been at least as great as that lost by vertical transport since the termination of weapon testing in 1958.

(*d*) The explanation by Stewart *et al.*[7] of the peaks in the fall-out of radioactive material of stratospheric origin reaching the ground in temperate and polar regions[7,22,23] is still valid. The circulation has fed debris from the lower equatorial stratosphere to that of temperate latitudes where conditions are more favourable for air transfer from stratosphere to troposphere. Staley[24] has shown that conditions favourable for this transfer are not uncommon at latitudes greater than 30°. Aircraft measurements[25] of the humidity and ozone distribution in the region of discontinuities in the tropopause have demonstrated that the transfer of air from stratosphere to troposphere is particularly strong in these regions. Transport along the isentropic surfaces often passing through such tropopause gaps is probable[26].

(*e*) The smaller quantity of fall-out in the southern hemisphere[22,23] can be explained by the fact that no significant injections of nuclear debris have been made south of the equator. The material placed in the lower northern equatorial stratosphere has been quickly transferred by the circulation to higher latitudes to the north. However, that debris produced in the stagnant stratosphere would be expected to diffuse to the south as well as the north. The debris at these heights can then feed the upper arms of the circulating air of the southern hemisphere and accounts for the comparatively small amounts that have fallen there.

The spring peak in the radioactive fall-out reaching the ground[27,28] and the total ozone[6] in extratropical regions are still considered to be the result of the subsidence of air from high over the winter pole. The sudden breakdown of the winter polar vortex may be associated with the onset of this subsidence[29].

(*f*) In August 1958, rhodium-102 was placed directly into the high equatorial stratosphere at heights of more than 100,000 and 250,000 ft. in rocket detonations. This rhodium-102 first became apparent[15] in ground air at Argonne, United States (42° N.), some thirteen months after its production in the upper atmosphere. This contrasts with a delay of 1–2 months[15,16] found for tungsten-185 injected lower in the equatorial stratosphere.

Although the presence of rhodium-102 is so far unsupported by other observers, this type of behaviour for high-altitude equatorial debris, compared with that from the lower stratosphere, is to be expected on the limited circulation model. The tungsten-185 debris is in the lower fast stratospheric circulation and so is transferred to middle latitudes quickly. The onset of the rhodium-102 in the ground air occurred in September. This suggests that subsiding polar air was not the source of this rhodium-102, but that the meridionally circulating flow was the mode of transfer to the lower temperate stratosphere. The upper arms of the circulation could have received the rhodium-102 by gravitational settling and by the limited turbulent vertical mixing from above. A steady increase in the rhodium-102 for the six months following its first detection in ground air suggests that the upper circulating air may already be sinking at these latitudes.

The difference of about one year in the time required for material from the two equatorial sources to reach temperate zones indicates the order of the difference in speed between the upper and lower arms of the circulation pattern.

The work of Burton and Stewart[29] on the concentration of natural radium *D* and radium *F* in the troposphere and lower stratosphere indicates a mean circulation from low to temperate latitudes in the lower stratosphere of about six months. This agrees well with the evidence in favour of a few months for the lower flow and about a year for the higher circulation.

(*g*) The most recent estimate of less than one year[32] for the stratospheric half-residence time is considered to refer to the mean life of the debris placed in the lower equatorial stratosphere. The shorter residence time[27,31,32] for material injected at high latitudes is not surprising, since when these injections are in the lower stratosphere, they simply by-pass the meridional circulation and are in regions favourable for air transfer from stratosphere to troposphere.

Equatorial debris above 80,000 ft. will have a much longer residence time and it may be that Libby's original estimate[33] of 10 ± 5 yr. applies to this material.

It might be expected that debris resulting from high-altitude events at high latitudes would have a shorter residence time than the equivalent equatorial debris, since it is produced directly in or near those regions scavenged annually by the 'Dobson' mechanism. Its actual residence time, however, will depend on the amount of mixing taking place toward the equator in the stagnant regions of the stratosphere over polar areas.

Concluding Remarks

Murgatroyd and Singleton[34] have calculated meridional and vertical velocities in the stratosphere by considering the transfer of heat from the radiational sources to the sinks in the stratosphere. Their findings are not inconsistent with the limited circulation pattern presented here. They do indicate that above 90,000 ft., in spring and summer, there is often a region with no meridional or vertical flow, while in winter there is a slow descending circulation toward the poles. The velocities given for this meridional flow show a decrease with increasing height in the lower stratosphere. This work also shows the presence, above the stagnant region, of very strong circulations.

It is not considered that the model sketched in Fig. 2 is invariable. It may be that the height of transition between meridional circulating to meridional stagnant air is variable in time and latitude. It is possible that the spring peaks are associated with a change in the height of this boundary in the polar regions. Measurements of the humidity in the lower stratosphere directly over the poles would be of considerable interest.

It would seem that the equatorial stratosphere does act as an efficient storage reservoir of radioactive material injected there during the course of weapon testing, if those injections are made at heights greater than about 80,000 ft. Lower than this, the stratospheric circulation is very effective at transferring material from equatorial to temperate latitudes. It may be that short-lived fission products from medium-sized events at these heights add more to the fall-out reaching the ground than those from much larger injections at greater heights.

[1] Brewer, A. W., *Quart. J. Roy. Met. Soc.*, **75**, 351 (1949).

[2] Brewer, A. W., Cwilong, B. M., and Dobson, G. M. B., *Proc. Phys. Soc.*, A, **60**, 52 (1948).

[3] Bannon, J. K., Frith, R., and Shellard, H. C., *Meteorological Office, Geophys. Memoirs*, No. 88 (1952).

[4] Murgatroyd, R. J., Goldsmith, P., and Hollings, W. B. H., *Quart. J. Roy. Met. Soc.*, **81**, 533 (1955).

[5] Helliwell, N. C., Mackenzie, J. K., and Ketley, M. J., *Quart. J. Roy. Met. Soc.*, **83**, 257 (1957).

[6] Dobson, G. M. B., *Proc. Roy. Soc.*, A, **236**, 187 (1956).

[7] Stewart, N. G., Osmond, R. G. D., Crooks, R. N., and Fisher, Miss E. M. R., *Atomic Energy Res. Est. Rep.* 2354 (1957).

[8] Brown, F., Goldsmith, P., Green, H. F., Holt, A., and Parham, A. G., *Tellus* (in the press).

[9] Feely, H. W., and Spar, J., *Nature*, **188**, 1062 (1960).

[10] Barclay, F. R., Elliott, M. J. W., Goldsmith, P., and Jelley, J. V., *Quart. J. Roy. Met. Soc.*, **86**, 259 (1960).

[11] Murcray, D. G., Murcray, F. H., Williams, W. J., and Leslie, F. E., *J. Geophys. Res.*, **65**, 3641 (1960).

[12] Murcray, D. G., Murcray, E. H., and Williams, W. J., *Univ. of Denver, Final Report Contract AF* 19 (604)-2049 (1960).

[13] Lab. of Astrophys. and Phys. Meteor., *Prelim. Rep. Solar Observations from a U-2 Observatory* (Johns Hopkins Univ., 1960).

[14] Mastenbrook, H. J., and Dinger, J. E., *N.R.L. Rep.* 5551 (1960).

[15] Gustafson, P. F., and Kerrigan, M. A., *Argonne Nat. Lab. Rep.* 6199 (1960).

[16] Walton, A., *Nature*, **188**, 220 (1960).

[17] United Kingdom Min. of Defence, press release (May 5, 1959).

[18] Hagemann, F., Gray, J., Machta, L., and Turkevich, A., *Science*, **130**, 542 (1959).

[19] Ramanathan, K. R., and Kulkarni, R. N., *Quart. J. Roy. Met. Soc.* **86**, 144 (1960).

[20] Paetzold, H. K., a paper presented at the Symp. Atmos. Chem. and Radioactivity, Int. Assoc. Met. and Atmos. Phys., Helsinki, 1960.

[21] Murgatroyd, R. J., and Goldsmith, P., *Prof. Notes, Met. Office*, London, **7**, No. 119 (1956).

[22] Crooks, R. N., Osmond, R. G. D., Fisher, Miss E. M. R., Owers, M. J., and Evett, T. W., *Atomic Energy Res. Est., Rep.* 3349 (1960).

[23] Lockhart, L. B., Patterson, R. L., Saunders, A. W., and Black, R. W., *N.R.L. Rep.* 5528 (1960).

[24] Staley, D. O., *J. of Met.*, **17**, 591 (1960).

[25] Murgatroyd, R. J., a paper presented at the I.U.G.G. Symp. on Atmospheric Ozone at Oxford (1959).

[26] Reed, R. J., and Danielson, E. F., *Arch. Met. Geophys. Biokl. A.*, **11**, 1 (1959).

[27] Peirson, D. H., Crooks, R. N., and Fisher, Miss E. M. R., *Atomic Energy Res. Est. Rep.* 3358 (1960).

[28] Gustafson, P. F., Brar, S. S., and Kerrigan, M. A., *Science*, **133**, 460 (1961).

[29] Burton, W. M., and Stewart, N. G., *Nature*, **186**, 584 (1960).

[30] Godson, W. L., *Quart. J. Roy. Met. Soc.*, **86**, 301 (1960).

[31] Feely, H. W., *Science*, **131**, 645 (1960).

[32] Martell, E. A., *Science*, **129**, 1197 (1959).

[33] Libby, F. W., *Proc. U.S. Nat. Acad. Sci.*, **45**, 738 (1957).

[34] Murgatroyd, R. J., and Singleton, F., *Quart. J. Roy. Met. Soc.*, **87** (1961).

ORIGIN OF THE BRITISH INSULAR RACES OF SMALL MAMMALS AND OF THE 'LUSITANIAN' FAUNA

By G. B. CORBET

Department of Zoology, British Museum (Natural History), London, S.W.7

In the early years of this century, Hinton and Barrett-Hamilton[1] put forward a theory which asserted that the populations of Microtine rodents in the Orkney, Hebridean and Channel Islands were relict forms, once present throughout mainland Britain in post-glacial times but displaced by later arrivals from the south. These island populations were believed to be most closely related to certain forms found in the Pyrenees, Alps and in Scandinavia, which were also considered to be relict. The principal evidence supporting this theory was the morphological resemblance between the island and alpine populations, and the presence of similar forms in Late Pleistocene deposits in southern England.

A re-assessment of the problem in the light of extensive new data, both geological and zoological, suggests that the majority of these island populations are not relict, but must be attributed to introduction by human agency. The relevant geological facts, now established independently of zoological evidence[2], are that at the maximum of the last glacial phase the ice extended as far south as Norfolk and South Wales at sea-level; the rest of Britain south of the ice consisted of tundra; and the maximum eustatic lowering of the sea-level was about 70–100 metres, that is, 35–50 fathoms. As an actual depression of the shore-line, this only applies to the unglaciated south. In the north it was accompanied by a very considerable isostatic depression of the land surface so that the depression of the shore-line was very much less; and it is therefore almost certain that the Shetlands, Orkneys and Outer Hebrides have never been connected to the mainland during or since the last glacial phase.

The fossil deposits said to support the relict theory (from fissure deposits at Ightham in Kent) are now seen to represent a very long period of interstadial and late glacial time, and provide no evidence of the presence of the 'relict' types, *Microtus corneri* Hinton and *Clethrionomys kennardi* Hinton, in the post-glacial colonization.

Extensive measurements of island and mainland voles, to be published separately, have now shown that the basic characters distinguishing the island forms are very few and merely represent extremes in the ranges of variation found on the mainland. These data do suggest the presence of a relict form of *Microtus*, but not of *Clethrionomys*, on the mainland. The *Microtus* from the north-west Highlands are believed to represent a relict population, of which the distinctive features (large size and complex first upper molars) have been replaced farther south to a variable extent by interbreeding with a later arrival (*M.a.hirtus* of the relict theory). The principal dividing line seems to be the Great Glen.

Theory of Human Introduction

In the case of those islands separated from the mainland by deep channels, the geological evidence alone is sufficient to show that all the mammalian species must have been introduced; but in order to distinguish between indigenous and introduced species on the more inshore islands, we must consider also the order in which the species colonized the country, and their chances of subsequent extinction on an island. The three rodent species, *Microtus agrestis*, *Clethrionomys glareolus* and *Apodemus sylvaticus*, are

Table 1. Distribution of Small Mammals on the Islands Discussed in the Text

	Microtus arvalis	*Microtus agrestis*	*Clethrionomys glareolus*	*Apodemus sylvaticus*	*Sorex araneus*	*Sorex minutus*	*Crocidura suaveolens*	*Crocidura russula*
Shetlands				+				
Orkneys	+			+		+		
Lewis*, Barra*, Man, Ireland				+		+		
Uist*, Eigg†, Muck†		+		+		+		
Raasay†, Skomer			+	+	+	+		
Mull†, Bute†		+	+	+	+	+		
Skye†, Islay†, Jura†, Gigha†, Arran†		+		+	+	+		
Scillies				+			+	
Jersey			+	+	+		+	
Guernsey	+			+				+

* Outer Hebrides. † Inner Hebrides.

characteristic of open grassland, scrub and closed woodland respectively. This distribution is related to their requirements of food and cover, which in turn are controlled by potential competition with each other. In northern Europe, *M. agrestis* alone occurs on the open tundra, *C. glareolus* appearing farther south, and *A. sylvaticus* yet farther south in the forest zone, whereas in Britain a similar sequence occurs in the transition from high to low altitude. It is therefore reasonable to assume that these three species would advance in the order quoted above at the time of the post-glacial colonization, with *M. agrestis* well ahead of the other species.

Many islands lack one or more of these species (Table 1). Because of their distribution by habitat, extinction due to climatic deterioration would affect *Apodemus* first and *Microtus* last. The chances of *Microtus* alone becoming extinct on any but the smallest islands are very small. The islands with which we are concerned are mostly large; for example, Lewis (870 sq. miles) and Raasay (25 sq. miles). Further, many of these islands lack the ground predators such as fox, cat, *Mustela* spp. and the adder *Vipera berus*, which are probably important enemies of *Microtus* and could conceivably cause extinction if the population was already very low.

Thus it can be postulated that the absence of *Microtus agrestis* from any large island indicates isolation since a time when the climate was severely arctic, and that any members of the fauna of the island which are characteristic of a more temperate climate must have reached the island after separation.

It is generally agreed that mice, including *Apodemus*, have been introduced by human agency; for example, to St. Kilda and Iceland. Trade among the British islands has taken place at least since mesolithic times and has involved the carriage of cattle and sheep (with accompanying fodder), building stone, timber, peat and roofing turf, all of which could have harboured voles and shrews as well as mice. Such trade is more extensive to small islands, which are deficient in natural resources, than to large ones like Ireland.

The difference of environment between mainland and island is often slight in terms of physical and botanical factors, but is vast in terms of mammalian neighbours; there are about 33 species of indigenous terrestrial mammals in northern France, 25 on the English mainland, and none on most of the islands. In particular, large size, the chief characteristic of most island forms, could have evolved rapidly due to the absence of ground predators. It is likely that the size of any species of small mammal is controlled by the interplay of opposing selective forces, intraspecific selection favouring large size, the presence of ground predators favouring small size, that is, the ability to escape into holes inaccessible to the predator. Rapid evolution would also be facilitated by the growth of a population from perhaps a single litter, allowing great possibilities of random divergence in the early stages of colonization. Examples of rapid evolution after undoubted introduction are provided by *Apodemus sylvaticus* on St. Kilda, and *Mus musculus* on North Bull Island in Dublin Bay, where a distinctive colour variety arose within a century[3].

Origin of the Individual Races

Microtus arvalis. This species is confined to Orkney and Guernsey, being absent from all the numerous western islands that are no more isolated, but are yet without *M. agrestis*. In fact there is no evidence, apart from these two cases, that it has ever occurred in the British Isles in post-glacial time. Orkney is separated from the mainland by a channel of 30–40 fathoms and its fauna as a whole suggests very early isolation. For example, there are no stoats, *Mustela erminea*. This species is widespread in the Arctic and is unlikely to have lagged behind its prey during the colonization, but equally unlikely to have become extinct on six separate islands in the continued presence of its prey. The *Microtus* themselves are absent from four large islands which are no more isolated and not separated by deeper channels than the others. Introduction from the continent of Europe could have taken place at any time since the Viking era when trade with Denmark, for example, was very extensive. Establishment would be easier in Orkney than in mainland Britain, because of the absence of competitors and ground predators.

The situation on Guernsey is similar. The absence of *M. arvalis* from all the other Channel Islands is difficult to explain on the relict theory since there is no evidence that they were isolated before Guernsey.

Microtus agrestis. The *Microtus* on the Outer Hebrides are clearly introduced since the channel separating the group from the mainland is everywhere deeper than 50 fathoms, which alone precludes contact under post-glacial conditions. Study of *M. agrestis* from the north-west Highlands of Scotland, that is, from north-west of the Great Glen, shows them to possess the complex variant of M^1 almost as universally as do the Outer Hebridean populations. It therefore seems likely that the latter have been introduced from the north-west coast.

The *Microtus* of the Inner Hebrides can be divided clearly into two groups having respectively a complex and a simple M^1 as follows: 'complex'—Skye, Eigg, Islay; 'simple'—Muck, Mull, Gigha, Arran. Those from Skye can be considered endemic since the channel is less than five fathoms deep and the fauna as a whole shows no impoverishment when compared with that

ACADEMIC PRESS New York and London

Announcing . . .

MOLECULAR BIOLOGY

An International Series of Monographs and Textbooks

Edited by NATHAN O. KAPLAN, *Brandeis University, and*
HAROLD A. SCHERAGA, *Cornell University.*

Volume 1

PROTEIN STRUCTURE

By HAROLD A. SCHERAGA

September 1961, 306 *pp.,* 64*s.*

The primary aim of this book is to present some of the quantitative aspects of the physicochemical approach to the protein structure problem, including both gross and internal structure. In discussing gross structure, polymer principles have been applied. In considering the more detailed aspects of internal structure, attention has been directed to interactions between polar groups and nonpolar ones. The material is developed from first principles, assuming only a background of elementary physical chemistry. The book has evolved from a series of lectures given for C. S. I. R. O. in Melbourne, based on research carried out in the author's laboratory during the past decade. (It will be of interest to all students and research workers engaged in the study of the structure and biological activity of proteins. It will also be of value to workers in other biological sciences in which the principles of protein structure and activity are of interest.)

Volume 2

POLYELECTROLYTE SOLUTIONS

A Theoretical Introduction

By STUART A. RICE, *University of Chicago, and*
MITSURU NAGASAWA, *Nagoya University*
With a contribution by H. MORAWETZ, *Polytechnic Institute of Brooklyn*

November 1961, 568 *pp., approx.* 115*s.*

This is the first monograph to deal exclusively with the study of the properties of solutions of polyelectrolytes. Emphasis is consistently placed on the interrelation of theory and experiment, on the construction of simple models for the interpretation, correlation, and prediction of both molecular and thermodynamic properties, and on the information derivable from the general theory of solutions without specific reference to models. The current status of our understanding of the properties of polyelectrolyte solutions is illustrated with carefully selected experimental data.

Volume 3

FLUORESCENCE ASSAY IN BIOLOGY AND MEDICINE

By SIDNEY UDENFRIEND, *National Institutes of Health, Bethesda, Maryland*

December 1961, *about* 450 *pp., approx.* 85*s.*

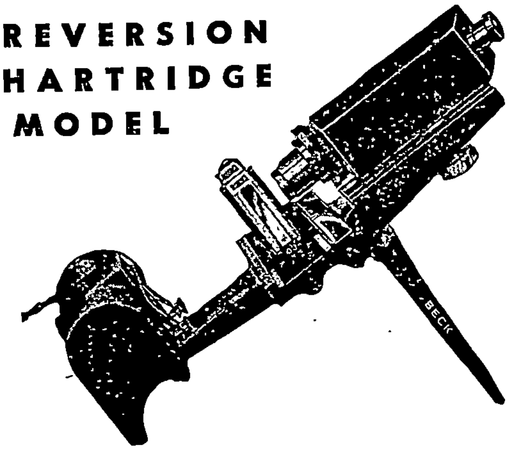
Besides providing a working knowledge of fluorescence theory and practice, this work is intended to convey the wide range of potential applications of this method to structural studies on proteins and other macromolecules, to research into enzyme-coenzyme-substrate interaction, to immunochemistry, to studies on endocrine secretions, and to many other areas of research and testing. "Biology" is used in a broad sense and includes applications in public health and sanitation, food inspection (including quality control), toxicology, detection of insecticide residues, and other specialized fields.

Telephone: WHItehall 6631

111 FIFTH AVENUE, NEW YORK 3 17 OLD QUEEN STREET, LONDON, S.W. 1

BECK SPECTROSCOPE

REVERSION HARTRIDGE MODEL



For all spectrometric work, absorption or emission, the reversion method gives the most accurate results. Readings can be obtained to an accuracy of 1 ÅU.

Full particulars forwarded on application

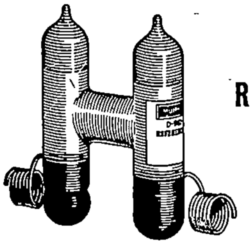
R. & J. BECK LTD.
69/71 MORTIMER STREET, LONDON, W.1

STABILITY WITH TIM

For long-term stable
D.C. Reference Voltages
use

MUIRHEAD

STANDARD CELLS



REFERENCE CELLS

They are Stable, Reliable, Robust, Repeatable—and the price is right. Saturated or Unsaturated types for industrial or academic purposes
WRITE FOR PUBLICATIONS 425 & 430

MUIRHEAD
Precision Electrical Instruments

488

MUIRHEAD & CO. LIMITED · BECKENHAM · KENT · ENGLAND
Phone: BEC 4888
MUIRHEAD INSTRUMENTS LIMITED · STRATFORD · ONTARIO · CANADA
Phone: 271-3880
MUIRHEAD INSTRUMENTS INC · 441 LEXINGTON AVE · N.Y.17 · U.S.A.
Phone: Murray Hill 2-8131

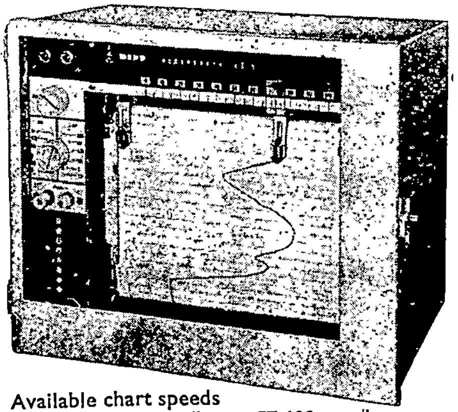
The Most Sensitive Recorder...

An instrument that records down to 50 microvolts full scale without an external amplifier

Micrograph Recorder
BD Series

This compact, ultra-sensitive Kipp Micrograph Recorder, BD Series, with 21 cm chart span, is accurate to within less than 1% of full scale deflection. Response is very rapid, full scale traverse being effected in 0.8 of a second. Up to four channels can be recorded by means of a new channel selector.

LINEARITY :
Error < 0.25% of full scale deflection
DEAD ZONE :
< 0.25% of full scale deflection



Available chart speeds range from 60 mm/hr. to 57,600 mm/hr.

Current range for full scale deflection	Voltage range for full scale deflection
0·1 μA	0·05 mV
0·25 „	0·1 „
0·5 „	0·25 „
1 „	0·5 „
2·5 „	1 „
5 „	2·5 „

Admissible external resistance :
on 0·1 μA range: 500–∞ Ohms
on all other ranges: 0–∞ Ohms

GD 782

Write for fully descriptive leaflet to :

SHANDON

SHANDON SCIENTIFIC COMPANY LIMITED
6 CROMWELL PLACE, LONDON, S.W.7
Tel: KNIghtsbridge 1131

of the mainland. Eigg is much more isolated (by 30–40 fathoms) with an impoverished fauna and therefore it is likely that the *Microtus* have been introduced (from the north-west Highlands). Islay, through Jura, is separated from the mainland (south of the Great Glen) by a shallow channel (5–10 fathoms) and therefore the *Microtus* population is likely to be indigenous. If so, being complex, it must be considered to be truly relict, that is, isolated before the arrival of the 'hirtus' characters. This places it in the same category as the north-west Highland form, except that in this case the isolation is complete instead of only partial. This interpretation is supported by the fact that the complex condition is more extreme and general than in any other population examined.

Muck is well isolated (30–40 fathoms) and M^1 is simple. It therefore seems likely that introduction has taken place either from the southern part of the mainland or from one of the more southern islands (for example, Mull). The *Microtus* of Mull have M^1 almost 100 per cent simple. The island is connected to the mainland on the south side of the Great Glen (through Lismore Island) by a submarine ridge 10–20 fathoms deep. It is therefore conceivable that it could have been populated before separation; but if that had been the case one would expect the population to be complex, as in the case of Islay. The most likely explanation therefore is that the *Microtus* have been introduced accidentally from the southern part of the mainland coast. The remaining islands of Gigha and Arran are probably in the same category. Arran in particular is well isolated (by 20–30 fathoms).

Microtus seem to be absent from the remaining large islands of the Inner Hebrides, namely, Raasay, Rhum, Tiree, Coll and Colonsay, some of which are no more isolated than Mull. To summarize, accidental introduction is postulated for five Inner Hebridean islands: Eigg, Muck, Mull, Gigha and Arran.

Clethrionomys glareolus. The islands of Raasay, Skomer and Jersey are similar and at the same time unique in having a large form of *Clethrionomys* with complex dentition (M^3) and no *Microtus*. The main argument in favour of accidental introduction is that *Clethrionomys* is unlikely to have colonized the country ahead of *Microtus*, but the latter is equally unlikely to have become extinct on the islands while *Clethrionomys* survived, especially in such marginal habitats to which *Microtus* is particularly well adapted. Absence of *Microtus* would favour adaptation to a grassland habitat, in particular adaptation to the tougher but more reliable and abundant food of *Microtus*. The complex dentition of these races represents a condition which is present, but in a smaller proportion of individuals, on the mainland, and the change in incidence of the complex condition could have taken place almost in one step if the colonizing parents happened to be complex. Such an occurrence in an isolated habitat on the mainland has already been recorded[4]. Once the *Clethrionomys* were established in all available habitats, subsequent colonization by *Microtus* would be considerably more difficult. The limitation of this type of *Clethrionomys* to these few islands is explained on the present theory by assuming that these were the islands on which *Clethrionomys* happened to have been introduced ahead of *Microtus*; it is inexplicable by the relict theory.

The *Clethrionomys* on Mull have not developed a complex dentition presumably because they were confined to their normal ecological niche by the prior presence of *Microtus*. If it is accepted that the latter have been accidentally introduced, as argued above, then the same must be said of the *Clethrionomys*.

Apodemus sylvaticus. *Apodemus* is more likely than the voles to be transported accidentally and at the same time is likely to have been a late, woodland colonizer. Its survival on small treeless islands does not contradict this, since there it is free from competition from voles. It is therefore postulated that all the following populations of *Apodemus* have arisen by accidental introduction: Shetland, Orkney, Outer Hebrides, all Inner Hebrides except Bute, Ireland, Isle of Man, Scillies and Channel Islands.

Sorex minutus. This species is very widespread on the islands and seems likely to be indigenous on many of them. This is not unexpected since its present range, extending to an altitude of more than 4,000 ft. in Scotland, suggests that it may well have been in the forefront of the post-glacial colonization. Introduction need only be postulated for Orkney, the Outer Hebrides and perhaps some of the more isolated Inner Hebrides. The presence of *S. minutus* in Ireland could be explained by early post-glacial entry from Scotland, but survival in the south of Ireland during the last glacial phase cannot be ruled out, nor can introduction.

Sorex araneus. From its distribution and present habitat this species appears to have been slightly in advance of *M. agrestis* in its post-glacial spread. Most of the small islands on which it occurs are not deeply separated from the mainland and therefore on these it may be indigenous (Raasay, Skye, Mull, Islay, Jura, Gigha, Bute, Skomer). It is only present on a very small proportion of the more isolated islands (Arran, Jersey) on which it was probably introduced.

Crocidura spp. This genus is present only on the Scilly and Channel Islands. It has a much more southern distribution than any of the others dealt with and is thus likely to have been a late colonizer. It is therefore considered to have been introduced on all these islands. The ubiquity of *C. suaveolens* on the Scilly group has been used as an argument against chance introduction[5]. But its usual habitat, among the kelp on the storm beaches, coupled with the extensive use made of the kelp for manure and reduction to ash, suggest clearly how it could have been distributed among the islands.

Origin of the Irish Fauna

The so-called 'Lusitanian element' in south-west Ireland has been a stumbling-block to all students of the history of the British fauna and flora. Recent authors have been forced to postulate glacial survival on land now submerged off south-west Ireland[6], or very early post-glacial colonization by land[7]. We may ask first why a land link should have allowed passage of such a non-athletic creature as the slug *Geomalacus maculosus*, which typifies the Lusitanian element, and not of such vertebrates as rodents and reptiles which abound in Iberia ? Alternatively, it is inconceivable that the vertebrates but not the invertebrates should have perished in a glacial re-advance. The present distribution of the Lusitanian species, confined to the warmest corner of Ireland, scarcely suggests great tolerance of peri-glacial conditions. On the other hand, trade between Iberia and Ireland has taken place since mesolithic times, and the size and habits of the Lusitanian species predispose them to such a form of transport.

The Lusitanian flora has probably had a similar origin, but in this case there is the additional possibility of carriage by birds which could have taken place at any time in the post-glacial period, Ireland and Iberia lying on a major migration route. In a recent survey of the botanical problem, Godwin[8], who dismisses glacial survival but accepts the idea of early post-glacial colonization by land, lists only one Lusitanian species from an early post-glacial horizon, namely, *Erica mackaiana* from the Atlantic period. This is a species which could easily have been carried by birds.

The absence of some of these species from the climatically similar south-west of England is to be expected, since there they would meet competition from related, indigeneus species which are absent from Ireland (or have only been introduced later from England).

The Irish fauna as a whole is of such an erratic composition as to suggest a series of chance introductions rather than an orderly sequence of arctic, followed by more temperate, faunas, until at some stage the supply has suddenly stopped due to severance of the land connexion. The absence of voles suggests that the last connexion (to Scotland) was severed at a very early climatic stage. However, a narrow channel or wide river would effectively bar small mammals while allowing unrestricted passage to many insects, birds and plants. Thus a south-flowing river could have allowed such selective colonization in the southern Irish Sea region while a truly terrestrial bridge in the north was inaccessible to most species because of the climatic severity.

---

[1] Hinton, M. A. C., *Proc. Geol. Assoc. London*, 21, 489 (1910). Barrett-Hamilton, G. E. H., and Hinton, M. A. C., *Ann. Mag. Nat. Hist.*, (8), 12, 361 (1913). Hinton, M. A. C., *Monograph of the Voles and Lemmings* (Microtinae), 226 (London, 1926).

[2] Zeuner, F. E., *The Pleistocene Period* (London, 1959).

[3] Jameson, H. L., *J. Linn. Soc. (Zool.)*, 26, 465 (1898).

[4] Corbet, G. B., *Proc. Zool. Soc. London* (in the press).

[5] Davis, J. L., *Ann. Mag. Nat. Hist.*, (13), 1, 97 (1958).

[6] Beirne, B. P., *The Origin and History of the British Fauna*, 88 (London, 1952).

[7] Beaufort, L. F., *Zoogeography of the Land and Inland Waters*, 48 (London, 1951).

[8] Godwin, H., *The History of the British Flora*, 345 (Cambridge, 1956).

THE DEVELOPMENT OF THE NEON GLOW LAMP (1911-61)

By PROF. H. E. WATSON
Emeritus Professor of Chemical Engineering, University of London

FOR ten years after its discovery (1898), the world's supply of pure neon was contained in two small tubes inverted over mercury which stood on Sir William Ramsay's mantelpiece, and experiments on its use as a commercial illuminant were scarcely to be expected. In 1908, however, Georges Claude designed an extra stage for his oxygen plant and produced, hourly, 12 litres of a gas containing 45 per cent of mixed helium and neon. While purifying some of this gas in Ramsay's laboratory, I noticed the great ease with which neon conducted the electrical discharge, and, two years later, working in the Cavendish Laboratory, I found that the cathode fall with aluminium electrodes in pure neon was 200 V. This indicated that, provided conditions were such that there was no positive column, a lamp would operate at approximately this voltage. Further measurements of the spark-potential confirmed this, and several experimental lamps were made, some of which would start with 150 V. and run at 85 V. The important feature of all of them was operation on the domestic voltage of 220, although in France and the United States, where the usual value was 110 V., they were not of great interest. A provisional patent was applied for in March 1911, but was not completed as I had to abandon this work on taking up an appointment abroad. These lamps, in which substantially the whole of the light comes from the negative glow, are known as 'glow lamps', or familiarly in recent years 'neons', and a useful property is that the glow follows the shape of the cathode and can thus be used for illuminated signs. In the long, tubular, high-voltage neon lights, the illumination comes from the positive column. These were first shown in public by Claude in 1910.

Experimental glow lamps were made by Dr. D. McF. Moore in the General Electric Laboratories, in Schenectady, from 1914 onwards, but even by 1920 no reliable lamp had been produced which worked on 110 V., and it was decided that this type of illuminant was of no great value. In Germany, on the other hand, the firm of Pintsch A.G. realized that although the efficiency of glow lamps, normally an important criterion, was low, there were many more uses than appeared at first sight. The smallest filament lamps for 220 V. then made were 15 candle-power, taking 20 W., and these were very expensive and fragile; in many cases, such as for nightlights and in mines, they could be replaced advantageously by 3-5 W. glow lamps. These were also valuable as indicators on switchboards. In consequence, their research department, under Dr. F. Schröter, undertook extensive investigations during the War, and in 1918 produced the first commercial glow lamps. These resembled filament lamps in size and were suitable for 220 V. In 1920 Osram G.m.b.H were making similar lamps designed by Dr. F. Skaupy at the rate of 500 a day.

It must be remembered that at that time very little was known about the mechanism of the discharge and that practically all the experimental work was empirical. The early lamps suffered from sputtering of the electrodes, which blackened the tube and absorbed the gas, sometimes in a few hours, while the methods of evacuation and removal of gases from the walls of the tube would now be considered crude. Troubles due to these causes had been met with and largely overcome by Claude and other makers of high-voltage tubes, but their remedies were not all suitable for the production of small lamps in quantity, and much new work became necessary. Freedom from diatomic gases had always been considered essential for preserving the colour of the discharge and obtaining the minimum spark-potential until Pintsch A.G. in 1917 showed that very small quantities of these gases not only did not affect the colour but also lowered the spark potential and reduced sputtering to a quite unexpected degree. Shortly afterwards, N.V. Philips recommended a helium–neon mixture containing up to 5 per cent of argon for the reduction of sputtering, and in 1926, T. E. Foulke at Schenectady, with far more theoretical knowledge at his disposal and after prolonged investigations, confirmed the value of a mixture of this nature.

Meanwhile, efforts were being made to find materials with a low cathode fall which would enable glow lamps to start and run on 110 V. d.c. Skaupy solved this problem in 1917 by coating metallic electrodes with barium azide and afterwards heating them, but simplification and improvement of the process took nearly five years. N.V. Philips in 1921 made tubes with cathodes of magnesium and beryllium alloys which would start below 110 V. and obtained improved results by introducing potassium nitrate. These results aroused great interest in the United States, where Foulke had been studying the effect of gas composition, as mentioned above. He soon succeeded in producing a tube with magnesium electrodes which would start with 70 V., and three years later he had developed two new coating processes now known as the 'oxide' and the 'azide'. The first started with barium carbonate, which was heated in the tube to 1,000° C. or more and activated by a discharge with a steep-fronted wave-form. The second was similar to Skaupy's but a little cæsium was added to the barium and the subsequent processes were strictly controlled. The result was a lamp starting with 45 V. a.c.

During this work, an outstanding design of lamp was evolved. The lamps, named *T*-lamps, were made from tubing ¼-in. or less in diameter, with two parallel wires as electrodes, the overall length being less than 1 in. These miniature lamps, bright enough to be seen as indicators and readily incorporated in instruments, caused an increased demand, but it was not until about 12 years ago in the United States and a few years later in Britain that a remarkable development took place, caused by the introduction of glow lamps into domestic appliances such as electric irons and refrigerators, and later into switches. The demand was far greater than had been foreseen and much ingenuity had to be exercised in developing machinery to carry out what was still a highly complicated process requiring the greatest precision. It is estimated that the world output of neon glow lamps is now about 80 million a year, and is increasing rapidly. Quite 80 per cent of these are of the *T* type. These can be sold for less than a shilling, are remarkably robust and reliable, and will run for their rated life of 25,000 hr. at a cost of twopence.

Within the past few years a demand has arisen for 'high brightness' lamps which can be seen readily in full daylight. This could not be met by minor modifications of existing processes and required a great deal of additional research, one item being a study of lanthanum and its alloys. Satisfactory lamps are now in production in several countries, but research is by no means at an end.

These notes are extracted from a longer account of the development which I have compiled, and it may be mentioned that a very valuable publication has just appeared dealing with the scientific basis of the glow discharge, with 156 references, written by M. P. Lemaigre-Voreaux as a D. ès Sc. thesis presented to the University of Lyons.

OBITUARIES

The Right Hon. the Lord McGowan, K.B.E.

LORD MCGOWAN OF ARDEER, who died on July 13, was honorary president of Imperial Chemical Industries, Ltd., of which he was chairman from 1930 until the end of 1950 and for the creation of which, with Sir Alfred Mond, afterwards Lord Melchett, he was mainly responsible.

Henry Duncan McGowan was born in Glasgow on June 3, 1874, and educated at Allan Glen's School, entering the Glasgow offices of the Nobel's Explosives Co. in 1889 as a junior clerk or office boy. He became assistant successively to the home and to the overseas sales manager, and then, in 1902, deputy assistant sales manager, and in 1909 assistant manager. In December 1915 he was appointed to the board and became primarily responsible for co-ordinating the Company's activities in relation to the requirements of the Ministry of Munitions. In 1918 he was appointed managing director, and in recognition of his services during the War he was created K.B.E. At the conclusion of the War he was responsible for the merger of all the important explosives and ammunition companies in Britain into Explosives Trades, Ltd., of which he became chairman and managing director. Earlier, in 1910, he had used the Hamilton Powder Co. as a nucleus for the amalgamation with three other explosives companies and the Dominion Cartridge Company of Quebec to form Canadian Explosives, Ltd., and in the winter of 1923–24 he also negotiated the formation of African Explosives and Industries, Ltd., in South Africa.

The creation in 1926 of Imperial Chemical Industries, Ltd., by the joint efforts of McGowan and Sir Alfred Mond, out of Nobel Industries, Ltd., Brunner Mond and Co., Ltd., the United Alkali Co., Ltd., and the British Dyestuffs, Ltd., was thus the crowning of his persistent efforts towards amalgamation and rationalization of the chemical industry of Britain, and McGowan became president and Mond chairman of the new Company. Mond (afterwards Lord Melchett), however, died in 1930, and McGowan succeeded him as chairman, and the growth and shaping of the enterprise were very largely his work. By 1938, however, he realized that it had become too large to be managed efficiently from the centre and that the advantages of large-scale production were in danger of being offset by rigidity, and McGowan initiated some degree of decentralization.

By temperament McGowan was something of an autocrat, but he was a good mixer and, working hard and thoroughly himself, he expected the same of others. What mattered was the way the job was done, and with marked ability to grasp the essentials of a problem, he was also swift to see and seize an opportunity. Melchett had been elected president of the Society of Chemical Industry in 1930 in view of the Society's jubilee meeting in London in the following year. On Melchett's death McGowan took his place, and his presidential address to the Society on "Science and Industry" in July 1931, and his Messel Memorial Lecture to the same Society at Cardiff three years later on "The Uneven Front of Research" display very fairly his outlook on science and technology.

He was created a baron in 1937 and received honorary degrees from the Universities of Glasgow (1934), Birmingham, Oxford, Durham and St. Andrews. He married in 1903, Jean Boyle Young, of Paisley, who died in 1952, and he is survived by two sons and two daughters, the title passing to his elder son, Harry Wilson McGowan.

R. BRIGHTMAN

Sir Hugh Warren

HENRY WILLIAM HUGH WARREN was born on June 30, 1891, and died on June 18, 1961. He is survived by Lady Warren and a daughter. An only son was killed serving with the R.A.F. in 1939.

Hugh Warren, in 1929, took over the Research Laboratory at the British Thomson-Houston Co., Ltd., Rugby, which, six years previously, he had helped the late R. C. Clinker to start. In 1938 he was appointed to the board of B.T.H. as director of research. He remained in charge of the Laboratory until 1945, becoming managing director of B.T.H. in that year and managing director of A.E.I., Ltd., in 1948, retiring in 1954. He received honorary degrees from the University of Bristol in 1942 and from Birmingham in 1946, and was knighted in 1951.

It is as leader of what is now the A.E.I. Research Laboratory, Rugby, a job he did for sixteen years, that 'H. W.' will be remembered. He was alert and interested in everything, intensely energetic and always desirous of presenting the work of his Laboratory in as impressive a manner as possible. His own subject was insulations. It was in 1916, on the then vital matter of the insulation of magnetos, that he showed the energy and enthusiasm to engage in, and pursue relentlessly, difficult technological problems that proved to be characteristic of the man. He directed with vigour the pioneer work of his Laboratory on sound reproduction, talking films and discharge lamps. During the War, starting with the *VFO* ('Valve for Oliphant') klystron, he built up a large effort on centimetric radar, including work on magnetrons and other microwave valves, radar sets and the silicon and germanium crystal valves which were the forerunners of the modern semiconductor devices.

He played a part of considerable significance in bringing about cordial and fruitful co-operation between teams of scientists from industry and those from Government and military establishments that frequently met together at Rugby to attempt to solve some of the problems of the times. Such meetings were memorable and, whether 'H. W.' took the chair or not, his presence with its sense of urgency and promise of achievement helped us all to accomplish things that seemed to border on the impossible. He enjoyed overcoming the difficulties of people as well as those of physics and production.

Hugh Warren was a member of many engineering institutions, councils and committees, and was the author of many technical papers and a comprehensive volume published in 1931 entitled *Electrical Insulating Materials*.

He loved writing, and would spend odd minutes as well as long hours on semi-technical essays, some of which were gathered together in a volume called *ON—Some Irresponsible Jottings, Scientific and Otherwise*, published for private circulation in 1944. He had a great sense of humour and was a pleasant companion and a kindly and courteous leader; he will be remembered with admiration and affection.

L. J. DAVIES

NEWS and VIEWS

Technical Managing Director of Reactor Centrum Nederland: J. Pelser

JOHANNES PELSER has been appointed technical managing director of the Reactor Centrum Nederland foundation in succession to J. J. van Rijsinge. Johannes Pelser was born at Dordrecht on March 21, 1925, and matriculated at the Christian Grammar School at Dordrecht; afterwards he studied at the Technical University at Delft, where he obtained his certificate for physical engineering in 1951. Following this he joined the Foundation for Fundamental Research on Matter, but was transferred to the Reactor Centrum Nederland foundation when it came into existence in 1955. While at the Foundation for Fundamental Research on Matter, he was seconded to the (Netherlands–Norwegian) Joint Establishment for Nuclear Energy Research at Kjeller, Norway. Here he worked for four years and took part in the research work with the nuclear reactor *Jeep*. A short time after the setting up of the Reactor Centrum Nederland, Pelser returned to the Netherlands and took an active part in the preparations for the building of a research centre at Petten. In 1957 he was sent to the United States as a representative of the Reactor Centrum Nederland to supervise the fabrication of the high-flux reactor. By the end of 1958 he was appointed head of the Reactor Department at Petten, taking charge of the preparation and experiments with, and the operation and maintenance of, not only the low-flux reactor but also the high-flux reactor.

Aircraft Propulsion at the College of Aeronautics, Cranfield: Prof. A. H. Lefebvre

PROF. A. H. LEFEBVRE has been appointed to the chair of aircraft propulsion at the College of Aeronautics, Cranfield, in succession to Prof. A. G. Smith, who is now head of the Mechanical Engineering Department, University of Nottingham (see *Nature*, 187, 743; 1960). After leaving Long Eaton Grammar School, Prof. Lefebvre served an apprenticeship at Ericssons Telephones, Ltd., where he was employed afterwards for several years on engineering production and inspection. He studied electrical engineering at Loughborough College and mechanical engineering at the University of Nottingham. He is a graduate of the University of London and a postgraduate student of the Imperial College of Science and Technology. Since 1952, Prof. Lefebvre has been with the Aero-Engine Division of Rolls-Royce, Ltd., where his main responsibilities have been in combustion research and in the design and performance analysis of gas turbine combustion systems. He is the author or co-author of several published papers on combustion and heat transfer, and is the United Kingdom editor of the new review series, *Progress in Combustion and Propulsion Science and Technology.* Until recently he was a member of the Combustion and Propulsion Panel of the Advisory Group for Aeronautical Research and Development of NATO, in which capacity he contributed as author and editor to several publications of the Advisory Group for Aeronautical Research and Development.

Photography at the Federal Technical College, Zurich : Prof. W. F. Berg

Dr. W. F. Berg has been appointed professor of the Photographic Institute of the Federal Technical College in Zurich, in succession to Prof. J. Eggert. Dr. Berg has already established an outstanding position as an authority on scientific aspects of photography. He started research work at the early age of sixteen in the X-ray laboratories of Siemens in Berlin under his father, Otto Berg, and graduated Dr. phil. of Berlin in 1932. The activities of the Nazis brought him to the University of Manchester in 1933, where he studied the growth of crystals, for which he was awarded a Ph.D. degree in 1936. In the same year he joined the Research Laboratories of Kodak, Ltd., where he soon began work on the fundamental processes in the formation of the photographic latent image. This work, on the temperature coefficient of sensitivity and the time required for build-up of the latent image, gave results in complete agreement with the theory of Gurney and Mott that the formation of the latest image was a two-stage process, and had much to do with the general acceptance of that theory. This work was followed by other investigations of the photographic process, less spectacular in their impact maybe, but not less important. This work was recognized by the award of a D.Sc. (Manchester) degree. For about a dozen years Dr. Berg has been in charge of aspects of research into photographic emulsions of more immediate practical application. His present appointment is a not unnatural result, not only of his authoritative position in photographic research work but also of his interest in education. He has been interested for many years in education in medical photography and is a member of the Education Committee of the Institute of British Photographers.

Editor of *Discovery* : Mr. Gerald Leach

Gerald Adrian Leach was recently appointed to the editorship of *Discovery.* Born in Colombo in 1933, Mr. Leach was educated at Glengorse School and Dauntsey's. He read for the Natural Sciences Tripos at Clare College, Cambridge, taking Part 1 in 1954, in physics, mathematics, geology and mineralogy, and Part 2 in 1955, in geology. Since Cambridge, he has been a full-time science writer and producer. In 1957 he worked on the *Manchester Guardian* as a general reporter and assistant to the Science Correspondent, and the following year was script-writer for scientific documentary films with the Film Producers Guild. In 1959 he wrote and presented 35 programmes in the Associated Television science series, "It Can Happen To-morrow". In October that year he joined the BBC Television Outside Broadcasts Department as assistant producer on the 1960 "Eye on Research" television series. In 1960 and early this year he helped to plan, and write the scripts for, Dr. Bronowski's series, "Insight".

British Association for the Advancement of Science

The annual report for 1960–61 of the Council of the British Association to the General Committee characterizes the year as one of major expansion with a total audience of 140,000, or nearly double that of any previous year, and a rapid growth in the demand for lectures and other forms of presentation of science (Pp. 31. London: British Association for the Advancement of Science, 1961). Three new Area Committees were established during the year, making a total of sixteen, and the Junior British Association meetings continued to expand, while an entirely new type of meeting was the science fair held in Manchester in March 1961. A grant of £6,000 from the Leverhulme Trust Fund enabled a research project for the study of the generating of mathematical insights to be initiated under Dr. Z. P. Dienes, and the work of the Unit is to last two years. During the year, films have been shown to audiences totalling 78,000 and other visual aids to audiences totalling 10,000. Some twelve receptions were organized, at which science teachers were shown a programme of films and other visual aids display. A survey of the use of visual aids by science teachers in schools in Essex was reported in *The Advancement of Science* for September 1960 and a more detailed report of the survey was in subsequent issues of the *Film User.* Following the Cardiff meeting, eight research committees were appointed on the recommendations of various sections and grants were made to three of these.

Successful Operation of *Nimrod* Injector

On August 1 the first proton beam accelerated to the design energy of 15 MeV. was obtained from the linear-accelerator injector which forms the first stage of *Nimrod,* the 7,000-MeV. proton synchrotron under construction at the Rutherford High-Energy Laboratory of the National Institute for Research in Nuclear Science, Harwell. The linear accelerator consists of a cylindrical copper cavity approximately 44 ft. long and 5·5 ft. in diameter, with 48 drift tubes spaced along its axis, the whole being enclosed in a vacuum envelope. Approximately 1 MW. of radio-frequency power is supplied to the cavity at its resonant frequency of 115 Mc./s. to set up the required accelerating fields across the gaps between drift tubes. The function of the drift tubes is to screen the protons from the field when its direction reverses every alternate half-cycle, and they also contain focusing magnets which constrain the protons to form a proper beam close to the machine axis. The drift tubes increase in length along the tank to correspond with the increase in proton velocity. Protons enter the linear accelerator with an initial energy of 0·6 MeV. from a pre-injection stage which is basically a conventional d.c. accelerator. Pulsed 15-MeV. beams will be delivered by the injector to the synchrotron at rates up to 2 per sec. and at pulse lengths up to 2 m.sec. Within the synchrotron, they will be constrained to a circular path by a suitably varying magnetic field. In this way they will make many revolutions, receiving a small acceleration at a particular point in the orbit until the output energy of 7,000 MeV. is reached. Construction work on *Nimrod* started in August 1957 and is expected to be completed during 1962.

Aeronautical Research in Asia and Africa

The International Civil Aviation Organization is to participate with the Governments of India, Morocco, Thailand and the United Arab Republic in setting up and operating an aeronautical research laboratory and three civil aviation training centres. The laboratory is to be at Bangalore, India, where an Aeronautics Department in the Indian Institute of Science already exists. The prime objective will be the investigation of flight problems related to the design, construction and operation of aircraft in India. The laboratory will also undertake research and development work in allied fields of importance in India's economy, such as wind power and industrial aerodynamics.

The three new civil aviation training centres are to be in Morocco, Thailand and Cairo (U.A.R.). All three aim to train personnel in aircraft maintenance and operation to the recognized International Civil Aviation Organization standard. These centres will be open to trainees of neighbouring countries. Thus, the Moroccan centre in association with a similar Tunisian establishment will serve other African countries; Thailand will cater for south-east Asia; and Cairo will be the centre for the Middle East. In addition, the International Civil Aviation Organization is to help the centre already operating in Mexico City to increase its capacity and raise the level of training. In this way it is hoped that the supply and standard of trained civil aviation personnel throughout the world may be satisfactorily increased and improved to meet the increasing demand from the still expanding field of air transport. The Governments concerned and the United Nations Special Fund will provide the necessary financial support. The International Civil Aviation Organization will administer the scheme and provide any necessary additional funds.

Sterilizing Materials for Hospitals

ALL new hospitals in the United Kingdom will include a central sterile supply department, and many existing hospitals are already planning or constructing such departments. A small book, edited by Brian Watkin, assistant editor of *The Nursing Times*, describes the experience which has already been gained in setting up central sterile supply departments (Pp. 58. London: Macmillan and Co., Ltd., 1961. 4*s.* 6*d.*). Such departments have not only produced considerable financial economies but also have enabled a limited supply of nurses to be used to the best advantage, as well as to reduce the incidence of hospital cross-infection. Unlike the United States, where each financially independent hospital has its own central sterile supply service, the grouping of hospitals in Britain under regional hospital boards means that the arrangements for central sterile supply departments may differ from group to group. This booklet describes some of the experiments which have taken place and will be of great value to those responsible for planning hospital services as well as the doctors and nurses who work in them.

The South African Museum

THE annual report of the South African Museum for 1959–60 records the completion of plans for the extension of the main Museum (Pp. ii + 19 + 4 plates. Cape Town: South African Museum, 1961). It also refers to the low scale of museum salaries and the consequent difficulty in obtaining personnel capable of assembling educational exhibits and undertaking research. The Board is endeavouring to establish equality of museum and university scales of pay. Several field trips were undertaken by the staff, most of them with the definite policy of collecting material to fill gaps in the collections. Remarkable progress has been made in the re-organization of several of the exhibition galleries, especially in the "Hall of Man", where the European Stone Ages are well illustrated, and a striking exhibit clearly shows the importance of South African ape men. An innovation in South African museums was the installation of earphones, by which visitors may, on inserting a coin, hear the calls of some common birds and at the same time see the animals in the case.

Modern Methods of Traffic Control

AN exhibition of modern traffic control arranged by the Westinghouse Brake and Signal Co., Ltd., will be on view at the Science Museum, South Kensington, during September 12–October 1, to coincide with the opening there, by Lord Brabazon of Tara, of the London conference of the International Commission for Transport Museums. It illustrates, by means of actual equipment and working models, a few of the types of device employed in controlling safely the more rapid flow of traffic on road and on rail. Particular attention is paid to those regions of British Railways undergoing electrification. The exhibition includes the 'Westronic' demonstration model, which shows how all the points and signals in a particular signalling location are controlled, some distance away, from a central control cabin, which also receives an indication of the condition of the remote points and signals and the position of trains passing through. There is also an automatic barrier machine and its associated equipment, which ensures that road traffic is stopped for the shortest possible time at level crossings. Other railway signalling equipment devised to prevent false operation or damage to it by interference from the traction system will be shown in model form. Among the road equipment is a road traffic-lane divider, for varying the width of traffic lanes at the approach to main junctions or cities according to the direction and volume of traffic.

British Regional Development Plan for Courses for the Diploma in Technology

A REGIONAL development plan for courses leading to the diploma in technology and the diploma in technology (engineering) has been drawn up by the Regional Advisory Council for Technological Education, London and the Home Counties, after consultation with industry, the Ministry of Education and the local education authorities in the area (*Regional Development Plan for Courses for the Diploma in Technology*. Pp. 16. London: Regional Advisory Council for Technological Education: London and Home Counties, 1961). It is the policy of the Council to encourage and build up such courses so as to secure maximum efficiency. The need for providing such courses in anticipation of the 1964 and 1965 period, when a major influx of eighteen- and nineteen-year-olds is expected, is kept in mind, as well as the requirements of industry. The development of 'end-on' as well as 'parallel' courses will be encouraged as one means of making the best possible use of available industrial training places, even though this may present problems of staffing and time-tabling in the technical colleges. Another method may be that of the 'thick' sandwich courses of at least three years full-time academic study, together with a minimum of one year of industrial training during the course, and the need for the continuing development of Higher National

Diploma courses will not be overlooked. Stress is laid on the need for flexibility in this development, of which a synopsis is included in the pamphlet, together with some notes on the particular courses.

The Harkness Fellowships of the Commonwealth Fund—New York

THIRTY Harkness fellowships of the Commonwealth Fund—New York are tenable during 1962 for education and travel in the continental United States. The fellowships are offered to candidates from the United Kingdom, Australia, New Zealand and Western Europe, who are British subjects and either are graduates or have experience in Government service, the professions, the arts, branches of business or industry or other comparable careers or vocations, and have the equivalent of a degree in qualifications conferred by professional bodies or an attested level of professional competence. Forms of application, which must be returned before November 15, can be obtained from the Warden, Harkness House, 38 Upper Brook Street, London, W.1, from whom further details can be obtained.

The Institute of Scientific Information

A BROCHURE describing the services, publications and techniques adopted by the Institute for Scientific Information (Philadelphia, Penn.) has now been issued by the Institute, to which Eugene Garfield, its director, contributes a foreword. He states that the primary purpose of the Institute is to increase the productivity of research through a programme of dissemination and retrieval of information, screening, coding and indexing systems, design, research and development. The brochure describes the scope of *Current Contents* and the *Index Chemicus*, the *Index Medicus* programme, the *Citation Index* project and the three projects still in development: the transliterating typewriter, the *National Newspaper of Science*, and the *National Directory of Science*.

Polish Textile Research

THE journal of the Politechnika Łodźka (*Zeszyty Naukowe Politechniki Lodzkiej*) is published in five sections, corresponding to chemistry, applied chemistry, electricity, mechanics, and textiles. Volumes 6 and 7 of the textile section have been issued, and provide some ten extensive papers on subjects ranging from the variable delivery of metering pumps to the cleaning of bast fibres by passing them through fluted rollers. The work is described in great detail (in Polish) with summaries in Polish, English and Russian. The reports are of high standard, and should be available in textile laboratories. Copies can be obtained from the Politechnika Łodźka, ul. Gdańska 155, Łodź.

Announcements

DR. FABIAN RECALDE, former director of the National Institute of Nutrition in Quito, Ecuador, has been appointed to the staff of the Food and Agriculture Organization as a nutrition officer. Dr. Recalde's task will be the co-ordination of field projects in Latin America that require joint action by the Organization and the United Nations Children's Fund. These projects chiefly concern improving material and child nutrition.

DR. E. L. POWERS, associate director, Division of Biological and Medical Research, Argonne National Laboratory, Illinois, will deliver the seventh Douglas Lea Memorial Lecture, entitled "New Considerations on Survival Curves and Target Theory", at a meeting of the Hospital Physicists' Association, at 5.15 p.m. on September 15, in the Chemistry Department, University of Leeds. Further information can be obtained from Dr. J. R. Mallard, Department of Physics, Hammersmith Hospital, Ducane Road, London, W.12.

THE fifth international conference on "Pigment Cell Biology" is being sponsored by the New York Academy of Sciences and will be held in New York City during October 11–14. The conference will deal with melanoma and closely related fields. Further information can be obtained from Vernon Riley, Sloan-Kettering Institute for Cancer Research, New York 21, N.Y.

THE fourteenth international instructional course on "Fine Measuring Instruments" is being held by Carl Zeiss, Jena, at the Imperial College of Science and Technology, London, during September 11–22. Further information can be obtained from Carl Zeiss Scientific Instruments, Ltd., 12*a* Golden Square, London, W.1.

THE newly formed American Society for Cell Biology is holding a meeting at the Edgewater Beach Hotel, Chicago, Illinois, during November 2–4. Three symposia are included in the programme, which will cover: cell continuity; cell diversification; characteristics of cell interfaces. Further information can be obtained from Montrose J. Moses, Acting Secretary, American Society for Cell Biology, Box 2982, Duke University School of Medicine, Durham, North Carolina.

THE Food and Agriculture Organization is sponsoring an international conference on "Fish in Nutrition" in Washington, D.C., during September 19–27. The programme will include: the role of fish in world nutrition; chemical composition of fish and fishery products; contribution of fish and fish products to national diets; fish and fishery products in animal nutrition; demand for fish as human food and possibilities for increased consumption. Further information can be obtained from the headquarters of the Food and Agriculture Organization, Viale delle Terme di Caracalla, Rome.

A COURSE for young naturalists with a strong interest in conservation is run annually by the Verein Naturpark in the Lüneburger Heide, North Germany. The participants are given first-hand knowledge of the theoretical and practical sides of conservation. Information can be obtained from the camp secretary of the International Youth Federation for the Study and Conservation of Nature, Reiner Lidauer, Paracelsusstrasse, 6, Salzburg, Austria, or from Derek S. Davis, 56 Altmore Avenue, East Ham, London, E.6, of the British Co-ordinating Group of the International Youth Federation.

ERRATUM. In the article entitled "Effect of Polyvinyl Alcohol on the Distribution of Iodine-131 in the Internal Organs, Fluids and Excreta of Rabbits", by H. F. Nowak and J. Kucharski, in *Nature* of August 12, p. 665, the sentence beginning at line 14 from the end should read: "Administration of iodine-131 with polyvinyl alcohol caused it to collect in smaller quantities in the parenchymal organs and thus the possibility of injury to these organs is considerably reduced".

INORGANIC POLYMERS

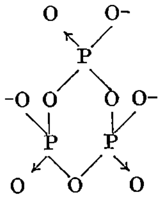
THE international symposium on "Inorganic Polymers", held at the University of Nottingham during July 18–21, attracted more than four hundred people, drawn from eighteen different countries. This response reflected the widespread interest in a subject virtually unmentioned a few years ago.

Contributions to the symposium were of two kinds: nine invited speakers gave full-length lectures, the texts of which are to be published by the Chemical Society; thirty-one shorter communications were presented and very briefly discussed. The most effective discussions were those which were held informally between sessions: these were facilitated by the fact that nearly all those attending were accommodated on the University campus. These social arrangements were excellent, and contributed in no small measure to the undoubted success of the meeting.

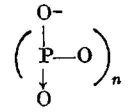
After the president of the Chemical Society (Sir Alexander Todd) had welcomed visitors in English, French, German and Russian, an introductory address was delivered by Dr. J. S. Anderson (National Chemical Laboratory). A main theme of this address was the problem, very fundamental to the symposium: "What is an Inorganic Polymer?" The theme recurred from time to time, and led to good-humoured exchanges suggesting rivalry between organic and inorganic chemists over polymers which might be described as organo-metallic, metallo-organic, or inorganic. Anderson pointed out that there are really two parts to this problem: (*a*) How large must the molecule be to be described as a polymer? (*b*) What restrictions of chemical composition are implied by the adjective 'inorganic'? On the first point, cyclic structures such as $(PNCl_2)_3$ occupied a prominent place in the discussions but are neither more nor less polymeric than, say, benzene. The second point is best dealt with by listing some of the types of polymer which were discussed during the meeting. They fall naturally into three classes: (1) polymers containing no carbon, for example, the chain forms of sulphur and selenium, $(PNCl_2)_n$, polyphosphates; (2) chain polymers in which the main chain does not involve carbon, for example, the siloxanes $(SiR_2—O—)_n$, where R is organic; (3) chain polymers in which carbon forms only a part of the main chain, for example, co-ordination polymers of the type $(—M—R—)_n$, where M is a metal and R an organic unit. The best compromise definition might insist on the absence of carbon from the main chain, and then include (2) but not (3). In point of fact, the wide range of materials surveyed by different speakers reflects the origin of much of the recent work in this field in a search for polymers capable of practical use at high temperatures. Since the object is to improve on the performance of organic polymers, large organic groups have been automatically excluded from consideration.

Before reporting on papers which dealt with specific polymers, one other general lecture may be mentioned. Prof. G. Gee (Manchester) directed attention to the large number of the systems under discussion which could exist in either chain or cyclic forms, the latter containing usually 6 or 8 atoms in the ring. He outlined a thermodynamic analysis of the equilibrium between ring and chain forms, pointing out that both heat and entropy of conversion ring/chain were likely to be small. In consequence, the equilibrium is readily displaced in either direction by small influences, and four classes of behaviour are possible: (i) chains unstable at any temperature; (ii), (iii) chains unstable below a 'floor temperature' or above a 'ceiling temperature'; (iv) an equilibrium chain concentration on any temperature. This analysis cannot yet be widely applied, due to lack of thermochemical data. In most of the systems described, equilibrium is not readily attained, and the product found in given circumstances is determined by kinetic factors.

One of the classical problems of inorganic chemistry is that of the structure of the many phosphates which have been described. A review of recent work in this field, given by Prof. E. Thilo (Berlin), showed that much confusion had been resolved by recognizing the existence of ring and chain forms, for example:

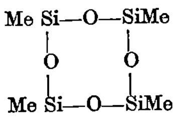


and



where n could reach values as high as 10^6. The chain forms may be either amorphous and soluble in water or crystalline and insoluble. The nature of the cation is of importance, in controlling the configuration of the chain and the crystal structure. The arsenates form a similar family, and mixed compounds, both cyclic and chain, can also be made.

Among the very few synthetic inorganic polymers which have already achieved industrial success the siloxanes occupy a prominent place. There have also been many attempts to modify the siloxanes by partial or complete replacement of silicon by other atoms. The lecture given by Prof. K. A. Andrianov (Moscow), who has been outstanding in this work, summarized some of his methods and results. Cyclic siloxanes such as $(SiMe_2O)_4$ are very well known, and Andrianov has described three-dimensional closed structures, including one which may be written as a cube having as its side:



Cyclic structures are readily opened by strong bases to give linear chains with terminal $—O^-$. Reaction of such a chain with an alkoxide of aluminium, titanium or boron is reported to lead to polycondensation, for example:

$$HO—(SiR_2O)_nH + Al(OR'')_3 \rightarrow HO—[(SiR_2O)_n—Al(OR')O]_x R$$

Vulcanizable elastomers having good physical properties have been reported.

A contributed paper by D. L. Alletson, A. G. Davies and B. N. Figgis (University College, London) described work on the dialkyl tin oxides, indicating the existence of tin analogues of both the ring and chain siloxanes. Four-membered rings are believed to occur among the stannoxanes.

Another well-known group of inorganic polymers are the phosphonitrilic compounds, of which the most familiar is the chloride $(PNCl_2)_n$, sometimes referred to as 'inorganic rubber'. In his lecture, Dr. C. P. Haber (U.S. Naval Ordnance Laboratory, California) summarized recent work, including several new general methods of synthesis. The usual procedure for the chloride involves reaction of ammonium chloride with phosphorus pentachloride (PCl_5), and can be modified to give $(PNR_2)_n$ by replacing PCl_5 by PCl_3R_2. This method always gives cyclic products; in the case of the chloride these are readily polymerized by heating. The chain polymer $(PNR_2)_n$ (R=Me, Et, Ph) cannot, however, be obtained in this way, as the ring does not open on heating. Reaction of $PClR_2$ with sodium azide leads, via R_2PN_3, to chain polymer. Substitution reactions can also be used to replace chlorine by other groups. By these various techniques, a considerable range of both cyclic and polymeric phosphonitrilic compounds have now been made.

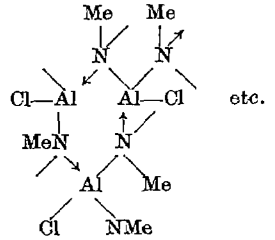
One of the most interesting problems associated with the phosphonitrilic compounds concerns their electronic constitution, that is, the nature of the P—N bond. In a contributed paper, N. L. Paddock (Manchester) reviewed this problem in the light of recent thermochemical and spectroscopic measurements and studies of base strength. There is no doubt that delocalized bonds are formed, both in rings and in the chain, by overlap of p orbitals of N with d orbitals of P. It is possible, on this basis, to understand why the stability of a PN ring is very much less dependent on its size than is that of a hydrocarbon $(CH)_n$. This problem was also referred to by Prof. A. B. Burg (Southern California) in his lecture. Discussing the polymerizability of the 6-membered ring $(PX_2N)_3$, Burg sought for factors tending to increase the size of the angle PNP, and on this basis deduced as the order of increasing polymerizability: $X = NR_2 <$ Me or Ph $<$ Cl $<$ F or CF_3.

Turning to BN polymers, Burg reported that the chain polymer $(Me_2N.BH_2)_n$ is unstable at room temperature, but the 6-membered ring ($n = 3$) is unchanged by heating at 300° C. The structure of this compound has been determined (by Lipscomb) and found to be of cyclohexane chair form, with ring angles of 113–114°. The corresponding phosphinoborine $(M_2PBH_2)_3$ is similar, with ring angles 118° (P) and 112° (B).

BN polymers were further discussed in the lecture by Prof. A. W. Laubengayer (Cornell) who represented three stages arising by progressive removal of RX from a co-ordination compound I of R_3N and BX_3:

$$\underset{\text{I}}{R_3N \to BX_3} \to \underset{\text{II}}{(R_2N \to BX_2)_n} \to \underset{\text{III}}{(RN—BX)_n} \to \underset{\text{IV}}{(BN)_n}$$

In series II, both B and N are approximately tetrahedral, but there is charge alternation in the ring or chain. Series III has as its parent member borazole $(NH—BH)_3$, which possesses a plane aromatic ring and closely resembles benzene. The corresponding chain is unknown: on pyrolysis borazole gives a variety of products, among which have been identified analogues of naphthalene and diphenyl. Boron nitride (IV) forms a layer structure very similar to graphite, but with B^- and N^+ alternating in adjacent layers. Aluminium–nitrogen polymers have been less studied, and there is no evidence for a borazole analogue. $(Me\ N.\ AlCl)_n$ is a very stable, insoluble material, possibly:

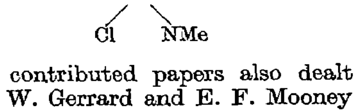


Several contributed papers also dealt with BN polymers. W. Gerrard and E. F. Mooney (Northern Polytechnic, London) reported that borazole ring formation could be prevented by bulky substituents (III, R = P or Bu, X = Ph). J. Dewing (Imperial Chemical Industries, Billingham) reported the polymerization of $Me_2N.BH_2$ by heating at 150° C. and 3,000 atm., to an amorphous, infusible, insoluble polymer, which slowly reverted to a cyclic dimer under ambient conditions.

The two remaining lectures, by Prof. J. C. Bailar (Illinois) and Prof. O. Schmitz-Dumont (Bonn), both dealt with co-ordination polymers. The underlying principles are straightforward, though their application to produce useful polymers has not yet been successful. The most useful technique involves polymerization by co-ordination, and requires the use of a bi- or poly-functional ligand. A very simple example is

$$Ag^+ + NH_2(CH_2)NH_2 \to \\ \to Ag^+ \leftarrow NH_2(CH_2)_2NH_2 \to Ag^+ \leftarrow$$

A metal of higher co-ordination number can form chelate rings; use of *bis*-diketones then leads to very stable polymers such as



where X is any linking unit such as $(CH_2)_n$ or C_6H_4. A variation on this procedure involves linking together, for example, by polycondensation, units which are already co-ordinated, that is, making the X bridges. It will be seen that the 'inorganic' content of such a structure is extremely small.

In this review of the symposium, no attempt has been made to mention all the contributors or all the topics discussed. It will be clear that the emphasis was on the preparation of polymers. The symposium offered a timely opportunity to take stock of the present position, and should lead to some reorientation of future effort. While there is clearly much synthetic work still to be done, increasing attention should now be given to reaction mechanisms and energetics, and to the physical study of the products. Prof. C. C. Addison and his colleagues on the organizing committee are to be congratulated on a most successful venture, which may well exercise an appreciable influence on the development of the subject.

G. GEE

SOCIETY OF EUROPEAN NEMATOLOGISTS

AT the invitation and under the presidency of Prof. J. van den Brande, the sixth international nematology symposium of the Society of European Nematologists was held during July 24–28, 1961, at the State Agricultural College in the ancient and beautiful city of Ghent. From some twenty-seven countries came about 140 participants, the largest contingents being those from Belgium (thirty-three), Great Britain (thirty, after deducting four for overseas students), Germany, West and East (nineteen), and Netherlands (seventeen).

After the inaugural presidential address and a stimulating special lecture by Prof. de Coninck on "Problems of Systematics and Taxonomy in Nematology today," there followed some fifty papers, grouped after the first day under two simultaneous sections, and each ostensibly limited to 20 min., including time for discussion.

Lack of space precludes a reference to every paper, but some of the trends and highlights can be briefly indicated.

In the field of taxonomy a paper by B. G. Chitwood (presented in his absence by A. L. Taylor) described and illustrated photographically the holotype and allotype specimens of most of the recognized *Meloidogyne* species and sub-species. These specimens were selected in 1960 by a committee of the American Phytopathological Society. Chitwood regards these taxa as possibly polyphyletic, originating as repetitive mutants ; at least one (*M. javanica javanica*) is probably a defective triploid.

W. C. Clark commented on the variability and accuracy of linear measurements used in systematics, and of ratios based on them. The de Man ratios, based on total length, are of little value when the latter is highly variable; also, they presuppose absence of allometric growth. H. Gysels showed that in *Panagrellus silusiae* post-embryonic growth is in fact allometric with respect to length: moreover, in the expression $y=Kx^a$, the index may change at different stages in the life cycle.

Among matters of life-history and biology, A. M. Shepherd showed that *Heterodera göttingiana* larvæ emerged from cysts in soil, in the presence of a host, even more readily than *H. rostochiensis*, but could not be made to emerge *in vitro*. In tissue-culture experiments with *Meloidogyne*, M. L. Schuster and R. M. Sandstedt found that movements of larvæ were consistent with a random walk and accidental contact with roots rather than with an attraction mechanism. W. H. Lavallee and R. A. Rhode, on the other hand, interpreted the rapid concentration of *Pratylenchus penetrans* around a host at one end of a box as due to attraction.

The orientation of *Ditylenchus dipsaci* to physical stimuli was discussed by H. R. Wallace : in gradients of temperature, moisture, and size of sand particles, the worms preferred 10° C., high moisture, and fine sand.

The feeding mechanisms of Rhabditids and Tylenchids were interestingly compared in a film by C. C. Doncaster. The movements of dye globules and air bubbles through the œsophagus were analysed, the bubbles showing change in size with pressure. He also showed a film on nematode predators.

The geographical or topographical distribution of nematode populations was discussed in several papers. M. Ritter was able to correlate the distribution of species of *Meloidogyne* in France with climatic zones. J. de Maeseneer reported on the distribution of some of the chief migratory root nematodes in Belgium. E. Gadea gave data on the free-living nematodes of freshwater and moss biotopes in Spain. B. Weischer has made the ecologically interesting discovery of two species of Desmoscolecidae in vineyard soils from a small area centred on Bad Ems: the point is that most other species of this family are marine. A. B. Oteifa usefully summarized the chief plant nematodes of crops in Egypt.

Changes in nematode populations were dealt with by S. Stemerding, who counted *Tylenchorhynchus dubius* and *Rotylenchus robustus* in weekly samples from a monoculture of peas. R. Gair *et al.* found differences between varieties of spring barley in their effect on rate of increase of *Heterodera avenae*. M. Oostenbrink presented the results of examining numerous samples from meadows and arable land in the Netherlands. It is clear that the heaviest populations of nematodes come from the grass/clover crop from meadows. Many of these nematodes are plant parasites and are important in two ways: some are harmful to the grass/clover crop itself, and some become harmful on arable crops when the meadow is ploughed. K. Kuiper is tracing the gradual invasion of new polder-soils from the Zuider Zee area by plant nematodes, and the factors responsible for this invasion.

In the field of pathology, H. Goffart has shown that one effect of *Ditylenchus dipsaci* in sugar beet is to increase the content of reducing sugars; this also occurs with *D. destructor* in nutrient-agar cultures and to a smaller degree with *Heterodera rostochiensis*. The effect is ascribed to enzyme action from the œsophageal glands.

The importance of nematodes as vectors of virus pathogens was stressed by R. S. Pitcher. All the known cases belong to the Dorylaimoidea and are of two types: some 'ring-spot' viruses carried by nematodes of the Longidorinae, and tobacco rattle virus carried by *Trichodorus*. H. H. Sol showed that *T. pachydermis* appears to be the only transmitter, nematodes extracted from infected soil retaining the virus for at least three weeks.

An interaction between *Verticillium albo-atrum* and *Pratylenchus penetrans* has been shown by W. B. Mountain and C. D. McKeen, the nematode being more prevalent in the presence of the fungus.

L. Roguska-Wasilewska found that *Heterodera schachtii* inhibited the respiration of rape far more than did the saprophagous *Eucephalobus elongatus*.

Other disease situations included: *Ditylenchus dipsaci* increasingly causing collar-rot of beet in Switzerland (A. Savary); *D. radicicola* of Dutch strain damaging *Poa annua* but not barley, whereas the Swedish strain behaves in the reverse manner (J. J. s'Jacob); *Radopholus similis* causing root-rot in banana in New South Wales (C. D. Blake).

Numerous papers dealt with control methods. Outstanding were a tabulated compilation of reported crop-yield increases as a result of using nematicides in the United States, comprising 853 comparisons (A. L. Taylor), and a succinct account of the way in which *Heterodera rostochiensis* is held in check on Long Island by nematicidal treatment (chiefly with dichloropropene), cultural methods and legal regulation, and the use of resistant varieties of potato in fields where the nematode population remains below the level of discovery (W. F. Mai and M. B. Harrison).

The diffusion of ethylene dibromide through soil was shown by F. Call and N. G. M. Hague to be largely determined by soil porosity; relating chemical to bioassay determinations, they found that very low concentrations persist for long periods. H. W. Nolte found only temporary benefit from using 'Vapam' against *Pratylenchus* in pine nurseries, and K. Raschke showed that reduction of hatching from *Heterodera* cysts after treatment with 'Trapex' was due not to delayed hatching but to death of larvæ.

Ultrasonic vibrations, tested by L. Kaempfe, gave disappointing results, especially against encysted *Heterodera* larvæ.

Radiotracer techniques using phosphorus-32 were applied by B. A. Oteifa and D. M. Elgindi to a study of galling in tomatoes by *Meloidogyne javanica* and its effect on uptake of nutrient, and by F. Sprau and A. Suesz to indicate that *Longidorus maximus* feeds on the roots of beet and is thus a parasite; and, using carbon-14, were adapted by B. M. Zuckerman to estimate the amount of plant fluid ingested by a single nematode.

At the biennial general meeting on July 27 members of the Society heard an encouraging report from the secretary, Dr. Lindhardt, who was able to show a steady growth in membership over the years:

1957: 141 members from 19 countries; 1959: 179 from 25 countries; 1961: 218 from 33 countries (of this last total, 166 come from Europe).

An invitation from Dr. John Grainger to hold the seventh symposium at Auchincruive in September 1963 was accepted with enthusiasm, and Prof. Nolte tabled an advance invitation to Aschersleben for 1965. It was decided to issue to members a duplicated 'Newsletter' under the editorship of Dr. Lindhardt, assisted by a corps of reporters.

A lavish social programme included a civic reception at the Town Hall, a dinner at the Castle of Laarne, and a lunch at the Park Hotel, Lokeren. There was also a programme of daily excursions for non-participating ladies.

Translator-in-chief, displaying equal facility in the three official languages besides his native Flemish, was Mr. R. Kips (secretary).

B. G. PETERS

A NEW LABORATORY FOR RESEARCH ON CREEP

ON July 6 the new Creep Laboratory of the British Electrical and Allied Industries Research Association was opened at Leatherhead by Dr. Charles Sykes, managing director of Thomas Firth and John Brown, Ltd. Its purpose is to carry out an extended programme of long-term co-operative research and investigation on the creep of steels in conditions of high temperature and mechanical stress.

Increase in the overall efficiency of generation of electrical power by steam now turns critically on improving the thermodynamic efficiency by operating at higher temperatures and pressures. The limiting factors in practice are set by materials capable of withstanding these conditions during long periods. The highest temperatures (above 600° C.) are found in superheater tubes, steampipes and control valves, but conditions are even more onerous in turbine rotors, blades and casings where tolerance to distortion is very small.

Historically, progress in steel development has occurred in three phases. Initially, power-station plant was constructed entirely of plain carbon steel; this was followed by the addition of small amounts of alloying elements which added strength to the steel. Now, in the third phase, special highly alloyed stainless steels are being developed. The cost of such steels is high and is reflected in the great increase in the proportion of cost of steam-raising equipment in modern power stations. Such increases must, in the long run, be offset by increased efficiency, and this can only be judged when full knowledge of the life and reliability of these new steels is available. Creep, which can be defined as permanent deformation of materials arising from continued stress, is of basic importance in this connexion.

The investigation of the properties of high-temperature steels for power stations was inaugurated as long ago as 1930 by the Steels for High Temperature Committee of the Electrical Research Association. Most of the investigations until recently have been carried out by the Metallurgical Division of the National Physical Laboratory and at the National Engineering Laboratory. In 1957 work was started at the Electrical Research Association to supplement that at the National Physical Laboratory. The National Physical Laboratory is, however, no longer able to cope with the vastly expanded programme, and through the joint efforts of the Committee it has been possible to centre all the work in the new buildings and equipment installed at Leatherhead.

The main Laboratory is 140 ft. long and 45 ft. wide. It has space for about 250 units for testing creep. Since these units dissipate considerable heat it has been necessary to install air-circulating equipment to maintain a suitable ambient temperature for instrumentation and personnel. The bulk of the units are designed to record either the creep or the time of rupture of standard samples cut and very accurately machined from bars of steel or from fabricated parts. At present there are fifty units of high sensitivity fitted with Martens optical extensometers. These machines are designed particularly for studying the creep behaviour of turbine rotor forgings, blades and casings, and are at present also being used for the creep of metal weld deposits and the parent metal. There are fifty large rupture machines, in which ten specimens can be stressed simultaneously end to end. They do not record the creep but only the time to rupture. They are used to provide long-time design data for high-alloy steel steampipes and superheater tubes. The stress applied to the specimens is varied so as to produce rupture times extending from about 300 to 10^5 hr. Graphs of rupturing time against stress are then used to predict the life under the stress conditions found in service. The variation from cast to cast within a particular specification is under investigation.

There are a further fifty dual-purpose creep/rupture units which can be used either as specimen rupture machines or as creep machines using dial gauge extensometers where less-accurate creep measurement is required. They are at present employed in measurements on turbine casings. One large machine with 15-ton loading has been installed for rupture-testing of full sections of steampipe.

The temperatures of the specimens are maintained by electric furnaces, the range of temperature of which at present extends from 450° C. to 700° C., but can be raised above this temperature when future requirements demand. The furnace temperature is maintained automatically by an electronic controller, to within ± 3° C. up to 600° C. and to within ± 4° C. above 600° C. Temperatures of the specimens themselves are measured by means of thermocouples (of which there are three on each specimen) in the high-sensitivity units. The thermocouple readings can be channelled selectively to a central control desk for manual measurement, but during critical periods they can be connected to one of 36 continuous recording charts situated in the Laboratory. In the rupture units there are five thermocouples distributed among the ten specimens. These, too, can be channelled to the control desk.

Another important project which is being undertaken in the new Laboratory is the testing to destruction of thick-walled tubes by applying steam pressure for long periods of time at temperatures encountered in service. This enables the creep data obtained in the Laboratory to be correlated with data on actual tubes so that the expected life in practice can be assessed. In testing creep in the Laboratory only a single uni-axial stress is applied whereas a steampipe is subjected to a complex stress system. Four such test rigs have been operating in the Electrical Research Association during the past three years. There are now fifteen rigs.

The data obtained in the new Laboratory will be subject to constant scrutiny as a means of providing more understanding of the mechanism of creep resistance of high-temperature steels. Knowledge of this kind will almost certainly be of benefit in the future development of steels for still higher temperatures, the demand for which is likely to continue for a long time.

CLIMATOLOGY AND CONSERVATION IN MUSEUMS

THE Commission for Museum Laboratories of the International Council of Museums and the International Study Centre for the Preservation and Restoration of Cultural Property have recently undertaken a world-wide inquiry into the subject of environment in relation to conservation. Replies from sixty-four institutions to a questionnaire on the subject, and further study of the situation thus revealed, form the basis of a 46-page report*. In it, museum curators, rather than scientists specializing in conservation, are provided with a useful compendium on the atmosphere and its contaminants, hygrometry, instrumentation and equipment for the control of museum climate. In addition there are several useful reference tables relating to conditions within and outside museums in various parts of the world.

As is proper for documents issued by an international organization, several authors from various European countries have contributed, but the actual publication consists of complete texts in French and in English. Taking into account the polyglot sources of the material, the effort can only be said to have been altogether praiseworthy. Moreover, the fact that the report is written for curators who must be assumed to have little knowledge of the physics involved, must have added to the task. Faced with this requirement, however, it is unfortunately all too easy to stray away from rigorous scientific exposition for the sake of a tempting analogy. For example, it may be acceptable in the introductory paragraph to compare inanimate objects with human beings in their reaction to the environment: "They live and breathe in the sense that there is continual if imperceptible change arising from their *adaptation* to the ambient conditions of the museum"; but, is it wise to persist with: "Some objects have a surprising capacity for *adapting* themselves to environment apparently without deleterious effect. Thus, a particular piece of wood or bronze may be recovered in remarkable condition after having been conserved for centuries in a swamp or bog; or a certain picture which has been exposed in a damp church may survive the ill-effects beyond all expectation. Let them be brought from their accustomed *habitat* into a museum, however, and the true state of affairs will soon be manifest, even where the atmosphere is rigorously conditioned" ? (p. 243). Again: "The crux of the matter is to be found in the past history of the specimen, its structure and the conditions to which it has already become *acclimatised*. As is well known, even the best museum conditions can be dangerous for a painted panel that has been normally housed in a cold, damp country house" (p. 253). This may or may not be a phenomenon which is observable under scientific control, but if it is, to imply a conditioning process in the biological sense only helps to perpetuate the notion which is sometimes present among curators that objects, particularly paintings, lose their 'resistance' in the soft environment of the air-conditioned gallery and decay, or alternatively, become unfit to withstand uncontrolled conditions.

Where the equilibrium that the object will attain in the museum results in a considerable change in moisture content, there will obviously be undesirable —often catastrophic—volume changes. Special techniques have been developed for dealing with such cases as, for example, water-logged wood. But it is difficult at first to entertain the idea that stabilizing the atmosphere at parameters well within those of the natural cycles can be harmful. Evidence on the alleged phenomenon is unfortunately biased for the simple reason that objects are closely and regularly inspected once they have entered the museum—but not before. Even so it is not without interest to proceed on hypothetical lines because it is the experience of every curator that certain objects prove intractable. It might be supposed, for example, that continual swelling and shrinking prevents embrittlement in organic materials. Another possibility is connected, not unreasonably, with gradients in moisture content within the object. Certain forms of deterioration in paintings (both panel and canvas) can be shown undoubtedly to result from the gradient set up when the back is in close proximity to what may be a cool and/or damp wall, while the front enjoys constant and ideal but different conditions. It cannot be assumed that such steady-state conditions are *a priori* more favourable on all counts than cyclic ones in which even a reversal of the gradient is possible. The transfer of soluble material, whether salts or adhesives, within the object is a source of breakdown, particularly at an interface where the structure is laminar. The report does not deal with this phenomenon beyond pointing out that the variation in climate within a room may be such as to produce a 12 per cent variation in relative humidity over 3 m. in height. The importance of moisture content gradients relative to the main hazard—the swelling and shrinking of hydrophilic gels—is a matter for investigation. The result may well explain the unexpected results of air-conditioning which sometimes disappoint the curator. In so many investigations connected with conservation the actual number of variables is difficult to define, and generalizations are often misleading. A case in point is the assumption (p. 247) that picture canvases become slack when the atmosphere is over-dry, as raw natural fibre textiles do. The converse is more often observed because the textile behaviour is opposed by that of the animal glue with which it is impregnated—old masters made use of this effect to tension their canvases.

---

* International Centre for the Study of the Preservation and the Restoration of Cultural Property, Rome. Works and Publications III: *Climatology and Conservation in Museums*. (Reprinted from *Museum*, 13, No. 4, 1960.) Pp. 201–289. (Rome: International Centre for the Study of the Preservation and the Restoration of Cultural Property, Via Cavour, 256, 1960. Also available at H.M.S.O.)

The report is generously illustrated with diagrams, photographs of instruments and conditioning apparatus, and with striking examples of objects which have suffered deterioration due to unfavourable environment. S. Rees Jones

VIEWING AND LISTENING

A YEAR ago the British Broadcasting Corporation published a report of an audience research inquiry under the title *The Public and the Programmes*. The interest which this aroused has encouraged the Corporation to make available other findings of its Audience Research Department. *Facts and Figures about Viewing and Listening** offers, in a series of twelve charts, some facts which are important to a proper understanding of what has been happening to viewing and listening habits in recent years.

Each chart has its accompanying commentary explaining the significance of the matter dealt with and directing attention to outstanding points.

Charts I and II show the extent to which the British public uses the three broadcasting services, BBC Sound, BBC-TV and ITV. This is assessed in two ways—in terms of the numbers who typically listen (or view) in the course of a single day (that is, the numbers of 'patrons'), and in terms of the average number who are listening (or viewing) at any given moment (that is, the 'average audience').

Chart I, "The Patronage of Broadcasting", shows the number of adult 'patrons' for each service quarter by quarter for the past three years and shows that the number of adult patrons of BBC Sound has not fluctuated very greatly (this, despite the continuing increase in television coverage). The numbers of adult patrons of both BBC-TV and ITV have shown a rising tendency, with a seasonal fluctuation. The number of BBC-TV adult patrons is consistently above that of ITV because, if for no other reason, though all viewers can see BBC-TV, some cannot yet receive ITV.

The curves for child-patrons show that a much smaller proportion of children than adults patronize Sound and a larger proportion of children patronize ITV than patronize BBC-TV.

In Chart II a different picture emerges when the services are compared in terms of 'average audience' than when they are compared in terms of 'patrons'. Thus, Sound's patrons exceed those of either television service, but its average audience is much smaller than that of BBC-TV or ITV. The explanation lies in the fact that the average patron of sound listens for a relatively small proportion of the time during which sound broadcasting is transmitted. Similarly, though there are more patrons of BBC-TV than of ITV, the former spend less time viewing (1 hr. 44 min. a day) than do the latter (2 hr. 12 min.); hence the number who are viewing ITV at any given moment (7,500,000) is greater than the number who are viewing BBC-TV (6,350,000).

Chart III shows the relationship, since 1952–53, between the amount of time (in the evening) during which television programmes were broadcast and the amount of time which the average viewer actually spent in viewing. Two points emerge from this comparison: (1) that an increase in the number of television programme hours in the evening is not followed by a proportionate increase in viewing; (2) that, since commercial television became available, there has been little difference between the amount of time spent in evening viewing by those who could receive both BBC and ITV and by those who could only receive BBC-TV.

These results suggest that the introduction of further television services (a third and perhaps a fourth) will be unlikely to cause an increase in the total amount of time which people devote to viewing.

Charts IV and V illustrate the way in which people with multichannel receivers distribute their viewing between BBC-TV and commercial television. Chart IV shows the proportions which, on the average day in each quarter of the past two years, confined their viewing to BBC-TV, viewed both BBC and ITV, confined the viewing to ITV, or did not view at all. The last of these categories tends to vary seasonally, being greatest in the July–September quarter. Apart from this, there has been a tendency in the past two years for fewer adults to confine their viewing to ITV and for considerably more to view both services. Among children, many more restrict their viewing to ITV than restrict it to BBC-TV.

---

* British Broadcasting Corporation. *Facts and Figures about Viewing and Listening*. In Twelve Charts with a Commentary. Pp. 31. (London: British Broadcasting Corporation.) 5s. net.

Chart V shows how Band 1/3 viewers have divided their evening viewing time between BBC and commercial television. The trends are remarkably similar: when commercial television first comes to a region there is usually a period of 'settling down' during which the proportion of time given to BBC-TV tends to fall. Then relative stability is reached.

Chart VI relates to the way evening viewing is divided between BBC and ITV according to the educational level of the viewer. Among those whose full-time education continued up to and beyond the age of seventeen years, roughly half view BBC-TV either virtually exclusively or more than they view Independent Television. Those in this group who view ITV exclusively or predominantly are a minority, 37 per cent. In the group whose full-time education was not continued beyond the age of fifteen or sixteen, about 30 per cent either always view BBC or view it more than ITV, whereas 52 per cent, always or more often, view ITV. In the group whose full-time education ceased at fourteen years or earlier the corresponding proportions are 20 per cent and 65 per cent respectively.

Chart VII shows the present comparative quantities of listening to sound broadcasting at different times of the day. The period when there is most listening is shown to be that between noon and 2 p.m. when the average audience is nearly 5·5 million. The main point brought out by the chart is that 'levels' of listening in the afternoon and evening are now far lower than those in the morning; in the evening sound broadcasting must compete with television.

The 1950–60 trends in the day-time and evening audiences for sound are shown in Chart VIII. During the period 6–11 p.m. Sound's audiences declined sharply each year with the result that, for every four people who were listening in January–March 1950, only one was doing so ten years later. Between 9 a.m. and 6 p.m., on the other hand, when the competition of television was less fierce, audiences for sound showed no comparable decrease. For every four people listening in the winter of 1950, three were doing so ten years later.

Chart X shows how much time 'listeners' and 'viewers' have spent with sound broadcasting in the evening each autumn since 1954. The average adult 'listener', far from reducing his evening listening, has tended to increase it in the past six years. He now listens for about 1 hr. (out of a possible 5) a night. The average 'viewer', on the other hand, has never listened much in the evening. This small average quantity of listeners guarantees Sound broadcasting a considerable audience in the evening. Even if the time should come when virtually everyone has a television set, the audience for sound broadcasting in the evening would still, at this rate of 'viewer-listening', be about one million persons.

Chart XI shows the extent to which, during October–December 1960, children aged five to seven, eight to eleven, and twelve to fourteen years viewed television between 5 and 6 p.m. and in the first half of the evening, 6–9 p.m. Each day between the hours of 5 and 9 p.m. the average child of five to seven years and the average child of eight to eleven years, watched television for more than two hours, while the average twelve to fourteen-year-old watched for nearly an hour and a half—and in each case the bulk of this time was spent in viewing programmes after 6 p.m.

Between 5 and 6 p.m., the older the child the greater is the share of such time when he views which he devotes to BBC-TV. During the early evening, on the other hand, the reverse is true: the older the child the smaller is the share of his viewing time which he spends with BBC-TV.

Chart XII shows that the size of an audience is, by itself, no sure guide to its popularity. The Chart illustrates this by showing that, during four typical weeks in mid-winter 1960, there were fourteen BBC-TV broadcasts each of which had an audience numbering approximately six million persons yet varied widely in the extent to which they were enjoyed. At one extreme one broadcast had a reaction index (the BBC's yardstick of 'enjoyment') of seventy-nine (out of a possible one hundred), while at the other was one with a reaction index of forty-nine. Thus, had 'audience size' alone been accepted as the criterion of popularity, these two broadcasts would have ranked as equal, whereas it is clear that those who saw Broadcast No. 1 enjoyed it far more than those who saw Broadcast No. 14.

EDUCATION OF BACKWARD CHILDREN IN BRITAIN

SINCE the War there has been a three-fold increase in the number of places available in special schools in Britain for educationally sub-normal children, the figure having risen from 11,000 in 1946, to 34,500 at the beginning of this year.

Educationally sub-normal pupils are defined as "pupils who, by reason of limited ability or other conditions resulting in educational retardation, require some specialized form of education wholly or partly in substitution for the education normally given in ordinary schools". They form one of ten categories of handicapped pupils for whom special education is provided. There is no overall national deficiency of places for blind, partially sighted, deaf, partially deaf, epileptic, physically handicapped, delicate and speech-defective children, although for some of these handicaps local improvements can be and are being made.

In a circular to local education authorities (11/61) Sir David Eccles, Minister of Education, estimates that a further 19,500 places will be needed to cater fully for all the educationally sub-normal children requiring education in special schools.

The Minister has already announced building programmes for the years up to March 1963 to provide about 10,000 of the places and intends to approve programmes, as soon as practicable, to provide the remainder.

The circular discusses the role of parents, teachers and doctors in discovering backwardness among children and in assessing their needs and the various measures that may be taken to enable them to live useful and independent lives. There has been a substantial increase in the rate at which children are brought forward for assessment since the War, during which time the school population has risen from 5 million in 1946 to 7 million in 1961.

Backwardness may be due to limited ability or other causes such as illness, a break in schooling or a disturbed home background. Many of these children will not need the specialized treatment given in special schools and can be given suitable education in ordinary schools. On the other hand, boarding schools or boarding homes are required for a small minority whose special education needs to extend to their life outside the classroom, for children whose home environment is such that they are unlikely to make good progress unless removed from it, and for those who do not live within reach of a special day-school. Each child's needs should be carefully assessed and should be kept under regular review.

The provision of extra places in special schools and the increasing interest in the needs of backward children in ordinary schools will require a substantial increase in the number of teachers for the educationally sub-normal. Teachers who wish to specialize in this work can attend one-year courses in the education of handicapped children, which are mainly concerned with backward children. In 1962 there will be 16 of these courses taking 250 teachers in all; in addition, a number of shorter courses is available.

The adjustment from school to working life is more difficult for these children than for those of normal ability but can be made easier by the advice of the youth employment officers in finding suitable jobs and by sympathetic guidance and help whether in the form of further education or informal activities arranged by their former schools.

THE BUILDING RESEARCH STATION

THE open days, July 10–13, held by the Building Research Station, Garston, Watford, Hertfordshire, were primarily for the benefit of the building industry—architects, builders, surveyors and building materials manufacturers—leaving aside research in structural engineering, but much of the work displayed was, as might be expected, of general scientific interest. Indeed, the open days were coupled with the official opening by Sir Harry Melville, secretary of the Department of Scientific and Industrial Research, on July 7, of a new laboratory for physical and chemical research on building materials. The building has been erected by the Ministry of Works (superintending architect, Mr. H. A. Snow; superintending engineer, Mr. M. Woolfson; main contractor, Wilson, Lovatt and Sons, Ltd.) as part of the general building programme for the Station. It replaces more than a dozen war-time huts and brings together in one building all the laboratory research on building materials that the Station carries out. In opening the laboratory, Sir Harry Melville referred to the frequently expressed view that good research results most frequently come from unpromising surroundings and primitive apparatus. "Nothing", he said, "can be further from the truth in these days." He went on to point out that the scope of the work of the Station had broadened enormously since its foundation forty years ago, but that materials research is still basic to the whole of the building industry.

Although, as Sir Harry Melville said, materials research is the oldest part of building research, it has always been prosecuted with the best scientific tools of the day and with the most up-to-date techniques. Thus at present electron microscopy, X-ray analysis, infra-red absorption spectroscopy, differential thermal analysis, high-temperature microscopy and microwaves all find application. The Station has indeed been a pioneer in the use of high-temperature microscopy directed to investigations of silicate minerals, and has developed a rapid and elegant technique for observing under the microscope reactions taking place at temperatures up to 2,400° C. A similar technique applied in the development of an X-ray camera for use up to 1,850° C. was also on view.

Micro-waves find application in equipment (now available commercially) designed to investigate the dampness of walls, a technique that is being further developed into a promising method of destroying dry rot by the heating effect of the waves, and radioactive techniques in the measurement of the wear of floor-surfacing materials.

Much of the research into building materials is devoted to a study of their fundamental physical and chemical properties, with the dual aim of providing a background of knowledge against which the durability of materials can be judged and of guiding new developments. Among the examples demonstrated were phase-equilibrium investigations on multi-component aqueous systems designed to increase understanding of the slow chemical reactions that materials undergo in use, and of the distribution of pore-size which has a close bearing on their resistance to frost. Laboratory abrasion tests for flooring materials have been notoriously unreliable, but following an investigation of the forces applied by the foot to the floor a new type of testing device was demonstrated that reproduces these forces.

New forms of construction make new demands on materials and throw up their own problems. Many curtain walling systems rely for their satisfactory performance on mastic materials for jointing. Thermal movements may be comparatively large and may make heavy demands on the mastic. Here the work of the Station has been mainly directed towards the assessment of the properties needed and the development of tests that can be used to evaluate the performance of jointing and caulking materials. The tests demonstrated dealt with rates of movement, stiffening with time, adhesion and load-bearing.

One of the great strengths of the Station is its ability to tackle most—if not all—aspects of problems involved in the development of new materials or processes. This is illustrated by its work on the perforated brick, the manufacture of which was demonstrated during the open days in the pilot-scale laboratory equipped with standard brick-making machinery. Not only has the Station been able to study the development of the design of the brick from the point of view of manufacture and æsthetic acceptability, but also it has been able to study strength, thermal properties, laying methods, rain resistance, and costs.

Visitors were able to see demonstrated the work of the Station on the packaging of building materials and the various units of mechanical plant developed for their handling; methods for fixing and joining plasterboard linings for house interiors, developed so as to remove as much of the wet process from building as possible; and the production of lightweight aggregate from pulverized fuel ash.

Of much current interest is the work on heating, as, for example, of flats, employing an electrical analogue, and of heat transmission through walls and roofs.

Equally topical is the survey being carried out of noise in London. One of the recording vans was on view, as was the main mobile acoustics laboratory, admirably equipped for almost any acoustical measurement and largely used for the measurement of sound transmission in buildings and for investigations into the laws governing the propagation of sound through the atmosphere.

The use of models finds considerable application in studies of the lighting of buildings, for, when suitably designed, they provide a ready means of obtaining both a factual and a subjective judgment of proposed schemes. The Station is active in establishing the principles on which permanent artificial lighting can be used to supplement day-lighting and thus give a new freedom to architectural planning. Closely associated with lighting is the use of colour in buildings: the one can modify considerably the effectiveness of the other. The range of colours for paint established as *B.S.* 2660 is now widely accepted, and the Station is engaged in trying to get the principles underlying the paint colours applied to building components such as tiles, linoleum, and plastics.

Allied to the physical investigations were the more subjective studies on the needs of users, illustrated by work on the design of drawing offices and of dwellings for old people.

Another use of three-dimensional models is found in the design of engineering services, and an exhibit showed ways in which they can help to resolve problems in the design of hospitals.

The economic aspects of building design and construction formed another exhibit on a topic of much importance. Here were shown investigations on the influence of the design of flats on their cost, the assessment of the comparative costs of different forms of urban development, and the programming of the sequence of work on building sites.

Altogether, there was much for the main target of the open days—architects and builders—to ponder over. The value of the open days for scientists is perhaps best exemplified by the director of a research organization in an entirely different industry, who went away having seen three pieces of work of immediate application in his own field. Some 3,500 visitors attended.

A NEW 'WEDGE' TYPE INSTABILITY OBSERVED IN A 'THETA' PINCH

By J. K. WRIGHT, R. D. MEDFORD, J. D. HERBERT and R. C. POTTINGER

Atomic Weapons Research Establishment, Foulness, Southend-on-Sea

RECENT experiments by Niblett and Green[1] have shown that flutes are developed on the outer surface of a plasma produced by a theta pinch and these are interpreted as magnetohydrodynamic Rayleigh–Taylor instabilities produced by the acceleration of the interface separating the driving magnetic field and the plasma. Josephson has recently reported another experiment in which he investigated a possible mechanism for neutron production in a theta pinch with reversed trapped magnetic field[2]. Niblett has said (private communication) that in his experiments with Green they made detailed magnetic probe measurements and these showed that in their experiment parallel trapped field was obtained on the second half-cycle. In both experiments high-speed camera shots were taken of the end view of the plasma during the second half-cycle of the discharge.

We have been studying similar phenomena in theta pinches but have made our observations during the first half-cycle of the discharge. The theta piece used was 7·5 cm. long, 3·4 cm. internal diameter and this was wound around the middle of a quartz discharge tube 22 cm. long, 3·0 cm. diameter filled with deuterium gas at pressures of the order of 1 mm. of mercury. Our condenser bank had a capacity of 40 μF. and was charged to a voltage of 10 kV. An explosive type switch discharged the bank through the theta piece. The parameters of the experiment were such that we obtained[3] a cylindrically converging shock wave on the first half-cycle with trapped magnetic field parallel to the driving field.

Photographs of this discharge have shown an interesting new phenomenon; during the first half-cycle of the discharge we see bright radial 'wedges' with less luminous plasma filling their interstices. Even with radial wedges present it appears that there is still magnetic field trapped by the imploding plasma. About the time of maximum compression it forces its way out of the centre by pushing the plasma in the interstices out towards the wall of the discharge tube, thereby forming flutes which appear to have trapped magnetic field inside them. These outgoing flutes grow into a fascinating octopus pattern similar to that observed by other workers and associated with a rotation of the whole plasma. In our experiment the flutes grow in amplitude following a curved line giving an impression of rotation.

The number of wedges seems to be determined by the irregularities in the system since it can be varied by deliberately introducing irregularities in the external conductors. Figs. 1, 2 and 3 show high-speed framing camera records (about 8 frames per μsec.) of the theta pinch on the first half-cycle of the discharge. Figs. 1 and 2 are of theta pinches fired in the *Prize*[4] apparatus (Fig. 8), where it will be noticed that there are 16 wedges, that is, half the number of wires in the return conductor normally used for the *Z* pre-ionizer. Fig. 3 is taken with the return conductor removed and the theta piece wound directly on to the discharge tube, and shows that the wedges are randomly spaced and fewer in number; Fig. 3 is particularly interesting because it shows the trapped magnetic field apparently pushing its way out of the plasma through the three gaps where the plasma luminosity is least. The resulting outgoing flutes are also seen to grow as a result of the pressure exerted by the trapped magnetic field. A similar effect is also seen in Figs. 1 and 2. The field is observed to be trapped inside the largest flute until the fourth camera frame of Fig. 2, that is about ½ μsec. after the first wedges become visible. In order for the diffusion time to be about ½ μsec., the electron temperature must be of the order of 5 × 10^5 °K.

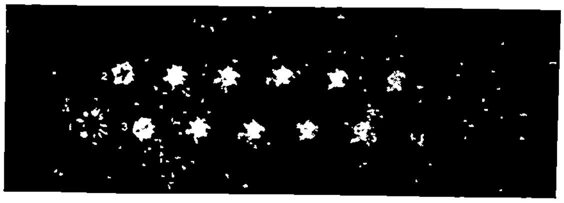


Fig. 1. Theta pinch with parallel magnetic fields. Ambient deuterium pressure 1 mm. mercury

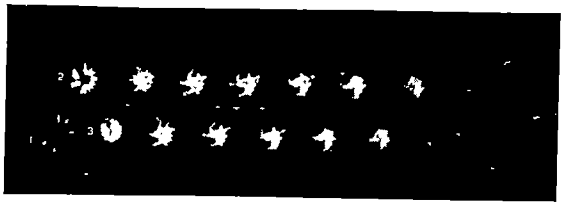


Fig. 2. Theta pinch with parallel magnetic fields. Ambient deuterium pressure 0·5 mm. mercury

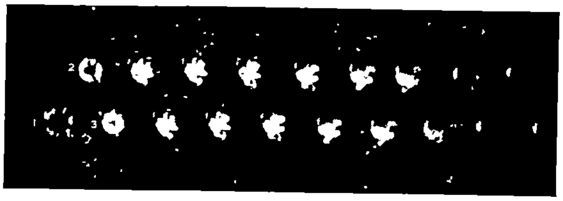


Fig. 3. Theta pinch with parallel magnetic fields. Ambient deuterium pressure 0·56 mm. mercury

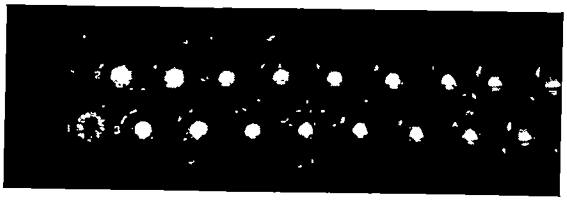


Fig. 4. *Prize* round ambient deuterium pressure 0·5 mm. mercury

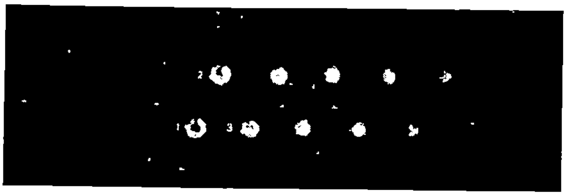


Fig. 5. Theta pinch with reversed trapped magnetic field. Ambient deuterium pressure 0·6 mm. mercury

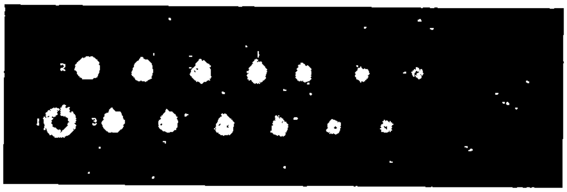


Fig. 6. Theta pinch with reversed trapped magnetic field. Ambient deuterium pressure 1 mm. mercury

The effect of reducing the trapped magnetic field by a considerable factor was studied by firing *Prize* with a 1 μF., 10 kV. condenser feeding the *z* pre-ionizer and a 40 μF., 10 kV. condenser bank feeding the theta pinch. Fig. 4 shows the result. It will be noticed that Rayleigh–Taylor type instabilities are formed during the implosion phase of the first half-cycle, but that they are damped out after the plasma converges on to the shock-tube axis; no outgoing flutes are observed after this time and better stability of the plasma is obtained.

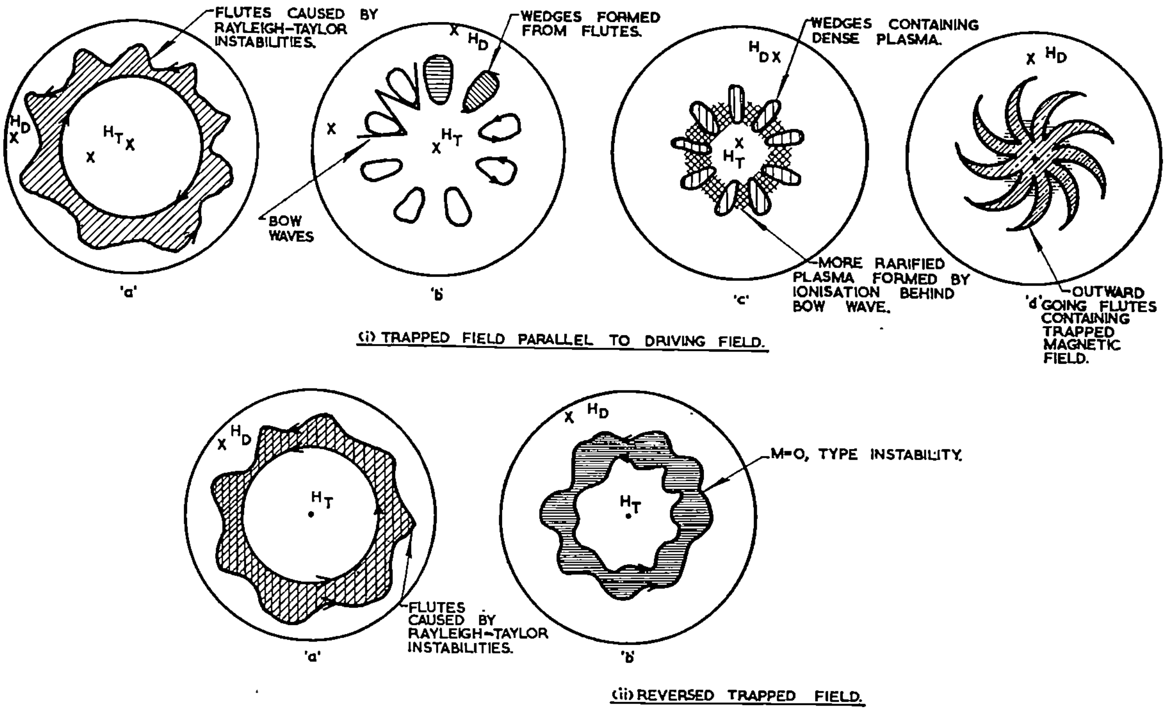

FLUTES CAUSED BY RAYLEIGH–TAYLOR INSTABILITIES.
H_D X
H_T X
X
'a'
WEDGES FORMED FROM FLUTES.
X H_D
X
X H_T
BOW WAVES
'b'
WEDGES CONTAINING DENSE PLASMA.
H_D X
X H_T
MORE RARIFIED PLASMA FORMED BY IONISATION BEHIND BOW WAVE.
'c'
X H_D
OUTWARD GOING FLUTES CONTAINING TRAPPED MAGNETIC FIELD.
'd'
(i) TRAPPED FIELD PARALLEL TO DRIVING FIELD.
X H_D
H_T
FLUTES CAUSED BY RAYLEIGH–TAYLOR INSTABILITIES.
'a'
X H_D
M=O, TYPE INSTABILITY.
H_T
'b'
(ii) REVERSED TRAPPED FIELD.

Fig. 7. Schematic diagram of instabilities observed in theta pinches

The next step was obviously to produce a reversed trapped magnetic field during the first half-cycle of the discharge. This was done with a pair of Helmholtz coils placed adjacent to the theta piece and fed from a critically damped circuit so that the rise time of the external field was 3 μsec. and the maximum field produced was 1,000 œrsted. When the externally produced field was at a maximum the theta discharge was initiated and the plasma produced had a reversed trapped magnetic field. Figs. 5 and 6 show the effect of a reversed trapped magnetic field on the discharge; it will be observed that there are no radial wedges or Rayleigh–Taylor type instabilities and that there is only one gap in the otherwise continuous plasma cylinder. The gap in the plasma is adjacent to the power feed to the theta piece so that it may be ignored as a wedge effect. Since there are no apparent Rayleigh–Taylor instabilities in Figs. 5 and 6 we assume that the radial velocity of the plasma is constant and that the acceleration required for the growth of instabilities is only present for a very short time during the initial formation of the imploding cylindrical shock. If the current sheet had accelerated for a longer time we would have expected to see the same type of instabilities as those observed by Niblett and Green[1].

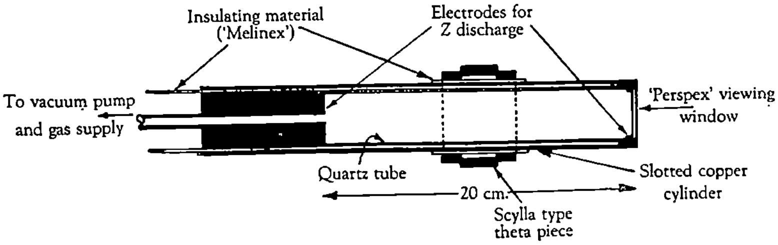


Fig. 8. Cross-section of *Prize*

Our experimental evidence suggests that the radial wedges are only formed when there is a trapped magnetic field in the same direction as the driving field, that is, when the azimuthal currents on the shock front and the current sheet have opposite directions of rotation. This hypothesis is also supported by the Niblett–Green[1] photographs, which show that radial 'wedges' are present when there is parallel trapped field. A possible reason for this is as follows: if the currents are in opposite directions Rayleigh–Taylor instabilities on the current sheet will produce flutes which can grow until they break up the plasma into radial wedges, each wedge carrying its own circulating current. The wedges travelling inwards at shock speeds produce bow waves which fill the interstices between the wedges with less highly ionized plasma. If the trapped field is in the opposite direction to the driving field the currents are in the same direction and the flutes produced by the instabilities in the current sheet are unable to break up the plasma, constrictions similar to the sausage type $m = 0$ instability observed by Josephson should then occur. An attempt to show the two effects graphically is made in Fig. 7.

Recent experiments on the *Scylla* apparatus[5], with the deuterium gas pre-ionized so that neutrons were observed in the first compression half-cycle, have shown that the neutron output was greatly enhanced by the inclusion, prior to the discharge, of a small axial magnetic field in the direction opposite to the compression field. We think there may be a connexion between this recent discovery and the instability mechanism discussed above.

We wish to thank Dr. R. Latham of the Imperial College of Science and Technology and Drs. H. R. Hulme and N. J. Phillips of the Atomic Weapons Research Establishment, Aldermaston, for stimulating discussions, and Messrs. A. Skinner and R. J. Abrey for the loan of their high-speed framing camera and their help and guidance in its use.

[1] Niblett, G. B. F., and Green, T. S., *J. Plasma Phys. and Thermonuclear Fusion*, 1, 42 (1960).
[2] Josephson, V., Dazey, M., and Wuerker, R., *Phys. Rev. Letters*, 5, 9, 416 (1960).
[3] Wright, J. K., Eeles, W. T., and Herbert, J. D., *Nature*, 183, 1665 (1959).
[4] Medford, R. D., Powell, A. L. T., Herbert, J. D., Pottinger, R. C., and Wright, J. K., *Nature*, 186, 706 (1960).
[5] Glasstone, S., and Lovberg, R. H., *Controlled Thermonuclear Reactions*, 420 (Van Nostrand, 1960).

A NEW TEST FOR DETECTING ANAPHYLACTIC SENSITIVITY: THE BASOPHIL REACTION

By Prof. WALTER B. SHELLEY and LENNART JUHLIN*

Department of Dermatology, University of Pennsylvania School of Medicine Philadelphia

EACH year many individuals in seeming perfect health meet sudden death as a result of anaphylaxis. Such overwhelming allergic reactions reflect an immediate type hypersensitivity to diverse drugs, foods, insect bites or indeed even cold. Of all these, penicillin is perhaps the most common cause of anaphylactic shock and death. Although much is known of anaphylaxis, no safe reliable predictive test is yet available. Skin tests (direct, passive, and passive cutaneous anaphylaxis), serological antibody studies, as well as animal smooth muscle reactions each suffer serious limitations in clinical practice.

In our attempt to develop a test for anaphylactic reactors, two well-documented observations appeared to be of signal interest. First, all the key signs of anaphylaxis are, or may be, induced by histamine. Secondly, the depot of histamine in the blood is the basophilic granulocyte[1]. Thus, it seemed likely to us that in anaphylaxis the basophil might release its stores of histamine, with consequent degranulation similar to that observed in its congener, the mast cell[2]. Transmutation of this idea into experimental data has required two years for developing a technique to handle and to concentrate this at once fragile and rare white cell of the blood. We are now able to examine a miniature *in vitro* anaphylaxis system consisting of the human basophil, blood and antigen. Such work reveals the basophil to be a cell with a cytological picture reflecting its functional state. Hence it can serve as a laboratory index of anaphylactic sensitivities. The following is a preliminary account of our results.

---

* Present address: University Hospital, Uppsala.

The majority of these observations were made on twenty consecutive penicillin-sensitive patients and an appropriate twenty non-sensitive healthy control subjects. Limited investigations were also performed on four additional patients who had experienced severe systemic reactions to cold, bee stings and prochlorperazine. The following general sterile technique was employed. Tests were not made on subjects after a fat meal, during corticosteroid therapy, systemic illness, urticarial or anaphylactic reactions. At such times the basophils were often inadequate for testing purposes. Specimens of venous blood (0·25 ml., 5 μgm. heparin sodium added), drawn with siliconized needle and syringe, were mixed ('Vortex Jr.' mixer) with 0·025 ml. of penicillin or appropriate antigen in a concentration of 10^{-3} in physiological saline solution. After standing in a polystyrene tube at room temperature (25° C.) for 15 min., 0·1-ml. samples were withdrawn in siliconized syringes and added to ice-cold fixative solution (ethyl alcohol 6 ml., chloroform 2 ml., and acetone 2 ml.). After overnight fixation, at +4° C. the cells were re-suspended and aliquots were filtered through a cellulose membrane filter (Schleicher and Schuell, coarse 'Cella *O*', 20 min.). The filter was then selectively stained with toluidine blue (30 sec., 1/300 aqueous, phosphate buffer *p*H 5·2) dehydrated in ethyl alcohol and cleared in xylene. After mounting, the basophils could be clearly seen, counted, typed and photographed. Routinely a standard count of 20 consecutive basophils was made. The basophils were classified into distinct categories on the basis of size, staining properties, number and location of basophilic granules. Such a 'basophil differential' permitted accurate assessment of the effect of exposure to given antigens. In essence, the technique enabled us to distinguish the depleted from the intact cell. Depleted cells appeared as basophils (*a*) with few granules, or (*b*) with small lightly stained granules, or (*c*) with an appreciable number of granules seen outside the cell. The preparations were deemed unsatisfactory when the control slide showed more than one-fourth of the cells 'fired'. Our initial investigations suggest that for a positive test at least half the basophils exposed to the test antigen should show evidence of degranulation (depletion). It is apparent that test antigens must not be intrinsically 'histamine liberators' and must be used in concentrations which have no effect on the granulation of basophils in normal controls.

Table 1 reveals that each of the five patients with a history of penicillin anaphylaxis showed significant degranulation of the basophils in the presence of penicillin *in vitro*. It is important to note that the episodes of anaphylaxis predated the testing by months, even as long as nine years. Thus, anaphylactic penicillin sensitivity may be remarkably persistent. In the fifteen who gave a history of urticaria associated with penicillin (various types and routes of administrations, over a ten-year period), five had a positive test as defined by us. This urticarial group is heterogeneous. Some members may be sensitive to procaine or other substances, rather than penicillin. Others may have lost their sensitivity over the years, and, although re-administration of penicillin might re-light it, they are nevertheless non-sensitive at the time of our testing. None of the controls was positive. This is significant since these individuals had each had penicillin without any clinical reaction. Some were actually taking it at the time of testing. It would seem desirous to correlate further these results of predictive testing of penicillin sensitivity with the clinical administration of penicillin. This may be done in animal work, but not in man because of the potentially grave consequences. We can rely at present only on the close correlations of history and *in vitro* studies.

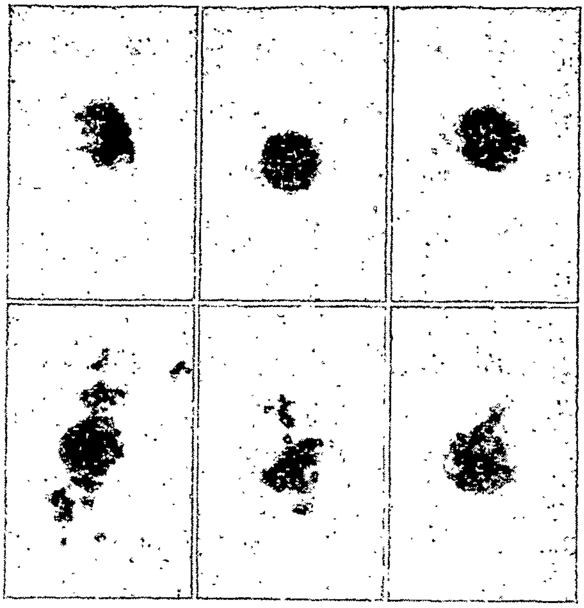


Fig. 1. Human basophils observed in an *in vitro* test for anaphylactic sensitivity. Upper three control cells show relatively intact structure. Lower three cells show varying degrees of explosive degranulation. The test is positive when more than 50 per cent of the cells show this depleted appearance after 15 min. exposure to test antigen in 10^{-4} concentration. (× 1,750)

Table 1 further shows that the results of the basophil test correlate well with the clinical histories of individuals who have experienced major or minor anaphylaxis as a result of immersion in cold water, yellow jacket bee sting, as well as another drug hypersensitivity, namely, prochlorperazine.

We feel that the test described aids in the detection of the anaphylaxis-prone individual. Nevertheless, negative data in this test reflect the patient's status only at the time of testing and do not anticipate any possible future sensitization. The test gauges the critical potential for anaphylaxis by determining the degree of anaphylactic sensitivity. Whether or not

Table 1

Clinical history	Antigen (10^{-4}) tested	No. of patients	Positive reactors
Penicillin anaphylaxis	Benzyl penicillin potassium	5	5
Penicillin urticaria (presumptive)	Benzyl penicillin potassium	15	5
Normals	Benzyl penicillin potassium	20	0
Cold anaphylaxis	+ 4° C. bath	2	2
Normals	+ 4° C. bath	10	0
Prochlorperazine urticaria	Prochlorperazine	1	1
Normals	Prochlorperazine	3	0
Yellow jacket Bee sting Anaphylaxis	Yellow jacket extract	2	2
Normals	Yellow jacket extract	2	0

a given patient actually experiences clinical anaphylaxis following antigen exposure rests on a large number of additional variables. The most important of these would seem to be the number of basophils and their histamine granule content at the time of exposure to the antigen. Even in the presence of a high degree of anaphylactic sensitivity, an antigen would not be expected to have much impact clinically if the basophil histamine load were light. Thus, the healthy, yet sensitized, adult with a full complement of loaded basophils is a prime candidate for anaphylaxis. Further work is in progress to learn more concerning the clinical significance of the rate of allergic basophil degranulation.

The parallel behaviour of the cutaneous mast cell in allergic reactions accounts for the development of urticaria. Here the histamine released has a local effect on the skin vasculature[3]. We suspect a high degree of correlation between the release of mast cell granules and basophil degranulation, but the dichotomy of the two systems may be apparent at times and thus account for negative skin tests in a known anaphylactic reactor.

'Basophil anaphylaxis' affords new cyto-immunological vistas since this target cell reaction can be observed in animals as well as in man. The data presented at least suggested that the basophil is the central cell of the anaphylactic state. It is hoped that the new technique described here will permit an intensive study of this heretofore elusive and labile cellular rarity of the blood stream.

This work was conducted under the sponsorship of the Commission on Cutaneous Diseases of the Armed Forces Epidemiological Board and was supported in part by the Surgeon General, Department of the Army, and in part by grant *PHS 2G*-62 from the National Institutes of Health.

---

[1] Valentine, W. N., Lawrence, J. S., Pearce, M. L., and Beck, W. S., *Blood*, **10**, 154 (1955).

[2] Boreus, L. O., *Acta Physiol. Scand.*, **48**, 431 (1960).

[3] Riley, J. F., *The Mast Cells* (E. and S. Livingstone, Ltd., Edinburgh 1959).

STEROIDS AS ICE NUCLEATORS

By RICHARD B. HEAD

Commonwealth Scientific and Industrial Research Organization,
Chemical Research Laboratories, Melbourne

MOST substances suggested as possible ice nuclei for cloud seeding are inorganic compounds of hexagonal structure with lattice parameters similar to those of ice. The possibility of using organic compounds as ice nuclei has not yet been explored. During an investigation of water-insoluble organic compounds, seven out of thirty steroids examined, the symmetry of which was mainly monoclinic or orthorhombic, were found to nucleate ice almost as effectively as silver iodide. The discovery of a class of organic materials capable of acting as ice nuclei appears to be novel.

Activities were determined by means of a microscope cold stage into which slides carrying steroid particles could be inserted. Provision was made for the introduction of moist air. The particles under test thus acted as freezing nuclei.

Although nucleations occurred on a proportion of crystals slowly grown from alcohol, as shown in Fig. 1 (androstenolone at −12° C.), none occurred on small needles or plates grown rapidly. Particularly active sites were engendered by quick recrystallization

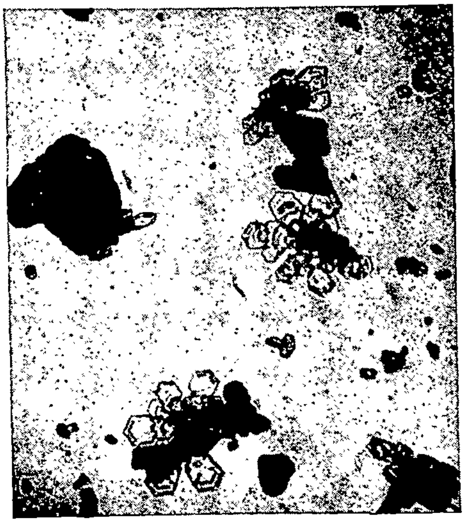


Fig. 1. Ice crystals growing on androstenolone at −12° C.

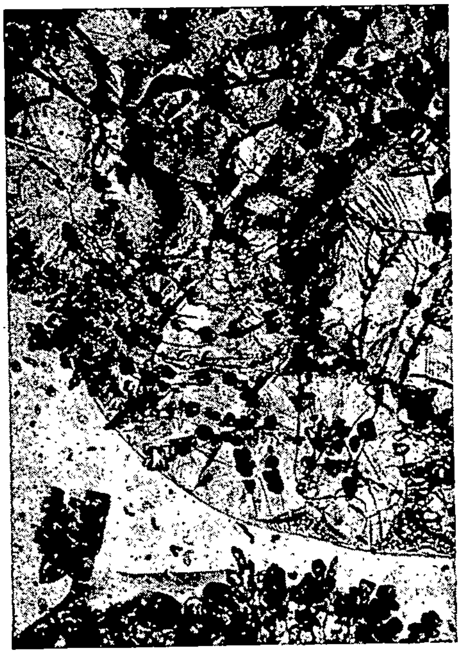
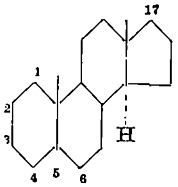


Fig. 2. A recrystallized droplet of androstenolone showing multiple nucleations on cracks

at the melting point in air or pure nitrogen, followed by quick cooling. This was done by heating a slide, powdered with crystals, at one end until about half the particles had melted. A line of particles which had recrystallized near the melting point was thus formed at the junction of the melted and unmelted portions, and these, together with the larger droplets which had recrystallized on cooling, showed multiple nucleations, especially at cracks. Fig. 2 shows part of a recrystallized droplet of androstenolone on which more than a hundred nucleations occurred at −7° C. Droplets smaller than about 0·03 mm. diameter were usually inactive.

The most active steroids were androstenolone (I), testosterone (II), 3α-methylcholestan-3β-ol (III), androsterone (IV), pregnenolone (V), androstan-3,17-dione (VI), and cholesterol (VII), for which the onset temperature was −5° C. Nucleation of all particles larger than 0·03 mm. diameter was complete at −7° C.



Compound	Position of C=C	Configuration at position 5	Substituent at C_3	Substituent at C_{17}
I	5–6	—	β-OH	=O
II	4–5	—	=O	β-OH
III	—	5α-H	αMe-β-OH	β-C_8H_{17}
IV	—	5α-H	α-OH	=O
V	5–6	—	β-OH	β-$COCH_3$
VI	—	5α-H	=O	=O
VII	5–6	—	β-OH	β-C_8H_{17}

Much lower activities were shown by androsta-1,4-dien-3,17-dione, cortisone, cholestenone, progesterone and œstrone, while no activity was shown by steroid esters, ergosterol, 17α-methyltesterone or 3β-methylcholestan-3α-ol, although the 3β-ol compound was very active. Substances with no groups capable of hydrogen bonding such as 3β-methylcholestane were inactive.

It is usual to associate nucleation with the similarity in spacing between the ice lattice and that of the nucleating substance. The exact crystal structures of many steroids are still unknown; but the survey by Bernal, Crowfoot and Fankuchen[1], and the detailed analyses of cholesteryl iodide[2], and lanostenyl iodoacetate[3], suggest that the molecules in the majority of steroidal structures are arranged in parallel stacks with 'head-to-tail' packing. One surface of the stack would thus present a pattern of alternating hydroxy groups and side-chains. It is presumed that nucleation takes place on this surface. The relationship between the cell dimensions of ice and those of the steroids is not obvious, but the cross-sectional dimensions of the steroid molecule, 5 ± 1 Å. × 7 ± 1 Å., deduced by Bernal *et al.*[1] suggest a possible compatibility with the prism face of ice (4·51 Å. × 7·35 Å.) although it is realized that this spacing is only relevant if the molecules are stacked approximately normal to the surface. The precise measure, however, would depend on the structural dimensions of the crystal form.

It has been shown that the most active substances have keto or hydroxy groups at position 3, and it is notable that three of them have keto groups at position 17 in addition. That these groups may be interchanged without loss of activity is illustrated by the comparison of androsterone versus testosterone. If it is assumed that the essential requirement for activity is a hydroxy or keto group at position 3, it is clear why no activity is shown by the steroid esters since esterification occurs at this position. The failure of 3β-methylcholestan-3α-ol and 17α-methyltestosterone to show activity is probably due to the shielding of axial hydroxy-groups by equatorial methyl groups.

It is interesting to find a class of compounds in which the presence of nucleation sites depends so much on the physical methods of sample preparation. The most active samples of cholesterol prepared appear to contain robust sites which will withstand radiation equivalent to a 100 min. of sunlight without losing activity. This may be due to the inaccessibility of other positions of attack if only the edges of the molecular stack are exposed.

[1] Bernal, J. D., Crowfoot, D., and Fankuchen, I., *Phil. Trans. Roy. Soc.*, A, **239**, 135 (1940).

[2] Carlisle, C. H., and Crowfoot, D., *Proc. Roy. Soc.*, A, **184**, 64 (1945).

[3] Fridrichsons, J., and Mathieson, A. McL., *J. Chem. Soc.*, 2159 (1953).

IDENTITY OF L-ASCORBIC ACID FORMED FROM D-GLUCOSE BY THE STRAWBERRY (*FRAGARIA*)

By Dr. FRANK A. LOEWUS and STANLEY KELLY

Western Regional Research Laboratory*, Albany 10, California

WORK in this Laboratory has shown that ascorbic acid recovered from ripening strawberries or germinating cress seedlings administered D-glucose-1-^{14}C is labelled primarily in carbon 1 (the carboxyl carbon)[1,2]. In these experiments, ascorbic acid was removed from the plant extract with an ion exchange resin, 'Dowex 1-formate', selectively eluted by means of a formic acid gradient, diluted with non-isotopic L-ascorbic acid, recrystallized from methanol and ethyl ether mixtures or from glacial acetic acid until there was little or no change in specific activity, and, finally, degraded chemically to determine the specific sites of ^{14}C labelling. A portion of the ^{14}C-containing ascorbic acid plus carrier L-ascorbic acid was oxidized with *p*-quinone to dehydroascorbic acid and then converted to its di-*o*-phenylenediamine derivative[3]. The free acid of this derivative, crystallized from water, had the same specific activity as the ascorbic acid. Conversion of this free acid to its lactone and recrystallization from methanol also yielded a product with the same activity.

---

* A laboratory of the Western Utilization and Research Division, Agricultural Research Service, United States Department of Agriculture.

When these results were published it was felt that these observations sufficed to establish the exact isomeric nature of the labelled ascorbic acid from the strawberry or the cress seedling as L-ascorbic acid. However, Takahashi, Mitsumoto and Kayamori recently published two notes[4] describing the formation of D-araboascorbic acid (D-*iso*ascorbic acid) by various species of the mould, *Penicillium*, when it was grown on D-glucose, D-gluconic acid or sucrose. They also found that *P. notatum* converted D-glucose-1-^{14}C to D-araboascorbic acid in which 80 per cent of the ^{14}C of the molecule was located in carbon 1. The discovery that *Penicillium* can convert D-glucose to D-araboascorbic acid suggests that a more critical appraisal should be made of the isomeric nature of ascorbic acid recovered from higher plants which have been administered specifically labelled D-glucose-^{14}C.

This communication presents the results of an experiment designed to compare L-ascorbic acid and D-araboascorbic acid as carrier substances in the successive recrystallization of labelled ascorbic acid obtained from a ripening strawberry administered D-glucose-6-^{14}C. D-Glucose-6-^{14}C (50 μc. at 7·7 mc./m.mole) was administered in 0·1 ml. of water through the cut stem of a strawberry in the green–white stage of ripening. It required 3 hr. for the aqueous solution to be taken up by the strawberry. After an additional 40 hr. with the stem immersed in distilled water, the fruit (less hull and stem) was frozen, mascerated in ethanol at − 40° and the ascorbic acid recovered without carrier dilution on a 'Dowex 1-formate' exchange resin. The ascorbic acid was selectively eluted with a gradient of 0 → 0·06 *N* formic acid and the fractions containing ascorbic acid, as characterized by titration of aliquots with 2,6-dichlorophenol indophenol, were pooled. These pooled fractions, which contained 5·6 mgm. of reducing material, were divided into two equal portions. To one was added 200 mgm. of D-araboascorbic acid and to the other, 200 mgm. of L-ascorbic acid. Each was evaporated to a syrup, taken up in glacial acetic acid and allowed to crystallize in the cold. Subsequent crystallizations were also done in glacial acetic acid. The radioactivities of each series of crystallizations are summarized in Table 1. With L-ascorbic acid as the carrier, the product after three crystallizations was only slightly less active than after the first crystallization. By contrast, nearly all the carbon-14 remained in the mother liquor when D-araboascorbic acid was used as a carrier. Less than 2 per cent of the specific activity found in the portion diluted with L-ascorbic acid was retained in the portion diluted with D-araboascorbic acid.

The identity of labelled ascorbic acid as L-ascorbic acid was also confirmed by preparing two derivatives of the product representing the third crystallization of the L-ascorbic acid-diluted portion and by comparing their activities with the original ascorbic acid. Both derivatives had specific activities similar to the ascorbic acid used in their preparation (Table 1). Derivatives of the carrier L-ascorbic acid and D-araboascorbic acid were also prepared and submitted to microanalysis.

The 5,6-*iso*propylidene L-ascorbic acid was prepared by the method of von Vargha[5] and recrystallized from absolute ethanol. Calculated for $C_9H_{12}O_6$: C, 50·0 per cent; H, 5·60 per cent. Found: C, 50·1 per cent; H, 5·67 per cent. Attempts to prepare 5,6-*iso*propylidene D-araboascorbic acid by the same procedure resulted in a product having the same melting-point and elemental analysis as D-araboascorbic acid and showing no melting-point depression when mixed with authentic D-araboascorbic acid. Calculated for $C_6H_8O_6$: C, 40·9 per cent; H, 4·57 per cent. Found: C, 41·0 per cent; H, 4·71 per cent. Apparently, the conditions sufficient for the formation of 5,6-*iso*propylidene L-ascorbic acid cannot effect the formation of 5,6-*iso*propylidene D-araboascorbic acid.

Table 1. RADIOACTIVITY OF CARRIER-DILUTED ASCORBIC ACID FROM A STRAWBERRY LABELLED WITH D-GLUCOSE-6-^{14}C

Diluent	L-Ascorbic acid		D-Araboascorbic acid	
	Amount recovered (mgm.)	Radioactivity, mμc./mmole of ascorbic acid	Amount recovered (mgm.)	Radioactivity, mμc./mmole of ascorbic acid
First crystallization	155	74·7	97	8·2
Second crystallization	99	68·7	79	2·0
Third crystallization				
First crop	48	66·5	56	1·0
Second crop	12	69·2		
5,6-*Iso*propylidene derivative		57·4*		
2,3-Diphenacyl derivative		64·2*		

* Activities corrected for a 5·76-fold dilution in the case of the diphenacyl derivative and an 11·7-fold dilution in the case of the *iso*propylidene derivative. These derivatives were wet combusted with Van Slyke–Folch reagent to carbon dioxide and counted in an internal gas phase proportional counter (ref. 14). Other activities were determined in a liquid scintillation apparatus using a liquid scintillation mixture of 0·6 gm. naphthalene, 14·5 ml. *p*-dioxane containing 0·4 per cent 2,5-diphenyloxazole and 0·01 per cent 1,4-*bis*-2(5-phenyloxazolyl)-benzene and the radioactive ascorbic acid in 0·5 ml. of water (ref. 15).

A second derivative, 2,3-diphenacyl L-ascorbic acid[6], was prepared according to the same procedure used to obtain diphenacyl malate[7]. The crude, moist, crystalline product was recrystallized immediately from ethanol in order to avoid the partial decomposition otherwise observed. Calculated for $C_{22}H_{20}O_8$: C, 64·1 per cent; H, 4·88 per cent. Found, L-isomer: C, 64·1 per cent; H, 5·02 per cent. M.p. 137–138°. D-isomer: C, 63·2 per cent; H, 4·99 per cent. M.p. 139–140°.

A portion of the recrystallized L-ascorbic acid diluted fraction was further diluted with authentic L-ascorbic acid (non-isotopic) and degraded[8,9], and the carbon-14 distribution results are given in Table 2. This pattern confirms one obtained previously from a strawberry labelled with D-glucose-6-^{14}C (ref. 10),

Table 2. DISTRIBUTION OF CARBON-14 IN L-ASCORBIC ACID-DILUTED PORTION AFTER THREE CRYSTALLIZATIONS

	Radioactivity*		Total ^{14}C (per cent)
	mμc./m.mole of carbon	mμc./m.mole of ascorbic acid	
Carbon 1 + 2	6·14	12·28	18
Carbon 3	5·25	5·25	8
Carbon 4 + 5	0·86	1·72	3
Carbon 6	47·18	47·18	71
Sum of degraded fragments		66·43	
Van Slyke–Folch wet combustion	11·08	66·48	

* Activities of the degraded fragments are corrected for a final 5·35-fold dilution of L-ascorbic acid after the third crystallization. All counting data were obtained with an internal gas phase proportional counter (ref. 14).

but more importantly, it provides a definitive reply to suggestions[11,12] that such a pattern could also be obtained if D-glucose-6-^{14}C were converted by two separate pathways to a mixture of L-ascorbic acid (labelled primarily in carbon 1) and D-araboascorbic acid (labelled primarily in carbon 6). The present results show that 71 per cent of the carbon-14 in the L-ascorbic acid remained in carbon 6, whereas the residual activity found in the D-araboascorbic acid after two recrystallizations may have been due to traces of L-ascorbic acid.

It may be concluded from this brief study that higher plants do contain an enzymic process capable of converting D-glucose to L-ascorbic acid over a pathway which preserves the carbon skeleton and does not invert the carbon chain yet does result in an epimerization at carbon 5 from the D to the L configuration. Tracer work on ascorbic acid biosynthesis in plants preceding the experiment just described has been reviewed in a paper that will be published shortly[13].

We acknowledge the help of Miss Geraldine Secor, who performed the microanalyses, and the suggestions of Dr. Peter Dayton, Goldwater Memorial Hospital, New York, concerning the preparation of 5,6-*iso*propylidene L-ascorbic acid.

[1] Loewus, F. A., Jang, R., and Seegmiller, C. G., *J. Biol. Chem.*, **222**, 649 (1956).
[2] Loewus, F. A., and Jang, R., *Biochim. Biophys. Acta*, **23**, 205 (1957).
[3] Hasselquist, H., *Arkiv Kemi*, **4**, 369 (1952).
[4] Takahashi, T., Mitsumoto, M., and Kayamori, H., *Bull. Agric. Chem. Soc., Japan*, **24**, 533 (1960); *Nature*, **188**, 411 (1960).
[5] von Vargha, L., *Nature*, **130**, 847 (1933).
[6] Vestling, C. S., and Rebstock, M. C., *J. Biol. Chem.*, **161**, 285 (1945).
[7] Loewus, F. A., Tchen, T. T., and Vennesland, B., *J. Biol. Chem.*, **212**, 787 (1955).
[8] Horowitz, H. H., Doerschuk, A. P., and King, C. G., *J. Biol. Chem.*, **199**, 193 (1952).
[9] Horowitz, H. H., and King, C. G., *J. Biol. Chem.*, **200**, 125 (1953).
[10] Loewus, F. A., Finkle, B. J., and Jang, R., *Biochim. Biophys. Acta*, **30**, 629 (1958).
[11] Mapson, L. W., *Proc. Intern. Cong. Biochem., Fourth Cong., Vienna*, 1958, 11, 1 (Pub. 1959).
[12] Isherwood, F. A., and Mapson, L. W., *Ann. New York Acad. Sci.*, **92**, 6 (1961).
[13] Loewus, F. A., *Ann. New York Acad. Sci.*, **92**, 57 (1961).
[14] Van Slyke, D. D., Steele, R., and Plazin, J., *J. Biol. Chem.*, **192**, 769 (1951).
[15] Werbin, H., Chaikoff, I. L., and Imada, M. R., *Proc. Soc. Exp. Biol. Med.* **102**, 8 (1959).

AMINO-ACID LIBERATION FROM PROTEINS BY ENZYME OR 'HEAT CATALYSED' HYDROLYSIS

By S. M. HARDY*

Research No. 5, The Hospital for Sick Children, Great Ormond Street, London, W.C.1

THE term 'heat catalysed' hydrolysis is introduced here for the first time to emphasize a certain phenomenon in the so-called acid/alkali hydrolysis of proteins which has been overlooked apparently or underestimated hitherto. The term is used to point out some interesting similarities between acid/alkali and enzymic hydrolysis, such as the similar parts played by the enzyme and by the heat in bringing about the liberation of amino-acid from the peptide chain. At the same time certain dissimilarities between the two processes will be indicated, and it will be shown how these may explain the significant differences in the physical parameters of the resultant amino-acids.

Analysis of urinary peptide and amino-acid excretion in children has been carried out recently using a high-tension electrophoretic apparatus which I have designed and constructed. This was of the free-floating type. It was kept at a constant temperature of 0° C. by a refrigerated *n*-hexane circulating system controlled by thermistors. The cooling was efficient enough to maintain the entire length of the electrophoretic paper at within 0·5 deg. C. of the same temperature. This was checked by the use of a series of ten specially designed thermometers which were placed in contact with the paper at 10-cm. intervals. Thus the temperature of the paper was measured throughout the entire operation.

Whatman No. 4 paper 1 cm. wide and 120 cm. in length was used. The paper was suspended horizontally through the centre of the cooling liquid with the nylon-sheathed ends dipping into the two buffer-electrode containers. The buffer used had a pH of 1·9 (acetic acid–formic acid–water, 3 : 1 : 16) with a conductivity of $0·3 \times 10^4$ micro-ohm. The power used was 200 V./cm. length of paper and thus 1·0 m.amp./cm. width of paper for 25 min. was produced. While this was taking place several hitherto undescribed phenomena in the behaviour of amino-acids at high field strengths were observed.

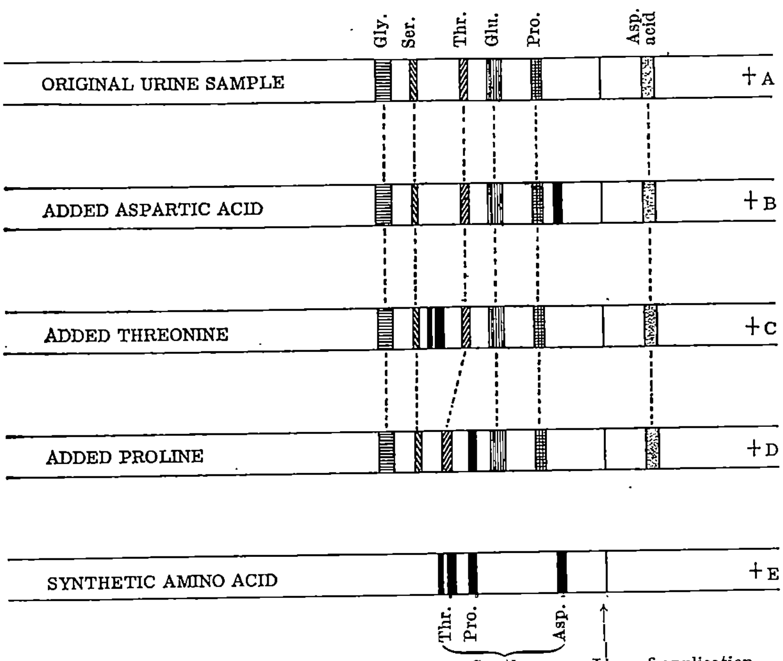
Among these was one which initially caused some difficulties, when it was attempted to identify the separated fractions by adding, to the ion-exchange resin-desalted urinary sample, commercial amino-acids of high purity which were known by previous chromatographic tests to be present in the urinary sample. The result was that these added amino-acids, instead of joining up with their urinary counterparts, and thus increasing the intensity of the subsequent colour reaction, produced separate additional fractions on their own.

The findings of an experiment in which three commercial amino-acids were added to the ion-exchange resin-desalted urinary sample are demonstrated in Fig. 1. The original urine of a healthy child yielded, according to previous chromatographic evidence, six amino-acids in ninhydrin-staining quantities; the same six amino-acids were also found to be separated on subsequent high-tension electrophoretic testing. Strip *A* represents the urinary sample, yielding aspartic acid, proline, glutamic acid, threonine, serine and glycine. On strips *B*, *C* and *D*, aspartic acid, threonine and proline were added to the urinary sample respectively. Finally, on strip *E* all three commercial amino-acids that were added can be seen in a simple buffer solution alone.

It is apparent that in no case have the added amino-acids joined up with their urinary counterparts. They have produced instead an additional fraction on their own account which is clearly identifiable.

---

* Present address: Department of Biochemistry, Dulwich Hospital, East Dulwich Grove, London, S.E.22.



200 V./cm. 1 m.amp. pH 1·9. 25 min.

Fig. 1

(Threonine has produced not one, but two fractions on strip *C*, as well as on strip *E*. This is thought to be due to the fact that this amino-acid is a racemic mixture of D- and L-threonines. This particular finding and the possible explanation for it will be discussed separately in a later paper.)

This test was repeated chromatographically, but no such behaviour was then observed. The commercial amino-acids which were added merely joined up and increased the ninhydrin colour reaction of the appropriate amino-acids.

After repeating these tests and obtaining the same results, it was concluded that the underlying explanation for this specific and unexpected behaviour must lie in the electric parameters of the respective amino-acids, since it was found to manifest itself only when they were subjected to a strong electric field.

The major factor determining the mobility of amino-acids in electric fields is the molecular electric charge of the amino-acid itself. It is assumed, therefore, from the findings described here that the molecular electric charge of urinary and commercial amino-acids must differ.

The literature has been searched for descriptions of similar experiences by others using high-tension electrophoretic techniques. It was found that the actual number of authors who describe their results in detail using various means of chemical identification of the separated fractions is very small, and that those who do so do not mention such difficulties as have just been described.

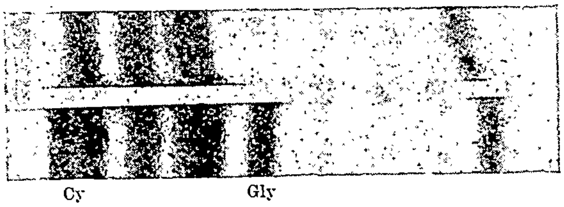


Fig. 2

In one single instance, however, in an article by Heilmeyer *et al.*[1], a photograph of an original high-tension electropherogram was found (Fig. 2). This illustration (Fig. 3 of the original article) shows two stained high-tension electropherograms. On the upper strip four amino-acids separated from the resin-desalted urine sample can be seen. Underneath is another strip, on which it can be seen that the actual number of separated fractions has increased by two. The caption states that in the case of the lower strip two commercial amino-acids, cysteine and glycine, have been added (these are marked Cy and Gly). However, there is no mention, in either the text or caption, of the fact that the number of separated fractions has increased. This increase in this case is thought to be a confirmation of my own findings.

The importance of this specific behaviour of amino-acids, the fundamental building blocks of proteins, in electric fields will become more evident if we consider that, in biological circumstances in living systems, proteins, peptides and amino-acids, as well of course as other metabolites, are under the permanent stress of electric fields applied across biological membranes. These electric fields are maintained by the difference in concentration of the electrolytes on the two sides of the membrane, and they amount to an average potential difference of 100 mV. Since the average thickness of such membranes is of the order of 10^{-6} cm., the average actual value of these biological membrane potentials is of the order of 100,000 V./cm. Thus it can be said that the electric field maintained by my high tension electrophoresis is somewhat sub-biological, being about a tenth of the average of the living cell. These biological potentials, however, are characteristic and inherent parts of the living systems and their changes are the foundations of life.

The importance of this biological electrical field has already been emphasized in a previous report[3]. Attention was directed to the fact that there may be a selective biological membrane transport of individual amino-acids due to these biological potentials.

It would therefore seem important to try to elucidate the real foundations of this unexpected behaviour of amino-acids derived from different modes of liberation.

Living organisms liberate these amino-acid metabolites from food proteins by the use of enzymes at the average body temperature of the living species. The amino-acids that are commercially available, on the other hand, are either liberated by acid/alkali or enzymatic hydrolysis of a readily hydrolysable protein, for example, casein, or else are the result of bacterial metabolic production. It was thought that a closer investigation of at least the two main types of liberating processes—the acid/alkali and the enzymic—might provide some explanation as to why commercial amino-acids bear a different molecular electric charge from their urinary counterparts.

In the enzymic hydrolysis of proteins, the protein substrate is first subjected to an adjustment of its original *p*H. This will be according to the optimum range of *p*H for the enzyme(s) to be used. This imposed change of *p*H can be either on the acid or on the alkali side. The importance of this *p*H adjustment is obvious from theoretical considerations, and it was assumed earlier that the carriers of catalytic activity of enzymes were mostly the zwitterionic forms of the molecule, since it was observed that in most cases the optimum *p*H coincided with the isoelectric point of the enzyme. This was first suggested by Michaelis and Davidsohn[4], but since then it has been discovered that this is often not the case, and the isoelectric point does not really coincide with the optimum *p*H; therefore, it is supposed that the activity is dependent on the ionization of certain groups on the enzyme molecule and not always necessarily in the zwitterionic form.

The importance of the effect of *p*H on enzymic activity from the point of view of the quality of hydrolysis is laid down in an excellent work by Wilson and Nachmanson[5], in which they elucidated the reaction mechanism of the acetylcholinesterase enzyme. Waley[6] and Dixon[7] have demonstrated the effect of *p*H in general on enzymic activity.

After the adjustment of the *p*H of the protein substrate, the chosen enzyme(s) is introduced, and at the optimum temperature, the process of enzymatic hydrolysis proceeds.

Since it is proposed to deal later on with the heat-catalysed acid/alkali hydrolysis of proteins, it is thought that the meaning of the term 'optimum temperature' should be looked at more closely. Temperature is surely one of the most influential and important factors governing reaction mechanisms specificially in biological processes. The optimum temperature is generally considered to be the deg. C. at which the maximum rate of reaction occurs, or in other words, at which the enzyme will liberate the greatest number of amino-acids from the protein substrate in a given time. As early as 1895, Tammann[8] suggested that enzymic processes show acceleration when the temperature is raised, but when a maximum point has been reached will slow down rapidly again on account of thermal destruction of the enzyme protein molecule itself, in which case irreversible changes take place in the molecular configuration of the enzyme which greatly affect its specific activity, and finally arrest it. It will be seen that this optimum temperature is a range of temperatures rather than a sharp point, since living systems are accustomed to certain degrees of change in their environmental temperature, which in turn influence their internal temperature; thus, their metabolic processes have acquired, during the course of evolution, a certain flexibility in their response to changes in temperature over a limited range. It is important to maintain the optimum temperature in enzymic processes, because if it falls or rises the inherent energy content of the reactive system would be either reduced or increased, thus causing variation in an important factor in the reaction mechanism.

It can be said with certainty at this stage that in enzymic hydrolysis, whether *in vitro* or *in vivo*, change of entropy does not occur appreciably; no mass resonance, rotation, translation or vibration of the electron system of the reacting molecules occurs and the force binding the enzyme to the substrate protein molecule acts by straightforward contact and is dependent among others on intermolecular distances. Charged groups on the substrate molecules become attached to groups on the enzyme molecules bearing opposite charges. This bond may be analogous to 'salt-bridge' formation in proteins. It is known that the K_S (dissociation constant of the enzyme-substrate complex) is infinite only for positively charged substrates of trypsin. Hydrogen bridges may also play a part. Protein molecules are capable of forming intramolecular hydrogen bridges, and with a suitable substrate it is plausible that intermolecular hydrogen bridges can also be formed. This is thought to be the case with the proteases. Van der Waals forces also come into play, for example, in the action of cholinesterase, where the K_S decreases and therefore the affinity increases with the increasing length of the hydrocarbon chain in the acyl esters of choline. Finally, it should be mentioned that the substrate can also react with the enzyme in the formation of covalent bonds.

In all these types of enzymic hydrolysis reactions the reacting substrate protein molecules utilize their molecular free-energy content, for no external energy is supplied to them in any form; the maintenance of a constant optimum temperature prevents them from losing energy unproductively.

It is also well known that enzymic hydrolysis of proteins can be repeated successfully with the same results so long as the delicate experimental conditions are adhered to strictly. The specific mode of action of the various enzymes has been described and explained by Neurath and Schwert[9], Smith[10] and Green and Neurath[11].

In the heat-catalysed hydrolysis of proteins the substrate protein undergoes the same preliminary treatment as in the enzymic process described above, namely, its *p*H is changed. But in this case, instead of introducing the enzyme into the *p*H-adjusted substrate, external energy is supplied in the form of excessive heat, and thus amino-acid liberation occurs.

A well-known phenomenon in chemistry is the increase in the rate of reaction which occurs when the temperature of the reactive mixture is raised. This increase cannot be accounted for solely by the increased number of molecular collisions because, as a rule, the rate of the reaction increases with the rising temperature far more sharply than does the frequency of the collisions. It will be remembered that reacting molecules having an average velocity do not generally produce chemical reactions since they do not have enough energy to develop into the transitional state. This transitional state, from the point of view of energy consumption in reactive phases, is the top of a curve up which the reacting molecules must have sufficient energy to climb before they can descend down the other side, releasing energy and forming the product. The rate of reaction is related then to the number of molecules that pass from the reactive side to the productive side of this curve in a given time. Raising the temperature will necessarily increase the number of molecules with sufficient energy to take part in this process.

The thermodynamic constants, for example, heat, entropy, etc., of activation and reaction supply a good picture of the stages of reaction in these cases. Kunitz[12] has provided evidence for the reversible denaturation by heat of crystalline trypsin inhibitor, a globular protein of 25,000 mol. wt. He found that when the native protein was denatured reversibly, the heat generated by the reaction was 57·3 k.cal./mole and the entropy change was 180 cal./°C./mole. There was a great increase in entropy and no increase in the number of molecules in the substrate heated.

This indicates that the denatured form of the molecule is formed from the native state by bond breakage, and thus it has greater freedom of movement than before. It is an interesting point that the total energy of the activated complex is almost the same as that of the denatured form and very different from that of the native form.

This activated complex is an intermediate form of the native molecule in its progression to the end product and its lifetime is about 10^{-13} sec. Once formed, it decomposes with a frequency of kT/h, where T is the absolute temperature, h is Planck's constant and k is Boltzmann's constant, that is, R/N the gas constant per molecule. The precise significance of the activated complex, however, can only be appreciated by extensive reference to the quantum theory and statistical methods, subjects which it is not intended to develop further here.

However, the entropy of the activated complex is still much less than that of the total completed reaction, that is, the sum of the entropies of the amino-acids liberated. Under the compelling influence of the constantly available supply of excess energy the activated protein molecule, while not gaining in its molecular free-energy content, steadily increases its entropy until such a state of instability is produced that some of its valence bonds will be broken. The remaining bonds will break spontaneously as time progresses.

It is reasonable to assume that the excess energy in the activated protein complex at the time of its final break-up will reside in the resultant amino-acids.

It has been found by Eyring and Stearn[13] using trypsin, and by Eisenberg and Schwert[14] using chymotrypsin, that the value of the thermodynamic constants of heat denaturation of proteins depends significantly on the pH of the substrate. This is interesting if it is considered together with the dependence of pH of the enzymic activity.

The fact that it has many drawbacks is a well-known characteristic of such a heat-catalysed liberation of amino-acids. These include structural modification, racemization, tautomerization, destruction and artefact production of various types of amino-acids, as have been described by Neuberger[15], Spies and Chambers[16], Dakin[17], Levene and Steiger[18], and Sanger and Thompson[19]. These authors are all in agreement, and they suggest that such amino-acids as are formed during heat-catalysed hydrolysis of proteins are chemically and biologically identical to those liberated by enzymatic hydrolysis. They admit that side-effects are unavoidable, and although they are to some extent undesirable, they account merely for some quantitative and qualitative loss or gain of some specifically known amino-acid.

In attempting to find the fundamental difference between enzymic hydrolysis or enzyme-catalysed hydrolysis and acid/alkali hydrolysis or heat-catalysed hydrolysis, it becomes apparent that it is to be looked for in the very similarity of the two methods. The two kinds of liberating agents, the heat and the enzyme, both produce the same result, namely, bringing about breakage of the terminal bonds of amino-acids: without them there would be no significant liberation of amino-acids. But if we go one step further and consider the way in which these two agents, to such an extent similar, achieve their final target, we must realize the essential dissimilarity between their activities.

What kind of factor is it then which can be pointed out as responsible for the dissimilarities in the molecular electric charges of the liberated amino-acids, since they are chemically and probably, so far as we know at present, biologically identical ? It is unlikely to be found in the similarities between the two methods. But in considering the differences between them, in terms of the utilization of energy, exchange of energy and the general relations between the energies involved in the processes, the real cause may be brought to light.

In enzymic processes, as I have already pointed out, there is no need whatever to provide energy externally, as there is in heat-catalysed hydrolysis. All the energy needed to proceed from one side of the 'hill' over the 'summit' of the intermediate complex to the resultant reactive side of the liberated amino-acids is obtained from the molecular free-energy content of the protein substrate itself, and of the enzyme without significant change in the entropy of the system and well within the limits of thermodynamics. This is why any kind of gross structural irregularity, artefact production or destruction of amino-acids during enzymic hydrolysis would not be expected. Everything proceeds along a fairly well-determined pathway in stages which can to some extent be calculated from the thermodynamic equations.

But this is not the case in heat-catalysed hydrolysis. In this method of liberation of amino-acids, excess energy is transferred to the protein substrate. An unlimited amount of energy is available for any type of energy-consuming reaction to take place. The intra- and inter-molecular bonding electrons of the protein substrate molecules are also excited by means of resonance to extreme levels. The heat energy is absorbed by the electron system and transformed into electrical form. The molecular electric charge of the resultant amino-acid ultimately depends on the behaviour of the electron pool. These electric effects are not great enough to cause alternations in the chemical behaviour of the molecules; but the physical means by which they are recognized, their molecular electric charge, is altered sufficiently to change their motility in an electric field, and thus make them behave differently from the chemically similar amino-acids formed by the enzymic liberating processes.

If this problem is considered more in terms of physics than of chemistry, it will be seen that the application of wave mechanics to the behaviour of electrons will lead to an understanding of the function of the ultra-structure of the living intracellular system, and to an appreciation of factors hitherto thought to be insignificant.

It is suggested therefore that, under the readily observable chemical changes in heat-catalysed hydrolysis, there lie appreciable variations in the world of physical parameters. There are a number of changes in the physical nature of the molecules which may occur, but which will not affect necessarily the simple chemical behaviour of the amino-acids. They will, however, become apparent when tested by sufficiently sensitive methods, for example, that of molecular electric charge measurement.

Unfortunately, it was beyond the scope of the present research to investigate the demonstration of the differences in molecular electric charge by physical means other than high-tension electrophoresis.

However, it is thought to be more than probable from what is known of the forces involved in crystallization, that the existence of differences in molecular electric charge on one or two sites of an amino-acid molecule would result in alteration, if only to a limited extent, of its crystalline structure. Nevertheless, I have been unable to find any reference in the literature to work on the crystalline structures of amino-acids derived from different means of liberation.

Another means by which it would apparently be possible to prove or disprove the above theory would be to measure the electron spin resonance of these differently liberated amino-acids. This excellent method, although in its pioneer stage, promises to become a most powerful tool for fundamental research, and is already finding its way into biological laboratories at the present time[20,21].

The measurement of the diamagnetic susceptibility of amino-acids[22] of different origin would also be a useful method of investigation. In 1936, Bauer and Ruskin[23] used this method with great success in establishing the differences between the diamagnetic susceptibility of proteins in living and non-living states. Work along similar lines is in progress, to interpret their results in the light of the present findings with regard to molecular electric charge and its connexion with the electrical functions of living systems, and will be described later in a separate publication.

I thank G. H. Zeal and Co., London, for their help in designing and supplying special thermometers for the observation of temperature variations along the entire length of the high-tension electrophoresis paper. This has enabled me to clear up an important point and one which has been in question for many years.

[1] Heilmeyer, L., *et al.*, *Klin. Wchschr.*, **32**, 833 (1954).

[2] Johnson, F. H., *et al.*, *The Kinetic Basis of Molecular Biology*, Chap. 2 (Wiley, New York, 1954).

[3] Hardy, S. M., *Nature*, **185**, 245 (1960).

[4] Michaelis and Davidsohn, *Biochem. Z.*, **28**, 1 (1910).

[5] Wilson, I. B., and Nachmanson, D., *Adv. in Enzymol.*, **12**, 259 (1951).

[6] Waley, S. G., *Biochim. Biophys. Acta*, **10**, 27 (1953).

[7] Dixon, M., *Biochem. J.*, **55**, 161 (1953).

[8] Tammann, G., *Z. physik. Chem.*, **18**, 426 (1895).

[9] Neurath, H., and Schwert, G. W., *Chem. Rev.*, **46**, 69 (1960).

[10] Smith, E. L., *Adv. in Enzymol.*, **12**, 191 (1951).

[11] Green, M., and Neurath, H., *The Proteins*, edit. by Neurath and Bailey, **2**, 1106 (Academic Press, New York, 1954).

[12] Kunitz, M. J., *J. Gen. Physiol.*, **32**, 241 (1948).

[13] Eyring, H., and Stearn, A. E., *Chem. Rev.*, **24**, 243 (1939).

[14] Eisenberg, M. A., and Schwert, G. W., *J. Gen. Physiol.*, **34**, 583 (1950).

[15] Neuberger, A., *Adv. in Protein Chem.*, **4**, 297 (1948).

[16] Spies, J. R., and Chambers, D. C., *Annal. Chem.*, **21**, 1249 (1949).

[17] Dakin, H. D., *J. Biol. Chem.*, **8**, 25 (1910).

[18] Levene, P. A., and Steiger, R. E., *J. Biol. Chem.*, **86**, 703 (1930); **98**, 321 (1932).

[19] Sanger F., and Thompson, E. O. P., *Biochim. Biophys. Acta*, **9**, 225 (1952).

[20] Pake, G. E., *et al.*, *Disc. Faraday Soc.*, **19**, 147 (1955).

[21] Sogo, P. B., and Tolbert, B. M., *Biol. Med. Physiol.*, **5**, 1 (1957).

[22] Perceau, R., *C.R. Acad. Sci., Paris*, **236**, 76 (1953).

[23] Bauer, B., and Ruskin, A., *Nature*, **138**, 801 (1936).

BIO-ASSAY OF ERYTHROPOIETIN IN MICE MADE POLYCYTHÆMIC BY EXPOSURE TO AIR AT A REDUCED PRESSURE

By Dr. P. MARY COTES[*]

Medical Unit, University College Hospital Medical School, London

AND

Dr. D. R. BANGHAM

National Institute for Medical Research, London

ERYTHROPOIETIN is characterized by its ability to induce an increase in the total red cell mass of an intact normal animal[1]. This property is seldom used for the estimation of erythropoietic activity in biological fluids from individual animals or human subjects, since it requires larger amounts of active material than are usually available. For this reason, methods of assay in common use employ test animals in which red cell formation has been suppressed[2,3] so that there is a low base-line from which to measure stimulation of erythropoiesis. Formation of new red cells is estimated by measurement of parameters which reflect maturation and division of bone marrow cells[4], hæmoglobin synthesis[5–7], or a mixture of these events. The assays in most general use are based on measurement of reticulocyte counts[8–12] or incorporation of iron-59 into circulating red cells[5–7]; the test animals are normal[10,11] or starved[3,5] or transfused-polycythæmic rats[7] or mice[8]. This article describes a system for the bio-assay of erythropoietin in mice in which endogenous red cell formation is suppressed by the polycythæmia which persists after animals have been exposed to air at a reduced pressure; incorporation of iron-59 into red cells is used as the response parameter to measure the stimulation of new red cell formation produced by the administration of exogenous erythropoietin.

The erythropoietin used in development and use of the assay was prepared from plasma of rabbits, monkeys or sheep, made anæmic by treatment with phenylhydrazine. The rabbit material was a filtrate made from plasma after heat treatment at *p*H 5·5 essentially as described by Borsook[13]; it was stored at − 30° C. The monkey material was prepared from the supernatant of plasma after treatment with 15 per cent w/v sodium sulphate. The sheep material was a freeze-dried powder (Lot No. *RO*77019), purified in the Armour Laboratories and kindly provided by Dr. White; it was made up in solution so that 1 ml. contained 8·0 'cobalt units'[14]. The human erythropoietin was unfractionated plasma from an anæmic patient with paroxysmal nocturnal hæmoglobinuria. The test animals were female mice of the MRC *TO* and Schneider strains and weighed 16–24 gm. Before the test, they were maintained for 14 days in a tank with air at a pressure of half an atmosphere, and an air flow through the tank sufficiently great to keep the carbon dioxide concentration below 1 per cent. Water and food (MRC cube diet 41) were supplied

---

[*] Beit Memorial Research Fellow.

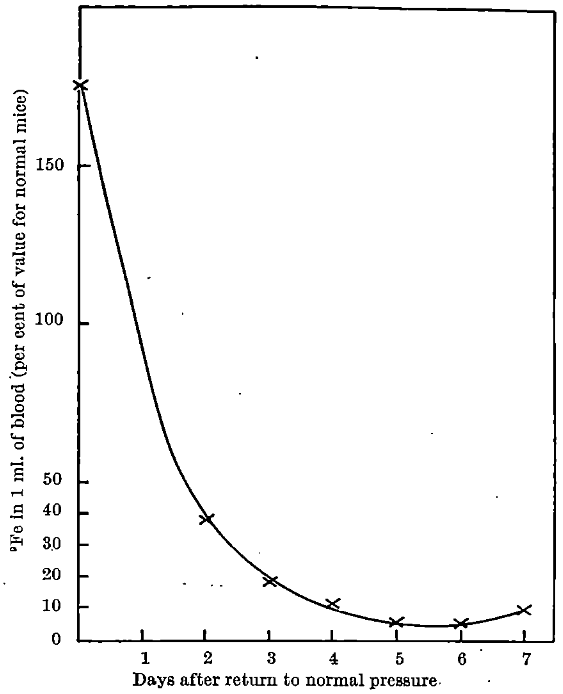


Fig. 1. Depression of red cell formation in mice returned to air at normal pressure after two weeks in air at half an atmosphere pressure
Appearance of a tracer dose of iron-59 in 1 ml. of blood of female *TO* mice (20 hr. after intravenous injection of $0\cdot1\mu$c. ^{59}Fe-citrate) at various time-intervals after return of the mice to normal pressure. Results expressed as percentage of iron-59 appearing in blood of normal mice

ad lib. and the tank was opened to the atmosphere for a short period each day for routine animal care. Hæmatocrits were estimated by a micro-method. Blood volume was measured using red cells labelled with chromium-51 which, before use, were screened by passage through a normal mouse to remove cells damaged during labelling. Red cell mass was derived from hæmatocrit and blood volume. The incorporation of iron-59 into red cells was measured 20 hr. after intravenous injection of a tracer dose of 0·1 μc. iron-59 citrate; a 20-hr. time-interval was selected for this measurement, as the appearance of iron-59 in red cells was then small and less than 1 per cent of the injected iron-59 remained in the plasma.

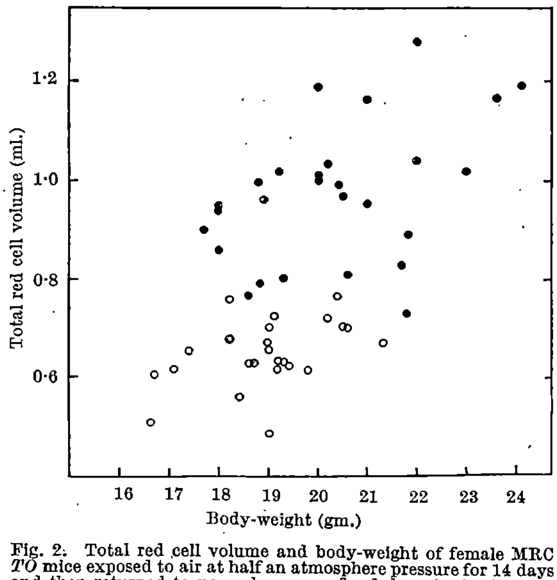


Fig. 2. Total red cell volume and body-weight of female MRC *TO* mice exposed to air at half an atmosphere pressure for 14 days and then returned to normal pressure for 6 days (series '14 + 6')
○, Normal; ●, '14 + 6'

Mice which had spent 14 days in air at half an atmosphere were studied at various times after they had been returned to normal pressure. Four or six days after the mice had been returned to normal pressure, the incorporation of iron-59 into red cells was depressed (Fig. 1), their red cell masses were increased (Fig. 2) and, in MRC *TO* mice at 4 days and 6 days, their hæmatocrits were above the normal range (Fig. 3). Blood volumes at (14 + 4) and (14 + 6) days were approximately 7·5 per cent of body-weight.

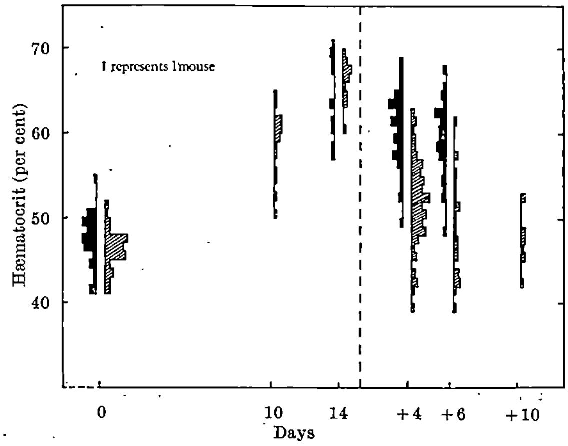


Fig. 3. Hæmatocrits of female MRC *TO* and Schneider mice exposed to air at half an atmosphere pressure for 14 days and then returned to normal pressure. Frequency distribution of hæmatocrits are shown as histograms
Black, *TO*; shaded, Schneider

On the basis of these results, the response to erythropoietin was investigated in mice which had spent 14 days in air at half an atmosphere pressure and which were killed 4 or 6 days after their return to normal pressure. These series are referred to as '14 + 4' and '14 + 6', respectively. The routine of the test is essentially similar to that used by other workers and is shown in Table 1. The response was taken as the percentage of the administered iron-59 present in the blood 20 hr. after intravenous injection of the iron-59; the blood volume was calculated as 7·5 per cent of body-weight. The log dose–response relationships obtained with small groups of mice treated with rabbit or sheep erythropoietin are shown in Fig. 4. From these graphs it may be seen that both the MRC *TO* and the Schneider mice are satisfactory test animals for the estimation of erythropoietin. *TO* mice, series 14 + 4, have been selected for our routine assay of erythropoietin. With rabbit erythropoietin, the log dose–response regression line did not differ significantly from linearity over a thirty-fold range of doses; its slope (b) was 16·03 and the index of precision (λ) was 0·35. Sheep erythropoietin tested in this system also gave a linear regression with slope 20·73 and index of precision (λ) 0·22. Over a longer range of doses, the log dose–response relationship was sigmoid in form. In this series the range of hæmatocrits of test animals was above the normal. Thus measurement of hæmatocrit was used to assess whether a test extract had

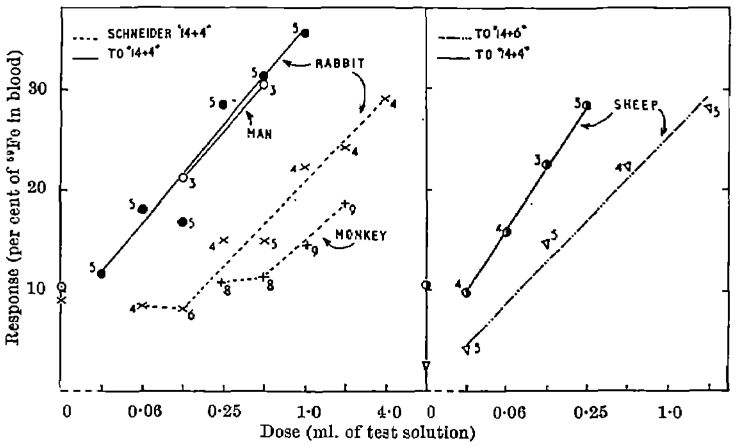


Fig. 4. Log dose–response regression lines obtained with MRC *TO* mice series '14 + 4' and '14 + 6' and with Schneider mice series '14 + 4' treated with preparations of erythropoietin from plasma of rabbit, sheep, monkey and man. Figures on the graph show numbers of animals used

caused hæmolysis, so that affected animals in which response might be increased by secretion of endogenous erythropoietin might be excluded from the test (cf. Winkert and Gordon[15]). In routine assays, results from mice with hæmatocrits below 50 per cent were discarded.

TO mice killed on day (14 + 6) may be advantageous for certain assays. These animals gave a base-line which was lower and more consistent than that obtained with the '14 + 4' series. They were sensitive to as little as 0·25 'cobalt units'[16] of erythropoietin and responded to this with a significant increase above the base-line incorporation of iron-59. The hæmatocrits of *TO* mice, series '14 + 6', overlapped little with the normal range and were also used to show whether treatment had caused hæmolysis. The Schneider mice, series '14 + 4', were not selected for routine assays because they were less sensitive to small doses of erythropoietin than the *TO* mice, and their range of hæmatocrits overlapped with the range for normal Schneider mice.

Data obtained with human erythropoietin administered to *TO* mice, series '14 + 4', and with monkey erythropoietin administered to Schneider mice, series '14 + 4' are also included in Fig. 4. These show that the system responds to erythropoietins prepared in various ways from four species, namely, rabbit, sheep, man and monkey; moreover, the slopes of the regression lines obtained with rabbit, sheep and human erythropoietin did not differ significantly ($P = 0{\cdot}35$).

Table 1. ROUTINE TEST PROCEDURE FOR USE OF MICE MADE POLYCYTHÆMIC BY EXPOSURE TO HYPOXIA

	Day of test	
	Series '14+4'	Series '14+6'
Mice maintained in air at half atmosphere pressure	0–14	0–14
Mice returned to normal pressure	14	14
First injection of test material (68 hr. before killing)	15	17
Second injection of test material (44 hr. before killing) (Each injection made up to 1 ml. with saline and given intraperitoneally)	16	18
^{59}Fe-citrate, 0·1 μc. in 0·2 ml. saline, injected intravenously (20 hr. before killing)	17	19
Weigh, bleed and kill mice (avertin anæsthetic)	18	20
Measure hæmatocrit and count ^{59}Fe present in sample of blood using a well-type scintillation counter		

This method has certain advantages over the methods in common use for estimation of erythropoietic activity. The labour and cost of transfusion, and the possibility of non-specific stimulation in a starved animal is avoided by the suppression of endogenous erythropoiesis with a polycythæmia of each animal's own red cells. Our test animal is a more physiological preparation than a starved or transfused animal. The mice are easily prepared in large numbers and the assay may thus be planned on a statistical basis. The system is at least as sensitive as other *in vivo* methods for the estimation of erythropoietin[17].

Garcia[6] has shown that a preparation of human urinary erythropoietin which stimulates incorporation of iron-59 into red cells in the starved rat, will also increase the total red cell mass in rats. Keighley *et al.*[18] have shown that one preparation of rabbit plasma erythropoietin induced an increase in the total red cell volume, hæmoglobin concentration and hæmatocrit of normal rats; it stimulated incorporation of iron-59 into red cells of normal, starved and hypophysectomized rats and caused a reticulocytosis in normal rats and polycythæmic-transfused mice. These test systems reflect various aspects of erythropoiesis. The incorporation of iron-59 into red cells measures chiefly hæmoglobin synthesis and only indirectly reflects division of erythropoietic cells. Reticulocytosis measures chiefly maturation of normoblasts, but it may be produced by release of reticulocytes. At the present time it is not known if these and other aspects of erythropoiesis are all controlled by one and the same 'erythropoietin' molecule. It is therefore essential that estimates of the relative erythropoietic activities of various preparations should be correlated by simultaneous studies using different assay systems.

[1] For review and refs., see Gordon, A. S., *Physiol. Rev.*, **39**, 1 (1959).

[2] Jacobson, L. O., Plzak, L., Fried, W., and Goldwasser, E., *Nature*, **177**, 1240 (1956).

[3] Fried, W., Plzak, L. F., Jacobson, L. O., and Goldwasser, E., *Proc. Soc. Exp. Biol., N.Y.*, **94**, 237 (1957).

[4] Matoth, Y., Biezunski, N., and Szabo, G., *J. Lab. Clin. Med.*, **51**, 420 (1958). Ross, W. F., and Gurney, C. W., *J. Lab. Clin. Med.*, **53**, 446 (1959). Gordon, A. S., Winkert, J., Dornfest, B. S., and Siegel, C. D., *Ann. N.Y. Acad. Sci.*, **77**, 650 (1959).

[5] Hodgson, G., Perreta, M., Yudilevich, D., and Eskuche, I., *Proc. Soc. Exp. Biol., N.Y.*, **99**, 137 (1958).

[6] Garcia, J. F., and Van Dyke, D. C., *J. App. Physiol.*, **14**, 233 (1959).

[7] Gurney, C. W., and Pan, C., *Proc. Soc. Exp. Biol., N.Y.*, **98**, 789 (1959).

[8] Lowy, P. H., Keighley, G., Borsook, H., and Graybiel, A., *Blood*, **14**, 262 (1959).

[9] Jacobson, L. O., Marks, E. K., Gaston, E. O., and Goldwasser, E., *Blood*, **14**, 635 (1959).

[10] Winkert, J. W., and Gordon, A. S., *Proc. Soc. Exp. Biol., N.Y.*, **104**, 713 (1960).

[11] Payne, R. W., Jones, N. F., and Hyde, R. D., *Nature*, **185**, 549 (1960).

[12] Erslev, A. J., *Ann. N.Y. Acad. Sci.*, **77**, 627 (1959).

[13] Borsook, H., Graybiel, A., Keighley, G., and Windsor, E., *Blood*, **9**, 734 (1954).

[14] Goldwasser, E., and White, W. F., *Fed. Proc.*, **18**, 236 (1959).

[15] Winkert, J. W., and Gordon, A. S., *Proc. Soc. Exp. Biol., N.Y.*, **104**, 713 (1960).

[16] White, W. F., Gurney, C. W., Goldwasser, E., and Jacobson, L. O., *Rec. Prog. Hormone Res.*, **16**, 219 (1960).

[17] Schlueter, R. J., Norgello, H., and White, W. F., *Proc. Soc. Exp. Biol., N.Y.*, **103**, 43 (1960).

[18] Keighley, G., Lowy, P. H., Borsook, H., Goldwasser, E., Gordon, A. S., Prentice, T. C., Rambach, W. W., Stohlman, jun., F., and Van Dyke, D. C., *Blood*, **16**, 1424 (1960).

TRYPTAMINE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

By Dr. J. R. VANE
Department of Pharmacology, Royal College of Surgeons, Queen Square, London, W.C.1

Dr. H. O. J. COLLIER and Dr. S. J. CORNE
Department of Pharmacological Research, Parke, Davis and Co., Hounslow, Middlesex

AND

Dr. E. MARLEY and Dr. P. B. BRADLEY
Department of Experimental Psychiatry, Medical School, University of Birmingham

THE actions and role of 5-hydroxytryptamine (5-HT) in the central nervous system are of great interest. Since injected 5-HT does not readily enter the central nervous system, Vane[1] suggested studying the central actions of related compounds such as α- or *N*-alkyl-tryptamines and their 5-methoxy derivatives, which are able to pass the blood-brain barrier and resist attack by monoamine oxidase (MAO). We describe here some effects of tryptamine, *dl*-α-methyltryptamine (MT), *dl*-α-ethyltryptamine (ET), *dl*-5-methoxy-α-methyltryptamine (MeOMT) and *N*,*N*-dimethyltryptamine (DMT) on the behaviour of animals and on their responses to other centrally acting drugs, on central electrical activity and on spinal reflexes. The 5-HT-like actions of these compounds on isolated smooth muscle have already been described[1,2].

Spontaneous Locomotion in Mice

Locomotion was measured as the number of breaks of an infra-red beam in modified Dews[3] activity boxes. Administered intraperitoneally to normal mice, MT, ET and, less strongly, MeOMT stimulated spontaneous locomotion, while DMT was inactive (Table 1). Tryptamine, 5-HT and iproniazid depressed locomotion, confirming the results of Brown[4]. Intravenously, however, tryptamine produced a brief episode of excitement followed by a prolonged period of depression.

Table 1. POTENCIES OF TRYPTAMINES AND OTHER COMPOUNDS IN MICE

Compound	Effective dose (mgm./kgm. i.p.) producing stimulation (*S*) or depression (*D*) of locomotion*	Effective dose (mgm./kgm. i.p.) producing reversal of reserpine ptosis‡
Tryptamine	50 (*D*)	190
α-Methyltryptamine	12·5 (*S*)	9·4
α-Ethyltryptamine	12·5 (*S*)	28·3
5-Methoxy-α-methyltryptamine	50 (*S*)	> 60 §
N,*N*-Dimethyltryptamine	No effect at 50	42·5
5-Hydroxytryptamine	50 (*D*)	> 300 ‖
Iproniazid	> 400 (*D*)†	No effect at 500
β-Phenylisopropylhydrazine	50 (*S*)	33
Amphetamine	2 (*S*)	5·8

* Locomotion was measured as the number of breaks of an infra-red beam in modified Dews (ref. 3) activity boxes. The effective dose is that which increased or decreased geometric mean activity count by a factor of 3 compared with that of control mice ($P < 0{\cdot}05$), when given within the dose-range 12·5, 25, 50, 100, 200 and 400 mgm./kgm. or in the case of amphetamine 1, 2 and 4 mgm./kgm.

† At this dose locomotion was depressed ($P < 0{\cdot}1$).

‡ Ptosis was induced by intraperitoneal injection of 4·0 mgm./kgm. reserpine 18 hr. before the test. The effective dose is that which in a group of 10 mice gives a score for reversal of ptosis of 10 out of a possible maximum of 20 on the scale 0 = full ptosis; 2 = eyes fully open; 1 = any intermediate state between 0 and 2.

§ At this dose a maximum score of 8/20 was obtained.

‖ At this dose a maximum score of 9/20 was obtained.

In another series of experiments, mice were given a larger dose of β-phenylisopropylhydrazine (PIH) than that necessary to inhibit brain MAO completely[5,6]. 18 hr. later, we treated them with MT, ET or iproniazid. Table 2 shows that MT and ET powerfully stimulated locomotion in normal mice and in mice after inhibition of brain MAO.

Table 2. EFFECTS OF α-METHYLTRYPTAMINE (MT), α-ETHYLTRYPTAMINE (ET) AND IPRONIAZID ON LOCOMOTORY ACTIVITIES OF NORMAL MICE AND MICE TREATED WITH MAO INHIBITOR (β-PHENYLISOPROPYLHYDRAZINE (PIH) 40 MGM./KGM. INTRAPERITONEALLY 18 HR. PREVIOUSLY)

Exp. No.	Treatment dose/kgm. i.p.	Mean activity count* and significance of difference	
		Without PIH	After PIH
1	Saline, 10 ml. MT, 20 mgm.	279 1,652 } $P < 0{\cdot}001$	236 2,771 } $P < 0{\cdot}001$
2	Saline, 10 ml. ET, 20 mgm.	102 1,355 } $P < 0{\cdot}001$	169 3,751 } $P < 0{\cdot}001$
3	Saline, 10 ml. Iproniazid, 100 mgm.	108 100 } $P > 0{\cdot}75$	236 145 } $P > 0{\cdot}10$

* Each value is the geometric mean of counts obtained over 4 hr. from 6 activity boxes, each containing 3 mice.

Anti-Reserpine Activity in Mice

Anti-reserpine activity was measured by the extent of reversal of ptosis produced by injecting reserpine 4·0 mgm./kgm. intraperitoneally 18 hr. previously. Table 1 shows that the tryptamines were active in this test, MT being the most and 5-HT the least potent. Amphetamine and PIH were also potent; but iproniazid ineffective. However, when given some hours before reserpine, iproniazid prevents ptosis[7].

Experiments on Unrestrained Conscious Cats

Intravenous tryptamine caused convulsions, tonic extension of the limbs, unsheathing of claws, hissing and snarling, piloerection, salivation, mydriasis and retraction of the eyelids: all these effects have already been described by Laidlaw[8]. The animals behaved as though angry or afraid. These reactions lasted for about 5 min., after which the animals were subdued for some hours. However, if MAO had been inhibited by injection of PIH (10 mgm./kgm. intraperitoneally 18 hr. beforehand), the phase of excitement following tryptamine lasted several hours. In untreated cats, MT or ET (5 mgm./kgm. intravenously) produced excitement lasting 4–5 hr.

Intraperitoneally, MT, ET or DMT (0·5–2·0 mgm./kgm.) caused recurrent extensor and flexor spasms of the limbs, ataxia, apparent reluctance to move and bewilderment. These effects, which lasted for more than 6 hr., were reduced or abolished within 2 hr. by intraperitoneal injection of 2 mgm. bromlysergic acid (BOL).

In cats with implanted cortical electrodes, MT, ET, MeOMT or DMT (2 mgm./kgm. intraperitoneally) did not produce electrocortical alerting. Within 15 min., however, bursts of 6–10 c./sec. high-voltage (250–400 μV.) electrocortical activity appeared and these were maintained unchanged for more than 6 hr.

Cat Encéphale Isolé Preparations

MT (0·5–2·0 mgm./kgm. intravenously) produced immediate and prolonged behavioural arousal. There was also very brief electrocortical alerting, which was replaced in 30 min. by bursts of high-voltage electrical activity, similar to that described above in conscious cats, or by high-voltage 2–4 c./sec. activity. These changes were found in all the cortical areas except the hippocampus. MT modified the normal reciprocal relationship which exists between electrocortical activity in the hippocampus and in other areas. The bursts at 2–4 or 6–10 c./sec. seen in the cortex could also be recorded from deep structures, such as the thalamic nuclei and the reticular formation in the brain stem. Subsequent intravenous injection of 2 mgm. BOL abolished or reduced the electrical patterns attributable to MT gradually over 1–2 hr.

In addition to changing spontaneous electrical activity, MT also affected evoked potentials. Thus the threshold for electrocortical arousal on stimulating the brain-stem reticular formation was raised from 0·4 to 1·2 V. and that on stimulation of the thalamic nuclei from 2·5 to 4·0 V. Similarly, the thresholds for eliciting recruiting and augmenting responses by low-frequency stimulation of the appropriate thalamic nuclei were raised as were the thresholds for arousal with tactile and auditory stimuli.

Spinal Reflexes

These were studied in spinal or chloralosed cats. In a polysynaptic reflex (twitch of the tibialis anterior muscle evoked by stimulation of the central end of the cut posterior tibial nerve), increase of both basal tension and peak twitch tensions with fused or partially fused tetani followed intravenous injection of tryptamine, MT, ET, DMT or MeOMT (0·5 mgm./kgm.). The facilitating effect of tryptamine was brief and larger doses abolished the response. However, with tryptamine after MAO inhibition or with MT alone, a tetanic response was followed by protracted augmentation of the twitch and a single sensory nerve stimulus produced repetitive muscle contractions. These compounds similarly facilitated a monosynaptic reflex (knee jerk).

Several observations showed that the facilitating effects of the tryptamines originated in the spinal cord: (1) these substances increased the spike potential recorded from the ventral root after stimulation of the corresponding dorsal root; (2) they were more potent when injected into a spinal artery; (3) they did not enhance responses of muscle to direct stimulation of the motor nerve or of the muscle itself; and (4) pressor effects of comparable size to those produced by tryptamines, but elicited by tyramine, adrenaline or noradrenaline, did not increase the reflexes.

Reduction of the spike potential after the initial increase sometimes followed injection of tryptamine. 5-HT (1 mgm./kgm. intravenously) enhanced both reflexes, but after a much longer latency, possibly because 5-HT does not easily cross cell barriers[1]. BOL (2 mgm. intravenously) and another specific antagonist of 5-HT—1-methyl-D-lysergic acid butanolamide[9] (UML-491)—at the same dose, reduced or abolished these reflexes, and also the facilitatory actions of the tryptamines.

Depression of both reflexes by acute or chronic reserpine or acute chlorpromazine treatment was reversed by intravenous injection of any of the compounds, MeOMT being the most potent.

Conclusions

In all the effects we have studied, tryptamine differed from MT and ET only in depressing spontaneous locomotion in mice, although for a brief initial period it caused excitement. Greig *et al.*[10,11] have claimed that MT and ET owe their central stimulatory action to inhibition of MAO, causing an accumulation of catechol amines or HT in the brain. However, two reasons show that MT and ET do not only or even mainly act in this way: (1) their stimulatory effect on locomotion was undiminished by prior inhibition of MAO (Table 2); and (2) they take effect rapidly. Another explanation of the action of substituted tryptamines has been advanced[12]—that they increase the availability of free HT by preventing its storage. However, this explanation seems unlikely to be important, since the substituted tryptamines act after reserpine, which has already inhibited storage of HT. These considerations suggest that the substituted tryptamines act directly on receptors in the central nervous system. Gaddum[13] has argued that tryptamine and 5-HT share common receptors in the gut, and Tedeschi, Tedeschi and Fellows[14] have advanced a similar hypothesis for the central nervous system. That substituted tryptamines as well as tryptamine and HT are antagonized by BOL and UML-491 suggests that they all act on the same receptors—the tryptamine receptors. That electrical responses to substituted tryptamines can be recorded from cortex, thalamus, brain stem and spinal cord suggests that tryptamine receptors are widely scattered throughout the central nervous system. In spinal reflexes in particular, the high potency of tryptamines and their antagonism by specific antagonists of 5-HT suggest that tryptamines naturally present may have a physiological function.

The substituted tryptamines were prepared in the Department of Chemical Research, Parke, Davis and Co., Hounslow. We wish to thank Dr. R. E. Bowman for helpful discussions, Dr. B. T. Warner for statistical advice and Mr. R. W. Pickering for experimental assistance. We also wish to thank Ciba Laboratories, Ltd., for supplies of reserpine and Sandoz Products, Ltd., for UML-491.

[1] Vane, J. R., *Brit. J. Pharmacol.*, **14**, 87 (1959).

[2] Barlow, R. B., and Kahn, I., *Brit. J. Pharmacol.*, **14**, 99 (1959).

[3] Dews, P. B., *Brit. J. Pharmacol.*, **8**, 46 (1953).

[4] Brown, B. B., *Arch. int. Pharmacodyn.*, **128**, 391 (1960).

[5] Horita, A., *J. Pharmacol.*, **122**, 176 (1958).

[6] Eltherington, L. G., and Horita, A., *J. Pharmacol.*, **128**, 7 (1960).

[7] Chen, G., and Bohner, B., *J. Pharmacol.*, **131**, 179 (1960).

[8] Laidlaw, P. P., *Biochem. J.*, **6**, 141 (1912).

[9] Doepfner, W., and Cerletti, A., *Intern. Arch. Allergy*, **12**, 89 (1958).

[10] Greig, M. E., Walk, R. A., and Gibbons, A. J., *J. Pharmacol.*, **127**, 110 (1959).

[11] Greig, M. E., Seay, P. H., and Freyburger, W. A., *J. Neuropsychiat.*, **2**, 131 (1961).

[12] Parkes, M. W., and Lessin, A. W., *Brit. J. Pharmacol.*, **17**, 3 (1961).

[13] Gaddum, J. H., *J. Physiol.* (Lond.), **119**. 363 (1953).

[14] Tedeschi, D. H., Tedeschi, R. E., and Fellows, E. J., *J. Pharmacol.*, **126**, 223 (1959).

INHERITANCE OF SUSCEPTIBILITY TO INDUCED PULMONARY TUMOURS IN MICE

By DR. D. S. FALCONER* and JOYCE L. BLOOM

Institute of Animal Genetics, Edinburgh

THE inheritance of cancer susceptibility is not amenable to study by the methods of Mendelian genetics, because many genes are involved which cannot be recognized individually, and any measure of the degree of susceptibility is subject also to non-genetic variation[1]. The methods of quantitative genetics must, therefore, be applied, and this article describes briefly the results of an investigation by these methods. Pulmonary tumours induced by urethane were chosen for study, because the manifestation of this form of cancer can be measured quantitatively, by the number of tumours present. All mice were given the same treatment: 0·1 ml. of 10 per cent urethane in distilled water injected intraperitoneally at 3 weeks of age, followed by a second injection of 0·28 ml. at 9 weeks. The mice were dissected at 23 weeks of age and the tumour nodules visible to the unaided eye on the surface of the lungs were counted. The number of tumours counted were transformed to square roots for the purpose of computation.

The object of the work was to determine the relative importance of heredity and environment as causes of the differences of tumour number between the individuals of a genetically heterogeneous, or random-bred, strain. The observed variance, that is, the phenotypic variance (V_P) among the individuals of a genetically heterogeneous strain is the sum of two principal components, the genetic variance (V_G) and the non-genetic, generally termed the environmental, variance (V_E); so that:

$$V_P = V_G + V_E$$

Then the ratio of the genetic to the total variance, V_G/V_P, measures the relative importance of genetic differences between individuals in determining the differences of tumour number between them. This partitioning of the variance can be made if a group of genetically homogeneous individuals is available, such as the F_1 of a cross between highly inbred lines. The variation among these, which contains no genetic component, provides a measure of the non-genetic variation; and, if this can be assumed to represent the non-genetic variation in the non-inbred strain, an estimate of the genetic variance can be obtained by subtraction. The genetic variance can be subdivided into an additive component (V_A), which is the variation chiefly responsible for the resemblance between relatives, and the remainder, or non-additive component (V_{NA}), which arises from dominance and other genetic complexities. The additive genetic variance can be estimated from the resemblance between relatives in a genetically heterogeneous strain. Thus the measurement of the variances of a genetically heterogeneous strain and of a genetically uniform group, together with the measurement of the resemblance between relatives in the heterogeneous strain, leads to a partitioning of the phenotypic variance of the heterogeneous strain as follows (for a more complete explanation, see ref. 2):

$$\underset{\text{(phenotypic)}}{V_P} \quad = \quad \underbrace{V_A + V_{NA}}_{\text{(genetic)}} \quad + \quad \underset{\text{(non-genetic)}}{V_E}$$

The ratio of the additive genetic variance to the total (V_A/V_P), known as the heritability, measures the extent to which differences of tumour number are transmitted from parents to offspring. Only in the absence of genetic complexities giving rise to non-additive variance does it measure the degree of genetic determination, or the relative importance of genetic differences in determining the phenotypic differences between individuals.

The genetically heterogeneous strain used in this work, known in the laboratory as *JC*, originated from crosses between several non-inbred strains and had been maintained for some thirty generations by random mating. The genetically uniform mice, from which the non-genetic variance was estimated, consisted of thirty F_1's made from six highly inbred strains crossed in all combinations, and the inbreds themselves. The variances of the inbreds did not differ from those of the F_1's, so there was no reason to exclude them. Counts were made on 723 F_1 and inbred individuals. The mean tumour number ranged from 0·9 to 25·4, with an overall mean of 11·7. (In what follows the F_1's and inbreds together will be referred to as the uniform groups.)

The results are shown in Table 1. The transformation to square roots did not fully succeed in rendering the variance independent of the mean, and the variance in the uniform groups tended to be higher in those with higher means. Since most of the groups had higher means than the heterogeneous strain, it was therefore necessary to adjust the estimate of the uniform group variance to allow for this difference. This was done from the regression of the standard deviation on the mean, which led to an estimate of the expected variance of uniform groups having the same mean tumour number as the heterogeneous strain. The unadjusted estimate of the mean variance in the uniform groups was 0·527, and the adjusted estimate was 0·325. The comparison of the variances of the heterogeneous strain and the uniform groups adjusted in this way leads to the conclusion that 58 per cent of the variation in tumour number in the heterogeneous strain

Table 1

Total phenotypic variance of random bred strains (V_P)	0·771 ± 0·042
Phenotypic variance of uniform groups (adjusted) (V_E)	0·325
Total genetic variance of random-bred strain ($V_G = V_P - V_E$)	0·445
Degree of genetic determination (V_G/V_P) = 0·445/0·771 =	58 per cent
Regression of offspring on mid-parent	0·323 ± 0·051
Correlation of full sibs	0·218 ± 0·044

---

* Agricultural Research Council, Unit of Animal Genetics.

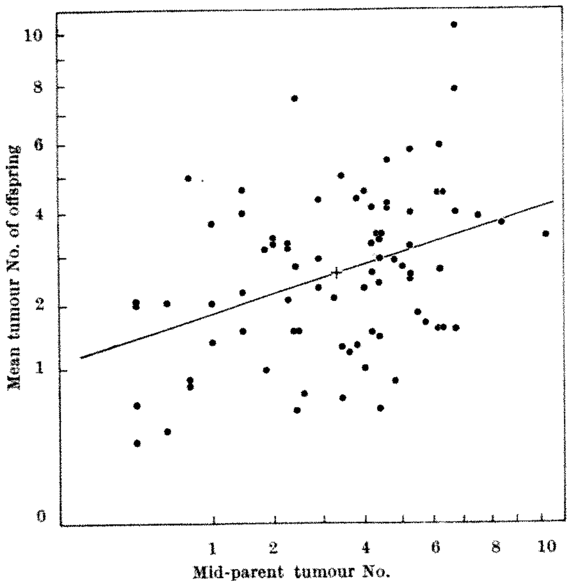


Fig. 1

was genetic in origin. This measures the relative importance of heredity as a cause of differences in tumour number.

The additive genetic variance in the heterogeneous strain was estimated from the regression of offspring on the mean of their two parents. Counts were made on 518 offspring in 79 families. The mean tumour number was 3·4. The correlation of full sibs (litter-mates) was also estimated. As a measure of the additive genetic variance this is liable to upward bias from non-additive genetic variance and from environmental causes of variation common to litter-mates, such as might arise from maternal effects or from the experimental procedure. The scatter diagram shown in Fig. 1 illustrates the resemblance between offspring and parents. Each point represents the mean tumour number among the offspring of one pair of parents plotted against the mean tumour number of the parents. The straight line is the computed regression of offspring on mid-parent (that is to say, the mean of the two parents), and the slope of this line measures the degree of resemblance between offspring and parents, which estimates the heritability (V_A/V_P). This was 32 ± 5·1 per cent. Thus, the heritability differs materially from the ratio V_G/V_P, which was 58 per cent. This indicates the presence of non-additive genetic variance arising from dominance and other genetic complexities in the action of the genes controlling tumour number. If there were no non-additive genetic variance and no maternal effects the full-sib correlation would estimate half the heritability. Doubling the observed correlation yields an estimate of 44 ± 9·2 per cent. This is in excess of the estimate from the offspring–parent regression, which is to be expected when non-additive variance is present.

These analyses show that about 58 per cent of the variation in tumour number between the mice of the strain studied was caused by genetic differences. The remaining 42 per cent, which is non-genetic in origin, can be subdivided by consideration of the fact that the number of tumours counted does not necessarily indicate precisely the true susceptibility of the individual. A number of individuals of exactly the same susceptibility would be expected to vary in the number of tumours present, because the occurrence of a tumour can be regarded as a chance event with a certain probability characteristic of the susceptibility of the individual. The variance of tumour number will, therefore, have a component of variance attributable to chance in addition to the variance arising from individual differences of susceptibility. There is evidence[3] that the chance variation follows a Poisson distribution; the variance attributable to chance will then be equal to the mean tumour number[4], when the mean and variance are computed from the untransformed counts. The mean of the (untransformed) tumour numbers in the random-bred strain was 3·4 and the variance was 8·5. The variance attributable to chance (that is, 3·4) therefore represents 40 per cent of the total variance of tumour number. This leaves only 2 per cent of the variance of tumour number to be attributed to non-genetic differences of susceptibility together with differences due to experimental error, such as might have arisen from variation in the dosage of urethane administered to different individuals. It is not altogether credible that non-genetic differences of susceptibility, together with experimental error, account for as little as 2 per cent of the variation. The chief source of possible error in this estimate lies in the adjustment of the variances of the uniform groups, and it is not a simple matter to attach a standard error to the adjusted estimate. A second source of possible error lies in the amount of variance to be attributed to chance. Until these sources of error have been excluded, or confirmatory evidence obtained from other experiments, only a qualitative conclusion can be accepted, namely, that environmental sources of variation are relatively unimportant, and an individual's genetic constitution is the major determinant of its true susceptibility to urethane as a carcinogenic agent.

The results of this work are summarized in Table 2, which shows the relative importance of heredity, environment and chance as causes of the differences between individuals in the number of tumours developed. The figures are subject to some possible error, but the following conclusions can be drawn. More than half the variation of tumour number among the mice of the strain investigated results from genetic differences between the individuals; but, as a result of genetic complexities, only about one-third of the variation is 'inherited' in the sense of being transmitted from parents to offspring. A major part of the non-genetic variation of tumour number is attributable to chance, and environmental differences between individual mice are relatively unimportant as determinants of an individual's susceptibility to the carcinogenic action of urethane.

Table 2

	Total (per cent)
Genetic variance of susceptibility	
Additive	32
Non-additive	26
Total	58
Non-genetic variance of susceptibility	2
Chance variance of tumour number	40
Total variance of tumour number	100

This work was carried out with the support of a grant from the Medical Research Council.

[1] Heston, W. E., *J. Nat. Cancer Inst.*, **3**, 69 (1942).

[2] Falconer, D. S., *Introduction to Quantitative Genetics* (Oliver and Boyd, Edinburgh, 1960).

[3] Polissar, M. J., and Shimkin, M. B., *J. Nat. Cancer Inst.*, **15**, 377 (1954).

[4] Kendall, M. G., *The Advanced Theory of Statistics*, second edit., **1**, 123 (Charles Griffin, London, 1945).

A MORPHOGENETIC EFFECT OF BETA-MERCAPTOETHANOL

Distribution of Water in the Embryo

CELLULAR mechanisms responsible for the uptake and distribution of water in the embryo play an important part in normal development[1]. Prof. Brachet's reports[2] on the effects of β-mercaptoethanol on the normal development of urodele embryos, which he attributed to its effect on the fibrous proteins responsible for morphogenetic movements, were therefore of particular interest, because two of the abnormalities he described—the collapse of the blastocœl and the failure of the neural tube to close—clearly resulted in an abnormal distribution of water in the embryo.

This prompted me to carry out a series of quantitative experiments to measure the effect of this reagent on the uptake and distribution of water during the formation of the blastula and gastrual of *Xenopus leavis* (Daud.). The results were surprising, for they showed that under the conditions of my experiments, $M/500$ mercaptoethanol had little or no effect on the morphogenetic movements themselves, but it did have a pronounced effect on the uptake and, particularly, the distribution of water in the embryo.

Having first established that the reagent had no effect on the reduced weight of the embryos by weighing single embryos on a Cartesian diver balance before and after treatment for 3 hr. with $M/100$ mercaptoethanol, it was assumed that the density of the embryos could change only by taking up or losing water. (It had previously been established that the reduced weight of normal embryos remained constant until the early tail-bud stage.) Thus, it was possible to follow the effect of the reagent on the water content of the embryo by measuring the density of treated and control embryos in a density gradient of colloidal thorium oxide in water[3]: and its effect on the volume of the cells themselves could be determined by repeating the process on open embryos from which the contents of the intercellular spaces had been removed. The results of one such experiment are shown in Table 1, and the corresponding volume changes, calculated from the mean densities and the mean reduced weight of the experimental embryos, are shown in the histogram (Fig. 1).

Table 1

Control embryos in buffered tap water pH 7·4 at 20° C. Treated embryos in buffered tap water pH 7·4 at 20° C. + $M/500$ β-mercaptoethanol

	Duration of treatment Age (hr.) In	Out	No. of embryos	Mean density (20° C.) at end of treatment Intact gm./ml.	Open gm./ml.
Control	—	—	5	1·0770	1·0887
Treated	2	10	5	1·0780	1·0887
Control	—	—	6	1·0620	1·0840
Treated	2	22	5	1·0732	1·0812

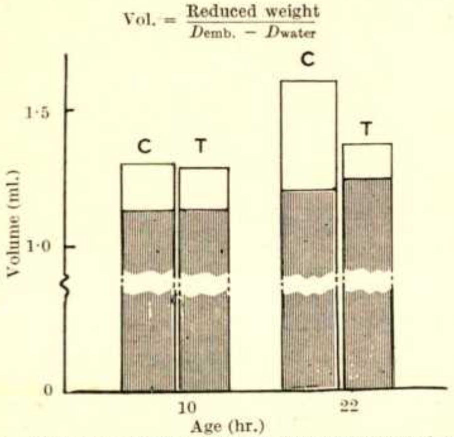


Fig. 1. Histogram showing effect of β-mercaptoethanol on the volume of *Xenopus* embryos. Shaded areas, vol. of cells; unshaded, vol. of intercellular cavities

It will be seen that at 10 hr. there is very little difference between control embryos and embryos laid at the same time which had been in a solution of $M/500$ mercaptoethanol from the four-cell stage onwards. The volume of the cells in both cases is the same. At 22 hr., however, there is a marked difference. The density of the treated embryos is very much greater than that of the controls, whereas the density of their cells is slightly less. This means that the volume of the intercellular cavities of the treated embryos is very much less than that of the controls (Fig. 1). The treated embryos differ from the controls in one other important respect—they lie upside down in the vitelline membrane, due to the presence of a liquid-filled cavity on their ventral surface. The presence of this cavity became more pronounced if treated embryos were removed from the reagent at this stage and transferred to tap water (Fig. 2).

Serial sections and time-lapse films clearly showed that this cavity was the cavity of the blastocœl which, having formed normally, failed to empty during gastrulation. The morphogenetic movements involved in the formation of the archenteron and its associated structures did not appear to be affected by the treatment, and all the structures normally found in transverse sections of normal embryos could be identified. The only obvious abnormality was that the archenteron cavity was empty. At a later stage the treated embryos also differed from the controls, as Brachet reported, in having open neural tubes. It should be mentioned in passing that nuclear counts from macerated embryos showed that treated and control embryos have the same number of nuclei.

It seems reasonable to conclude, therefore, that the primary effect of the mercaptoethanol is not, as Brachet has suggested, on the mechanisms responsible for the morphogenetic movements themselves, but on the mechanisms responsible for controlling the

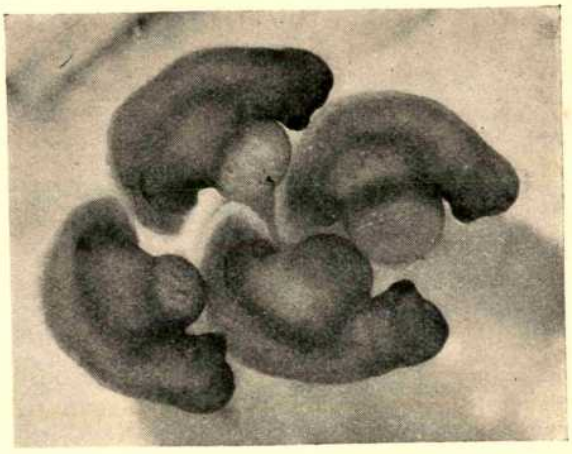


Fig. 2. *Xenopus* embryos showing persistent blastocœl cavities after 15 hr. in tap water following treatment for 20 hr. with $M/500$ mercaptoethanol at 20° C.

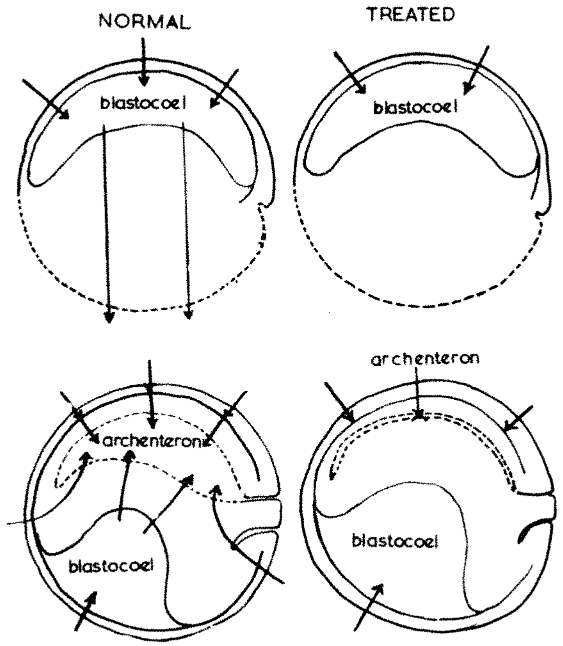


Fig. 3. Diagram to illustrate the effect of the net water fluxes in normal and treated *Xenopus* embryos

distribution of water in the embryo. This in turn may be the result of its effect on the bonding of specific proteins, for as I have shown[1], the distribution of water in the embryo is probably due to the difference in polarity of cellular mechanisms located in the cell membranes, and not to simple osmotic and hydrostatic gradients.

Thus, in the normal embryo, these mechanisms are thought to be polarized in such a way that there is a net influx of water through the cells of the animal pole and a net efflux through the vegetative pole cells, the difference between the two fluxes being responsible for the accumulation of water in the blastocœl, and after invagination for the transfer of water into the archenteron. The failure of treated embryos to transfer water from the blastocœl into the archenteron may therefore be explained by supposing that the reagent reduces the endodermal efflux to zero, leaving a small ectodermal influx to account for the formation of the blastocœl which, as the experiments show, is unaffected (Fig. 3).

This work is being reported in more detail elsewhere[4].

Peter Tuft

Department of Zoology,
University of Edinburgh,
West Mains Road, Edinburgh, 9.

[1] Tuft, P. H., *Proc. Roy. Phys. Soc. Edin.*, **26**, 42 (1957).

[2] Brachet, J., *Nature*, **181**, 1736 (1958); **184**, 1074 (1959).

[3] Lovtrop, S., *C.R. Lab. Carlesberg*, Serie Chem., **27**, 137 (1950*b*). Salzen, E., *J. Embryol. Exp. Morph.*, **8**, 139 (1960).

[4] Tuft, P. H., *Proc. Roy. Phys. Soc. Edin.*, **28**, 123 (1961).

Distribution of Water in the Chick Blastoderm

It has been shown experimentally that the effect of β-mercaptoethanol on the morphogenesis of the anuran *Xenopus leavis* is largely due to an abnormal uptake and distribution of water in the treated embryos. This was explained by suggesting that the reagent affects the mechanisms responsible for controlling the water content of the embryonic cells in such a way that the normal net efflux of water through the endoderm cells is abolished, so preventing the transfer of water into the archenteron, leaving a small residual ectodermal influx to account for the formation of the blastocœl, which is unaffected[1].

The complexity of the geometrical relations between ectoderm and endoderm in the amphibian embryo makes it difficult to test this hypothesis. In the early chick embryo, on the other hand, the problem is greatly simplified because the ectoderm and endoderm are merely parallel sheets of cells. Moreover, their polarity has been demonstrated experimentally by New, who has shown that water is transferred through the chick blastoderm from the ectodermal to the endodermal surface[2]. Thus not only is the polarity the same as that postulated for the *Xenopus* embryo, but there must also be a similar difference in the magnitude of the fluxes to account for the small accumulation of liquid between the layers which occurs during the development of the normal blastoderm.

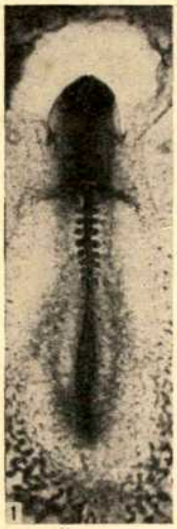
Accordingly, one of us (A. J.) has carried out a series of experiments to test the effect of β-mercaptoethanol on early chick embryos.

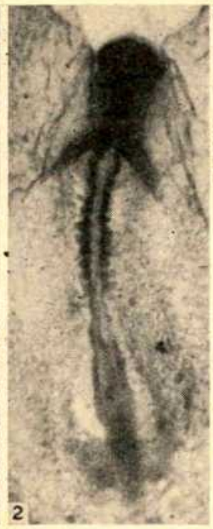
Chick embryos were explanted from eggs at the definitive streak stage (Hamburger and Hamilton stage 4)[3] and cultured *in vitro* using the technique described by New[5]. The β-mercaptoethanol was diluted with liquid egg albumen containing 10 per cent sterile saline solution (0·9 per cent) to give a final concentration of 4 or 5 m*M*, and 0·5 ml. of this solution was spread around the plastic ring on which the embryos were cultivated; in control experiments a solution of liquid egg albumen containing 10 per cent saline was applied in the same way. After treatment, the embryos were incubated for another 20–24 hr., that is, until the controls had reached stages 10–12 (Fig. 1). Some embryos were also treated *in ovo* at stage 4 by injecting 0·25 ml. of 400 m*M* solution of the reagent. These embryos were then allowed to develop for another 20–24 hr. before being explanted.

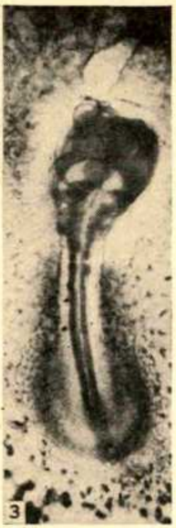
Macroscopic examination of treated embryos showed abnormal swelling of the blastoderm on either side of the mid-line and enlargement of the proamnion and the head. The neural tube in all cases failed to close, often along its entire length (Figs. 2 and 3).

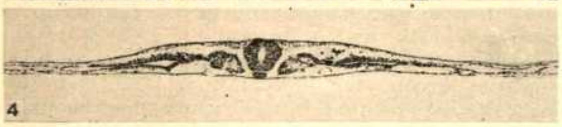
In transverse sections it was seen that the swelling of the blastoderm was the result of abnormally large spaces between the ectoderm and mesoderm, and between mesoderm and endoderm. This was especially noticeable in the region of the posterior somites and farther towards the caudal end of the embryo (Figs. 4 and 5). The extra-embryonic cœlom was also widened along the whole length of the embryos. In the cephalic region, the mesenchyme spaces were enlarged, and the mesenchyme cells were dispersed more than in the normal embryo. The increase in size of the head in treated embryos was in part the result of a considerable dilation of the main blood vessels (ventral and dorsal aortic roots) accompanied by a dilation of the heart ventricle.

The neural plate of treated embryos, especially where it failed to close along its entire length, sometimes showed necrotic changes in the median part of the neural groove, or scattered over the whole tissue. The notochord was sometimes poorly formed and not clearly detached from the surrounding mesenchyme cells, and finally, in some embryos, well-defined blisters were found in both the ectoderm and endoderm.









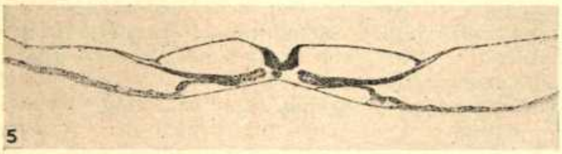


Fig. 1. Control chick embryo at stage 10 (× 15)
Fig. 2. Chick embryo after treatment with $M/250$ solution of β-mercaptoethanol. Note the open neural tube and widened omphalomesenteric veins (× 15)
Fig. 3. Chick embryos treated with $M/200$ solution of β-mercaptoethanol. Note the enlarged head, and open neural tube (× 20)
Fig. 4. Transverse section through a control embryo (stage 10) at the level of the last somite (× 40)
Fig. 5. Transverse section through an experimental embryo at the same level after treatment with $M/200$ β-mercaptoethanol (× 40)

These results show that in the chick, as in *Xenopus*, β-mercaptoethanol causes an abnormal distribution of water in the embryo: a distribution, which is in accord with the hypothesis outlined earlier. Thus if mercaptoethanol reduces the endodermal efflux in the chick as it does in *Xenopus*, the residual ectodermal influx would account for the abnormal accumulation of water between the two layers that has been observed.

The effect of the reagent on the volume of the cœlom is less easily explained. However, the fact that the myocœl when it forms is not abnormally large, although the extra embryonic cœlom is always greatly distended, suggests that the mesodermal cells are normally polarized centripetally with respect to the net transfer of water; but because the lateral plate mesoderm is so closely associated with the ectoderm and endoderm, the layers of cells which result—the somatopleur and splanchnopleur—have the same polarity as the ectoderm and endoderm respectively and are affected in the same way.

A further interesting feature of these experiments is the appearance of the neural plate in transverse sections of treated embryos; this suggests that its failure to close may simply be a mechanical consequence of the increased tension in the ectoderm caused by the abnormal accumulation of water. Attempts to test this hypothesis by puncturing the ectoderm of embryos that had been treated *in ovo* (see earlier) were inconclusive. The neural plate did not close up immediately the tension in the ectoderm had been relieved, although there was a limited closure after 2 hr. The embryos in these experiments were of course relatively old, and it is possible that permanent damage had occurred in the neural tissues. Moreover, the reagent may affect the differential water uptake of the cells on the upper and lower surfaces of the neural plate itself; a differential which must exist in the normal embryo to account for the changes in cell volume which occur during invagination.

In view of these results, it is suggested that the effects of other specific morphogenetic inhibitors, particularly those which tend to alter the S—S S—H equilibrium of the cells, may be the result at least in part of their effect on the distribution of water in the embryo and the mechanisms by which it is controlled.

A detailed account of this work will be published elsewhere.

A. JURAND

Institute of Animal Genetics,
University of Edinburgh,
West Mains Road, Edinburgh, 9.

PETER TUFT

Department of Zoology,
University of Edinburgh,
West Mains Road, Edinburgh, 9.

[1] Tuft, P. H., preceding communication, and *Proc. Roy. Phys. Soc. Edin.*, **28**, 123 (1961).
[2] New, D. A. T., *J. Embryol. Exp. Morph.*, **4**, 226 (1956).
[3] Hamburger, V., and Hamilton, H. L., *J. Morph.*, **88**, 49 (1951).
[4] New, D. A. T., *J. Embryol. Exp. Morph.*, **3**, 326 (1955).

IONIC CURRENTS IN MEMBRANE OF ACTIVE MUSCLE FIBRE

By DR. H. JENERICK

Department of Physiology, Emory University, Atlanta 22, Georgia

A NERVE or muscle fibre immersed in a large volume of fluid and carrying a propagated action potential at velocity s, will obey the following relationship, usually termed the cable equation:

$$(a/2Rs^2)\mathrm{d}^2V/\mathrm{d}t^2 = C\ \mathrm{d}V/\mathrm{d}t + I_i \qquad (1)$$

where a is the radius of the fibre, R is the resistivity of the cytoplasm, V is the membrane voltage, t is time, C is the membrane capacitance, and I_i is the membrane ionic current density. As a rule, this equation cannot be integrated since I_i is an unknown function of voltage and time. However, if the first and second time-derivatives of the action potential can be determined as functions of time, appropriate substitution into (1) will lead to a numerical solution for the membrane ionic currents associated with the action potential.

During investigation in this laboratory of the action potential of frog sartorius fibre, it was realized that much of the needed information lies in a record of the action potential in phase-plane co-ordinates, as in Fig. 1. Trans-membrane voltage (V) was recorded on the horizontal axis of the oscilloscope, while dV/dt (obtained electronically) was recorded simultaneously on the vertical axis. The entire spike-potential record develops clockwise from the initial spot at the left (resting potential − 92 mV.). The farthest horizontal excursion to the right is the peak of the spike (28 mV. overshoot). The large spot at the centre recorded the outside potential (zero) when the electrode was dislodged by the twitch. (The small peaked curve in the centre is the transient of the electrode pull-out; neither it nor the high-frequency hash on the entire record is significant.) The various regions of the phase plane record chosen for the analysis described in the text are labelled, and the arrows indicate the approximate boundaries of each region. This particular record was rotated slightly clockwise by a 3° phase-shift introduced by the differentiating network, and the inflexion point of the leading edge (at the top of the record) seems to occur at smaller membrane potentials than might be expected. It was possible, after analytical functions were fitted to various portions of the record, to obtain a description of the action potential in terms of several differential equations; that is, a set of functions where $dV/dt = f(V)$. These were integrated mathematically for $V = f(t)$, which in turn were differentiated successively for dV/dt and d^2V/dt^2 as $f(t)$, the functions required for the solution of the cable equation. It was necessary to separate the record by trial and error into several regions as indicated in Fig. 1, and to fit individual functions to each section, since no single function has been found which describes the entire course of the action potential. (Regions a and d are linear, b is closely fitted by a parabolic expression, and c by a modified cubic.) Once these functions were obtained from each record, they were integrated and differentiated as described, and yielded a set of functions suitable for direct substitution into the cable equation. Taking t as the independent variable, V and I_i were calculated point by point every 10–50 μsec. throughout the spike potential. The calculations for one record take about 2 days to complete, and this article summarizes results obtained in separate experiments from six typical fibres.

The list of fitted functions and derived equations describing the muscle spike-potential is lengthy (eighteen in all) and will not be presented here. However, the equation for region a is of particular importance and will be discussed briefly. As the action potential shown in Fig. 1 invaded the recording site, the advancing foot which develops exponentially in time[1] was necessarily transformed in the phase plane into a straight line of slope k_1 (ref. 2). It is suggested in refs. 1 and 2 that k_1 is equal to $(2RCs^2/a)$. The cell constants are thus obtained directly from this one portion of the phase plane record and permits a revision of (1) to:

$$I_i/C = (d^2V/dt^2)/(k_1) - dV/dt \qquad (2)$$

After selecting a value of 6μF./cm.² for the membrane capacitance from the average of published data, all calculated derivatives were corrected to give the membrane ionic current density I_i in m.amp./cm.², as illustrated in Fig. 2; also a portion of the spike-potential reconstructed in its usual form from the differential equations is shown. Fig. 2 represents a reconstruction of the spike potential and membrane ionic current obtained from the phase-plane record of Fig. 1. (Time in msec. Inward current in m.amp./cm.² is plotted as positive.) Calculations were carried to within a few mV. of the beginning of the decay of the after-potential. Since the time-constant of the after-potential is close to or equal to that of resting membrane, it would appear that the conductances of the active membrane have subsided, and the remainder of the action potential is not relevant to the present work. It should be emphasized that, as a result of setting appropriate boundary conditions for the equations describing each region of the phase plane record, the derivatives and hence the potential and the ionic current are continuous in time across each junction point and throughout the entire portion of the reconstructed spike-potential.

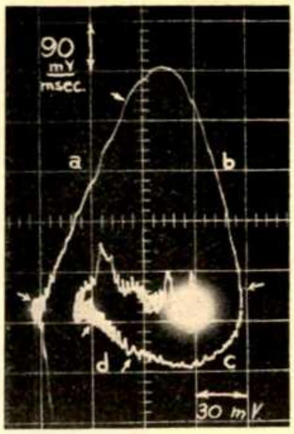


Fig. 1. Propagated action potential of a single surface fibre of the frog sartorius muscle recorded by a penetrating micro-electrode and displayed in the phase-plane
Conditions: 24° C., sodium chloride 110 mM, potassium chloride 2·5 mM, calcium chloride 4 mM, and phosphate buffer 1 mM at pH 7·3

The largest ionic current appears to be associated with the upstroke of the muscle fibre spike-potential,

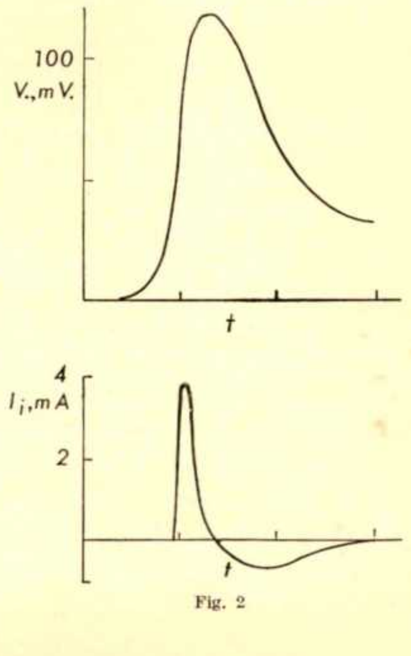


Fig. 2

as in the squid giant axon, and lasts somewhat less than 0·5 msec. at room temperature. Graphical integration of Fig. 2 predicts a net entry of positive charge during initial portions of the spike of about 7×10^{-12} mole/cm.[2] followed by a net exit of about 6×10^{-12} mole/cm.[2] during the terminal phase. These charges should balance, and the discrepancy is attributed to the difficulty involved in fitting a cubic equation to region *c* which is largely generated by the outward currents. Region *b* can be fitted more accurately and more confidence is placed in the corresponding portion of the inward current plotted against membrane voltage in Fig. 3. The solid curves plot the relation between the inwardly directed membrane ionic current/cm.[2] and the membrane voltage during the upstroke of two spike-potentials obtained from the same muscle under different conditions. The largest current was obtained when the muscle was immersed in a Ringer fluid containing 110 m*M* sodium chloride while the other was obtained after the concentration of sodium chloride was reduced to 55 m*M* by replacement with choline chloride. The dashed lines are extrapolated to the potential at which the ionic currents would equal zero. The calculation for the magnitude of ionic current was based on the assumption of a membrane capacitance of 6 μF./cm.[2]. The actual value of the capacitance enters the calculations only as a scale-factor for the ordinate and will not affect the extrapolation to + 37·5 mV. (110 m*M* sodium chloride) or + 22·5 mV. (55 m*M* sodium chloride). Note that the firing potential at − 45 mV. is not affected by the concentration of sodium ions. Although the method in itself cannot distinguish between currents carried by sodium or potassium ions, the results which followed experimental alterations of the Ringer fluid suggest that the sodium ion may carry a significant fraction of the current during the upstroke of the muscle fibre spike. For example, as shown in Fig. 3, reduction of the external concentration of sodium ion from 110 m*M* to 55 m*M* caused a decrease of 15 mV. in the extrapolated intercept as compared with the 16·5 mV. shift demanded by the resulting decrease in the sodium potential. On the other hand, analysis of other experiments clearly showed that increases of external concentration of potassium ions from 2·5 m*M* to 10·5 m*M* or of concentration of calcium ions from 1 m*M* to 10 m*M* have a negligible effect on this intercept, although the ionic current magnitudes were significantly modified. While these latter ions might be expected to alter the membrane conductances, they should not affect the sodium potential. In addition, the approximate, extrapolated zero-current potentials from the five records obtained from fibres immersed in 110 m*M* sodium chloride Ringer fluid range from + 38 to + 42 mV., and when substituted into the diffusion potential equation predict internal concentrations of sodium ions of 22–25 m*M*. These records were obtained from fibres isolated for at least 1 hr., and these concentrations seem quite consistent with published data.

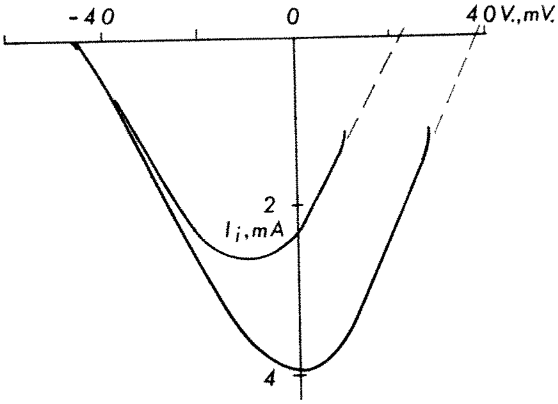


Fig. 3

Because the intercept at zero current behaves as described it has been tentatively assumed that this may be the sodium potential. If so, the ionic currents associated with this portion of the spike-potential should be carried predominantly by the sodium ion. It is recognized that the behaviour of this part of the voltage-current plot may be a fortuitous result of the chosen functions and this is being investigated.

This work has been aided by grants from the National Institutes of Health (*B*–861 and *B*–3272) and from the McCandless Fund of Emory University.

---

[1] Tasaki, I., and Hagiwara, S., *Amer. J. Physiol.*, **188**, 423 (1957).

[2] Jenerick, H., *Proceedings of the First National Biophysics Congress*, 377 (Yale Univ. Press, 1959).

ENLARGEMENT OF SALIVARY GLAND IN MICE TREATED WITH ISOPROPYLNORADRENALINE

By Dr. K. BROWN-GRANT

Department of Physiology, Medical School, Birmingham 15

SELYE *et al.*[1] have recently reported that large doses of *iso*propylnoradrenaline (*iso*proterenol) stimulate the growth of the salivary glands of the rat. In view of the similar sex dimorphism and the similar response of the submandibular glands of both rats and mice to sex hormones and thyroxine[2], the effect of this catechol amine on the glands of mice has been investigated.

Nineteen male albino mice of the Parkes strain (mean initial body-weight 30 gm., range 28–34 gm.) were used; 7 served as controls and the others were injected twice daily intraperitoneally with *iso*propylnoradrenaline sulphate (40 mgm. per 100 gm. body-weight in 0·2 ml. of water). On the fifth day of treatment, five of the animals died. The dose was reduced to 30 mgm. per 100 gm. body-weight and the treatment continued for a further two days. On the eighth day the control animals and the survivors of the treated group were injected subcutaneously with 4 μc. of carrier-free ^{131}I-iodide in a volume of 0·1 ml. and killed 2 hr. later. The external lacrimal, sublingual, submandibular and parotid glands were removed, weighed rapidly to the nearest 0·1 mgm. and one gland of each pair placed in 10 per cent neutral formol-saline for fixation. A serum sample was obtained and the second submandibular gland was dissolved in 3 N sodium hydroxide. Radioiodine contents were measured in an $M6$ liquid Geiger counter and the concentration ratio (counts/sec./100 mgm. gland: counts/sec./100 mgm. serum) was calculated.

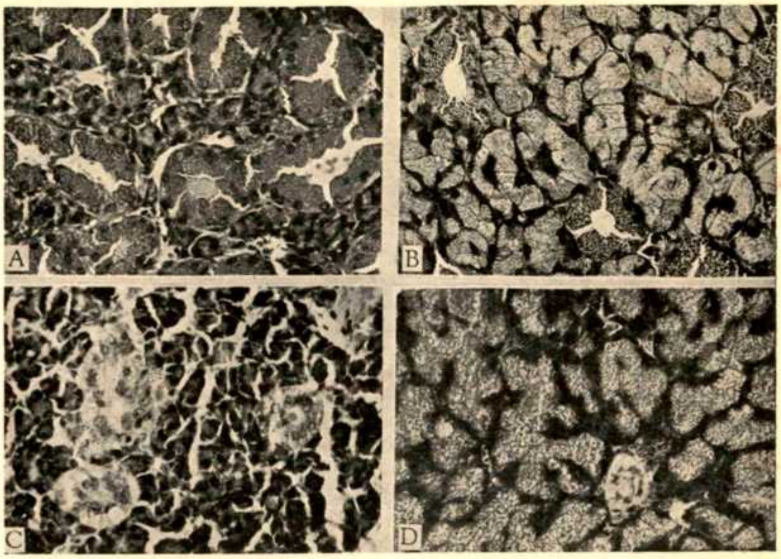


Fig. 1. 7-μ sections, stained hæmatoxylin and eosin. (× *c*. 200.) *A*, Normal mouse submaxillary gland. Note predominance of granulated tubules in the field; *B*, treated mouse submaxillary gland. Note increase in size of acinar cells. Granulated tubule size maintained. Cell granules are prominent; *C*, normal parotid gland. Small basophilic acinar cells; tubule cells show well marked striations with round central nuclei; *D*, treated parotid gland. Note acinar cell enlargement with displacement of nuclei. Ducts compressed with loss of basal striations in cells

The results obtained are shown in Table 1. Significant increases in the weight of the submandibular and parotid glands were observed in mice dying after 5 days treatment and in mice killed on the eighth day. The further increase between the fifth and eighth days was significant in the case of the parotid gland but not in that of the submandibular gland. In confirmation of the findings of Selye *et al.*[1], the weight of the external lacrimal gland was not affected by this treatment. The sublingual gland weight was not increased in these mice and the submandibular gland weight at the eighth day was only 150 per cent of the control value as compared to a parotid weight of 550 per cent of the control value. Selye *et al.*[1] state that the parotid, submandibular and sublingual glands were enlarged to approximately the same degree in female rats treated with a similar dose of *iso*propylnoradrenaline for 17 days, but do not give figures for the individual glands. The difference could be due to the difference in species, sex or duration of treatment.

Histological examination (paraffin embedding, 7-μ sections stained with hæmatoxylin and eosin) of the submandibular and parotid glands of all the control mice and of all those killed after 8 days treatment revealed marked differences. The most striking feature of both submandibular and parotid glands was a great increase in the size of the acinar cells; the cytoplasm was filled with basophilic secretory granules; the nuclei often appeared compressed and were displaced to the periphery of the acini. Intercellular canaliculi were prominent and contained material with the same staining properties as that seen in the major intralobular ducts. Intercalated ducts which are not obvious in the normal gland were prominent in the treated animals because of the distension of their lumina by secreted material. The condition of the major intralobular ducts differed in the two glands; the striated ducts of the parotid were found to be compressed when the diameter was measured from the base of the tubule cells. The lumen, however, was dilated with basophilic secretion; the tubule cells themselves were flattened, the normally round, central nuclei appeared as ellipsoids with their long axes parallel to the length of the tubule. The normal basal striations of the cytoplasm were much less obvious and in some cases absent. The convoluted granular tubules (serous tubules) of the submandibular gland which predominate in the normal male gland were less obvious in the treated animals because of the great increase in size of the acinar cells. Examination of individual tubules did not show any change in external diameter or in the size of individual tubule cells comparable with the changes in acinar cells. A possible increase in the number of large, intensely eosinophilic granules was suspected in some of the glands from treated animals; but considerable variation within both control and experimental groups and within the same gland made a decision on this point difficult. Sections stained by the periodic acid–Schiff method and counterstained with aniline blue and orange *G* showed that some acinar cells in the normal parotid contained periodic acid–Schiff-positive granules lying at the luminal borders of the cells. A faint positive reaction was observed diffusely in the cytoplasm of the majority of acinar cells in the treated gland. The normal submandibular acinar cells showed areas of periodic acid–Schiff-positive material in the cytoplasm; in the treated gland the acinar cell cytoplasm was uniformly positive; the contents of the ducts gave a strong positive

Table 1. GLAND WEIGHTS (MGM.) AND THE SUBMANDIBULAR GLAND/SERUM CONCENTRATION RATIOS FOR IODINE-131 IN NORMAL AND *ISO*PROPYLNORADRENALINE-TREATED MICE

Figures are means ± S.E.M. Not significant (N.S.) indicates a value for P of greater than 0·05 in the t test for the significance of the difference of group means

	No.	External lacrimal	Sublingual	Parotid	Submandibular	Concentration ratios iodine-131
Controls	7	27·4 ± 2·1	25·5 ± 1·8	88·4 ± 7·7	152·4 ± 6·6	3·34 ± 0·24
Died after 5 days treatment	5	21·1 ± 2·0	31·4 ± 4·9	315·8 ± 22·1	197·8 ± 5·8	—
P values for difference from controls		N.S.	N.S.	< 0·001	< 0·001	—
Killed on eighth day	7	25·1 ± 1·6	26·5 ± 1·6	474·9 ± 19·4	228·3 ± 16·9	3·90 ± 0·64
P values for difference from controls		N.S.	N.S.	< 0·001	< 0·01	N.S.
P values for difference from animals dying at fifth day		N.S.	N.S.	< 0·001	N.S.	—

reaction. The mitotic proliferation reported by Selye *et al.*[1] in the rat was not seen in these mouse glands. Mitotic figures were searched for, but were extremely rare.

The histological picture is interpreted as showing an intense stimulation of the glands resulting in an increase in size of the acinar cells which are swollen with secretory material; many of the intralobular duct lumina are also distended with secretion. There is no histological evidence of a comparable stimulation of duct cells in the parotid glands or in the intercalated ducts, striated intralobular ducts or interlobular ducts of the submandibular gland. There is some evidence of stimulation of the cells of the serous tubules, which are not compressed like the cells of other ducts, and many show an increase in the number and size of their secretory granules. The gland/serum concentration ratio for radioiodide is not significantly altered (Table 1). This observation may be related to the histological evidence of stimulation of the convoluted granular tubules, as these are the elements in the gland which are thought to be responsible for the high concentration of iodide in the saliva and in the gland itself relative to the serum[3]. The maintenance of the normal gland : serum concentration ratio, despite the marked increase in the acinar cell mass, is also suggestive of a possible stimulation of the serous tubule cells.

No evidence of hyperplasia of the glands was seen in the present experiments, nor is it necessary to invoke this to explain the increase in weight. The relative width of the acinar cells in the normal and treated glands was measured in representative sections and was found to be in the range of 1 : 1·6–1 : 1·8. Assuming the cells to be cuboidal and the specific gravity to be unchanged, such an increase in size in cell dimensions would result in a weight ratio of about 1 : 4·9, roughly what was found in the parotid glands, where acinar cells predominate over tubules. The smaller increase in weight in the submandibular glands, despite an equally striking increase in the size of the acinar cells, may be due to the far greater proportion of the normal gland weight that is contributed by the tubules which do not increase in size during treatment with large doses of *iso*propylnoradrenaline. Although the weights of the sublingual and external lacrimal glands were not increased, histological examination showed that these glands, too, had been affected. There was evidence of a much less-marked and more variable increase in size of the acinar cells with the accumulation of secretory material within the cells and in the duct lumina.

The changes observed in the salivary glands of these mice are consistent with an intense, but not unexpected, stimulation of acinar cell secretory activity by these very large doses of *iso*propylnoradrenaline. This, rather than a sialoadenotrophic action of this compound, such as has been reported in rats[1], appears to be the most probable explanation for the dramatic changes in weight observed.

My thanks are due to Prof. J. Orr, Dr. F. Jacoby and Dr. Kathleen Hall for their advice, and to Mr. D. Varley and Miss Rita Hore, who prepared the sections and photomicrographs for me.

[1] Selye, H., Veilleux, R., and Cantin, M., *Science*, **133**, 44 (1961).
[2] Grad, B., and Leblond, C. P., *Endocrin.*, **45**, 250 (1949).
[3] Logothetopoulos, J. H., and Myant, N. B., *J. Physiol.*, **134**, 189 (1956).

STIMULATION OF TURNIP YELLOW MOSAIC VIRUS PROTEIN PRODUCTION BY 2-THIOURACIL

By Dr. R. I. B. FRANCKI and Dr. R. E. F. MATTHEWS
Botany Department, University of Auckland, and Plant Diseases Division, Department of Scientific and Industrial Research, Auckland,

THE uracil analogue, 2-thiouracil, reduces the rate of multiplication of several plant viruses[1]. When tobacco mosaic virus multiplication is inhibited by 2-thiouracil, the analogue is incorporated into the virus ribonucleic acid (RNA)[2]. Both whole virus containing 2-thiouracil and RNA prepared from it by the phenol method[3] appear to be less infectious than normal virus. In the experiments reported here we have found that, while 2-thiouracil suppresses multiplication of turnip yellow mosaic virus (TYMV) nucleoprotein, it causes a spectacular increase in the production of virus protein shells containing no RNA. The analogue is not incorporated into the RNA of complete virus in detectable amounts.

The strain of TYMV used and the methods of inoculation and culture in Chinese cabbage plants have been described[4]. We have found that Chinese cabbage plants are much less susceptible to gross leaf injury and inhibition of growth by 2-thiouracil than many other species, such as tobacco. Plants to be treated with 2-thiouracil were sprayed daily with a 0·001 *M* solution of the analogue, controls being sprayed with water. To reduce variability in the sample, groups of 6–18 plants were used for each treatment and one or more disks were taken from each leaf and pooled at each time of sampling. In most experiments the disks were ground immediately after sampling and the sap pressed through muslin. The sap was then examined in a Spinco model *E* analytical ultracentrifuge either without further treatment or after heating at 55° for 5 min.—a treatment which destroys the integrity of the plant ribosomes. Sap from healthy Chinese cabbage plants sprayed with 2-thiouracil and heated in this way, shows no peaks sedimenting at the rates of the virus protein or nucleoprotein. In some of our early tests samples of leaf were frozen before examination. We abandoned this procedure, however, since we found that freezing may convert a substantial proportion of the empty protein shell fraction and a lesser amount of the nucleoprotein to a heterogeneous material sedimenting at about the same rate as the plant ribosomes.

Quantitative estimates of the concentration of components were made by measuring areas under the schlieren curves. Areas were corrected for radial dilution, but not for the Johnston–Ogston effect. Difficulty in assessing the correct base line in the schlieren patterns from crude plant extracts leads to significant ambiguity when the areas of the peaks are small. The base line is easier to determine when heat-clarified sap is used, but heat-treatment leads consistently to a slight reduction in the area of the empty protein shell peak. We do not yet know whether this reduction is due to error in base line determination or to destruction of a small proportion of the empty shells. However, this source of error is negligible for the major effects described here. Areas were translated into concentrations in mgm./ml. using experimentally determined factors for the virus protein and nucleoprotein. In some experiments an independent estimate of the virus nucleoprotein material was made by the serological–chromatographic procedure[5].

Earlier experiments[6] in which rather crude methods were used, had suggested that the ratio of virus protein to nucleoprotein in plants infected with TYMV was fairly constant, but our examination of heat-clarified sap in the ultracentrifuge has shown that this is not so. In normal infections the virus nucleoprotein fraction is the first detectable component (6–8 days after infection in inoculated leaves and 9–11 days in systemically infected leaves). The virus protein peak appears a few days later, and for several days thereafter the virus protein/nucleoprotein ratio rises, reaching a maximum of about 0·4–0·6 and then falling steadily to about 0·1–0·3.

Fig. 1 illustrates the substantial increase in the area of the virus protein peak that is brought about by appropriate treatment of plants with 2-thiouracil. Our evidence that the material constituting this increased peak is largely or entirely TYMV protein is as follows: (1) it has the expected sedimentation coefficient; (2) the material composing the peak is as stable to heating at 55° as the virus protein peak from untreated plants; (3) the peak, along with the virus nucleoprotein peak, is quantitatively removed by the addition of a slight excess of antiserum prepared by the injection of rabbits with highly purified TYMV B_1 nucleoprotein[4]; (4) both the ethanol-ammonium sulphate[7] and pH 4·8 (ref. 4) procedures for purifying TYMV are known to be effective in removing plant proteins and nucleoproteins. When TYMV is isolated by either of these methods from sap such as that shown by Fig. 1*A*, the ratio of the virus protein to nucleoprotein peaks remains substantially unchanged through all steps in the isolation; (5) when purified virus preparations made from infected plants suitably treated with 2-thiouracil are fractionated by sedimentation into dense cæsium chloride solutions[4], the opalescent band sedimenting in the position of the empty protein shell is greatly increased in amount relative to the B_1 nucleoprotein. The material isolated in this way has an ultra-violet absorption spectrum indistinguishable from the normal virus protein.

Under conditions where 2-thiouracil causes a large increase in the content of empty virus shells, it has no detectable effect on the areas of the plant ribosome peak, or the fraction I (ref. 8) protein peak.

The effect of 2-thiouracil treatment on the amounts of TYMV protein and nucleoprotein produced in the leaf depends strongly on the time after infection at which treatments are begun (Fig. 2). If spraying is started within a few days after inoculation, nucleoproduction is strongly suppressed, while protein production is not markedly affected. This leads to a large increase in the protein/nucleoprotein ratio, although there is a net decrease in the rate of production of total virus protein (empty protein shell + protein in the nucleoprotein fraction). If treatment is begun when the virus infection is well established, 2-thiouracil causes a striking rise in the production of empty protein shells. The production of nucleoprotein is not as strongly depressed as when treatments are started earlier. The net effect is a substantial increase over untreated plants in the content of total virus protein material.

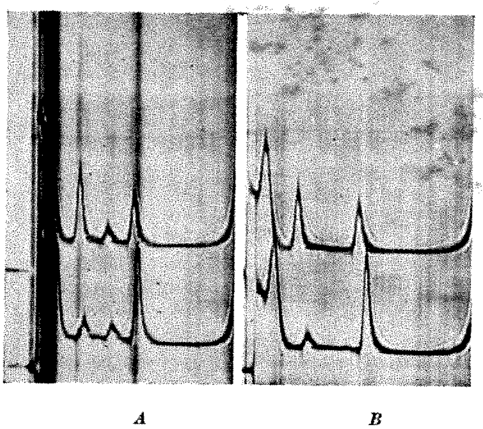


Fig. 1. Schlieren diagrams of expressed sap from Chinese cabbage plants infected with TYMV. *A*, untreated sap after centrifugation at 35,600 r.p.m. for 14 min.; *B*, sap heated to 55° for 5 min. and then centrifuged at 31,410 r.p.m. for 21 min. Plain cell (lower pattern) contains sap from control plants. Wedge cell (upper pattern) contains sap from plants sprayed daily with 2-thiouracil for 10 days. For Fig. 1*A* peaks in order from left are: (*a*) fraction I plant protein; (*b*) TYMV empty protein shell; (*c*) plant ribosomes; (*d*) TYMV nucleoprotein

Spraying infected plants daily with 0·001 *M* uracil beginning 13 days after inoculation causes an increase in the rate of production of virus nucleoprotein, but the protein/nucleoprotein ratio is not affected. Thus the availability of uracil may be a factor limiting TYMV production. If, however, plants are sprayed with a mixture of 2-thiouracil and uracil (both 0·001 *M*), uracil does not annul the effect of 2-thiouracil. Rather the production of excess protein shells reaches substantially higher levels than with 2-thiouracil alone. The stimulation of virus protein synthesis can, however, be halted by washing the leaves with water and stopping application of 2-thiouracil.

Experiments in which plants were supplied with sulphur-35 as inorganic sulphate, as described in ref. 4, suggest that the rapid increase in empty protein shells caused by 2-thiouracil is due to the formation of new protein rather than 'polymerization' of pre-existing sub-units of the virus shell. For example, in one test, two virus-infected plants, one

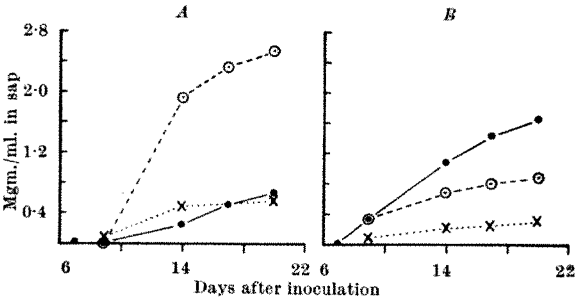


Fig. 2. Effect of 2-thiouracil on TYMV protein and nucleoprotein production. *A*, virus protein shells; *B*, protein in virus nucleoprotein; ●, control sprayed with water; ×, sprayed daily with 2-thiouracil beginning 1 day after inoculation; ○, sprayed daily with 2-thiouracil beginning 9 days after inoculation

of which had been treated with 2-thiouracil for 3 days, were placed in the sulphate solution labelled with sulphur-35 for 4 hr. before gathering. The protein in the isolated protein and B_1 nucleoprotein fractions had the following relative specific activities (expressed as c.p.m./mgm. protein): control protein, 7,570; control nucleoprotein, 4,570; 2-thiouracil protein, 42,500; 2-thiouracil nucleoprotein, 1,200. This differential labelling, together with the observed variations in the ratio of protein shells to nucleoprotein in untreated plants and the accumulation of protein shells following treatment with 2-thiouracil, all lend support to the view[4,9] that the empty protein shells are precursors of complete virus.

In all experiments in which concentrations of the virus components were measured in expressed sap, the nucleoprotein fraction, while consisting largely of the B_1 component[4], also included the B_2 and possibly the B_0 components. We have found that, by sedimenting purified virus preparations in the analytical centrifuge at high concentrations (10 mgm./ml.) in a medium of cæsium chloride of density = 1·36, the B_2 component migrates, as a peak with measurable area, ahead of the B_1 component. The concentration of B_1 in the preparation is estimated from the area of the peak obtained in a second run at lower concentration (2–3 mgm./ml.) in 0·1 *M* sodium chloride. Using this procedure, we have been able to estimate the ratio B_2/B_1. This ratio was consistently less in preparations from plants where empty protein shell was accumulating rapidly and where the rate of nucleoprotein production was reduced by treatment with 2-thiouracil. For example, in a control virus preparation where the ratio of protein to nucleoprotein was 0·25, the ratio B_2/B_1 was 0·042. The corresponding figures for a preparation from plants treated with 2-thiouracil for 4 days were protein/nucleoprotein 0·88; B_2/B_1 0·029. This reduction in the ratio B_2/B_1 would be consistent with the suggestion[4] that B_2 may be a stage in the assembly of complete virus from empty protein shells.

2-Thiouracil does not appear to be incorporated into the virus RNA. In one test where plants were treated with 2-thiouracil labelled with sulphur-35 (Amersham code *SJ*50) no significant radioactivity could be detected in either whole purified virus preparations or in the isolated B_1 component under conditions in which we would have detected incorporation of 2-thiouracil equivalent to 0·01 per cent of the virus uracil. In this experiment the ratio protein/nucleoprotein at the beginning of treatment was 0·19 and at time of collecting (after 8 days) was 0·73.

The reason why 2-thiouracil causes protein shells to accumulate is at present quite obscure. The phenomenon bears at least a superficial resemblance to the action of proflavine on certain viruses attacking *Escherichia coli*[10]. 2-Thiouracil may have its effect in part either by inhibiting virus RNA synthesis or, if virus RNA is made, by preventing its incorporation into the protein shell. However, neither of these effects could explain the appearance of a greater total of virus protein material in leaves treated with 2-thiouracil. Lack of incorporation of the RNA might be due to the production of either faulty RNA or faulty virus protein shells or both of these. As yet, we have no evidence as to whether the protein which accumulates is biologically functional or not.

It should be possible to determine whether free virus RNA accumulates at a rate equivalent to the increase in protein shells since after treatment for a few days with 2-thiouracil the amounts present would be about 0·5 mgm. of RNA per gm. fresh weight of leaf.

This work was supported in part by a grant from the Rockefeller Foundation.

[1] Commoner, B., and Mercer, F. L., *Arch. Biochem. Biophys.*, **35**, 278 (1952). Bawden, F. C., and Kassanis, B., *J. Gen. Microbiol.*, **10**, 160 (1954). Jeener, R., *Biochim. Biophys. Acta*, **23**, 351 (1957).

[2] Jeener, R., and Roseels, J., *Biochim. Biophys. Acta*, **11**, 438 (1953). Matthews, R. E. F., *ibid.*, **19**, 559 (1956).

[3] Francki, R. I. B., *Virology*, **10**, 374 (1960).

[4] Matthews, R. E. F., *Virology*, **12**, 521 (1960).

[5] Matthews, R. E. F., *Virology*, **1**, 165 (1955).

[6] Matthews, R. E. F., *Virology*, **5**, 192 (1958).

[7] Markham, R., and Smith, K. M., *Parasitology*, **39**, 330 (1949).

[8] Singer, S. J., Eggman, L., Campbell, J. M., and Wildman, S. G., *J. Biol. Chem.*, **197**, 233 (1952).

[9] Jeener, R., *Biochim. Biophys. Acta*, **13**, 307 (1954).

[10] de Mars, R. I., Luria, S. E., Fisher, H., and Levinthal, C., *Ann. Inst. Pasteur*, **84**, 113 (1952).

ROOT TEMPERATURE AND SYMBIOTIC NITROGEN FIXATION

By A. H. GIBSON

Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra

THE effect of physical environment on nodule formation and symbiotic nitrogen fixation has received little attention, although such factors as soil and air temperatures, light intensity and moisture conditions appear to be important. Investigation of the effects of these factors has begun, with the initial emphasis on effects of root temperature on symbiotic nitrogen fixation by *Trifolium subterraneum* (subterranean clover).

Hely (personal communication) has observed that during winter periods nodulated clover plants make poor growth compared with plants receiving combined nitrogen. Meyer and Anderson[1] have shown that moderately high temperatures specifically inhibit nitrogen fixation, although Mes[2] suggested that their conclusions should be restricted to subterranean clover. The aim of this investigation was to find the most effective combinations of host and bacterial strain suitable for the various ranges of environmental conditions found in the field. This article reports two experiments with two varieties of *T. subterraneum* inoculated with each of two strains of *Rhizobium trifolii*.

The plants were grown on mineral nutrient agar, free from available combined nitrogen, the enclosed tube technique[3] being modified so that only the roots grew inside the tube. Two days after sowing surface-sterilized pre-germinated seeds, the plants were either inoculated or left uninoculated as controls; 13 days later the inoculated plants were well nodulated. At this time a uniform population of plants was selected, and ammonium nitrate added to the controls. One-fifth of the plants were collected for initial nitrogen-level determinations, and the remainder transferred to a controlled environment unit in which the tubes were suspended in temperature-controlled water-baths (temperature variation ± 0·3 deg. C. in the tubes). The source of light was a bank of 'Warm White' fluorescent, and incandescent, tubes giving an intensity of 1,000 ft.-candles, and a photoperiod of 16 hr. For all plant tops the phototemperature was 22–23° C., and the nyctotemperature 10–11° C. The tubes were re-filled with sterile nutrient solution when necessary and ammonium nitrate added to the controls at regular intervals to give the following amounts of combined nitrogen: 4 mgm. for root temperatures 5° and 9·5° C., and 6 mgm. for 14° and 18° C. in experiment 1 ; 9 mgm. for 18°, 22°, 26° and 30° C. in experiment 2. The ten replicates of each treatment were collected after growth for 20 days in the unit, and the dry weights and total nitrogen determined.

The values in Table 1 express nitrogen fixed during the temperature treatments as a percentage of the combined nitrogen assimilated by the control plants; these values provide an index of the effectiveness of the symbiosis[4]. In experiment 1, the Tallarook controls at 18° C. appeared to be slightly deficient in nitrogen, although the amount assimilated was less than that added. For experiment 2, higher levels of ammonium nitrate were added to the controls, but the amounts assimilated were probably excessive, as shown by: (1) a percentage nitrogen of 4·3–4·7 in all control treatments compared with a normal level of 3–3·5 per cent; and (2) a close similarity in total dry weight of the controls and the most effectively nodulated treatments (Tallarook inoculated with *TA*1). The excessive assimilation of combined nitrogen probably explains the generally lower values for the index of effectiveness in experiment 2, relative to experiment 1, in Table 1.

At root temperatures of 5° and 9·5° C., Tallarook inoculated with strain *TA*1 is able to fix nitrogen as effectively as the controls can assimilate combined nitrogen, whereas the remaining three combinations of host and strain are less effective. As the temperature rises, the effectiveness of these three associations improves. Above 22° C., the effectiveness of nitrogen fixation by strain *NA*30 on both varieties is reduced considerably, while the effectiveness of strain *TA*1 remains comparatively constant. The results obtained with strain *NA*30 are similar to those reported by Meyer and Anderson[1], who also used this strain (personal communication). However, the results with strain *TA*1 suggest that their postulate that moderately high temperatures specifically inhibit symbiotic nitrogen fixation requires further elucidation.

Table 1. EFFECTIVENESS OF SYMBIOTIC NITROGEN FIXATION AND COMBINED NITROGEN UPTAKE (MGM. AMMONIUM NITRATE – NITROGEN/PLANT) AT DIFFERENT ROOT TEMPERATURES

Root temp. (°C.)	Uptake of ammonium nitrate		Index of effectiveness			
			Variety Tallarook		Variety Dwalganup	
	Tallarook	Dwalganup	Strain *TA*1	Strain *NA*30	Strain *TA*1	Strain *NA*30
Exp. 1						
5·0	0·99	0·90	97·6	49·7	42·6	50·0
9·5	2·67	2·07	104·2	72·8	58·4	67·7
14·0	4·46	3·58	90·2	79·7	65·1	75·8
18·0	4·55	3·76	111·0	86·4	63·8	82·8
Exp. 2						
18·0	5·96	4·83	72·4	56·1	45·1	57·2
22·0	9·05	6·89	74·3	51·0	48·7	49·1
26·0	7·41	6·54	85·8	32·7	39·7	35·7
30·0	7·00	2·68	74·9	9·9	58·8	18·8

The index of effectiveness is derived from $\frac{\text{nitrogen fixed}}{\text{ammonium nitrate uptake}} \times 100$

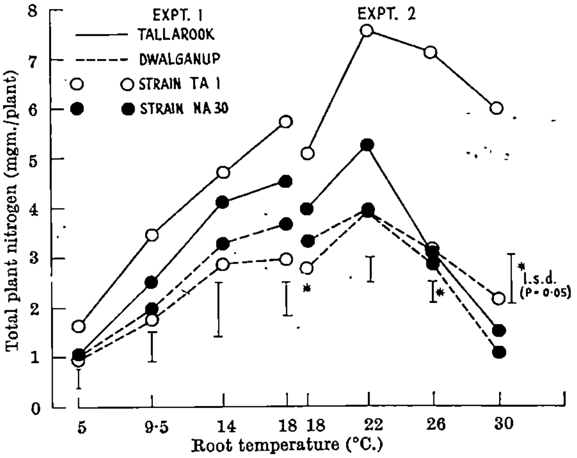


Fig. 1. Effect of root temperature on total plant nitrogen

*Owing to significant differences between error variances, no L.S.D. values are shown for the treatment means at 18° C., nor for the Tallarook means at 26° and 30° C. in experiment 2.

It would appear that the temperature at which nitrogen fixation is affected, relative to plant growth on combined nitrogen, may depend on the bacterial strain, or at least on the host × strain combination. However, it is possible, as found with these experimental conditions, that fixation of nitrogen by some combinations of host and strain is relatively insensitive to higher temperatures, when compared with the uptake of combined nitrogen; for example, Dwalganup controls made poor growth at 30° C., yet the index of the effectiveness of nitrogen fixation by this host, inoculated with *TA*1, remains similar to that at 18–26° C.

Jones and Tisdale[5] showed that four species of nodulated legumes differed in their tolerance of high soil temperatures. Mes[2] observed that maximum nitrogen fixation by several species of nodulated legumes was attained at different day temperatures, and also at different night temperatures. In neither case were the effects of bacterial strain examined. In the present investigation, temperature influenced the relative effectiveness of two strains of *Rhizobium trifolii* on two varieties of the one host species (Fig. 1). With the variety Tallarook, there is a marked divergence in the level of total plant nitrogen for the two strains of *Rhizobia* as the root temperature rises above 22° C. In addition, there is a temperature × strain interaction with the variety Dwalganup, with *NA*30 being the better strain below 22° C., and strain *TA*1 superior at 30° C. This reversal of the order of effectiveness of the two strains on Dwalganup has been observed repeatedly.

The demonstration of temperature × strain, and host × strain, interactions within the species *T. subterraneum* indicates that these three factors should be considered in the selection of strains of *Rhizobia*, if the full potential of the legume-rhizobia symbiosis is to be attained.

[1] Meyer, D. R., and Anderson, A. J., *Nature*, **183**, 61 (1959).
[2] Mes, M. G., *Nature*, **184**, 2032 (1959).
[3] Thornton, H. G., *Proc. Roy. Soc.*, B, **106**, 110 (1930).
[4] Boyes, J., and Bond, G., *Ann. App. Biol.*, **29**, 103 (1942).
[5] Jones, F. R., and Tisdale, W. B., *J. Agric. Res.*, **22**, 17 (1921).

LETTERS TO THE EDITORS

COSMOLOGY

An Unsteady Universe with Excess of Charge

RECENTLY, Lyttleton and Bondi[1] put forward the suggestion that the outflow of matter in the universe is caused by a net charge on the hydrogen atom, and that in order for the universe to remain steady in the large, there is creation of matter. This analysis was simplified by me[2] with the aid of Watson's creation potential[3].

Suppose, however, that the universe is not steady, but is uniform, being composed of hydrogen atoms of mass m and charge e. Following the arguments of Milne[4], if the velocity of any particle, the position vector of which with respect to the origin is $\mathbf{r}$, is $\mathbf{r}/t$ each particle in a Newtonian system can equally be regarded as the centre of the universe. The associated acceleration is:

$$\frac{d\mathbf{v}}{dt} = (\mathbf{v} \cdot \nabla)\mathbf{v} + \frac{\partial \mathbf{v}}{\partial t}$$

$$= (\alpha^2 - \alpha)\frac{\mathbf{r}}{t^2}$$

The proof is given by Milne for the case $\alpha = \frac{2}{3}$, and the extension is obvious.

Maxwell's modified equations are[3]:

$$\nabla \times \mathbf{E} + \frac{\partial \mathbf{B}}{\partial t} = 0, \quad \nabla \cdot \mathbf{B} = 0,$$

$$\nabla \times \mathbf{H} - \frac{\partial \mathbf{D}}{\partial t} = \mathbf{J} - \frac{1}{\mu_0}\nabla N, \quad \nabla \cdot \mathbf{D} = \rho + \varepsilon_0 \frac{\partial N}{\partial t}$$

If a solution be taken for which $\mathbf{H}$ is zero as previously[2];

$$\nabla \times \mathbf{E} = 0, \quad -\frac{\partial \mathbf{D}}{\partial t} = \mathbf{J} - \frac{1}{\mu_0}\nabla N,$$

$$\nabla \cdot \mathbf{D} = \rho + \varepsilon_0 \frac{\partial N}{\partial t}$$

Eliminating N, it follows that:

$$\nabla(\nabla \cdot \mathbf{D}) - \mu_0\varepsilon_0 \frac{\partial^2 \mathbf{D}}{\partial t^2} = \nabla \rho + \mu_0\varepsilon_0 \frac{\partial \mathbf{J}}{\partial t}$$

$$= \nabla \rho + \mu_0\varepsilon_0 \frac{\partial}{\partial t}(\rho\mathbf{v})$$

$\mathbf{v}$ being the flow velocity of the electric charge density.

If m be the mass of a hydrogen atom, then:

$$m\frac{d\mathbf{v}}{dt} = e^*\mathbf{E} = e^*\frac{\mathbf{D}}{\varepsilon_0}$$

where:

$$e^* = e\left(1 - \frac{\gamma m^2 4\pi\varepsilon_0}{e^2}\right)$$

that is, if the electric repulsion between two particles is greater than the gravitational attraction between them, e^*/e is positive, and vice versa.

It follows, therefore, that:

$$\mathbf{D} = \frac{\varepsilon_0 m}{e^*}\frac{d\mathbf{v}}{dt} = \frac{\varepsilon_0 m}{e^*}(\alpha^2 - \alpha)\frac{\mathbf{r}}{t^2}$$

This field satisfies $\nabla \times \mathbf{E} = 0$ identically.

On the assumption that the universe is uniform in the large, $\nabla \rho$ vanishes; also $\nabla \cdot \mathbf{r} = 3$ and so $\nabla(\nabla \cdot \mathbf{r}) = 0$.

$$\mu_0\varepsilon_0 \frac{\partial}{\partial t}\left(\rho\,\alpha\frac{\mathbf{r}}{t}\right) = \frac{\varepsilon_0 m}{e^*}(\alpha^2 - \alpha). - \mu_0\varepsilon_0\frac{6\mathbf{r}}{t^4}$$

whence:

$$\rho = \frac{2\varepsilon_0 m(\alpha - 1)}{\varepsilon^* t^2}$$

The rate of production of charge per unit volume is given by:

$$\nabla \cdot (\rho\mathbf{v}) + \frac{\partial \rho}{\partial t} = \nabla \cdot \left\{\frac{2\varepsilon_0 m(\alpha - 1)\alpha\mathbf{r}}{e^* t^2}\right\} - \frac{2\varepsilon_0 m(\alpha - 1).2}{e^*\ t^3}$$

$$= \frac{2\varepsilon_0 m}{e^* t^3}(\alpha - 1)(3\alpha - 2) = \frac{\rho}{t}(3\alpha - 2)$$

The rate of creation of atoms per unit volume is, of course, obtained by dividing by e. The cases where the production-rate are zero $\alpha = 1$, and $\alpha = \frac{2}{3}$. In the first case, the acceleration of the nebulæ $\frac{d\mathbf{v}}{dt}$ is zero[5] which would correspond here to the electric repulsion being exactly equal and opposite to the gravitational attraction, thus balancing it out. The second case corresponds to Milne's Parabolic Universe[6].

The recession-rate of the nebulæ is given also by $\mathbf{v} = \frac{\mathbf{r}}{\tau}$, where τ is the inverse of Hubble's constant. Thus, the time at present is $\alpha\tau$, and the present rate of production of charge density is $\rho/\tau\left(3 - \frac{2}{\alpha}\right)$. Corresponding to this is a present rate of production of mass density $\frac{\rho_m}{\tau}\left(3 - \frac{2}{\alpha}\right)$ where ρ_m is the present mass density. Now

$$\rho_m = \frac{m}{e}\rho = 2\varepsilon_0 m^2 \frac{e}{e^*}\frac{\alpha - 1}{t^2}$$

and at present,

$$\rho_m = 2\varepsilon_0 m^2 \frac{e}{e^*}\ \frac{\alpha - 1}{\alpha^2\tau^2}$$

There is experimental evidence[7] that:

$$\frac{8}{3}\pi\gamma\rho_m\tau^2 = U^{-1}$$

where U is a number the value of which is about unity and so it follows that:

$$\frac{e^2}{4\pi\varepsilon_0\gamma m^2} = \frac{4}{3}\ U\frac{\alpha - 1}{\alpha^2} + 1$$

The left-hand side is the ratio of electric repulsion to gravitational attraction, and must be positive, and so $\frac{4}{3}U\frac{(1 - \alpha)}{\alpha^2} < 1$. Now e is determinate in principle as indicated by Lyttleton and Bondi, and ρ_m and τ (and hence U) are also determinate from astronomatical observations. It follows, therefore, theoretically that α can be found, and hence the present rate of production of matter.

The observations made at time t of matter at a distance R, will refer to the matter at a time $t - R/c$. It follows, therefore, that the observed mass density at a distance R is:

$$2\varepsilon_0 m^2 \frac{e}{e^*}\frac{\alpha - 1}{(\alpha\tau - R/c)^2} = \rho_0\left(1 - \frac{R}{\alpha c\tau}\right)^{-2}$$

where ρ_0 is the local mass density at present. Also the observed recession velocity is:

$$\frac{\alpha R}{\alpha\tau - R/c} = \frac{R}{\tau}\left(1 - \frac{R}{\alpha c\tau}\right)^{-1}$$

Both these quantities, the observed mass density and the observed recession velocity, are in principle obtainable (albeit the experimental techniques are not good enough at present) and so α is obtainable in principle, and a test of this theory will be possible once the data are known with sufficient accuracy.

Ll. G. Chambers

Mathematics Department,
University College of North Wales,
Bangor.

[1] Lyttleton, R. A., and Bondi, H., *Proc. Roy. Soc.*, *A*, **252**, 313 (1959).

[2] Chambers, Ll. G., *Nature* **191**, 262 (1961).

[3] Watson, W. H., *Proc. Second Symp. American Mathematical Soc.*, 49.

[4] Milne, E. A., *Relativity, Gravitation and World Structure*, 306 (Oxford, 1935).

[5] Milne, E. A., *Relativity, Gravitation and World Structure*, 75 (Oxford, 1935).

[6] Milne, E. A., *Relativity Gravitation and World Structure*, 304 (Oxford, 1935).

[7] For example, Klein, O., *Proc.* 1958 *Solway Conf.*, 36.

ASTRONOMY

The Alpha Virginis (Spica) Nebula

An extensive ultra-violet nebula surrounding Spica has been reported by Kupperian, Boggess, and Milligan[1]. Their rocket-borne photon counters yielded an isophotal map of roughly circular extent 22° in diameter, increasing in brightness from the outside towards Spica but reaching the instrumental saturation-level for the central area. If the nebula were due to Lyman-α radiation it would be reasonable to assume that H_α should also be detectable, but no nebula has ever been detected by conventional photographic means. This may have been due to the fact that the strong image of Spica drowns the faint nebula close to the star. On the red plates of the Palomar Sky Survey, the over-exposed image of Spica covers more than 10 min. of arc, while on the blue plates it is nearly three times larger in diameter. The spectrum of Spica itself does not show H_α in emission[2].

Recently, Johnson[3] reported negative results in H_α of the nebula by means of direct and spectroscopic exposures centred on the brightest ultra-violet isophote about 10 min. of arc west of Spica.

In order to examine the area immediately surrounding the star a number of long exposures have been taken during the past 18 months by means of a novel device which might be called a 'stellar coronagraph', attached to the 30-in. refractor at Allegheny Observatory. This device, to be described in detail in a later communication, prevents the stellar image from reaching the emulsion and obscures a circular area 1 mm. (14·6 sec. of arc) in diameter at the focal plane, thus completely eliminating the over-exposed stellar image and halation pattern. To cover the range of H_α, Eastman 103*a*-*E* plus Corning 2403 filter was exposed to a limiting stellar magnitude of $m_{pg} \approx 17$. A few blue exposures on 103*a*-*O* without filter were also taken. The useful field is only 6 × 8 min. of arc with the 14·6 sec. diameter blank space at the centre. None of the exposures reveals any trace of nebulosity.

This method looks promising for detecting circumstellar envelopes and faint companions close to bright stars and should yield best results on large reflectors with large plate scales.

It is a pleasure to thank Dr. N. E. Wagman for making the telescope available for these studies.

Walter A. Feibelman

1063 Findley Drive,
Pittsburgh 21, Pa.

[1] Kupperian, jun., J. E., Boggess, III, A., and Milligan, J. E., *Astrophys. J.*, **128**, 453 (1958).

[2] Struve, O., Sahade, J., Huang, S.-S., and Zebergs, V., *Astrophys. J.*, **128**, 310 (1958).

[3] Johnson, H. M., *Pub. Astro. Soc. Pac.*, **73**, 73 (1961).

GEOPHYSICS

Height of Night-time *F* Layer Irregularities at the Equator

It has been established by observations at low latitudes that there exists near the equator a belt in the *F* region in which large irregularities in electron density give rise to equatorial spread *F*[1] severe radio star scintillations[2] and the scatter of radio waves[3]. Work by Hewish[4] and Briggs[5] on extra-terrestrial radio sources shows how the height of the irregularities may be inferred from observations of the diffraction pattern on the ground. But the methods used are rather indirect, and therefore subject to some uncertainty. More recently Cohen and Bowles[6], using radar techniques, arrived at an estimation of irregularities of height for equatorial locations. It is not certain that these irregularities are to be identified with those responsible for satellite and radio star scintillation.

Work on these disturbances now being pursued at University College, Ibadan, and at the University of Ghana, Accra, shows that the irregularities in the pattern on the ground are elongated in the direction of the Earth's magnetic field, that the axial ratio is greater than 6:1, and that the smaller dimension is of the order of 0·5 km. (J. R. K. and G. S. K., unpublished). These results are deduced from the study of satellite and radio star transmissions through the disturbed region. It is desirable to determine the heights of the irregularities by a direct method. The procedure adopted consists in the observation at two separated stations of the diffraction pattern produced on the ground when the radio transmissions from an Earth satellite pass through the irregularities. The velocity of the pattern over the ground is determined, and by relating this velocity to the known height and velocity of the satellite, it is possible to determine the effective height of the diffracting screen. Since the velocity of drift of the diffracting screen itself is known to be of the order of 150 m./sec. (J. R. K., unpublished) it can be neglected without introducing an appreciable error.

The observations were made at Legon, near Accra, Ghana (5° 38′ N.; 0° 11′ W.), and the satellite used was *Tiros II* (1960 π 1). Since its apogee and perigee distances are 724 and 624 km. respectively, it is normally above the electron-density maximum in the *F* layer. The orbit is inclined to the equator at an angle of 48·6°. The signal used was the transmission on 108·03 mc./s. At each station the signal was received on a half-wave dipole, and fed to a crystal-controlled converter ahead of a Collins type *R*.390*A*/*URR* radio receiver. To achieve a satisfactory signal to noise ratio it was necessary to employ a bandwidth of 100 cycles per second. Tuning was done manually at both receivers throughout each observation. In each case the receiver *B.F.O.* was used to give a 2,000 c.p.s. note, which was transmitted via telephone cable to a central recording location. The amplitudes of the signals were then displayed on a double-beam oscilloscope and recorded photographically. A number of observations were made with an east–west base-line 1 km. in length. Later a 2·4 km. base-line was used, inclined 48° to the equator in the plane of the satellite orbit. Observations were limited to night-time passes in the period June 21–July 13, 1961.

If the irregularities occur in a thin layer at a height z and the satellite is moving horizontally at a height h and a velocity V, it can be shown that the diffraction pattern will move across the ground with a velocity $V\frac{z}{h-z}$ (ref. 7). In the case of a satellite moving in an elliptical orbit around the Earth, the expression is more complicated; but the velocity and direction of movement of the pattern on the ground can still be derived from a knowledge of the orbit. The time-shifts observed in the fading at the two aerials depend on the relative direction of the velocity of movement of the pattern, the line joining the two aerials, and the direction of elongation of the pattern on the ground. The irregularities have been assumed to lie along the magnetic lines of force and, to a first order of approximation, to be infinitely elongated.

If the layer of irregularities is not thin, the form of the pattern will change slowly as the pattern moves across the ground, in a manner depending on the thickness. This will cause the maximum correlation of the signals at the two aerials to be less than unity. A further decrease will occur since the pattern is not infinitely elongated and the two aerials do not lie precisely along the direction of motion of the pattern. From observations of this decrease with different aerial orientations, it is in principle possible to separate these two effects and determine the thickness as well as the height of the layer of irregularities. In practice, due to the limitations of the equipment available, the results obtained even with the aerials lying almost along the direction of motion of the pattern were not accurate enough to give more than an upper limit to the thickness. The decrease in correlation also reduces the measured time-shift slightly, and a correction was applied for this.

The rate of fading of the signals was normally of the order of 5 cycles per second during periods of scintillation when measurements were made. Time-delays found over the two paths were about 0·2 and 0·4 sec. The records were normally taken for about 5 sec., and were read at intervals of 20 m.sec. Cross- and auto-correlation functions were calculated for the amplitudes of the signals received at the two aerials. Ten records were analysed, and the results are summarized in Table 1.

Table 1

Date	U.T.	Distance	Direction of irregularities observed	Height of base of *F* layer	Height of irregularities
22/6/61	22:01:03	1,169	1° W. of N.	350 km.	424 km.
22/6/61	22:02:01	1,658	6° E.	350	401
23/6/61	20:56:04	1,171	37° E.	360	416
24/6/61	21:27:02	913	34° W.	300	402
24/6/61	21:29:00	1,171	8° W.	300	396
24/6/61	21:30:06	1,545	7° E.	300	380
9/7/61	02:33:54	697	151° E.		395
10/7/61	01:22:30	788	9° E.		427
12/7/61	00:51:07	415	12° E.	260	354
12/7/61	00:52:07	292	46° E.	260	314

The distance given in column 3 refers to the distance along the ground from the observer to the point vertically below the irregularities observed. Column 5 shows the virtual height of the base of the *F* layer from an ionogram taken at Accra at the time of the observation. On all occasions on which satellite scintillations were observed, spread *F* and radio star scintillation were also present. It will be noted that the effective height of the irregularities always lies between 50 km and 100 km. above the base of the *F* layer. The value of height given above may be compared with the results of Cohen and Bowles[6], who found that the irregularities which they observe lie at or near the base of the *F* layer.

From the maximum values of the cross-correlations observed the elongation of the irregularities is found to be greater than 7 to 1, and the thickness of the scattering layer to be less than 120 km.

The programme of research of which this forms a part is financed by the Air Research and Development Command of the U.S. Air Force under contract No. *AF* 61 052–421.

G. S. Kent

Department of Physics,
University College,
Ibadan, Nigeria.

J. R. Koster

Department of Physics,
University of Ghana,
Legon, Accra, Ghana.

[1] Lyon, A. J., Skinner, N. J., and Wright, R. W. H., *J. Atm. and Terr. Phys.*, **19**, 145 (1960).
[2] Koster, J. R., *J. Atm. and Terr. Phys.*, **12**, 100 (1958).
[3] Koster, J. R., and Wright, R. W., *J. Geophys. Res.*, **65**, 2303 (1960).
[4] Hewish, A., *Proc. Roy. Soc.*, A, **214**, 494 (1952).
[5] Briggs, B. H., *J. Atm. and Terr. Phys.*, **12**, 34 (1958).
[6] Cohen, R., and Bowles, K. L., *J. Geophys. Res.*, **66**, 1081 (1961).
[7] Kent, G. S., *J. Atm. and Terr. Phys.*, **16**, 10 (1959).

A Method of Estimating Ionospheric Absorption Heights From Recovery Times

It is generally agreed that the absorption of radio waves in the ionosphere occurs at heights below about 100 km. In a particular instance it is usually possible to deduce the approximate height by indirect reasoning. For example, Hultquist[1] has used indirect evidence to place the abnormal absorption which follows intense solar flares at heights as low as 50 km. As yet there are too few direct measurements (using rockets) to provide a complete and consistent picture. This communication suggests a new method of estimating the height of the regions responsible for abnormal absorption of the kind associated with auroral disturbances.

Fig. 1 shows two examples of absorption as recorded by a riometer operating at a frequency of 30 Mc./s.

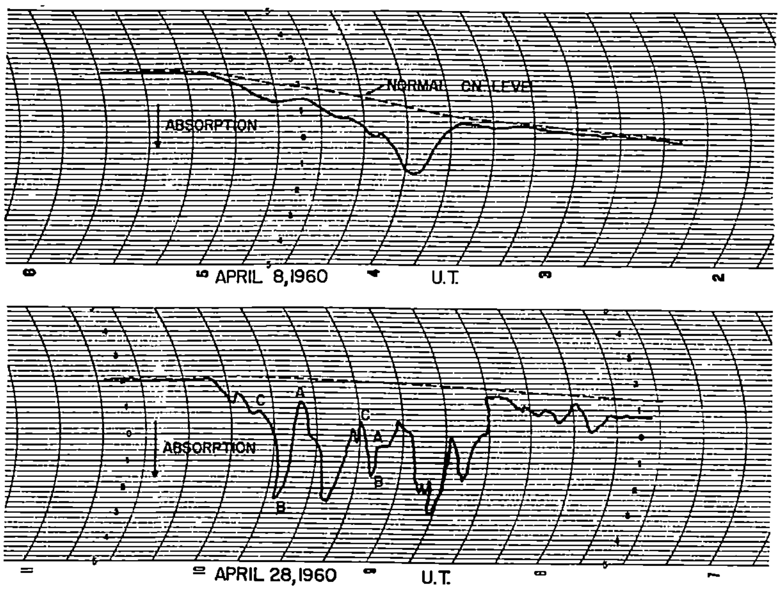


Fig. 1. Typical riometer records during abnormal disturbances. The upper trace shows a weak disturbance. The bottom trace was obtained during a severe disturbance

In each case the normal level of the cosmic noise signal in the absence of absorption is shown by the broken line. Sudden onsets of absorption (marked *AB* in Fig. 1) are frequently followed by rapid recoveries (*BC*). If it is assumed that the ionizing agency ceases to operate at the instant of peak absorption the recovery can be used for estimating the height of the region. The method depends on the fact that the absorption (expressed in decibels) suffered by a radio wave in the lower ionosphere is proportional to the number of electrons encountered (per unit area of the wave-front) in the absorbing region and is a function of the collisional frequency of the electrons.

Appleton investigated the disappearance of ionization in the lower ionosphere[2]. For small variations the ionization (electron density) should decay exponentially with a time constant:

$$T = (2\alpha N)^{-1} \text{ sec.}$$

where N is the average electron density, and α is an effective recombination or loss-rate coefficient. The quantity T is also known as the relaxation time of the ionospheric region concerned. For the present purpose it is assumed that the absorption occurs in a layer having a thickness of about one scale height which is taken to be 6·5 km. throughout the region of interest.

The electronic collisional frequencies given by Chapman and Little[3] were used to deduce electron densities required at various heights and were combined with the values of α given by Rumi[4] for day-time and night-time conditions to calculate the relaxation times (for 1 decibel of absorption) at various heights in the lower ionosphere. The relaxation times are plotted as a function of height in Fig. 2. In order to use the graphs it is necessary first to divide the observed time constant by the average absorption (expressed in decibels). Clearly, the observation would be meaningful only if the decay were measured over a change in absorption that represents only a small part of the total absorption present. There is an ambiguity in the heights deduced from Fig. 2; but this ambiguity usually can be resolved by reasoning based on the general characteristics of the regions concerned and of the absorption event. Heights deduced by the method outlined here have been compared with those determined by means of a three-frequency riometer method[5] and found to be in good agreement. I am indebted to Dr. A. Kavadas for very useful discussions and to Dr. P. A. Forsyth for valuable suggestions during the course of this investigation.

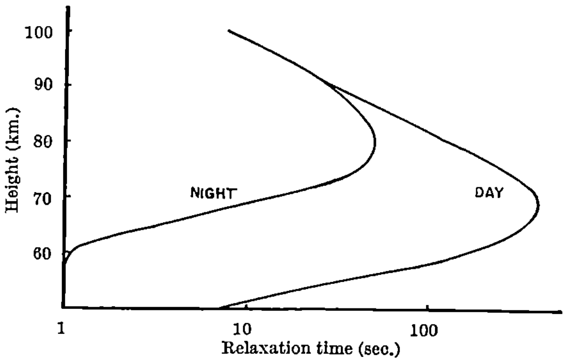


Fig. 2. Relaxation times as a function of height

This work was done at the University of Saskatchewan and was supported in part by Defence Research Board grant 9511-25 and in part through a National Research Council grant *T*-123.

SYED ZIAUDDIN*

Division of Pure Physics,
National Research Council,
Ottawa.

* On leave of absence from the University of Mysore.

[1] Hultquist, B., *Tellus*, **14**, 332 (1959).
[2] Appleton, E. V., *J. Atm. Terr. Phys.*, **3**, 282 (1954).
[3] Chapman, S., and Little, C. G., *J. Atm. Terr. Phys.*, **10**, 20 (1957).
[4] Rumi, G. E., *J. Geophys. Res.*, **65**, 3625 (1960).
[5] Ziauddin, S., and Forsyth, P. A., *J. Geophys. Res.*, **66**, 2315 (1961).

PHYSICS

Ignition of Gas by Sparks produced by breaking Wires at High Speed

INVESTIGATIONS of the conditions for ignition of inflammable gas by electrical sparking have usually been carried out with discharges produced in one of two ways: (i) by discharging a capacitor charged to a high voltage between thin fixed electrodes separated by several millimetres; (ii) by breaking low-voltage circuits at the contacts of a mechanical switch (or 'break-flash'). It has been found that less electrical energy need be dissipated in the discharge to ignite the gas if the first of these methods is adopted; for example, an 8·3 per cent mixture of methane in air can be ignited by about 0·3 mJ. by the first method, whereas an energy of about 2 mJ. is required if the second method is used.

However, recent work both in Great Britain and in the U.S.S.R. has made use of the discharge which results when a wire carrying a current is broken in gas. The first experiments in this laboratory made use of comparatively thick wires (0·022 in. and 0·012 in. in diameter), broken so that their ends separated at about 400 cm./sec.; this is not very different from the speed of separation of electrodes of the standard break-flash[1] used in statutory testing of electrical apparatus for intrinsic safety. The currents and energies necessary to ignite gas in these conditions were only slightly smaller than those necessary with the standard break-flash. However, Berz[2] has found that, if very fine wires are broken at about the same speed, the minimum igniting currents become markedly smaller, and that they fall as the diameter of the wire is reduced. He showed that for 0·001-in. diameter tungsten wire the minimum igniting energy for an 8·3 per cent mixture of methane in air is comparable with that indicated by high-voltage experiments. He concluded that such substantial reductions in minimum igniting-energy could be obtained only when the 'electrodes' (that is, the broken wire-ends) were small compared with the critical size of the initial flame kernel in the gas (see, for example, Lintin and Wooding[3]).

It seemed likely, however, that the quenching effect of bulky electrodes could be reduced if they were rapidly removed from the kernel of the growing flame, and that the minimum igniting energies indicated by high-voltage experiments might be approached by breaking thick wires, provided that the speed of separation of the wire-ends after the break was made sufficiently high. It has been known for some time that variations in the speed of separation of electrodes of the standard break-flash apparatus (usually operated at about 300 cm./sec.) have an effect on the minimum igniting currents obtained with it, but we are not aware of previous experiments at much higher electrode speeds. Separation speeds of about 2,000 cm./sec. have now been achieved with a breaking-wire apparatus, partly based on a method developed by Gavril'chenko[4], in which a previously notched copper wire is broken by being stretched between pairs of rollers rotating at different speeds. The machine, an early model of which will be described elsewhere, differs from that of Gavril'chenko in that the length of wire stretched is comparatively long (about 35 cm.). As the wire is stretched, elastic energy is stored in it; when the wire breaks this is converted into kinetic energy and the ends of the wire separate rapidly. Although the speed of separation is substantially unaffected by the length of wire stretched, the distance over which this speed is maintained is approximately proportional to the length of wire, and in the present apparatus the separation speed of some 2,000 cm./sec. is maintained for a few millimetres.

Tests show that, although the wire is bulky (diameter 0·012 in.) compared with the wires broken by Berz, similar minimum igniting currents are obtained. Thus for a particular set of circuit conditions, a minimum igniting current of 110 m.amp. is obtained with the machine, compared with 110 m.amp. obtained by Berz for 0·005-in. steel wire, 95 m.amp. for 0·001-in. platinum wire, 82 m.amp. for 0·001-in. tungsten wire, and 185 m.amp. for the standard break-flash apparatus.

Further experiments in which notched wires 0·012 in. in diameter were broken manually (by anchoring one end and pulling the other with a pair of pliers) indicate even smaller minimum igniting currents of about 100 m.amp. for the same circuit conditions. It has not yet been possible to reproduce this manual process in a mechanized form, and further investigations are in progress.

These experiments suggest that it may be possible to design a breaking-wire apparatus which will be completely automatic and which will yield minimum igniting energies comparable with those indicated by high-voltage experiments. From a more fundamental point of view, they confirm that disturbance of the growing flame kernel by the electrodes plays a part in increasing the minimum energy necessary for ignition. If this disturbance is reduced, either by using very fine wires as in Berz's experiments, or by removing the electrodes rapidly from the flame kernel as in those reported here, energies approaching the minimum may be achieved.

R. L. GORDON
H. LORD
D. W. WIDGINTON

Safety in Mines Research Establishment,
Ministry of Power, Sheffield, 1.

[1] Lloyd, H., and Guénault, E. M., *Safety in Mines Res. Estab. Res. Rep.*, No. 33 (1951).

[2] Berz, I., *Combustion and Flame Quarterly*, **3**, 131 (1959).

[3] Lintin, D. R., and Wooding, E. R., *Brit. J. App. Phys.*, **10**, 159 (1959).

[4] Gavril'chenko, L. I., *Voprosy Gornoi Electromekhaniki, Trudy Mak NII. Ugletekhizdat. M.*, **9**, 94 (1959).

Damping without Influence on Frequency

IN a recent communication[1], R. Parks and L. Maunder have directed attention to the fact that the period of oscillation of a gyrostat is not affected by damping if this were applied to both axes in a certain ratio (equal to the ratio of the moments of inertia of the uncoupled axes).

It is possible to re-formulate this condition as one of equal loss angles of the energy stores; with proper interpretation the same interesting and potentially useful result holds for ordinary (non-gyroscopic) systems and it can be extended to systems with more than one degree of freedom.

For a system with one degree of freedom the assertion will appear as an obvious conclusion from the Parks–Maunder result if we follow the approach of ref. 2, for there it is shown how the system considered by these authors can be transformed into a dynamically equivalent system consisting of a mass and a spring which are both subject to damping. The argument is based on the fact that, 'seen' across a gyrostatic coupler, a mechanical impedance Z looks like an admittance:

$$Y = Z/C^2n^2 \qquad (1)$$

where Cn is the moment of momentum of the gyrostat rotor around its axis of rotation. From this it follows that the mass impedance Bp attached to axis 2 of the gyrostat appears from axis 1 as the admittance $D_2p = Bp/C^2n^2$ of a spring; the viscous damper of constant c_2, which is linked co-resistively[3] to the mass, transforms into a viscous damper of constant $K_2 = C^2n^2/c_2$, which is linked co-yieldingly[3] to this spring. As the combined impedance of the uncoupled axis 2 amounts to $Bp + c_2$, we need not in fact even evaluate these individual components but can write directly from equation 1 the value of the impedance which the coupler transfers to axis 1 as $C^2n^2/(Bp + c_2)$. The governing equation for axis 1 then reads:

$$Ap + c_1 + \frac{C^2n^2}{Bp + c_2} = 0 \qquad (2)$$

This is, of course, equivalent to the equation derived in ref. 1 by purely algebraic manipulation.

To prove the general assertion, assume first that we deal either with a non-gyroscopic system consisting of masses and springs only, or with a gyroscopic system which, according to ref. 2, can be transformed into an equivalent system of this kind. Let $p_l = j\omega_l$ be one of the n frequencies for which the governing equations for this loss-less system are identically fulfilled. The value ω_l enters these equations only via the expressions for the mass impedances $Z_{mlh} = p_l m_h = j\omega_l m_h$ or the spring admittances $Y_{slh} = p_l D_h = j\omega_l D_h$, or via the reciprocals.

The corresponding expressions for the damped system are:

$$Z'_{mlh} = p_l m_h + K_h = p_l m_h + \alpha m_h, \text{ say, and}$$
$$Y'_{slh} = p_l D_h + x_h = p_l D_h + \alpha D_h \qquad (3)$$

for the conditions considered, where α is a constant, usually positive. It will be seen that for values $p'_l = j\omega_l - \alpha$, we get:

$$Z'_{mlh} = (j\omega_l - \alpha)m_h + \alpha m_h = Z_{mlh}$$
$$Y'_{slh} = (j\omega_l - \alpha)D_h + \alpha D_h = Y_{slh} \qquad (4)$$

This means that for this particular value of the complex frequency the coefficients of the governing equation(s) for the damped system are the same as for the undamped system. These equations are therefore solved by the same set of complex phasors as before, and the new case differs from the old one merely in so far that these phasors now describe a damped oscillatory motion of the form u_l = real part of U_l. exp($j\omega t$). exp($-\alpha t$) instead of U_l.exp($-j\omega t$).

If it is not possible or not desirable to transform a gyroscopic system into an equivalent non-gyroscopic system we have to consider the contributions arising from the gyroscopic coupling terms, that is, in the present notation from terms due to asymmetrical mutual coupling impedances of the form $Z_g = \pm C^2 n^2$. These impedances do not change when damping is added to the system nor—being independent of frequency—are they influenced by the change from p_l to p_l'. Hence the conclusion reached from equations (4) is not disturbed and the assertion remains valid.

A. Bloch

Hirst Research Centre,
The General Electric Company, Ltd.,
Wembley, Middlesex.

[1] Parks, R., and Maunder, L., *Nature*, **190**, 710 (1961).
[2] Bloch, A., *Phil. Mag.*, **35**, 315 (1944).
[3] Bloch, A., *Proc. Inst. Elect. Eng.*, **92**, (Part I) 157 (1945).

Cryoscopic Effect of Separation and Concentration of Solutes

The normal method of concentrating a substance in solution is by distillation. In the course of certain experiments it was found that it is possible to do so to a certain extent by refrigeration.

A column of skim milk in a 250-ml. cylinder was frozen between −5° and −10° C. The frozen column was taken out and allowed to thaw at room temperature (24°–27° C.) in about 2·5 hr. The melted column showed a graded separation, ranging from solid concentration at the bottom to a thin, watery layer at the top. Portions of 10 ml. were carefully collected from the top and bottom of the column with the least possible disturbance, and analysed for total solids and milk sugar.

Table 1. CONCENTRATION OF SKIM MILK BY CRYOSCOPY (gm./100 ml.)

Portion	Total solids	Lactose
Top	3·6	2·23
Bottom	30·8	7·31
Milk before freezing	8·1	4·63

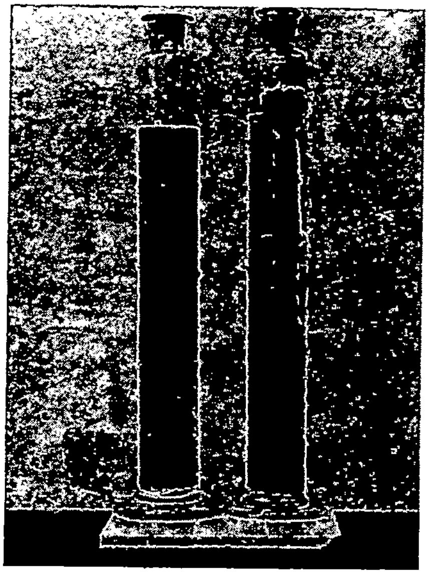


Fig. 1. Columns of potassium permanganate. Left, solution before freezing; right, after freezing. Note the cone of concentrated solute in the frozen column

These figures clearly bring out the high concentration of the solids at the bottom of the column on freezing. It is also noteworthy that the degree of concentration differs from constituent to constituent.

Use of whole milk interferes only with the visible separation of the solids, since the watery top-layers are masked by a plug of light fat.

Table 2. CONCENTRATION OF WHOLE MILK BY CRYOSCOPY (gm./100 ml.)

Portion	Total solids	Fat	Solids-not-fat
Top	8·439	3·24	5·199
Bottom	30·66	7·45	23·21
Milk before freezing	14·03	5·2	8·83

The 'cryoscopic effect', as the phenomenon may be called, is common to crystalloids as well. Solutions of copper sulphate, potassium permanganate and potassium ferrocyanide, similarly frozen and thawed, yield a higher concentration of the salts at the bottom of the column, at once evident by the greater intensity of colour. The disparity in concentrations of the salts between the top and bottom layers of the melted column can be seen in Table 3.

Table 3. CONCENTRATIONS OF CRYSTALLOIDS BY CRYOSCOPY (gm./100 ml.)

Portion	Copper sulphate	Potassium permanganate	Potassium ferrocyanide
Top	2·328	0·035	0·382
Bottom	7·950	0·230	6·410
Solution before freezing	2·739	0·052	1·008

The frozen column of potassium permanganate exhibits a vivid concentration gradient of the salt in the form of a purple cone with its base at the bottom. It is perhaps typical of cryoscopic concentration of solutes. Possibly this can be verified by using X-rays or by the tracer technique in relatively opaque frozen columns of other solutes.

In order to obtain a three-dimensional picture of the concentration of solutes in the frozen column, annular portions of the melts (simultaneously with analyses of top and bottom portions in another column) were analysed, as the thawing progressed.

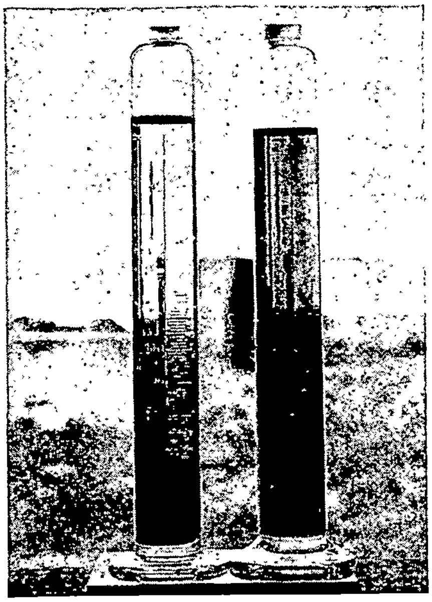


Fig. 2. Columns of potassium permanganate. Left, after freezing and thawing. Note heavy concentration of solute at the bottom. Right, original solution without freezing. Dark streaks in the centre of the columns are due to photographic effects

The average concentrations of salt in the concentric layers of the column from outside inwards, as well as the concentrations of the top and bottom in the second column of each salt, are given in Table 4.

Table 4. CONCENTRATION OF ANNULAR LAYERS AND VERTICAL PORTIONS OF FROZEN COLUMNS OF SOLUTIONS (gm./100 ml.)

Portion	Copper sulphate	Potassium permanganate	Potassium ferrocyanide	Skim milk
Outer layer	4·05	0·091	2·192	11·49
Thawing time (min.)	30	35	28	40
Middle layer	2·775	0·08	—	8·962
Thawing time	60	75	—	85
Inner layer	1·495	0·06	0·840	5·814
Thawing time	135	110	65	145
	Top and bottom portions in second column			
Top	2·328	0·035	0·382	2·378
Bottom	7·95	0·23	6·410	26·918
Solution before freezing	2·739	0·052	1·008	9·2834

These results give an insight into the pattern of concentration of solutes on freezing. The heaviest concentration seems to be a hollow ring at the bottom of the column. It is probable that a predictable relation exists between the solute concentration at the bottom of the frozen column and the concentration of the original solution, the molecular weight of the substance, the height and diameter of the frozen column.

The cryoscopic phenomenon may be of general use in analytical procedures and manufacturing processes, particularly where dilute solutions are involved and distillation is unsuitable. It may be possible to concentrate the heavy water fraction in water by repetitive cryoscopy for the atomic energy industry.

I thank Mr. A. E. Owen for his assistance in the analyses of the samples, and Drs. Anantakrishnan and Sethu Rao for experimental facilities at the National Dairy Research Institute, Bangalore.

K. S. RANGAPPA

Central Water and Power Commission,
New Delhi.

CHEMISTRY

Differential Thermogravimetry versus Differential Thermometry in studying the Pyrolysis of Polymers

ORDINARY chemical methods of analysis are impractical for studying the pyrolysis of highly cross-linked polymers, because such materials are extremely infusible, insoluble and non-volatile. Techniques such as differential thermometry and differential thermogravimetry are therefore being used more and more for this purpose. Differential thermometry measures the net heats of reaction produced by chemical change as a function of temperature, whereas differential thermogravimetry measures loss of weight. Since the endothermic breaking of and the exothermic forming of bonds occur simultaneously in the pyrolysis of substances, differential thermometric information can be misleading. For example, if, at a given temperature, the heats of the endothermic reactions were exactly equal to the heats of the exothermic ones, a differential thermometric peak would be absent. This would lead to the false conclusion that no thermal decomposition had occurred. Changes in specific heat and in thermal conductivity of a polymer during pyrolysis also complicate the differential thermometric picture, especially when quantitative information is desired. Since differential thermogravimetry measures losses of weight during pyrolysis rather than heat changes, it is not subject to the above-mentioned complications, and should therefore give a more reliable quantitative estimate of the relative thermal stabilities of different polymers.

An example of this difference in reliability of the two methods may be had by comparing the corresponding data obtained for two epoxide polymers having different chemical structures. These polymers were prepared by treating dipentene dioxide in one case and vinyl*cyclo*hexene dioxide in the other case with one mole of methyl nadic anhydride per epoxy equivalent, using 1 per cent N,N-dimethylbenzylamine as a catalyst.

Both the differential thermometric and differential thermogravimetric experiments were performed at a heating rate of 5 deg. C./min., the former in nitrogen at atmospheric pressure and the latter *in vacuo*. Descriptions of the apparatus and procedures have been given previously[1,2]. The differential thermogravimetric curves in Fig. 1 clearly show that the vinyl*cyclo*hexene dioxide polymer is more thermally stable than the dipentene dioxide one. The former polymer shows a maximum rate of loss of weight at 375° C., whereas the latter one shows a maximum rate at 265° C. This large difference in thermal stability of the two polymers is not revealed by the differential thermometric curves in Fig. 2. Actually, the differential thermometric peaks at approximately 275° C. for each of the two polymers indicate that the vinyl*cyclo*hexene dioxide polymer has its maximum rate of decomposition at the same temperature as the dipentene dioxide polymer. Furthermore, if the two methods are compared for only one polymer, the differential thermometric data can still be unreliable. For example, the differential thermometric curve for vinyl*cyclo*hexene dioxide indicates that much more decomposition occurred at 275° C. than at 360° C. This indication is also contrary to the more reliable differential thermogravimetry data.

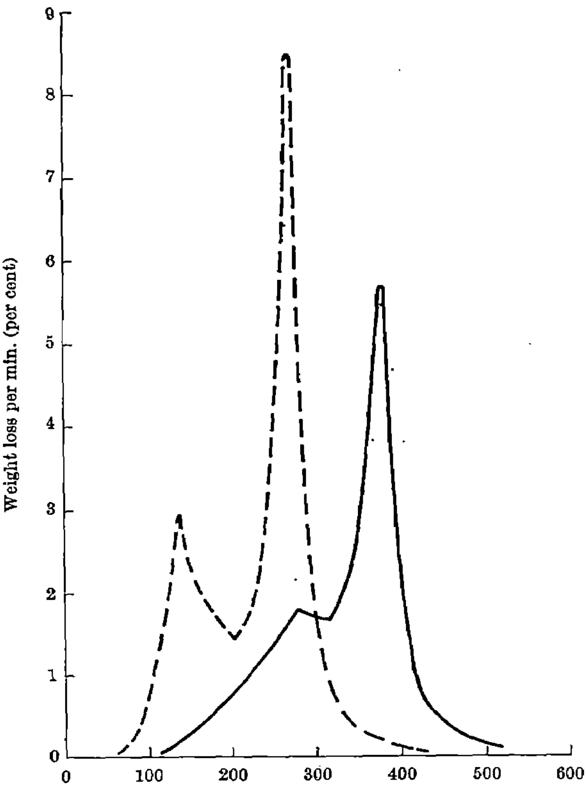


Fig. 1. Differential thermogravimetric curves. – – –, Dipentene dioxide polymer; ——, vinyl*cyclo*hexene dioxide polymer

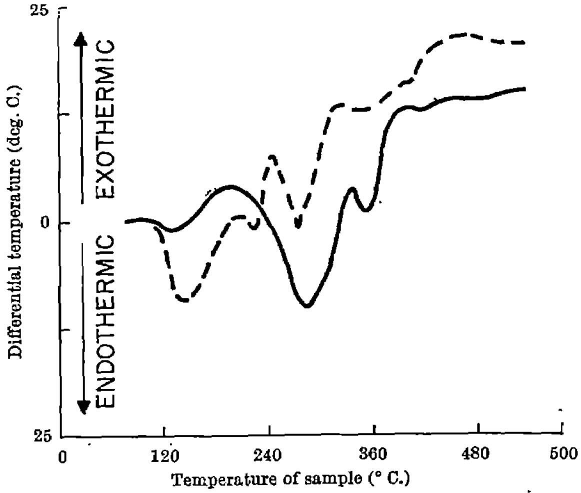


Fig. 2. Differential thermometric curves. – – –, Dipentene dioxide polymer (3·3 gm.); ——, vinyl*cyclo*hexene dioxide polymer (1·6 gm.)

Despite these shortcomings of differential thermometry as a tool for studying the pyrolysis of polymers, it can sometimes be very useful in revealing, or in clarifying, certain mechanisms of thermal degradation. For example, it has been shown by differential thermometry that isomerization of residual epoxy groups in certain epoxide polymers plays an important part in the decomposition mechanism[2]. It is therefore recommended that differential thermogravimetry be used to obtain quantitative information on the pyrolysis of polymers, especially highly cross-linked ones, and that differential thermometry be used as a qualitative adjunct.

HUGH C. ANDERSON

Non-Metallic Materials Division,
U.S. Naval Ordnance Laboratory,
White Oak, Silver Spring,
Maryland.

---

[1] Anderson, Hugh C., *J. App. Polymer Sci.* (in the press).

[2] Anderson, Hugh C., *Anal. Chem.*, **32**, 1592 (1960).

Ionization Potentials of *cis* and *trans* Dichloro- and Dibromo-ethylenes

THE ionization potentials of pure *cis* and *trans* dichloro- and dibromo-ethylenes have been obtained by electron impact and by photon impact methods[1]. Table 1 gives my results and those obtained by Walsh[2] from Rydberg series.

Table 1. IONIZATION POTENTIALS (IN EV.) OF *cis* AND *trans* DICHLORO- AND DIBROMO-ETHYLENES

Substances	Walsh's results	Electron impact method	Photon impact method
cis $C_2H_2Cl_2$	9·66	9·67 ± 0·05	9·66 ± 0·02
trans $C_2H_2Cl_2$	9·96	10·00 ± 0·05	9·64 ± 0·02
cis $C_2H_2Br_2$		9·69 ± 0·05	9·45 ± 0·01
trans $C_2H_2Br_2$		9·54 ± 0·05	9·46 ± 0·01

I have observed in the photon impact efficiency curves the presence of singular points which I believe correspond to vibrationally excited states of the molecular ion. An example of this is given here for *trans* dichloroethylene (Fig. 1).

The singular points are as follows: *cis* dichloroethylene (9·77 ± 0·02 eV.); *trans* dichloroethylene (9·81 ± 0·02; 9·97 ± 0·03 eV); *cis* dibromoethylene (9·62 ± 0·02; 9·79 ± 0·02 eV.); for the *trans* dibromoethylene no sharp point appears.

From the values of the ionization potentials of ethylene, methyl chloride, and ethyl chloride, which are respectively 10·51, 11·28 and 10·97 eV. (ref. 3), it must be concluded that the electron

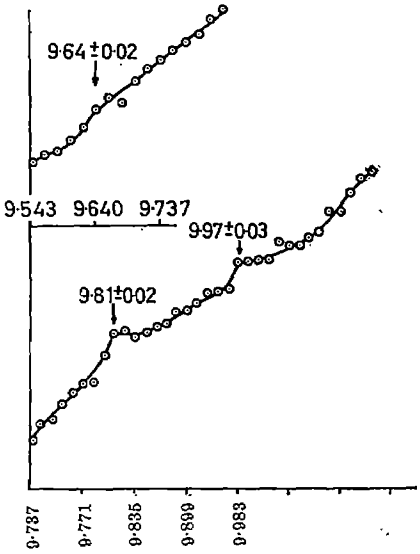


Fig. 1. Curve of photo-ionization efficiency (arbitrary scale) against photon energies (electron volts) for *trans* dichloroethylene

removed in the case of the dihalogenoethylenes is a πC=C electron. This and the order of magnitude of the jumps between two consecutive singular points indicate that the vibration involved is probably the C=C stretching vibration.

The electron impact and photon impact values are in rather good agreement for *cis* dichloro- and *trans* dibromo-ethylene, but an important discrepancy exists for *trans* dichloro- and *cis* dibromo-ethylene. From the vibrational separations of about 0·17 eV. observed for both substances, we conclude that the electron impact data correspond to the appearance of vibrationally excited states of the ion ($v=2$ for *trans* dichloroethylene and $v=1$ for *cis* dibromoethylene) and that the two other ions appear in the $v=0$ vibrational level for both photon and electron ionization.

Discrepancies of this nature between photon impact and electron impact ionization potentials are quite frequent and the interpretation given here seems to be of great generality. It conforms to the ideas of Mulliken[4], who considers photon impact potentials as "adiabatic" and electron impact potentials as "vertical" ones.

Further, from the equality of the formation enthalpies of the ions[1], it may be assumed that only one equilibrium configuration of the nuclei exists for the ion coming from a *cis* or from a *trans* isomer. This is in agreement with the theoretical work of Mulliken[5] on the $C_2H_4^+$ ion, the configuration of which he considers to be a crossed one.

By treating the dichloroethylene molecule and ion as diatomic vibrators with respect to the C=C bond, it may be shown that the results given here can be interpreted by assuming that the distance C=C is 0·035 Å. larger in the *cis* molecule and 0·065 Å. larger in its ion than in the corresponding *trans* dichloroethylene states.

I am indebted to the British Council and to the Patrimoine of the University of Liège for a grant, and to Prof. W. C. Price for permitting me to carry out the photo-ionization measurements in his laboratory at King's College, London. I wish to thank Prof. L. D'Or for the active interest he has shown in this work.

J. MOMIGNY

Faculty of Sciences,
University of Liège.

[1] Momigny, J., *Acad. Roy. Belg., Bull. Classe Sci.* (Nov. 1960).
[2] Walsh, A. D., *Trans. Farad. Soc.*, **41**, 35 (1945).
[3] Watanabe, K., *J. Chem. Phys.*, **26**, 1 (1957).
[4] Mulliken, R. S., *J. Chem. Phys.*, **1**, 491 (1933).
[5] Mulliken, R. S., and Roothaan, C., *Chem. Rev.*, **41**, 35 (1945).

Rapid Determination of Ytterbium in Aqueous Solutions and in Thulium Oxide by Neutron Activation

IT is known that when natural ytterbium is irradiated with neutrons, ytterbium isotope of 6 sec. half-life is formed[1], and that this emits γ-rays of 0·104 MeV. and of 0·212 MeV.

In the present work, a sample (0·07 ~ 0·3 gm.) is placed in the nuclear reactor *JRR*-1 for 6 sec. to be irradiated with neutrons at a flux of 3×10^{11} neutrons per cm.^{2}/sec. and then the decay of the mixture of activities produced is determined by use of a 2-in. thick, well-type sodium iodide crystal attached to a 256-channel pulse-height analyser. In each of successive γ-spectra obtained, the height of the photopeak of the 0·104-MeV. γ-ray from 6 sec. ytterbium isotope is measured, and the decay curve is made by plotting the log peak height against time. Identification was by the γ-energy and the half-life. A calibration curve was prepared by activating and counting ytterbium solutions containing known amounts ranging from 0·01 to 1 mgm. of ytterbium.

Table 1. RESULTS OF ANALYSES OF THULIUM OXIDE

Sample	Sample weight used (mgm.)	Ytterbium found (per cent)	Dysprosium found (p.p.m.)
I	122	0·057	5·6
	76	0·062	6·2
II	124	0·083	5·5
	70	0·075	6·0

In the analyses of an aqueous sample, the coefficient of variation of single analyses was 5 per cent, where the content of ytterbium was 0·064 per cent and eight aliquots was used each of which was 0·25 ml. in volume. Since interfering activities were practically negligible and activities produced were on a level very suitable to the counter used, this value is felt to be the approximate lower limit of the coefficient of variation in this method.

In thulium oxide samples the resolution of the decay curve showed two components: a 6-sec. and a 1·3-min. The latter was identified to be dysprosium-165*m*. Dysprosium was then determined quantitatively by use of a calibration curve made by the method similar to that for the case of ytterbium mentioned above. Results of analyses of thulium oxide samples as fine powder are shown in Table 1.

Since ytterbium found and dysprosium found in Table 1 are nearly independent of sample weight used, it is deduced that self-shielding for neutrons in these samples was negligible. It has also been deduced from decay curves that, when a thulium oxide powder contains 6 p.p.m. dysprosium, the lower limit of the detection of ytterbium is about 0·01 per cent.

By a similar method using a 0·212-MeV. photopeak instead of a 0·104-MeV. photopeak reliable analyses of decay curves could not be attained.

I am indebted to Prof. Shoji Makishima for helpful suggestions, to Dr. Toshio Nakai for permission to use laboratory facilities, and to Dr. Yuichiro Kamemoto for help in the γ-ray spectrometry.

MINORU OKADA

Government Chemical Industrial
Research Institute,
1-chome, Hon-machi, Shibuya-ku,
Tokyo.

[1] Strominger, D., Hollander, J. M., and Seaborg, G. T., *Rev. Mod. Phys.* **30**, 585 (1958).

Stability Constant of Copper(II) Complexes of Reduction Products of 2,2'-Dipyridyl

THE ligand 2,2'-dipyridyl (DP), its derivatives and analogues form an extremely interesting and important class of ligands. Their complexes possess some particular properties, presumably as a consequence of π-electron system of the ligand. To elucidate the role of the π-electron system, the study of complex-forming properties of the reduction product of 2,2'-dipyridyl was undertaken. Although we are at the very beginning of a systematic investigation, nevertheless, we achieved some results worthy of publication.

2,2′-Dipiperidyl (DPP) was prepared from DP by hydrogenation at 100° C. under 120 atmospheres pressure, using Raney-nickel catalyst. The yield exceeds 90 per cent. The boiling-point of DPP at 6 mm. of mercury pressure is 100–105° C. 2,2′-Pyridylpiperidyl (PPP) was prepared also from DP by tin-hydrochloric acid reduction; after the reduction PPP was extracted from the alkalized solution by chloroform. The yield is about 70 per cent, the boiling point 265–266° C.

The acidic dissociation constants were determined by potentiometric titrations: 10^{-2} mole/l. solutions of ligands were titrated with 1 mole/l. hydrochloric acid at 20° C.; the solutions were of 0·1 mole/l. for potassium chloride. The values of acidic dissociation constants are shown in Table 1. For comparison acidic dissociation constants of DP (ref. 1) are also given.

Table 1

	K_1	K_2	K_1K_2	K_1/K_2
DP	1·59	$4 \cdot 27 \times 10^{-5}$	$6 \cdot 77 \times 10^{-5}$	$3 \cdot 72 \times 10^{4}$
PPP	10^{-2}	$7 \cdot 95 \times 10^{-10}$	$7 \cdot 94 \times 10^{-12}$	$1 \cdot 26 \times 10^{7}$
DPP	10^{-7}	$6 \cdot 90 \times 10^{-11}$	$6 \cdot 90 \times 10^{-18}$	$1 \cdot 45 \times 10^{3}$

As can be seen, the basicity of nitrogens increases with hydrogenation. It is interesting that the ratio K_1/K_2 is nearly the same in the case of DP and DPP, while it differs strongly from PPP due to the inequality of nitrogens in this compound.

The stability constants of copper(II) complexes of DPP and PPP were determined also by potentiometric titrations under the same conditions as the acidic dissociation constants of ligands. The values are tabulated in Table 2.

Table 2

	K_1	K_2	K_1K_2
PPP	—	—	$1 \cdot 59 \times 10^{12}$
DPP	$3 \cdot 16 \times 10^{10}$	$2 \cdot 75 \times 10^{7}$	$8 \cdot 69 \times 10^{17}$

It is worth mentioning that the ratio K_1/K_2 is exactly the same as in the case of N,N'-dimethylethylenediamine copper(II) complex studied by Irving and Griffiths[2].

In the case of PPP the formation function does not show a step at $\bar{n} = 1$. So the product, K_1K_2 could only be calculated.

M. T. Beck
Institute for Inorganic and Analytical Chemistry,
M. Halmos
Institute for Organic Chemistry,
University of Szeged.

---

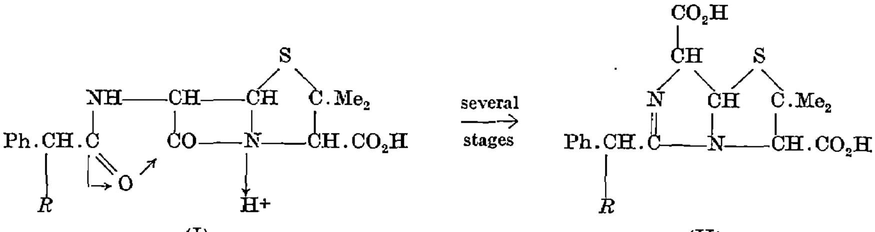
[1] Perkampus, H. H., and Köhler, H., *Z. Elektrochem.*, 64, 365 (1960).

[2] Irving, H., and Griffiths, J. M. M., *J. Chem. Soc.*, 213 (1954).

Some Novel Acid-stable Penicillins

Rapid inactivation of penicillins *F*, *G*, *K* and *X* in acid solution was demonstrated at an early date[1], and was widely assumed to be a general property until 1954, when phenoxymethylpenicillin (penicillin *V*) was shown to be relatively stable towards mineral acids[2]. The recent isolation in these Laboratories of 6-aminopenicillanic acid[3] permitted the synthesis of many hitherto inaccessible penicillins, including an extended series of acid-stable α-aryloxyalkylpenicillins[4] of which one, α-phenoxyethylpenicillin, is now in clinical use. Both penicillin *V* and its newer homologues are well absorbed from the gastro-intestinal tract and hence can be given by mouth, but they show useful antibacterial activity only against Gram-positive bacteria. Benzylpenicillin (penicillin *G*) has a broader antibacterial spectrum, but is not suitable for oral administration. We therefore prepared a series of α-substituted benzyl penicillins (I) in the hope of finding an acid-stable penicillin active against Gram-negative as well as Gram-positive bacteria.

The α-methoxy-, α-chloro-, and α-benzyloxycarbonylamino-compounds were prepared by treating 6-aminopenicillanic acid in aqueous sodium bicarbonate with an acetone solution of either the chloride or the mixed ethoxyformic anhydride of the appropriate α-substituted phenylacetic acid. The resulting penicillins were extracted from the acidified reaction mixture into ether, then re-extracted into aqueous sodium bicarbonate and freed from water by evaporation at low temperature and pressure. Hydrogenation of an aqueous solution of the sodium salt of α-benzyloxycarbonylaminobenzylpenicillin (I; *R* = $NH.CO_2.CH_2Ph$) over a palladium catalyst gave α-aminobenzylpenicillin[5].



(I) (II)

Specimens (50 mgm.) of each of the penicillins (I; *R* = H, OMe, Cl, and NH_2) were added to a mixture of 0·2 *N* aqueous hydrochloric acid (25 ml.) and ethanol (25 ml.) maintained at 35° C. and aliquot portions of each solution were withdrawn at intervals and the penicillin content determined by colorimetric assay with hydroxylamine[6]. The half-life of each penicillin was then read from the straight-line plot of log concentration against time. The results shown in Table 1 indicate that α-methoxy-, α-chloro-, and α-amino-benzylpenicillin are all much more stable to acid than is benzyl penicillin itself, while the chloro- and amino-compounds are also more stable than penicillin *V* (half-life 160 min.).

Table 1

	R = H	*R* = OMe	*R* = Cl	$R = NH_3^+$
Half-life of penicillin (I) in 50 per cent aq. EtOH at *p*H 1·3 and 35° C.	3·5 min.	77 min.	300 min.	660 min.
pKa of side-chain acid, $\mathrm{Ph.\underset{R}{CH}.COH}$, in 50 per cent aq. EtOH	5·6	4·6	4·0	3·2

Evidently benzylpenicillin can be rendered more stable to acids by the introduction into the α-position of the side-chain of electron-attracting substituents which might be expected to hinder the electron displacements initiating rearrangement to the isomeric biologically inactive penillic acid (II)[7]. Such stability would be expected to vary with the inductive effect of the substituent and hence to be roughly proportional to the strength of the side-chain acid from which the penicillin is derived. Support for this idea was obtained by potentiometric titration of the appropriate α-substituted phenylacetic acids against standard alkali in 50 per cent aqueous ethanol. Correlation of the *pKa* values calculated by the half-neutralization method with the acid half-lives of the corresponding penicillins is shown in Table 1.

As well as being the best of the new penicillins from the point of view of stability to acid, α-aminobenzylpenicillin also showed useful activity against a wide range of Gram-positive and Gram-negative bacteria both *in vitro*[8] and *in vivo*[9] and was well absorbed from the gastro-intestinal tract[9]. The epimer derived from D(–)α-aminophenylacetic acid was more active against most organisms than that from the L(+)-acid. Details of the preparation of it and some of its analogues will be published elsewhere.

We thank Dr. G. N. Rolinson *et al.* and Mr. D. M. Brown *et al.* for the bacteriological and chemotherapeutic testing of the penicillins and Mr. D. Neal for experimental assistance.

F. P. DOYLE
J. H. C. NAYLER
H. SMITH
E. R. STOVE

Organic Chemistry Department,
Beecham Research Laboratories, Ltd.,
Brockham Park,
Betchworth,
Surrey.

[1] Benedict, R. G., Schmidt, W. H., and Coghill, R. D., *J. Bact.*, **51**, 291 (1946); cf. Clutterbuck, P. W., Lovell, R., and Raistrick, H., *Biochem. J.*, **26**, 1907 (1932).

[2] Brandl, E., and Margreiter, H., *Oesterr. Chem. Z.*, **55**, 11 (1954).

[3] Batchelor, F. R., Doyle, F. P., Nayler, J. H. C., and Rolinson, G. N., *Nature*, **183**, 257 (1959). Doyle, F. P., Nayler, J. H. C., and Rolinson, G. N., Brit. Pat. Spec. No. 870,396 (1961).

[4] Perron, Y. G., Minor, W. F., Holdrege, C. T., Gottstein, W. J., Godfrey, J. C., Crast, L. B., Babel, R. B., and Cheney, L. C., *J. Amer. Chem. Soc.*, **82**, 3934 (1960).

[5] Doyle, F. P., Nayler, J. H. C., and Smith, H., Brit. Pat. Spec. No. 873,049 (1961).

[6] Ford, J. H., *Indust. Eng. Chem.*, Anal. Ed., **19**, 1004 (1947).

[7] Johnson, J. R., Woodward, R. B., and Robinson, R., in *The Chemistry of Penicillin*, 445 (Princeton Univ. Press, 1949). Abraham, E. P., *Giornale di Microbiologica*, **2**, 102 (1956).

[8] Rolinson, G. N., and Stevens, S., *Brit. Med. J.*, ii, 191 (1961).

[9] Brown, D. M., and Acred, P., *Brit. Med. J.*, ii, 197 (1961).

Crystallization of Isotactic Polypropylene using a Light Depolarization Technique

THE intensity of depolarized light transmitted by a thin film of isotactic polypropylene, crystallizing isothermally on a polarizing microscope hot stage was followed using a photomultiplier unit with an appropriate recorder system[1,2]. For the slower rates of crystallization, the cathode ray oscillograph–camera arrangement was replaced by a Honeywell Brown m.V. recorder. All measurements were made within the linear operating range of the equipment.

Dried sections (150–200μ) of unpigmented polymer chip of intrinsic viscosity 2·7 dl./gm., measured in tetralin at 135° C., were mounted between cover slips using *MS* 550 silicone oil as an immersion medium to reduce oxidation of the sample during melting. Specimens were melted at 270 ± 3° C. for 30 min. followed by rapid transfer (< 1 sec.) to the microscope hot stage maintained to within 0·5° C. of the desired crystallization temperature. Typical sigmoidal curves of the change in photomultiplier output with time were obtained and used as a measure of the extent of the crystallization. Results were fitted to Avrami equations after expressing them as a proportional change (I_c–I_t) relative to the overall primary change (I_c–I_0) associated with any isothermal[2]. I_c, I_t and I_0 are respectively equal to the final, intermediate and initial photomultiplier outputs. For the lower crystallization temperatures (< *c.* 80° C.) secondary or 'after' crystallization behaviour is frequently pronounced.

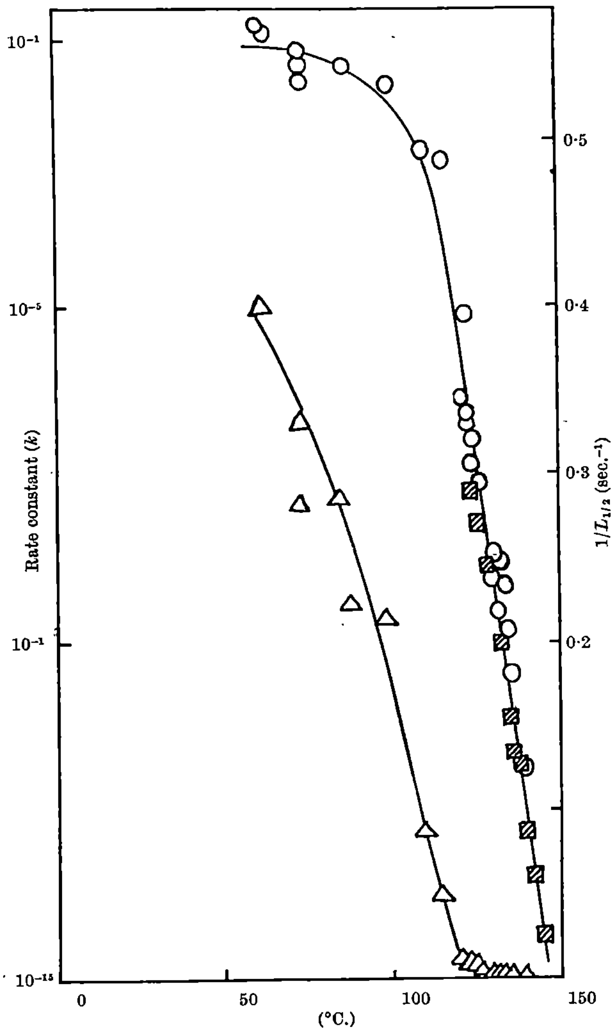


Fig. 1. Crystallization parameters for isotactic polypropylene polymer. Triangles, reciprocal of the half-times of crystallization temperatures (this work); circles, rate constants versus crystallization temperatures (this work); below and at 115° C. K is expressed in sec.$^{-2}$; above 115° C. K is expressed in sec.$^{-3}$

A change in the mechanism of nucleation occurs in the region of 115° C. Above this crystallization temperature spherulitic growth occurs from predetermined or simultaneous nuclei, while below 115° C. the structures formed are granular, and birefringent and nucleation is sporadic. The rate constants calculated from light depolarization isothermals (Fig. 1) agree well with published values derived from dilatometric measurements[3] for an isotactic polypropylene of similar intrinsic viscosity. The rate measurements presented here establish the utility of the technique for studying crystallization behaviour in polymers, and also its applicability over a wider temperature-range than most other established methods. Strain birefringence limits the use of the method at large degrees of supercooling where poor heat transfer is also significant[2].

J. H. Magill

Research Department,
British Nylon Spinners, Ltd.,
Pontypool, Mon.

[1] Magill, J. H., *Nature*, 187, 770 (1960).
[2] Magill, J. H., *Polymer*, 2, 921 (1961).
[3] Marker, L., Hay, P. M., Tilley, G. P., Early, R. M., and Sweeting, O. J., *J. Polymer Sci.*, 38, 33 (1959).

BIOCHEMISTRY

Synthesis of Succinate from Acetate

In a previous communication[1], evidence was presented that an enzyme system, isolated from pig heart, catalysed the oxidation of acetate to succinate. Aconitase, assayed by the method of Racker[2], was not detectable and the addition of malate to the incubation mixture did not increase the incorporation of acetate into succinate. Consequently, the formation of succinate was attributed to a Thunberg condensation and the slow rate (7 mμmoles/hr./mgm. protein) was explained by the unfavourable equilibrium[3].

This explanation has been tested by attempting to measure the formation of acetate-^{14}C from succinate-2.3-^{14}C with preparations capable of incorporating acetate-^{14}C into succinate. Such experiments failed to demonstrate any significant formation of acetate, acetyl co-enzyme A or acetyl phosphate (when a preparation of transacetylase was added to the incubation mixture). These results indicate the absence of the Thunberg reaction and suggest that succinate is formed via the established reactions of the Krebs cycle—the slow rate being due to the extremely low activity of aconitase. Support for this view comes from the finding (Dr. T. M. Sutherland, private communication) that the incorporation of acetate into succinate disappears after dialysis of the enzyme system, but is restored by the addition of oxaloacetate.

It thus appears that the interpretation of the data in terms of a Thunberg condensation, for which I was solely responsible, was erroneous.

D. D. Davies

Botany School,
University of Sydney.

[1] Davies, D. D., *Nature*, 181, 339 (1958).
[2] Racker, E., *Biochim. Biophys. Acta*, 4, 211 (1950).
[3] Seaman, G. R., *J. Biol. Chem.*, 228, 149 (1957).

Conversion of Fibrin to Desmofibrin

In experiments on fibrin stabilization great difficulty has been encountered in obtaining a substrate free from fibrin-stabilizing factor (FSF)[1-5].

Such a substrate can be obtained from a fibrinogen preparation made by various methods, for example, from Kekwick's preparation[6] or, from oxalated plasma clotted with citrate thrombin[7]. From a clot mechanically released from a solvent or serum, a 2–4 per cent fibrin solution in urea solution at *p*H 5·2 (20 gm. urea in 100 ml. acetic buffer adjusted to *p*H 5·2 with acetic acid) is prepared.

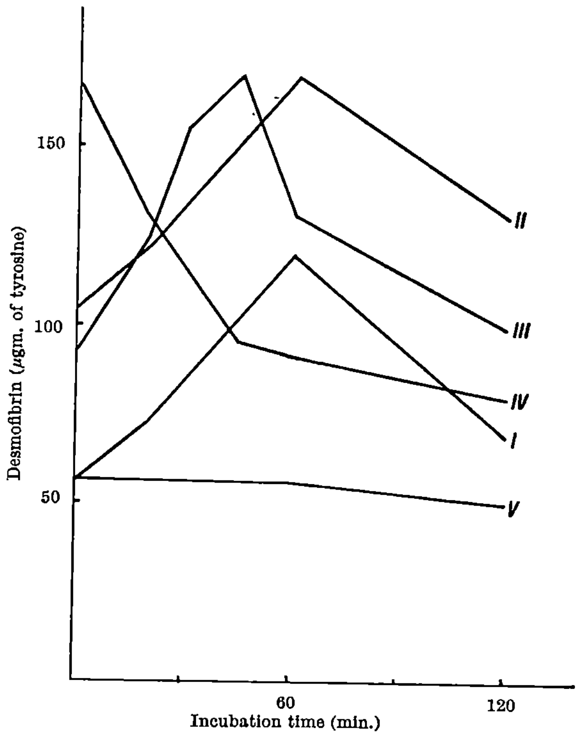


Fig. 1. Thrombinic activation of fibrin stabilizing factor in the presence of calcium. Incubated mixture: thrombin + FSF + calcium chloride. Curves I, II, V, 0·05 unit thrombin/ml.; curve III, 0·1 unit thrombin/ml.; curve IV, 1·0 unit thrombin/ml.; curves I, III, IV, V, 20 min.; curve II, 45 min. reaction of fibrin with incubated mixture; curve V, incubated mixture: thrombin + FSF without calcium chloride (calcium chloride was added just before mixing with fibrin monomer)

A fibrin solution from which the precipitates, regarded as being denatured, had been removed by centrifugation, was diluted 25–50 times in Palitzsch buffer solution[8] at *p*H 7·6, in which a fibrin network was immediately formed. This procedure of dissolving and repolymerizing of fibrin was repeated ten times.

Traces of thrombin could be identified in the diluent after the third repolymerization and removal of the clot. In subsequent operations, it was not possible to demonstrate the presence of thrombin, though it is known to be absorbed on the fibrin[9].

The product finally obtained is considered to be a fibrin monomer and it possesses the following properties: it clots immediately after dilution and neutralization, the time of polymerization depends on the concentration of salt and is lengthened if distilled water is used for dilution, the addition of thrombin has no effect on polymerization-time, provided that the concentrations of salt are constant.

The clot, that is, the fibrin polymer obtained from the monomer described here, is soluble in urea solution and in mono-chloracetic acid even when thrombin and calcium are added.

Stabilized fibrin polymer or desmofibrin is obtained by adding FSF, thrombin and calcium.

The fibrin polymer stabilization time and the amount of desmofibrin obtained in a definite period of time (for example, 20 min.) are proportional to the amounts of FSF added. With a constant FSF fibrin polymer, stabilization time and the amount of desmofibrin formed depend on the amount of thrombin added.

The experiment shown in Fig. 1 was carried out in two stages. In the first stage, three test-tubes containing a buffer solution at pH 7·6, FSF derived from platelets[10], and calcium in equal concentrations with citrate thrombin[7], the activity of which was, in the first test-tube 0·05 unit/ml., in the second, 0·1 unit/ml., and in the third 1 unit/ml. of the mixture, were incubated. At various intervals during incubation the incubated mixture was added to prepared test-tubes containing fibrin monomer.

In the second stage at various intervals after mixing the fibrin monomer with the incubated mixture the reaction was interrupted by adding mono-chloracetic acid and the amount of desmofibrin contained in the tubes was measured[11].

The experiment shows that during the reaction with FSF, when calcium and thrombin 0·05 unit/ml. and 0·1 unit/ml. are present, the fibrin-stabilizing activity increases and, during further incubation, it decreases (curves I, II, III).

Where 1 unit/ml. of thrombin is present, the high fibrin-stabilizing activity of the incubated thrombin, FSF and calcium is observed at the beginning of incubation (curve IV).

On comparing curves I and II, it will be seen that where 0·05 unit/ml. of thrombin is present, the peak of fibrin-stabilizing activity is observed 60 min. after incubation of thrombin, calcium and FSF. The conversion of fibrin polymer into desmofibrin takes place gradually, since after a 20-min. reaction of the incubated mixture with the fibrin polymer (curve I) it contains 120γ, after 45 min. (curve II) fibrin polymer contains 170γ of tyrosine expressed as the equivalent of desmofibrin.

During the incubation of thrombin and FSF without calcium, the fibrin-stabilizing activity does not increase (curve V). Stabilization of the fibrin polymer with this mixture and the addition of calcium correspond at the onset of incubation to that of the control in which all elements required in order to obtain desmofibrin are mixed simultaneously and, after further incubation, the stabilizing activity of the incubated mixture is lower than that of the control.

The experiment indicates that the stabilized fibrin, that is, desmofibrin, is a product of two-step reaction: in the first step thrombin acting on fibrinogen, not necessarily in the presence of calcium, produces the fibrin and by acting on FSF in presence of calcium thrombin, produces an active fibrin-stabilizing factor.

In the second step, during reaction of fibrin with active fibrin-stabilizing factor, desmofibrin is formed.

The problem of the significance of calcium during the reaction of fibrin polymer with active fibrin-stabilizing factor has not yet been elucidated. Active fibrin-stabilizing factor created during the time of reaction with thrombin in the presence of calcium, after addition of sodium citrate, results in desmofibrin during the reaction with fibrin polymer.

With the same conditions, but with potassium oxalate added to the active fibrin-stabilizing factor, desmofibrin is not produced in the reaction with fibrin polymer.

The two-step formation of desmofibrin is as follows:

First step: (a) fibrinogen + thrombin → fibrin

$$(b)\ \text{FSF} + \xrightarrow[\text{Ca}^{++}]{\text{thrombin}} \text{active fibrin-stabilizing factor}$$

Second step:

$$\text{fibrin} + \xrightarrow[\text{Ca}^{++}\,(?)]{\text{active fibrin-stabilizing factor}} \text{desmofibrin}$$

The fact that the formula given above differs from other known findings[12] can be explained as being the result of using fibrinogen preparations containing FSF instead of the sol of fibrin monomer free from fibrin-stabilizing factor, for work on fibrin-stabilization.

K. BULÚK
T. JANUSZKO
J. OLBROMSKI

Department of General and
Experimental Pathology,
School of Medicine,
Białystok, Poland.

[1] Laki, K., and Lorand, L., *Science*, **108**, 280 (1948).
[2] Ferry, J. D., Miller, M., and Shulman, S., *Arch. Biochem. Biophys.*, **34**, 424 (1951).
[3] Loewy, A. G., and Edsall, J. T., *J. Biol. Chem.*, **211**, 829 (1954).
[4] Lorand, L., and Dickenman, R. C., *Proc. Soc. Exp. Biol. and Med.*, **89**, 45 (1955).
[5] Buluk, K., and Małofiejew, M., *Roczn. A.M. w Bialymstoku*, **1**, 159 (1955).
[6] Kekwick, R. A., Mackay, M. E., Nance, M. H., and Record, B. R., *Biochem. J.*, **60**, 671 (1955).
[7] Kowarzyk, H., Rechnic. M., Siwińska, M., and Czerwińska, B., *Arch. Immunol. i Terapii Dośw.*, **6**, 475 (1958).
[8] Palitzsch, S., *Biochem. Z.*, **70**, 341 (1915).
[9] Landaburu, R. H., and Seegers, W. H., *Amer. J. Physiol.*, **198**, 173 (1960).
[10] Buluk, K., *Pol. Tyg. Lek.*, **10**, 191 (1955).
[11] Buluk, K., *Roczn. A.M. w Bialymstoku*, **3**, 17 (1958).
[12] Lorand, L., and Jacobsen, A., *J. Biol. Chem.*, **230**, 421 (1958).

Catalysis of Incorporation of ^{14}C-Uridine into Rat Lens Ribonucleic Acid by Rat Lens Enzyme System

MAMMALIAN lenses may now be considered to be centres of the active metabolism of protein on the basis of the recent findings of Devi, Lerman and Sarkar[1] on the existence in rat lens of a very active system which catalyses the incorporation of amino-acids into lens ribonucleic acid (RNA) and proteins, and on the basis of the occurrence of many proteases in rat lens as demonstrated by Zeller and Devi[2] and Devi[3,4]. The relatively high concentration of RNA in rat lens as reported by Dische and Devi[5] and its key role in protein synthesis as shown by various investigators[6,7] has stimulated us to examine whether or not there also exists in rat lens any system capable of synthesizing RNA.

Rats 6–8 weeks old and weighing approximately 100–120 gm. each were given intraperitoneal injections of 0·2 ml. of 2-^{14}C-uridine (specific activity $2·2 \times 10^6$ c.p.m.) prepared in normal saline. The animals were killed at different time-intervals after injection. The lenses from each rat were immediately removed and homogenized in 1 ml. of 0·25 M sucrose in the cold and to the latter 4 ml. of 5 per cent cold trichloracetic acid (TCA) were added. The TCA-insoluble material was afterwards washed three times with cold 5 per cent TCA, once with alcohol, once with methanol/chloroform/ether (2 : 1 : 1 proportion) mixture at 50° C. and then twice with ether. The TCA-insoluble, lipid-free material was plated and assayed for radioactivity as previously described by Devi, Lerman and Sarkar[1] in the gas-flow windowless counter with an efficiency of 30 per cent.

In experiments where the incorporation of ^{14}C-uridine into RNA was studied *in vitro*, the lenses of 10 such rats were pooled and homogenized at 0° C. in 10 ml. of cold 0·25 M sucrose medium containing 0·025 M potassium chloride, 0·035 M potassium bicarbonate and 0·004 magnesium chloride[8]. The reaction mixture

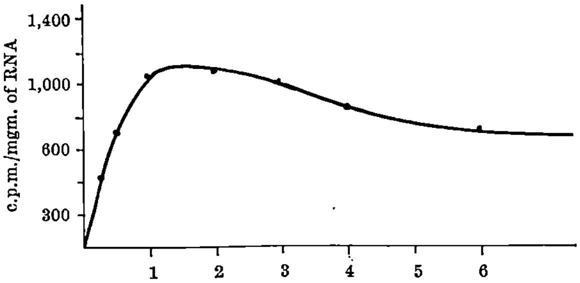


Fig. 1. Carbon-14 uridine incorporated into rat lens RNA (*in vivo*)

containing 0·5 ml. lens homogenate, 2 µmoles of adenosine triphosphate (ATP), 2 µmoles of Mg^{++}, 20 µmoles of *tris* buffer, *p*H 8·0, and 0·05 ml. 2-^{14}C-uridine, specific activity 0·55 × 10^6 c.p.m. in a total volume of 1 ml. was incubated for 30 min. at 37° C. The reaction was terminated by adding 4 ml. of cold 5 per cent TCA. The procedures for washing, plating, and counting were similar to those described above. In some experiments the insoluble material washed three times with TCA was heated in 5 per cent TCA at 90° for 15 min. and then centrifuged. 1 ml. of the TCA-extract obtained was dried and afterwards assayed for radioactivity. ^{14}C-uridine, when injected into rats, appreciable counts can be recorded in TCA-insoluble material. The incorporation reaches the peak within 60–90 min.; then very slowly and gradually declines. Even 24–48 hr. after injection, TCA-insoluble material shows 60–70 per cent of the radioactivity noted at 60–90 min. These results are shown in Fig. 1.

The ability of the lens homogenate to catalyse the incorporation of ^{14}C-uridine into lens RNA under various experimental conditions is shown in Table 1. This system requires ATP and is dependent on Mg^{++}. Omission of any one of the components from the reaction mixture results in a poor incorporation of ^{14}C-uridine into RNA. Incubation of the homogenate with (50 µgm./ml.) crystalline pancreatic ribonuclease (RNase) (Worthington) for 30 min. prior to the addition of ATP, Mg^{++}, ^{14}C-uridine and *tris* buffer renders the system inactive. If, on the other hand, the lens homogenate is first treated with 50 units of venom phosphodiesterase (Biochemical Research) and then incubated with the other components of the reaction mixture, the extent of uridine incorporation into RNA remains practically the same.

The lens homogenate, on exhaustive dialysis at 2–4° C. against 0·02 *M tris* buffer *p*H 7·4 containing 0·01 *M* ethylenediamine tetraacetate (EDTA), becomes inactive in catalysing the incoporation of ^{14}C-uridine into RNA, but the activity can be restored by adding Mg^{++} (Table 2). Mn^{++} can replace Mg^{++} to the extent of 70–80 per cent and Co^{++} to the extent of 35–40 per cent, while other divalent metallic ions such as Zn^{++}, Ca^{++} and Fe^{++} are comparatively poor activators. These metals do not, however, exert any inhibitory effect as the extent of incorporation of carbon-14 into RNA remains the same in the presence of these metals provided Mg^{++} is also present. All these divalent metallic ions begin to exhibit an inhibitory effect when their concentration is raised above 10 µmoles in the test system. It should be of some interest to recall and compare the remarkable effect of Co^{++} in promoting the incorporation of amino-acids into lens RNA and proteins and the strong inhibition of the Co^{++} effect exerted by Ni^{++} (ref. 1) with the effect these metals have on this system which lends support to the conception that Co^{++} is highly specific in protein synthesis.

Table 1. INCORPORATION OF ^{14}C-URIDINE INTO RAT LENS RNA (*in vitro*)

Components of the reaction mixture	c.p.m./mgm. of RNA
Complete system	11,650
Complete system − Mg^{++}	2,060
Complete system − ATP	4,625
Complete system + RNase (50 µgm./ml.)	2,850
Complete system + venom phosphodiesterase (50 units/ml.)	9,210

The complete system contained 0·5 ml. of dialysed rat lens homogenate, 2 µmoles of ATP, 2 µmoles of Mg^{++}, 20 µmoles of *tris*, *p*H 8·0 and 0·05 ml. of ^{14}C-uridine, specific activity 0·55 × 10^6 c.p.m.

Table 2. EFFECT OF METALLIC IONS ON THE INCORPORATION OF ^{14}C-URIDINE INTO RAT LENS RNA

Metals	c.p.m./mgm. of RNA	
(Mg^{++}, 2 µmoles, other metals, 5 µmoles)		
1. Lens homogenate + ATP + Mg^{++}	11,680	
2. Lens homogenate + ATP + Ca^{++}	2,250	
+ Mg^{++}		11,025
3. Lens homogenate + ATP + Fe^{++}	2,040	
+ Mg^{++}		10,965
4. Lens homogenate + ATP + Co^{++}	4,080	
+ Mg^{++}		12,005
5. Lens homogenate + ATP + Mn^{++}	9,294	
+ Mg^{++}		12,608
6. Lens homogenate + ATP + Zn^{++}	2,060	
+ Mg^{++}		10,985

The addition of chloramphenicol (50–100 µgm./ml.) does not block the incorporation of ^{14}C-uridine into RNA; but the addition of 5-bromouracil, 5-fluorouracil or 2-thiouracil (at a level of 40–50 µgm./ml.) inhibits the incorporation only to the extent of 25–35 per cent. No further inhibition is noted with higher concentration of these analogues of this pyrimidine. The purine analogues such as 8-azoguanine and 8-azoadenine cannot inhibit the incorporation of uridine into RNA more than 20–30 per cent.

These results clearly demonstrate the existence of an active system in rat lens capable of synthesizing RNA. Previously it was possible to demonstrate the presence of an active protein-synthesizing system in rat lens[1]. These results seem to be consistent with the assumption that in rat lens and also in lenses of other species of animals metabolism of proteins and nucleic acids is an active dynamic process.

ANIMA DEVI
N. K. SARKAR

Department of Medicine,
College of Physicians and Surgeons,
Columbia University,
620 W., 168th Street, New York City
and Biochemistry Department,
Laval University, Quebec City, Canada.

[1] Devi, Anima, Lerman, Sidney, and Sarkar, Nirmal, *Nature*, **190**, 1193 (1961).

[2] Zeller, E. A., and Devi, Anima, *Amer. J. Ophthal.*, **44**, Part 2, 281 (1957).

[3] Devi, Anima, *Amer. J. Ophthal.*, **48**, Part 2, 29 (1959).

[4] Devi, Anima, *Amer. J. Ophthal.*, **51**, Part 2, 145 (1961).

[5] Dische, Z., and Devi, Anima, *Amer. J. Ophthal.*, **51**, Part 2, 132 (1961).

[6] Brachet, J., in *The Nucleic Acids*, edit. by Chargaff, C., and Davidson J. N., 2, 476 (Academic Press, New York.)

[7] Caspersson, T., *Symposia Soc. Exp. Soc.*, 1, 127 (1947).

[8] Hogland, M. B., Stephenson, M. L., Scott, J. F., Hecht, L. I., and Zamecnik, P. C., *J. Biol. Chem.*, 231, 241 (1958).

Serum Lipids in Relation to the Intake of Alcohol and Sodium Chloride

THE question whether variation in the intake of alcohol or sodium chloride has any marked influence on serum lipids is of considerable interest in human pathology. Whereas several investigators have studied the long-term influence of these factors, especially on vascular changes, relatively few reports have been

TABLE 1. SERUM LIPIDS IN RELATION TO INGESTION OF ETHYL ALCOHOL

Patient, age and sex			Diagnosis	Equiv. dose of 100 per cent alcohol (gm.)	Time	Total lipid (mgm./100 ml.)	Cholesterol (mgm./100 ml.)	Phospholipids (mgm./100 ml.)	Blood alcohol (parts/1,000)
A.J.	86	*F*	Cerebral arterio-sclerosis	45	8.00	840	299	340	
					9.00	855	297	331	1·53
					12.00	815	289	330	0·68
					14.00	855	292	321	0·29
P.T.	84	*M*	Bronchitis	45	8.00	695	241	269	
					9.00	705	246	279	0·91
					12.00	715	244	253	0·32
					14.00	710	248	262	0·00
B.L.	30	*M*	Normal	45	8.00	525	188	192	
					9.30	525	186	178	0·45
					12.30	620	223	229	0·00
J.A.	82	*M*	Coronary sclerosis	67·5	8.00	780	284	283	
					9.00	690	255	255	1·30
					12.00	650	239	272	0·57
					14.00	770	263	289	0·24
J.J.	71	*M*	Hypertrophy of the prostate	45	8.00	625	205	240	
					9.15	670	205	253	1·09
					12.00	690	207	259	0·70
					14.00	685	221	263	0·42
C.J.	72	*M*	Coronary sclerosis	45	8.00	495	181	208	
					9.00	555	199	215	0·78
					12.00	465	170	201	0·26
					14.00	455	187	223	0·00
C.J.	72	*M*	Coronary sclerosis	45	7.50	470	168	168	
					9.30	340	141	149	0·67
					12.00	420	158	168	0·17

TABLE 2. SERUM LIPIDS IN RELATION TO LOW- AND HIGH-INTAKE OF SODIUM CHLORIDE

Patient, age and sex			Diagnosis	Sodium chloride (gm./day)	Date of beginning and and end of regimen	Date of blood sample	Total lipid (mgm./100 ml.)	Serum: Total cholesterol (mgm./100 ml.)	Phospho-lipids (mgm./600 ml.)
N.C.	69	*M*	Senile dementia	Normal	–Nov. 15	Nov. 13	650	270	235
				0·5	Nov. 16–Nov. 29	Nov. 20	670	259	248
						Nov. 27	645	265	256
				8	Nov. 30–Dec. 13	Dec. 4	555	238	213
						Dec. 11	625	252	221
				Normal	Dec. 12–	Dec. 18	590	244	215
K.S.	74	*M*	Sequelæ hæmorrh. cerebri	Normal	–Nov. 15	Nov. 10	515	199	214
				0·5	Nov. 16–Nov. 29	Nov. 17	505	180	206
						Nov. 24	535	173	196
				8	Nov. 30–Dec. 13	Dec. 1	580	188	214
						Dec. 8	470	187	210
				Normal	Dec. 14–	Dec. 15	495	165	173
V.K.	79	*M*	Hypertension	Normal	–Nov. 22	Nov. 20	520	167	176
				0·5	Nov. 23–Dec. 6	Nov. 27	550	197	207
						Dec. 4	570	205	223
				8	Dec. 7–Dec. 20	Dec. 11	630	213	215
						Dec. 18	580	222	229
				Normal	Dec. 21–	Jan. 5	520	186	214
T.N.	89	*F*	Arteriosclerosis	Normal	–Nov. 22	Nov. 17	575	202	249
				0·5	Nov. 23–Dec. 6	Nov. 24	620	229	225
						Dec. 1	575	189	192
				8	Dec. 7–Dec. 20	Dec. 8	515	196	218
						Dec. 15	570	202	222
				Normal	Dec. 21–	Jan. 5	520	186	216

published on the variations in serum lipids caused by a single dose of alcohol and by short periods of high or low intake of sodium chloride. The present communication deals with some experiments aimed at elucidating these problems.

Experiments with alcohol. Six persons participated in this experiment : 1 normal person, 30 years of age, and 5 geriatric patients. One dose (100–150 ml.) of brandy (45 per cent v/v) was given at 8 a.m. to the fasting person together with 4 plain biscuits (24 gm.). Blood samples were taken immediately before and 1, 4, and 6 hr. after. Total lipids, cholesterol and phospholipids were determined in all the samples. Alcohol was determined in the samples after dosing, determinations being carried out at the Institute of Forensic Medicine, University of Copenhagen, by courtesy of Prof. H. Gormsen.

Furthermore, in all samples prothrombin-proconvertin, serum transaminase, alkaline phosphatases and icterus index were determined, and thymol- and Takata-Ara tests were carried out. The liver function as judged by these examinations was normal and did not show any variation during the experiment.

The results of the serum lipid and alcohol determinations are presented in Table 1. In no case were significant changes in total lipids, total cholesterol and phospholipids observed after the ingestion of ethanol in the quantities used. This seems to be in agreement with observations of Grande *et al.*[1], who did not find any significant increase in serum cholesterol when 0·45 gm. ethanol/kgm./day was given for three weeks, and an increase of only 18 mgm. cholesterol per 100 ml. serum when 1·35 gm./kgm./day was given for one week. Under quite different conditions, namely acute alcoholism with damage to the liver, Epstein and Greenspan[2] found a reduction of the cholesterol esters in blood plasma.

Experiments with sodium chloride. Four geriatric patients participated in this experiment. They were given a diet low in sodium chloride (less than 0·5 gm. sodium chloride/day) for two weeks and thereafter the usual hospital food (5 gm. sodium chloride/day) plus 3 gm. sodium chloride in tablets/day for the following two weeks.

Serum total lipids, total cholesterol and phospholipids were determined once before the experiment, twice during each experimental period, and once after the experiment. The results were presented in Table 2. The values for total lipid, total cholesterol and phospholipids did not change significantly from one period to the other. This finding does not necessarily contradict the possibility that a diet rich in sodium chloride may contribute to the development of atheromatosis, as has been claimed, for example, in rats[3].

ERLING LUND
TORBEN GEILL

Geriatric Unit,
Old Peoples Town,
Copenhagen.

HENRIK DAM
GUNHILD KRISTENSEN

Department of Biochemistry and
Nutrition,
Polytechnic Institute,
Copenhagen.

[1] Grande, F., Hay, L. J., Heupel, H. W., and Amatuzio, D. S., *Circ. Res.*, **8**, 810 (1960).

[2] Epstein, E. Z., and Greenspan, E. B., *Arch. Intern. Med.*, **58**, 860 (1936).

[3] de Langen, C. D., *Symposium on Arteriosclerosis*, 352 (Benno Schwabe and Co., Basel and Stuttgart, 1957).

Eucollagen

As protein chemistry becomes more complex and large units become molecularly subdivided, new terms are required for adequate description. Prefixes such as α-, β-, pseudo- are well known. In collagen research, terms have been invented to cover new concepts, such as Orekhovich's procollagen[1], Banga and Balo's metacollagen[2] and the tropocollagen units of Schmidt, Gross and Highberger[3].

None of these terms was adaptable to the highly modified forms of collagen described by me[4], and so the term 'eucollagen' was used[5] in this connexion.

The *Shorter Oxford English Dictionary* describes 'Eu' as a prefix representative of Greek, used mostly in words of Greek derivation. ευ κόλλα λεν is derived wholly from the Greek and clearly means 'easily producing glue'. This is an exact property entering into the definition and the term was chosen for this precise reason.

Unaware, at the time, of the original publication[5] on eucollagen and of subsequent references[6] to this term by Kelly, Little and Courts; Reed and Stainsby Crosby and Stainsby; and by Tristram, a second interpretation of eucollagen was used by [the late] W. T. Astbury[7] to name a hypothetical limiting structure for collagen, in which glycine occurs at intervals of three residues and hydroxyproline at intervals of ten residues. This unit is considered to be responsible for the 28·6 Å. X-ray repeat.

So far as the modified collagen[5] is concerned, many of the properties suggest a retention of the collagen helical configuration but absence of segmented spacings. A substantial reduction in molecular chain weight to parent gelatin size occurs. The hydrothermal stability falls to a minimum value and the material undergoes transition to a random coil structure entirely by a hydrogen bond-breaking mechanism. It is suggested, therefore, that use of the term 'eucollagen' for such material is both etymologically and scientifically in order.

A. COURTS

British Gelatine and Glue Research Association,
2a Dalmeny Avenue,
London, N.7.

[1] Orekhovich, V. N., *Second Intern. Congr. Biochem. Comm.*, 106 (1952).

[2] Banga, I., and Balo, J., *Connective Tissue*, 254 (Blackwell, 1957).

[3] Schmidt, F. O., Gross, J., and Highberger, J. H., *Proc. U.S. Nat. Acad. Sci.*, **39**, 459 (1953).

[4] Courts, A., *Nature*, **178**, 405 (1956); **182**, 439 (1958).

[5] Courts, A., *Biochem. J.*, **74**, 238 (1960).

[6] Little, Kelly and Courts, *Lancet*, 1125 (1959). Reed and Stainsby, *J. Sci. and Indust. Res.*, A, 30, 132 (1961). Crosby and Stainsby, *Nature*, **190**, 80 (1961). Tristram, *Nature*, **187**, 998 (1961).

[7] Astbury, W. T., *J. Soc. Leather Tr. Chem.*, **45**, 186 (1961).

Some Properties of the Deoxyribonucleic Acid of Phage Alpha

WE wish to report some results concerning the unique properties of the deoxyribonucleic acid (DNA) isolated from the phage α grown on its host, *Bacillus megatherium*, strain Paris. This phage and its host have been studied by Drs. S. Aurisicchio, A. Celano, A. Coppo, P. Donini and F. Graziosi, and kindly supplied to us by them. These workers had found that the decay of phosphorus-32 incorporated in the DNA of the phage led to a time-dependent loss of viability similar to that of the phage φ*X*-174 (Graziosi,, F., personal communication). Since the DNA of the latter phage has been shown to be single-stranded[1], the DNA of phage α was expected to display similar properties. However, as will be indicated later, many of its properties resemble those of double-stranded DNA with the notable exception that, on denaturation, there are formed two denatured units with distinct buoyant densities in a cæsium chloride gradient.

After gathering and purification of phage α, DNA was isolated by the phenol method of Mandell and Hershey[2]. Using as much caution as possible to avoid shear degradation, a mean sedimentation coefficient of 40*S* (extrapolated to infinite dilution and corrected to water at 20° C.) was obtained for this type of preparation. Exposure to heat resulted in an absorbance–temperature profile similar in the sharpness of its rise of absorbance to that of the DNA of the *T* phages of *Escherichia coli*, with a T_m (midpoint of the rise of absorbance) of 86·5° C. in 0·15 *M* sodium chloride plus 0·015 *M* sodium citrate. This T_m corresponds to a base composition of 41 mole per cent guanine plus cytosine (ref. 3 and Marmur, J., and Doty, P., unpublished work). When phage α DNA is denatured and then renatured under optimal conditions[4], it exhibits an absorbance–temperature profile similar to that of the native; the curves can be superimposed to the extent of approximately 85 per cent in the region of the T_m.

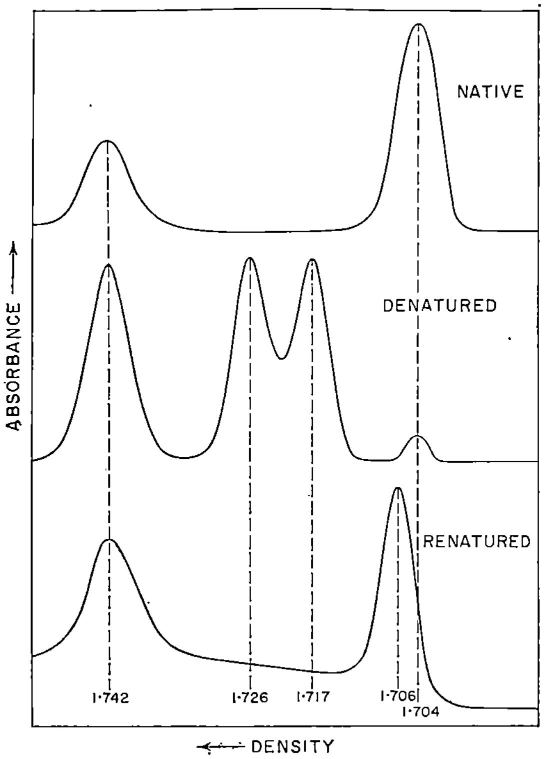


Fig. 1. Buoyant densities of native, denatured and renatured phage α DNA. Microdensitometer tracings were made of the ultra-violet absorption photographs taken after 23 hr. at 44,770 r.p.m. (25° C.). The density 1·742 represents that of the standard, *Pseudomonas aeruginosa* nitrogen-15 DNA, which is used to calculate the densities of the other bands

Native and denatured samples of phage α DNA were then examined for their properties in cæsium chloride density gradient centrifugations. The results are shown in Fig. 1. Native phage α DNA was equilibrated in a single band with a buoyant density of 1·704 gm./c.c. This density corresponds to a base composition of 44 mole per cent guanine plus cytosine (refs. 5, 6 and Schildkraut, C. L., Marmur, J., and Doty, P., unpublished work). However, a thermally denatured aliquot gave rise to two equal distinct bands with densities of 1·717 gm./c.c. and 1·724 gm./c.c. When the denatured material was renatured, again one band was obtained. The density of this band (1·706 gm./c.c.) is slightly higher than that of the native DNA, a result most likely due to the persistence of some denatured unmatched ends.

The two distinct denatured units, obtained on thermal denaturation (and on formamide denaturation as well), might result from a variety of factors. The two hypotheses which at the present time we favour as explanations of these results may be summarized as follows: (1) That there is a difference in the base compositions of the two units or strands which causes one or both of them to have a different density from that usually obtained for a DNA of this overall base composition (42·5 per cent, obtained by chemical analysis, Graziosi, F., personal communication). The Watson–Crick structure does not exclude this possibility even for deoxyribonucleic acids in which adenine = thymine and guanine = cytosine (as has been shown for this DNA, Graziosi, F., personal communication). (2) That an unknown material, *X*, is attached to one of the two units causing it to have a lighter or heavier buoyant density.

In considering the first hypothesis, it is possible to calculate the percentage of guanine plus cytosine for each of the denatured bands from their buoyant densities. This corresponds to 42 mole per cent for the lighter and 49 mole per cent for the heavier band. The lighter denatured band then has, by this calculation, a guanine plus cytosine content closely approximating that obtained by chemical analysis and by the T_m of the absorbance–temperature profile. However, the average composition calculated from the densities of the two bands (45 per cent) is close to that corresponding to the buoyant density measured for the native material. Since the lighter denatured band approximates the average base composition of the double-stranded material, we feel inclined to favour hypothesis (2). Some unknown material firmly attached to one of the strands causes it to exhibit a higher buoyant density, resulting in the two-band pattern observed by thermal and formamide denaturation. If only one of the two strands of the DNA molecule were essential for the replication of the phage, it would then be possible to explain the high phosphorus-32 suicide of the phage.

This investigation was supported in part by research grants (*RG* 7985, 13990 and *G* 16360) from the National Institutes of Health and from the National Science Foundation.

S. Cordes *
H. T. Epstein
J. Marmur

Departments of Biochemistry and Biology,
Brandeis University,
Waltham,
Massachusetts.

* Pre-doctoral Fellow of the National Institutes of Health.

[1] Sinsheimer, R. L., *J. Mol. Biol.*, **1**, 43 (1959).
[2] Mandell, J. D., and Hershey, A. D., *Anal. Biochem.*, **1**, 66 (1960).
[3] Marmur, J., and Doty, P., *Nature*, **183**, 1427 (1959).
[4] Marmur, J., and Doty, P., *J. Mol. Biol.* (in the press).
[5] Rolfe, R., and Meselson, M., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1039 (1959).
[6] Sueoka, N., Marmur, J., and Doty, P., *Nature*, **183**, 1429 (1959).

An Inhibitor of Liver Alcohol Dehydrogenase in Preparations of Reduced Diphosphopyridine Nucleotide

Different samples of reduced diphosphopyridine nucleotide (β-DPNH, Sigma Chemical Co.) have been found to give significantly different maximum rates of reduction of aldehyde catalysed by liver alcohol dehydrogenase. The crystalline enzyme was prepared from horse liver[1] and measurements of initial rates were made with a recording fluorimeter[1,2] at 23·5°. In Table 1 relative values of initial rates with $1 \cdot 3 \times 10^{-3}$ *M* acetaldehyde and 2×10^{-5} *M* DPNH in *p*H 6 phosphate buffer (ionic strength 0·1) are collated with assays of the samples of DPNH. The percentage by weight of anhydrous disodium salt was calculated from direct extinction measurements at 340 mμ (column 2), and also from the decrease of absorption of light at this wave-length ($\Delta E340$) accompanying complete oxidation of the coenzyme by excess acetaldehyde and liver alcohol dehydrogenase (column 3), using in each case the value 6·22 (ref. 3) for the millimolar extinction coefficient. The second value is taken to be the true DPNH content. The difference between the two values is due to the presence of a substance which closely resembles DPNH in both light absorption and fluorescence emission, but is not coenzymically active with liver (or yeast) alcohol dehydrogenase. The results indicate that the samples which give the higher initial rates have the greater coenzyme content and a smaller excess of total adenine over coenzyme (column 4). Ribose and total phosphate analyses were also made ; calculating the total adenine content from the extinction at 260 mμ (*p*H 7) and a millimolar extinction of 15 (ref. 4), the molar ratios of ribose and phosphate to adenine were 2·1 ± 0·1 for all the samples.

With butyraldehyde as substrate, similar differences in initial rates at *p*H 6 were found. At *p*H 7 the differences were smaller, and with acetaldehyde at *p*H 10 all the DPNH samples gave the same initial rates. With yeast alcohol dehydrogenase (Worthington Biochemical Corporation) the rates of reduction of acetaldehyde at *p*H 6 were the same for all the DPNH samples. It appears that the more impure samples at least contain a potent and relatively specific inhibitor of liver alcohol dehydrogenase in neutral and acid solution.

Table 1. ASSAY VALUES AND RELATIVE INITIAL RATES OF OXIDATION OF DPNH SAMPLES BY ACETALDEHYDE AND LIVER ALCOHOL DEHYDROGENASE AT *p*H 6·0

Sample	Assay, percentage DPNH(Na_2)		$E260/\Delta E340$	Initial rate (arbitrary units)
	$E340$	$\Delta E340$		
1*	73	66	3·6	57
2	78	73	3·0	68
3	85	80	2·85	96
4	88	81	2·75	100

* Sample 1 not designated as the β-isomer.

The following compounds were tested as inhibitors with negative results : nicotinamide and its oxidized and reduced N-methyl derivative, adenosine, adenosine di- and tri-phosphate, yeast adenylic acid, and α- and β-DPN. Digests of DPN and DPNH with nucleotide pyrophosphatase[5], which yield adenosine monophosphate and nicotinamide mononucleotide, were also not significantly inhibitory. Treatment of DPNH with 0·1 *N* hydrochloric acid and DPN with 0·1 *N* sodium hydroxide produced potent inhibitors, however ; both these treatments form adenosine diphosphate ribose[6], and this compound was isolated from acidified DPNH and from digests of DPN with the nucleotidase of *Neurospora crassa*[7]. A concentration of 8×10^{-6} adenosine diphosphate ribose produced 40 per cent inhibition in the rate of reduction of acetaldehyde at *p*H 6 under the conditions described here and using the purest DPNH preparation, but had no inhibitory effect at *p*H 10. It did not affect the reaction catalysed by yeast alcohol dehydrogenase at *p*H 6. Digestion of the compound with nucleotide pyrophosphatase destroyed its inhibitory power. Direct evidence of the presence of adenosine diphosphate ribose in DPNH samples has been obtained by paper electrophoresis, but it is not yet possible to say whether the amount is sufficient to account for the observations.

The remarkably potent and *p*H-dependent inhibition of liver alcohol dehydrogenase by adenosine diphosphate ribose has obvious possibilities for work on coenzyme binding. It also seems probable that discrepancies between the kinetic data for this enzyme[1,8,9], which were particularly large as regards the maximum rate of aldehyde oxidation in acid solution, were mainly due to the presence of different amounts of inhibitor in the coenzyme samples. Highly purified coenzyme samples are evidently essential for valid kinetic studies, especially if conclusions about the mechanism are to be drawn from relations between the forward and reverse rates and equilibrium constants[8,10,11]. It remains to be seen whether these observations and conclusions are relevant to other dehydrogenases.

I am indebted to Dr. N. O. Kaplan for a gift of α-DPN and to Dr. V. Massey for a preparation of *Neurospora* nucleotidase.

KEITH DALZIEL*

Department of Biochemistry,
University of Sheffield.

---

* Sorby Research Fellow of the Royal Society.

[1] Dalziel, K., *Biochem. J.*, **80**, 440 (1961).

[2] Theorell, H., and Nygaard, A. P., *Acta Chem. Scand.*, **8**, 877 (1954).

[3] Horecker, B. L., and Kornberg, A., *J. Biol. Chem.*, **175**, 385 (1948).

[4] Burton, K., in *Data for Biochemical Research*, edit. by Dawson, R. M. C., Elliott, D. C., Elliott, W. H., and Jones, K. M., 74 (Oxf. Univ. Press, 1959).

[5] Kornberg, A., and Prieer, W. E., *J. Biol. Chem.*, **182**, 763 (1950).

[6] Chaykin, S., Meinhart, J. O., and Krebs, E. G., *J. Biol. Chem.*, **220**, 811 (1956). Kaplan, N. O., Colowick, S. P., and Barnes, C., *ibid.*, **191**, 461 (1951).

[7] Kaplan, N. O., Colowick, S. P., and Nason, A., *J. Biol. Chem.*, **191**, 473 (1951).

[8] Theorell, H., Nygaard, A. P., and Bonnichsen, R., *Acta Chem. Scand.*, **9**, 1148 (1955).

[9] Theorell, H., *Adv. Enzymology*, **20**, 31 (1958).

[10] Alberty, R. A., *J. Amer. Chem. Soc.*, **75**, 1928 (1953).

[11] Dalziel, K., *Acta Chem. Scand.*, **11**, 1706 (1957).

A New Universal Buffer System

DURING investigations of the effect of *p*H on the activity of certain enzymes it was found necessary to use a single buffer solution for measurements at widely different *p*H values. For various reasons the universal buffer solutions of Prideaux and Ward[1], and Britton and Robinson[2] were unsuitable, and so the following solution was devised, the *p*H value of

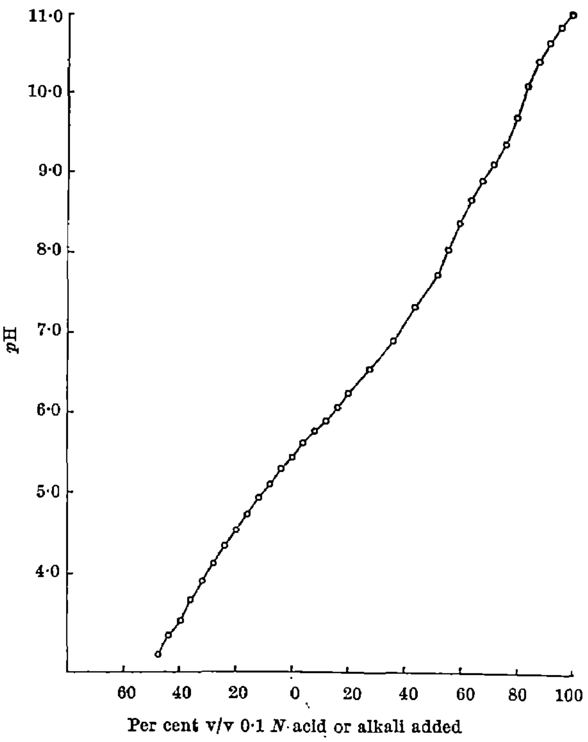


Fig. 1

which was found to vary almost linearly with added acid or alkali.

0·1 mol. of each of the following was dissolved separately in 250 ml. water, the solutions being mixed in the order shown just prior to use: sodium carbonate, 2-amino-2-methyl propan-1 : 3-diol[3] (Eastman-Kodak reagent); sodium dihydrogen orthophosphate; citric acid.

Van Slyke[4] and Britton and Robinson[2] deduce that in mixtures of buffer substances a constant buffering power over the working pH range will be obtained if the consecutive pK values of the acids involved differ by not more than 1·2. In the present system the pK values are deduced to be[3,5]:

1·0; 3·1: 4·7: 5·4: 6·4: 7·1; 8·8; 10·4 and 12·3.

These approximate to the ideal system, and the buffering power in fact remains nearly constant between pH 2 and 12 (Fig. 1).

D. A. Ellis*

Department of Biochemistry,
University of Leeds.

* Present address: Midland Centre for Neurosurgery, Holly Lane, Smethwick, Staffs.

[1] Prideaux, E. B. R., and Ward, A. T., *J. Chem. Soc.*, 125, 426 (1924).
[2] Britton, H. T. S., and Robinson, R. A., *J. Chem. Soc.*, 1457 (1931).
[3] Gomori, G., *Proc. Soc. Exp. Biol. Med.*, 62, 33 (1946).
[4] Van Slyke, D. D., *J. Biol. Chem.*, 52, 558 (1922).
[5] *Handbook of Chemistry and Physics*, thirty-second ed., 1507 (Chemical Rubber Pub. Co., Cleveland, Ohio, 1950).

RADIOBIOLOGY

Determination of Cæsium-137/Potassium Ratios in Diet and in the Human Body by Urine Analysis

A radiochemical method[1] has been developed for the determination of cæsium-137 in urine. Acidified urine containing cæsium carrier is passed through a column of ammonium phosphomolybdate mixed with an equal weight of asbestos to give adequate porosity. The ammonium phosphomolybdate acts as a highly selective cation-exchanger and retains the cæsium quantitatively, even at comparatively high flow-rates. Other cations, including sodium, potassium and the alkaline earths, are not retained to any significant extent. After all the sample has passed through the column, the ammonium phosphomolybdate is dissolved in sodium hydroxide solution and the cæsium precipitated as the cobaltinitrite. The removal of final traces of potassium and rubidium from the cæsium is ensured by its subsequent precipitation as the bismuth iodide complex and finally as the chloroplatinate, which is filtered, dried and beta-rays counted. The recovery of carrier normally exceeds 80 per cent. The sensitivity of this method makes it particularly useful for the determination of fall-out cæsium-137 in urine, as current concentrations are low and are likely to decline still further.

The cæsium-137 contents of seven urine samples collected in February, March and April 1961 were determined by this method. These samples were prepared by bulking at least 10 specimens obtained from individuals, mostly resident in Berkshire, with no known exposure to cæsium-137 other than fall-out levels in their diet. Potassium was estimated in the same samples by flame photometry. The results of these analyses are given in Table 1.

Table 1. CÆSIUM-137 AND POTASSIUM CONCENTRATIONS IN BULKED URINE SAMPLES

Date of collection	Cæsium-137 ($\mu\mu$c./l.)	Potassium (gm./l.)	Ratio ($\mu\mu$c./gm.)
21.2.61	29·9	2·47	12·1
1.3.61	21·1	2·00	10·6
7.3.61	26·8	2·73	9·8
14.3.61	24·3	1·73	14·0
20.3.61	24·6	1·66	14·8
10.4.61	26·2	2·07	12·7
28.4.61	21·7	2·07	10·5
	Mean 24·9	Mean 2·10	Mean 12·1

In vivo cæsium-137/potassium ratios were determined by Rundo, J. (private communication, 1961) in 14 residents of Oxfordshire and Berkshire during March 1961. The mean value for this group was 36 $\mu\mu$c./gm., which is almost exactly three times the value for this ratio in urine given in Table 1. Values for the ratio :

$$\frac{\text{cæsium-137/potassium in the body}}{\text{cæsium-137/potassium in urine}}$$

have also been determined for a number of individuals by Rundo and by Miller and Marinelli[2]. These varied from 1·9 to 4·6, indicating that individuals may show a considerable departure from the average value of 3 obtained in the present comparison.

As both cæsium and potassium are completely absorbed from the human gastro-intestinal tract, the cæsium-137/potassium ratio in urine will be equal to that in the diet and should also be one-third of that in the body, under equilibrium or near equilibrium conditions. This is in agreement with the measurements of McNeill and Trojan[3], who measured the cæsium-137/potassium ratio in the diet of Toronto residents and in the residents themselves, using a γ-spectrometric technique. They found that the body/diet ratio was 3: 1.

The analysis of bulked urine samples appears to provide a simple method for assessing current cæsium-137/potassium ratios in both diet and the human body, so long as dietary-levels are not changing too rapidly. By analysing a bulked sample, the difficulty in selecting representative aliquots of various items of diet for analysis is avoided.

The possibility of monitoring strontium-90 in diet by urine analysis has been proposed by Schulert[4], whose measurements show that the strontium-90/calcium ratio in diet is approximately twice that in the urine. Both strontium-90 and cæsium-137 may be estimated on the same sample by adding strontium and cæsium carrier to urine before passing it through the ammonium phosphomolybdate column. While cæsium is retained on the column, both strontium and calcium pass through and may be co-precipitated from the effluent as the oxalates. Separation of the strontium from the calcium and determination of the strontium-90 can then be effected by well-established techniques.

We wish to thank Mr. W. H. A. Raymond of the Medical Research Council Radiobiological Unit, Harwell, who carried out potassium determinations in the urine samples.

A. Morgan
Gloria M. Arkell

Atomic Energy Research Establishment,
Harwell,
Didcot.

[1] Arkell, G. M., and Morgan, A., *Atom. Energy Res. Est., Harwell, R.* 3675 (1961).
[2] Miller, C. E., and Marinelli, L. D., *Argonne Nat. Lab. Rep. ANL*-5919, 74 (1958).
[3] McNeill, K. G., and Trojan, O. A. D., *Health Phys.*, 4, 109 (1960).
[4] Schulert, A. R., *Nature*, 189, 933 (1961).

Effect of Irradiated Normal Tissues on the Growth of Mouse Carcinoma

In 1932 it was observed by Geoffrey Keynes[1], working on the disappearance of human mammary carcinoma by surrounding the growth with radium needles, that on cutting out a piece of the tumour after the needles had been in place for ten days, some of the malignant cells were alive and became grafted in the overlying biopsy skin-incision, although the rest of the tumour from which the piece had been removed eventually disappeared. This suggests that the surrounding normal cells might have been acted on by the radiations so as to continue to pour out a chemical substance which led to the death of the cancer cells[2].

It was decided to search for evidence of the presence of such substances in irradiated normal tissues. The material tested has been bovine and rabbit muscle, lamb's thymus and mouse meat. The irradiated tissue was supplied either solid as food or in water as a drink. The tumour used was one of two mice carcinomata, Bashford 63 (also labelled *X*), or Compton 22, which arose in our laboratory spontaneously and so far has not been described. The general method of experimenting was to implant grafts from the same fragment of tumour subcutaneously into 10 mice. From these animals, 1–5 were taken at random for testing the irradiated material. The others were kept as controls. Both control and test animals were fed on the pellets customarily used in laboratories, but the test animals had either (*a*) the irradiated material, if solid, added to the ground-up pellets or (*b*) had a watery extract of the irradiated material substituted for their drinking water. There was no restriction of the amount the animals were allowed to eat or drink.

Experiments showed that unirradiated meat or meat extracts added to the normal diet of the mice had no effect on tumour growth; and that animals receiving irradiated food showed no alteration in weight compared with the controls.

In 23 experiments of this nature, 16 by the feeding procedure and 7 by administering the material by drinking, the results were as follows: (*a*) in 6 there was no evidence of inhibition; (*b*) 17 showed disappearance of the growth or inhibition in some degree.

Among the failures was a drinking experiment where the material had been heated to 80° C. In three others the material had received doses of 1,000 r., 6,000 r. and 7,500 r., which we consider as inadequate, while in one, although the dose given to the food 120,000 r. was suitable, the animal was fed for only 3 days. For the sixth failure we have no explanation.

Experiments 619 (feeding with lamb's thymus, radiation dose 600,000 r.) and 664 (drinking experiment with a watery extract of beef muscle, radiation dose 150,000 r.) are examples of findings.

Experiment 619 (Fig. 1) illustrates the disappearance of 3 treated tumours and, while feeding was proceeding, inhibition in 2 others.

In the experiment 664 (Fig. 2), No. 2 was treated from the fifth day after implantation of the graft, for 7 days. The tumour disappeared. No. 1 was allowed to grow until the eleventh day and then given the drink for 7 days. It showed some initial shrinking in size but then resumed growth and caught up with the controls by the eighteenth day. This suggests that there is a simple relation between the amount of active agent necessary to cause disappearance of the growth and the weight of the tumour (proportional to r^3 in the case of a sphere). Below this critical amount, inhibition only can occur and the growth of the tumour soon gets the upper hand. The amount of active agent in our extracts is likely to be small, so that the animal may not drink enough to absorb the critical amount for disappearance of the tumour.

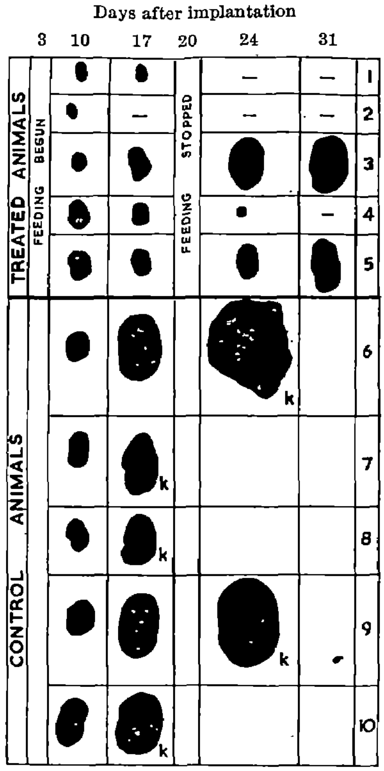


Fig. 1. Experiment 619, tumour 63. *K*, killed for tumour propagation purposes

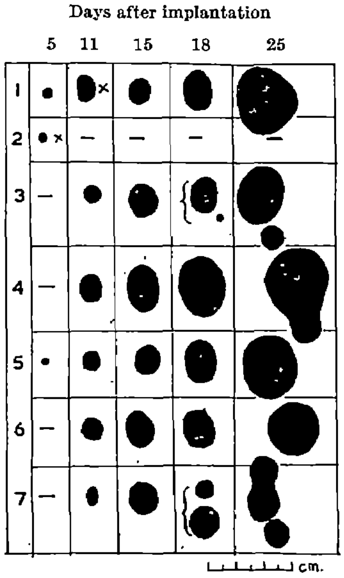


Fig. 2. Experiment 664, tumour *X*. *X*, treated animals ; the other animals are controls

Graphs of the rate of tumour growth in controls and in treated animals where the tumour is inhibited but does not disappear show the same general character; but the growth-time curve for the inhibited tumour shows a variable delay before it turns upward.

Assuming the tumours to be spherical in shape and that the inhibitory effect is due to direct destruction of tumour cells, theoretical curves have been plotted for different amounts of active agent given. It appears that for complete disappearance of a tumour a dose is required which will destroy at least 37 per cent of it.

The inhibitor seems to be diminished in activity by heating above 60–65° C. and destroyed by an X-ray dose greater than from 10^6 to 2×10^6 r.

A similar phenomenon in plants has been reported from the U.S.S.R. A. M. Kuzin[3] has found evidence that irradiating the normal cells of a plant produced substances which had an inhibitory effect on cell division in other parts of the plant which had not been exposed to X-rays. He isolated from the irradiated part a mixture of polyphenols which proved to be the active substance.

Arthur Compton
Charles A. Pannett

Wright-Fleming Institute,
St. Mary's Hospital,
London.

[1] Keynes, Geoffrey, *Brit. J. Surg.*, 19, 415 (1932).

[2] Pannett, C. A., *Lancet*, 829-830 (April 20, 1957).

[3] Kuzin, A. M., Radiobiology Symposium, Moscow, Oct. 18–20, 1960, reported in *The New Scientist*, p. 1200 (Nov. 3, 1960).

PHYSIOLOGY

Spontaneous Spike-Discharges of Vascular Smooth Muscle

I reported in refs. 1 and 2 that trans-membrane potentials of smooth muscle fibre of the small blood-vessels, distributed in frog tongue and web, had been studied with the aid of an intracellular micro-electrode technique. The results of these experiments were as follows: (1) The resting potential of smooth muscle fibres was found to be negative on the inside, against the external medium; the mean value was − 40·5 ± 5 mV. and fluctuations of the resting potential were rarely observed. (2) Single spike-potentials were evoked by single induction shocks, which were about 60 mV. in magnitude and 200 msec. in duration.

Though these potentials usually had the shape of simple spikes, some of them showed the so-called 'complex shape'[3,4] provided with some humps on the phase of either depolarization or repolarization, or on both, and showed graded response. Another type of action potential was obtained from smooth muscle fibres in the remarkably contractile region of the bifurcation of small blood-vessels or in blood capillaries that were highly sensitive to electrical or chemical stimulation; in this type the magnitude was about 60 mV. and the duration 50 msec. This evidence suggested that there were two sorts of muscle fibres, distinguishable in contractility, in the small blood-vessel system.

(3) The mean value of the resting potential of endothelial cells in blood capillary wall was − 47 ± 3·7 mV., but spike-potential, elicited by electrical stimulation, had not been observed.

In the present work, the spontaneous spike-discharges of vascular smooth muscle of frog were observed with the intracellular micro-electrode. The recordings *in situ* were carried out in the following

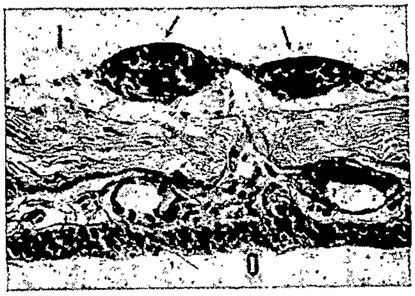


Fig. 1. Photomicrograph showing a transverse section of a blood-vessel in the skin of the lateral abdomen of a frog. Note the absence of visible connective tissues in the surface of the blood-vessels. The arrows mark blood-vessels (venules containing red-stained corpuscles). *I* and *O* are the inside and outside respectively of the skin. Stained with hæmatoxylin–eosin. (× 100)

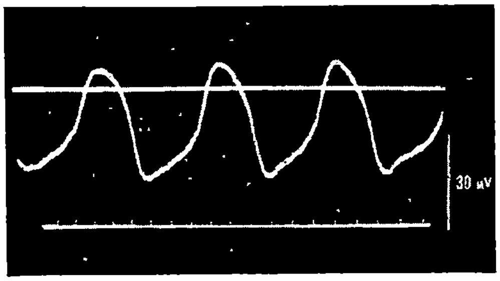


Fig. 2. A series of the spike-discharges recorded intracellularly from a vascular smooth muscle fibre (venule). In this record the resting and action potential are −26 and 36 mV. respectively. The spikes show an overshoot of about 10 mV. and a time-course of about 200 msec. Note the initial slow potential (pre-potential), from the summit of which the spike arises. The horizontal line indicates zero potential. Calibration, 30 mV.; time mark, 30 msec.; temperature, 29° C.

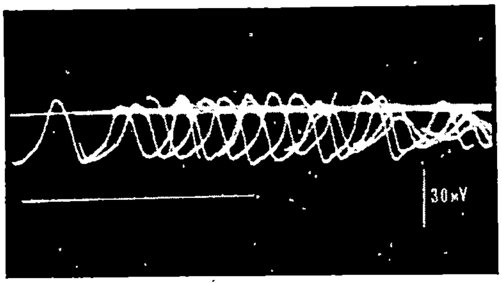


Fig. 3. Typical record of spike-discharges obtained from a muscle fibre with an intracellular micro-electrode during spontaneous activity (arteriole). This continuous record was taken repeatedly with several sweeps on a slow-moving film. The horizontal line indicates zero potential. Calibration, 30 mV.; time mark, 30 msec.; temperature, 26° C.

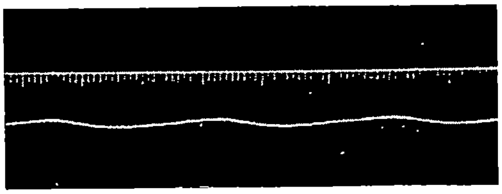


Fig. 4. A vascular pulsation curve recorded from the same specimen as in Fig. 3, in which the period is about 900 msec. This curve is shown to compare with the time-course of the spike. Time mark, 30 msec.

way: the frog was immobilized by fixing the dorsal side down with a metal cover in a lighted chamber. The skin of the abdomen was incised both along the mid-line and at the flank so as to be turned over, and was spread out under moderate tension on a cork board immersed in Ringer solution. A micro-electrode was then inserted into the blood-vessel wall from the outside with the aid of a Peterfi-type micro-manipulator under the binocular microscope. The micro-electrode filled with 3 M potassium chloride solution was used to record the potential, a relatively high-resistance micro-electrode (35–50 $M\Omega$) being used for the purpose of minimizing injury to the muscle cell. For the arteriole, the tip of a micro-electrode had to be thinner than for the venule, in order to penetrate the wall safely. For the convenience of good penetration small blood-vessels, with very thin connective tissue membrane covering the surface as shown in Fig. 1, of 50–100μ in diameter, were selected. The control of vascular pulsation was made possible by applying adequate pressure to the proximal part of the same blood-vessel at some distance from the point of impalement.

All experiments were carried out at temperatures between 25° and 32° C. When the tip of a micro-electrode penetrated into a muscle fibre of a small blood-vessel, the resting membrane potential showed a negativity against the external medium, the value of which was about − 25 mV. During the observation, repetitive bursts of spike-discharges occurred sometimes spontaneously at regular spike-intervals, and lasted for several seconds or longer. The typical record is shown in Fig. 2. In this record the resting and action potentials were − 26 and 36 mV. respectively, the resting potential being measured at maximum polarization between one spike and another. The action potentials had an overshoot of about 10 mV. and a time-course of about 200 msec. They were invariably provided with an inflexion of the rising phase. This indicated that the spike configuration consists of two components, namely, an initial slow potential and a large simple spike.

The slow potential resembled in appearance pre-potentials of other smooth muscles (tænia coli[3], pregnant uterus[5], etc.) or excitatory post-synaptic potentials of various neurones[6,7]. The spike-potential recorded appeared to be conducted excitation in view of the initial slow potential and it is probable that the excitation is propagated from one unit to another to some extent in the blood-vessel. It is with this point taken into account that the mechanism of vascular activity is now under investigation by me.

With the spike-discharges, there was a progressive decrease of the amplitude towards the end, finally reaching a certain depolarization-level of the resting potential. Though the spontaneous discharges were observed in both arterioles and venules, no significant difference was noted between them.

S. FUNAKI

Department of Physiology,
Osaka Medical College,
Japan.

[1] Funaki, S., *Proc. Jap. Acad.*, **34**, 534 (1958).

[2] Funaki, S., *Electrical Activities of Single Cells*, 233, edit by Katsuki, Y. (1960).

[3] Holman, M. E., *J. Physiol.*, **141**, 486 (1958).

[4] Bülbring, E., Burnstock, G., and Holman, M. E., *J. Physiol.*, **142**, 420 (1958).

[5] Goto, M., and Woodbury, J. W., *Fed. Proc.*, **17**, 58 (1958).

[6] Woodbury, J. W., and Patton, H. D., *Symp. Quant. Biol.*, **17**, 185 (1952).

[7] Tauc, L., *J. Physiol.*, **47**, 286 (1955).

Stimulation of Motor Nerve Terminals

KNOWLEDGE of prejunctional events in neuromuscular transmission is at present indirect in origin and largely speculative in content. This is because there has been no method of directly recording from, or stimulating, the very small motor nerve endings. Using a glass micro-electrode in the isolated rat diaphragm phrenic nerve preparation we have succeeded in stimulating single nerve fibres very close to, or at, the neuromuscular junction. The recording chamber and bathing solution have been previously described[1].

The stimulating electrode (filled with 4 M sodium chloride, 1·5–3μ tip and 1–3 megohm resistance) was located at neuromuscular junctions by using it to record extracellular end-plate potentials in curarized ($2\text{–}4 \times 10^{-6}$ gm./ml.) or magnesium-poisoned (11 m.mole/l.) preparations (Fig. 1*A*). With this method extracellular end-plate potentials are recorded only when the tip is very close to the junction. When localized in this way the electrode was connected to two Grass stimulators which delivered pairs of pulses separated by varying intervals (1–200 msec.). These pulses were recorded across a known small resistance in the stimulating circuit and monitored on the C.R.O. screen. In response to stimulation small all-or-nothing potentials could be recorded in the phrenic nerve (Fig. 1*B*). The position of the electrode tip was very critical in that movements of only 10–15μ sideways or upwards caused the response to stimulation to be lost. Moving the electrode downwards resulted in impalement of the muscle fibre. As the diameter of the neuromuscular junction in the rat diaphragm is 20–30μ (ref. 2) we feel that we are stimulating structures very close to the nerve terminals.

The changes in threshold after one supra-threshold conditioning stimulus have been examined in both magnesium-poisoned and curarized preparations. The results (Fig. 1*C*) indicate a refractory period of 1·5–3 msec. followed by a period of supernormality lasting 15–30 msec. There is often a slight subnormality for about 50 msec. after the supernormal period (Fig. 1*C*). Following 2–5 conditioning volleys the period of supernormality is reduced and the subnormality increased in magnitude and duration. These excitability changes of the terminals are very similar in time course to those described for the parent fibres, the mammalian *A* fibres[3]. After a brief (0·1 msec.) sub-threshold conditioning pulse changes in threshold—an initial fall and later recovery—can be detected for 2·5–3·0 msec. in both magnesium-poisoned and curarized preparations.

We have further found that the excitability of the terminals can be markedly altered by drugs. A comparison of magnesium and curare at the same neuromuscular junctions has shown that curare reversibly reduces the supernormal period found after one conditioning stimulus both in curarized and magnesium-poisoned preparations. Prostigmine in concentrations as low as 10^{-7} gm./ml. produces a reversible increase in refractory period followed by an increase in the magnitude and duration of the supernormal period (Fig. 1*C*).

These findings are of interest in two connexions. First, prolongation of the refractory period and thus presumably of the spike potential in the terminals suggests that part of the effect of prostigmine on the end-plate potentials is caused by a prolongation of

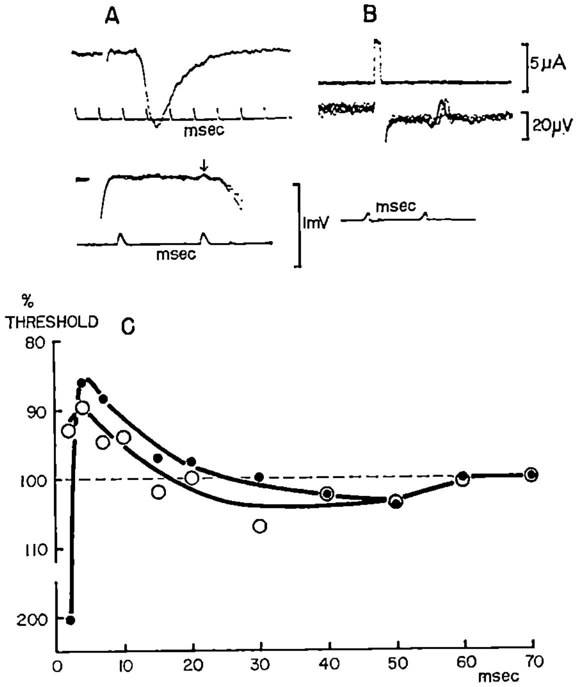


Fig. 1*A*, Extracellular end-plate potentials recorded with a 4-*M* sodium chloride electrode, neuromuscular transmission being depressed by magnesium chloride 11 m.mole.; *B*, action potential in phrenic nerve in response to threshold stimulation (with square pulse of 0·06 msec. duration) at same neuromuscular junction as 1*A*; *C*, changes in threshold after one conditioning stimulus. Ordinate: threshold current at given interval as percentage of unconditioned threshold current. Abscissa: interval between conditioning and testing pulses. ○, magnesium chloride 11 m.mole values before and after prostigmine bromide, averaged; ●, magnesium chloride 11 m.mole and prostigmine bromide 2 × 10^{-6} gm./ml.

the period of transmitter release. Secondly, the prostigmine-induced increase in negative after-potential provides an adequate mechanism for the initiation of the antidromic discharges found in motor nerves after intra-arterial administration of this drug[4].

J. I. HUBBARD
ROBERT F. SCHMIDT

Department of Physiology,
John Curtin School of Medical Research,
Australian National University,
Canberra.

[1] Liley, W., *J. Physiol.*, 132, 650 (1956).
[2] Cole, W. V., *J. Comp. Neurol.*, 108, 445 (1957).
[3] Gasser, H., and Grundfest, H., *Amer. J. Physiol.*, 117, 113 (1936).
[4] Masland, R., and Wigton, R., *J. Neurophysiol.*, 3, 269 (1940).

Potential Changes produced by Application of Current Steps in Motoneurones

IN cat spinal motoneurones, when current steps are applied across the membrane through the intracellular micro-electrode, the membrane potential changes in an approximately exponential way, but with a certain deviation. Thus, the soma-dendritic membrane of the motoneurone has been assumed to behave as a resistance and a capacitance in parallel[1,2], and the deviation has been attributed to the cable-like property of dendrites, the dominance of which in forming the effective surface area of motoneurones has been emphasized recently[3]. As will be described, however, the potential produced by current steps in motoneurone membrane shows a considerable overshoot, which has so far been overlooked because the current steps used previously were generally too brief to reveal the later decline of the potential. This electrical behaviour of the membrane suggests that the equivalent electrical circuit of the membrane is not a simple resistance-capacitance network.

Single micro-electrodes filled with 3-mole/l. potassium chloride solution were inserted into motoneurones in the lumbosacral region of the cat spinal cord. Rectangular currents were applied to the impaled cell membrane through the micro-electrode, and changes in the membrane potential thereby induced were recorded through the same micro-electrode by compensating for the voltage set up across the micro-electrode resistance. The applied current was monitored by the potential drop across a 10-M.ohm resistor inserted in series between the micro-electrode and the 100-M.ohm resistor, the latter being connected to the current generator. After d.c. amplification, the potential changes in the membrane were recorded photographically from a cathode ray oscilloscope trace.

Fig. 1 *A* and *B* shows an example of the potential change thus induced by rectangular current pulses of about 50 msec. duration. The current strength was about half the rheobase for initiating the *IS–SD* spike potential. Both depolarizing (*A*) and hyperpolarizing (*B*) potentials rose in an approximately exponential way to a maximum at about 15 msec., there being a later decline during the continuation of the currents. After cessation of currents, the membrane potentials rebounded in the opposite direction before returning to the original level, as seen with slower sweep velocity in Fig. 1 *C* and *D*. In Fig. 1 *E* and *F* the potential induced by current steps reached a steady level in about 100 msec. after the summit at about 15 msec. The rebound, after current ceased, followed a course similar to that of the initial hump at the onset of the current. The potentials caused by depolarizing and hyperpolarizing currents were virtually mirror images. In these records, care was taken to eliminate distortion by electrode polarization[1]. Traces in Fig. 1 *G–L* were obtained just after the withdrawal of the micro-electrode to the extracellular position by applying the same currents used in *A–F*, and they showed that electrode polarization was not responsible for the overshoot at the current onset and the undershoot when current ceased. The net changes in the membrane potential are illustrated in Fig. 1 *N* by subtracting the extracellularly recorded electrode polarization from the corresponding intracellular potential in the same motoneurone as shown in Fig. 1 *E* and *F*. Measurements of the threshold changes give further evidence that these observed potential changes are taking place across the cell membrane. Short depolarizing pulses (duration, 0·2 msec.) were applied to the membrane through the micro-electrode. The threshold strength was measured as the current exciting the cell in about 50 per cent of the trials[1,2]. The decrease or increase of the threshold strength of pulses during the depolarizing or hyperpolarizing current steps is plotted in Fig. 1 *M*. As shown by dashed lines through the plotted points, the change of the threshold was closely related to the observed change in the membrane potential in *N*.

Changes in membrane potential resembling those of Fig. 1 have been observed constantly in all fifteen

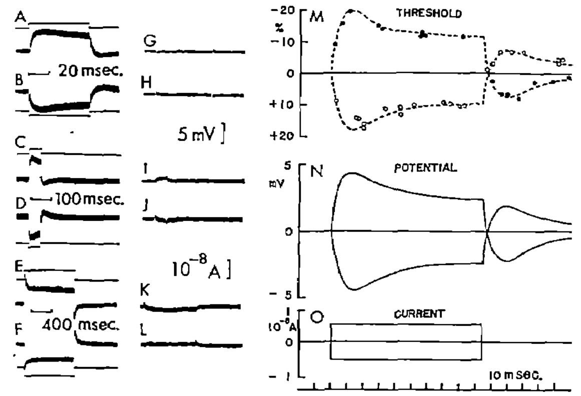


Fig. 1. *A* to *F*, depolarized (*A*, *C*, *E*) and hyperpolarized (*B*, *D*, *F*) potentials in the cat motoneurone membrane were induced by rectangular current pulses. The applied currents were registered in the companion traces of each record. *G* to *L*, electrode polarization recorded in the extracellular position by applying the same currents that were used for obtaining the intracellular records arranged to the left in *A–F*. *M*, Increase (+) and decrease (−) of the threshold strength of short pulses during conditioning by hyperpolarizing (○) and depolarizing (●) currents which are shown in O. *N*, change in the membrane potential by the conditioning currents. Note that records *A–D* were obtained in a different cell from that for *E*, *F*, *M* and *N*. The voltage and current calibrations apply to the appropriate records *A* to *L*

motoneurones examined so far. When current steps of from 2 × 10^{-9} amp. to 6 × 10^{-9} amp., in each case corresponding to about half the rheobase for each motoneurone, were applied, the polarized potential reached the summit at 16 msec. on the average (range, 10–22 msec.) after the onset and then declined by 30 per cent on the average (range, 17–46 per cent) of the peak value in the following interval of about 100 msec. This electrical behaviour of the motoneurone membrane may be explained by assuming that an inductive reactance is involved in the membrane, as has been proposed for squid giant axon[4,5], and for frog nerve[6]. Since the over- and under-shoots of polarized potentials are considerable, the analysis of the time-course of the rise or fall in potential caused by the current steps can no longer be based on the previously assumed resistance-capacitance model. The small undershoot following the monosynaptic excitatory post-synaptic potential, which summates to a large undershoot after a repetitive series of post-synaptic potentials[7], appears to be another example of the undershoot described here. During a depolarizing steady current, the change in membrane threshold is correlated accurately with the change in the membrane potential and is not strictly an example of accommodation of the membrane as has hitherto been assumed[1,2].

T. Araki
M. Ito
T. Oshima

Department of Physiology,
John Curtin School of Medical Research,
Australian National University,
Canberra.

[1] Frank, K., and Fuortes, M. G. F., *J. Physiol.*, **134**, 451 (1956).
[2] Coombs, J. S., Curtis, D. R., and Eccles, J. C., *J. Physiol.*, **145**, 505 (1959).
[3] Rall, W., *Science*, **126**, 454 (1957); *Exp. Neurol.*, **1**, 491 (1959).; **2**, 503 (1960).
[4] Cole, K., *J. Gen. Physiol.*, **25**, 29 (1941).
[5] Hodgkin, A. L., and Huxley, A. F., *J. Physiol.*, **117**, 500 (1952).
[6] Monnier, A. M., *Arch. Sci. Physiol.*, **3**, 371 (1949).
[7] Coombs, J. S., Eccles, J. C., and Fatt, P., *J. Physiol.*, **130**, 374 (1955).

Effects of Corticotrophin, Hydrocortisone and Methopyrapone on the Circulating Eosinophiles in Man

2-METHYL-1,2-*bis*(3-pyridyl)-1-propanone (methopyrapone) has been shown to stimulate the secretion of corticotrophin (ACTH) in the pituitary by inhibition of production of cortisol. 11-Desoxycortisol (compound *S*) is a precursor of cortisol and 11β-hydroxylation of compound *S* converts it into cortisol. Methopyrapone (*SU*-4885) inhibits 11β-hydroxylation of 11-deoxycortisol[1]. The result is the rise of compound *S* and fall of cortisol in the adrenal cortex and the blood. Secretion of corticotrophin or its administration by the parenteral route stimulates the adrenal cortex. The resultant release of hydrocortisone and other glucocorticoids from the adrenal cortex causes a marked eosinopenia. A clinical trial was undertaken to evaluate and compare the effects of exogenous administration of ACTH and hydrocortisone and endogenous secretion of corticotrophin by methopyrapone. This work is a part of our neuroendocrinological investigation of the role of methopyrapone and phrenotropic drugs in man[2].

Twenty normal human subjects volunteered for clinical trial. One group of five received 25 units of ACTH parenterally, the second group of five, 80 mgm. hydrocortisone equally divided, orally and parenterally, and the third group of five, 1 gm. methopyrapone orally and 1 gm. dissolved in 250 ml. 0·9 per cent saline infused in 1 hr. Five subjects acted as controls. Using phloxine as a staining medium, circulating eosinophile counts of the venous blood were done just before and 3 hr. after administration of the drug.

Table 1 shows the analysis of variance of control versus drugs and among drugs, and is significant in the comparisons. Tables 2–5 indicate the percentage fall in the circulating eosinophiles 3 hr. after the administration of the drug. The mean percentage fall was 83·3 with ACTH, 80·7 with hydrocortisone, 24·4 with methopyrapone and 1·8 in controls. In one control there was a rise of 22 per cent in the circulating eosinophiles.

Previous work has shown that the parenteral administration of 25 units of ACTH in man resulted

Table 1. ANALYSIS OF VARIANCE OF CHANGES IN EOSINOPHILE COUNT AFTER 3 HR.

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Treatments			
Control versus drugs	1	9,702·816	9,702·816
Among drugs	2	11,426·534	5,713·267
Individuals	16	17,943·6	1,121·475
Total	19	39,072·95	

$F1,16 = 8{\cdot}651$ $P < 0{\cdot}01$ (control versus drugs)
$F2,16 = 5{\cdot}094$ $P < 0{\cdot}05$ (among drugs).

CIRCULATING EOSINOPHILE COUNTS (PER MM.^{3})

Table 2

Subjects	Basis	3 hr. after 25 units (*I.M.*) ACTH	Change (per cent)
(1)	139	6	−95·7
(2)	116	17	−85·3
(3)	94	55	−41·5
(4)	56	6	−89·1
(5)	133	11	−91·7
		Mean	80·7

Table 3

Subjects	Basis	3 hr. after 80 mgm. hydrocortisone	Change (per cent)
(1)	161	28	−82·6
(2)	55	17	−69·1
(3)	44	5	−88·6
(4)	50	5	−90·0
(5)	122	17	−86·1
		Mean	83·3

Table 4

Subjects	Basis	3 hr. after 2 gm. methopyrapone	Change (per cent)
(1)	83	61	−26·5
(2)	111	50	−54·9
(3)	144	138	−4·2
(4)	244	239	−2·0
(5)	67	44	−34·3
		Mean	24·4

Table 5

Subjects	Basis	3 hr. after basis of control group	Change (per cent)
(1)	189	172	−9·0
(2)	117	105	−10·2
(3)	50	61	+22·0
(4)	339	310	−8·5
(5)	144	139	−3·5
		Mean	1·8

in a mean percentage fall of 62·2 in circulating eosinophiles in 3 hr. Hydrocortisone in 80-mgm. dosage also caused a mean fall of 75·6 per cent[4]. The results of the present experiment indicate the similar significant eosinopenic response to the exogenous ACTH and hydrocortisone. An infusion of 1 gm. methopyrapone in 250 ml. 0·9 per cent saline in 2 hr. has been reported to raise 17-hydroxycorticoids in bloods significantly within 4 hr.[5]. The rise in 17-hydroxycorticoids is attributed to uninhibited pituitary secretion stimulating the production of Porter-Silber chromogens.

The eosinopenic response to ACTH and hydrocortisone is uniformly high and without significant difference (Tables 2 and 3). The eosinopenic response to methopyrapone versus control is significant, but low in comparison with that for groups treated with ACTH and hydrocortisone (Tables 4 and 5). Methopyrapone stimulates the secretion of corticotrophin in the pituitary to such an effective level as to cause a significant fall in the circulating eosinophiles.

We acknowledge the receipt of ACTH from Armour, hydrocortisone from Merck, Sharp and Dohme and methopyrapone from Ciba. We wish to thank Dr. E. I. Burdock for help with biometrical work.

N. J. Kothari
J. C. Saunders

Rockland State Hospital,
Orangeburg, and
College of Physicians and Surgeons,
Columbia University, New York.

[1] Liddle, G. W., Island, D., Lance, E. M., and Harris, A. P., *J. Clin. Endocrinol. and Metab.*, 18, 906 (1958).

[2] Saunders, J. C., Kothari, N. J., and Lourie, I., *Proc. Third Ann. Western Pharm. Soc.* (in the press).

[3] Snedecor, G. W., *Statistical Methods*, fifth ed., Chapter 10 (Iowa State College Press, Ames, Iowa, 1956).

[4] Kothari, N. J., Saunders, J. C., and Kline, N. S., *Psychopharmacologia*, 2, 22 (1961).

[5] Bissell, G. W., Scott, A. L., Farnsworth, W. E., and Winkler, I., *N.Y. State J. Med.*, 59, 3596 (1959).

Precorticotrophin in Blood

The existence of precorticotrophin, precursor of corticotrophin, has been demonstrated by Dasgupta and Young[1]. Extracts of ox anterior pituitary, otherwise inactive, acquire a corticotrophic activity detectable by Sayers' test (adrenal ascorbic acid depletion) when the medium is acidified (*p*H 3) or when urea (3–8 *M*) is added to it. These findings have been confirmed by Dixon *et al.*[2], who observed the presence of precorticotrophin in pig pituitary also (personal communication). Earlier, Moruzzi *et al.*[3] reported the presence of an 'inactive' form of corticotrophin (activable by treatment with oxycellulose) in the blood of patients suffering from various types of adrenal insufficiency, including Addison's disease; they called it 'activable' corticotrophin. The possibility that 'precorticotrophin' may be comparable with 'activable' corticotrophin and that the former may be in circulation even under normal physiological conditions was discussed by Dasgupta[4]. The present report is concerned with the actual demonstration of precorticotrophin in normal rabbit blood.

When fresh serum obtained from normal rabbit blood is diluted 15–20 fold by adding 0·9 per cent sodium chloride solution no activity is detected when tested by the method of Sayers as modified by Chowdhuri[5]. The modification consists of using morphinized (morphine sulphate 1·5 mgm. per 100 gm. body-weight by subcutaneous route 2–3 hr. prior to administration of test doses) rats as test animals instead of hypophysectomized ones. When, however, the serum is either acidified with 2 *N* hydrochloric acid to *p*H 3 or treated with urea (3 *M*) and then diluted, adrenal ascorbic acid depleting activity is found to be present in the final test solutions (Table 1).

In a second series of experiments, instead of fresh serum, freeze-dried serum was used as the test material. The solid residue left after freeze-drying serum is dissolved in distilled water such that 1 ml. of the solution corresponds to 2 mgm. of the solid. The clear solution is divided into three portions. The first portion is left untreated, the second portion is acidified to *p*H 3 and the third is treated with urea (3 *M*). Each of these three portions is next separately diluted 20-fold with distilled water and its adrenal ascorbic acid depleting activity determined. Whereas no corticotrophic response is produced by the untreated solution, those treated show definite corticotrophic activity (Table 1).

These results provide evidence suggesting the existence in normal blood of a precorticotrophic factor inactive *per se* but giving rise to corticotrophin under the influence of *p*H adjustment (*p*H 3) or urea.

The extent of dilution (15–20 fold) of the serum was determined by trial such that the concentration of endogenous corticotrophin present in the serum was reduced to a very low value and caused no depletion of adrenal ascorbic acid. However, the same serum, despite dilution, was found to maintain the concentration of endogenous precorticotrophin at a sufficiently high level as to give in consequence of *p*H adjustment or treatment with urea a positive response in the Sayers test. The serum, therefore, seemed to be behaving in this respect in a manner analogous to the

Table 1. Mean Response in Sayers' Test produced by Normal Rabbit Serum

	Fresh serum			Freeze-dried serum		
	Untreated (control)	Adjusted to *p*H 3	Treated with urea	Untreated (control)	Adjusted to *p*H 3	Treated with urea
Mean response (ascorbic acid change mgm./100 gm. adrenal) ± Standard error	−4·4 ± 3·34 (20)	−95·7 ± 13·00 (14)	−87·2 ± 8·82 (10)	−0·125 ± 4·86 (8)	−92·0 ± 23·96 (5)	−55·6 ± 16·18 (5)

Figures within parentheses indicate the total number of observations. Test solutions were injected intravenously at a dose of 0·25 ml./100 gm. body-weight.

extracts of ox anterior pituitary prepared by Dasgupta and Young[1], Dixon *et al.*[2] and Dasgupta[4].

Since the freeze-dried serum residue redissolved in distilled water behaved like fresh serum under the same experimental conditions (Table 1), it appears that the precorticotrophic factor of blood survives a single freezing treatment. Moreover, the serum sample was prepared 4–6 weeks before biological testing and this indicates that the circulating precorticotrophic factor is fairly stable in dry conditions.

I am grateful to Dr. B. Mukerji, director, and to Dr. A. B. Kar, assistant director, for their interest in this work.

P. R. DASGUPTA

Central Drug Research Institute,
Lucknow.

[1] Dasgupta, P. R., and Young, F. G., *Nature*, **182**, 32 (1958).

[2] Dixon, H. B. F., Góth, A., and Young, F. G., *Acta Physiol. Acad. Sci. Hung.*, **15**, 133 (1959).

[3] Moruzzi, G., Rossi, C. A., Montanari, L., and Martinelli, M., *J. Clin. Endocrinol.*, **14**, 1144 (1954).

[4] Dasgupta, P. R., *Bull. Nat. Inst. Sci. India*, No. 17, 117 (1960).

[5] Chowdhuri, B. N., *J. Indian Med. Assoc.*, **32**, 470 (1959).

PHARMACOLOGY

Augmentation of Toxicity of Monoamine Oxidase Inhibitor by Thyroid Feeding

THE administration of desiccated thyroid or thyroxine modifies the susceptibility of animals to the poisonous effects of a number of substances. Thyroid hormone is known to increase the toxicity of fluoride[1], endotoxin[2], alloxan[3], cocaine[4], morphine[5], anoxia[6,7], ozone[8], dinitrophenol[9] and adrenaline[10,11]; furthermore, it prolongs the action of certain barbiturates[12]. Conversely, a diminished sensitivity to the toxicity of a number of nitriles has been reported[13,14].

As a consequence of certain work on subacute thyroid and monoamine oxidase inhibitor interaction, apparent augmentations were observed in the mortality of hyperthyroid rats receiving high daily parenteral doses of a hydrazide (iproniazid) or a non-hydrazide (*N*-methyl-*N*-benzyl-2-propynylamine hydrochloride; '*MO*–911') enzyme inhibitor. The latter drug was first described by Taylor *et al.*[15,16]. To evaluate this possible hormonal sensitization, four groups of fasted male rats (Wistar strain) (172 gm. mean weight), eight animals to a group, were injected intraperitoneally with doses of '*MO*–911' differing by 0·03 log unit. Four other groups (127 gm. mean weight), previously made hyperthyroid by a 7-day desiccated thyroid (2 per cent) *ad lib.* diet, were injected in an identical manner. Nine hyperthyroid rats (130 gm. mean weight) were injected solely with normal physiological saline solution and served as controls. All animals were housed at a temperature of 25–27° C. and were provided prior to monoamine oxidase inhibitor medication with oral vitamin A and D (oleum percomorphum) supplementation at 3-day intervals. The acute toxicity results are given in Table 1.

Table 1. ACUTE TOXICITY OF 'MO–911'

Treatment	Dose (mgm./kgm.)	Response (12 hr.)	Dead (per cent)
'MO–911' intraperitoneal	187·5	4/8	50·0
	175·0	2/8	25·0
	163·0	3/8	37·5
	152·4	2/8	25·0
'MO–911' intraperitoneal + thyroid (2 per cent) diet	187·5	8/8	100·0*
	175·0	8/8	100·0*
	163·0	8/8	100·0*
	152·4	8/8	100·0
Thyroid diet controls + 0·14 ml. normal saline intraperitoneal	—	0/9	0

* All deaths within 2 hr. of dosing.

Because a perfect statistical analysis of the respective LD_{50}'s was not possible due to the limited number of animals employed and the one group aberrantly responding to '*MO*–911' at 175 mgm./kgm., only an approximation to this dose for the enzyme inhibitor in the euthyroid animals could be made; this value was found to be 183·5 ± 15·0 (*S.E.*) mgm./kgm. using a graphic method of analysis[17]. It should be noted that in the thyroid-'*MO*–911' treated group all deaths, except one at the 152·4 mgm./kgm. level, occurred within 2 hr. These results show rather conclusively that the acute intraperitoneal toxicity of '*MO*–911' in hyperthyroid rats is markedly increased and that the LD_{50} of the drug lies well below 152 mgm./kgm. in these circumstances.

The cause of this augmented lethality is not easily understood, but because of the early onset of malaise and death, a direct sensitivity to the toxic effects of the monoamine oxidase inhibitor molecule *per se* seems to be the most likely explanation. However, these effects may be possibly related to monoamine oxidase inhibition coupled with the similar enzymatic *in vivo* effects of the thyroid feeding[18,19] resulting in an acute liberation of unbound catecholamines, a diminished ability of the animal to cope with stress and a predisposition to fatal thyrotoxicosis. In any event, this potentiation of toxicity may well explain the reported psychotherapeutic incompatibility of simultaneous medication with an irreversible amine oxidase inhibitor and thyroid extract[20,21].

A more detailed account of these experiments will be published elsewhere.

The '*MO*–911' was generously provided by Dr. G. M. Everett, of Abbott Laboratories, Chicago.

RICHARD N. CARRIER
PAUL V. BUDAY*

Department of Pharmacology,
University of Rhode Island,
Kingston, Rhode Island.

* Present address: Medical Communication Department, Medical Research Section, Lederle Laboratories, Pearl River, New York.

[1] Phillips, P. H., English, H., and Hart, E. B., *J. Nutr.*, **10**, 399 (1935).

[2] Kroneberg, G., and Potzsch, E., *Arch. Exp. Path. Pharmak.*, **216**, 233 (1952).

[3] Houssay, B. A., and Sara, J. G., *Rev. Soc. Argent. Biol.*, **21**, 81 (1945).

[4] Glaubach, S., and Pick, E. P., *Arch. Exp. Path. Pharmak.*, **162**, 537 (1931).

[5] Hunt, R., and Seidell, A., *J. Pharmacol.*, **2**, 15 (1910).

[6] Smith, A. U., Emmens, C. W., and Parkes, A. S., *J. Endocrin.*, **5**, 186 (1947).

[7] Smith, A. U., *J. Endocrin.*, **5**, 64P (1947).

[8] Fairchild, E. J., and Stokinger, H. E., *Fed. Proc.*, **20**, 203 (1961).

[9] Glaubach, S., and Pick, E. P., *Schweiz. Med. Wschr.*, **64**, 1115 (1934).

[10] Kroneberg, G., and Huter, F., *Klin. Wschr.*, **29**, 649 (1951).

[11] Kroneberg, G., *Arch. Exp. Path. Pharmak.*, **216**, 240 (1952).

[12] Conney, A. H., and Garren, L., *The Pharmacologist*, **2**, 82 (1960).

[13] Hunt, R., *J. Biol. Chem.*, **1**, 33 (1905).

[14] Seyle, H., *J. Clin. Exp. Psychopath. Quart. Rev. Psychiat. Neurol.*, **19**, 97 (1958).

[15] Taylor, J. D., Wykes, A. A., Gladish, Y. C., Martin, W. B., and Everett, G. M., *Fed. Proc.*, **19**, 278 (1960).

[16] Taylor, J. D., Wykes, A. A., Gladish, Y. C., and Martin, W. B., *Nature*, **187**, 941 (1960).

[17] De Beer, E. J., *J. Pharmacol.*, **85**, 1 (1945).

[18] Spinks, A., and Burn, J. H., *Brit. J. Pharmacol.*, **7**, 93 (1952).

[19] Zile, M., and Lardy, H. A., *Arch. Biochem. Biophys.*, **82**, 411 (1959).

[20] Bailey, S. d'A., Bucci, L., Gosline, E., Kline, N. S., Park, I. H., Rochlin, D., Saunders, J. C., and Vaisberg, M., *Ann. N.Y. Acad. Sci.*, **80**, 652 (1959).

[21] Kline, N. S., *J. Neuropsychiat.*, **2**, 158 (1961).

High Concentration of (−)-Noradrenaline in *Portulaca oleracea* L.

Portulaca oleracea L. is one of the plants reputed to be of value in the treatment of cardio-vascular diseases in Jamaican folk-lore[1]. It was therefore examined for the possible occurrence of pharmacologically active substances.

A crude protein-free extract was prepared as follows: the fresh plant was macerated in cold 2 *N* hydrochloric acid with a Waring blender. Protein was precipitated by adding ethanol (to approximately 75 per cent) and the supernatant was evaporated at low temperature, under reduced pressure, and in the absence of oxygen to remove the ethanol. The concentrate was diluted with water to an appropriate volume. A strong pressor response was observed when this crude extract was injected intravenously into a dog anæsthetized with nembutal. Because of this response, the possibility of the presence of catechol amines was examined.

The crude extract was examined by paper chromatography using three solvent systems as indicated in Table 1, common catechol amines being used as markers. When the paper was developed with potassium ferricyanide reagent[2], four spots were observed. Three of these spots corresponded to markers of dopa, noradrenaline, and dopamine while the fourth spot is as yet to be identified. The areas of these spots on chromatograms demonstrated approximately equal quantities of noradrenaline and dopamine to be present while the amount of dopa was much smaller.

In order to separate these catechol amines the following procedure was used: the crude protein-free extract was adjusted to *p*H 4 with 10 per cent sodium hydroxide solution and passed through a column of 'Dowex 50–*x*4' (hydrogen form). The catechol amines in the column were eluted with 2 *N* hydrochloric acid. The fraction which contained mainly noradrenaline was subjected to chromatography on sheets of Whatman 3*MM* paper using *n*-butanol/acetic acid/water (4: 1: 5) as the solvent system. The appropriate strips from these sheets (located by trial cuttings developed with the ferricyanide reagent) were cut and eluted with 2 *N* hydrochloric acid. The eluates were pooled and were freeze-dried to a brown powder which gave a single spot on the paper in the same position as a marker of noradrenaline. Quantitative comparison of the extracted material with synthetic noradrenaline hydrochloride on paper revealed that equal amounts of each gave comparable spot areas.

Table 1. PAPER CHROMATOGRAPHY OF CATECHOL AMINES OF THE CRUDE EXTRACTS OF *Portulaca oleracea* L.

System	R_F value						
	Spot I	Dopa	Spot II	Noradrenaline	Spot III	Dopamine	Spot IV
n-Butanol/acetic acid/water (4 : 1 : 5)	0·21	0·21	0·34	0·34	0·41	0·42	0·61
Phenol–water (HCl vapour)	0·16	0·14	0·23	0·23	0·41	0·41	0·54
n-Butanol–0·5 *N* HCl	0·17	0·18	0·12	0·12	0·20	0·20	0·33

The biological activity of the extracted material was then examined. It was found to give a pressor response comparable with that of an equal amount of synthetic (−)-noradrenaline hydrochloride when administered intravenously into dogs anæsthetized with nembutal. This result not only supports the chromatographic evidence for the identity of noradrenaline but also suggests that the noradrenaline present is the biologically active (−)-isomer.

Dopa and dopamine are known to be common plant constituents, but noradrenaline in plants has only been reported previously in banana, plantain, and in potato[3–5]. However, the concentration of noradrenaline found in *Portulaca* is much greater than that in any of these plants. Both by paper chromatography and biological estimation it was found that one of the crude extracts contained noradrenaline approximately equivalent to 2·5 mgm./gm. of fresh plant. It is of interest to note that the concentration of noradrenaline in this plant might be greater than that extractable from suprarenal glands of mammals.

This work forms part of a pharmacological and chemical analysis of some West Indian medicinal plants and is supported by the Tropical Products Institute, Department of Scientific and Industrial Research.

P. C. FENG

Department of Pharmacology,

L. J. HAYNES
K. E. MAGNUS

Department of Chemistry,
University College of The West Indies,
Jamaica.

[1] Asprey, G. F., and Thornton, P., *W.I. Med. J.*, **4**, 152 (1955).
[2] James, W. O., *Nature*, **161**, 851 (1948).
[3] Waalkes, T. P., Sjoerdama, A., Creveling, C. R., Weissbach, H., and Udenfriend, S., *Science*, **127**, 648 (1958).
[4] Udenfriend, S., Lovenberg, W., and Sjoerdama, A., *Arch. Biochem. Biophys.*, **85**, 487 (1959).
[5] Foy, J. M., and Parrott, J. R., *J. Pharm. Pharmacol.*, **12**, 360 (1960).

HISTOCHEMISTRY

Sialic Acid as a Structural Component of Some Mammalian Tissue Cell Surfaces

WHEN human erythrocytes are treated with trypsin, a sialomucopeptide is liberated from their surfaces[1]. It is a common observation that a mucoid material is liberated from mammalian cells by trypsin treatment; the release of this material is associated with a considerable loss of cellular dry mass[2], and alteration of the stickiness of cells for various surfaces *in vitro*[3]. Attempts to investigate the chemical nature of the liberated mucoid[4] have been largely unsuccessful, partly owing to the lack of sensitivity of the tests, and partly because the mucoid cannot be obtained free of cells.

In view of the possibility that the structure of ovine salivary gland mucoprotein put forward by Gottschalk[5] is a general model for the molecular arrangement of mucoprotein at the cell surface, it is important to know whether sialic acid is present at the surfaces of mammalian tissue cells, and if so whether it occurs as a structural element as opposed to a cellular excretion.

A method has recently been developed for measuring the strength of attachment to glass of tissue cells in culture[6]. It consists of growing cells covered by a fluid medium, in circular culture vessels; a rotating disk is lowered into the fluid covering the cells and a shearing stress, transmitted through the fluid, tends to detach the cells from their glass substratum. With a knowledge of the angular velocity of the rotating disk, the viscosity of the culture medium, the distance separating the bottom of the rotating disk from the cells, and the distance of the cells from the centre of the disk, it is possible to apply accurately reproducible and approximately known detaching pressures to the cells. The cells are counted before and after the application of the shearing stresses, and the percentage of cells detached is determined.

It was found that when a cell is removed from a glass substratum under these conditions, separation is not cleanly at the cell surface/substratum interface, but occurs in the cell surfaces, parts of which are left on the glass[7]. Thus this type of peeling-test provides a measure of the cohesiveness or shear-strength of cell surfaces, rather than measurement of the strength of adhesion between cell and substratum. This differentiation between cohesion and adhesion is in accordance with work done in other fields[8,9].

Recently, an attempt has been made to identify structural components in the cell surface, by incubating cultures with enzymes and determining the resulting change in the shear-strength of the cell surfaces as reflected in the percentage of cells detached by various shear stresses.

For these experiments three 'permanent' strains of cells were used, two (16*R* and 16*C*) obtained from late rat fœtal skin and the other from bovine kidney cortex, and also freshly isolated fibroblasts from rat fœtal skin. It was found that in all these cultures crude receptor-destroying enzyme (Burroughs Wellcome) obtained from *Vibrio choleræ*[10,11] weakened the cohesive strengths of the cellular surfaces. These experiments were not entirely satisfactory as a means of identifying sialic acid, however, since this crude enzyme preparation may also contain a mucinase, a trypsin-like enzyme, and an *N*-acetylneuraminic acid aldolase, as well as 0·01 per cent 'Thiomersal' (sodium ethyl-mercuri-thiosalicylate) preservative.

A very small amount of pure recrystallized neuraminidase[12] was then obtained, which was just sufficient to do one series of confirmatory tests on the 16*R* strain of cells. The cells were grown on glass in synthetic medium 199 (Glaxo) plus 15 per cent bovine serum for 18 hr.; they were washed gently with Hanks's balanced saline solution at *p*H 6·9 and counted in delineated areas. Neuraminidase (10,000 units per ml.) in Hanks's fluid was then applied for 60 min. at 37° C., after which the cells were exposed to a shearing stress of approximately 32 dynes/cm.^{2} for 60 sec. and again counted. 16 observations, each on groups of 200–300 cells, were made on the experimental cultures, and eight on the controls which were incubated with Hanks's fluid only. In the neuraminidase-treated group 37·7 per cent ± 2·3 (*S.E.*) of cells were detached as opposed to 21·0 per cent ± 6·0 in the control group. A *t*-test made on the data gave the following results: $t = 3{\cdot}13$, d.f. $= 22$, $0{\cdot}01 > P > 0{\cdot}001$.

The crystalline neuraminidase used in the present experiments has been very carefully prepared and is very specific in its action[13], which is 'the hydrolytic cleavage of the glycosidic bond joining the keto group of *N*-acetyl-neuraminic acid to D-galactosamine'[14].

It is therefore concluded that sialic acid is an important structural component of the cell surface in one strain, and possibly also in the three other cell types treated with the crude enzyme preparation only.

My thanks are due to Dr. G. L. Ada of the Walter and Eliza Hall Institute, Melbourne, for providing me with the sample of crystalline neuraminidase. I am also indebted to my colleague, Dr. Mary R. Daniel, for providing me with the cells.

LEONARD WEISS

Strangeways Research Laboratory,
Cambridge.

[1] Cook, G. M. W., Heard, D. H., and Seaman, G. V. F., *Nature*, **188**, 1011 (1960).

[2] Weiss, L., *Exp. Cell Res.*, **14**, 80 (1958).

[3] Weiss, L., *Exp. Cell Res.*, **17**, 508 (1959).

[4] Weiss, L., M.D. thesis, University of Cambridge (1958).

[5] Gottschalk, A., *Nature*, **186**, 949 (1960).

[6] Weiss, L., *Exp. Cell Res.*, Supp., **8**, 141 (1961).

[7] Weiss, L., *Exp. Cell Res.* (in the press).

[8] Bowden, F. P., and Tabor, D., *Proc. Second Intern. Congr. Surface Activity*, edit. by Schulman, J. H., **3**, 386 (Butterworth, London, 1957).

[9] Bikerman, J. J., *Proc. Second Intern. Congr. Surface Activity*, edit. by Schulman, J. H., **3**, 427 (Butterworth, London, 1957).

[10] Burnet, F. M., and Stone, J. D., *Austral. J. Exp. Biol. Med. Sci.*, **25**, 227 (1947).

[11] Burnet, F. M., McCrea, J. F., and Stone, J. D., *Brit. J. Exp. Path.*, **27**, 228 (1946).

[12] Ada, G. L., and French, E. L., *Nature*, **183**, 1740 (1959).

[13] Ada, G. L., French, E. L., and Lind, P. E., *J. Gen. Microbiol.*, **24**, 409 (1961).

[14] Gottschalk, A., *Biochem. Biophys. Acta*, **23**, 654 (1957).

HÆMATOLOGY

Immunoelectrophoretic Examination of Agammaglobulinæmia

IT is now well established that congenital agammaglobulinæmia is associated with multiple serum protein deficiencies; the sera of these patients lack $7S$ γ-globulins, $19S$ γ-globulins or β_2M globulin, and β_2A globulin[1]. Good *et al.*[2,3] have recently reported that the sera of patients with agammaglobulinæmia contain three proteins not found in normal human sera and they have termed them β_2K, β_2E and γ_1A globulins; their work was performed with antiserum from a horse immunized against human serum by Dr. P. Grabar. A review of our own immunoelectrophoretic patterns as well as those of Grabar *et al.* has failed to reveal any protein constituent other than C-reactive proteins in the sera of agammaglobulinæmic patients which is not ordinarily detectable in normal sera. Consequently the work reported here was undertaken to re-investigate this matter.

The micromethod of Scheidegger was used for immunoelectrophoresis of sera from normal and agammaglobulinæmic individuals[4]. Electrophoresis was carried out for 3½ hr., at 10 V./cm. in an agar gel containing a borate buffer of 0·1 ionic strength at *p*H 8·6. Following electrophoresis, horse antiserum against whole human serum obtained from Dr. Grabar was placed in a long well cut into the agar to one side of the patient's serum but parallel to the axis of the electrophoresis, and horse antiserum against human γ-globulin prepared by us was placed in an identical well placed on the opposite side of the patient's serum (Fig. 1). Both electrophoresis and the diffusion of antisera into the agar occurred at room temperature. The immunoelectrophoretic patterns were then observed at regular intervals for 48 hr.

Fig. 1 shows that all the bands of precipitation which were obtained with agammaglobulinæmic sera were also obtained with normal sera. The band corresponding to β_2K was elicited with both antisera,

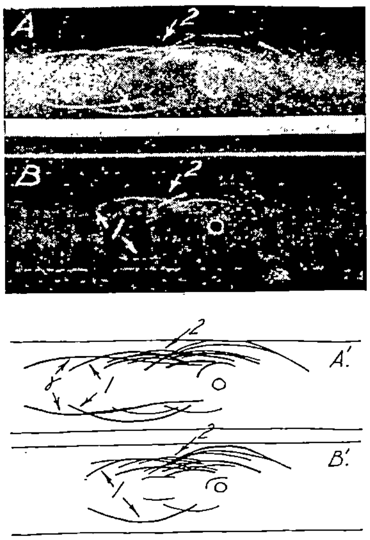


Fig. 1. Immunoelectrophoresis of sera from a normal person (*A*) and from a patient with congenital agammaglobulinæmia (*B*); the arrows marked '1' indicate the protein designated β_2K, those marked '2' indicate that designated β_2E or β_2X and those marked 'γ' indicate the γ-globulins. Figs. *A'* and *B'* are tracings of the precipitation bands seen in Figs. *A* and *B*. Antiserum in the well at upper edge of each figure was horse antiserum versus whole human serum and antiserum in lower well was horse anti-human γ-globulin. Time for diffusion of antiserum was 24 hr.

but the β_2E band was evident only with the antiserum against whole human serum. The identity of the β_2K line formed by each of the two antisera was established by the merging of the ends of these curvilinear precipitation bands. The distance of the vertex of the β_2K band from the electrophoretic axis was the same with both normal and agammaglobulinæmic sera, which suggested that the concentration of the protein component in both sera was similar.

One ml. aliquots of each antiserum were adsorbed with either 0·15 ml. of normal human serum or with 0·15 ml. of a 1:10 dilution of normal human serum. In both instances the adsorbed antisera no longer formed β_2K or β_2E bands with either normal or agammaglobulinæmic sera. These results indicated that normal serum contained β_2K and β_2E globulins in quantities sufficient to remove their respective antibodies from the horse antisera.

The protein which had been designated γ_1A by Good *et al.* had previously been noted by Heremans, who labelled this band of precipitation γ_x-globulin[5]. Heremans noted its occurrence in a variety of pathological states and has shown that it is probably C-reactive protein. The French workers had also noted the band of precipitation designated as β_2E by Good and had variously labelled this band β_2X or β_2B; Heremans stated that it was present in all normal sera which are fresh. A protein corresponding to that called β_2K globulin by Good *et al.* was demonstrated by Scheidegger in the sera of normal persons as well as in the serum of a patient with a dysgammaglobulinæmia characterized by the absence of β_2A and β_2M globulins from the serum. The same horse antiserum against human γ-globulin that we used in the present work had been used in Scheidegger's study[6].

These findings indicate that there is no definite evidence as yet for the presence of abnormal globulins in the sera of patients with agammaglobulinæmia or for the presence in such sera of globulins not demonstrable in normal persons.

This work was supported by grants from the National Institutes of Health, United States Public Health Service.

Fred Rosen*
Charles A. Janeway
David Gitlin†

Department of Pediatrics,
Harvard Medical School,
and the Children's Hospital Medical Center,
Boston, Mass.

* Research Fellow, U.S. Public Health Service.

† Established Investigator, American Heart Association.

[1] Gitlin, D., in *Analyse Immuno-Electrophorétique*, edit. by Grabar, P., and Burtin, P. (Masson, Paris, 1960).

[2] Bridges, R. A., and Good, R. A., *Ann. N.Y. Acad. Sci.*, **86**, 1089 (1960).

[3] Good, R. A., Zak, S. J., Condie, R. M., and Bridges, R. A., *Pediat. Clin. N. Amer.*, **7**, 397 (1960).

[4] Scheidegger, J. J., *Intern. Arch. Allergy App. Immunol.*, **7**, 103 (1955).

[5] Heremans, J., *Les Globulines Sériques du Systeme Gamma* (Masson, Paris, 1960).

[6] Giedion, A., and Scheidegger, J. J., *Helvet. Paediat. Acta*, **3**, 241 (1957).

Nature of the Coating Globulin causing a Positive Antiglobulin Consumption Test

The direct antiglobulin consumption test has been developed in several laboratories and has been applied chiefly to the detection of antibodies that sensitize thrombocytes and leucocytes[1–6].

The test is based on the following principle: Pure, thoroughly washed suspensions of leucocytes and thrombocytes obtained from the blood to be examined are added to an antiglobulin serum of known titre and are left at room temperature for 30 min. The supernatant fluid is then tested for its antiglobulin titre against red blood cells coated with incomplete anti-*D* antibodies. If the cells have been sensitized *in vivo*, a significant drop in the antiglobulin titre occurs, while normal cells do not cause any reduction in titre.

The nature of the coating globulin causing a positive antiglobulin consumption test of thrombocytes and leucocytes has not been defined (ref. 6 and Dausset, J., personal communication), although some attempts have been made in this connexion with the globulin coating of red blood cells causing a positive Coombs test[7,8]. In the present work some experiments are reported which suggest that the coating substance causing a direct antiglobulin consumption test of thrombocytes and leucocytes may be a normal plasma protein attaching itself to the cells under certain conditions. The coating substance must not be an antibody produced as a result of an antigenic stimulus.

Preparation of the anti-human globulin serum, the coating of erythrocytes with incomplete anti-*D* antibodies, the anticoagulant and the washing fluid, as well as the separation of the cells from the blood, were performed as described in ref. 6.

The direct antiglobulin consumption test[6] was performed with some minor changes. To two tubes, each containing 0·5 ml. of anti-human globulin serum, 0·1 ml. of repeatedly washed thrombocytes and 0·1 ml. of repeatedly washed leucocytes, respectively, were added and re-suspended in the serum by gentle shaking. The respective blood cell concentration of the samples in the antiglobulin serum was as follows: thrombocytes 50,000–100,000/mm.^{3}, and leucocytes 40,000/mm.^{3}. The tubes were kept at room temperature (20°–25° C.) for 30 min. They were then centrifuged for 10 min. at 3,000 r.p.m. and the supernatants titrated against $O\ Rh^+$ erythrocytes, 'sensit-

Table 1. Results of Direct Antiglobulin Consumption Test with Leucocytes and Thrombocytes incubated in Autoplasma for Various Times at Different Temperatures

	Separated immediately	After 2 hr.	After 4 hr.	After 6 hr.	After 8 hr.	After 10 hr.	After 12 hr.	After 24 hr.
Leucocytes,								
6° C.	−	−	−	−	±	+	+ +	+ + +
25 °C.	−	−	±	±	+ +	+ + +	+ + +	+ + +
37° C.	−	−	±	+ +	+ + +	+ + +	+ + +	+ + +
Thrombocytes,								
6° C.	−	−	−	−	±	±	+ +	+ + +
25° C.	−	−	+	+	+ +	+ + +	+ + +	+ + +
37° C.	−	±	+ +	+ +	+ + +	+ + +	+ + +	+ + +

+ + +, Strong antiglobulin consumption, that is, positive test.
+ +, +, ±, Degrees of antiglobulin consumption.
−, No consumption, that is, negative test.

ized with incomplete anti-*Rh* antibodies. The titre was expressed in terms of the last tube showing macroscopical agglutination.

Whole blood taken into siliconized syringes containing disodium sequestrene as anticoagulant in a proportion of 1 : 9 was divided into aliquots of equal amounts. From the first the thrombocytes and leucocytes were separated immediately and washed three times. The second sample was kept for various times at either 6° C., 25° C. or 37° C. before the cells were separated and washed. The direct antiglobulin consumption test was then performed at the same time on the cells which had been separated at once from the blood and on those that were incubated before separation. Results of such experiments are summarized in Table 1. It will be seen that a direct relationship could be demonstrated between the temperature, time of incubation and the degree of the antiglobulin consumption. If the cells were immediately separated from the blood after its withdrawal, the antiglobulin test was always negative, while the test became positive after keeping the blood for 4–6 hr. at 37° C. or 8–10 hr. at room temperature and more than 10 hr. in the refrigerator.

Table 1 gives typical results of one such experiment. Twenty blood samples from normal healthy donors were examined and all of them showed similar results, although the degree of the globulin coating *in vitro* of the thrombocytes and leucocytes varied significantly from sample to sample.

The results reported indicate that leucocytes and thrombocytes may absorb *in vitro* plasma proteins, which adhere to the cells very strongly, so that even repeated washings do not separate them. The absorbed protein causes a positive direct antiglobulin consumption test, indistinguishable from the test obtained when cells are sensitized *in vivo* due, presumably, to an immune mechanism.

It is tempting to believe that the globulin coating of the leucocytes and thrombocytes *in vitro* was achieved as a result of some damage to the cell in its abnormal surroundings. The fact that at 37° C. the sensitization by autoplasma proteins was quicker than at 6° C. points to the same conclusion.

If the results obtained *in vitro* may be compared with *in vivo* processes, we may assume that bacterial toxins, breakdown products of drugs, high temperature or any other damage to the cells in the body may change them in such a way as to prepare them to absorb irreversibly normal autoplasma proteins.

The finding, therefore, of a positive antiglobulin consumption test indicates only that the cells are coated with globulin, which may either be an antibody or a normal protein absorbed on a damaged cell.

The clinical significance of the coating as a result of an antibody or the coating as a result of the interaction of the cells with normal autoplasma proteins is not yet clear, although it may be very similar.

This work has been aided by a grant from the Leukemia Research Foundation, Chicago.

D. Nelken

Department of Clinical Microbiology,
Hadassah University Hospital and the
Hebrew University-Hadassah Medical School,
Jerusalem, Israel.

[1] Moulinier, J., *Rev. Franç. Etudes Clin. et Biol.*, 1, 355 (1956).

[2] Butler, K. R., and Moeschlin, S., *Intern. Arch. Allerg. App. Immunol.*, 9, 222 (1956).

[3] Van Loghem, J. J., Van Der Hart, M., Hijmans, W., and Schuit, H. R. E., *Vox Sang.*, 3, 203 (1958).

[4] Dausset, J., and Brecy, H., *Vox Sang.*, 3, 197 (1958).

[5] Steffen, C., *J. Lab. Clin. Med.*, 55, 9 (1960).

[6] Nelken, D., Gurevitch, J., and Gilboa-Garber, N., *Lancet*, i, 742 (1961).

[7] Fudenberg, H., Barry, J., and Dameshek, W., *Blood*, 13, 201 (1958).

[8] Weiner, W., *Blood*, 14, 1057 (1957).

PATHOLOGY

Development in Mice of Prolonged Non-specific Resistance to Sarcoma Implant and Staphylococcus Infection following Repository Injection of Lipopolysaccharide

Previous reports have indicated that the injection of lipopolysaccharides (endotoxin) of Gram-negative organisms exert a profound effect on many vital functions of an animal[1,2]. Particularly striking has been the development of a non-specific resistance against infection[3,5]. More recently, resistance against implantation of malignant tumours has also been demonstrated following treatment with agents stimulating the reticulo-endothelial system, including lipopolysaccharides[6,7]. However, use of lipopolysaccharides clinically has, so far, been frustrated in view of the hazards of fatal shock and/or infection, since the action of the endotoxin is biphasic[4]. For several hours following the injection of the lipopolysaccharide, resistance to infection is markedly decreased, but if the animal survives the shock and other physiological derangements, the resistance to infection is increased manyfold. The administration of lipopolysaccharide is particularly hazardous in experimental animals with latent or low-grade infections, converting these into acute processes often terminating in death[8].

To overcome the hazards of shock and the decreased resistance developed during the negative phase, which is an expression of excess dosage or limited tolerance, it was thought advisable to administer the lipopolysaccharide in repository form. Prepared as a water-in-oil emulsion in which mineral oil is the outer phase, the endotoxin is released slowly owing to the poor metabolism of the oil.

It is the purpose of this communication to summarize the results of experiments with emulsified lipopolysaccharide, in which single large doses (2–4 × *LD* 50) were injected into mice, with the survivors being challenged afterwards in small groups at varying intervals, either by a sarcoma 180 implant, or by an intravenous injection of a virulent staphylococcus. Tables 1 and 2 show the number of animals originally injected with lipopolysaccharide and the number that survived; the number of days post-injection when challenged; the number of survivors for each group; controls (mice receiving the same challenge but pre-treated only with saline); and the increases in the size of the liver and spleen of the endotoxin-treated animals as compared with

Table 1. Survival of Mice following Implantation of Sarcoma-180 after Priming with a Single Injection of Emulsified Endotoxin *

Day of challenge post-injection of endotoxin	No. of animals and material injected	No. of survivors	Weight of organs (mgm.)†	
			Spleen	Liver
2	6 Endotoxin	2	305	1,175
	6 Saline	0	306	1,180
13	5 Endotoxin	2	336	1,710
	5 Saline	0	313	1,550
30	10 Endotoxin	8	528	2,059
	10 Saline	2	370	1,804
44	10 Endotoxin	5	650	2,151
	10 Saline	2	412	1,850
61	10 Endotoxin	6	720	2,320
	10 Saline	2	423	1,872
92	17 Endotoxin	5	745	2,410
	10 Saline	1	525	1,976

* *E. coli* lipopolysaccharide *O*26 (*B*6 Difco). 110 mice injected with 2 mgm./0·1 ml. of emulsion—67 survived.

† One of each of the treated and control groups was killed before challenge.

the controls. One of each group was killed on the varying days of challenge for these measurements, without challenge by either implant or infection.

Analysis of Tables 1 and 2 shows the following: The intramuscular injection of emulsified endotoxin in 1-mgm. doses resulted in the death of only 5 out of 135 animals. On the other hand, 2-mgm. doses resulted in the loss of 43 of 110 mice injected initially. Deaths here, however, cannot be attributed solely to the dosage since on other occasions the same dosage of better emulsions (droplet size 1–2μ) did not kill. The emulsion employed in this instance had shown droplets of about 4μ, which may have contributed to the higher number of deaths.

Resistance against implantation of the sarcoma becomes evident in the pre-treated animal when challenged on the second day post-injection, reaching a peak about the 30th day, and maintaining this resistance as long as 92 days (Table 1). Resistance against the *Staphylococcus* challenge was more striking since 114 of 115 control animals died, whereas the survival-rate in the sarcoma implantation was 20 per cent in 3 control groups. The highest peak of resistance (80 per cent) was noted against sarcoma implant, whereas the resistance against the *Staphylococcus* reached a peak of 60 per cent. However, when consideration is given to the dose of lipopolysaccharide administered and the survival-rate of the control animals, the resistance against the infection becomes most significant.

The enlargement of both liver and spleen following a single repository injection is noteworthy, since the development of non-specific resistance is asserted to be due to the stimulation of the reticulo-endothelial system[3]. These experiments show, however, that resistance is displayed long before there is maximum enlargement of these organs.

Table 2. Survival of Mice following an Intravenous Injection of Hæmolytic *Staphylococcus* after Priming with a Single Injection of Emulsified Endotoxin *

Day of challenge post-injection of endotoxin	No. of animals and material injected	No. of survivors	Weight of organs (mgm.)†	
			Spleen	Liver
8	10 Endotoxin	6	306	1,132
	10 Saline	0	300	1,052
14	10 Endotoxin	4	490	1,192
	10 Saline	0	401	1,094
26	20 Endotoxin	12	540	1,590
	20 Saline	0	420	1,261
40	20 Endotoxin	10	601	1,622
	20 Saline	1	463	1,321
55	20 Endotoxin	8	632	1,810
	20 Saline	0	451	1,681
78	15 Endotoxin	1	459	1,960
	15 Saline	0	450	1,875
90	20 Endotoxin	0	467	1,910
	20 Saline	0	455	1,891

* *E. coli* lipopolysaccharide *O*26 (*B*6 Difco). 135 mice injected with 1 mgm./0·1 ml. of emulsion—130 survived.

† One of each of the treated and control groups was killed before challenge.

Samuel J. Prigal

Department of Medicine (Allergy),
N.Y. Medical College,
Flower-Fifth Avenue Hospital,
New York.

[1] Bohme, D., *Res. Bull.*, **4**, 3 (1958).

[2] Benacerraf, B., and Sebestyen, M. M., *Fed. Proc.*, **16**, 860 (1957).

[3] Biozzi, G., Benacerraf, B., and Halpern, B. N., *Brit. J. Exp. Path.*, **36**, 226 (1954).

[4] Dubos, R. J., and Schaedler, R. W., *J. Exp. Med.*, **104**, 53 (1956).

[5] Landy, M., *J. Exp. Med.*, **103**, 823 (1956).

[6] Biozzi, G., Stieffel, C., Halpern, B. N., and Mouton, D., *Ann. Inst. Pasteur*, **94**, 681 (1958).

[7] Old, L. J., Clarke, D. A., Benacerraf, B., and Goldsmith, M., *Ann. N.Y. Acad. Sci.*, **88**, 264 (1960).

[8] Schaedler, R. W., and Dubos, R. J., *J. Exp. Med.*, **106**, 719 (1957).

The Effect of Various Diets upon Sexual Maturity of Mice

It was demonstrated previously that eggs are carcinogenic in mice[1]. On the other hand, it was found that chicks maintained on an egg diet become sexually mature and the hens begin to lay eggs 6–8 weeks earlier than the controls (chicks of the same breed maintained on a Purina mash diet[2]). The question was consequently raised whether these two phenomena, carcinogenicity and sexual precocity, are caused by a single, or by two different components of the egg. It was important, therefore, to determine the effect of egg-yolk and egg-white upon sexual maturity of mice and compare it with the carcinogenic effect.

Mice of the *T.M.* strain were used in the present experiments. This is an inbred strain of mice developed in the School of Tropical Medicine, and in the 13 years of its existence no malignancies were observed. On a diet supplemented with eggs, however, the mice of this strain developed as high an incidence of cancer as the *A* strain[2]. The mice were divided into 4 groups and at the age of 4 weeks they were placed on the following diets. Group 1, the control, was maintained on the Rockland rat diet, no supplement. For the other 3 groups the Rockland rat diet was supplemented with raw egg-yolk (group 2), hard-boiled egg-white (group 3), or a mixture of lard, cholesterol and sugar (group 4). One egg-yolk, one egg-white or half a gm. of lard mixed with 50–100 mgm. of cholesterol and with half a gm. of sugar were given daily per cage containing 8 mice. The mice of the 4 groups were bred and their offsprings, which were maintained on the same diets, were used in the present experiments.

The age of the mice at the time they gave birth to the first litter varied considerably. As shown in Table 1, the mice of the second group, on the Rockland rat diet supplemented with raw egg-yolk, were the most precocious. They gave birth to the first litter at the average age of 66 days, or 8 days earlier than the controls. On the contrary, the mice on the diet supplemented with egg-white were delayed in their sexual development; they first reproduced at the average age of 94 days, or 21 days later than the controls. These differences between the two experimental groups and the controls appear to be highly significant. The mice of the fourth group, on the diet supplemented with lard, cholesterol and sugar, first reproduced at the average age of 75·4 days, or one

Table 1

Diets	Degrees of freedom	Mean ± S.E.M.*	Variance	Pooled variance (control and experimental)	Standard error of difference between means	Difference between control and experimental means	t	P
1 Rockland rat diet	40	73·5 ± 8·3	68·96	—	—	—	—	—
2 Rockland rat diet + yolk	44	65·8 ± 5·6	31·47	49·32	1·5	7·7	5·13	< 0·1%
3 Rockland rat diet + white	43	94·5 ± 20·5	422·26	251·99	3·4	21·0	6·18	< 0·1%
4 Rockland rat diet + lard and cholesterol	44	74·6 ± 8·4	70·56	69·80	1·8	1·1	0·61	> 20·0%

$$^{*}\,\text{S.E.M.} = \frac{\sqrt{(X - \bar{X})^2}}{n - 1}$$

day later than the controls. This difference, however, does not seem to be statistically significant.

In summary, the yolk of eggs accelerates sexual development, while the white slows it down. Since preliminary results of other experiments indicate that not only the egg-yolk but also the egg-white is carcinogenic, the conclusion can be drawn that the carcinogenic and sex-stimulating substances in eggs are not the same. There is, however, the possibility that a single substance, present in both the egg-white and egg-yolk, is carcinogenic and stimulates sex development, but a second factor present in the egg-white inhibits sexual maturity thus masking the latter effect. Recently, work has been started with alcohol and ether extracts of whole eggs and of yolk. Some of the mice on the alcohol extract have already died of cancer. The same extract, however, does not seem to cause precocious sexual development, which indicates that our first assumption is correct; namely, that there are two different substances in eggs, one carcinogenic and the other one stimulating sex development.

J. SZEPSENWOL

Department of Anatomy,
School of Medicine, University of Puerto Rico,
San Juan, Puerto Rico.

[1] Szepsenwol, J., *Proc. Soc. Exp. Biol. and Med.*, **96**, 332 (1957); **102**, 748 (1959).

[2] Szepsenwol, J., *Poultry Sci.*, **37**, 1350 (1958).

Effect of Alpha-Amylase on the Ehrlich Mouse Ascites Tumour

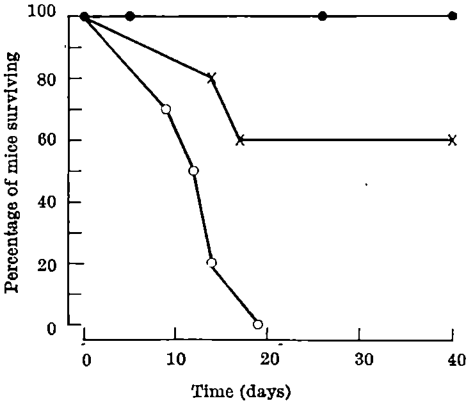
EVEN after incubation in saline at 37° for as long as 24 hr., about $8{\cdot}5 \times 10^{6}$ Ehrlich ascites tumour cells will produce tumour and cause death in 20–26 days when re-injected into new hosts. As part of an attempt to pin-point *in vitro* specific types of damage and then to test the virulence of these damaged cells by subsequent inoculation into mice, Ehrlich ascites tumour cells were exposed to the action of the enzyme α-amylase.

A number of white Swiss mice were inoculated intraperitoneally with 0·3 c.c. of fresh ascitic fluid. After seven days, the ascitic fluid was withdrawn, centrifuged for 10 min. at 4,000 rev./min. and then washed three times with triple volumes of 0·85 per cent saline. 1 c.c. of these preparations of packed tumour cells contained about 34×10^{7} cells (counted in a routine hæmocytometer and diluted in 0·1 per cent citric acid). The packed cells were suspended in twice their volume of saline and incubated at 37° in a water-bath equipped with a shaker following the addition of 25 mgm. of α-amylase (three times recrystallized, Worthington) in 0·5 c.c. water for each 34×10^{7} cells. The control cell flasks received 0·5 c.c. distilled water; 500 units of penicillin per c.c. of incubation mixture were always added. After 3 and 7 hr. respectively, the cell suspensions were diluted with saline, so that 1 c.c. contained $8{\cdot}5 \times 10^{6}$ cells. This amount was injected intraperitoneally into 15 white Swiss mice (weight 20–22 gm.) and their survival-times were followed.

A representative experiment is shown in Fig. 1. Six such experiments were carried out. Mice injected with the 0-, 3- and 7-hr. control mixture showed approximately the same survival-rates, apparently because of the large inoculum of tumour cells (at 0 hr. about 1/10 of this amount would have sufficed for production of ascitic tumour). All mice died of ascites tumour within 20 days after inoculation and 50 per cent mortality occurred on about the twelfth day. Mice injected with cell preparations incubated with α-amylase for 3 hr. showed a 40 per cent tumour mortality within 40 days. Increasing the incubation time with enzyme to 7 hr. resulted in a further loss of virulence, since none of the mice inoculated with these preparations developed an ascitic tumour within 40 days. β-amylase was found to have no effect on the virulence of these tumour cells.

Cell counts carried out at the beginning and end of the incubation showed no appreciable change in number of cells.

DNA and RNA were determined at 0 hr. and after 7 hr. incubation by Schmidt and Thannhauser's method[1]. The DNA phosphorus ranged from $1{\cdot}6$ to $2{\cdot}2 \times 10^{-9}$ mgm./cell and the RNA phosphorus from $3{\cdot}5$ to $4{\cdot}9 \times 10^{-9}$ mgm./cell in different preparations. No appreciable decrease in the tumour cell DNA and RNA content was noted in any experiment.



Percentage survival of mice inoculated with the following cell mixtures. ○—○, Untreated cells incubated for 7 hr.; ×—×, cells incubated with α-amylase for 3 hr.; ●—●, cells incubated with α-amylase for 7 hr.

Cytological preparation from representative incubation mixtures were stained by the Papanicolaou technique and examined without knowledge as to the treatment previously given. Depending on the appearance of their nuclei, cells were considered to be either viable or non-viable. There was no appreciable difference in the proportion of non-viable cells between preparations made from the 0-hr. control mixture and those made after 3 hr. incubation, either with or without α-amylase. There was, however, a marked increase in the ratio of non-viable to viable cells both in the treated and untreated mixtures after 7 hr. incubation. The difference between the two groups at 7 hr. was slight ; about 30 per cent of the control preparations appeared to have remained viable compared with about 20 per cent of the cell suspensions treated with amylase.

Because of the frequent assumption that viable cells are resistant to eosin, all these cell suspensions were examined by Schrek's method[2]. The 0-hr. preparations contained 93–95 per cent, the 7-hr. control preparation about 80–90 per cent and the 7-hr. preparations incubated in the presence of α-amylase about 80–95 per cent resistant to eosin cells. No appreciable amount of material giving a positive reaction with periodic acid–Schiff reagent was found in cell smears from any of the preparations.

Regardless of the unaltered DNA and RNA contents or the lack of apparent cytological distinctions between treated and untreated cells, the tumour cells were impaired in their growth ability after incubation with enzyme for 3 or 7 hr., even after they were returned to an optimal nutritional milieu.

These experiments suggest that a polysaccharide containing 1,4 glycosidic linkages may be important for the virulence of Ehrlich ascites tumour cells.

This work was supported in part by a grant (C–3538) from the U.S. Public Health Service, National Cancer Institute and a grant from the American Cancer Society (Massachusetts Division).

F. STECKERL
A. OFODILE
R. R. CAMPBELL
G. H. FRIEDELL

Department of Medicine and Pathology,
Boston University School of Medicine,
Boston, Mass.

---

[1] Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, **161**, 83 (1945).
[2] Schrek, R., *Amer. J. Cancer*, **28**, 389 (1936).

Evidence that the L-Asparaginase Activity of Guinea Pig Serum is responsible for its Antilymphoma Effects

THE inhibitory action of guinea pig serum on the cells of three transplantable mouse and rat lymphomas *in vivo* was described some years ago in papers from this Laboratory[1,2]. In a continuation of this work, evidence has now been found that the L-asparaginase activity of guinea pig serum is responsible for the antilymphoma effect. The evidence has been obtained in two ways: first, by a study of the induction of resistance to guinea pig serum by 6*C3HED* cells grown in tissue culture; and secondly, by a direct comparison of L-asparaginase activity in various preparations of guinea pig serum with their tumour inhibitory properties.

It was found that 6*C3HED* cells obtained from ascites tumours, and shown to be sensitive to inhibition by guinea pig serum, could be cultivated relatively simply in Eagle's medium[3], which contains L-glutamine, but no L-asparagine. For an initial period of 2–4 weeks growth of the cultured cells was poor, as indicated by a low rate of acid production and diminished cell counts, but with adaptation to the medium the cultures became considerably more vigorous. When, however, cells from the 9 cultures examined were removed after 2–4 weeks incubation and implanted into *ZBC* mice the tumours produced all showed complete resistance to guinea pig serum. Furthermore, the tumour cells having once acquired resistance maintained this character permanently both in prolonged culture and in serial transplantation through *ZBC* hosts. In these features, and particularly in the complete degree of resistance to guinea pig serum, the cultured cells strongly resembled resistant sublines of 6*C3HED* cells obtained by Kidd and Holmquist from tumours treated *in vivo* with small quantities of guinea pig serum[4].

However, the presence of L-asparagine in the medium profoundly modified the characteristics of the culture. In four separate experiments cultures were prepared in parallel using Eagle's medium and a modification containing 200 mgm./l. of both L-glutamine and L-asparagine. The cultures grown in Eagle's medium behaved in the manner already described, initially they grew poorly and when tumour cells were tested after 3 weeks in culture they proved to be fully resistant to guinea pig serum. But in the modified medium growth was more vigorous from the beginning. Cells from the four cultures have been implanted into mice after periods of 12, 11, 9 and 9 weeks incubation respectively. They remain fully sensitive to supression by guinea pig serum.

Modifications of Eagle's medium were also prepared to contain L-aspartic acid, L-alanine, L-arginine, glycine, L-histidine, L-leucine, L-lysine, L-methionine, L-phenylalanine, proline, and L-tyrosine at concentrations of 200 mgm./l. But in these, cultures showed the same growth lag as observed in the original medium and the development of guinea pig serum resistance was completely uninfluenced.

These experiments therefore show the intimate relationship between the metabolic changes induced in 6*C3HED* cells by the absence of an external source of L-asparagine, and the development of resistance to guinea pig serum.

Now guinea pig serum is a particularly rich source of L-asparaginase activity, as was shown by Clementi[5], but activity was not detected in the sera of numerous other species examined. Similarly, Kidd[1] found that antilymphoma activity was confined to guinea pig serum. A comparison of these two activities in preparations derived from guinea pig serum showed a striking correspondence.

Electrophoresis of guinea pig serum by a modification of the starch-block technique of Kunkel and Slater[6] showed that L-asparaginase activity was confined to the α_2 globulin fraction. When eluates from the starch block were injected into groups of 3 *ZBC* mice immediately following the subcutaneous implantation of 5 × 10^4 6*C3HED* cells of the original guinea pig serum sensitive line into each groin, a marked delay in the appearance of tumours occurred in animals receiving fractions which possessed L-asparaginase activity. No other fraction produced any tumour inhibition. Thus, from the fractionation shown in Fig. 1, eluates in tubes numbered 27–30 caused a delay in the first tumour appearance of 3, 5, 7, and 4 days respectively, compared with untreated controls and animals receiving other eluates, in which tumours first appeared in all groups by the

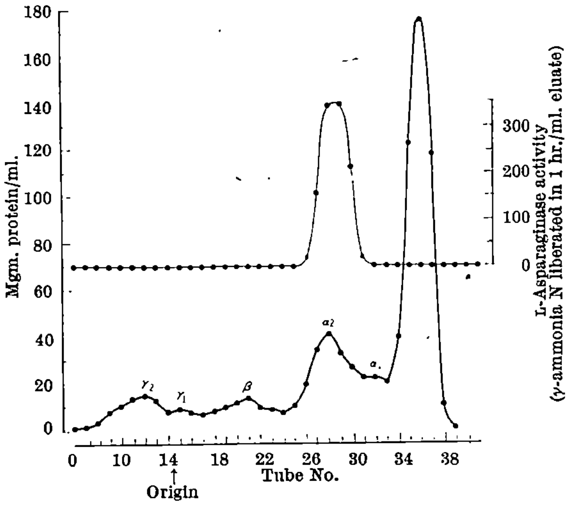


Fig. 1. Electrophoretic pattern of guinea pig serum (in 0·1 *M* veronal buffer, *p*H 8·5) with the localization of L-asparaginase activity

11th day following implantation. Fractions possessing L-asparaginase activity produced complete suppression of many tumours and others appeared at irregular intervals after considerable delay.

Further experiments were performed to examine the stability of L-asparaginase activity in samples of guinea pig serum to changes in temperature and *p*H. Assays of residual enzyme activity provided an accurate correlation with the effectiveness of these samples in producing tumour inhibition.

Ainis *et al.*[7], using the Murphy-Sturm lymphosarcoma, showed that serum from new-born guinea pigs possesses only very weak antilymphoma activity. This finding was confirmed using lymphoma 6*C3HED*. Assays of neonatal serum showed that L-asparaginase activity was only present at 3–15 per cent of adult values.

These experiments provide strong evidence that the inhibitory action of guinea pig serum on 6*C3HED* cells is due to its L-asparaginase activity. It is probable that the antilymphoma action on other sensitive cell strains is by a similar mechanism, and indicates that a distinct form of L-asparagine metabolism is possessed by this group of tumours.

These findings will be published in detail elsewhere. Further experiments are in progress to prepare more highly purified enzyme preparations and to examine the function of L-asparagine in tumour cell metabolism.

I am grateful to Dr. John G. Kidd for his advice and encouragement and to Mrs. P. Moy for much valuable technical assistance.

This work was supported by grants from the American Cancer Society, the National Cancer Institute and Mr. John W. O'Boyle.

J. D. Broome

Department of Pathology,
Cornell University Medical College,
1300 York Avenue,
New York, 21.

[1] Kidd, John G., *J. Exp. Med.*, **98**, 565 (1953).
[2] Kidd, John G., *J. Exp. Med.*, **98**, 583 (1953).
[3] Eagle, H. J., *J. Exp. Med.*, **102**, 595 (1955).
[4] Kidd, John G., *J. Exp. Med.*, **108**, 665 (1958).
[5] Clementi, A., *Arch. Internat. Physiol.*, **19**, 369 (1922).
[6] Kunkel, H. G., and Slater, R. J., *Proc. Soc. Exp. Biol. and Med.*, **80**, 42 (1952).
[7] Ainis, H., Kurtz, H. M., Kramer, P. I., Weimer, H. E., Ryan, R. M., and Jameson. E., *Cancer Res.*, **18**, 1309 (1958).

BIOLOGY

Excretion of Inulin by Malpighian Tubules

In an earlier investigation[1] it has been shown by the use of isotopically labelled compounds that the wall of the Malpighian tubule of the stick insect is permeable to urea and to certain sugars and amino-acids. At that time labelled inulin was not available.

Five Malpighian tubules of the stick insect *Dixippus morosus* were isolated in drops of medium containing one part of stick insect Ringer, one part of stick insect serum, a small quantity of the antibiotic oxytetracycline and inulin labelled with carbon-14 at a concentration of about 0·2 per cent. Urine was collected as described elsewhere[1]. The count-rates of equal volumes of urine and medium were compared (Table 1).

Table 1

Tubule	Urine (*U*) (c.p.m.)	Medium (*P*) (c.p.m.)	*U*/*P*
1	406	8,679	0·047
2	331	8,828	0·038
3	363	9,165	0·040
4	431	9,984	0·043
5	582	9,752	0·060
			Mean = 0·046

In subsequent experiments urine and medium were examined by paper chromatography for evidence of metabolization of inulin; no evidence was found.

The *U*/*P* ratio for inulin may be compared with the *U*/*P* ratios for other carbohydrates: glucose, 0·75; fructose, 0·58; sucrose, 0·58 (ref. 1). On the theory that soluble constituents of the medium enter the urine by diffusion through the wall of the Malpighian tubule[1], this much lower *U*/*P* ratio for inulin is understandable in view of its much greater molecular weight.

J. A. Ramsay
J. A. Riegel*

Zoological Laboratory,
University of Cambridge.

* Postdoctoral Research Fellow of the U.S. Public Health Service. Present address: Department of Biology, Queen's University, Kingston, Ontario, Canada.

[1] Ramsay, J. A., *J. Exp. Biol.*, **35**, 871 (1958).

Composition of the Skin Follicle Population in Seven Rajasthan Sheep Breeds

Early workers found that the wool follicles of domestic sheep are mainly arranged in groups of three primaries with their associated secondaries[1]. The existence of a number of groups with only two or one primary (*P*) follicles was also recognized. The proportion of these 'incomplete' trio groups was considered to be so small as to make a negligible difference in the primary lateral/primary central (*PL*/*PC*) ratio. Ross[2] found a *PL*/*PC* ratio of 1·9, hence most workers have assumed the *PL*/*PC* ratio to be equal to 2.

Counts of trio, couplet, and solitary groups, and the number of associated secondary (*S*) follicles were made at superficial levels on whole sections of biopsy skin samples (taken with a trephine, 1 cm. in diameter) of about two-year-old ewes, of seven Rajasthan breeds. The results are given in Table 1.

Table 1. PERCENTAGES OF DIFFERENT FOLLICLE GROUPS, S/P AND PL/PC FOLLICLE RATIOS, AT MID-SIDE OF RAJASTHAN SHEEP BREEDS

Breed	No. of sheep	Percentage of total number of groups				Mean S/P ratio					PL/PC
		More than 3	Trio	Couplet	Solitary	More than 3	Trio	Couplet	Solitary	All follicles	
Chokla	7	1·8	64·0	12·6	21·6	2·02	2·54	2·65	2·99	2·58	1·41
Magra	7	0·2	60·2	15·5	24·1	2·75	2·26	1·98	2·49	2·28	1·36
Nali	7	2·7	62·8	13·9	20·6	1·22	2·58	2·57	3·00	2·55	1·45
Jaisalmere	8	0·2	64·3	17·7	17·8	3·87	2·46	2·40	3·36	2·53	1·46
Marware	6	0·4	63·1	12·9	23·6	2·25	2·12	2·14	2·66	2·19	1·39
Malpura	7	0·3	46·3	19·6	33·8	1·75	1·78	1·57	1·78	1·74	1·12
Sonadi	8	0·2	37·3	23·6	38·9	1·00	1·14	1·01	1·19	1·13	0·98

The ratio of PL to PC follicles varies from 0·98 to 1·41 in these Indian breeds and is consistently lower than the figure 1·9 observed by Ross[2] in New Zealand Romney N-type sheep. Further, the proportions of trio groups, which varies from 37 to 64 per cent, is much lower than the figures of more than 90 per cent for Romney N-type sheep (Ross[2], quoted by Fraser and Short[3]).

In the hairy group (Malpura and Sonadi) the PL/PC ratio, the mean S/P ratios and percentage of trio groups is lower and of couplet and solitary groups is higher than in the carpet wool group (Chokla, Magra, Nali, Jaisalmere and Marware).

The trio percentage is higher in the carpet wool group and lower in the hairy wool group than the total of couplet and solitary groups. In these hairy breeds, the trio arrangement is apparently not a predominant feature of the grouping of the P follicles.

As adult maturity is approaching, it becomes progressively more difficult to establish the essential arrangement of the primary follicles in their original trio, couplet or solitary configuration. Hence these results need to be corroborated by qualitative and quantitative histological examination of appropriate pre-natal material, from the different breeds, covering the transition from the pre-trio to the post-trio stage in development of follicle population.

In general, the mean S/P ratio of the solitary groups is greater than that of both couplet and trio groups. Differences in S/P ratio between trio and couplet groups are usually small compared with the differences between solitary and trio or couplet groups.

The mean follicle densities of Sonadi (lowest among these breeds) are compared with those of Chokla (highest) in Table 2.

The PC density can be taken as indicating the density of follicle groups, and hence it is seen that the increase in total follicle density from Sonadi to Chokla is associated with substantial increases in both PL follicles (57 per cent) and S follicles (228 per cent) per group, but a much smaller (14 per cent) increase in the density of the groups themselves. The total population of both P and S follicles has also increased from Sonadi to Chokla, with only a very small increase (7 per cent) in the total number of follicle groups.

Stephenson[4] speculated that "central primary anlagen, once present, may inhibit the initiation of new central primaries within a certain radius". The initiation of new PC follicles may possibly depend on skin expansion taking place simultaneously at the required rate. Since PC follicles directly influence the initiation of PL follicles, for a given rate of skin expansion, the rate of formation of P follicles may vary from different positions, and such variation may also occur between different fœtuses. It may be that variations in formation of P follicle are greater for lateral than for central primaries.

Table 2

	PC	PL	S	$PC + PL + S$
Chokla	1·6	2·2	9·5	13·3
Sonadi	1·4	1·4	2·9	5·7
Sonadi adjusted to body-weight of Chokla	1·5	1·5	3·2	6·2

Two probably independent mechanisms seem to influence the number of S follicles per P: one which provides for an increase of S follicles in those breeds with higher PL densities and another which allows the initiation and development of more S follicles around couplet and solitary primaries.

Helpful comments from Mr. H. B. Carter, of the Animal Breeding Research Organization, Edinburgh, Dr. A. G. Lyne and Dr. B. F. Short, of the Commonwealth Scientific and Industrial Research Organization, Australia, and Mr. N. L. Narayan, of the Animal Husbandry Department of Rajasthan, are acknowledged with thanks.

SUNANDA NARAYAN

Sheep Research Station,
Office of the Deputy Director
(Sheep and Wool),
Jaipur.

[1] Carter, H. B., *Anim. Breed. Abstr.*, **23**, 101 (1955).

[2] Ross, J. M., *Austral. J. Agric. Res.*, **5**, 484 (1954).

[3] Fraser, A. F., and Short, B. F., *Tech. Paper* No. 3 (C.S.I.R.O., Australia, 1960).

[4] Stephenson, S. K., *Austral. J. Agric. Res.*, **10**, 453 (1959).

Sex Composition of Lamprey Populations

SINCE 1947 records have been kept of the size and sex composition of annual spawning populations of *Lampetra planeri* in the River Yeo in Somerset. In a previous communication[1] it was reported that up to 1954 there had been marked variations in the sex ratio from year to year and that in general these changes were associated with fluctuations in the relative abundance of this small isolated population. Observations made in subsequent years have confirmed the existence of a positive correlation between abundance and the ratio of males to females; that is, the bigger the population the larger has been the proportion of males. This trend is emphasized in Fig. 1, where the total annual catches have been plotted against the number of males and females separately and the corresponding regression lines fitted to the observations. The lowest observations, which lie close to the intersection of the two regression lines, give sex ratios of 1·2 ♂/♀ for the smallest populations of 50–60, while the highest observed sex ratio of 3·4 corresponds to a relative population of about 200.

In the course of the work on the landlocked sea lamprey (*Petromyzon marinus*) in the Lake Huron–Michigan watersheds, undertaken by the U.S. Depart-

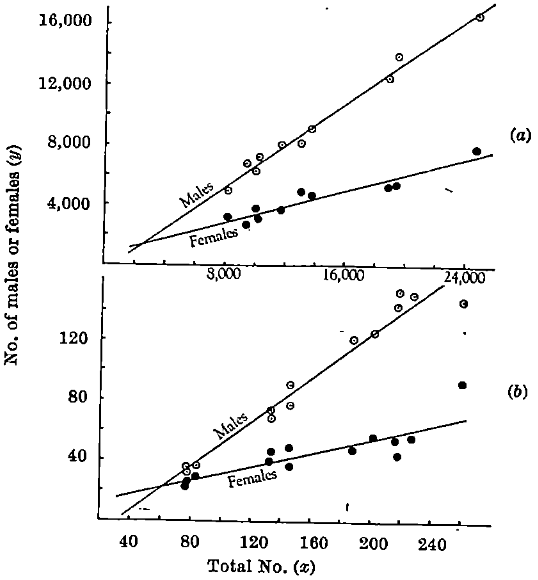


Fig. 1. Changes in the sex ratio of lamprey populations with relative abundance. *a*, *P. marinus* (Oqueoc River, 1944–57): males, $y♂ = -468+0{\cdot}71x$; females, $y♀ = 632+0{\cdot}27x$. *b*, *L. planeri* (R. Yeo, 1947–60): males, $y♂ = -9{\cdot}0+0{\cdot}74x$; females, $y♀ = 10{\cdot}5+0{\cdot}25x$

ment of the Interior, Fish and Wildlife Service, a somewhat similar situation has come to light in the upstream-migrants of this parasitic species. The data on relative abundance and sex composition for the Oqueoc River, supplied by Dr. V. C. Applegate, have been plotted in the same way as the observations on *L. planeri* (Fig. 1) and the corresponding regression coefficients calculated. The similarity in the curves is indeed remarkable; for the male *planeri* the slope of the regression line as given by the regression coefficient *b* is 0·74 compared with 0·71 for *P. marinus*, while the corresponding values for the females are 0·25 and 0·27 respectively. The correspondence is the more striking when it is borne in mind that, whereas the observations on *P. marinus* are based on very large numbers (8,000–24,000), the information on the brook lamprey populations has been limited to annual catches of 50–200.

In view of this correspondence, it is tempting to search for a common explanation for these changes in the sex composition of the spawning populations. However, it is important to recognize that the pattern of changes in relative abundance have been somewhat different in the two species. In the brook lampreys of the Yeo, there have been no continuous upward or downward trends in population size, but rather a series of annual fluctuations with a distinct tendency for large and small populations to follow one another. In the Great Lakes, on the other hand, there appears to have been a continuous increase in the lamprey population during 1944–49 and during this period the relative abundance increased from an estimated 3,300 in 1944, to 24,600 in 1949. In this phase, the sex ratio also increased from 1·65 ♂/♀ in 1947 to 2·13 in 1949. The highest sex ratios were recorded from 1950–52 (2·56—2·58 ♂/♀) but in subsequent years the excess of males tended to decrease and in 1957 the sex ratio was 1·56, that is, slightly lower than in 1947, when records were first made. Although there have been fluctuations in relative abundance, the general trend of the population during the period 1950–57 has been downwards.

Such a close parallel in the sex composition of spawning populations is the more surprising in view of the great divergence in the life-cycle of the two species. Whereas in *L. planeri* spawning takes place within about six months of metamorphosis, in *P. marinus* a period of parasitic life in the Great Lakes, of about two years duration[2], is interposed between metamorphosis and spawning. The existence of a similar relationship between sex ratio and population size, in spite of geographical differences and diversity of life-cycle, suggests that whatever factors may be responsible, their operation should be sought in those phases of the life-cycle which are common to both species. Moreover, since any attempt to account for this relationship must start from the assumption that the sexes are present in approximately equal proportions during the larval stages[3], this would appear to limit the causal period to the critical stage of metamorphosis or to the period of sexual maturity.

The observed variations in the sex ratio could conceivably be explained by assuming that the mortality-rate is dependent on density and higher in the female lamprey than in the male, but while this would be plausible in the large parasitic populations of *P. marinus*, it is unlikely that it could apply to the small Yeo population of brook lampreys which do not feed in the adult state. Moreover, in view of the differences in environment and size of the two forms, similar patterns of mortality due to predation could almost certainly be excluded.

In a detailed study of the brook lamprey population of the River Yeo, which will be published elsewhere, it has been suggested that the spawning class consists of more than one age group and that fluctuations in abundance may be due to differences in the annual recruitment of these age groups. If this hypothesis is extended to include a differential recruitment of the two sexes with respect to their age class, it is possible to explain the sequences of high and low populations and high and low sex ratios which have been observed in brook lamprey populations during 1947–60. A similar situation, it is thought, might exist in the landlocked sea lamprey, a possibility which could be verified by determining the distribution of the sexes in large numbers of the downstream migrants.

I should like to acknowledge with gratitude the help received from Dr. V. C. Applegate of the U.S. Department of the Interior, Fish and Wildlife Service, both in supplying specimens and in furnishing the information on the sea lamprey.

M. W. Hardisty

College of Science and Technology,
Bristol.

[1] Hardisty, M. W., **173**, 874 (1954).
[2] Applegate, V. C., *U.S. Dep. Interior, Fish and Wildlife Serv., Spec. Sci. Rep.* 55 (1950).
[3] Hardisty, M. W., *Nature*, **186**, 988 (1960).

Isolation of *Plasmodium knowlesi* from Philippine Macaques

Between October 1959 and November 1960 blood films were examined for parasites from 164 Philippine long-tailed macaques (*Macaca irus*) at the University of California Medical Center. Ten of the animals were found infected with species of *Plasmodium*. Morphological investigations suggested that several species were present either as single infections or, in several monkeys, as multiple infections. Sub-inoculations into uninfected monkeys were used to help determine the species. Blood from two animals (*MK* 116 and *MK* 119, originally from the Cebu area of the central Philippines) was also sent to the Laboratory of Parasite Chemotherapy in Memphis. This communication has been prepared primarily to record the isolation of *Plasmodium knowlesi* from one of the Philippine macaques. This finding represents a considerable extension of the known range of this parasite which is transmissible to man experimentally and perhaps also in Nature.

On November 15, 1960, 2 ml. of infected blood (scanty plasmodia on thick films) from *M. irus*, *MK* 119, were injected intraperitoneally into an uninfected immature rhesus (*M. mulatta R* 614). Daily blood films from *R* 614 remained negative, and after three weeks it appeared that the transfer had been unsuccessful. On December 5, 1960, *R* 614 was sub-inoculated by intraperitoneal injection of 2 ml. of blood, this time from *M. irus MK* 116. Again the blood was only lightly infected with plasmodia. On the following day a patent plasmodial infection appeared in *R* 614 (5 parasites/1,000 red cells). It later became clear (see later) that this infection was caused by parasites inoculated from *MK* 119 three weeks earlier. The infection evidently became patent as the result of the stimulus due to foreign protein of the second injection of *M. irus* blood.

From December 6 until the work was terminated, blood films were made from *R* 614 every four hours. During a nine-day period beginning on the first post-inoculation day, the plasmodia followed a well-defined 48-hr. asexual cycle with maturation of schizonts being a maximum in the early morning hours, between 1 a.m. and 5 a.m. The blood parasites during this phase of the infection were characteristic of *Plasmodium cynomolgi*, a parasite which has been recorded from Philippine macaques on at least one previous occasion (Garnham, P. C. C., personal communication). Parasitæmia rose to a peak of 75 parasites/1,000 red cells on the eighth day after the *MK* 116 inoculation. On the ninth, tenth, and eleventh days after this inoculation, the parasite counts were much lower, but gametocytes were prominent, at one time comprising 62 per cent of the total count of parasites.

On the eleventh day after inoculation, as the *P. cynomolgi* infection rapidly waned, a second species of *Plasmodium*, morphologically characteristic of *Plasmodium knowlesi*, suddenly appeared. It became apparent later that this second parasite resulted from the *MK* 116 sub-inoculation. The parasites of this species appeared in rapidly increasing numbers during the next three days. Two complete 24-hr. asexual cycles were observed, with maximum maturation of schizonts at about midday. On the evening of the fourteenth day after inoculation, the parasite count was 160/1,000 red cells. The monkey was severely anæmic, pale, and listless. At this time virtually all parasites in the blood-stream appeared to be *P. knowlesi*. 1 ml. of blood was inoculated into a clean *M. irus* (*MK* 117, previously treated with chloroquine and primaquine) to preserve the strain. This transfer was successful. At the same time 50 mgm. of sulphadiazine was administered intramuscularly in an attempt to save the life of the rhesus. This was unsuccessful; the monkey died from the overwhelming *P. knowlesi* infection during the night.

At the same time that these investigations were carried out, *P. cynomolgi* was isolated from *MK* 119 blood at the Memphis laboratory (confirming the suspicion that this species in rhesus *R* 614 had been derived from the first sub-inoculation), and *P. knowlesi* and *P. inui* were isolated from *MK* 116 blood. *P. knowlesi* became patent in Memphis rhesus *X* 509 five days after injection of blood from *MK* 116. For about a week small numbers of parasites were present. A very heavy *P. knowlesi* infection then developed which was successfully treated with chloroquine (50 mgm. a day intramuscularly for three days). Prior to treatment a second rhesus (*X* 536) was inoculated from *X* 509 and *P. knowlesi* infection developed rapidly. Treatment was carried out with sulphamerazine (250 mgm. a day by stomach tube for five days). The *P. knowlesi* parasites disappeared and typical *Plasmodium inui* became evident. Quartan periodicity was demonstrated by segmenter counts of daily blood films.

While *P. inui* was not detected in California rhesus *R* 614 in the face of the vigorous *P. knowlesi* infection, three of the nine other *M. irus* found infected appear to have plasmodia morphologically resembling *P. inui*. *P. inui* has been isolated from Philippine macaques on at least one previous occasion[1].

Plasmodium knowlesi has been recorded previously only from Malaya. The present report extends the known range of the parasite considerably. It is of interest, however, that a variant of *P. knowlesi* was discovered in Formosan macaques (*M. cyclopsis*) by Yokogawa *et al.* in 1941[2]. This variant, *Plasmodium knowlesi* var. *arimai*, was reported to have a 24-hr. asexual cycle, but to be only slightly or moderately pathogenic for rhesus monkeys, and non-infective for man. The Philippine strain of *P. knowlesi*, although not studied in man as yet, is certainly highly virulent in the rhesus and thus appears to be more closely related to the Malayan strain than to the Formosan variant.

Further work on these plasmodia is in progress at both laboratories; for convenience they have been termed the Cebu strains of *P. knowlesi*, *P. inui*, and *P. cynomolgi* respectively.

F. L. LAMBRECHT
F. L. DUNN

George Williams Hooper Foundation,
University of California Medical Center,
San Francisco.

D. E. EYLES

Laboratory of Parasite Chemotherapy,
National Institute of Allergy and
Infectious Disease,
Memphis,
Tennessee.

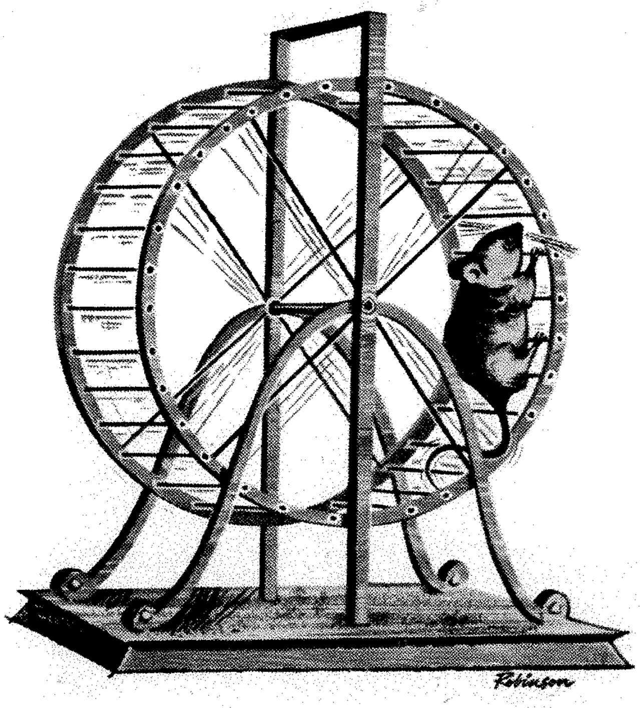
---

[1] Mohiuddin, A., *Rivista di Malariologia*, **36**, 203 (1957).

[2] Yokogawa, S., Kobayashi, H., Ro, M., and Yumoto, Y., *Taiwan Igakkai Zasshi*, **40**, 2173 (1941). Hsien-chen, Hsieh, *Formosan Science*, **14**, 477 (1960).

Excretory Ducts in the Genus *Notopterus* (Lacepede)

THE reduction in size of the cœlom in the genus *Notopterus* has resulted in certain interesting modifications of the alimentary canal, swim-bladder, kidneys and gonads. An anomalous feature has also been observed in the organization of the kidney ducts and urinary bladder.



IT'S A SMALL WORLD

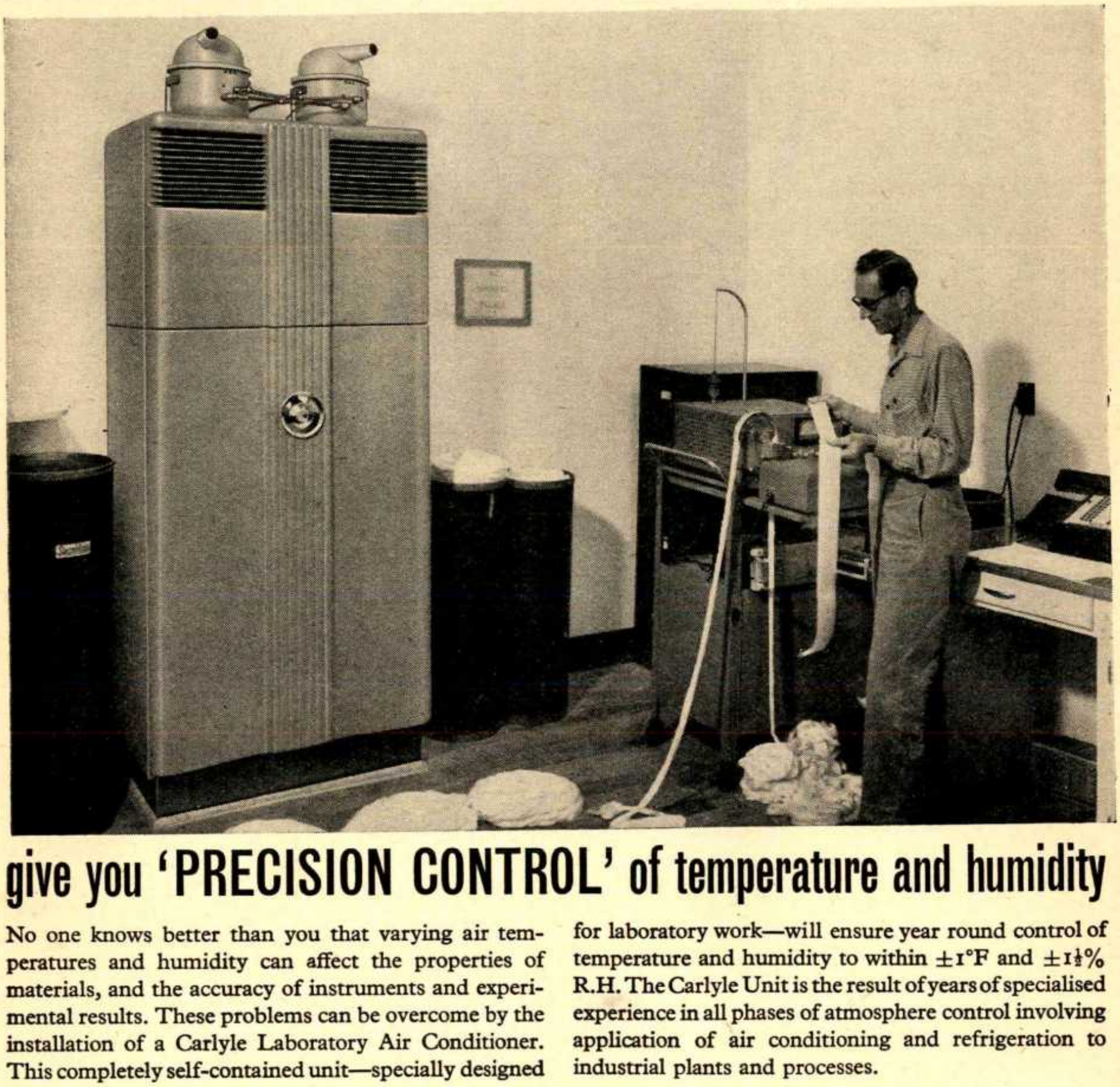
AND MARCHON SURFACTANTS PLAY A BIG PART IN IT

The mouse runs hard in order to stay in the same place. That is how Marchon keeps its lead in the detergent industry. The Marchon factory at Whitehaven produces an ever-increasing range of detergent raw materials to meet the expanding—and exacting—needs of industry all over the world. Marchon is, of course, able to keep its leading position because it supplies high quality materials at economic prices.

Marchon

MARCHON PRODUCTS LIMITED WHITEHAVEN, ENGLAND. MEMBER OF THE ALBRIGHT & WILSON GROUP OF COMPANIES

CARLYLE
LABORATORY CONDITIONERS



give you 'PRECISION CONTROL' of temperature and humidity

No one knows better than you that varying air temperatures and humidity can affect the properties of materials, and the accuracy of instruments and experimental results. These problems can be overcome by the installation of a Carlyle Laboratory Air Conditioner. This completely self-contained unit—specially designed for laboratory work—will ensure year round control of temperature and humidity to within ±1°F and ±1½% R.H. The Carlyle Unit is the result of years of specialised experience in all phases of atmosphere control involving application of air conditioning and refrigeration to industrial plants and processes.

Capacities from 29,000 to 80,000 Btu/hr

Larger capacities are also available on special order. We shall be glad to co-operate with organisations in recommending the unit most suitable for their purpose.

CARLYLE

CARLYLE AIR CONDITIONING & REFRIGERATION LTD

1 King Street, St. James's, London S.W.1 Tel: WHItehall 5356

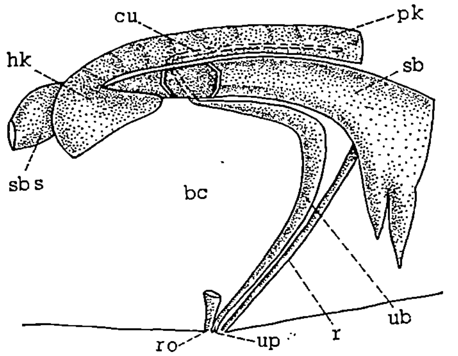


Fig. 1. Lateral view of the excretory organs and swim-bladder in *Notopterus*. *bc*, body cavity; *cu*, common ureter; *hk*, head kidney; *pk*, posterior kidney; *r*, first radial bone; *ro*, rectal opening; *sb*, swim-bladder; *sbs*, sub-spherical sac of swim-bladder; *ub*, urinary bladder; *up*, urinogenital papilla

The typical teleostean excretory organ consists of a pair of kidneys lying retroperitoneally beneath the vertebral column. Each kidney is differentiated into an anterior non-excretory part, the head kidney and a posterior functional part, the mesonephros. The ureter of each kidney proceeds backwards along the dorsal surface of the cœlom to open independently into the urinary bladder. In *Notopterus*, however, the ureters lie deep within the kidney. The two ureters join posteriorly to make a U-shaped bend whence a common, relatively thick-walled ureter runs forward superficially over the entire length of the left mesonephros. The common ureter then enters the swim-bladder, which lies immediately below, courses along its inner partition wall and pierces its tunica and fibrous peritoneal wall to open into a long, curved, tubular urinary bladder which extends as far as the middle of the body cavity.

The fusion of the ureters into a long common duct, its recurrent course along the kidney cephalad and its passage through the swim-bladder before it opens into the urinary bladder in both *Notopterus notopterus* and *Notopterus chitala*, the two available species in India, have not been reported earlier. The arrangement of the ureters and urinary bladder differs fundamentally from that of the typical teleostean excretory ducts.

Bridge[1], who worked on the swim-bladder of *Notopterus borneensis*, did not mention this feature. Also, Srivastava[2] and Dehadrai[3,4], who worked on the swim-bladder of *N. chitala*, failed to notice its association with the excretory system. It is inferred that an intense compression of the body and reduction of the cœlomic cavity have necessitated this change in the organization of kidney ducts and urinary bladder in the genus.

My sincere thanks are due to Dr. M. Chandy for her guidance and to Prof. B. R. Seshachar for helpful suggestions. I am also grateful to the Colombo Plan Authorities for the award of a Technical Co-operation Scheme Fellowship.

MADHU SUDAN SHARMA *

Department of Zoology,
University of Delhi,
Delhi, India.

* Permanent address: Department of Zoology, T. C. College, Tribhuvan University, Kathmandu, Nepal.

[1] Bridge, T. W., *J. Linn. Soc. (Zool.)*, **27**, 503 (1899–1900).
[2] Srivastava, P. N., *Proc. Nat. Acad. Sci. India*, **25**, B, 46 (1955).
[3] Dehadrai, P. V., *J. Zool. Soc. India*, **9** (1), 50 (1957).
[4] Dehadrai, P. V., *Nature*, **185**, 929 (1960).

Stimulants of Cell Division in Developing Fruits

STIMULANTS of cell division have been detected in a number of plant tissues, but little progress has been made in identifying the active substances involved[1]. Work on tobacco pith tissue culture[2] has recently shown that apple fruitlets collected while undergoing cell division contain particularly active stimulants of cell division. We have confirmed this, obtained further information about these stimulants and found that fruitlets of other species are also rich sources of factors stimulating cell division. Some properties of the factors in plum fruitlets have been determined.

The method of detection of these stimulants used in our experiments depended on observing the growth by cell division of explants from secondary phloem of carrot roots in the presence and absence of fruit extracts. Liquid culture methods[3] were employed. Ferric sodium ethylenediaminetetraacetate[4,5] was used as source of iron. All media contained 2 p.p.m. of indole-3-acetic acid, were adjusted to *p*H 6·4 and sterilized by autoclaving. Apple fruitlet extracts were active only in the presence of indole-3-acetic acid.

Sturmer Pippin apple fruitlets showed evidence of an active factor promoting cell division at the time of probable cessation of division (30 days from fertilization[6]) and again 24 days later. Activity was also detected in Granny Smith fruit 58 days from fertilization. No such activity, however, was detected in Sturmer Pippin fruit 110 days from fertilization or in mature fruit. The continued presence of stimulants of cell division in apple fruitlets after the cessation of intense cell division suggests that division is not regulated solely by the presence or absence of such stimulants. We have some evidence that extracts of more mature fruit inhibit the stimulative activity of the fruitlet extracts.

Stimulants of cell division were present in unfertilized fruitlets about to drop and in unfertilized fruitlets artificially held on the tree (by spraying with 2-benzthiazolyloxyacetic acid). Thus, while the development of an apple fruit is normally dependent on successful fertilization of a flower, the presence of stimulants of cell division in the developing receptacle is not entirely dependent on fertilization. Yet excision of the developing seeds, which are known to yield active extracts, renders the growth in sterile culture of fruitlet explants more dependent on the supply of a stimulant of cell division. Hence the developing fruit may depend in part on the developing seeds for a supply of such stimulants. The presence of stimulants has also been detected in other parts of the developing apple cluster (cluster base, fruitlet stalks and petals) and at times in leaves.

The presence of stimulants of cell division in fruitlets of other species collected within a period of 30 days from fertilization has been detected by the same method. In cold 70 per cent ethanol extracts of plum (diameter 7–11 mm.), peach (5–8 mm.) and pear (6–10 mm.) the activities equalled or exceeded that in similar apple fruitlet extracts and were about 40–50 per cent that of coconut milk. Less marked activity was detected in quince and pumpkin fruitlet extracts. No activity was detected in extracts of immature avocado or tomato fruit.

Some properties of the active substances in plum fruitlets have been determined. When the 70 per cent ethanol extracts were evaporated *in vacuo* at

Table 1. Weight and Cell Number of Carrot Tissue after Growth on Media containing Plum Fractions.

Medium*	Final weight of explants† (mgm.)	Final cell number per explant† × 10^{-3}
Basal (*A*)	8·4	57
A + coconut milk (10 per cent by volume)	101·5	483
A + complete water-soluble fraction	13·7	252
A + fraction *AP*	55·6	461
A + fraction *EP*	4·6	43
A + *AP* + *EP*	17·6	279

* All plum fractions assayed at concentrations equivalent to 1 gm. fresh tissue per 100 ml. of medium.

† After growth for 21 days; each value is the mean for 20 explants.

40° C. and the residue extracted with water, all the activity was located in the water-soluble fraction. The activity of this fraction could not be accounted for by its content of amino-acids or chlorogenic acid, substances reported[1,7] to stimulate slightly the growth of carrot tissue. Attempts to concentrate the activity of this fraction by adsorption on, and elution from, charcoal were unsuccessful, even when the strongly adsorbing sites were blocked with palmitic acid. Extraction of an aqueous solution of the water-soluble fraction three times with a double volume of ethyl acetate at *p*H 3·2 yielded an aqueous phase (fraction *AP*) with enhanced activity and organic phases (fraction *EP*) containing factors which retarded both cell division and cell enlargement. The influence of these fractions on the growth of carrot explants is indicated in Table 1.

Fraction *EP* was rich in polyphenolic compounds, two of which have been tentatively identified by paper chromatography as catechin and *epi*-catechin. These two substances have been found to retard the growth of carrot tissue. The phenolic components of fraction *EP* may account for the inhibitory action of this fraction. Ethyl acetate extraction would appear to be a simple, effective method for removing some growth inhibitors from plant extracts containing natural stimulants of cell division. (*Note added in proof.* Sumicki and Kawarda have also used ethyl acetate for this purpose, *Plant Growth Regulation*, 483; Iowa State Univ. Press, 1961).

Extraction by butanol and ion-exchange investigations have both provided evidence for the presence in the plum extract of at least two synergistic factors promoting division. Fraction *AP* was further fractionated by running first through 'Zeo-Karb 225' (H) and then through 'Amberlite *IR*-4*B*' or 'De-Acidite *G*' (free base). Activity was confined to the fraction obtained by elution of the 'Zeo-Karb' with 1·5 *N* ammonia after first removing adsorbed material with 70 per cent alcohol, and to the fraction not adsorbed on either resin. Activity of each was slight, but in combination they yielded 90 per cent of the original activity.

The factors in plum fraction *AP* differ from kinetin both chemically and biologically. Extraction with diethyl ether at *p*H 7 yielded inactive ether extracts, all the activity being accounted for by the extracted aqueous phase. Kinetin would be extracted by ether[8]. Fraction *AP* will not replace kinetin in satisfying the non-photosynthetic red-light requirement of *Spirodela oligorrhiza* (analogous to that of *Lemna minor*[9]) and in this respect resembles coconut milk. The factors in plum extract also resemble those in coconut milk in that they can be divided by ion exchangers. However, the 'neutral' fraction from coconut milk is itself completely inactive[1], whereas that from plum extracts promotes division slightly. The activity (increase in cell number per unit dry weight) of plum fraction *AP* is ten times that of coconut milk.

D. S. Letham
E. G. Bollard

Fruit Research Division,
Department of Scientific and Industrial Research,
Auckland, New Zealand.

[1] Steward, F. C., and Shantz, E. M., *Ann. Rev. Plant Physiol.*, **10**, 379 (1959).

[2] Goldacre, P. L., and Bottomley, W., *Nature*, **184**, 555 (1959).

[3] Caplin, S. M., and Steward, F. C., *Nature*, **163**, 920 (1949).

[4] Street, H. E., *J. Nat. Cancer Inst.*, **19**, 467 (1957).

[5] Street, H. E., McGonagle, M. P., and McGregor, S. M., *Physiol. Plant.*, **5**, 248 (1952).

[6] Bain, J. M., and Robertson, R. N., *Austral. J. Sci. Res.*, B, **4**, 75 (1951).

[7] Shantz, E. M., and Steward, F. C., *Ann. Bot.*, N.S., **23**, 371 (1959).

[8] Miller, C. O., Skoog, F., Von Saltza, M. H., and Strong, F. M., *J. Amer. Chem. Soc.*, **77**, 1392 (1955).

[9] Hillman, W. S., *Science*, **126**, 165 (1957).

Flower Initiation in Kent Wild White Clover (*Trifolium repens* L.), under Controlled Environmental Conditions

Field experiments on seed production in white clover carried out here during 1958–60 have emphasized the importance of time of flowering in relation to grazing management[1].

Under natural field conditions the first signs of flower initiation can usually be seen during the third week in April, although in spaced plants flower initials are laid down earlier. During this period longitudinal sections of the apex of the stolon show the developing inflorescence as a flattened mass of meristematic tissue in the axil of the subtending leaf close to the growing point of the stolon. This meristematic region rapidly develops into a cone-like structure, differentiating individual florets from the base upwards[2,3].

Little critical work has been done on the environmental factors controlling flower initiation in white clover. Laude[4] investigated the effect of photoperiod on flowering in Ladino white clover, and compared plants overwintered outdoors with plants overwintered in heated glasshouses. Cains[5] has observed the effects of seed vernalization on flowering in white clover. Britten[6] has shown that warm-day, cold-

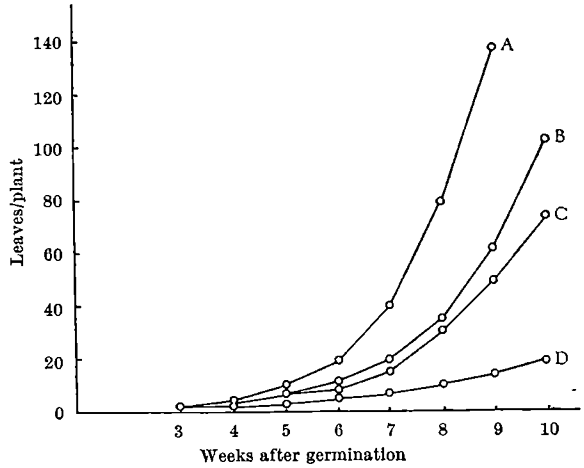


Fig. 1. Photoperiod and vegetative growth. Vegetative growth, measured as leaves per plant, of white clover seedlings grown under four different photoperiods. *A*, 24 hr. continuous illumination; *B*, 20 hr. a day; *C*, 16 hr. a day; *D*, 8 hr. a day

Table 1. Photoperiod and Flowering

Photoperiod	Time to first-flowering (days)	No. of inflorescences per plant
24 hr.	67	8·0
20 hr.	102	5·0
16 hr.	115	2·0
8 hr.	—	0·0

night temperatures can promote flowering in certain 'non-flowering' clones of white clover.

In the present investigations the effect of photoperiod and vernalization on the growth and development of wild white clover plants has been investigated, using controlled growth-room conditions (unpublished results).

Length of day has been found to be the chief environmental factor controlling flowering in white clover. When plants were grown from seed under photoperiods of 8 hr., 16 hr., 20 hr. and continuous illumination at 500 foot-candles and 65° F., flower initiation was observed only in plants in photoperiods of 16 hr. or more. In day-lengths greater than 16 hr. there was a quantitative response in both vegetative growth (Fig. 1) and reproductive development (Table 1) reaching a maximum under continuous illumination. This long-day flowering response in white clover confirms the findings of Laude[4], although he obtained relatively little difference in response to photoperiods of 15½ hr. or greater.

Cold temperatures during seed germination were not found to be essential for subsequent flowering, since some plants grown from non-vernalized seed completed their full cycle of development under long-day photoperiods. However, seed vernalization at 38° F. for 15 days resulted in earlier flowering and increased the number of inflorescences per plant, when grown on under natural long-day photoperiods in the early summer. Vernalization at 38° F. for longer, or shorter, periods than 15 days appeared to depress flowering ability.

Preliminary results for the interaction of seed vernalization and photoperiodism suggest that vernalization becomes more important in promoting flowering in the shorter photoperiods. In this respect, white clover appears to behave similarly to subterranean clover, as reported by Evans[7].

Once flowering has taken place, vegetative plant material has been induced to flower again by limited periods of cold temperature. Vegetative stolon cuttings were given successively longer periods of cold temperature in an unlit refrigerator at 38° F. When grown afterwards in the growth-room at 65° F., flowering occurred earliest under long-day photoperiods from plants which had received 1–2 weeks of cold temperature.

Under field conditions there would appear to be a positive flowering response as a result of the cold temperature received during the autumn and early winter. Plants that had received successively longer periods of natural low temperature during the autumn tended to flower earlier when brought into the growth-room and grown under long-day photoperiods at 65° F. This effect of cold temperature appeared to be satisfied by the beginning of January, since plants brought in thereafter showed little further increase in flowering response.

In the field it has been observed that some plants initiate flowers early in the spring and continue producing flowers for several weeks, whereas other plants may start flower initiation later in the spring and continue flowering for only a few days. This wide range of response is probably genetical and offers considerable scope for plant selection for time of flowering.

These findings have shown that Kent wild white clover is a long-day plant and that flowering can be stimulated by limited periods of cold temperature.

R. J. Haggar

Department of Agriculture,
Wye College
(University of London),
Near Ashford, Kent.

[1] Wye College, *Guide to Dept. Agric.* (1960).

[2] Erith, A. G., *Monograph on White Clover* (Duckworth and Co., London, 1924).

[3] Aitkin, Y., *Nature*, **187**, 622 (1960).

[4] Laude, H. M., *Agron. J.*, **50**, 223 (1958).

[5] Cains, D., *N.Z. J. Sci. Tech.*, **22**, A, 279 (1941).

[6] Britten, E. J., *Science*, **131**, 100 (1960).

[7] Evans, L. T., *Austral. J. Agric. Res.*, **10**, 1 (1959).

MICROBIOLOGY

Metabolic and Genetic Investigations of Nutritionally Exacting Strains of *Staphylococcus pyogenes*

Coagulase-positive strains of *Staphylococcus pyogenes*, taken at random from material phage-typed at Hammersmith Hospital and at the Central Public Health Laboratory, Colindale, were tested for their nutritional character. All but 50 of the 926 strains grew well on a defined medium (based on that of Fildes *et al.*[1]) containing valine, leucine, glycine, proline, aspartic acid, methionine, phenylalanine, arginine, histidine, cysteine, thiamine and nicotinic acid in a glucose-citrate-salts-agar base. Of the 50 exacting strains, all but 4 grew on the medium further supplemented as follows : tryptophan, 12 strains ; threonine, 6 strains ; alanine, 4 strains; tyrosine, 3 strains; lysine, 2 strains; tryptophan and tyrosine, 9 strains; tryptophan and *iso*leucine, 2 strains; tryptophan and biotin, 2 strains; threonine and glutamic acid, 1 strain; biotin, 2 strains; riboflavine, 1 strain; hypoxanthine, 2 strains.

Non-exacting variants were obtained from many of the exacting strains when large inocula were plated on the defined medium. The strain requiring threonine and glutamic acid gave variants able to dispense with threonine, and others able to dispense with glutamic acid, but none able to dispense with both amino-acids. This suggests that the two requirements result from two genetic lesions, each capable of repair by mutation. Some strains yielded only small-colony-type non-exacting variants, like those resulting from suppressor mutations in some nutritionally exacting mutants of *Salmonella typhimurium*.

Of the strains responding to tryptophan, some responded to anthranilic acid, indole or tryptophan, some to indole or tryptophan, some only to tryptophan; these categories correspond to classes of auxotrophic mutants in *Salmonella typhimurium* with blocks at different points in the biosynthetic path to tryptophan. None of the strains requiring both tryptophan and tyrosine responded to shikimic acid, the common precursor of the three aromatic amino-acids (of which phenylalanine was present in the standard medium); furthermore some of these strains utilized indole, and some indole or anthranilic acid, in place of tryptophan. It is therefore unlikely that they owe their double requirements to a single block in the synthesis of the aromatic amino-acids.

Of the 7 strains responding to threonine, or threonine plus glutamic acid, 2 only responded to homoserine, a precursor of threonine and methionine in *Escherichia coli*[2]. These 2 strains, but not the other 5, failed to grow on threonine-supplemented defined medium from which methionine had been omitted. These 2 strains, needing either threonine and methionine or homoserine, are, we infer, unable to convert aspartic acid to homoserine; and the other 5 threonine-requiring strains unable to convert homoserine to threonine.

We attempted to obtain non-exacting forms from the exacting strains by phage transduction, using typing phage 53, and other typing phages serologically related to it, obtained by growth on their respective propagating strains, all of which grew on our defined medium; Morse[3] showed that phage 53 obtained by induction of a lysogenic strain transduced fermentation and drug-resistance characters. Transduction of the non-exacting character was observed in all the strains responding to threonine, and in some with requirements for tryptophan, tyrosine, alanine, lysine or hypoxanthine. In the threonine-exacting strains with a phage-bacterium input ratio of 5 : 1 about 10^{-7} transductant colony was obtained per phage particle; the rate was lower in the other strains. No minute colonies, such as result from abortive transduction, were seen.

Phage 53 was grown on the two strains responding to threonine or homoserine, and on spontaneous non-exacting variants of them. When either of the exacting strains was treated with phage grown on the other, the yield of non-exacting colonies was only about one-fifth of that evoked by phage grown, instead, on the non-exacting variant. We infer that these 2 strains have non-identical genetic lesions, at sites very closely linked so that their probability of co-transduction is about 0·8. As phage 53 could not be grown on the 5 strains responding only to threonine these strains could only be used as receptors. When any of the 5 was treated with phage grown on either of the 2 strains responding to threonine or homoserine about half the colonies obtained by plating on defined medium supplemented with homoserine had the requirement for homoserine (or threonine) of the donor strain. We infer that the sites of the genetic lesions in the 5 threonine-requiring strains are closely linked to the sites in the homoserine-responding strains, so that the probability of co-transduction is about 0·5.

Thus examination of the minority of strains of *Staph. pyogenes* which are nutritionally more exacting than the majority has provided evidence that the biosynthetic paths to tryptophan and to threonine are similar to those in *Salm. typhimurium* and *Esch. coli*; and that typing phage 53 and some serologically related phages can transduce various nutritional characters. The observed linkage between loci for successive steps in the conversion of aspartic acid to threonine has been found also in *Salm. typhimurium*[4].

JANICE B. EDGAR*
B. A. D. STOCKER

Guinness-Lister Research Unit,
Lister Institute of Preventive Medicine,
Chelsea Bridge Road, London, S.W.1.

---

* Now Mrs. R. K. Haylock, Pathology Department, Royal Children's Hospital, Carlton, N.3, Victoria, Australia.

[1] Fildes, P., Richardson, G. M., Knight, B. C. J. G., and Gladstone, G. P., *Brit. J. Exp. Path.*, **17**, 481 (1936).

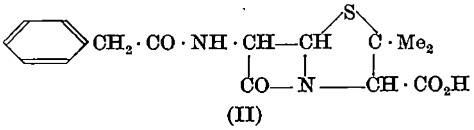
[2] Roberts, R. B., *et al.*, *Carnegie Inst. Wash. Publ.*, **607** (1955).

[3] Morse, M. L., *Proc. U.S. Nat. Acad. Sci.*, **45**, 722 (1959).

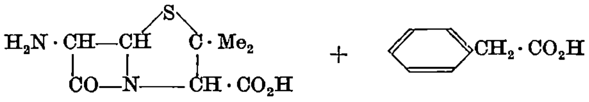
[4] Glanville, E. V., and Demerec, M., *Genetics*, **45**, 1359 (1960).

A Method for isolating Bacteria capable of producing 6-Aminopenicillanic Acid from Benzylpenicillin

BATCHELOR *et al.* discussed the isolation of 6-aminopenicillanic acid (I) from a submerged culture of *Penicillium chrysogenum*[1]. Recently, it was shown that (I) could be prepared by the microbial hydrolysis of benzylpenicillin (II)[2–4].



(II)



(I) (III)

While engaged in the study of resolving amino-acids by metabolism or hydrolysis of soil bacteria on acyl derivatives of DL-amino-acids[5–7], we have found that more than eight strains (*KT* 514, *KT* 513, *KT* 515, etc.) of soil bacteria capable of producing (I) from (II) by metabolism or enzymic hydrolysis could be isolated from four different field areas by using a synthetic medium containing (II) as the sole source of carbon.

The constituents of the culture medium used in the experiments are as follows : potassium benzylpenicillin, 0·93 gm. (0·0025 mole) ; NH_4Cl, 0·1 gm.; K_2HPO_4, 0·1 gm. ; $MgSO_47H_2O$, 0·05 gm. ; 1 per cent $CaCl_26H_2O$, 2 drops ; 1 per cent $FeCl_36H_2O$, 1 drop ; distilled water, 100 c.c. ; *p*H 7·0–7·2. All strains (*KT* 514, *KT* 513, *KT* 515, etc.) could be cultivated successively in the synthetic medium containing phenylacetic acid (III) as well as (II) as the sole source of carbon. They seemed to be rod-shaped in the phenylacetic acid medium, but they had a tendency to form filaments in the benzylpenicillin medium.

When 18·6 mgm. of potassium benzylpenicillin was incubated with 20 mgm. of bacterial acetone powder in 2 c.c. of 0·1 *M* potassium phosphate buffer at *p*H 7·8 and 37° C. for 4 hr. in the presence of toluene, the reaction mixture was found to contain 1·12–0·17 mgm. (that is, 10·4–1·6 per cent of the theoretical quantity) of (I), and (III) (Table 1). (I) was determined by treating a centrifuged sample with phenylacetyl chloride and bio-assaying the (II) formed by a plate diffusion assay. (III) was tested by the method of Pan *et al.*[8].

When ammonium chloride was removed from the benzylpenicillin medium, *KT* 514, *KT* 513, *KT* 515, and *KT* 512 could not or only just be cultivated, and then they scarcely utilized (I) as the sole source of

Table 1. PRODUCTION OF 6-AMINOPENICILLANIC ACID FROM BENZYLPENICILLIN BY HYDROLYSIS OF BACTERIAL ACETONE POWDER

Strains of soil bacteria	6-Amino-penicillanic acid (per cent)	Phenyl-acetic acid	Strains of soil bacteria	6-Amino-penicillanic acid (per cent)	Phenyl-acetic acid
KT 502	2·6	+	*KT* 513	2·3	+
KT 504	6·5	+	*KT* 514	1·6	+
KT 510	5·2	+	*KT* 515	10·0	+
KT 511	10·4	+			
KT 512	7·8	+	*KT* 517	2·6	+

Table 2. Production of 6-Aminopenicillanic Acid from Benzylpenicillin by Metabolism of Bacteria

Strains of soil bacteria	Fermentation time (day)	6-Aminopenicillanic acid (per cent)
KT 514	3	6·0
KT 513	3	5·5
KT 515	3	3·7
KT 512	3	1·5

carbon in the presence of ammonium chloride. Consequently, it is to be understood that *KT* 514, *KT* 513, *KT* 515, and *KT* 512 may produce (I) from (II) by utilizing (III), which was formed from (II) by the action of their 'penicillin acylase', as the sole source of carbon and ammonia as the sole source of nitrogen. A loop of the culture fluid was inoculated into 5 c.c. of the culture medium containing potassium benzylpenicillin and incubated at 25° C. for three days. The fermentation liquor contained 1·62–0·4 mgm. (that is, 6·0–1·5 per cent of the theoretical amount) of (I) (Table 2). The production of (I) was assayed by the microbiological determination mentioned here.

Thus, a new method to isolate bacteria capable of producing (I) from (II) was established.

Yukio Kameda
Yukio Kimura
Etsuko Toyoura
Tomoo Omori

Laboratory of Antibiotics,
Faculty of Pharmacy,
Kanazawa University, Japan.

[1] Batchelor, F. R., Doyle, F. P., Nayler, J. H., and Rolinson, G. N., *Nature*, **183**, 257 (1959).

[2] Rolinson, G. N., *et al.*, *Nature*, **187**, 236 (1960).

[3] Claridge, C. A., *et al.*, *Nature*, **187**, 237 (1960).

[4] Huang, H. T., *et al.*, *J. Amer. Chem. Soc.*, **82**, 3790 (1960).

[5] Kameda, Y., *et al.*, *Nature*, **169**, 1016 (1952); **170**, 888 (1952); **181**, 1225 (1958); **182**, 453 (1958).

[6] Kameda, Y., *et al.*, *Yakugaku Zasshi*, **78**, 202, 748, 754, 759, 763, 765, 767, 769 (1958); **80**, 362 (1960).

[7] Kameda, Y., *et al.*, *Chem. Pharm. Bull.*, **6**, 321, 394, 395, 441 (1958); **7**, 702, 785, 787, 789 (1959).

[8] Pan, S. C., and Perlman, D., *Anal. Chem.*, **26**, 1432 (1954).

Leptospirosis of White Laboratory Rats

Leptospiral infection of white laboratory rats was first reported by Uhlenhuth and Zimmermann[1]. Later it was observed in some stocks in Germany, Holland and Denmark[2]. The infection seemed to be present inapparently in white rats, although some observers emphasized the high incidence of albuminuria in these populations. The examination of single isolated strains and the results of serological screening tests suggested *Leptospira icterohaemorrhagiae* as the causative agent. It was generally assumed that the infection originated from wild rats in the animal house. Human cases were regularly detected in these institutes showing mainly the picture of Weil's disease with a high fatality-rate.

Our investigations in Hungary in 1959 and 1960 showed that leptospiral infection often occurs in large stocks of Wistar rats. The animal sera gave positive sero-reactions of high titre with the sero-types of the *icterohaemorrhagiae* sero-group; with other serotypes only occasional cross-reactions were present. The frequency of sero-positivity varied in the different populations, but in some stocks it ranged to 100 per cent in old animals. The positive sero-reactions indicated that the rats were carriers and *Leptospirae* could be easily cultured from the kidney of these animals. Study of the isolated strains showed that they represent one sero-type, which is closely related to the sero-types of the *icterohaemorrhagiae* sero-group, but they could be differentiated in cross-absorption tests. Therefore the name *Leptospira budapest* was given this sero-type, indicating the place of the first description.

Epidemiological investigations suggested the endemic spread of the infection in different white rat populations. In agreement with our former observations, typical *icterohaemorrhagiae* strains could be isolated from wild rats in Hungary and *Leptospira budapest* was encountered hitherto only in white laboratory rats.

The widespread incidence of leptospiral infection in white rats directs attention to the paramount importance of this problem in institutions where white rats are regularly used for experimental and routine work. Epidemiological surveys and serological screening tests generally revealed human cases in such places, occurring mainly among personnel of the animal house. The presence of the infection in the stocks may affect the experimental results in diets, kidney and liver function tests, determination of the basal metabolism and in histological examinations.

M. Füzi
R. Csóka

Institute of Microbiology,
University of Medicine,
Budapest.

[1] Uhlenhuth, P., and Zimmermann, E., *Deutsch. Med. Wschr.*, **59**, 1393 (1933).

[2] Korthof, G., *Nederl. Tidschr. Geneesk.*, 81, 4571 (1937). Roelcke, K., *Z. Hyg.*, 121, 369 (1938). Blumenberg, W., *Erg. Hyg. Bakt. Immf. Exp. Ther.*, **22**, 168 (1939). Welcker, A., *Zbl. Bakt., I.O.*, **141**, 400 (1938). Jensen, B. H., *Zbl. Bakt. Ref.*, **135**, 366 (1940). Schüffner, W., *Z. Immforsch.*, 99, 323 (1941); **104**, 237 (1943). Scheunert, A., and Gebauer, A., *Z. Immforsch.*, 112, 351 (1955).

GENETICS

Stabilizing Selection for Heading Time in a Bulk-Hybrid Population of Barley

Several workers[1-3] have recently pointed out the importance of the stabilizing type of natural selection, in which the intermediate phenotype has an adaptive advantage over the deviants from such a centrally located norm, thus resulting in a rather stable population mean and a continually diminishing population variance. Robertson[4] has analysed the theoretical implications of two different models of stabilizing selection: the intermediate optimum model of Wright[5], where the more fit individuals are phenotypically intermediate, and the homeostatic model of Lerner[6], in which the favoured individuals are more fit since they are heterozygotes. The latter model involves true overdominance at one or more loci governing fitness or its various components. Hence, it would be of interest to investigate an actual example of stabilizing form of selection for its fit to one or the other model. The present communication summarizes evidence for stabilizing selection for heading time (recorded as the number of days before the first spike comes out of the flag leaf) in a bulk-hybrid population of barley that was derived from a composite cross and propagated over 18 generations under natural self-fertilization without conscious selection, and it further provides some information on the discriminating choice between the two models.

The experimental material consisted of the random samples of single plants in the bulked F_3, F_5, F_6, F_{13}, F_{15}, and F_{18} generations along with all the thirty

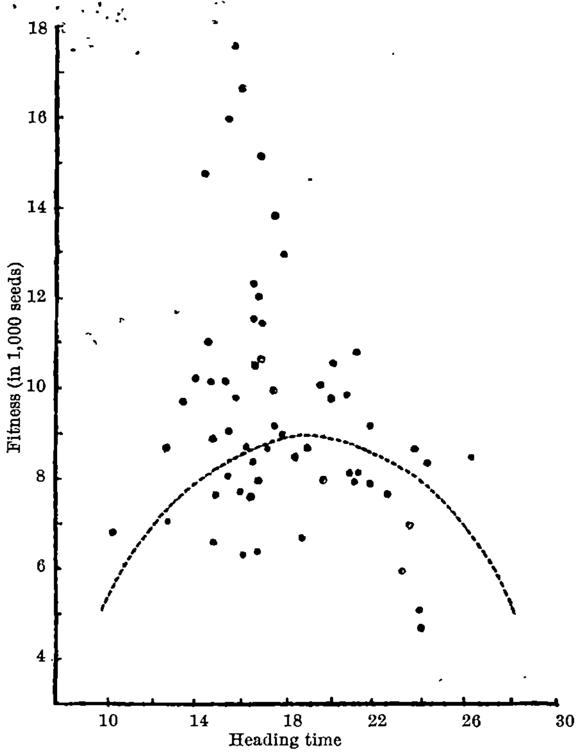


Fig. 1

parents of the composite cross, all planted together in a five-times replicated randomized block design. Table 1 gives the estimates of overall generation means and variances. A relatively small change in the mean towards earliness together with a consistent and striking diminution of the variance clearly suggest a strong pressure of stabilizing selection for intermediate metric values of heading date, since in absence of selection, theoretically, continued inbreeding is expected to bring about an increase in total variance through having subdivided a population into many familial lines. Wright[7] showed that with all genetic variance additive, the among-family-means and within-families variances are simple, linear functions of the inbreeding coefficient, F, such that $\sigma_m{}^2$ (among-families variance) $\propto 2F$, and $\sigma_w{}^2$ (within-families variance) $\propto (1-F)$. The relative proportions of $\sigma_m{}^2$ and $\sigma_w{}^2$, as estimated from progenies of 63 randomly chosen individuals in each of the F_3, F_6, F_{13}, and F_{18} generations, are given in Table 2. For sake of comparison, their estimates from the thirty parental cultures are also included. The values of $\sigma_m{}^2$ indicate a steady decrease in the amount of genetic heterogeneity due to the extinction of certain families under inbreeding and selection. The within-families variance, $\sigma_w{}^2$, is smallest in the parental (P) generation, and although it decreased slowly with inbreeding generations, yet F_{19} gave a higher value of $\sigma_w{}^2$ than P, an interesting observation that might be accounted for by a certain amount of heterozygosity being retained by natural selection even after as many as 18 generations of 98–99 per cent selfing occurring in this material[8]. The frequency distribution for the individual progenies shows only continuous variation which might imply that perhaps none of the segregating and overdominant loci could be singled out as being the major one. It is significant to note, however, that earlier, Jain and Allard[8] reported evidence for a distinct heterozygote advantage at four different marker loci segregating in the present material. The homeostatic model of stabilizing selection deals with precisely such truly overdominant loci.

Table 1. ESTIMATES OF OVERALL GENERATION MEANS AND VARIANCES

Statistic	Generation F_3	F_5	F_8	F_{13}	F_{15}	F_{18}
Mean (days)	18·86	16·94	17·42	16·27	16·47	16·42
Variance	31·76	24·06	25·14	21·75	21·05	18·00

Table 2. ESTIMATES OF AMONG-FAMILIES ($\sigma_m{}^2$) AND WITHIN-FAMILIES ($\sigma_w{}^2$) VARIANCES

Statistic	Generation F_4	F_7	F_{14}	F_{19}	P
$\sigma_m{}^2$	745·61	462·58	528·13	398·36	1,280·97
$\sigma_w{}^2$	10·70	9·80	8·91	7·10	5·28

A further consequence of the homeostatic model can be verified from the present experiment. As pointed out by Robertson[4], unlike its alternative model, the homeostatic model implies that homozygosis would never be complete and that the fitness (measured by the number of seeds produced) and mean heading time of a family would not be correlated. The fitness – heading time graph for F_{19} progenies (Fig. 1) actually shows a very wide scatter of points with reference to the parabolic curve expected under the intermediate optimum model. The absence of any significant correlation here may therefore be taken as further evidence for heterozygote advantage as the basis of stabilizing selection. A detailed report of this experiment is hoped to be published elsewhere.

I wish to thank Mr. C. A. Suneson of the United States Department of Agriculture for providing the seed material, and Prof. R. W. Allard of the University of California, Davis, for his helpful supervision and criticism. This work was carried out at the University of California, Davis, California.

S. K. JAIN

Division of Botany,
Indian Agricultural Research Institute,
New Delhi.

[1] Schmalhausen, I. I., *Factors of Evolution*, 1327 (Blakiston, Philadelphia, 1949).

[2] Mather, K., *Cold Spring Harbor, Symp. Quant. Biol.*, **20**, 158 (1955).

[3] Waddington, C. H., *The Strategy of Genes* (1958).

[4] Robertson, A., *J. Genetics*, **54**, 236 (1956).

[5] Wright, S., *J. Genetics*, **30**, 243 (1935).

[6] Lerner, I. M., *Genetic Homeostasis*, 133 (John Wiley and Sons, New York, 1954).

[7] Wright, S., *Ann. Eugenics*, **15**, 323 (1951).

[8] Jain, S. K., and Allard, R. W., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1371 (1960).

Pulmonary Tumours and Group VII in the House-Mouse

CERTAIN aspects of the experiments reported in Dr. J. A. H. Tatchell's recent communication[1] are likely to result in misunderstanding of the genetic nature of the inheritance of susceptibility to pulmonary tumours in mice. Heston[2] has established that the inheritance of susceptibility to pulmonary tumours in mice is multifactorial in nature and therefore the further investigation of this problem requires an approach designed to take this into account. The experiment reported by Dr. Tatchell does not appear to do so.

I agree that Heston's[3–5] evidence proves the presence of a factor in linkage group VII, but it has not been shown that this is the only factor influencing susceptibility to pulmonary tumours in this linkage group; nor has the possible existence of background effects resulting from segregating loci present in other linkage groups been taken into account. The validity of estimating the recombination frequency depends on the supposition that the classification by the presence or absence of tumours corresponds with the segregation of a single Mendelian gene, and Dr. Tatchell has not given any supporting evidence for this. Even if the classification resulted in a 1 : 1 segregation in backcross matings, this in itself would not be sufficient evidence because, as Wright[6] showed in a well-known paper on polydactyly in guinea pigs, a polygenic character with a threshold, and all-or-none expression, can "simulate one-factor Mendelian heredity to a remarkable extent" in F_1, F_2 and backcross segregations. But the single-factor interpretation "breaks down completely in the tests of the supposed segregants". In addition, because of the segregation of genes at other loci, some mice with the linkage group VII gene may have no tumour, and some mice without the gene may have tumours. Dr. Tatchell states that there was certainly some degree of misclassification but does not indicate how much. Without a knowledge of the accuracy of the classification it is impossible to estimate the true recombination frequency: the results obtained are equally compatible with either loose linkage and accurate classification or close linkage and inaccurate classification.

A further complication is that the treatment used resulted in a 50 per cent loss of experimental animals, which appears to be excessively high; no information is given as to whether or not this loss is randomly distributed between the mutant groups. If the loss were not randomly distributed, so high a mortality might have seriously affected segregation ratios and the apparent recombination frequencies.

In my opinion consideration of these criticisms leads to the conclusion that the approach that Dr. Tatchell has used in this experiment is not valid for this character.

JOYCE L. BLOOM

Institute of Animal Genetics,
West Mains Road,
Edinburgh, 9.

---

[1] Tatchell, J. A. H., **190**, 837 (1961).

[2] Heston, W. E., *J. Nat. Cancer Inst.*, **3**, 69, 79 (1942).

[3] Heston, W. E., *J. Nat. Cancer Inst.*, **2**, 127 (1941).

[4] Heston, W. E., Deringer, M. K., Hughes, I. R., and Cornfield, J., *J. Nat. Cancer Inst.*, **12**, 1141 (1952).

[5] Heston, W. E., *Nature*, **172**, 1007 (1953).

[6] Wright, S., *Genetics*, **19**, 537 (1934).

THE points raised by Dr. Bloom were either fully covered in my communication or else were unsuitable for inclusion in a preliminary account.

I stated that Heston's work *suggests* the presence of one major factor in linkage group VII without excluding minor factors; I did not claim that there is definitely only one factor involved, and I pointed out that his work does not exclude some interaction.

I agree that a polygenic character can "simulate one-factor Mendelian heredity to a remarkable extent", but Fisher's[1,2] later work on polydactyly in mice has clearly shown not only that a major gene can be isolated from its modifiers by refined genetic techniques but also that the demonstration of linkage with another factor is unassailable confirmation of its existence. He has also shown the necessity for having both phases before the evidence for linkage itself is conclusive. This is precisely why I have stated, on one hand, that the lack of linkage between the possible factor (*Tm*) and sex, where both phases are available, is proved, while on the other hand, the putative linkage between *Tm* and group VII, where the coupling phase only is available, is still uncertain. The further experiments in this series are an attempt to give the second phase (repulsion), as stated in my communication. This attempt is itself a test of "the single-factor interpretation" because the repulsion heterozygotes will be "supposed segregants", and thus, if they in turn give single-factor ratios, this will be evidence, additional to linkage, of the presence of the major factor.

The degree of modification of *Tm*, and thus the degree of misclassification, should also be more accurately measured by this further experiment. However, misclassification on the scale proposed by Dr. Bloom would already have been revealed by the more critical analysis which, as I have stated, I will give in my more detailed account. As this analysis agrees well with the simpler treatment, I felt that the latter was more suitable for publication in a preliminary communication, particularly as this was the only one used by previous workers. With minimal misclassification and sufficient numbers, it may now be claimed not only that Heston's "association" with group VII is strongly confirmed but also that, with the choice of factors having reduced known weight interaction, the case for linkage is strong enough to be worth pursuing.

I regret that my footnote to Table 2 did not indicate that the apparently high mortality-rate was due to my having to kill large numbers of my stock because of circumstances beyond my control and unconnected with the experiment. It includes also, of course, the low random litter mortality present in any breeding stock. The mortality-rate after injection was, in fact, extremely low.

In my opinion the application of genetic techniques and mathematical analyses used successfully in other apparently polygenic situations is essential to the discovery of a single major factor in cancer work and that no advance in this direction can be made without it.

J. A. H. TATCHELL

Department of Genetics,
University of Cambridge.

---

[1] Fisher, R. A., *Nature*, **165**, 407 (1950).

[2] Fisher, R. A., *Heredity*, **7**, 91 (1953).

Distribution of X-ray- and Nitrous Acid-induced Mutations in the Genetic Fine Structure of the ad_7 Locus of *Schizosaccharomyces pombe*

GENETIC research of the past ten years has shown that the classical gene can be subdivided by mutation, recombination and function. The region of a chromosome which determines the production of one enzyme can be inactivated by mutation at many independent sites which are separable by recombination. One well-known example is the *r*II region of phage *T*4 (ref. 1. For other genes analysed in micro-organisms see Pontecorvo[2] and Demerec and Hartman[3]).

In fungi, one of the most extensive intragenic recombination analyses has been performed by Leupold in the ad_7 locus of the yeast *Schizosaccharomyces pombe*[4a-c]. Leupold has obtained 153 ad_7 mutants by ultra-violet irradiation. These mutants need adenine for growth and accumulate a red pigment. Most of the ultra-violet mutants cannot grow without adenine at any temperature tested, but six mutants are sensitive to temperature (no growth at 25° C., growth at 35° C.). Using intragenic recombination analyses, Leupold has constructed a linear map of the ad_7 locus (Fig. 1*a*). The ultra-violet mutants are not randomly distributed on the map. There are a few 'hot spots', and the mutation sites appear to be concentrated in several parts of the map. Each ultra-violet mutant seems to be a 'point mutation'.

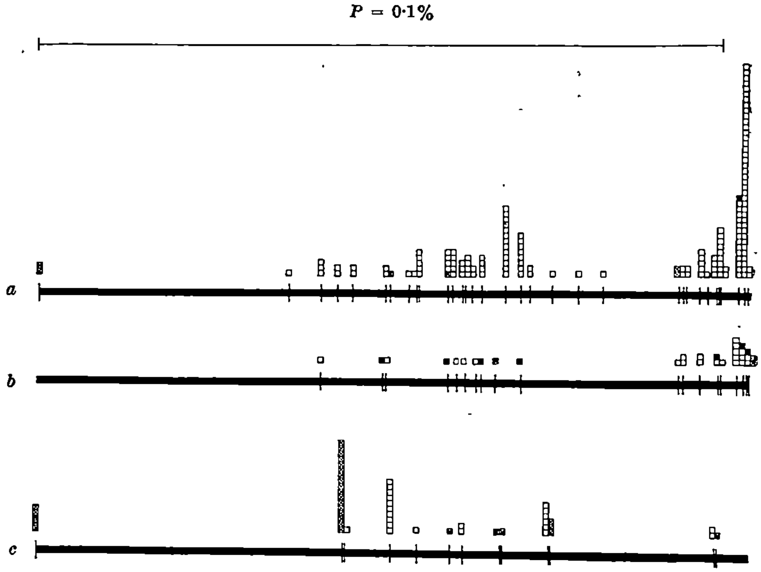


Fig. 1. Map of the ad_7 locus of *Schizosaccharomyces pombe* showing the distribution of the mutants induced by *a*, ultra-violet light (according to Leupold (ref. 4*c*); *b*, X-rays; *c*, nitrous acid. The map has a length of about 0·1 Morgan unit. ☒, Temperature-sensitive; ■, mating type

It was of interest to test the action of other mutagenic agents on the ad_7 locus. First, I chose X-rays. They are known to produce chromosomal aberrations, for example, deletions, and I wanted to see whether gross changes of corresponding types are produced on the intragenic level as well. Secondly, I chose nitrous acid. Nitrous acid causes inactivation and mutations in tobacco mosaic virus[5], bacteriophages[1,6], and bacteria[7]. *In vitro*, it deaminates the purine and pyrimidine bases of the ribonucleic acid[5] and deoxyribonucleic acid (DNA)[8]. Thus nitrous acid is a very interesting mutagen which is able to react directly with the genetic substance by a known mechanism. With respect to the ad_7 locus, one would expect to obtain mutations which are distributed evenly within the map, without the expression of 'hot spots'. In bacteriophage *T*4, however, Benzer[1] has found that the intragenic distribution of nitrous acid-induced *r*II mutants is non-random, suggesting that mutation-rates at individual sites may depend not only on the nature of the base pair involved but also on its position with respect to neighbouring base pairs. In cellular organisms, the situation is complicated further by the fact that the mutagenic action of nitrous acid may in part be an indirect one, affecting the DNA through changes in metabolism rather than by a direct deamination of its amino-bases.

In my X-ray experiments most of the mutants were produced with 180 keV. X-rays at a dose of 30,000 r. For the experiments with nitrous acid we used cells which were starved for 6 hr. in saline. The cells were incubated in acetate buffer *p*H 4·0 plus 0·03 *M* sodium nitrite at 30° C. for 3–5·25 min. This results in an inactivation of about 40–90 per cent. The treated cells were plated on yeast-extract agar. Following incubation at 30° C. the plates were scored for red colonies. I obtained 32 ad_7 mutants with X-rays and 51 with nitrous acid. The mutants were examined by the methods described by Leupold[4a]. The results of the intragenic recombination analyses are represented in Fig. 1*b* and 1*c*.

Each of the 32 X-ray mutants appears to occupy just one site only, no multi-site mutations having been obtained; they have to be considered as 'point mutations'. The distribution of the mutants in the map is similar to that of the ultra-violet mutants. Thus, no striking difference is observed in the distribution of ad_7 mutants induced with ultra-violet light or X-rays. Seven X-ray mutants have not only mutated in the ad_7 locus but also from mating type + to mating type −. It is not yet clear whether there is any causal connexion between the ad_7 mutations and the mutations concerning the mating type.

The ad_7 mutants obtained with nitrous acid are quite different from the ultra-violet and X-ray mutants. 29 out of the 51 mutants are sensitive to temperature. It is very striking that they show pronounced 'hot spots' different from those of the ultra-violet and X-ray mutants (Fig. 1*c*). It should be mentioned especially that no nitrous acid-induced mutations were found at the 'hot spots' on the right end of the ultra-violet map, and that a 'hot spot' of temperature-sensitive mutants is present in the left part of the map, at a site at which neither ultra-violet nor X-ray mutations has been observed.

The fact that nitrous acid-induced mutants show 'hot spots' may be interpreted as indicating that, under our experimental conditions, the nitrous acid has not attacked the DNA directly or only partially so. The mutagenic action of nitrous acid may to a certain degree be an indirect one, inactivating the ad_7 locus at specific sites partially different from those attacked by ultra-violet and X-rays.

The greater part of the work reported was performed during a research visit at the Institute of General Botany, University of Zurich, Switzerland, and has been supported by grants of the Deutsche Forschungsgemeinschaft, the Schweizerische Nationalfonds, and the Julius-Klaus-Stiftung, Zurich.

HERBERT GUTZ

Institut für Gärungsgewerbe der
Technischen Universität,
Berlin, N 65.

[1] Benzer, S., *Proc. U.S. Nat. Acad. Sci.*, **47**, 403 (1961).

[2] Pontecorvo, G., *Trends in Genetic Analysis* (New York, 1958).

[3] Demerec, M., and Hartman, P. E., *Ann. Rev. Microbiol.*, **13**, 377 (1959).

[4] Leupold, U., *a*, *Schweiz. Z. allg. Path. Bakt.*, **20**, 535 (1957); *b*, *Cold Spring Harbor Symp. Quant. Biol.*, **23**, 161 (1958); *c*, *Arch. Julius-Klaus-Stiftung Zürich* (in preparation).

[5] Schuster, H., Gierer, A., and Mundry, K. W., *Abh. Deutsch. Akad. Wiss., Berlin*, 76 (1960).

[6] Vielmetter, W., and Schuster, H., *Z. Naturforsch.*, **15**, b, 304 (1960).

[7] Kaudewitz, F., *Abh. Deutsch. Akad. Wiss., Berlin*, 86 (1960).

[8] Schuster, H., *Z. Naturforsch.*, **15**, b, 298 (1960).

FORTHCOMING EVENTS

Thursday, September 14—Friday, September 15

Society of Chemical Industry, Food Group (at the Royal Society of Medicine, Wimpole Street, London, W.1), at 9.30 a.m. daily—Symposium on "Recent Advances in Processing Cereals".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Experimental Officer (with a general degree with zoology, botany and chemistry) in the Department of Insecticides and Fungicides—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (September 15).

Lecturer (with high academic qualifications and also teaching or research experience) in Inorganic and Physical Chemistry—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (September 15).

Lecturer (with suitable academic and/or industrial experience) in Mathematics—The Secretary, Royal College of Science and Technology, Glasgow (September 16).

Scientific Officers/Senior Scientific Officers (with a first- or second-class honours degree in a science or engineering subject, and a wide and intelligent interest in scientific matters and an aptitude for administrative work) at the Headquarters Office of the D.S.I.R., Regent Street, London, for scientific administrative work—The Establishment Officer, Headquarters Office, D.S.I.R., 5–11 Regent Street, London, S.W.1, quoting HQ.4 (September 16).

Assistant Hydrographer (marine zoologist or a geographer with suitable training and experience), for work in connexion with an investigation by a small team of the physical, chemical and biological conditions of inshore waters, with particular reference to the biology of sessile marine organisms—The Registrar, University College of North Wales, Bangor, North Wales (September 18).

Director of Combined Studies in the Faculty of Science, to supervise admissions to and the progress of students in the courses leading to the newly instituted degree in which two science subjects are studied to a higher level—The Registrar, The University, Leeds 2 (September 18).

Head (well qualified in botany or zoology and with appropriate teaching, research and industrial experience) of the Department of Biology—The Principal, Brunel College of Technology, Woodlands Avenue, Acton, London, W.3 (September 18).

Research Student in Psychology—The Registrar, The University, Hull (September 18).

Histological Technician, Experimental Officer grade, to be in charge of a service to research workers involving a wide range of methods for research programmes—The Secretary, Institute of Animal Physiology, A.R.C., Babraham, Cambridge (September 23).

Research Assistant in the Department of Botany, Gatty Marine Laboratory, St. Andrews, to assist in cultural, cytological and taxonomic investigations of marine algæ—The Secretary of University Court, College Gate, St. Andrews (September 23).

Assistant Lecturers (2) in Organic, Inorganic or Physical Chemistry—The Registrar, The Manchester College of Science and Technology, Manchester 1 (September 25).

Lecturer (with an honours degree in electrical engineering or physics and experience in some branch of electrical or electronic engineering) in the Department of Electrical Engineering—The Registrar, The University, Leeds 2 (September 25).

Senior Lecturer or Lecturer in Applied Mathematics at the University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (September 25).

Demonstrator in Agriculture—The Assistant Bursar, The University, Reading (September 29).

Principal Lecturer in Polymer Chemistry and Technology in the Department of Chemistry, Metallurgy and Textiles, to teach in H.N.C., Grad. R.I.C. and Dip.Tech. standard courses—The Registrar, Lanchester College of Technology, Priory Street, Coventry (September 29).

Junior Lecturer (preferably interested in cytology, radiation effects, or other aspects of biology with an application to medicine) in the Department of Zoology—The Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1 (September 30).

Lecturer in Cytogenetics in the Department of Agronomy, Waite Agricultural Research Institute, University of Adelaide, Australia, to undertake cytogenetic studies as part of a cereal breeding and genetic research programme—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

Lecturer in Theoretical Physics; a Lecturer in Physics; and Assistant Lecturers in Physics—The Registrar, The University, Manchester 13 (September 30).

Plant Physiologists (2) (honours graduates with research experience in plant physiology) at the Tobacco Research Institute, Division of Plant Industry, C.S.I.R.O., Mareeba, to conduct research into the physiology of the tobacco leaf within one of the following fields: (*a*) morphogenesis, particularly leaf development; (*b*) water relations; or (*c*) photosynthesis and translocation—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 815/33 (October 2).

Lecturer in Biochemistry at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 13).

Chair of Chemistry in the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

Lecturer in Mathematics at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 16).

Lecturer or Senior Lecturer in Mathematics at the University of Tasmania, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 16).

Lecturer in Civil Engineering at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 20).

Lecturer/Senior Lecturer in Applied Mathematics at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 20).

Senior Lecturer in Physics in the School of General Studies, Australian National University, Canberra—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 21).

Fellow in the Department of Demography, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 27).

Assistant Lecturer (Grade B) in Mechanical Engineering—Clerk to the Governing Body, Borough Polytechnic, Borough Road, London, S.E.1.

Chemist (male, with an honours degree in chemistry or an equivalent qualification, and not less than two years postgraduate experience or training) in the Department of Agriculture, Tanganyika, for research work on coffee, including soil and plant analysis connected with nutritional problems and soil survey work—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting BCD.63/8/020/T.

Fishery Research Officer (male, aged 22–35, with a good honours degree in natural science (zoology), some training in fish ecology, and preferably experience of handling nets and some acquaintance with hydrographic gear) in Nyasaland, for research into the biology and ecology of Lake Nyasa fish—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting BCD.152/4/01/T.

Instrument Makers or Toolmakers, for varied and interesting work in a small workshop engaged on scientific research—The Administrator, Medical Research Council, Radiobiological Research Unit, Harwell, Didcot, Berkshire.

Lecturer (with a good honours degree in physics and preferably a Ph.D. degree, experimental research experience in some aspects of plasma physics and an interest in high-current research work) in the Department of Natural Philosophy—The Secretary-Treasurer, Royal College of Science and Technology, George Street, Glasgow, C.1.

Physicists or Engineers in the Nuclear Physics Department, to assist in the bubble chamber development programme—Prof. D. H. Wilkinson, Clarendon Laboratory, The University, Oxford.

Research Assistant (with experience of numerical analysis and of high-speed computers) in the Mathematics Department, to be responsible for the day-to-day administration of a National-Elliott 803 digital computer, assist potential users, both from within the College and from outside, and to formulate and programme their problems—Chief Education Officer, Brunel College of Technology, Woodlands Avenue, Acton, London, W.3.

Research Assistants in (*a*) Metallurgy and (*b*) Chemistry—The Registrar, Bradford Institute of Technology, Bradford 7.

Research Associate and a Research Assistant, to join in an investigation of the synthesis and degradation of sulphate esters in mammalian tissues—Prof. Brian Spencer, Trinity College, Dublin, Republic of Ireland.

Research Fellow or Assistant (preferably with experience in programming for a digital computer) in the Department of Aeronautics and Astronautics, for work in the field of hypersonic gas dynamics—The Secretary and Registrar, The University, Southampton.

Scientists, Scientific Officers or Senior Scientific Officers (with a first- or second-class honours degree or equivalent, particularly in chemistry, physics, mathematics or engineering), for work in research and development establishments in the South of England—The Chief Scientist, The War Office, BN., London, S.W.1.

Senior Agricultural Officer (under 55, with a degree or diploma in horticulture or agriculture, a good knowledge of the production of tropical fruit and food crops and temperate vegetables, and preferably a knowledge of applied entomology and agricultural extension methods) in the Bahamas to encourage local production of subsistence crops and tropical fruits and advise on control measures of plant pests—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting RC.213/8/01/T.

Senior Lecturer in Solid Mechanics in the Department of Aircraft Design—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

Senior Research Assistant (with a good honours degree in physics or chemical or mechanical engineering) in the Department of Fuel Technology and Chemical Engineering—The Registrar, The University, Sheffield.

Senior Technical Assistant (preferably with experience in the use of transistors, familiarity with nuclear instrumentation, and some knowledge of workshop practice) in the Physics Department, to help in the design and construction of electronic and other instruments to be used in rockets and artificial Earth satellites—The Secretary and Registrar, The University, Southampton.

Senior Technician in the Chemistry Department, to organize and develop the department stores, and to prepare for their expansion in a new building to be occupied in 1965—The Registrar (CSTS), Queen Mary College (University of London), Mile End Road, London, E.1.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

The Lister Institute of Preventive Medicine. Report of the Governing Body, 1961. Pp. 29. (London: The Lister Institute of Preventive Medicine, 1961.) [67

The Natural Rubber Producers' Research Association. Twenty-third Annual Report. Pp. 49. (London: The Natural Rubber Producers' Research Association, 1961.) [67

Review of Coal Tar Technology, Vol. 12, Part 2: July–December 1960. Pp. 75–158. (Gomersal, Leeds: Coal Tar Research Association, 1961.) 20*s*. per volume. [67

Royal Observatory Bulletins. No. 24: Photometry of the Cluster NGC 2477. By O. J. Eggen and R. H. Stoy. Pp. E27–E35+1 plate. 2*s*. 6*d*. net. No. 35: Time Service 1960, July–September. Pp. B41–B57. 4*s*. net. (London: H.M. Stationery Office, 1961.) [67

National Physical Laboratory, Teddington. Paper D–1: Jet Engine Noise Reduction. By T. J. Hargest. (Presented at a Conference on "The Control of Noise" held at the National Physical Laboratory, Teddington, 26–28 June, 1961.) Pp. 6+6 figures. (Teddington, Mddx.: National Physical Laboratory, 1961.) [67

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1033, Vol. 253 (6 July 1961): Dynamical Theory of Electron Diffraction for the Electron Microscope Image of Crystal Lattices. 1: Images of Single Crystals. 2: Image of Superposed Crystals (Moiré Pattern). By H. Hashimoto, M. Mannami and T. Naiki. Pp. 459–516. 26*s*. No. 1034, Vol. 253 (6 July 1961): Steady-State Thermoelasticity for Initially Stressed Bodies. By A. H. England and A. E. Green. Pp. 517–542. 9*s*. No. 1035, Vol. 253 (6 July 1961): Analysis of the Naphthalene Vapour Absorption Bands at 3200 Å. 1: Naphthalene *h*–8. By D. P. Craig, J. M. Hollas, M. F. Redies and S. C. Wait, Jr. 2: Naphthalene *d*–8. By D. P. Craig and J. M. Hollas. Pp. 543–583. 17*s*. (London: Royal Society, 1961.) [67

Welsh Plant Breeding Station, University College of Wales. Report for 1960. Pp. 129+2 plates. (Plas Gogerddan, near Aberystwyth: Welsh Plant Breeding Station, 1961.) 10*s*. [127

Henry Wiggin and Co., Ltd. The Properties of Applications of 'K' Monel Heat-Treatable Corrosion-Resisting Alloy. Pp. 17. (London and Birmingham: Henry Wiggin and Co., Ltd., 1961.) [127

The Empire Cotton Growing Corporation. Progress Reports from Experiment Stations, Season 1959–60. Northern Nigeria. Pp. 22. 2*s*. 6*d*. Tanganyika Territory—Lake Province. Pp. 13. 2*s*. 6*d*. Nyasaland. Pp. 10. 2*s*. 6*d*. The West Indies. Pp. 10. 2*s*. 6*d*. (London: Empire Cotton Growing Corporation, 1961.) [127

Department of Scientific and Industrial Research: Laboratory of the Government Chemist. Report of the Government Chemist, 1960. Pp. iv+79. (London: H.M. Stationery Office, 1961.) 5*s*. net. [127

Standing Commission on Museums and Galleries. Sixth Report, 1959–60. Pp. iv+64. (London: H.M. Stationery Office, 1961.) 4*s*. net. [127

Library Association Pamphlet No. 21: University Extra-Mural Libraries. By E. P. Pritchard. Pp. 32. 5*s*. (L.A. Members, 3*s*. 6*d*.) Library Association Pamphlet No. 22: A Soviet View of British Libraries. By V. I. Shunkov, G. G. Firsov and N. I. Tyulina. Pp. 32. 5*s*. (L. A. Members, 3*s*. 6*d*.). (London: The Library Association, 1961.) [127

Department of Scientific and Industrial Research. Radio Research 1960: The Report of the Radio Research Board and the Report of the Director of Radio Research. Pp. iv+23+4 plates. (London: H.M. Stationery Office, 1961.) 2*s*. 6*d*. net.

Vision Research, Vol. 1, Nos. 1/2 (June, 1961). An International Journal. Pp. iv+201. Annual subscription rates: (A) for libraries, government establishments, research laboratories, etc. £10 (30 dollars); (B) for individuals who place their orders directly with the publisher and certify that the journal is for their personal use £3 10*s*. (10 dollars). (London and New York: Pergamon Press, 1961.) [127

The Nuffield Foundation. Report for the year ended 31 March 1961. Pp. xv+210. (London: The Nuffield Foundation, 1961.) [127

Political and Economic Planning. Occasional Paper No. 14: Agriculture, the Commonwealth, and EEC. Pp. 61. (London: Political and Economic Planning, 1961.) 6*s*. [127

Building Research Station Digest. (Second Series.) No. 12: Structural Design in Architecture. Pp. 4. (London: H.M. Stationery Office, 1961.) 4*d*. [127

Other Countries

United States Department of the Interior: Geological Survey Water-Supply Paper 1369-C: Surges in Natural Stream Channels. By S. E. Rantz. Pp. iii+77–90. (Washington, D.C.: Government Printing Office, 1961.) 15 cents. [67

Organization for European Economic Co-operation: European Nuclear Energy Agency. New Trends in Atomic Research and their International Significance. By Dr. L. Kowarski. Pp. 36. (Paris: Organization for European Economic Co-operation, European Nuclear Energy Agency, 1961.) [67

Transactions of the American Philosophical Society. New Series, Vol. 51, Part 4: Gladstone–Gordon Correspondence, 1851–1896—Selections from the Private Correspondence of a British Prime Minister and a Colonial Governor. Edited with Introductions by Prof. Paul Knaplund. Pp. 116. (Philadelphia: American Philosophical Society, 1961.) 2.50 dollars. [67

Instituut voor Toegepast Biologisch Onderzoek in de Natuur (ITBON), Arnhem. Werkzaamheden in 1960. (Annual Report of the Institute for Biological Field Research.) Pp. 38. (Arnhem: Instituut voor Toegepast Biologisch Onderzoek in de Natuur, 1961.) [67

United States Department of the Interior: Geological Survey. Bulletin 1081-F: Geology of the Southern Part of the Lemhi Range, Idaho. By Clyde P. Ross. Pp. iv+189–260+plates 7–10. (Washington, D.C.: Government Printing Office, 1961.) [67

Forschungsberichte des Landes Nordrhein-Westfalen. Nr. 974: Behmen Luftelektrische Faktoren Einfluss auf die Aktivitätswechsel Kleiner Insekten, Insbesondere auf die Häutungs- und Reproduktionszahlen von Blattläusen Von Dr. Else Haine. Pp. 80. Köln und Opladen: Westdeutscher Verlag, 1961.) 24.30 D.M. [67

Publications de l'Institut National pour l'Étude Agronomique du Congo. Série Scientifique, No. 90: Relations entre le Couvert Végétal et la Microfaune: Leur Importance dans la Conservation Biologique des Sols Tropicaux. Par M. E. Maldague. Pp. 122. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1961.) 120 francs. [127

Bulletins de la Société Royale de Zoologie d'Anvers. No. 21: Recherches Anatomiques sur Micropolamogale Ruwenzorii. 1: La Morphologie Extreme, les Viscéres et l'Organe Génital Mâle. Par Dr. W. N. Verheyen. Pp. 15+5 planches. 2: La Myologie de la Tête, du cou et de la Patte Antérieure. Pp. 27+10 planches. (Anvers: Société Royale de Zoologie d'Anvers, 1961.) [127

How to Fight Cancer. By Frank Totney. Pp. ii+65. (Durban: Frank Totney, 26 Musgrave Centre, Musgrave Road, 1960.) 35 cents: 3*s*. 6*d*. [127

Records of the Australian Museum. Vol. 25, No. 4: A Review of the Australian Species of Clusiidae (Diptera, Acalyptrata). By David K. McAlpine. Pp. 63–94. Vol. 25, No. 5: The Ecology, Morphology, Distribution and Speciation of a New Species and Subspecies of the Genus *Egernia* (Lacertilia: Scincidae). By Harold G. Cogger. Pp. 95–105+2 plates. (Sydney: The Australian Museum, 1960.) [127

World Meteorological Organization. Annual Report, 1960. Pp. xx+142+50. (Geneva: World Meteorological Organization, 1961.) 10 Swiss francs. [147

Organization for European Economic Co-operation. Agriculture: Production and Consumption Figures. (O.E.E.C. Statistical Bulletins.) Pp. 47. (Paris: Organization for European Economic Co-operation, 1961.) 2 N.F.; 3*s*.: 0.50 dollars; 2 Swiss francs; 1.70 D.M. [147

Institut Royal Météorologique de Belgique. Contributions No. 66: Effects de l'Ultraviolet Lointain Solaire sur l'Atmosphère de la Terre et des Autres Planètes. Rapport introductif par Dr. Marcell Micolet. Pp. 319–368. Publications, Série A, No. 18: Rayonnement a Uccle, Annéé 1959. Pp. v+60. Observations Ionospheriques, Station de Dourbes. Avril 1960. Pp. 26. Mai 1960. Pp. 26. Juin 1960. Pp. 26. Juillet 1960. Pp. 26. (Uccle-Bruxelles: Institut Royal Météorologique de Belgique, 1960 et 1961.) [147

The Annual of Czechoslovak Medical Literature, 1958. By the National Medical Library. Pp. xxiii+624. (Praha: Státni Zdravotnické Nakladatelstvi, 1960.) [177

United States Department of the Interior: Geological Survey Bulletin 1082–I: Geology and Mineral Deposits of the St. Regis-Superior Area, Mineral County, Montana. By Arthur B. Campbell. Pp. iv+545–612+plates 28–33. Bulletin 1082–K: Chromite and other Mineral Deposits in Serpentine Rocks of the Piedmont Upland, Maryland, Pennsylvania, and Delaware. By Nancy C. Pearre and Allen V. Heyl, Jr. Pp. vii+707–833+plates 40–47. Water-Supply Paper 1490: Geology and Ground-Water Resources of Platte County, Wyoming. By Donald A. Morris and Horace M. Babcock. With a section on Chemical Quality of the Water by Russell H. Langford. Pp. vi+195+plates 1–4. Water-Supply Paper 1519: Geology and Ground-Water Resources of the Owl Creek Area, Hot Springs County, Wyoming. By Delmar W. Berry and Robert T. Littleton. Pp. iv+58+plates 1 and 2. Water-Supply Paper 1622: Surface Water Supply of the United States, 1959. Part 1–B: North Atlantic Slope Basins, New York to York River. Prepared under the direction of J. V. B. Wells. Pp. xi+537. (Washington, D.C.: Government Printing Office, 1960 and 1961.) [177

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Geological Maps—Preliminary Series. Map 8–1961: Miminiska, Ontario. (Sheet 52P.) Map 18–1961: North Caribou Lake. (Sheet 53B). (Ottawa: Geological Survey of Canada. [177

International Scientific Radio Union. Proceedings of the 13th General Assembly held in London, September 5–15, 1960. Vol. 12, Part 1: Commission I on Radio Standards and Measurements Methods. Pp. 106. (Brussels: International Scientific Radio Union, 1961.) [177

New Zealand Forest Service: Forest Research Institute. Technical Paper No. 35: The Evolution of Marsupial Reproduction. By R. I. Kean. Pp. 40. (Wellington: Government Printer, 1961.) [177

Southern Rhodesia. Report of the Trustees and Director of the National Museum of Southern Rhodesia for the year ended 31st December, 1960. Pp. 22. (Bulawayo, Salisbury, and Umtali: National Museums of Southern Rhodesia, 1961.) [177

Records of the Australian Museum. Vol. 25, No. 6: Some Australian Tingidae (Hemiptera), including New Genera and New Species. By Carl J. Drake. Pp. 107–114+plates 3–6. Vol. 25, No. 7: A Remarkable Ritual Gallery of Cave Paintings in Eastern New South Wales. By Frederick D. McCarthy. Pp. 115–120+plates 7 and 8. (Sydney: The Australian Museum, 1961.) [177

Metropolitan Life Assurance Company. Statistical Bulletin, Vol. 42 (May 1961): Marriages in the United States and Canada. Trends in Blood Pressure of Individuals. Large Accident Toll in the Vacation Season. The Causes of Premature Death. Pp. 12. (New York: Metropolitan Life Insurance Company, 1961.) [277

United States Department of the Interior: Geological Survey. Bulletin 1121-C: Eolian Deposits of the Matanuska Valley Agricultural Area, Alaska. By Frank W. Trainer. Pp. iii+35. (Washington, D.C.: Government Printing Office, 1961.) [277

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

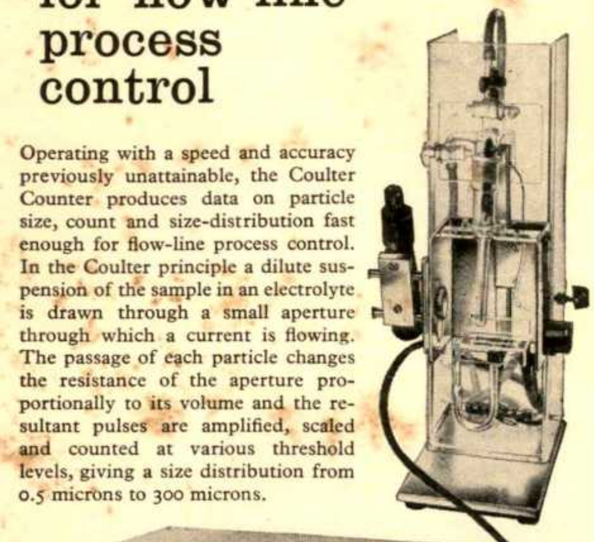
Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office

COULTER COUNTER

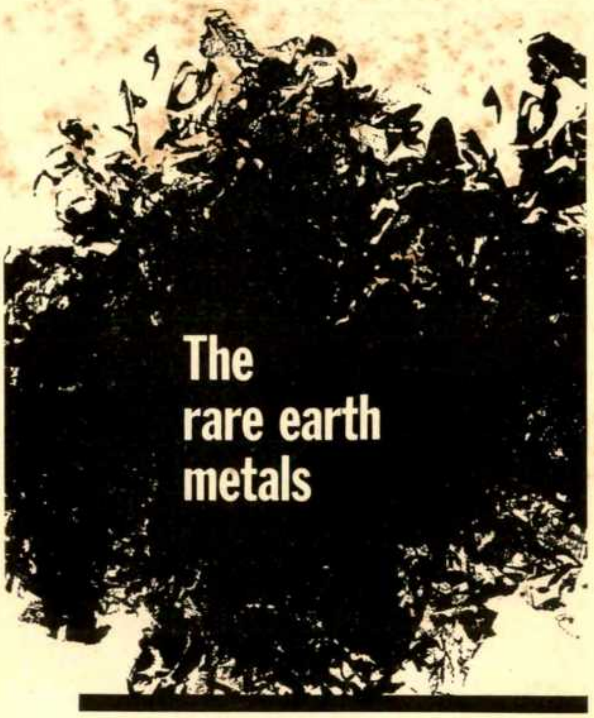
for accurate particle-size distribution data fast enough for flow-line process control

Operating with a speed and accuracy previously unattainable, the Coulter Counter produces data on particle size, count and size-distribution fast enough for flow-line process control. In the Coulter principle a dilute suspension of the sample in an electrolyte is drawn through a small aperture through which a current is flowing. The passage of each particle changes the resistance of the aperture proportionally to its volume and the resultant pulses are amplified, scaled and counted at various threshold levels, giving a size distribution from 0.5 microns to 300 microns.



Over 1,500 Coulter Counters are already in service in industry and medicine. Full details and technical literature will be sent on request.

COULTER ELECTRONICS LTD
2-4 ASHWELL STREET,
ST. ALBANS, HERTFORDSHIRE
Telephone: ST. ALBANS **50828/9**



SCANDIUM
YTTRIUM
LANTHANUM
CERIUM
PRASEODYMIUM
NEODYMIUM
SAMARIUM
EUROPIUM
GADOLINIUM
TERBIUM
DYSPROSIUM
HOLMIUM
ERBIUM
THULIUM
YTTERBIUM
LUTETIUM

In the Johnson Matthey laboratories techniques have been developed for the preparation of scandium, yttrium and the fourteen elements of the rare earth group as metals in a state of high purity.

All metals are available in ingot form and, with three exceptions at present, can be supplied as sheet or foil. Several metals can also be extruded into rod form and drawn to wire.

Further particulars will gladly be given on application

Specialised Products of

Johnson Matthey

JOHNSON, MATTHEY & CO., LIMITED
73-83 HATTON GARDEN, LONDON, E.C.1 Tel: Holborn 6989
Vittoria Street, Birmingham, 1. Central 8004
75–79, Eyre Street, Sheffield, 1. Sheffield 29212

RE11/694

APPOINTMENTS VACANT

AIR MINISTRY

require in

SCIENTIFIC ADVISER'S DEPARTMENT

GRADUATES in PHYSICS, MATHEMATICS and ENGINEERING for appointment as SCIENTIFIC OFFICERS or SENIOR SCIENTIFIC OFFICERS. Successful applicants will be engaged on OPERATIONAL RESEARCH either at Air Ministry in Whitehall or at one of the R.A.F. Command H.Q. or Establishments.

OPERATIONAL RESEARCH at Air Ministry is mainly concerned with the assessment of future operational needs and problems connected with the weapons of the future, while at Commands the emphasis is on obtaining the maximum efficiency from existing weapons systems and the evaluation of their behaviour under operational conditions. In either sphere, scientists work in close co-operation with officers of the R.A.F. and have contacts with other Research Establishments and firms engaged on defence work. There are also opportunities for visits abroad.

CURRENT VACANCIES are for posts at:

(a) Air Ministry, Whitehall, where the need is mainly for Scientific Officers who will be concerned with studies of future R.A.F. offensive and defensive systems, of the interplay of offence and defence, of nuclear submarine detection, and of tactical aircraft and weapons systems.

(b) Bomber Command, High Wycombe, Bucks, working on the evaluation, use and operation of airborne and ground electronic equipment, the effectiveness of the Medium Bomber Force, and in computer organization and programming.

(c) Fighter Command, Stanmore, Middx, working on problems concerned with air defence and tactical operations of military aircraft in support of ground forces, requiring studies of aircraft performance, weapons systems, tactics and operating procedures.

(d) Transport Command, Upavon, Wilts, where a Scientific Officer will be concerned with broad studies of aircraft characteristics and aircraft utilization to meet the needs of the Military Air Transport Force, and studies with the specialist R.A.F. staffs on specific technical and navigational problems.

SUCCESSFUL CANDIDATES may expect to spend a reasonable time in their first appointments but will later have opportunities for broadening their experience in other branches of the Department.

PROSPECTS for able scientists are good. PROMOTION to higher grades depends basically on merit.

QUALIFICATIONS. Candidates should hold a First- or Second-class Honours Degree in Physics, Mathematics, or possibly Engineering. Senior Scientific Officer Candidates should be at least 26 and have had not less than three years appropriate postgraduate experience. SALARY SCALES are: S.S.O., £1,342 to £1,654; S.O., £738 to £1,222, but S.O.s with suitable experience may start above the minimum. Scales in London are somewhat higher. INTERVIEWS for appointment will be held in London. Forms from Ministry of Labour, Technical and Scientific Register (K), 28 King Street, London, S.W.1, quoting A.347/1A. Closing date October 24, 1961.

REPUBLIC OF THE SUDAN
MINISTRY OF EDUCATION

Applications are invited from those suitably qualified for the post of Vice-Principal of the Khartoum Technical Institute, Sudan. Candidates should hold a good university degree and preferably have higher qualifications and Associate Membership of one of the Engineering Institutions. A sound technical background and experience in a similar capacity is also desirable. The appointment would be on contract for a period up to five years, salary by arrangement according to qualification and experience. A cost of living allowance of approximately £S.187 per annum is also payable and an initial outfit allowance of £S.50 is paid on appointment. A house would be provided at an annual rent not exceeding 7¼ per cent of the official's salary. Leave would accrue at the rate of seven days per month. There is no income tax in the Sudan at the present time. (£S.1 equals £1 0s. 6d.)

Further information and application forms can be obtained from the Appointments Section, Sudan Embassy, 3 Cleveland Row, St. James's, London, S.W.1. Closing date September 30, 1961.

NATIONAL INSTITUTE for RESEARCH IN NUCLEAR SCIENCE

SENIOR MATHEMATICIAN

A senior mathematician is required to be the Head of a new mathematical laboratory which is to be built by the National Institute for Research in Nuclear Science at their Rutherford High Energy Laboratory, Harwell, to provide a service to the Atomic Energy Research Establishment, the Universities, the Rutherford Laboratory and Government Departments. The mathematical laboratory will have an "Atlas" computer and an estimated total staff between 30 and 40. Administrative services will be provided by the Rutherford Laboratory.

Under the direction of the Atlas Computer Committee of the National Institute for Research in Nuclear Science the duties of the successful applicant will be to plan and control:

(a) The setting up and running of the laboratory and the direction of the staff, including operational and programming staff and a small mathematics research section.

(b) The installation and operation of the Atlas computer and peripheral equipment.

(c) The arrangements for the use of the facilities by the Atomic Energy Research Establishment, the Universities, the Rutherford Laboratory and Government Departments, and for the sale of any spare time on commercial terms.

Candidates should have a good honours degree in mathematics, and must be capable of organizing a service to a wide variety of users on a fair and convenient basis. Extensive experience in the control of a mathematics laboratory and in the operation of fast computers is essential. A thorough familiarity with the major computational requirements of scientific research in the country would be a great advantage.

The post will carry a salary of not less than £3,350 per annum. There is a contributory superannuation scheme and assistance is given with housing.

Applicants quoting the names of two professional referees and giving brief details of career should be addressed to the Secretary, Rutherford High Energy Laboratory, Harwell, Didcot, Berks, from whom further details may be obtained.

MATHEMATICIAN

Team engaged in theoretical and applied petroleum research requires a Mathematician for further mathematical-model interpretation of engineering and geochemical problems involving extensive use of differential equations and numerical procedures. IBM-1620 and McBee LGP-30 computers available with access to larger machines when necessary. Participation in these projects with freedom to publish offers good opportunity for professional development.

Applications, with a résumé and an indication of salary required, should be addressed to: Research Council of Alberta, 87th Avenue and 114 Street, Edmonton, Alberta, Canada. Att: G. W. Hodgson, Petroleum Division.

WESTFIELD COLLEGE
(UNIVERSITY OF LONDON)
HAMPSTEAD, N.W.3
CHEMISTRY DEPARTMENT

Applications are invited for two appointments as Senior Research Assistant and Research Assistant to work on Optical Rotatory Dispersion. Salaries from £900 per annum (senior) and £600 per annum (junior) according to qualifications and experience.

Applications should be sent to Professor W. Klyne, Westfield College, Hampstead, N.W.3, from whom further particulars may be obtained.

A PHYSICAL CHEMIST REQUIRED BY Ministry of Aviation at the Rocket Propulsion Establishment, Westcott, Bucks, to lead small team investigating properties of materials at very high temperature with special emphasis on high temperature-resistant ceramics. Experience in the field of solid state physics advantageous. Appointment in grade of Senior Scientific Officer: minimum age 26. Qualifications: degree or Dip.Tech. with first- or second-class honours in chemistry or equivalent qualification, and at least three years' postgraduate research experience. Salary scale £1,342 to £1,654, with superannuation under F.S.S.U. Opportunity for candidates under 31 (normally) to compete for established (non-contributory pensionable) posts. Forms from Ministry of Labour, Technical and Scientific Register (K), 28 King Street, London, S.W.1, quoting F531/1A.

ST. BARTHOLOMEW'S HOSPITAL, London, E.C.1, requires Basic Grade Biochemist in October for work on steroids. This post is suitable for a graduate in biochemistry and will in the first instance involve routine chemical estimation of steroids. A suitable candidate will after a probationary period be able to assist in research and work for a higher degree. Salary £650 to £700 (plus London weighting) depending on qualifications.—Applications to the Clerk to the Governors, within fourteen days of the date of this advertisement.

INDUSTRIAL RELATIONS IN THE WELFARE STATE

IN his book, *Nationalized Industry and Public Ownership*, published last year, in dealing with labour relations, Prof. W. A. Robson wrote that those who emphasized the need to imbue the employees of public corporations with the spirit of public service were right from every point of view. Without it, nationalization cannot succeed, but we must not expect that nationalization by itself will transform the outlook or influence the motives of those who work in an industry. What we in Britain should seek is to awaken in the work-people a strong desire to serve the public as well as they possibly can, together with the desire to improve their own standard of living.

Prof. Robson recognized then quite frankly that for such aims to be realized the unions must be willing to abandon some of their most cherished prejudices and to change some of their obsolete practices; that they need to abandon their defensive negative, demanding attitude, conditioned largely by the capitalism which produced them, and display qualities of constructive leadership, understanding and co-operation with management. In this way they can respond to the challenge to co-operate in the administration of vast industries operated for the benefit of the community. Especially, he added, the unions need to improve the quality of their own officers. Even the largest and wealthiest unions are handicapped by a lack of skilled staff—economists, statisticians and research workers. They seem reluctant to appoint men who have had a university education, even when they are their own members who have won scholarships paid for by the union.

In the past year it has become more and more apparent that the unions, as Prof. Robson wrote, must consider the part they have to play in the sphere of nationalized industries, and recognize the need to revise their own organization, attitudes and practices so as to enable them to play it more effectively. Nor is this need confined to the nationalized industries. It is manifestly true of the shipping and shipbuilding industries, as was fully recognized in a debate on those industries in the House of Commons in July.

An admirable exposition of the function of labour and the trade unions in industry and commerce is contained in a paper by Mr. W. J. Carron, president of the Amalgamated Engineering Union, read before the Royal Society of Arts last December and now published (*J. Roy. Soc. Arts*, **109**, 248; 1961). Mr. Carron recognized the difficulty of apathy among union members, but here, as in the matter of restrictive practices, co-operation is necessary; and the better the understanding between the two sides of industry the more readily restrictive practices on either side, and perhaps also apathy, are likely to be removed. If there is one thing in fact that recent economic debates have emphasized, it is that trade unions, no less than other groups, whether scientists, technologists, teachers or others, are all part of the community and suffer with it when one section, luckier or less scrupulous than the rest, takes more than its fair share.

What is needed in fact is, as was admitted in the House of Commons, a climate of opinion which recognizes that higher wages or salaries must be earned and related to productivity; a system enlisting the co-operation of both trade unions and employers, making it in the interest of the unions to eliminate restrictive practices and the employers to show the maximum enterprise. To create such a climate of opinion or understanding is a major task, and might well be described as one objective of the industrial code which Lord Fleck advocated in his presidential address to the Industrial Co-partnership Association on July 9 and in a speech in the House of Lords on June 14. Studies such as that of Mr. D. F. Macdonald on *The State and the Trade Unions** are to be welcomed for the same reason, since they contribute to an impartial appreciation both of past mistakes and the action which now needs to be taken. Mr. Macdonald brings out particularly well the changed position of the unions in the social scene to-day, and the way in which the intimate concern to the community of their work and methods has stimulated such close scrutiny.

Mr. Macdonald does not intend to offer any constructive proposals. He shows that the trade unions cannot afford to ignore any attempts at a wage-policy and to condemn or belittle an independent body which studies and makes pronouncements on the wages question as part of a review of national economic considerations. The carping at the Council on Prices, Productivity and Income has probably done more harm to its trade union critics in the eyes of the public than to the prestige of the Council. Mr. Macdonald recognizes also that the principle of compulsory membership, or 'the closed shop', may call for safeguards to protect the individual as well as the public interest against group pressure; but he does not discuss the safeguards which from time to time have been suggested, and which might both safeguard the individual and offer a more effective redress of grievances that under present conditions are liable to result in unofficial strikes.

Some of these problems were considered in a debate on industrial relations in the House of Commons on March 17, when Mr. R. Marsh, in opening the debate, suggested that a rationalized trade union structure must precede the creation of a wages structure. He also maintained that most of the problems arose from a lack of joint consultation and of understanding on both sides. Sir Richard Glyn agreed that a more rational trade union movement is essential for a planned industry and a planned economy, but probably the keynote of this debate was the stress laid by both sides of the House on training for management. The right type of management—the right kind of men and the right kind of spirit—is an essential element. Although the limitations of management were equally recognized in this constructive debate, it was clear that given management of this type, there would be no failure to tackle the problem of re-training skilled men whose skills were made obsolete or redundant through technological advance.

---

* The State and the Trade Unions. By D. F. Macdonald. Pp. vii + 199. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1960.) 21*s*. net.

The recognition of the importance of sound industrial relations to the economic life of Britain and to the maintenance and improvement of the British standard of living obviously lies behind the idea of an industrial code which Lord Fleck proposed in the House of Lords debate on co-operation in industry. Earlier, in his Fawley Lecture, Lord Fleck had suggested that if at least ten universities, including colleges of advanced technology, were to develop departments specifically concerned to promote good industrial relations, it would be worth while attempting from such sources to draft a broad measure of industrial philosophy. Once again, in the debate in the House of Lords, there was a good deal of stress on education on both sides, Viscount Amory calling for positive and dynamic co-operation and team-work, for spontaneous respect and concern for efficiency, and for pride in the job, both individually and collectively; and Lord Citrine, who doubted whether there are really any new methods open to us in combating apathy, suspicion and hostility in this field, thought that the decisive factor is to re-establish the leadership of the Trades Union Congress.

This large measure of agreement on both sides as to the need for a fuller understanding of these problems, for a climate of opinion in which more effective co-operation could flourish and for inspired leadership was reflected in subsequent debates on the economic situation in both Houses of Parliament. Mr. Hugh Gaitskell, in a notable speech on July 18, appealed for an attack on the complacency of some managements and the appalling indifference of some workers, and the conditions which foster such situations. He recognized that this was much harder than the adoption of a greater measure of planning, the more positive attitude to particular industries, or the increased degree of professionalism in business and exporting on which he also insisted. To some extent the Government has already moved in this direction, but it cannot fairly be claimed that the Government has yet provided the leadership which is needed and which was even more powerfully adumbrated by Mr. Harold Wilson in speaking on the economic situation on July 26. The necessary climate of opinion is not to be created by appeals to materialism, selfish acquisitiveness, or social emulation. There must, he argued, be some planning, but we need also a sustained increase in production and greater determination to control rising costs; and he admitted frankly that we cannot afford, year by year, an increase in economic rewards—profits, salaries, rents or wages—greater than the increase in productivity which provides the means to all rewards.

This is, of course, an argument for giving much higher priority to increased productivity, and Mr. Wilson believes that it is well within the capacity of Britain to achieve an increase, year by year, of 4 or 5 per cent in productivity and of 4 or 5 per cent in wages. It was as part of a wide attack on the spirit of lethargy which pervades too much of British industry that he advocated the ending of restrictive practices wherever they occur which hold back the national inventiveness and skill, and it is in this context that the trade unions must be regarded to-day. Mr. Nigel Birch, in the same debate, made an equally forthright appeal for an attack on industrial inefficiency, wherever encountered, and for a policy of encouraging understanding by the fullest possible publicity. Mr. R. Carr also stressed this need for the consistent and clear exposition of policy and for a far-reaching attack on restrictive practices. He made, too, a number of constructive suggestions for dealing with the shortage of skilled labour and encouraging companies which are progressive and adventurous, and some of these suggestions were welcomed on the Opposition side of the House.

These suggestions, however, are scarcely relevant in the present context. What is relevant, however, is the measure of agreement between political parties as to objectives and as to the need for the Trades Union Congress to play its part and secure the shedding by the unions of outmoded practices and ideas. That is admittedly essentially a matter for the unions themselves, and whether Parliament can do anything to help, that can only be done with their co-operation. In his classic work on *The English Constitution*, Walter Bagehot wrote of the House of Commons that its teaching function was the one which was worse performed: "A great and open council of considerable men cannot be placed in the middle of a society without altering that society. It ought to alter it for the better. It ought to teach the nation what it does not know".

In some of its recent debates—on science and on economic affairs, for example—the House of Commons has shown the extent to which, even in the conditions of to-day, it can exercise that educative function. It can still do much to create public understanding. It could go far to create the climate of opinion which will accept the harsh measures that may be needed. When leadership is not forthcoming from the Government, it may well be able once again to stimulate it as well as elicit the response required to sustain the measures of policy that a situation demands. Further, the trade unions themselves may well provide a classic example of the way in which Parliament through its educative function may stimulate both the leadership needed and the support which will make the appropriate measures effective. At a time like this, the Parliamentary and Scientific Committee has something to contribute to this educative function of Parliament. It is partly a means of informing Parliament, and professional men should be careful to use it responsibly and imaginatively to the maximum extent; but it is also a means through which Parliament can exercise that function in a wider if specialized field.

BALLISTIC MISSILE AND SPACE TECHNOLOGY

Ballistic Missile and Space Technology

Proceedings of the Fifth Symposium held at Los Angeles, California, August 1960. Edited by Donald P. LeGalley. Vol. 1: Bioastronautics and Electronics, and Invited Addresses. Pp. xiii + 494. Vol. 2: Propulsion and Auxiliary Power Systems. Pp. x + 439. Vol. 3: Guidance, Navigation, Tracking, and Space Physics. Pp. x + 450. Vol. 4: Re-entry and Vehicle Design. Pp. x + 422. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 9 dollars per volume; 36 dollars per set.

A SERIES of symposia on ballistic missile technology was started in June 1946 by the Ramo-Wooldridge Corporation and the Ballistic Missile Division of the United States Air Force; the fifth symposium was held in August 1960 at the University of Southern California in Los Angeles. To those who know the extensive campus of this University and the enormous size of the United States missile industry it will be no surprise to learn that more than one thousand scientists and engineers from all parts of the United States attended this symposium, at which seventy-four unclassified and fifty-one classified papers were presented after selection from two hundred and fifty-five manuscripts submitted. It has become a feature of the United States' so-called 'aerospace' programme that huge gatherings of scientists come together at frequent intervals for the presentation of papers covering a field so vast that it is obvious that no single participant could appreciate more than a small fraction of the specialized subjects involved. Since most of the time at symposia is spent in discussion outside the formal sessions, this plethora of papers is perhaps of little importance. Another feature is, however, of more serious significance, and the four volumes which report in full the proceedings last year exhibit it only too clearly. The frequency and size of these gatherings are now so great that there are just not enough papers of good quality to go round; difficulties with military security no doubt also play a part. The result is a number of contributions of limited interest, which explore in detail some byway which only the author really wishes to tread.

Nevertheless, the present volumes of proceedings are, in total content, of considerable interest and value to workers in missile and satellite technology. This is especially so as the editor and publisher succeeded in producing them within a very few months of the actual conference, thus allowing us to gain an up-to-date view of the extremely varied topics which are the concern of the industrial, government and university research laboratories and development teams engaged in U.S. ballistic missile and space activities. The emphasis throughout is towards space topics; this is shown by the fact that only twenty-two out of the seventy-four papers deal with subjects which have any significant relevance to non-space missile applications. Few of the authors take a basic approach to their subject although here and there one finds interesting items such as the study reported in Vol. 1 of an experimental investigation of gyro spin-axis bearing lubrication; or the careful theoretical treatment of the equations of motion of a satellite in a gravitational field with particular reference to attitude stabilization and control system analysis in Vol. 3. Vol. 1 is devoted to bioastronautics and electronics and includes also the general addresses given to the symposium by such prominent speakers as Dr. T. Keith Glennan, John H. Rubel, General Bernard Schriever and Dr. Simon Ramo. A topical note is struck by Ramo, in a style familiar to all who have known him during the past fifteen years in the missile business, by his paper on "The Life and Times of the Crash Program". This diverts attention from missiles to the great and growing need for improvements in United States' educational methods, the shortage of teachers and the potential applications of electronic teaching devices for technical subjects; one awaits the howl of protest from protagonists of the humanities against this further indication of the narrowness of scientific educational methods. Vol. 2 covers propulsion and auxiliary power systems; Vol. 3 guidance, navigation and space physics. Re-entry and vehicle-design problems are dealt with in Vol. 4.

The text is reproduced by the photo-lithographic process; illustrations and diagrams are clear and graphs are particularly well reproduced with scales which allow accurate interpolation. W. H. STEPHENS

TECHNOLOGY OF CHEMICAL PROCESSING

Encyclopedia of Chemical Technology

Edited by Raymond E. Kirk and Donald F. Othmer. Second Supplement Volume edited by Anthony Standen. Pp. xi + 970. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 175s.

CHEMICAL technology preserves its position at the spearhead of advancing standards of living. Not only has man distinguished himself as a toolmaker, but also he is an enthusiastic converter of the materials in his environment. Chemical reaction, chemical plant and processing are all basic to this vast enterprise of our era.

The very range of chemical technology makes it extremely difficult for a worker to find readily information related directly or indirectly to his pursuit. Established work in physics, chemistry, applied mathematics and even biological science is of interest to him. Knowledge of these is likely to result in unexpected innovation. For example, the theory of turbulent diffusion has enabled those concerned with the dynamics of chemical reactors to refine the calculations for their designs. Often such refinements lead to simpler methods of operation, better yields and improved quality of products.

As a compendium of information for chemical technologists, the Kirk–Othmer encyclopædia is maintaining its traditional calibre, with the appearance of a second supplementary volume. The function of these supplements is to deal with recent developments. This is done in a comprehensive yet fairly readable manner. The range of material and level of appeal are extensive. Fundamental theory is presented; so too are various chemical processes, modern unit operations, techniques for analysing problems in processing, and there is also information which should be of interest to the technologist concerned with economics and planning. Since there are fifty-eight articles, a few selected more or less at random will help to illustrate the diversity.

The comparatively new field of irreversible thermodynamics is discussed by H. Balescu, very appropriately, since the Belgian school has been very active in promoting this work. He shows how the equations of change are used together with Gibbs's fundamental formula of classical thermodynamics in deriving a formulation for production of entropy. This expression, bilinear in the forces and the flows caused by them, is the central concept in irreversible thermodynamics. Onsager's phenomenological principle is described, followed by a section on application. We see that in cases where chemical kinetics becomes so complicated that it can no longer be used exactly, thermodynamics can be a valuable tool in providing a relationship between rate-constants.

A chemical process of great technological importance these days is the manufacture of halogenated butyl rubber. This confers properties such as resistance to degrading by oxidation, adherence and ability to co-vulcanize with other polymers. Yields and mechanisms of reaction are described for processes involving various halogenating agents; methods of curing with zinc oxide and amines are discussed.

Another commercial enterprise of note is the manufacture of polypropylene. Production of crystalline polymers on an industrial scale began toward the end of 1957. Chemical and physical properties are discussed; a very useful table shows a comparison of polypropylene and high-density polyethylene. In the section on Ziegler-type catalysts it is interesting to note that the question whether growth of polymer occurs on the transition metal or on aluminium is still obscure. Effects of processing variables on polymerization are discussed; so too are techniques of moulding and extruding the final products.

A unit operation which promises to become a standard technique in chemical technology is that of separation by diffusion. An article deals with separation, usually of helium or hydrogen, by diffusion through membranes composed of a substance in the glassy state. The theoretical treatment is particularly good.

The use of radioisotopes for analysing the operation of chemical processes is described. Apart from the determination of levels of liquids or fluidized solids in vessels, continuous chemical analyses can be carried out; they are also used to detect leaks in buried pipes or in heat exchangers. Another application is to investigate the extent of reverse flow in chemical reactors.

An example of information useful to the industrial economist is an article on synthesis gas. After a description of various processes, which includes typical data indicating performance of the gas, the investment needed to produce hydrogen is shown graphically as a function of rates of production.

This supplementary volume certainly has a wealth of information, and may be recommended to the chemical technologist, whatever his pursuit. It is a pity that 'technologese' is not avoided throughout the book; one flinches, for example, at reading the word 'recognization'.

R. H. VILLET

AMINO-ACIDS

Chemistry of the Amino Acids

By Jesse P. Greenstein and Milton Winitz. Vol. 1: Pp. xxi + 1–760*d*. Vol. 2: Pp. xi + 761–1816*b*. Vol. 3: Pp. xiii + 1817–2872. (New York and London: John Wiley and Sons, Inc., 1961.) £40 the three volumes.

A NEW comprehensive treatise of more than 2,800 pages on the chemistry of amino-acids is a major event in the chemical and biochemical literature. The first text-book in the English language specializing on amino-acids was *The Biochemistry of the Amino Acids*, by Mitchell and Hamilton, published in 1929. It was a book of 600 pages, and more than half of it was concerned with physiology and biochemistry. Nine years later its place was taken by *The Chemistry of Amino Acids and Proteins*, edited by C. L. A. Schmidt, a treatise of a little more than a thousand pages written by a team of eighteen authors. In 1951, D. M. Greenberg edited a similar multi-author text-book on amino-acids and proteins of about 900 pages which included the metabolic aspects. After an interval of ten years in which the literature has grown at an exceedingly rapid rate, the late Dr. Jesse P. Greenstein, in collaboration with Dr. Milton Winitz, has written a new monumental work, arranged in three volumes and fifty-two chapters. The approach is essentially that of the organic chemist, and unlike its predecessors it deliberately omits most aspects of metabolism as this field has been recently dealt with in Dr. Alton Meister's book published not long ago from Dr. Greenstein's laboratory.

The bulk of the work deals with the physiologically occurring amino-acids but a number of related substances are also discussed, and apart from the purely chemical features a few physiological aspects, for example renal clearance and active transport, are included. The first of the three volumes deals with the general and physical chemistry of amino-acids. There is a full discussion of nomenclature, classification and configuration. The physical chemistry covers *inter alia* the dipolar ion concept, thermodynamics and solubility. Other chemical features which are extensively described are the metal complexes in which amino-acids take part, salt formation, effects of radiation, the general principles of α-amino-acid synthesis and of the resolution of isomers. The second volume covers chemical procedures for the synthesis of peptides, the analytical procedures including manometry, titration methods, isotope dilution, chromatography, micro-biological assay and spectrophotometry. The sequential analysis of peptides is also extensively treated. One chapter deals with the enzymes involved in the determination, characterization and preparation of amino-acids. The third volume is mainly concerned with a description of the preparation of the individual amino-acids. Apart from the protein-bound amino-acids there is an exhaustive treatment of other amino-acids and their derivatives. These include the large number of new amino-acids which have been discovered in biological material in recent years, thanks largely due to the chromatographic techniques and to the isolation of numerous special peptides and proteins produced by micro-organisms (for example, antibiotics).

The senior author unfortunately did not live to see the publication of this book. He died suddenly in February 1959 as a result of a cerebral hæmorrhage when the book had been virtually completed. A brief biographical sketch by the junior author prefaces the book and imparts something of the unusual gifts of the mind and character of the senior author. The book bears throughout the mark of Dr. Greenstein's distinguished style, outstanding in its clarity, accuracy, liveliness, elegance and beauty. A sample of this, conveying also the intentions of the authors, is the following passage from the preface:

"Let it be admitted that we have been entranced by the spectacle of these many and diverse phenomena. In attempting to transfer these pleasurable emotions to paper, and to portray accurately the work of so many able investigators, we were well aware of the magnitude and difficulty of the task which we set for ourselves, and of the impossibility of perfection. With filial piety and in justice to those who have built this field, as well as to convey a sense of perspective, we have stressed where we could the historical development of each subject. The literature of science is generally curt and colorless, except when enlivened at times by the statement of controversial opinion; on occasion an expression of notable and graceful generosity or of far-seeing imagination finds its way into this literature, and such examples have been quoted where pertinent in the pages to follow. The topics covered in this work have generally been those wherein a certain stability has been attained and are thereby suitable for comprehensive treatment—not that progress has ceased in such topics, but rather that nothing has so far emerged to render any substantial portion of them obsolete or questionable."

The references to the historical developments and the quotations from earlier writers are a particular asset of the book. Those from foreign languages are given in the original, with a translation in an appendix of the book. They add to the enjoyment and interest of a standard work which for many years to come will be an invaluable source-book. H. A. Krebs

INORGANIC CHEMISTRY

Inorganic Chemistry

By Jacob Kleinberg, William J. Argersinger, Jr., and Ernest Griswold. Pp. viii+680. (Boston: D. C. Heath and Company; London: George G. Harrap and Co., Ltd., 1960.) 75*s*. net.

The past few years have witnessed radical changes in the teaching of inorganic chemistry to undergraduate classes. These may largely be attributed to a fruitful interaction, at the research level, between physical and theoretical chemistry on one hand, and descriptive inorganic chemistry on the other. It has thus been possible to stimulate the undergraduate by presenting inorganic chemistry as a subject which makes full demands on his powers of comprehension and reasoning.

A new single-volume text on inorganic chemistry is therefore to be welcomed at this time. Drs. Kleinberg, Argersinger and Griswold have attempted to tackle the subject so as to reflect these present-day changes in its teaching. To this end, the book is divided into three parts. Part 1 (211 pages) is devoted to a brief account of the development of atomic theory, to elementary quantum mechanics, then on to the problem of the electronic configuration of atoms and the consequence of this on chemical and physical properties, especially from the point of view of chemical periodicity, and finally a section on chemical binding and molecular structure. Part 2 (80 pages) is entitled "Some Aspects of Chemical Behaviour", and consists of two chapters, on co-ordination compounds and a particularly good one on acid-base relationships and reactions in non-aqueous systems. Part 3 is divided into three sections. The first deals with the representative elements (196 pages), the second (136 pages) with the transition elements (the descriptive chemistry is presented here with more detail than is usual), and the third (58 pages) with the lanthanides and actinides.

The authors have deliberately chosen to omit sections on nuclear chemistry, the inert gases, and organo-metallic compounds. The last of these topics was considered to be one which, in order to have covered it adequately, would have increased the scope and size of the text beyond limits that they had set themselves. I feel that this decision was unfortunate. The field of organo-metallic chemistry is one which has in many ways been in the forefront in the recently much talked of renaissance of inorganic chemistry, and its inclusion in this text need not have occupied more than 30 or 40 pages. I would also have wished to see sections on crystal chemistry, physical methods of structure-determination (for example, infra-red, visible, and ultra-violet spectra are rarely mentioned), and reaction mechanisms. Very few of the discoveries or developments of the past five years are included. The problem of bonding is dealt with almost wholly in terms of valence bonds and the treatment is obsolete in many instances. In addition, it would have been preferable to have introduced much more physical and theoretical chemistry into Part 3 and thus emphasize the essential unity of the subject.

The authors are to be congratulated on the general presentation of their book, which is well written. It can, with few reservations, be recommended for use at least up to Part 1 honours B.Sc. standard.

M. F. Lappert

A MUCH-STUDIED PROTOZOON

The Biology of Stentor

By Vance Tartar. (International Series of Monographs on Pure and Applied Biology. Division: Zoology, Vol. 5.) Pp. x+413. (London and New York: Pergamon Press, 1961.) 75*s*. net.

The ciliate protozoon *Stentor* is large enough to be operated on by hand (with skill) and is remarkably amenable to surgery. For these reasons it has been the object of numerous morphogenetic studies, culminating in Tartar's own outstanding contribution. As the author of this book, Tartar shows his over-riding interest in cell morphogenesis.

Fission, re-organization (the "physiological regeneration" of Schwartz) and regeneration are related, in that each involves the formation of new adoral membranellæ (which appear as a primordium) and mouth structures and the fusion and renodulation-of the macronucleus. Although the study of *Stentor* has contributed important information about the mechanism of cleavage, the events leading to fission have not been elucidated. More is known about re-organization and regeneration. Tartar rejects several theories and concludes with Schwartz that re-organization is induced by a certain departure from the normal macronucleo-cytoplasmic ratio and permits a re-adjustment. He does not reveal whether this re-adjustment is merely one of area, or how it affects the quantities of deoxyribonucleic acid and other macronuclear constituents. Regeneration is induced by damage to the feeding organelles or when these are defective. Tartar interprets grafting experiments in terms of two cytoplasmic states: activation, which favours the formation of a primordium, and inhibition, which blocks this process. The fusion and renodulation of the macronucleus and mitosis of the micronuclei are considered by Tartar to be set in motion by the onset of cytoplasmic activation, for which the presence of the macronucleus is itself necessary. There is some evidence to suggest that the differentiation of mouth parts at the posterior end of the primordium is induced by the surrounding protoplasm, probably in relation to a gradient.

Perhaps Tartar's greatest achievement concerns the induction of the primordium itself. The precise localization of the primordium in relation to the pattern of stripes in the ectoplasm had already been noted by earlier workers, but Tartar has postulated that a primordium is formed in a region of contrast in stripe width, and has adduced effective experimental evidence in support of this hypothesis. This brings us back to gradients within the cell cortex, and so to the kinetosomes, linearly ordered, prolonged outwards as cilia, presumably themselves the site of important aspects of ciliary activity, and thus perhaps the dominant feature in cell gradients and 'molecular ecology'. All these and other aspects of morphogenesis are treated very fully; Tartar's book is of the nature of a detailed, lucid, and comprehensive review of these topics.

What then of other aspects of the biology of *Stentor*? These are in general summarized very competently and usefully from the literature, though with something slightly less than the critical approach of the cytophysiologist. Even the arrangement is peculiar. Behaviour is treated rather well near the beginning, followed by fine structure, including contractility; but ciliary co-ordination is exiled to a hotch-potch chapter far removed. Some important aspects of fine structure are summarized in a single stereogram; but it is doubtful whether the reader can appreciate or assess the evidence without electron micrographs such as those by Randall and Jackson. Sleigh's work on membranellar beat is faithfully retailed, but the co-ordination of body cilia is quite inadequately discussed, with no mention of the possibility of surface depolarization and no reference to pertinent work on other Protozoa.

Considerable space is given to the function of the macronucleus and to interspecific grafts, and useful comparisons are drawn with experiments on amoebae. The species of *Stentor* are briefly but usefully described and are illustrated in colour. In the penultimate chapter the simple secrets of microsurgery are very helpfully revealed.

This book is well produced apart from a number of obvious misprints. Tartar writes concisely and has given us excellent measure. *The Biology of Stentor*, though a book for the specialist, is indispensable to every zoological, cytological and embryological library, and many professional cytologists and protozoologists will want to own it. J. A. KITCHING

BASIC APPROACHES TO STRUCTURAL ANALYSIS

Energy Principles in Applied Statics

By T. M. Charlton. Pp. vi + 112. (London: Blackie and Son, Ltd., 1959.) 25*s*. net.

IN many institutions a student learns to determine the deflexion of nodal points in a pin-jointed framework and the forces in members of a statically indeterminate framework by use of Castigliano's theorems. There is no doubt that many students (and engineers) never realize that the solution is valid only when the load-deflexion relationship for the particular structure is linear. For most of the materials and proportions encountered in traditional practice, this assumption can be made with confidence and, frequently, the various design codes which an engineer must follow have implicitly made the initial step. With the development of new materials, and with the increasing awareness by the structural designer of the importance of ranges of mechanical behaviour outside those previously considered, it is desirable that the teaching schools, and the practising engineers, should look more closely at the fundamentals of the methods of analysis used.

During the past ten years, Mr. Charlton has been the author of a number of articles in the technical Press, in which he has treated several of these fundamental theorems of classical analysis. His recent book brings together much of that early work, and, by concentrating on the simplest plane pin-jointed systems, emphasizes the concepts involved in the basic equations of structural equilibrium and compatibility, and directs attention to the general applicability of approaches to analysis using complementary energy and the method of virtual work.

The differences between the various approaches may not be apparent in some cases to a superficial reader, and five of the seven chapters conclude with brief paragraphs which place each section in its proper position within the framework of the whole text. Many readers would prefer that these signposts occurred at the beginning rather than the end of the course, and at both, rather than either; but since the halting places are so well chosen the point becomes one of minor significance only.

Throughout the text the author speaks in terms of stiffness and flexibility coefficients and the final chapter is devoted mainly to the properties of these coefficients for linear systems. These concepts are becoming increasingly valuable with the introduction of the electronic computer in structural analysis, since the necessary equations may be set up in a systematized manner and lend themselves to solution by matrix methods on the machine.

The book is no vade-mecum for practising structural designers and does not purport to be. It would be, however, of great benefit to the good student and, one suspects, to most teachers of the theory of structures for, with both, fundamental principles are frequently obscured by an undue concentration on the techniques of analysis.

R. C. COATES

Pulp and Paper

Chemistry and Chemical Technology. By James P. Casey. Vol. 2: Papermaking. Second edition, revised and enlarged. Pp. xxiii + 581–1249 + 147. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 188*s*.

THE scope of the second volume of this work is indicated in the review of Volume 1 (*Nature*, **190**, 380; 1961). Much the same general comments apply to Volume 2. Thus, British readers will be surprised at the absence from the chapter on fibre bonding of any reference to the important 1957 Cambridge symposium on that subject.

However, this should not be allowed to detract from a recognition of the general excellence and high standard of this work as a whole. It contains a tremendous amount of information of a fundamental nature covering paper-making operations from stock preparation to supercalendering, and taking in water usage and disposal, microbiological considerations and beater and wet-end additives.

Incidentally, it is completely self-contained in its subject-matter, so that there is no necessity to possess Volume 1 in order to derive the fullest benefit from Volume 2. This fact may appeal to many in Britain who are more interested in paper making than in pulping and to whom the cost of these books must appear very high.

JULIUS GRANT

Gas Chromatography Abstracts, 1958

Pp. vii + 262. 42*s*.

Gas Chromatography Abstracts, 1959

Pp. ix + 164. 42*s*.

Edited by C. E. H. Knapman, assisted by C. G. Scott. Sponsored by the Gas Chromatography Discussion Group associated with the Hydrocarbon Research Group of the Institute of Petroleum. (London: Butterworths Scientific Publications, 1960.)

THE two volumes of gas chromatography abstracts have been sponsored and prepared for publication by the Gas Chromatography Abstract Editorial Committee of the Gas Chromatography Discussion Group. The first is a combination of bibliographies compiled independently by three members of the Committee and covers the literature until the end of 1958. The 1,468 abstracts are listed alphabetically according to the first author. Considering the somewhat limited resources, reliance has had to be placed on abstracting journals particularly in connexion with foreign publications. This cannot be said of the second volume, which is compiled from abstracts obtained from 28 abstractors who between them have covered more than 110 journals. Although no precise date is given, the 717 abstracts in this volume appear to cover the literature until the end of 1959.

The abstracts in both volumes, although short (averaging 30–50 words), are very concise and to the point. Easy reference is made possible by author and subject indexes, the latter in particular being most comprehensive, enabling a complete survey of the literature on any topic to be carried out with great rapidity. Although the difficulties of compiling works of this type must be very great, few errors appear to have crept in. One criticism, however, is the frequent omission of any indication of the language of the article abstracted.

These volumes were compiled by the voluntary efforts of gas chromatographers, who, seeing a need for an efficient abstracting system, pooled their resources and produced their own. Their achievement deserves the highest praise and will certainly be welcomed by many wishing to keep abreast of this rapidly expanding field.

A. GOLDUP

South African Animal Life

Results of the Lund University Expedition in 1950–1951, Vol. 7. Edited by Bertil Hanstrom, Per Brinck, and Gustaf Rudebeck. Pp. 488. (Stockholm: Almqvist and Wiksell, 1960.) 75 Sw.kr.

VOLUME 7 of *South African Animal Life*, in which results of the Lund University Expedition (1950–51) are reported, maintains the high standards of the previous volumes in this series. Groups dealt with are preponderantly from among the Coleoptera, Diptera, Hymenoptera and Hemiptera. There are also chapters on the Oligochaeta, Decapoda, Crustacea and on the Isoptera. The authors are again recruited from many countries and seats of learning, and of the twenty-seven articles three are written in French, eight in German and sixteen in English.

O. LOWENSTEIN

A Collection of Tables and Nomograms for the Processing of Observations made on Artificial Earth Satellites

By I. D. Zhongolovich and V. M. Amelin. (Mathematical Tables, Vol. 12.) Issued by the Institute of Theoretical Astronomy, Academy of Sciences of the U.S.S.R. Pp. iv + 188. (London and New York: Pergamon Press, 1961.) 100*s*. net.

THIS volume is a welcome addition to the very few existing mathematical tables which are designed to help in the calculation of artificial satellite orbits. The main table gives the true anomaly and the ratio of radial distance to semi-major axis, for a complete range of values of both mean anomaly (at 1° intervals) and eccentricity (from 0 to 0·76 at intervals of 0·01). Other tables give period and semi-major axis in terms of the mean angular motion, solutions of the equations used in calculating geocentric latitude and longitude, and the main secular perturbations of orbits. Finally, there are nomograms to determine the angle of elevation of a satellite. The intervals of tabulation and the number of figures in the tabulated quantities have been well chosen, and the layout of the tables is pleasing.

The tables themselves have been reproduced from the Russian original by a photographic process, and only the 10 introductory pages needed translating. Despite its minimal contribution to the subject-matter, the Pergamon Press has chosen to charge £5 for the volume, although it was advertised at 70*s*. This high price would be more justifiable if the 10 introductory pages were perfect, but they are not. There are irritating errors, such as the inconsistency of printing the symbol v as a Greek ν in the introduction but not in the tables. Also, the translation is at times sadly deficient. For example, the key sentence defining the units—"n is expressed in degrees per mean solar day and is given to 0·01 min. . ."—is nonsensical because of the omission (after 'day') of some 15 words, which give the tabulation interval for n and state that the orbital period is in minutes. These shortcomings do not, however, seriously mar the value of the volume to the specialist.

D. G. KING-HELE

STRUCTURE OF THE NORTHERN APENNINES

By Sir EDWARD BAILEY, F.R.S.
Formerly Director of H.M. Geological Survey
AND
Prof. W. J. McCALLIEN
University College of Ghana

IN 1957 we spent a full month in Florence, Spezia and Levanto, and have paid a few shorter visits since. In addition we have made an intensive study of publications, especially sheets of the Italian Geological Survey 1 : 100,000 map. We also have had the advantage of discussions with Prof. G. Merla and Dr. A. Valduga at the University of Florence; and we are specially grateful to the latter for conducting us over the exposures of the famous Carrara window. As a result of our inquiry we have come to conclusions that are in some respects novel. We consider that the rocks of the Northern Apennines, in large part Ligurian, can be conveniently assigned to four structural units as follows:

(1) The lowest unit is only seen in the Carrara window. Its rocks, as exposed, reach from Palæozoic, through Mesozoic to Tertiary, with a Miocene greywacke, termed Macigno, as their top member. The window interpretation advanced by S. Lencewicz[1] is now accepted by everyone.

(2) The next unit frames the window, and is widely, though intermittently, exposed in all directions therefrom. Its rocks, like those of unit 1, reach from Palæozoic through Mesozoic to Tertiary, with Miocene Macigno as top member.

(3) Above comes the Ligurian nappe, an old name, but used by us here in a somewhat extended sense. Its main rocks are a Jurassic submarine volcanic group, a Cretaceous shale group and an Eocene limestone, called Alberesi; and there is locally a further repetition of Macigno. The volcanic group, in addition to gabbro and ash, abounds in serpentinite, pillow-lavas and radiolarian chert, an assemblage which, here and elsewhere, has been called the Steinmann trinity. Detached masses of these rocks, sometimes of mountainous proportions, sometimes mere boulders, are distributed over an area measuring 200 km. by 100 km.; and everywhere they manifestly overlie Tertiary sediments, the Macigno of unit 2. They were for many years supposed to be themselves Tertiary, until Steinmann[2] demonstrated their Jurassic age. Ever since, they have ranked as particularly clear examples of older rocks transported for an amazing distance over younger rocks. Steinmann, coming from the complicated Pre-Alp nappe of Simmenthal in Switzerland, regarded the transport as a subterranean tectonic operation, a view with which we agree.

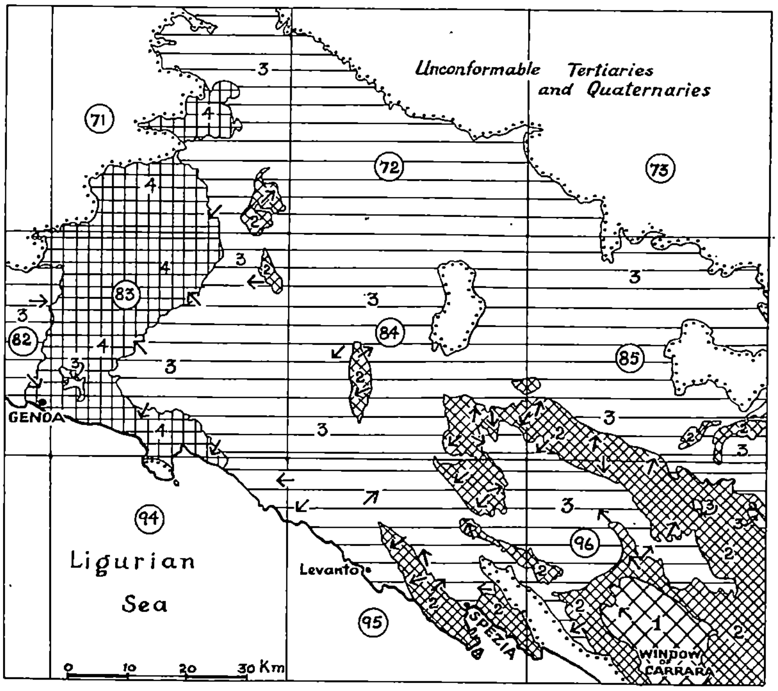


Fig. 1. Geological map showing four structural units in the Northern Apennines. Numbers in circles refer to map sheets of Italian Geological Survey 1 : 100,000. The other numbers are those of the structural units

A difficulty concerns the remarkable discontinuity of the igneous outcrops. L. W. Collet[3], a specialist in nappe structures, attributed their separation in large measure to erosion, and has called them klippes, in the sense of the klippe outliers of the Lucerne district of the Alps. This is clearly a mistake, for the masses, large and small, lie already separate in a matrix furnished by the Cretaceous shale, itself broken to pieces. The whole constitutes a *mélange* in the sense E. Greenly has applied the term to the Gwna *Mélange* of Anglesey. C. I. Migliorini[4], followed by Merla and others, has gone on to interpret the Ligurian *Mélange* as a product of superficial stage-by-stage landslipping, supposed to have taken place from a series of anticlines, uplifted successfully farther and farther to the north-east.

A strong objection to this interpretation is, in our opinion, furnished by the restriction of the igneous enclosures to the shale outcrops, where they occur by the thousand. They are very seldom, if ever, found in the associated, far-travelled, and supposedly land-slipped Alberesi. Our view is that the *Mélange* is due to underground flow of the mobile shale material during nappe formation. Another argument against the superficial landslip interpretation follows from unit 3 showing a layered structure within itself, which can be traced through a succession of folds, persisting for many kilometres. Thus in the frame of the Carrara window the structural succession in unit 3 is Ligurian *Mélange*, on Alberesi, on Ligurian *Mélange*, on Macigno of unit 2; while on the two sides of the Spezia anticline (see Sheet 95 of the Italian map) Alberesi is poorly represented, and the structural sequence is, broadly speaking, Macigno, on Ligurian *Mélange*, on Macigno of unit 2.

(4) The top unit may be called the Genoa nappe, and consists of a great sheet of Alberesi, strikingly discordant to the contents of unit 3.

We hope shortly to furnish detail sufficient to establish the truth of the above outline sketch. Meanwhile, like P. Fallot, we may direct attention to a recent account by T. Rocco[5] of boring and geophysical exploration in the Po Valley in pursuit of oil. The Po Quaternary sometimes exceeds 2,000 m. in thickness, and the late-Tertiaries below it furnish another 8,000 m. These sediments are gently folded and are supposed to occupy the foredeep of the Apennines. If so, we suggest that the Po Valley is homologous to the Swiss Plain, and that the articulation of the Alps with the Apennines is homologous with the articulation of the Juras with the Alps.

[1] Lencewicz, S., *Estr. R. Soc. Sci. di Varsavia* (1917).

[2] Steinmann, G., *C.R. Fourteenth Intern. Cong.*, Madrid, 1926, 2, 637 (1927).

[3] Collet, L. W., *The Structure of the Alps*, 266 (London, 1935).

[4] Migliorini, C. I., *Rep. Eighteenth Intern. Cong.*, London, 1948, 13, 186 (1952).

[5] Rocco, T., *Proc. Fourth World Petroleum Cong.*, Rome, 1955, 1, 675 (1956).

ORGANIC CONSTITUENTS OF METEORITES

By MICHAEL H. BRIGGS

Department of Chemistry, Victoria University of Wellington, New Zealand

PROF. J. D. BERNAL has recently commented[1] on the significance of carbonaceous meteorites in theories on the origins of terrestrial life. He points out that the reported[2] organic constituents of these meteorites may have played a part in the formation of life on Earth, or may be derived from some extra-terrestrial life-form. Alternatively, the meteorites could be fragments of Earth that broke away before the development of life, but after the abiotic formation of the complex compounds that are widely assumed to have given rise to the first terrestrial life-forms by a process of chemical evolution.

In this article I wish to discuss my own analytical results on a carbonaceous meteorite, and then to attempt to assess the available evidence concerning the origin and importance of meteorite organic matter.

Investigations of the organic constituents of meteorites have so far revealed materials of little biochemical interest. This could be due to the methods of extraction. Mueller, for example, removed compounds from the Cold Bokkeveldt meteorite with a variety of organic solvents, while Nagy *et al.* resorted to high-temperature distillation. Yet biochemicals are characteristically very soluble in water, and Calvin *et al.* have shown that some of the carbon in both the Orgueil and Murray meteorites is removable with water. With this in mind I have prepared hot-water extracts of a carbonaceous meteorite that fell at Mokoia, near Wanganui, New Zealand, in 1908.

The Mokoia meteorite consists mainly of two pieces (Fig. 1), with a total weight of approximately 10 lb. These two stones were found two days after impact. Consequently there is no chance that they picked up organic matter by prolonged contact with the soil. Since discovery the meteorite has been in the Wanganui Museum. The circumstances of discovery[3], together with the chondritic structure (Fig. 2), render the meteoritic nature of the stones unquestionable. Additional evidence is provided by the chemical composition (Table 1) and the X-ray diffraction analysis (Fig. 3).

Small samples of the meteorite have been supplied by the New Zealand Geological Survey. These fragments have been extracted by the following method:

The sample was first washed with distilled water and the upper 2 or 3 mm. removed from all over the surface by abrasion. In this manner the chances of extracting any surface organic compounds picked up by the stones from perspiration were minimized. The remainder of the sample was crushed to a fine powder by pestle and mortar, and the powder extracted with distilled water in a Soxhlet apparatus for two days. After filtration and evaporation of the solution so obtained, a pale brown residue was secured. This residue represented approximately 2 per cent by weight of the original meteorite fragment. The elemental composition of this residue varied quite appreciably from one meteorite fragment to the next. One residue contained approximately 6 per cent carbon, 1·3 per cent hydrogen, with traces of nitrogen. Spot tests carried out to detect inorganic constituents gave negative results for carbonate,

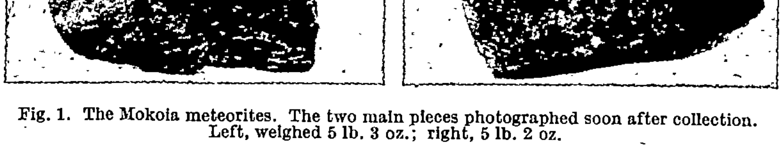


Fig. 1. The Mokoia meteorites. The two main pieces photographed soon after collection. Left, weighed 5 lb. 3 oz.; right, 5 lb. 2 oz.

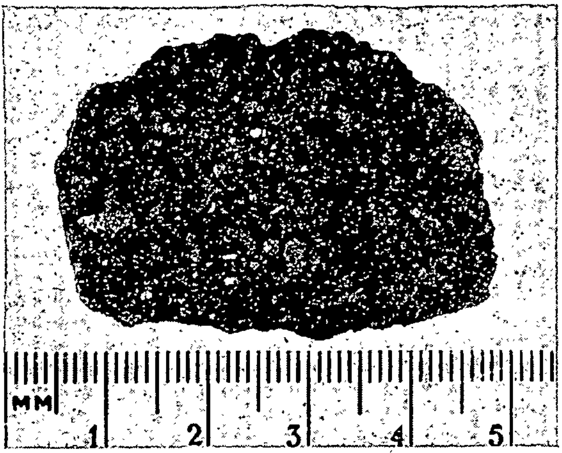


Fig. 2. Section of the Mokoia meteorite. Close-up of a small fraction (c. 40 mm. across) of the meteorite

Table 1. Composition of a Fragment of the Mokoia Meteorite

	Per cent
Fe_3O_4	36·95
FeS	5·64
NiO	2·20
MnO	trace
SiO_2	37·55
Al_2O_3	2·62
CaO	3·50
MgO	6·30
P_2O_5	0·64
Na_2O	2·86
CoO	trace
C	1·25
Total	99·51

This analysis is by B. C. Aston and is reported by Marriner in ref. 3. Analyses on fragments used in the present work gave carbon values of 1·36 per cent.

bicarbonate, nitrate, nitrite and ammonium. It was therefore concluded that the residue contained water-soluble organic compounds, with some organic nitrogenous substance present in trace amounts.

Inorganic constituents actually detected by spot tests were the following: magnesium, sodium, potassium, sulphate, phosphate, arsenate and chromate. Negative results were obtained in tests for all other common inorganic anions and cations.

To identify the organic compounds present has so far proved an impossible task, due mainly to the very small quantities available for investigation. However, by the use of ultra-violet and infra-red spectroscopy, and of paper chromatography, some tentative conclusions have been drawn.

Small amounts of the residue have been submitted to paper chromatography in a variety of solvents. By the use of sprayed reagents several spots have been developed and their R_F values calculated. Of the many compounds detected in this manner, four of the most interesting will be described.

Compound I is strongly fluorescent in the short ultra-violet (below about 320 mμ) and may be detected on paper by this property. From the shape of the spots in several solvents it is possible that this is more than one substance. The descending R_F value at room temperature on Whatman No. 1 paper in water-saturated phenol is about 0·9. The compound also reacts very slowly on paper with picric acid to give a yellow-orange colour.

Compound II is also strongly fluorescent in short ultra-violet, but unlike compound I has a very low R_F in water-saturated phenol (less than 0·1). Nor does this spot react with picric acid.

The fluorescence of both these compounds is unaffected by *p*H. Both dissolve readily in ammonia, but not in hydrochloric acid.

Compound III may be detected on paper by the two tests normally used in the detection of purines on chromatograms. When sprayed with silver nitrate solution, then bromophenol blue, then washed with water, a very pale blue spot remains. When sprayed with silver nitrate, then potassium chromate solution, then washed in dilute nitric acid, a pale red spot remains that slowly fades. These two tests give a spot with the same R_F value in all solvents so far used. The spot cannot be detected on paper by examination in short ultra-violet, but if the chromatogram is first sprayed with a very dilute solution of fluorescein, the spot can be detected by its weak absorbance of ultra-violet. The R_F values of this compound on Whatman No. 1 paper at room temperature are: 0·1 *N* ammonium hydroxide (ascending) 0·87; 86 per cent *n*-butanol (descending) 0·23; *n*-butanol–water–acetic acid (descending) 0·13.

Compound IV was detected in only one meteorite fragment and not in two others. It was observed on paper chromatograms which had been allowed to run overnight so that the solvent (86 per cent *n*-butanol with 5 per cent by volume conc. ammonia solution) had run off the end of the paper. The compound consequently has a very low R_F in this solvent. The wet paper chromatograms showed no markings, but on allowing the papers to dry at room temperature a dark red spot appeared with a 'tailed' appearance. This experiment was successfully repeated several times on the residue from one fragment. The spot could be drawn down from the origin only by allowing the solvent to run for many hours. Acid solvents were without effect; only ammoniacal solvents were successful. On this basis it is possible that the red colour is produced by a reaction of some organic constituent of the meteorite residue reacting with magnesium hydroxide formed *in situ* by the basic solvent. Quite a variety of organic compounds will form red complexes with magnesium.

While it is impossible to identify any of these compounds from this information, tentative hypothe-

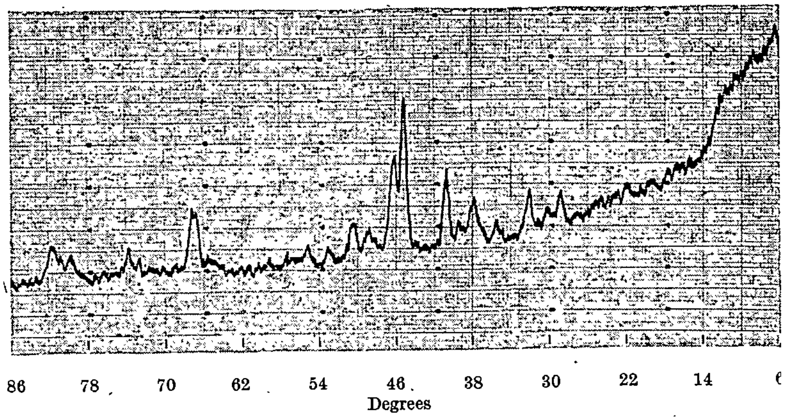


Fig. 3. X-ray diffraction analysis of the Mokoia meteorite. Determined on the powdered meteorite; wide slit, iron tube

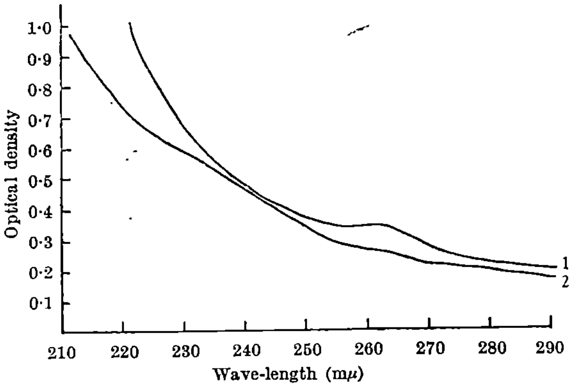


Fig. 4. Ultra-violet spectrum of meteorite extract. Curve 1 at pH 7; curve 2 at pH 2

ses concerning their constitution can be postulated. Thus from its reactions compound III must be able to form a silver compound capable of reacting with bromophenol blue or chromate to give a coloured complex. This immediately suggests a purine or related imidazole compound. Similarly, the fluorescent properties of compounds I and II suggest some conjugated ring system; while the water-solubility of all indicate the presence of polar groupings, such as carboxyl, hydroxide and amine. The low quantity of nitrogen in the residue indicates that amines can be present only in trace amounts.

Further information concerning the organic constituents of the residue is provided by ultra-violet and infra-red absorption spectra. In the ultra-violet the most characteristic property of the spectrum (Fig. 4) is a high end-absorption. However, the absorption curve varies somewhat with the pH of the solution. In neutral solutions there is a significant increase in absorption around 260 mμ as compared with the spectrum of acid solutions; suggesting an acidic chromophore.

Little information is given by the infra-red absorption spectrum of the intact residue; beyond a weak C—H absorption around 2,900 cm.$^{-1}$, a weak C=O absorption around 1,725 cm.$^{-1}$, and a strong double absorption at 860 and 880 cm.$^{-1}$. Most of the spectrum consists of strong, broad absorptions due to sulphate and water (even in carefully dried samples). One residue sample gave a sharp absorption at 1,395 cm.$^{-1}$; but this was not detected in other samples from different meteorite fragments. It is possibly significant that the residues giving this absorption also contained compound IV.

In an attempt to secure more information the residues were fractionated with different solvents. Cold water was found to dissolve only a part of the residue. After evaporation this cold-water-soluble portion was extracted first with chloroform, then with ethanol. All solvents used were carefully re-distilled analytical reagents that gave no residues on evaporation. The original hot-water-soluble extract of the meteorite was thus separated into four parts: cold-water-insoluble (residue *A*), chloroform-soluble (residue *B*), ethanol-soluble and chloroform-insoluble (residue *C*), and cold-water-soluble, ethanol-insoluble, and chloroform-insoluble (residue *D*). The infra-red absorption spectrum of each of these residues was determined, and the results are shown in Fig. 5.

Residue *A* shows few organic absorptions. There are a weak C=O and C—H absorptions, and absorption maxima at 2,500, 2,350, 880 and 800 that could be due to organic compounds. Residues *B* and *C* show the spectra with the greatest detail. Again C—H and C=O absorptions are present as well as a large variety of other peaks due to organic compounds. Residue *B* was not fluorescent. Residue *C* was strongly fluorescent and contained compounds I, II and III. After extraction with the two

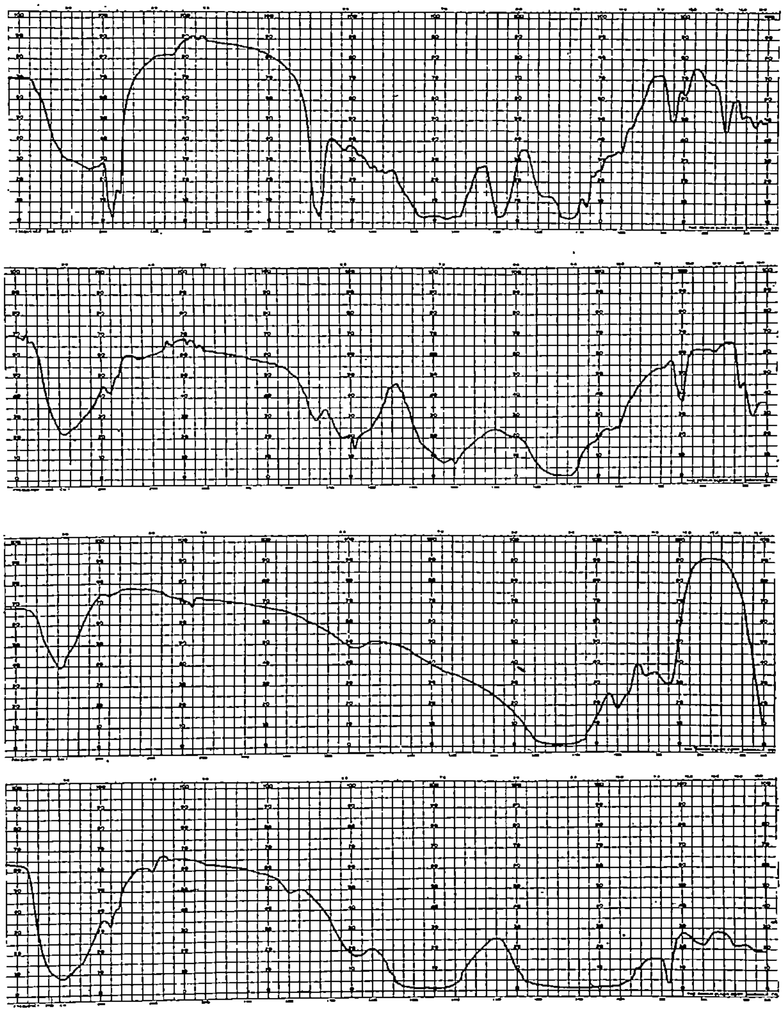


Fig. 5. Infra-red spectra of various fractions of the Mokoia meteorite extract. Spectra in descending order: chloroform-soluble fraction; ethanol-soluble, chloroform-insoluble; residue after chloroform and ethanol extraction; hot-water soluble, cold-water insoluble

organic solvents, residue *D* obviously contains little organic material, though a very weak C—H is present.

Residue *A*, which did not contain either compound I or II, was weakly fluorescent; indicating the presence of a third fluorescent compound.

Considering the evidence given here as a whole, it is apparent that the Mokoia meteorite contains a variety of quite complex organic compounds. Preliminary attempts to extract the carbon from the ground meteorite residues after water extraction with organic solvents have proved successful, and indicate the presence of water-insoluble organic material.

The problem raised by this, and other, work on meteorites is to provide a satisfactory explanation of the origin of these organic compounds. It is quite clear that, at least in the Mokoia case, the compounds are actual constituents of the meteorite and not terrestrial contaminants. The circumstances of discovery, preservation and the lack of such common soil compounds as amino-acids all render a contamination hypothesis untenable.

There would seem only two main possibilities for the origin of the meteorite compounds. Either they are the decomposition products of an extra-terrestrial life-form, or they are abiotic compounds formed early in the development of the solar system.

It has recently been demonstrated[4] that the cosmic-ray exposure ages of stony meteorites are all less than about 2×10^9 years, while the solidification ages are around $4{\cdot}5 \times 10^9$ years. This can be interpreted as evidence that the meteorites are fragments of a larger body that has disintegrated. While the cosmic-ray exposure ages of carbonaceous meteorites have not been determined, their overall similarity to non-carbonaceous chondrites makes the hypothesis that the two groups have cosmic-ray ages that are approximately the same at least plausible. Now the Earth has borne life for more than 2×10^9 years[5], hence, if carbonaceous meteorites have a cosmic-ray age less than this, it is unlikely that the organic compounds carried by these meteorites played any part in terrestrial biopoiesis.

However, several workers[6] have cast doubt on the interpretation of cosmic-ray ages, and propose that the meteorites and asteroids are not fragments of a larger body but have always been small objects. Several authors[7] also have suggested that the major features of Earth and Moon were produced by meteorite collisions. If this is true, it is evidence of the existence in the past of meteorites before the beginnings of terrestrial life.

The problem of the origin of the meteorite compounds is closely bound up with the larger problem of the origin of meteorites. If these bodies are fragments of a larger mass that no longer exists, then the compounds could be the remains of some exotic life-form. However, if the meteorites are not fragments of this nature, the compounds can only be the products of reactions of substances adsorbed on to surfaces. The numerous recent demonstrations[8] that highly complex compounds can be formed from simple materials by discharges and radiation suggest that organic syntheses could occur within a large cloud of meteorites in space.

Prof. J. Oro has recently suggested[9] that biochemical compounds would be formed at the point of impact of a comet with a planet. The asteroids, which obviously present a much larger target for comets, must have been in such collisions many times. Consequently the meteorite organic compounds could be the results of such collisions.

It is apparent that two major advancements remain to be made in this field. First, the chemical nature of the meteorite compounds must be established and mechanisms for their synthesis devised. Secondly, the cosmic-ray exposure ages of the carbonaceous chondrites must be measured to allow an estimate of the significance of these compounds in the early history of the solar system to be made.

I wish to thank Dr. W. A. Watters of the New Zealand Geological Survey for supplying samples of the Mokoia meteorite and for helpful advice; Dr. B. Cleverly and Dr. P. Grant of the Dominion Laboratory for the infra-red spectroscopy; Dr. N. F. Curtis and Mr. D. A. House for assistance with the inorganic spot tests; Dr. A. T. Wilson for assistance with the X-ray diffraction determination and for numerous helpful discussions; and Mr. D. Ward for helpful discussions on the infra-red analyses.

[1] Bernal, J. D., *Nature*, **190**, 129 (1961); *The Times Sci. Rev.*, No. 40, 3 (1961).

[2] Smith, J. L., *Amer. J. Sci.*, **11**, 388, 433 (1876). Mueller, G., *Geochim. Cosmochim. Acta*, **4**, 1 (1953). Nagy, B., Meinschein, W. G., and Hennessy, D. J., *Ann. N.Y. Acad. Sci.*, **93**, 25 (1961). Calvin, M., and Vaughn, S. K., *Univ. Calif. Lawrence Radiation Lab. Rep.*, No. 8993 (1959); *Proc. First Space Sci. Symp.*, 1171 (1960). Calvin, M., *Univ. Calif. Lawrence Radiation Lab. Rep.*, No. 9440 (1960); *M and B Lab. Bull.*, **4**, 34 (1960).

[3] Marriner, G. R., *Trans. Proc. N.Z. Inst.*, **42**, 176 (1910).

[4] Fisher, D. E., *Nature*, **190**, 244 (1961).

[5] Briggs, M. H., *Evolution*, **13**, 416 (1959).

[6] Fireman, E. L., and DeFelice, J., *Geochim. Cosmochim. Acta*, **18**, 183 (1960). Mason, B., *J. Geophys. Res.*, **65**, 2965 (1961).

[7] Gilvarry, J. J., *Nature*, **188**, 911 (1960); **190**, 1048 (1961).

[8] Miller, S. L., and Urey, H. C., *Science*, **130**, 245 (1959). Wilson, A. T., *Nature*, **188**, 1007 (1960).

[9] Oro, J., *Nature*, **190**, 389 (1961).

THE WORLD UNIVERSITY SERVICE

By HILDA M. BROWNE

General Secretary

FOR three generations since 1920, the promotion of international co-operation and mutual service between and within universities and centres of higher learning throughout the World has been a task carried out by the organization known first as European Student Relief, then as International Student Service, and finally, since 1951, as World University Service. Its material aid programme has been complemented by the facilities it offers for the exchange of knowledge and experience gained in solving basic problems within its sphere. Non-partisan in its attitude, it makes no discrimination as to race, colour, religious belief or political creed.

The officers of the Co-operating Committee for Great Britain and Northern Ireland are: *president*, Sir Walter Moberly; *chairman*, Prof. W. R. Niblett; *hon. treasurer*, Sir Robert Mayer; *general secretary*, Miss Hilda M. Browne, World University Service, 59 Gloucester Place, London, W.1. The address of the International Headquarters is 13 Rue Calvin, Geneva.

Appearing in September

Aircraft Stability and Control

A. W. BABISTER, *James Watt Engineering Laboratory, Glasgow University*

A comprehensive treatment of the whole subject of aircraft stability and control. The theory of longitudinal stability and control and lateral stability and control is developed from first principles, and then it is applied to problems of practical interest and importance. A numerical example is given, and the problems of stability and control discussed. 700 *pages* £7 7*s. net* ($21.00)

Experimental Methods in Combustion Research

A Manual Edited by **J. SURUGUE**

This loose-leaf AGARD Manual presents current and comprehensive data on experimental methods used and recommended by experts in solving combustion problems. Included is Experimental determination of local concentration in flames—R. M. Fristom; The energy balance in the combustion process—A. S. Leah; and Modern methods in high speed photography—J. S. Courtney-Pratt.
300 *pages.* £5 5*s. net* ($15.00)

The Chromatography of Steroids

I. E. BUSH, *Professor of Physiology, University of Birmingham*

This book reviews the available methods for the chromatography of steroids, giving particular attention to the application of the theory of partition chromatography to the behaviour of steroids in chromatographic systems.
448 *pages.* 80*s. net* ($12.50)

Absorption Spectra and Chemical Bonding in Complexes

C. K. JØRGENSEN

In this book the molecular orbital theory is shown to be the appropriate description of complexes, while the ligand field theory (in its electrostatic model) and the valency-bond model (especially Pauling's hybridisation hypothesis) are shown to be inadequate for the description of the energy levels and for the chemical bonding. Chapters on atomic spectroscopy and complex chemistry are included and a large number of references are given. This book will interest inorganic chemists and non-nuclear physicists at a fairly advanced level in addition to theorists of chemical physics.
350 *pages* 70*s. net* ($10.00)

Genetical Variation in Human Populations

Edited by G. A. HARRISON, *Department of Anatomy, University of Liverpool*

Contains the papers presented at the latest Symposium of the Society for the Study of Human Biology, including Blood Groups, Abnormal Haemoglobins and Erythrocyte Enzyme Deficiency Traits, Haptoglobins and Transferrins, Urinary Amino-Acids, and Determatoglyphic Patterns and Pigmentation.
121 *pages* 35*s. net* ($6.00)

The Relation between the Photo-Production and the Scattering of π-Mesons

N. F. NELIPA

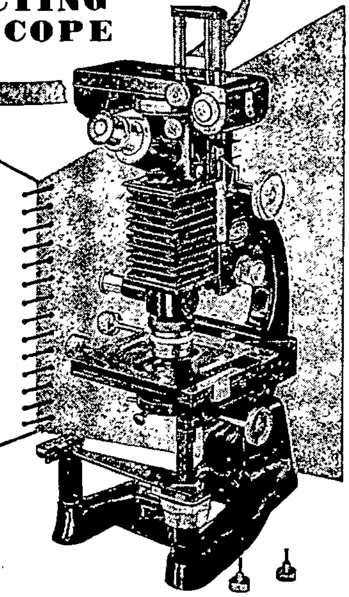
A full multipole analysis of the transition amplitude for photoproduction of mesons, describing in particular the relationship of this process to that of meson nucleon scattering. This book is a valuable complement to that by Baldin, Goldanskii and Rozenthal—*Kinematics of Nuclear Reactions*—(Pergamon Press 1960).
108 *pages.* 30*s. net* ($5.00)

All books gladly sent on 14 days' approval

PERGAMON PRESS
OXFORD LONDON NEW YORK PARIS

Headington Hill Hall, Oxford. 4 & 5 *Fitzroy Square, London, W.*1. 122 *East 55th Street, New York* 22, *N.Y. Gauthier-Villars,* 55 *Quai des Grands-Augustins, Paris* 6

BECK
REFLECTING MICROSCOPE



for work in visible, ultra-violet and infra-red light, utilizing the range of reflecting objectives, with powers of X15 to X172

Full particulars from
R & J BECK LTD., 69/71 MORTIMER ST., LONDON, W.1.

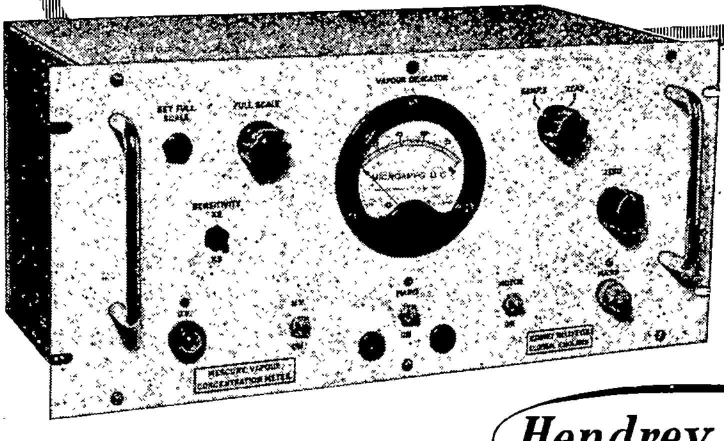
GREEN'S PURE FILTER PAPERS

Ashless, Qualitative,
Hardened, Commercial, etc.,

Write at once for
Price List N.G.

J. BARCHAM GREEN LTD.,
MAIDSTONE ENGLAND

Essential *for sampling and monitoring . . .*



HENDREY
Mercury Vapour Concentration Meter
Type E.3472

Measures concentration of mercury vapour present in air. Essential where quantities of mercury are regularly used or where danger exists of leakage of mercury vapour from apparatus.

Two sensitivities are available at the touch of a switch, giving either full-scale meter reading at a concentration of 770 microgrammes per cubic metre of air or full-scale meter reading at a concentration of 200 microgrammes. Terminations provided at rear for operation of a recorder to give continuous readings of concentration present.

Hendrey Relays

HENDREY RELAYS LTD · BATH ROAD · SLOUGH · BUCKS. *Telephone: Burnham 609/611*
MANUFACTURING ELECTRICAL ENGINEERS CONTROL & LABORATORY APPARATUS
On Admiralty, Principal Ministries and Post Office Lists. A.R.B. and M.O.A. Approved Inspection

The World University Service programme of aid, material and intellectual, is planned by mutual agreement among peers; need does not create inequality within the Service. The problems of a community, as they are seen by those who experience them, are presented for solution to the International Assembly, which consists of representatives, senior and student, drawn from the forty-two countries in which there are national World University Service committees. By accepting a project for inclusion in its programme, the Service can publicize the need and draw to it government and public interest and help.

In this way, health services have been established in countries where ill-health saps the student potential; hostels and student centres have been set up to improve living conditions; educational equipment and scholarships have been provided and funds created for the benefit of refugees, both student and teacher, in flight from man-made and natural disaster.

In Africa this year, the World University Service will assist community development projects on literacy, hygiene and construction which will bring together manual workers and members of the university community in a common effort and so restore the relationship with their own people of the small *élite* in the European-type university colleges. A five-year plan is being developed for the establishment of health services at university colleges in Basutoland, the Congo, Ethiopia, Kenya, Rhodesia and the Sudan, where the increasing influx of students will make medical examination even more essential in the future than it is now. Pius XII College, Basutoland, the only non-segregated college in South Africa, is being besieged by applicants who must be rejected for lack of space. The World University Service is to help with a vast project to raise sufficient funds for building a new Department of Education for which the organization will provide furniture and equipment, and aid will be given to a co-operatively run bookstore supplying text-books and materials not easily obtainable from the usual commercial sources. University College, Ibadan, Nigeria, is to open a co-operative hostel, to relieve the shortage of accommodation which is limiting acceptances. Medical equipment will be supplied for the student health service at the University of Khartoum, and financial help given towards a co-operative book-store there also. For many years the Service has given aid to the African Medical Scholarships Trust Fund, which enabled non-European students to study medicine at the University of the Witwatersrand, and latterly to the Non-European Students Fund which helps students at the University of Cape Town. The passage of the Extension of University Act, limiting the African Medical Scholarships Trust Fund and the Non-European Students Fund to continued support of students already enrolled at the two Universities, was followed by the establishment of the South African Committee for Higher Education, the funds of which are enabling non-Europeans to work externally for university degrees. The World University Service International South African Student Fund is supporting the South African Committee for Higher Education, making supplementary awards to students at Cape Town and providing emergency assistance to university personnel suffering through apartheid.

Direct health projects in Asia, where so much has already been done as the consequence of a student health conference held in 1951, include the establishment of the first health centre at the Chinese Language Colleges in Hong Kong, of student wards at the Banares Hindu University and the University of Bombay, of a comprehensive health service at the latter University, of out-patient care for tubercular students in Japan, where the Service already has two sanatoria, and of a clinic at Seoul, Korea. In addition, medical equipment and supplies, and X-ray apparatus, will be sent to Pakistan and Vietnam, and financial support given for the expansion of the student tuberculosis ward at Masan, Korea, and for further development of the anti-tuberculosis campaign in Thailand.

Outside the field of medicine, other preventive measures must be taken in Asia to combat the danger of ill-health arising out of poverty and malnutrition. Co-operative projects, which provide living accommodation, food, books, stationery, etc., at prices well below the commercial level, can do much to lessen the cost of living for the student. Following a conference on co-operative techniques organized in January 1959 in Madras at which experts addressed the representatives of ten south-east Asian countries, co-operative cafeteria, hostels, student centres, libraries, book-stores, book banks and employment bureaux have been set up by World University Service committees in Burma, India, Indonesia, Japan, Nepal and Pakistan.

Since the end of the Second World War, one of the main duties of European World University Service committees, including that of Great Britain, has been the care of refugee students and staff members in their countries. The two major crises in the past few years have been primarily the Hungarian uprising, when more than 7,000 staff members and students fled to Austria and Yugoslavia, and the flight of Algerian students from France and their native country into Europe, Tunisia and Morocco. Both called for strenuous efforts by the Service to raise funds on the local level and from trusts and foundations in order that staff members might gain the necessary qualifications for continuation of their work in the country of their adoption and that students might have the opportunity of completing their interrupted studies. In Great Britain, the Hungarian refugee settlement programme has provided free scholarships for university study for 119 students, and supplementary grants for those studying at technical and other colleges of higher education. More than £36,000 has been spent by the Service on scholarships and material for Algerian refugee students, many of whom are still in dire need.

Gradually, from the narrow front of Vienna in 1920, the field of activity has spread and moved, as the need for the World University Service help has declined in one area and grown in another. The demand for help always exceeds the supply of funds, which, apart from those drawn from major foundations and non-government agencies for very large-scale special projects, are derived from the community most concerned with higher education.

In Great Britain it is agreed that education should be given according to the capacity of the individual: we must also face our responsibility for the practice of this creed in the emerging and less-prosperous lands, particularly those having direct links with our own. Until they approach our level of economy, World University Service can do much to remove the barriers to teaching and learning set up by poverty, ill-health and lack of equipment.

OBITUARY

Dr. G. C. J. Dalton

It was with deep regret that atomic energy scientists throughout the world learnt of the death of Dr. G. C. J. Dalton on July 17.

Dr. Dalton was born in Te Awamatu in the centre of the North Island of New Zealand just a little more than forty-five years ago. After a distinguished university career at Auckland and Canterbury University Colleges, Dalton was awarded a Rhodes Scholarship in 1936. At about this time he characteristically played in a major rugby football match while suffering from influenza; in his subsequent weakened condition he contracted poliomyelitis which resulted, after a year's illness, in the loss of the use of one of his legs. This did not deter him, and his strong traits of courage, fortitude and determination enabled him to overcome this physical disability. It did not prevent him from breaking his studies at Oxford to take an active part with the Royal Air Force in the radar defence of Britain, to enjoy rowing at Oxford, tennis at Harwell and swimming in Sydney. These brave and unselfish characteristics again revealed themselves during his fatal illness when he continued his work long beyond the time when he could humanly or medically be expected to do so.

Dalton claimed that as the engineer had to design equipment to operate, "he had to know his physics better than the physicist". Dalton himself certainly showed a penetrating and critical approach to the basic scientific problems, and his mastery of these, coupled with his considerable mathematical ability, enabled him to make major contributions to both research and teaching in the newer engineering developments, such as atomic energy.

These broad scientific interests coupled with a critical and imaginative mind showed themselves to full advantage in his initiation of the fast reactor programme at Harwell, in academic life as dean of the Faculty of Engineering in the University of Auckland, in the formulation of the Australian high-temperature gas-cooled reactor programme; and from 1960 as director of the Australian Atomic Energy Research Establishment.

To quote Sir Mark Oliphant: "Dr. Dalton was a warm friend of all who thought about the new ideas in engineering, helpful and generous of his time and effort, inspiring to young and old, and completely devoted to his work, his family and his ideals".

His loss is indeed a grievous one to Australia, to atomic energy and to his many friends and colleagues scattered throughout the Western world.

C. N. Watson-Munro

NEWS and VIEWS

New Appointments at Associated Electrical Industries, Ltd.: Mr. L. J. Davies, C.B.E.

Mr. L. J. Davies has recently been made responsible for the direct supervision of the four Research Establishments of Associated Electrical Industries, Ltd., at Aldermaston, Harlow, Manchester and Rugby. Mr. Davies was educated at Oxford, going there with an open exhibition in natural science, taking his first degree and carrying out postgraduate work, joining British Thomson-Houston Co., Ltd., in 1924. For the past sixteen years he has been director of research at Associated Electrical Industries (Rugby), Ltd. During this period, under his guidance, many changes have been made in the scope and nature of the work and in the laboratory buildings, culminating in the setting up and occupation last year of completely new buildings on an eight-acre site reserved for research. For a period his interests were concerned with lamps and lighting. He was closely connected with the introduction into Britain of the fluorescent lamp and in a form that made it suitable for production for factory lighting during the Second World War. He was awarded in 1942 (with co-authors) the Crompton Premium of the Institution of Electrical Engineers for a paper on this subject. He is a past president of the Illuminating Engineering Society, a member of Council of the British Electrical and Allied Industries Research Association, and chairman of the Board of Governors of Rugby College of Engineering Technology. He was twice Faraday lecturer of the Institution of Electrical Engineers, and was made C.B.E. in 1960.

Dr. J. E. Stanworth

Dr. J. E. Stanworth has been appointed as director of research of Associated Electrical Industries, Ltd., in succession to Mr. L. J. Davies. Dr. Stanworth is a native of Lancashire, and was initially educated at Nelson Secondary School, from which he went to the University of Sheffield to gain a B.Sc. (Tech.) degree with honours in 1935 and his M.Sc. (Tech.) degree in 1937. He joined the British Thomson-Houston Co. as glass technologist in 1937. He spent his first years at Rugby in research on glasses and on glass structure, eventually leading a team on this subject partly in the research laboratory and partly in the glass works of the Company. He then took over wider duties involving the supervision of the research of a large number of groups engaged in investigating new materials and their applications, particularly in the fields of semiconductors, materials for nuclear power reactors, lamps, high-temperature materials and hard materials. Dr. Stanworth was awarded the degree of D.Sc.(Tech.) by Sheffield in 1958. He is a Fellow of the Royal Institute of Chemistry, the Institute of Physics and the Physical Society, and the Society of Glass Technology.

Medical Research Council: New Members

Prof. M. L. Rosenheim (professor of medicine in the University of London at University College Hospital Medical School) and Prof. Wilson Smith (lately professor of bacteriology in the University of London at University College Hospital Medical School) have been appointed members of the Medical Research Council from October 1, 1961.

New Research Group

The Medical Research Council has recently agreed to establish a Research Group at the University of Durham on demyelinating diseases. The Group, to be situated in the William Leech Research Laboratories, Royal Victoria Infirmary, Newcastle upon Tyne, will be under the direction of Dr. E. J. Field, senior lecturer in neuropathology, King's College Medical School, and consultant neuropathologist at the Infirmary; Dr. H. G. Miller, physician in neurology to the Infirmary, will act in an advisory capacity on the clinical side.

Feldberg Foundation for Experimental Medical Research

A foundation to endow an Anglo-German lectureship has recently been established by Prof. W. Feldberg, a senior member of the Medical Research Council's scientific staff at the National Institute for Medical Research. Prof. Feldberg has set up this Foundation out of funds awarded to him by the Federal German authorities as compensation for professional damages. It will be known as the Feldberg Foundation for German–English Scientific Exchange for Experimental Medical Research and will be administered by a Board of Directors consisting of three German and three British scientists. Each year the Board will award a prize to one German and one British research worker of distinction, and in recognition of this gift the German recipient will give a lecture in Britain and the British recipient a lecture in Germany. The prizewinners for 1961 are Sir Lindor Brown, Waynflete professor of physiology in the University of Oxford, and Prof. Herbert Hensel, director of the Physiological Institute of the University of Marburg. In November of this year, Prof. Hensel will lecture at University College, London, on "The Electrophysiology of Thermoreceptors", and Sir Lindor Brown will lecture at Heidelberg on "The Adrenergic Neurone". The German Federal Republic recently conferred a decoration, the 'Grosses Verdienstkreuz', on Prof. Feldberg in recognition of this generous endowment.

Change of Address

The Medical Research Council's headquarters office will shortly be moving to new accommodation, and from September 18 its address will be: 20 Park Crescent, Regent's Park, London, W.1. Telephone: MUSeum 5422.

The International Atomic Energy Agency

The International Atomic Energy Agency, Vienna, has issued a short pamphlet explaining the purpose which the Agency serves and describing its origin, structure and growth and what it has achieved since it came into being in October 1957. By April 1961 more than 600 senior workers from 50 countries had completed their training or were receiving it in 26 countries under the Agency's programme of nuclear science fellowships. The exchange and diffusion of information is another major programme under which four large conferences were held in 1959 and 1960, while under its technical assistance schemes, early in 1961, 30 Agency experts were serving in 16 countries and 7 composite teams had visited 31 countries. Another statutory function is to arrange for supplies of nuclear fuel at the request of member States, and the Agency has also formulated standards and measures of safety for various types of nuclear operations and has worked to establish internationally agreed legal obligations to compensate for damage caused by nuclear accidents. Besides research in its own laboratories on the preparation of standards of radioactive sources, calibration of nuclear equipment and analysis and handling of nuclear materials, the Agency sponsors research projects in member States.

Nuclear Energy Training Courses

As part of its expanding training programme, the International Atomic Energy Agency is organizing, in co-operation with some Member States and specialized agencies of the United Nations, four courses on various practical applications of nuclear energy during September 1961–February 1962. The first such course, on the "Use of Radioisotope Techniques in Soil–Plant Aspects of Agricultural and Forestry Research", held by the Agency jointly with the Food and Agriculture Organization and in co-operation with the Government of the Netherlands, at Wageningen, began on September 4 and will last until October 27. The course is under the direction of Prof. A. C. Schuffelen, head of the Laboratory of Soils and Fertilizers, Agricultural University, Wageningen, Netherlands, and Dr. C. H. Fuller, Brookhaven National Laboratories. A four-month advanced international training course on "Biological Effects of Radiation", commencing October 24, will be conducted by the Agency and the Government of Israel at the Radioisotope Training Centre near Rehovoth. Dr. Peter Alexander, of the Royal Cancer Hospital, London, United Kingdom, will be scientific adviser for this course. A four-week international training course on "Radiation Health and Safety", organized by the Agency and the World Health Organization in co-operation with the Government of Japan, will be held in Chiba City, Japan, during October 24–November 21. This course will be directed by Dr. J. N. Stannard, University of Rochester. A regional training course in the "Medical Applications of Radioisotopes" will be held by the Agency in co-operation with the Government of the United Arab Republic during November 4–December 28 in Cairo. Dr. William J. MacIntyre, University Hospitals of Cleveland, will be general adviser to the course. The courses in Israel and the United Arab Republic are financed from funds provided under the United Nations expanded programme of technical assistance. The sponsoring organizations will provide fellowships and some scientific equipment for the four courses. Further information can be obtained from the headquarters of the International Atomic Energy Agency, Vienna.

Glass in the Electronics Industry

In many ways the lamp and vacuum tube industry is peculiarly dependent on glass as a raw material. Until the advent of semiconductors it was probably true to say that every lamp and electronic device contained several glass-to-metal seals, and the new vapour lamps, particularly the sodium and mercury vapour lamps, only became possible after the development of suitable new glasses to contain the metal vapours. It is appropriate, therefore, that the Philips organization should have devoted a double number of the *Philips Technical Review* to the material glass (22, Nos. 9 and 10, 281; 1960–61). There is a most interesting introductory paper on the history of glass, which is beautifully illustrated with colour reproductions of ancient pictures showing the use of glass and monochrome illustrations of ancient pieces of glass, including one of a Roman cage cup which consists of a series of circles of glass joined together but which has been carved out of the solid. Of particular note are two carefully written articles, one describing the development of automatic bulb-blowing machinery and the other dealing with the automatic control of modern glass-melting tank furnaces. There is a brief reference to the Glass Development Centre opened at Eindhoven in 1957; this is a well-designed laboratory which has its own pilot plant capable of taking to the manufacturing stage any new glasses and articles made therefrom. The more fundamental investigations of glass which are carried out in the central research laboratories are represented by a characteristic article on the structure of glass from J. M. Stevels.

Ferro-Alloy Industry in India

SINCE the turn of the last century, technological developments in the production of ferro-alloys have been remarkable both in range and quality. The National Metallurgical Laboratory, Council of Scientific and Industrial Research, India, has embarked on some major research and development projects to investigate the suitability of Indian raw materials for production of a range of ferro-alloys to exacting specifications and to study the economical application of the latest metallurgical techniques.

A symposium on the ferro-alloy industry in India will be held at the National Metallurgical Laboratory, Jamshedpur, during February 1962. The symposium will cover the following: (1) survey of raw materials in regard to quality and quantity for production of different types and grades of ferro-alloys in India, including auxiliary requirements such as fluxes, fuels, electric power, transport, etc.; (2) beneficiation of raw materials for indigenous ferro-alloy industry based on physical, chemical and thermal beneficiation techniques and ore-dressing cycles, formulation of optimum flow-sheets and assessment of recovery yields and economics; (3) evaluation of electro-thermal, pyrometallurgical techniques and other specific processes and developments in the production of different types and grades of ferro-alloys in relation to possibilities for application in India in the context of indigenous resources; (4) study of the basic physico-chemical and thermodynamic principles involved in the production of ferro-alloys, including theoretical investigations on slag and metal equilibria and related fundamental hypotheses; (5) latest advances in technology of production of ferro-alloys considered in relation to the laboratory-scale, pilot-plant and industrial prototype production trials; (6) utilization of by-products obtained in ferro-alloy industries and overall economics of integrated production cycles; (7) standard specifications for different grades of indigenous ferro-alloys based on corresponding specification of the raw materials needed; (8) present status of ferro-alloy industry in India and future expansion plans to be projected during India's successive Five Year Plans in the general background of trends in world production. Invitations are being extended to technologists, metallurgists and research scientists in India and abroad to attend the symposium and contribute technical papers for discussion. The symposium will provide an international forum for establishing personal contacts and facilitating exchange of ideas on the development of ferro-alloy industry in general and that of India in particular.

Sub-bituminous Coals as Oil-well Drilling Mud-thinners

MODERN procedure in drilling oil-wells, especially the rotary system, is to employ a heavy 'mud' fluid, for example, bentonite, to counteract hole-pressure, act as drilling-bit lubricant, assist in removal of rock-cuttings, and to seal borehole walls. It is customary to introduce additives or thinners to control the rheological properties of drilling mud, which normally tends to a progressive contamination, hence thickening, by rock-powder derived from cuttings. Such additives may be of chemical origin, but also natural humic acid types are employed (in the United States under the common but perhaps somewhat misleading name of 'lignite'). The active components of this type occur in naturally weathered or artificially oxidized coals, from which they can be extracted with aqueous solution. Several Alberta coals are rich in humic acids, and in a recent publication of the Research Council of Alberta, *Alberta Subbituminous Coals as Drilling Fluid Thinners*, by E. J. Jensen (Information Series No. 33. Pp. 25. Edmonton: Research Council of Alberta, 1961), it is concluded that deposits of suitable coals requiring no treatment but pulverization occur abundantly in this Province; that they possess good thinning qualities and are characterized by high cation-exchange capacity and fixed carbon : volatile matter ratio of less than unity. The amount of coal required for maximum thinning is 2–4 per cent by weight of the drilling mud. Further investigation by the Research Council into this interesting development is foreshadowed in this publication, which also includes relevant analytical and experimental data on these Alberta coals as potential sources of drilling mud thinners.

The Le Play Society

SIR JOHN RUSSELL's presidential address to the concluding conference of the Le Play Society at Wadham College, Oxford (April 9–13), has now been published by the trustees of the Frederick Soddy Trust (Pp. 22. Hove, Sussex: The Frederick Soddy Trust, 9 The Drive, 1961). After reviewing briefly P. G. F. Le Play's methods of studying a country and its people and the developments due to Patrick Geddes, Sir John describes the characteristic activities of the Society, including its summer journeys abroad, its classes on the technique of surveying and field surveys by groups of students and the annual conferences. The changed conditions after 1945 limited considerably the character of its post-war visits, quite apart from the great increase in costs, and Sir John also discusses in some detail the drastic changes in environment that modern technology has made possible, for example, in the Zuider Zee and elsewhere in Holland, and in Jutland and the Channel Isles. While the Society can look back with satisfaction on the part it has played in fostering and stimulating interest in survey work, especially the interactions of place, work, and folk, the work has grown beyond its powers, although both name and work will live through the Frederick Soddy Trust. Sir John concludes with a warm tribute to the work of Miss Margaret and Miss Alice Tatton.

Research into Problems of Ageing

THE second compilation of reviews of selected papers reporting research in ageing, supported by grants from the United States National Institutes of Health or conducted in the Institutes' laboratories, has been made from reviews of more than 500 papers published during the year 1960. These articles range from fundamental work on the biology of ageing to studies concerned with the physical, psychological, and social problems of ageing people. The number of grants made to medical schools, research hospitals, and other research institutions for investigations on problems of ageing has been mounting steadily. To-day, 16 million dollars of support is being provided by the National Institutes of Health for more than 700 projects in ageing. The compilation, entitled *Research Highlights in Ageing*, can be obtained from the Superintendent of Documents, U.S. Government Printing Office, Washington, 75, D.C., price 25 cents. (United States Department of Health, Education, and Welfare: Public Health Service Publication No. 854. Pp. vii+50. 1961.)

The Plastics Institute

MR. L. M. READ, administration and services manager, Research and Development Laboratories, DCL Plastics Group, has recently been appointed chairman of the Council of the Plastics Institute. Mr. Maldwyn Jones, development director of the Plastics Division of Imperial Chemical Industries, Ltd., has been appointed vice-chairman. A Building Sub-Committee to deal with all matters consistent with the objects of the Institute relating to the development of the use of plastics in building has been set up. The following are members: H. D. Atkin (Allied Structural Plastics, Ltd.), G. A. Britton (John Laing Research and Development, Ltd.), Dr. W. B. Brown (Monsanto Chemicals, Ltd.), Dr. W. Dawson (Imperial Chemical Industries, Ltd., Plastics Division), D. Gonda (EPS (Research and Development), Ltd.), D. B. Honeyborne (Building Research Station), D. S. Mahon (Bakelite, Ltd.), Dr. Z. S. Makowski (Imperial College of Science and Technology), H. Stanley Smith (Richard Costain, Ltd.), K. Turner (chartered architect), V. H. Wentworth (Monsanto Chemicals, Ltd.).

The Night Sky in October

NEW moon occurs on Oct. 9d. 18h. 53m. U.T. and full moon on Oct. 23d. 21h. 31m. The following conjunctions with the Moon take place: Oct. 5d. 21h., Regulus 0·2° N.; Oct. 7d. 08h., Venus 0·5° S.; Oct. 17d. 05h., Saturn 3° S.; Oct. 17d. 14h., Jupiter 3° S.; Oct. 26d. 17h., Aldebaran 0·6° S. In addition to these conjunctions with the Moon, Mercury is in conjunction with Mars on Oct. 11d. 20h., Mercury being 4° S. Mercury is too close to the Sun for observation. Venus is a morning star, rising at 3h. 15m., 3h. 55m. and 4h. 45m. on October 1, 15 and 31, respectively. Its stellar magnitude on October 15 is − 3·4, the visible portion of the apparent disk increases during the month from 0·870 to 0·934, and its distance from the Earth increases from 131 to 144 million miles. Mars is too close to the Sun for observation. Jupiter is an evening star, setting at 23h. 25m., 22h. 35m. and 21h. 40m. on October 1, 15 and 31, respectively. It is near the border of Sagittarius and Capricornus; its stellar magnitude is − 2·0 on October 15 and its distance from the Earth is 450 million miles. Saturn is an evening star, setting at 23h. 05m., 22h. 10m. and 21h. 10m. at the beginning, middle and end of the month, respectively. It is in Sagittarius; its stellar magnitude is + 0·7 on October 15 and its distance from the Earth is 920 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Oct. 20d. 21h. 55·5m., χ Aqr. (*D*); Oct. 27d. 2h. 40·1m., 318*B*. Tau. (*R*). There is also an occultation of Venus: Oct. 7d. 6h. 31·0m., (*D*); Oct. 7d. 7h. 56·1m., (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The Orionid meteors are active during October 15–25, maximum activity being on October 20. The radiant is at R.A. 6h. 24m., Dec. + 15°, and conditions for observation are moderately favourable.

Announcements

DR. G. G. MACFARLANE, at present deputy director of the National Physical Laboratory (see *Nature*, 184, 1844; 1959), has been appointed director of the Royal Radar Establishment at Malvern, in succession to Mr. W. J. Richards.

As from August 30, the address of the headquarters of the Department of Scientific and Industrial Research became State House, High Holborn, London, W.C.1. Telephone: Chancery 1262.

A CONFERENCE of professors of mathematics from the universities of Great Britain is being convened by the Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom at the end of September to consider the teaching of mathematics. The proceedings will be private.

THE Royal Entomological Society of London is organizing a symposium on "Insect Polymorphism", to be held at the Imperial College of Science and Technology, South Kensington, during September 21–22. Further information can be obtained from the Registrar, Royal Entomological Society of London, 41 Queen's Gate, London, S.W.7.

THE second summer research institute of the Australian Mathematical Society will be held at the Australian National University, Canberra, during January 3–February 1, 1962. Analysis and number theory will be the main fields of research, and Prof. A. Erdelyi (California) will be the guest lecturer. Further information can be obtained from the director of the Institute, Prof. E. S. Barnes, Department of Mathematics, University of Adelaide, Adelaide, South Australia.

A SYMPOSIUM on "Optical Character Recognition" will be held during January 15–17, 1962. It will be jointly sponsored by the Information Systems Branch of the Office of Naval Research Information Centre of the National Bureau of Standards, and will be held in the Department of the Interior Auditorium on C Street, between 18th and 19th Street, N.W., Washington, D.C. Further information can be obtained from Miss Josephine Leno, Code 430A, Office of Naval Research, Washington 25, D.C.

ERRATUM. The article entitled "Reactions of Salt Solutions in Calcium Chloride on Mortar and Concrete", appearing on p. 125 of the July 8 issue of *Nature*, refers to two original articles appearing in the *Australian Journal of Applied Science* (11, No. 4, December 1960).

FROM RADAR TO SPECTROSCOPY: NEW REGIONS OF THE SPECTRUM*

By PROF. D. J. E. INGRAM

Department of Physics, University College of North Staffordshire, Keele

THE applied research that was concentrated on radar and microwave electronics during the War has now produced a new branch of research in pure science; it has opened what we might call a new 'window' in the spectrum of the electromagnetic radiations which stretch from the very energetic γ-rays at one end to the long wave-length radio waves at the other. This is one of the most striking examples of how development in applied physics can sometimes initiate a major advance in fundamental research and thus reverse the normal trend in scientific development.

The study of the emission and absorption of various types of radiation in the different regions of the electromagnetic spectrum may be taken as a reflexion of the progress of the whole history of physics, from its initiation with the classic experiment of Newton in which he split white light into its component colours. The detailed measurements on the spectral lines of different atoms in the visible region, followed by the beautifully simple explanation of all these complex frequencies by Niels Bohr in terms of quantitized orbiting electrons, are other major steps along this pathway. Somewhat earlier, too, the work of Madame Curie and others had shown that the nucleus itself would give off radiations of very short wave-length, and a study of these γ-rays has proceeded ever since to obtain as much information as possible about the nuclear forces themselves.

The electromagnetic spectrum can still be taken as the background to the advances in physics of recent years, and it can be said in all fairness that the two fields in which physics has made its greatest advances since the War have been, first, that of high-energy nuclear physics, with its study of radiations of very high energy produced by large accelerating machines; and secondly, radiophysics—or the study of the discrete radiations absorbed and emitted in the long wave-length region of the spectrum.

Radio astronomers have made particular use of this latter region of the spectrum; they have followed one discrete emission line in their investigations, as well as pursuing their general study of the radio noise, spread evenly across the spectrum, which can be detected from the remoter galaxies near the edge of the observable universe. This discrete spectral line of 21-cm. wave-length comes from the atomic hydrogen present in the arms of our own and other galaxies; and it is used by the radio astronomers to map the features of our own galactic system, and also to measure its speed of rotation from the slight changes that occur in the wave-length.

The terrestrial physicist is also very interested in this spectral line, although he uses it for exactly the opposite purpose: instead of following it out into the nearly infinite depths of space like the radio astronomer, he can pursue its origin back into the very heart of the atom, and deduce the nature and magnitude of the forces that are interacting there. It is possible to do this because the frequency, or wave-length, of the radiation emitted by an atom is related directly to the changes of energy which take place in it when the radiation is emitted. It follows that a careful study of the wave-lengths of these different radiations will therefore give very precise information on the energy-states existing within the molecules and atoms themselves, and, from this, on the nature of the actual forces which hold the atoms together.

The great advantage of carrying out spectroscopy in the radar or micro-wave regions is that very well-defined and highly stabilized wave-lengths are available, and these can be measured to accuracies of better than one part in a thousand million. An immense wealth of information is therefore available from the fine and hyperfine patterns that arise on these spectra, and which are produced by interactions with the nuclei of the different atoms. This was discovered very soon after the end of the War, when Bleaney and his co-workers in Britain, and Good in the United States, first applied the war-time radar techniques to a study of ammonia and other molecules.

Another branch of microwave spectroscopy, which was somewhat slower in starting than the work on gases, but which has now outstripped it, and developed widely in a variety of different directions, is that of electron resonance. In this type of experiment a magnetic field is applied across the specimen at the same time as it is bathed with the microwave radiation, and the energy jumps of the unpaired electrons can then be followed very precisely.

Some of the more practically minded might well ask at this point what relevance such investigations have to everyday life. One can usually justify most scientific investigations on two grounds: first, from an innate sense of curiosity, the desire to find out and unravel the truth so far as we can go; and secondly, from the fact that very useful and practical applications usually derive from this 'pursuit of truth' on the way. So it is with microwave spectroscopy. The war-time developments in radar opened up a vast unexplored region of the electromagnetic spectrum, and we have been eagerly and enthusiastically exploring all its features. This has afforded some new ideas and results which have proved to be of immense practical value.

First, the measurement of frequency and time—we find that the vibrations of the atoms are more regular and reliable than the rotation of the Earth and movement of the stars, and our standard time signals are now, in fact, controlled by microwave frequencies linked to the oscillations of cæsium atoms. A second application of increasing importance is the use of electron resonance as a probe for unpaired electrons in all kinds of systems—those formed by transient chemical reactions, those produced by irradiation damage, and, more recently, in biological systems involving cancerous growth or other abnormal metabolic activity.

---

* Substance of the Kelvin Lecture delivered on August 31 at the Norwich meeting of the British Association.

One final example will serve to turn the wheel full circle and show how the latest results of the pure physicists in this field have now produced a new device which promises to be of immense help to the microwave engineer. This is the maser, which uses the atoms themselves to produce microwave amplification and oscillation, rather than any radio circuits or electron beams. Thus the debt to the applied scientist has been repaid, and this new line of research continues to develop and flourish as the different sides go on together.

BRITISH WELDING RESEARCH ASSOCIATION: NEW LABORATORY

THE official opening by Lord Mills of a new laboratory block of the British Welding Research Association at Abington Hall, Cambridge, on July 12, marked an important stage in the development of the Association's Research Station. Since 1946, when Abington Hall was first taken over by the Association, much of the research work has had to be carried on in some of the original outbuildings of the estate and in wooden huts remaining from war-time military occupation. The first new laboratory was built during 1951–52, to accommodate large-scale equipment for fatigue researches, and this was followed in later years by the construction of a second research block which permitted the integration at Abington of the metallurgical work previously housed in London. The present scheme represents the completion of a further stage that was put in hand three years ago with the building of a welding construction shop that now forms part of the new laboratory opened by Lord Mills.

The new block is a two-storey building, the steel frame of which, in common with the earlier laboratories of the Association, is of welded construction and designed by the plastic theory. The ground-floor, of 15,000-sq. ft. area, accommodates research sections working on the design of pressure vessels and pipe-lines, the brittle fracture behaviour of steel, and the development and application of resistance welding methods. It also contains a machine shop. Office and conference rooms are provided on an upper floor of 8,200 sq. ft. area. The laboratory was designed by Messrs. H. C. Hughes and P. Bicknell, of Cambridge, and erected by Messrs. Rattee and Kett, Ltd., also of Cambridge.

It is appropriate to recall that the pressure, brittle fracture and resistance welding investigations were among the first projects to be inaugurated at Abington. Each, therefore, has a fairly extensive background of development and, clearly, each is seen to retain an important future in the welding research programme as a whole.

The subject of the design of welded pressure vessels was recently discussed at a symposium organized by the Institution of Mechanical Engineers in collaboration with the Association. With a theme of research towards better design, an excellent forum was provided for the presentation and consideration of the Association's particular contribution in this field, which has always been strongly directed towards defining the means of achieving more economical design. This has involved a lengthy and painstaking programme of stress analysis, for example, of the stress distributions set up by typical nozzle attachments, in which the deliberate choice of the examination of large-scale specimen vessels was made and to a large extent made possible by the co-operation of manufacturers of pressure vessels. If, to-day, the experimental approach in this field can be more elegant, it would be difficult to say that this possibility does not arise from the knowledge and confidence won from the large-scale testing work. The answers from such work appear, in any event, to have arrived at a time when, to judge from the mood at the symposium of the Institution of Mechanical Engineers, arguments for advancing code-of-practice design methods in the United Kingdom are likely to be sustained, particularly since much has been done by the Association to translate its accumulated research results into a simple design process.

Equipment for the static and pulsating pressure-testing of large vessels has been moved into the new laboratory, where work will continue on the current phase of the programme, in which attention is being given to some further aspects of the concentration of nozzle-stress factors and also to the important problem of the comparative merits of low-alloy steels in pressure-vessel service. A development foreshadowed in the exhibits displayed by this section on the Association's open day is the study of the behaviour under repeated pressure of vessels at high temperature.

The adjacent section of the new laboratory is concerned with the brittle fracture of welded steel structures and accommodates testing rigs for carrying out the Wells's wide-plate test. Basically, this is a tensile test, giving a stress–strain record to fracture, but the rig is designed specifically to accommodate wide (typically 36 in.), thick plates with the facility for cooling to sub-zero temperatures. The specimen plates contain a longitudinal butt weld, and an artificial notch is placed in the zone of the weld, where it is subjected to the heating cycle and the high, local, residual stresses induced by welding. As a means of studying the influence of important technological factors, for example, quality of steel, type of electrode, stress-relieving treatment, etc., the test has already given results of considerable significance to present practice in heavy engineering in which the brittle fracture problem can in certain circumstances be critical. This work has been done with rigs of 700 and 2,000 tons capacity, counterparts of which now exist in other countries, and the satisfactory performance of these has permitted the Association to advance the design to a new rig, exhibited in model form, that will be used for the study of low-alloy steels and which will have a capacity of 4,000 tons tensile load.

Fundamental aspects of brittle fracture in steel, with particular reference to fracture initiation and propagation, are also being investigated. The determination of a generalized resistance of the material embodied in the measurable quantity fracture toughness, has proved a difficult experimental problem in relation to propagation of fracture. Recent work on measurements of transient force against time in the Charpy (impact) test has indicated, however, that this test might well be capable of modification for the particular purpose. The examination of fracture surfaces for the detection of slip patterns, using suitable materials and etching and microscope techniques, has proved extremely fruitful in the study of fracture initiation. By causing the arrest of fracture in a Charpy test at an extremely early stage, a trigger mechanism has been revealed that appears in accord with Cottrell's hypothesis (*Trans. Amer. Inst. Met. Eng.*, **212**, 192; 1958) of a crack resulting from the intersection of two mutually perpendicular slip bands (see *Nature*, **190**, 432; 1961).

The third research section of the new laboratory of the Association is devoted to work on the resistance group of welding processes, namely, spot, projection, seam welding, etc. These methods are normally used in the quantity production of sheet metal components, in which field the problem of maintaining consistency of quality under rapid fabrication conditions often demands a critical examination of material and process control. The section is equipped with a range of modern welding machines and with ancilliary apparatus for the measurement of the important process variables. In addition, techniques have been developed for the direct measurement of temperatures during spot welding. The problem is complicated by reason of the fact that the generation of heat in the production of a spot weld takes place at the interface region of two overlapping sheets, and temperature gradients are severe. A considerable amount of work has been done by the Association to overcome the difficulty by using buried thermocouples, and the results will be of importance in extending the welding process to harder steels, for which a knowledge of heating and cooling conditions is of particular importance.

The investigations mentioned and those being conducted in other departments are described in the sixteenth annual report of the British Welding Research Association, which has just been issued. For the year under review, the Association's income was £265,379, an increase of £53,252 over the year 1959–60. This increase is, in itself, greater than the total income of the Association in the year when the research station was inaugurated, and is evidence of the rapid growth of the Association to a position in its field which is probably unequalled outside the U.S.S.R.

R. P. NEWMAN

THE NUFFIELD FOUNDATION

THE sixteenth annual report of the Nuffield Foundation*, covering the year ended March 31, 1961, in which grant allocations exceeded £2 million, comments on the extent to which the Foundation sometimes participates in the management of the programmes it supports, thus keeping closely abreast of present-day thought and discovery in its fields of interest. Sometimes its neutrality and independence enable it to take the initiative in bringing together many varied, and sometimes conflicting, interests. Again, it can also, as in the establishment with the Joseph Rowntree Memorial Fund of a National Institute for Social Work Training, or in planning a medical school for the University College of Rhodesia and Nyasaland, draw on its long and varied experience to render important advisory services. Furthermore, its activating role is illustrated by the promotion, at the invitation of the Colonial Office, of an informal but widely representative study of the problems associated with the provision of an adequate and proper supply of British television material to television stations overseas, particularly to those in the less-developed countries. Two conferences were convened for such a purpose by the Foundation, and the resulting document, summarizing the broad conclusions of the study, and making detailed and costed proposals for action, may result in the establishment of a special organization in Great Britain to promote the supply of British television material to overseas countries by all possible means.

Of the allocation of £165,000 for science, including technology, £50,000 went to the Madingley Field Station for the Study of Animal Behaviour, Cambridge, for new buildings and recurrent expenses up to ten years, and £36,000 over five years to the Department of Medical Biochemistry and Pharmacology, Birmingham, for work under Prof. A. C. Frazer on the biological effects of oxidized fats. An additional grant of £3,500 a year for four years to the Department of Chemistry, King's College, Newcastle upon Tyne, in support of Prof. J. Baddiley's work on the structure of cell walls will enable investigations into the biochemical aspects of the cell wall and teichoic acids to be initiated, and a grant of £11,500 over three years is in support of the microbiological aspects of Prof. C. H. Hassall's work at the University College of Swansea on the biosynthesis of complex phenolic compounds; a grant of £4,950 to the Gatty Marine Laboratory, University of St. Andrews, will provide Dr. A. J. Matty with research assistance over three years in his investigations of the thyroids of lower vertebrates.

In technology, a grant of £3,250 for five years was made to the National Trades Technical Societies of Sheffield to assist the introduction of their organization and educational services for adult workers in industry to other industrial areas; £15,000 to the Loughborough College of Technology will provide four postgraduate studentships in ergonomics for each of the years 1961–64 in the newly created Department of Ergonomics and Cybernetics; £5,500 to the Department of Mathematics, Royal Technical College, Salford, is to finance an investigation of mathematics in the electrical engineering industry; and an additional £1,200 to the Department of Chemistry and Food Technology, Borough Polytechnic, will allow Mr. P. D. S. Wood to continue the work on lipids until Easter 1962.

Of grants totalling £600,000 for medical projects, £220,000 was to meet the capital cost of the Nuffield Institute of Nuclear Medicine and running costs in its early years, while, also at the Middlesex Hospital Medical School, up to £45,000 during its first seven years goes to the establishment of a new research unit in biology as applied to medicine. £60,000 to the University of Manchester is to provide the Department of Audiology and Education of the Deaf with more accommodation for clinical and research work for the young deaf child, and a further grant of £29,350 over three years is to go to the audiology unit at the Royal Berkshire Hospital for work on hearing and deafness in early infancy. A capital grant of £70,000 has been made for an extension to the Liverpool School of Tropical Medicine to further co-operation with schools of tropical medicine and endemic disease in Ghana and Thailand, and a further £40,000 to the Zoological Society of London for building the Nuffield Institute of Comparative Medicine. A grant of £30,000 was made to the Central Council for Health Education for an investigation into the present patterns of sexual behaviour and attitudes towards sex and marriage among young people between the ages of thirteen and twenty, which is expected to take three years to complete; one of £6,600 over three years to cover the salary of a research biochemist to work under Prof. G. Belyavin at University College Hospital Medical School on the response of selected cell lines to infection with influenza virus; and another of £3,200 a year for two years to the Institute of Orthopædics to enable Dr. C. H. Lack to expand his work on the role of plasmin and plasmin inhibitors in the breakdown of connective tissue matrix, especially that of cartilage.

---

* The Nuffield Foundation. Report for the year ended 31 March, 1961. Pp. xv+210. (London: The Nuffield Foundation, 1961.)

A grant of £25,000 has been made to the London School of Hygiene and Tropical Medicine, mainly to cover the first year's cost of a training course in industrial hygiene under the direction of Prof. R. S. F. Schilling, head of the School's newly created Department of Occupational Health, and the Foundation has also set aside £250,000 specifically for the further development in Britain of practical schemes of co-operation in industrial health and occupational hygiene. Of this, £15,000 has been allocated to provide the Central Middlesex Industrial Health Service, Ltd., with a permanent clinic, and £20,000 on a tapering basis for the re-organization and reconstitution of the Slough Occupational Hygiene Service, on the understanding that the unit can be made self-supporting within five years.

From the £165,000 for social research and experiment, £12,500 for a period of four years went to the scheme for an experimental family welfare centre at Stevenage New Town to cover the salary of the organizer, rent, maintenance and administrative expenses; £2,000 for a study by Prof. T. Ferguson of Glasgow young people who have been in the statutory care of the local authority and particularly of their assimilation into the community; £20,000 over three years for an inquiry by the Nature Conservancy and the Department of Estate Management, University of Cambridge, into the management of common lands; £9,500 over three years to the Women's University Settlement for developments in the after-care of discharged prisoners; £7,600 to the University of Cambridge for an investigation by Mr. Z. A. Silberston into the economic impact of the patent system in Britain; £5,000 to the Institute of Strategic Studies for the development of the Institute's Library, and £5,000 over five years to the London School of Economics and Political Science to enable Prof. W. A. Robson's group to undertake a more comprehensive study of local government in Greater London.

Among grants totalling £220,000 in education, special attention is directed to two of the smallest: £300 towards the cost of a five-day residential meeting at the Battersea College of Technology, arranged by Dr. L. R. B. Elton, to consider policy and future action in regard to the proposed revision of syllabuses and teaching methods for Ordinary- and Advanced-level physics for the General Certificate of Education; and £1,500 in support of experiments in the teaching of French at East Ham Grammar School for Boys and Talbot County Primary School, Leeds. Grants were made of £100,000 to the Royal National Institute for the Blind for the change-over of the Nuffield Talking-Book Library from records to long-playing tapes; £20,000 to the University College of North Staffordshire for provision of multiple copies of texts and other documentation for the foundation year with an additional £5,000 towards the cost of an assessment of the foundation year; £30,000 over five years to the University of Cambridge for the continuation of its aerial reconnaissance over the British Isles; £6,650 over two years to the Department of Extra-Mural Studies, University of Liverpool, for a Senior Research Fellow to undertake a review of the nature and extent of adult educational provision at the present time; £4,250 for a critical analysis by the Acton Society Trust of management education courses; and a capital grant of £5,000 to Westminster School to form the "Tizard Research Fund" to assist research activities by the science staff of the School. The grant to Prof. P. L. Krohn's gerontological research unit at the University of Birmingham has been renewed a third time at the rate of £7,000 for two years, and an additional £1,800 allocated for Mr. F. Le Gros Clark's studies of women of mature age in paid employment and of occupational opportunities for people in retirement.

Grants totalling £385,000 for the Commonwealth overseas, distributed in forty grants to fifteen different countries, include £85,000 to finance for the next ten years a scheme at the University of Cambridge for a Nuffield Unit of Tropical Animal Ecology; £35,000 over five years to the Plunkett Foundation for Co-operative Studies for training courses aimed at raising agricultural standards in Africa; £10,000 for a survey with the Colonial Office of the incidence of deafness in East and West Africa; £18,000 over six years to the Cotton Research Station, Namulonge, Uganda, for a study of soil fertility in East Africa; £29,000 over five years to the East African Institute of Malaria and Vector-Borne Diseases for study of the effects of malaria on African vital statistics; up to £20,000 for angio-cardiological research equipment for the University College Hospital, Ibadan; a further £16,400 over two years to the University of Edinburgh to continue the study under Dr. K. L. Little of urbanization and the effects of industrialization in Sierra Leone. A further £19,000 was offered to the University of New South Wales in support of the Nuffield research chair of mechanical engineering for an additional five years; £8,000 over three years to the University of Adelaide for Prof. D. Rowley's studies of natural immunity to infectious disease; and £10,000 to the Young Women's Christian Association, Belize, British Honduras, for repayment of a loan for a community centre and hostel for girls.

Allocations to fellowships and scholarship schemes totalled £205,000. United Kingdom awards in medicine included three Heinz fellowships of the British Pædiatric Association, four scholarships at University College, Ibadan, two medical fellowships, and four scholarships and three fellowships in dentistry. One scholarship and two bursaries in biology were awarded, and four senior awards made in sociology. Under the schemes for the Dominions and Colonies there were eleven awards in medicine, fourteen in natural science (including one in engineering) and seven in the humanities and social sciences, in addition to ten Canadian travel grants and twelve farming scholarships (three for Africans). Two travelling fellowships were given to Indian Civil servants and fifteen Royal Society and Nuffield Foundation Commonwealth bursaries were awarded, while 124 grants were made from the Elizabeth Nuffield Educational Fund covering a wide range of projected careers.

EFFECTS OF IONIZING RADIATION ON ORGANIC AND BIOLOGICAL SOLIDS

THE Société de Chimie Physique and the British Biophysical Society combined during July 3–4, in the Laboratoire de Chimie Physique, 11 rue Pierre Curie, Paris, to hold an informal discussion on the effects of radiation on organic and biological solids.

Opening the meeting on the first day, Prof. R. L. Platzman (Argonne National Laboratory, Illinois) outlined very clearly the present state of the theory of primary processes, and the recent developments due to Prof. U. Fano. In systems containing strongly interacting atoms, interaction may influence the primary energy absorption. Molecular structure, and particularly the presence of π-electrons, may affect the proportion of energy absorbed by different components in the primary process. In simple molecules, for example, methane, there is no evidence for such selective mechanism. More information is needed, in particular about the influence of water molecules, before this theory can be applied to biological materials.

Prof. M. Magat (Laboratoire de Chimie Physique, Paris) reviewed reactions in the solid state, in particular low-temperature radiation-induced changes such as polymerization, radical transfer and aromatic ring-opening, which occur by free-radical mechanisms. Members of the Paris group dealt in detail with these reactions, for example, Dr. E. Migirdycian and Dr. S. Leach showed that benzene, if irradiated in the solid state, de-cyclizes and adds on aliphatic groups derived from the ethanol with which it is mixed in solid solution. Similar ring-opening was reported by Dr. K. Hyashi and Dr. S. Okamura (Osaka Laboratories, Japanese Association for Radiation Research on Polymers, Osaka) in the case of lactones, when it is followed by linear polymerization, and favoured by a well-ordered crystalline state.

A feature of the meeting was the use of electron-spin resonance to identify and measure the free radicals produced by irradiation. Drs. R. Bensasson, C. Chachaty, M. Bodard and R. Marx (Laboratoire de Chimie Physique, Paris) used the technique to establish the free-radical nature of low-temperature radio-polymerization of such monomers as acrylonitrile, and Dr. H. Szwarc (Institut de Chimie Physique, Paris) used it effectively to demonstrate the rapid recombination of radicals which occurs on heating irradiated cyclohexane through its transition point at 186° K. However, other factors may be involved, apart from a phase-change, in facilitating radical reactions in the solid. Dr. E. Collinson and Prof. F. S. Dainton (University of Leeds), while demonstrating that scavenging of radicals is more effective in the solid and with aromatic compounds, conjectured that this could be due to the π-electron configuration of the phenyl groups. They also found that a glassy solid behaves more like a liquid than a crystalline solid. The important distinction between aromatic and aliphatic compounds was further emphasized in work by Dr. R. Mason (Imperial College of Science and Technology, London) on post-irradiation thermo-luminescence, when the intensity of emission was found to be a thousand-fold greater in aromatic than in aliphatic amino-acids.

Prof. J. A. V. Butler (Chester Beatty Research Institute, London) introduced the second session by stressing the need for a re-appraisal of such terms as 'direct' and 'indirect' effect, 'target', etc., and emphasized that in living systems, the balance is dynamic, and that radiation need be damaging at only one of a large number of places.

A question of continuing interest is the role of oxygen in radiation damage, and a number of contributions dealt with this factor. Drs. A. Müller, G. Hotz, and K. G. Zimmer (Institute of Radiobiology, Nuclear Research Centre, Karlsruhe) found a modified electron-spin resonance signal when bacteriophage was pressed into pellets in such a way as to expel oxygen, and Dr. L. Ehrenberg (Institutionen för organisk kemi och biokemi, University of Stockholm) on irradiating *Agrostis stolonifera* seeds could distinguish two types of electron-spin resonance signal, one of which is closely correlated with oxygen, and is decreased by pre-heating, that is, when oxygen is expelled. Dr. E. L. Powers (Argonne National Laboratory, Illinois) described recent work on bacterial spores, which offer a convenient experimental system for radiation work. Part of the damage is toxic only in the presence of oxygen. This can be estimated directly, and it is also possible to observe the effect of heat by using high linear energy transfer radiation, when annealing occurs during irradiation. The heat generated during heavy-ion bombardment may be an important and neglected factor, and in the work of Dr. T. Brustad (Norsk Hydro Institute for Cancer Research, Oslo), to be mentioned later, may in part explain the apparent lack of effect of additives.

The nature of the increased damage brought about by oxygen is much under discussion, and whereas the signals observed by Dr. L. Ehrenberg are not characteristic of peroxy-formation, Prof. G. Stein, (Hebrew University, Jerusalem) found a signal when polymethylmethacrylate was irradiated in the presence of oxygen which appears to be peroxidic. This system, moreover, provides a convenient method of measuring rates of oxygen diffusion. In certain circumstances, the influence of oxygen may be minimized by limited diffusion; for example, A. B. Robins and Prof. Butler (Chester Beatty Research Institute, London) found that a high dose-rate can obscure the oxygen effect completely in the case of trypsin.

The use of electron-spin resonance in free-radical investigations has already been mentioned, and Prof. D. J. E. Ingram (University College, Keele) outlined how this can be used in the study of proteins, where electron trapping may occur. It may be possible to correlate denaturation of a protein with an interference in this trapping process since the electron-spin resonance signal does not disappear on warming in the normal way. However, it must be remembered that when applying electron-spin resonance to biological materials, water, for example, plays a part which is little understood.

Sulphur compounds are of much interest on account of their use as protective agents. Dr. R. Koch (Freiburg) investigated more than a hundred compounds by electron-spin resonance, but no simple correlations between structure and function can be seen at the present time. The radical nature is considered to be localized on the sulphur atom, and Dr. P. Alexander (Chester Beatty Research Institute, London) and Mr. M. G. Ormerod (Royal Military College of Science, Shrivenham) provided evidence that in the case of nucleoprotein protected by cysteamine, such localization can occur, minimizing the damage. There seems little doubt that the radicals produced on irradiation of keratin are cystine-like (Dr. A. Redhardt and Prof. B. Rajewsky, Max Planck Institute for Biophysics, Frankfurt), but unaccountably, cysteine and related compounds do not appear to protect by a factor of more than two in a system such as yeast, when judged by quenching of electron-spin resonance signals (Dr. B. Smaller, Argonne National Laboratory, Illinois).

In general, it appears that dry solids are more sensitive than wet solids or solutions, and Dr. R. A. Braams (Physical Laboratory, University of Utrecht) reported a ratio of three between dry and wet tendon. Dr. Robins and Prof. Butler found solid trypsin to be more sensitive than its solutions, possibly due to the self-annealing processes in the presence of water. The last-mentioned authors also reported that the radiosensitivity of trypsin is modified by the medium from which it is evaporated and a low *p*H leads to a highly sensitive state, possibly by aggregation. It is significant that at high linear energy transfer Brustad obtained target sizes in excess of the accepted molecular weight for some enzymes; this also raises the question of energy transfer in the solid state. The effect of added materials depends on their being intimately mixed with the substrate, and the state of the solid is thus again seen to be important.

Two papers were presented dealing particularly with chemical changes on irradiation. Dr. W. M. Garrison (Crocker Laboratory, Lawrence Radiation Laboratory, Berkeley) found good evidence for the conversion of the —NH—CH— configuration in amino-acids into keto-acids, and as little as 10^{-8} mole can be detected as derivatives. Dr. H. A. Dieu (Laboratoire de Chimie Physique, Université de Liège) pointed out that irradiation of ovalbumin leads to a more ready attack by proteolytic enzymes, and an 'unmasking' of sulphydryl groups with the heaviest doses. Moreover, since the rate of subsequent attack by pepsin depends on the dose, and that by trypsin does not, it is probable that the aromatic amino-acids have been preferentially destroyed. In the myoglobin molecule it appears that possibly the leucine residues are the foci of damage, in particular those in more-exposed parts of the molecule, and Mr. C. C. F. Blake and Dr. D. C. Phillips (Davy Faraday Laboratory, Royal Institution, London) provided evidence for this in which the effect of radiation was studied directly by X-ray crystallography.

In summing up, Dr. L. H. Gray (Radiobiology Unit, Mount Vernon Hospital, London) stressed that physical theories, such as that of Prof. U. Fano, must be cautiously applied to biological systems, bearing in mind their complexity and such unknown factors as water content. It appears that the degree of crystallinity of the solid phase may be of great importance, and electron-spin resonance has helped to emphasize this. Concerning the role of oxygen, the accessibility of this gas may be a factor that has not received enough attention hitherto.

Finally, I was struck by the different interpretations that were placed on electron-spin resonance signals, and feel that this useful technique needs to be used with reserve when drawing conclusions.

A. B. Robins

BACTERIA OF THE INTESTINE

THE summer conference of the Society for Applied Bacteriology was held at Wye College, Ashford, Kent, during July 11–13, and included a symposium on "Bacteria of the Intestine".

Dr. H. Williams Smith, in his paper entitled "The bacterial flora of the fæces of animals with particular reference to its development in the young", described experiments on the development of normal flora in healthy animals. Rectal swabs were shaken in phosphate buffer, and after passing the suspension through muslin, colony counts of *Escherichia coli*, staphylococci and *Clostridium welchii* were made.

Calves, lambs, piglets and a baby were examined daily for 2–3 weeks, weekly for three weeks, monthly for nine months and twelve months (baby). One rabbit was examined weekly.

In the calf, *E. coli*, streptococci and *Cl. welchii* predominated on the first day, but bacteroides domination began at two days and these organisms rapidly multiplied up to fourteen days, when lactobacilli followed. Counts slowly dropped to 10^7–10^8/gm. of fæces after thirty weeks.

Similar results were obtained from lambs and piglets, except that streptococci dominated later with *E. coli* in lambs, and the piglet flora was dominated ultimately by lactobacilli. Results for the baby were similar also, with counts of 10^{11} for *E. coli* at first, followed by a bacteroides count of 10^{10}; *Cl. welchii* rose sharply at twenty-five weeks when the child started eating solid food. The counts remained high after a year; this was different from the animals. *Staph. aureus*, 10^2–10^6/gm, was frequently found in the baby but not in the animals. The rabbit showed a constant bacteroides count of 10^9; the lactobacilli and streptococcal counts were 10^4 and 10^5. Gram-negative anaerobic cocci up to 10^8–10^9, and yeasts in older calves, were also found. There was a constant population of *E. coli* but frequent changes in phage types.

Adult animals, including cats and dogs, showed a high count of *Cl. welchii* which varied in degree according to the diet, being alternately high and low when fed on meat or bread. Similarly, if pigs were starved and fed alternately the count went down and up accordingly. The floras of adult guinea pigs were dominated by lactobacilli.

Dr. H. Haenel ("Micro-ecological rules in the composition of the intestinal flora of man") said that before birth the baby was germ-free, at birth infection was acquired from the mother's vagina and fæces, and after 3–4 days lactobacilli predominated. The effect of the mother's milk was to replace aerobic lactobacilli by *Lactobacillus bifidus* and to depress clostridia but not *E. coli.* After weaning, *L. bifidus* stayed at a high level, but aerobic lactobacilli and clostridia returned later. Similar results were obtained for poorly developed children in hospital. Factors ruling the flora were easily disturbed; when stools were stored the lactobacilli diminished and streptococci increased and under abnormal conditions there was a reduction of lactobacilli and an increase in proteolytic organisms.

Antibiotics such as terramycin abolished *L. bifidus* and there was an increase in streptococci and aerobic lactobacilli, with an occasional predominance of *Proteus*; the normal flora returned quickly when the antibiotic was stopped. Under antibiotics salmonellæ disappeared but they could return later; they sometimes shared the normal flora without disturbance. After gastrectomy, the anaerobic lactobacilli disappeared, *E. coli* counts became higher than normal; enterococci, clostridia and other organisms increased also and the large intestine showed high counts. In the adult the flora was stable. Normally the small intestine was nearly sterile, and the large intestine showed mostly anaerobic lactobacilli. Under abnormal conditions there could be a predominance of *E. coli* in the small and no anaerobic lactobacilli in the large intestine; the flora might be the same throughout the intestine with anaerobic lactobacilli absent.

Dr. Anne Dickinson and Dr. G. Mocquot ("Studies on the bacterial flora in the alimentary tract of pigs: 1, Enterobacteriaceae and other Gram-negative bacteria"), observed pigs of 20–100 kgm. in individual pens; the diet was supplemented with antibiotics in some animals. The object was to identify Enterobacteriaceae and other Gram-negative flora isolated on deoxycholate citrate agar after 24–28 hr. at 37° C. Details were given of ten groups classified mainly on morphology, colonial appearance, motility, whether mucoid or not, glucose agar stab cultures, lactose fermentation, growth at 45° C., indole, lysine decarboxylase, urea hydrolysis, production of hydrogen sulphide, liquefaction of gelatine, and ornithine and ammonia. There was no change in the distribution of types under antibiotic feeding. Group 1 usually dominated and the other groups varied for no apparent reason.

Drs. P. Raibaud, M. Caulet, J. V. Galpin and G. Mocquot ("2. Streptococci: selective enumeration and differentiation of the dominant groups") described the isolation of 415 strains of group D streptococci from cæcal and fæcal swabs, using their own medium and also those described by Barnes, Slanetz and Kjellander; thirty-eight types could be distinguished by fermentation reactions differing in at least one carbohydrate. Urea hydrolysis was used for differentiation and the results were regarded as of taxonomic significance. The predominant streptococci were related to *Streptococcus bovis* or *Strep. equinus*, but the classification of these organisms is at present extremely confused.

Dr. M. Lev's paper on "Germ-free animals and their use in elucidating the action of the gut flora on the host" was read by Dr. Cuthbert. Using an apparatus consisting of a nylon tube over a frame, all of which could be autoclaved, chickens and turkeys were hatched from washed and sterilized eggs and the young mammals, monkeys and guinea pigs removed by cæsarean section. Sterilized foods with and without antibiotics were given through tubes, and the effect on the germ-free birds and animals noted. Normal chicken fæces fed to germ-free chicks depressed growth, but feeding penicillin depressed *Cl. welchii* and stimulated growth. Feeding *Cl. welchii* to germ-free chicks depressed growth, but the effect was partly reversed by penicillin in the diet; growth was not increased when germ-free animals were given penicillin. Toxins of *Cl. welchii* caused the gut to thicken and this was prevented by penicillin. Feeding lactobacilli, streptococci and *E. coli* to germ-free creatures had no effect, but the addition of *Cl. welchii* depressed growth, which again was partly reversed by penicillin.

Deficiency in vitamin A led to infection, and gross deficiency caused death; lesions could be seen in the germ-free animals. Germ-free animals on a vitamin K-deficient diet suffered prolonged gut bleeding and the blood had no clotting power. Menedian (four vitamins) did not restore the clotting power, and it is suggested, therefore, that gut bacteria are needed to convert the menedian to some form which can be used.

Entamoeba histolytica did not attack the germ-free gut but survived for a few days only when *coli-aerogenes* were present; they did not survive when autoclaved cæcal contents were fed. There appeared to be complex factors involved in harmless organisms promoting a lethal effect in contact with pathogens. *Shigella flexneri* did not attack the normal guinea pig, but killed the germ-free animals; this was prevented by prior feeding with *E. coli,* non-colicine forming and not demonstrably toxic *in vitro*.

Germ-free animals were equally affected by hæmorrhagic shock caused by the endotoxins from gut Gram-negative flora. Germ-free chicks after five weeks exposure to conventional conditions suffered severe setbacks. Tests are needed on synthetic diets.

Dr. Joan Taylor, describing the "Host specificity and enteropathogenicity of *Escherichia coli*", said that the typing of pathogenic *E. coli* was based on '*O*' (somatic), '*K*' (surface) and '*H*' (flagellar) antigens. Infantile diarrhœa was due to types 0111, 055 and 026 mainly, which were found in all parts of the world. 0128 and 0127 could be found in the United Kingdom and United States respectively, but they were now beginning to cross-spread.

Epidemiological correlation confirmed the role of these organisms in infantile diarrhœa. With the three major types, it was possible to follow the rise in numbers until diarrhœa occurred, beginning when there was a pure culture and ending when other organisms were re-established. But there were similar occurrences with no symptoms. Volunteer feeding had confirmed the pathogenicity of 0111 in students, prisoners and a baby, and of 055 more feebly in prisoners.

Calf scours was related to *E. coli,* particularly 078, which was widely distributed, and may become septicæmic. Swine œdema due to 0138 and 0139 was frequent in the United States, United Kingdom and Canada. Enteritis in lambs due to type 078 occurred in the United Kingdom and Australia; hen septicæmia was caused by type 02.

There was little information about the occurrence of these organisms in healthy animals, but the human types 026, 055, 0111 were found in cats, dogs, and kittens without symptoms. Surveys suggested limited distribution in different species and disease in some only.

Infection experiments were carried out on alternate loops of sterile gut, in anæsthetized rabbits; the rabbits were killed 24 hr. later by injection of air. Inflammatory reactions with exudations occurred in the infected loops, but non-infected loops were sterile and normal. Many strains from human babies, also chicks, water, calf and swine strains, gave no reaction, although some calf-strains were positive. Living cells were necessary for infection.

Dr. J. G. Collee, Miss J. A. Knowlden and Dr. Betty Hobbs, in their paper on "Studies on the growth, sporulation and carriage of *Clostridium welchii* with special reference to food poisoning strains", observed that typical *Cl. welchii* type *A* strains were completely β-hæmolytic, and that strains were called non-hæmolytic if the hæmolysis was not complete. Food poisoning strains were non-hæmolytic and resisted 100° C. for 1 hr. 80/80 samples of fæces yielded *Cl. welchii*. 39/50 (78 per cent) stool samples from students gave non-hæmolytic strains, but spores from three (6 per cent) strains only were remarkably heat-resistant, as demonstrated by tests on fæces and tests on sporing cultures. Previous reports had given 2 per cent as the average carrier-rate of non-hæmolytic heat-resistant types in normal people; hospital personnel had shown 20 per cent or more. Heat-resistance tests for spores in stock strains revealed the protective effect of cooked meat, which was most apparent with heat-resistant strains. Preliminary findings indicated that the heat-resistant property of typical food-poisoning strains was genetically determined and was not primarily dependent on the presence of large numbers of spores.

Food-poisoning strains seldom produced spores in cooked foods and it was suggested that food should be streaked on neomycin blood agar for non-hæmolytic anaerobic colonies, which should be picked off, grown in Ellner's sporulation medium and the heat-resistance tested. It should be noted that hæmolysis on blood agar was not entirely consistent and spore yields from Ellner's medium were not reliable. Confirmation was given by slide agglutination with the thirteen antisera available; serological cross-reactions and untypable strains still occurred occasionally. It was possible to type 75 per cent of 298 strains submitted during 1960-1961.

An unusual growth pattern at 50° C., the Phœnix phenomenon, was observed with both heat-sensitive and heat-resistant strains; there was no difference between the growth curves of food-poisoning and non-food-poisoning hæmolytic strains over the normal range of temperature, and both could grow under nearly similar conditions, which accounts for occasional outbreaks caused by the heat-sensitive β-hæmolytic types. These strains were unlikely to survive cooking, but may be protected mechanically by lack of heat penetration or may be introduced and find a favourable anaerobic environment during the cooling period.

It was stressed that the relationship of these observations to the bulk cooking of meat and its known association with *Cl. welchii* poisoning of food merited further investigation.

Dr. Betty Hobbs, describing "The public health significance of *Salmonella* carriers in livestock and birds", considered the carriage of salmonellæ by animals and birds in relation to the contamination of foods provided for human consumption; human carriers now appeared to be less important than animal carriers. Particular attention was directed to poultry and egg products and to the contamination of boneless and carcass meats, horse meat and pet food. A parallel was shown between *Salmonellae* isolated from abattoirs and in local populations.

It was suggested that improved conditions and speed of transport for animals passing from farms to abattoirs and more care and attention over hygiene in processing may reduce the spread of infection among animals and the spread of contamination on carcass meat; also, that the pasteurization of egg products and pelleting of animal foodstuffs, both of which eliminated *Salmonellae*, would help markedly to reduce the introduction of *Salmonellae* into bakeries and farms. The increase in salmonellosis due to exogenous types was most probably due to the introduction of egg products, meat, and now coconut contaminated with these types.

Drs. R. Buttiaux and D. A. A. Mossel ("The significance of organisms of supposed fæcal origin in food and water") suggested that the enumeration of organisms indicative of fæcal contamination was necessary because: (*a*) direct tests for pathogens were difficult and statistically uncertain, and (*b*) repeated absence of indicators gave virtual certainty of absence of fæcal contamination, and also (*c*) guaranteed absence of non-pathogenic fæcal contamination. Human indicator organisms had been preferred hitherto but animal indicators might be equally or more important.

The test should be quantitative and foods classified as stable, that is, dried, or perishable, raw or heated. The indicator organisms should be frequent in the gut and scarce outside, but they must be able to survive outside the enteric environment.

E. coli I was common in fæces but not outside. *E. coli II* was fairly common in fæces but occurred elsewhere also. *Klebsiella*, non-motile and urease-positive, were frequent in human stools, also in swine stools but seldom elsewhere. Lancefield Group *D* streptococci were common in animals but little was known about their survival in the environment. *L. bifidus* and bacteroides were unsuitable.

Dr. J. H. McCoy, introducing the discussion, emphasized the fact that examination of water was chiefly concerned with measures against typhoid fever, dysentery and cholera and none of the bacteria responsible was carried by animals; thus interest was centred on human contamination. The survival of *E. coli* in water meant recent pollution; if these organisms were absent there had been time for pathogens to die, as the survival-rate of *E. coli* was similar to that of pathogens. The treatment of sewage aimed to reduce nuisance, not bacteria; *Salmonellae* could be isolated easily from treated sewage. In food the organisms had opportunity for growth, hence numbers of fæcal organisms at time of delivery bore no relation to the degree of original contamination. In untreated food the presence of intestinal organisms led to an assessment of cleanliness and not to safety; therefore there should be direct examination for pathogens also.

Dr. N. P. Burman, who continued the discussion, showed tables which gave the optimum growth temperature characteristics of coli-aerogenes from various sources and also the coli-aerogenes and streptococcal flora of shallow wells and mains. IMVIC types other than *E. coli* could be found in a variety of places, for example Wilson's irregular type 6.

Coliforms were stimulated by copper treatment, and *Cl. welchii* could be found in chlorinated water supplies. Streptococci found in river waters were mostly animal types, although many strains on membrane filters at 37° C. were untypable; these were eliminated if incubation at 37° C. was exchanged for 45° C. after 4 hr.

In more general discussion, Dr. Blanche Koelensmid and others expressed satisfaction at the authoritative indication that fæcal bacteria in foods cannot be regarded with the same significance as in water.

VITAMIN B_{12} AND INTRINSIC FACTOR

ABOUT 150 leading workers from forty-five laboratories in fifteen different countries attended the second European Symposium on "Vitamin B_{12} and Intrinsic Factor", organized by Prof. J. Kühnau and Drs. W. Friedrich and H. C. Heinrich, and held in the Institute of Physiological Chemistry in the University of Hamburg during August 2–5. Prof. Kühnau, in his opening address, emphasized the importance of the newly revealed structure of vitamin B_{12} coenzyme, endowing it with remarkable activity. In the first symposium at the same place five years ago a notable feature was the disclosure of the structure of vitamin B_{12}, the culmination of several years work by Dr. Dorothy Hodgkin *et al.* at Oxford. This was then the most complex molecule of which the structure had so far been elucidated. In this second symposium, the climax of years of patient work by Drs. Hodgkin and P. G. Lenhert came just in time for the disclosure of the structure of a still more complex molecule, that of a vitamin B_{12} coenzyme, the form in which the vitamin appears mainly to exist in the animal and human body.

Crystals of the 5 : 6-dimethylbenziminazole cobamide coenzyme were grown and photographed with copper X-radiation while in contact with their mother liquor. A complete X-ray analysis of the structure revealed some interesting differences from the structure of cyanocobalamin, which at present is the official form of the vitamin. The new unsaturated pentose previously reported to be liberated from the coenzyme by mild acid hydrolysis has now been found to be an artefact produced during the hydrolysis from ribose through which adenine is linked to the cobalt in the molecule. There may be a free valency in the coenzyme molecule that could play a vital part in metabolic reactions yet to be elucidated.

These differences in chemical composition and molecular structure between vitamin B_{12} and its coenzyme are reflected in differences in their absorption spectra. H. A. Barker *et al.*, at the University of California, where pioneer work on the coenzyme was done, found that in place of the characteristic sharp band of cyanocobalamin at 361 mμ the coenzyme shows a broader band at about 380 mμ and a very slight band at 340–345 mμ. The fairly broad cyanocobalamin band with maxima at about 520 and 550 mμ is replaced by a very broad B_{12} coenzyme band with maximum at about 520 mμ and a slight shoulder at about 495 mμ. The cyanocobalamin band at about 278 mμ is replaced by an inflexion at 288 mμ in alkaline solution shifting to 284 mμ in acid solution and considered specific for the coenzyme. Characteristic differences in absorption spectra of purine- and benzimidazole-containing coenzyme analogues appear to depend on differences in the strength of the bond between the base and cobalt. There is evidently scope for further spectroscopic studies, including a search for isosbestic points that are so prominent in mixtures of cyano- and hydroxocobalamin, now considered to be artefacts obtained from B_{12} coenzyme in the liver and other body stores. E. E. Gabbe and H. C. Heinrich (Institute of Physiological Chemistry, University of Hamburg) emphasized the importance of the freedom of B_{12} coenzyme from cyanide, which they investigated in experiments using radioactive preparations.

B_{12} coenzymes catalyse various reactions that can be used for their enzymic assays. These include conversion of glutamate to β-methylaspartate by the bacterial glutamate isomerase system, the methylmalonyl isomerase reaction and the conversion of certain glycols to deoxyaldehydes. Experiments with deuterium oxide exclude reaction mechanisms involving the intermediate formation of a free proton. These reactions have been studied in bacterial extracts but the methyl-malonyl isomerase reaction occurs in higher organisms.

These findings apply to different B_{12} coenzymes, of which several have been isolated by H. A. Barker *et al.* They appear to be the most abundant corrinoid compounds in various bacteria (for example, *Propionibacterium*) and in mammalian, including human, livers. However, D. Perlman and his colleagues at the Squibb Institute for Medical Research, New Jersey, obtained only 5,6-dimethylbenzimidazolyl cobamide coenzyme from 30 cultures of *Streptomyces*.

B_{12} coenzymes are highly sensitive to light and cyanide. Photolysis yields hydroxocobalamin, which the minute traces of cyanide always present in blood can partly convert to cyanocobalamin. Polarographic studies of these reactions have been carried out by K. Bernhauer *et al.* at Stuttgart. While body tissues usually contain a mixture of hydroxo- and cyanocobalamins, the latter, being at present the officially accepted form of vitamin B_{12}, has been chosen as the standard of comparison with B_{12} coenzyme by a number of investigators. M. E. Coates *et al.* (National Institute for Research in Dairying, Shinfield) detected no difference between the absorption of B_{12} coenzyme and that of cyanocobalamin (both labelled with cobalt-58) through rat intestine in presence of rat stomach extract. Chick assays showed the coenzyme to have only 78 per cent of the activity of cyanocobalamin when administered intramuscularly, but the coenzyme appeared more effective than cyanocobalamin in restoring hatchability in depleted chick embryos. On the whole the coenzyme does not seem to have any therapeutic advantage over cyanocobalamin.

A. W. Johnson (University of Nottingham), described synthetic approaches to the corrin ring system by Sir Alexander Todd *et al.* (Cambridge). Using radioactive delta-amino-lævulinic acid, porphobilinogen has been shown to be a common biogenetic precursor of vitamin B_{12} and of the natural porphyrins. Biosynthesis by different pathways has been studied by K. Bernhauer *et al.* (Stuttgart) and by A. Di Marco *et al.* (Laboratori Richerche Farmitalia, Milan).

A valuable indication of an important biological function of vitamin B_{12} was obtained by L. A. Manson (Wistar Institute, Philadelphia), who observed deoxyribose synthesis by vitamin B_{12} in a murine leukæmia cell of thymic origin which by leading to nucleoprotein synthesis may explain one of the roles of the vitamin in cellular metabolism. B. Connor Johnson *et al.* (University of Illinois), using the DR-aldolase system, found no relationship between B_{12} deficiency and deoxyribose biosynthesis by C_2 + C_3 condensation, and when investigating the role of B_{12} in thymidine synthesis showed that carbon-14 in glutamate was incorporated into the DNA-thymine of regenerating liver. H. R. V. Arnstein and A. M. White found cyanocobalamin to increase biosynthesis of phenylalanine, tyrosine and valine and concluded that neither general inability to oxidize and reduce one-carbon units nor to synthesize thymine can represent the main metabolic lesions in vitamin B_{12} deficiency.

In the search for specific effects of vitamin B_{12} deficiency much attention has been directed to the production of antimetabolites to the vitamin which could be used to produce and investigate controlled deficiencies. E. Lester Smith (Glaxo Laboratories, Ltd., Greenford) found the amides of the monocarboxylic acid, preferentially produced on mild hydrolysis of B_{12}, to prevent conversion of the vitamin to its functional form, but while they competed effectively with B_{12} for uptake by intrinsic factor and inhibited enzyme systems *in vitro* they produced no changes in the blood or bone marrow. W. Friedrich, H. C. Heinrich and P. Riedel (Hamburg) succeeded in producing still more effective antimetabolites from factor V_{1a} isolated from sewage sludge. The resulting alkanolamine B_{12} analogues showed very high inhibitory activity on the growth of *E. coli* and *Ochromonas malhamensis*. H. Heinrich related B_{12} specificity to the structure and position of the alkanolamine, nucleotide, and side chains in the corrin ring. Some attention is now being directed to human dietary deficiency of vitamin B_{12} which may occur in vegans (strict vegetarians consuming no animal food) and was first described by F. Wokes, J. Badenoch and H. M. Sinclair to the International Congress of Nutrition in Amsterdam in 1954. F. Wokes (Vegetarian Nutritional Research Centre, Watford, Herts) and A. D. M. Smith (Middlesex Hospital Institute of Clinical Research, London) gave at the Hamburg B_{12} Symposium further details of a B_{12} deficiency characterized by low serum B_{12}-levels and neurological manifestations with lesions in the peripheral and central nervous systems, well marked in some vegans, and of slight occurrence in vegetarians with low intakes of animal protein and vitamin B_{12} from eggs, milk and cheese, as well as in about 1 per cent of the general population. In India, where the Vegetarian Nutritional Research Centre has been asked to help, strict vegetarians consuming scarcely any animal food encounter much more marked B_{12} deficiency than is found in vegetarians consuming dairy produce, while meat-eaters encounter no vitamin B_{12} deficiency. To avoid widespread protein malnutrition in India an extra 2 million tons of protein will be needed annually, which should be fortified with B_{12} to improve its biological value.

Vitamin B_{12} seems to be missing from all staple vegetable proteins. Slight traces in certain leaf proteins (for example, alfalfa, turnip tops) are not significant sources in human diets. However, A. Pfau and G. Kallistratos (Max-Planck Institute for Animal Breeding and Nutrition, Mariensee, near Hanover) found that broad bean (*Vicia faba*) plants can take up vitamin B_{12} from nutrient solutions, about 27 per cent being absorbed in 24 hr. from solutions containing physiological concentrations of B_{12} labelled with cobalt-60. The absorbed B_{12} was found mainly in the roots (87 per cent of total absorbed) and much less in the leaves (3 per cent). In the ensuing discussion F. Wokes reported that in an investigation at Watford, using plants of Russian comfrey (*Symphytum peregrinum*), 200 μgm. per gm. total solids were found in roots and 76 μgm./gm. in total solids in leaves after 24 hr. immersion of the undamaged roots in 50 ml. of aqueous solution containing 40 mgm. B_{12}. These findings may find application in manuring, especially with sewage sludges rich in B_{12}.

The Symposium agreed that the term hydroxocobalamin should be used to describe products in which the cyanide group of cyanocobalamin is replaced either by —OH (in alkaline solution) or by [H_2O], hitherto termed aquocobalamin (in acid solution). Hydroxocobalamin obtained by photolytic decomposition of vitamin B_{12} coenzyme is present in body tissues in amounts at least equal to those of cyanocobalamin. R. F. Schilling *et al.* (University of Wisconsin) found hydroxocobalamin to be absorbed as efficiently as cyanocobalamin and better retained. After intramuscular injection hydroxocobalamin gives much higher serum B_{12}-levels than are given by cyanocobalamin. (G. B. J. Glass and colleagues, New York Medical College.)

Hydroxocobalamin is at present excluded by stringent spectroscopic tests from the cyanocobalamin which is recognized officially as vitamin B_{12} in leading pharmacopœias. Because of the increasing importance attached to hydroxocobalamin its acceptance as an alternative and perhaps also improved form of vitamin B_{12} seems likely to take effect during the near future, partly for the more efficient treatment of pernicious anæmia, but especially to cope with demyelinating diseases or tobacco amblyopia in which cyanide may be an important factor.

Vitamin B_{12} deficiency occurring through lack of intrinsic factor has led to intense efforts to purify and isolate intrinsic factor. L. Ellenbogen (Lederle Laboratories, American Cyanamid Co., Pearl River, New York) reported on the purest preparation so far obtained, active in doses of 50 μgm. or less, with very high binding capacity for B_{12} and B_{12} coenzyme, very low carbohydrate content, no peptic activity and only trace amounts of blood group substances. W. W. Bromer and E. O. Davisson (Lilly Research Laboratories, Indianapolis) obtained intrinsic factor preparations, effective in treatment of pernicious anæmia in daily doses of less than 100 μgm., by *XE*-64, followed by calcium phosphate gel, chromatography, combination with B_{12}, ultra-centrifugation and electrophoresis. R. Gräsbeck and colleagues from the Minerva Institute for Medical Research, Helsinki, used DEAE cellulose column chromatography with gradient elution and found one major B_{12}-binding peak that, under present conditions, is sole carrier of intrinsic factor activity. A 'releasing factor' similar to that in the fish tapeworm which liberates B_{12} bound to human gastric juice has been found in human small intestine, kidney, liver, thyroid, heart muscle, brain, spleen and lung, and has been partially purified with the aid of DEAE cellulose chromatography.

Radioactive vitamin B_{12} used in many investigations on its absorption, storage and utilization is available with different labellings. R. Gräsbeck (Helsinki) described how earlier use of cobalt-60 involved large doses of radioactivity. These are considerably reduced when it is replaced by cobalt-58 but solutions of this are unstable. The recently introduced cobalt-57 has several advantages in clinical diagnosis, when either urinary or fæcal excretion, hepatic uptake, plasma radioactivity or total body retention may be measured. Different forms of radioactive B_{12} have also been used to study complex problems concerned with the action of vitamin B_{12} as well as difficult questions regarding the possible role of vitamin B_{12} and its absorption with or without the aid of intrinsic factor.

The last five papers dealt with relationships between vitamin B_{12} and folic acid. J. R. Guest and D. D. Woods (Microbiology Unit, Department of Biochemistry, University of Oxford) described metabolic interrelationships in the synthesis of methionine by *E. coli* and postulated two pathways for the methylation of homocysteine, the first relatively independent of cobalamin metabolism and with a strict requirement for a conjugated folic acid co-factor, the second dependent on cobalamin but functioning with pteroylmonoglutamates as well as conjugate forms. L. Jaenicke (Biochemical Institute, Munich) described how the structure of different forms of folic acid affects their efficiency in the first pathway. D. L. Mollin and A. H. Waters (Postgraduate Medical School, London) found uncomplicated folic acid deficiency completely cured by very small doses of folic acid (50–150 μgm. daily) in persons with megaloblastic anæmia due to idiopathic steatorrhœa who absorb B_{12} normally, obtain adequate amounts of B_{12} in their diets and excrete very large amounts of formimino-glutamic acid in their urine. In patients with idiopathic steatorrhœa and tropical malnutrition syndrome there is defective absorption of both folic acid and vitamin B_{12}. The very low serum B_{12}-levels may be raised (but not to the normal range) by small doses of folic acid which completely remove other effects of deficiency. E. V. Cox *et al.* (General Hospital, Birmingham) found serum iron high in untreated megaloblastic anæmia and reduced to normal after satisfactory therapy with B_{12} or folic acid. Blood 'folic acid' was temporarily increased, especially in patients with adequate iron utilization and status. In some patients with subnormal serum B_{12}-levels, folic acid administration raises these levels; in others it either has no effect or still further reduces them.

Round-table discussions, to which all members listened, were held on the nomenclature of B_{12} vitamins, intrinsic factor assays and standardization of diagnostic radioactive B_{12} absorption tests, vitamin B_{12} pool, turnover-time and requirements, stability of radioactive B_{12} and B_{12} therapy, under the guidance of Dr. H. A. Barker, Prof. K. Bernhauer, Dr. Dorothy Hodgkin, Prof. A. W. Johnson, Dr. S. K. Kon, Dr. Rosenblum, Dr. R. P. Schilling, Dr. Lester Smith and Prof. L. S. Witts. Full details will be published in the Symposium Proceedings.

MEDIATION OF DIVALENT METAL IONS IN THE BINDING OF TETRACYCLINE TO MACROMOLECULES

By DR. KURT W. KOHN

Clinical Pharmacology and Experimental Therapeutics Service, General Medicine Branch, National Cancer Institute, Bethesda, Maryland

SEVERAL biological and biochemical effects of the tetracycline antibiotics depend on the presence of divalent metal ions. These effects include the inhibition of certain enzymes[1], the precipitation of β-lipoproteins[2], and the localization of tetracyclines in tumour tissues[3,4]. These phenomena probably involve the formation of tetracycline-metal chelates[5]. Calcium chelation may account for the binding of tetracyclines in newly formed bone[6] and for the interference with calcification caused by these antibiotics[7].

The metal ions may act in these systems by simultaneously binding tetracycline and suitable macromolecular sites. In order to test the possibility that such metal-bridge complexes may generally form with macromolecules having binding sites for divalent cations, we studied the interaction of tetracycline with deoxyribonucleic acid (DNA) and with serum albumin in the presence and absence of divalent cations.

Equilibrium Dialysis. Macromolecule solutions (1·0–1·5 ml.) in bags of ¼-in. transparent cellulose tubing were dialysed (40 hr. at 4°) against 15 ml. of buffers containing tetracycline and various concentrations of divalent metal ions. The transparent cellulose bags were weighed before and after dialysis and appropriate corrections were made for any changes in macromolecule concentration. Donnan effects were estimated to be negligible. Tetracycline concentrations inside and outside the dialysis bags were determined by fluorometry of extracted calcium–barbital complexes[8].

The preparations of DNA used were relatively undenatured, as indicated by the absence of any lowering of optical density (260 mμ) in response to 0·01 magnesium chloride[9]. Crystalline human serum albumin was dissolved in 0·15 *M* sodium chloride containing 0·01 *M* ethylenediaminetetraacetic acid and dialysed extensively at 4° against 0·12 *M* sodium chloride plus 0·03 *M* sodium bicarbonate–carbon dioxide buffer at *p*H 7·4. The concentration of the dialysed albumin solution was determined gravimetrically on heated trichloracetic acid precipitates[10]. (Salmon sperm DNA and crystalline human serum albumin were purchased from the Mann Research Laboratories, New York. Calf thymus DNA was obtained from the Worthington Biochemical Corp., Freehold, New Jersey.)

Little or no tetracycline became bound to either DNA or albumin in the absence of divalent metal

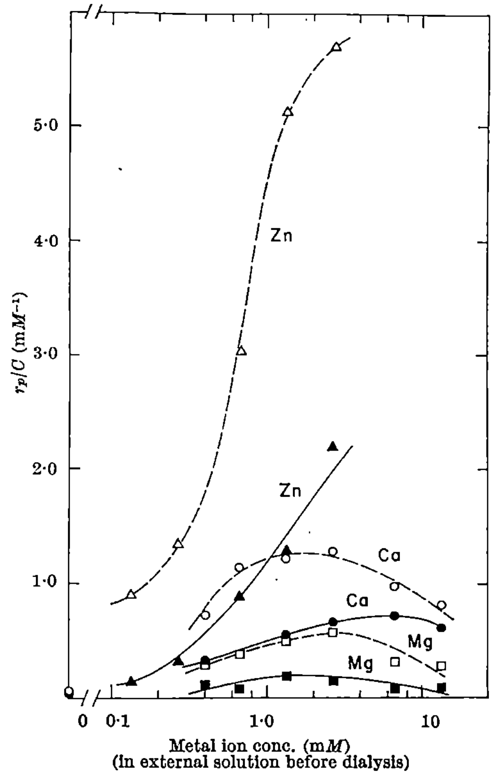


Fig. 1. Effect of divalent metal ions on the extent of tetracycline binding by 'native' (——) or heat-denatured (– – –) DNA, as determined by equilibrium dialysis of salmon sperm DNA solution (2 mgm./ml. in 10^{-2} M potassium chloride) against 10 volumes of 10^{-5} M tetracycline in 0·04 M *tris*-hydrochloric acid buffer at pH 7·8 (23°). Ionic strength 0·06 was maintained with potassium chloride. (r_p = mean number of tetracycline molecules bound per DNA phosphorus; C = concentration of unbound tetracycline at equilibrium)

ions. Binding to DNA did take place in the presence of zinc, calcium, manganese or magnesium, in decreasing order of effectiveness (Fig. 1). Heat-denatured DNA (100° for 15 min.) exhibited markedly increased binding. The results were qualitatively similar for DNA derived from salmon sperm or calf thymus. In the case of serum albumin, tetracycline binding occurred in the presence of zinc, but no significant binding was detected with calcium (Fig. 2).

At the low concentrations used, the amount of tetracyline bound was proportional to the concentration unbound. The maximum binding, represented by the highest points on the curves with zinc in Figs. 1 and 2 corresponded to 1 molecule tetracycline bound per 120 nucleotide units of heat-denatured DNA and 1 molecule bound per 40 molecules of albumin, respectively.

Fluorescence. The tetracycline chelates of calcium and zinc fluoresce in aqueous solution. DNA and albumin were observed to enhance this fluorescence. Tetracycline (10^{-5} M) was titrated with zinc chloride or calcium chloride at several pH values between 6·5 and 8·0, and fluorescence intensity (excited at 405 mμ, observed at 530 mμ) was plotted against the amount of metal added. The presence of DNA or albumin in such titrations caused much greater fluorescence to appear on addition of the metal (Fig. 3). The macromolecules did not, however, increase fluorescence in the absence of divalent metal ions. DNA and albumin gave qualitatively similar results. Heat-denatured DNA produced greater enhancement of fluorescence than did the undenatured material.

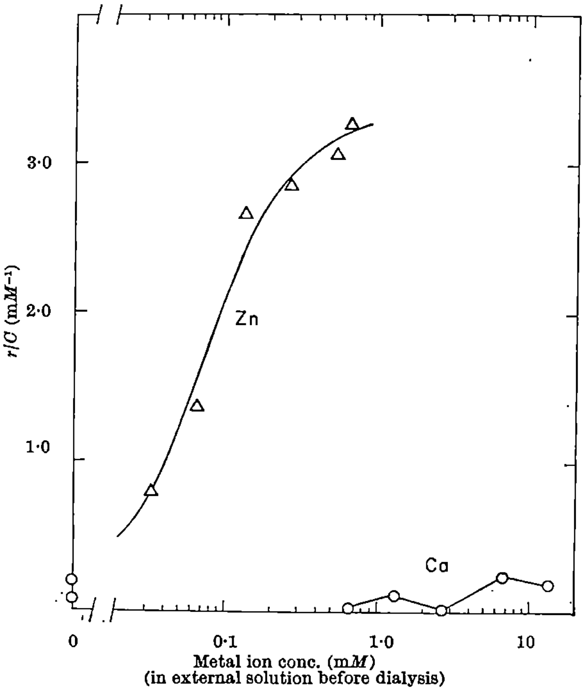


Fig. 2. Binding of tetracycline to human serum albumin. Equilibrium dialysis of 1·0 ml. protein solution (*c*. 30 mgm./ml.) against 15 ml. of 10^{-5} M tetracycline at pH 7·52 ± 0·04 (23°) (0·12 M sodium chloride, 0·03 M sodium bicarbonate–carbon dioxide buffer). (r = moles of tetracycline bound per mole of protein; C = concentration of unbound tetracycline at equilibrium)

In other experiments, the concentration of macromolecules was varied while other factors were held constant (Fig. 4). Zinc or calcium were present in sufficient excess to assure nearly complete chelation of unbound tetracycline. Fluorescence enhancement

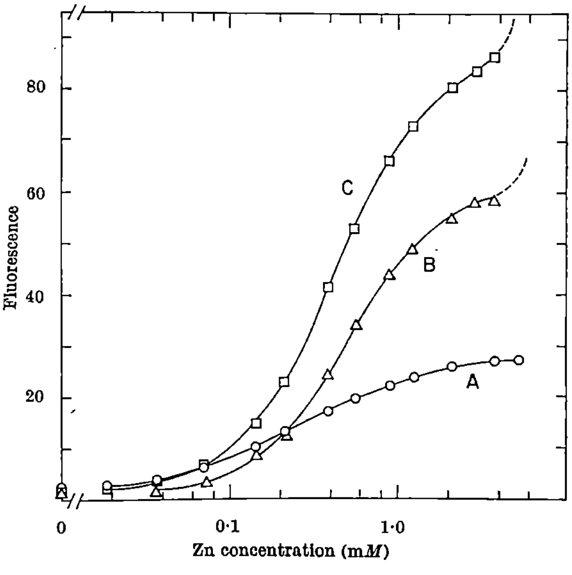


Fig. 3. Fluorescence titration of tetracycline with zinc chloride (0·06 M NaAc–HAc buffer, pH 6·65 ± 0·04). A, in the absence of DNA; B, in the presence of salmon sperm DNA (1·4 mgm./ml.); C, same with heat-denatured DNA. (Above 4 mM zinc, the DNA solutions became turbid and the fluorescence rose sharply)

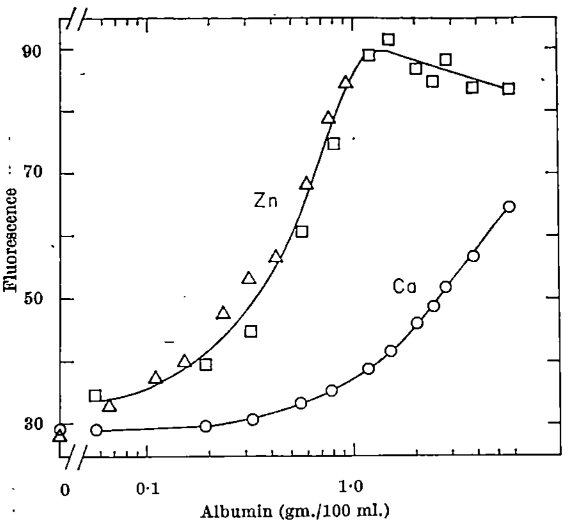


Fig. 4. Enhancement of fluorescence of tetracycline chelates by human serum albumin. Solutions of 10^{-5} *M* tetracycline in 5·8 gm. per cent albumin at *p*H 7·6 (0·04 *M* sodium bicarbonate–carbon dioxide, 0·12 *M* sodium chloride) and in buffer without albumin were mixed in various proportions. Both solutions also contained either 10^{-3} *M* zinc chloride or 10^{-3} *M* calcium chloride. (Activation at 405 mμ, fluorescence measured at 530 mμ)

occurred at much lower macromolecule concentrations in the presence of zinc than with calcium. The results for DNA and albumin were again qualitatively similar. The rise in fluorescence occurred at lower concentrations with denatured DNA than were required with 'native' DNA.

The enhancement of tetracycline chelate fluorescence produced by DNA and serum albumin might be explained by: (1) binding of tetracycline chelates to specific macromolecular sites; (2) electrostatic attraction of tetracycline chelates into the hydration layers of the macromolecules; or (3) increases in viscosity of the solutions. Electrostatic effects alone cannot account for the large differences in the effectiveness of albumin (or DNA) in enhancing the fluorescence of the zinc versus calcium chelates (Fig. 4), since, under the conditions of these experiments, nearly all the unbound tetracycline was in the form of 1 : 1 chelates which should have the same charge with zinc as with calcium. If viscosity were the major factor, undenatured DNA solutions, which have much higher viscosity than denatured solutions, should produce the greatest increases in fluorescence. The reverse was found to be the case. It appears, therefore, that the changes in fluorescence result mainly from binding which, at least in the case of the zinc complexes, occurs at specific macromolecular sites.

The divalent metal ion probably mediates this binding by serving as a connecting bridge between macromolecule and tetracycline. This conforms with previously reported instances of metal-mediated binding of chelating agents to proteins[11,12].

The results of both the dialysis and fluorescence experiments indicate that the binding of tetracycline chelates to DNA is increased after heat-denaturation. This is in accord with the increase in cation-binding ability brought about by heat-denaturation of DNA[13]. Serum albumin was observed to bind tetracycline in the presence of zinc, but not of calcium. The albumin sites for zinc are probably imidazole groups[14] which would be expected to have very little affinity for calcium. These relationships support the picture of a bond between DNA (or albumin) and the metal component of the tetracycline chelate.

The results strengthen the view that the tetracyclines may exert some of their biological effects by complexing with macromolecules through metal ions. The binding of tetracyclines in human plasma, for example, may be through this mechanism, since purified serum albumin, by itself, does not bind these compounds.

[1] Rokos, J., Burger, M., and Prochezka, P., *Nature*, **181**, 1201 (1958); *Antibiot. Chemother.*, **9**, 600 (1959).
[2] Lacko, L., Korinek, J., and Burger, M., *Clin. Chim. Acta*, **4**, 800 (1959).
[3] Rall, D. P., Loo, T. L., Lane, M., and Kelly, M. G., *J. Nat. Cancer Inst.*, **19**, 79 (1957).
[4] Lane, M., Titus, E., and Loo, T. L. (unpublished experiments indicating that the tetracycline complex in tumours is dissociated by EDTA).
[5] Albert, A., *Nature*, **172**, 201 (1953); **177**, 433 (1956).
[6] Milch, R. A., Rall, D. P., and Tobie, J. E., *J. Bone and Joint Surg.*, **40**, A, 897 (1958).
[7] Bevelander, G., Nakahara, H., and Rolle, G. K., *Developmental Biol.*, **2**, 298 (1960).
[8] Kohn, K., *Anal. Chem.*, **33**, 862 (1961).
[9] Shack, J., *J. Biol. Chem.*, **233**, 677 (1958).
[10] Hoch, F. L., and Vallee, B. L., *Anal. Chem.*, **25**, 317 (1953).
[11] Klotz, I. M., and Loh Ming, W. C., *J. Amer. Chem. Soc.*, **76**, 805 (1954).
[12] Vallee, B. L., Coombs, T. L., and Williams, R. J. P., *J. Amer. Chem. Soc.*, **80**, 397 (1958).
[13] Zubay, G., and Doty, P., *Biochim. Biophys. Acta*, **29**, 47 (1958).
[14] Gurd, F. R. N., and Goodman, D. S., *J. Amer. Chem. Soc.*, **74**, 670 (1959).

DAMAGE IN STAINLESS STEEL DUE TO FISSION FRAGMENTS

By P. R. B. HIGGINS and A. C. ROBERTS

Metallurgy Division, Atomic Energy Research Establishment, Harwell, Berks

An investigation has been made by transmission electron microscopy on the changes in the microstructure of stainless steel bombarded by fission fragments at 60° C. The steel used was a niobium-stabilized 20 per cent chromium, 20 per cent nickel austenitic stainless steel which was received as hot-rolled strip, 0·020 in. thick. The steel had been vacuum-melted twice prior to rolling and its analysis is given in Table 1.

The material was reduced to foil 0·001 in. thick by successive heat treatments and cold rolling. This was followed by a solution heat treatment at 1,145° C. for 20 min. and a water quench. The foils were then thinned electrolytically in a stabilized phosphoric–sulphuric acid polishing solution (solution kindly given by Messrs. Electropol, Ltd., Farnham, Hants), and given an electrolytic cleaning treatment in a mixture of 75 per cent sulphuric acid, 18 per cent glycerol and 7 per cent water. The thickness of the prepared foils was determined by measurements of

Table 1

	Cr	Ni	C	Mn	S	P	Si	Nb	Fe
Chemical composition (per cent)	19·6	20·1	0·02	0·72	0·004	0·008	0·69	0·44	~58·4

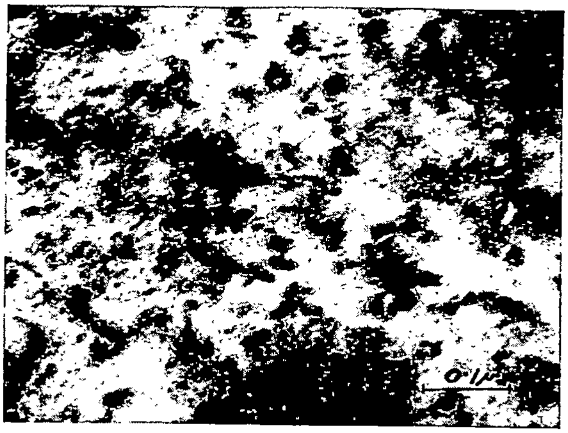


Fig. 1. Damage observed in stainless steel after an irradiation of 8·4 × 10^{15} fission fragments cm.$^{-2}$ at a rate of 1·6 × 10^{10} fragments cm.$^{-2}$ sec.$^{-1}$

slip traces and found to be a few hundred angstrom units.

Individual foils were held between strips 0·001 in. thick of either natural uranium or 93 per cent uranium-235 by an aluminium clamp. This assembly was sealed in silica and irradiated in the *Bepo* reactor in a thermal neutron flux of 1·3 × 10^{12} n. cm.$^{-2}$ sec.$^{-1}$. After irradiation it was necessary to clean the steel surface in the cleaning bath already described; however, this treatment did not remove a significant fraction of the steel from the surface. The steel was then examined by transmission microscopy in an Elmiskop I operating at 100 kV.

The fission-rate (R) in uranium of unit area and thickness y is given by $R = \varphi\sigma y n$, where φ is the thermal neutron flux, σ is the capture cross-section of uranium-235 (590 barns), and n is the number of uranium-235 atoms per unit volume.

Since the distance travelled by a fission fragment in uranium is about 4 × 10^{-4} cm., it is only necessary, in the arrangement used, to consider the fissions occurring in a layer of this thickness next to the steel surface, and geometrical considerations show that fragments from approximately 30 per cent of these fissions will pass through the foil. Each fission results in two fragments, and since there is uranium on both sides of the steel the flux of fission fragments is given by: $\varphi_f = 4{\cdot}8 \times 10^4 \varphi\sigma n$ cm.$^{-2}$ sec.$^{-1}$.

For natural uranium this gives a flux of 1·2 × 10^8 fragments cm.$^{-2}$ sec.$^{-1}$ and for 93 per cent uranium-235 a flux of 1·6 × 10^{10} fragments cm.$^{-2}$ sec.$^{-1}$.

Small defects, about 25 Å. in diameter, are first seen in the steel after a fission fragment dose of 1·8 × 10^{14} fragments cm.$^{-2}$. Increasing the dose results in an increase in size and number of defects until they are resolvable as dislocation loops, similar to those observed in other face-centred cubic metals after neutron irradiation or quenching from a temperature near the melting point. Fig. 1 shows a typical damaged area after a dose of 8·4 × 10^{15} fragments cm.$^{-2}$ at the higher dose-rate.

The steel will also be subjected to neutron irradiation, and to determine how much of the observed damage was due to neutron irradiation similar foils were irradiated at 60° C. in a thermal neutron flux of 1·3 × 10^{12} neutrons cm.$^{-2}$ sec.$^{-1}$ (epithermal flux 3 × 10^{11} neutrons cm.$^{-2}$ sec.$^{-1}$) for 700 and 2,000 hr. No defects were observed in the electron microscope, but tensile tests show a characteristic increase in yield stress.

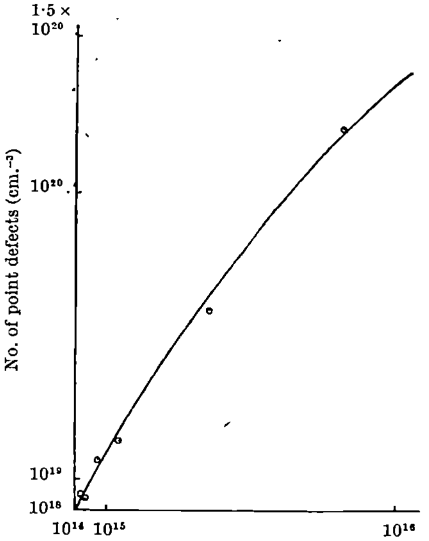


Fig. 2. Increase in concentration of point defects with dose of fission fragments

An estimate of the number of defects observed in the foil has been made by measurement of the integrated area of the defects and a knowledge of the thickness of the foil. Fig. 2 is a plot of the concentration of point defects against fission-fragment dose at a dose-rate of 1·6 × 10^{10} fragments cm.$^{-2}$ sec.$^{-1}$. It will be seen that saturation has not been reached at a concentration of 1·2 × 10^{20} point defects cm.$^{-3}$. A possible reason for such a large number of defects without saturation has been put forward by Barnes (unpublished work), who suggests that the observed defects are due to interstitial atoms[1]. However, after a dose of 1·7 × 10^{16} fragments cm.$^{-2}$, the dislocation loops appear to have grown to form the dislocation network shown in Fig. 3, which may mean that saturation has been reached at this dose.

The number of pairs of point defects produced by each fission event has been obtained from the slope of the initial part of the curve of Fig. 2, which gives a maximum of 45. This is 2 or 3 orders of magnitude lower than the estimated number of defect pairs

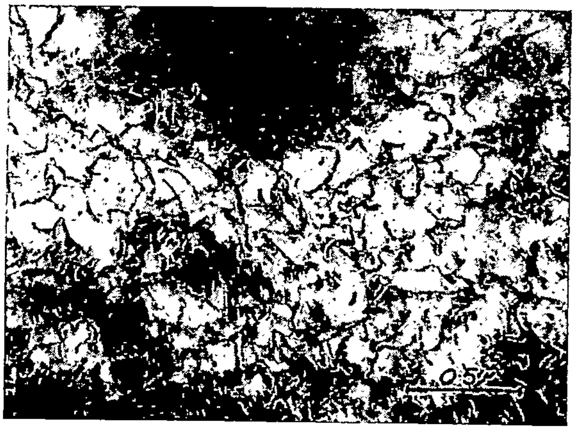


Fig. 3. Dislocation network observed in stainless steel after an irradiation of 1·7 × 10^{16} fission fragments cm.$^{-2}$ at a dose-rate of 1·6 × 10^{10} fragments cm.$^{-2}$ sec.$^{-1}$

produced by a fission event[2]. A higher number of defect pairs per fission event can be obtained by either irradiating at a higher temperature or at a lower dose-rate. For example, after irradiating the steel at a rate of $1 \cdot 2 \times 10^8$ fragments cm.$^{-2}$ sec.$^{-1}$ at 60° C., the number of pairs of defects per fission is 250. This last figure is of the same order as that found in uranium by B. Hudson, K. H. Westmacott and M. J. Makin (unpublished work), where the observed number of point defects will account for the irradiation growth observed experimentally.

The low number of defects per fission event obtained with the specimens subjected to the higher dose-rate at 60° C. is thought to be due to a large number of unobservable defects being present. Higher temperatures, or lower dose-rates, allow these small defects to grow to observable sizes.

Further experiments to study the effects of dose-rate and temperature of irradiation are in progress and will be reported later.

[1] Barnes, R. S., and Mazey, D. J., *Phil. Mag.*, **5**, 1247 (1960).

[2] Seitz, F., *Discuss. Farad. Soc.*, **5**, 271 (1949). Cottrell, A. H., *Metal. Rev.*, **1**, 490 (1956).

ELIMINATION OF THE ABSORPTION CORRECTION IN THE VISCOMETRY OF DILUTE SOLUTIONS OF HIGH POLYMERS

By H. van OENE and PROF. L. H. CRAGG

Department of Chemistry, University of Alberta, Edmonton

IN a recent paper[1] we directed attention to the fact that values of both intrinsic viscosity $[\eta]$ and the viscosity slope constant k', obtained in the usual way, may be significantly in error because of an apparent reduction in capillary radius. As Öhrn[2] has demonstrated, this apparent reduction in radius may be due to absorption of polymer from solution; it may also be due in part to other effects[1]. Öhrn has described a method of evaluating the 'absorption correction', but it is discouragingly demanding of time and careful experimentation. We have devised a means of calculating intrinsic viscosity which makes an 'absorption correction' unnecessary even when the 'absorption' effect is large.

Öhrn has shown that the absorption effect on the relative flow-time t_r may be expressed thus:

$$t_r = \eta_r(1 + 4a/R) \qquad (1)$$

where η_r is the relative viscosity (the ratio of the viscosities of solution and solvent) and a is the apparent reduction in R, the radius of the capillary.

From this it follows that, in sufficiently dilute solutions:

$$t_{sp}/c = (1 + 4a/R)[\eta] + (k' + 4a/R)[\eta]^2c + 4a/R\,.\frac{1}{c} \qquad (2)$$

and

$$\frac{\ln t_r}{c} = [\eta] - (\tfrac{1}{2} - k' - 4a/R)[\eta]^2c + 4a/R\,.\frac{1}{c} \qquad (3)$$

for, in such solutions:

$$\frac{\ln t_r}{c} = \frac{\ln(1 + t_{sp})}{c} = \frac{t_{sp}}{c} - \frac{\tfrac{1}{2}(t_{sp})^2}{c}$$

Subtracting (3) from (2), we obtain:

$$\frac{t_{sp}}{c} - \frac{\ln t_r}{c} = 4a/R\,.[\eta] + \tfrac{1}{2}[\eta]^2c$$

The correction term $4a/R$ has disappeared from the slope. Hence a value of $[\eta]$ that is unaffected by 'absorption' can be evaluated from the slope of the plot of:

$$\left(\frac{t_{sp}}{c} - \frac{\ln t_r}{c}\right) \text{vs. } c$$

We applied this procedure to Öhrn's very precise data[2] which he obtained with a solution of a polystyrene fraction in toluene. His measurements were made in three different viscometers over a very wide range of concentrations, 0·000470–0·196 gm./dl. Values of t_{sp}/c, $(\ln t_r)/c$, and $\dfrac{t_{sp}}{c} - \dfrac{\ln t_r}{c}$, calculated from the data for the concentration range 0·00232–0·196 gm./dl. (ref. 2, Table 5), are collected in Table 1. The marked effect of absorption at the low concentrations is clearly evident in the values of $\dfrac{t_{sp}}{c}$ and $\dfrac{\ln t_r}{c}$, as is the greater effect in a capillary of smaller

Table 1. VALUES OF t_{sp}/c, $\ln t_r/c$, AND $(t_{sp}/c - \ln t_r/c)$, CALCULATED FROM ÖHRN'S POLYSTYRENE–TOLUENE DATA (ref. 2, Table 5)

c (gm./dl.)	Viscometer S_1			Viscometer S_2			Viscometer S_3		
	t_{sp}/c	$\ln t_r/c$	$t_{sp}/c - \ln t_r/c$	t_{sp}/c	$\ln t_r/c$	$t_{sp}/c - \ln t_r/c$	t_{sp}/c	$\ln t_r/c$	$t_{sp}/c - \ln t_r/c$
0·196	2·512	2·042	0·469	2·530	2·055	0·475	2·547	2·066	0·481
0·134	2·408	2·087	0·321	2·429	2·103	0·326	2·452	2·120	0·332
0·0865	2·345	2·135	0·210	2·373	2·158	0·215	2·399	2·180	0·219
0·0552	2·335	2·196	0·139	2·374	2·231	0·143	2·416	2·268	0·148
0·0230	2·324	2·263	0·061	2·399	2·335	0·064	2·483	2·415	0·068
0·0145	2·363	2·324	0·039	2·469	2·425	0·044	2·597	2·549	0·048
0·00732	2·458	2·436	0·022	2·650	2·624	0·026	2·877	2·847	0·030
0·00567	2·496	2·478	0·018	2·734	2·712	0·022	3·007	2·982	0·025
0·00232	2·832	2·823	0·009	3·319	3·306	0·013	3·914	3·896	0·018

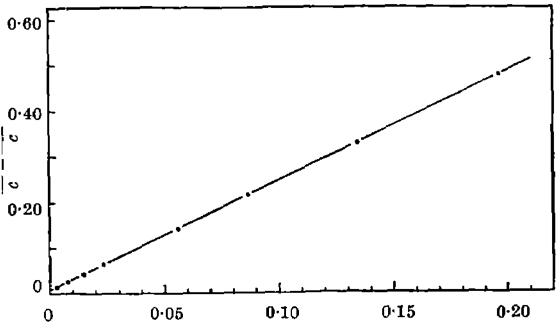


Fig. 1. Plot of ($t_{sp}/c - \ln t_r/c$) vs. c for data obtained in viscometer S_2. (The S_1 and S_3 data give equally good straight lines parallel to, but slightly displaced from, this line)

radius. A typical plot of $\frac{t_{sp}}{c} - \frac{\ln t_r}{c}$ vs. c is given in Fig. 1.

If the method is valid, it should give the same value of [η] for each of the three viscometers even though, because of differing radii, the absorption errors in the three viscometers are very different. In Table 2 are the results obtained, by least-squares analysis, for the data in the concentration-range 0·0230–0·196 gm./dl.

Table 2

Viscometer	Diameters (mm.) 2R	Slope $\frac{1}{2}[\eta]^2$	[η]
S_1	0·5	$2{\cdot}34_7$	$2{\cdot}16_8$
S_2	0·3	$2{\cdot}36_9$	$2{\cdot}17_7$
S_3	0·2	$2{\cdot}37_6$	$2{\cdot}18_8$
	Average	$2{\cdot}36_4$	$2{\cdot}17_5$

The agreement among these values of [η] is excellent, the maximum difference being 1 per cent (or less than the experimental error in the usual determination of [η]). Öhrn's corrected values, obtained by evaluation of a and R and application of his correction formula, is 2·14. It differs from our average value and from the value we obtained from the measurements in S_1 (the viscometer in which absorption was least and the capillary radius of which corresponds most closely to that of the viscometers generally used) by less than 2 per cent.

For concentrations less than 0·023 gm./dl., high-precision data are needed, for otherwise the difference between $\frac{t_{sp}}{c}$ and $\frac{\ln t_r}{c}$ would be within the experimental error of either. Since Öhrn's measurements were very carefully made, we calculated values of [η] for the concentration ranges 0·196–0·00232 gm./dl. and 0·0230–0·000232 gm./dl., obtaining, respectively, 2·18 and 2·21 dl./gm. Even the latter value is within 3 per cent of Öhrn's value, 2·14.

It is evident that our method makes possible a simple and rapid calculation of a 'true' intrinsic viscosity even when 'absorption' effects are so prominent as to introduce large errors into the values obtained by the usual methods or even to make it impossible to apply the usual methods. It is not necessary either to know the radius of the capillary in the viscometer or to measure (or estimate) the thickness of the absorbed layer. Furthermore, in any dilute-solution-viscosity study in which 'absorption' is suspected, a comparison of the value of [η] obtained by this method with that obtained by the usual methods $\left(\text{from values of } \frac{t_{sp}}{c} \text{ or } \frac{\ln t_r}{c}\right)$ will indicate whether or not the 'absorption effect' is significant.

Grateful acknowledgment is made of a grant-in-aid from the National Research Council of Canada and of the award of a fellowship (to H. v. O.) by the Shell Oil Company of Canada, Ltd.

---

[1] Cragg, L. H., and van Oene, H., *Canad. J. Chem.*, 39, 203 (1961).

[2] Öhrn, O. E., *Arkiv Kemi.*, 12, 397 (1958).

COLORIMETRIC DETECTION OF PENICILLINS AND CEPHALOSPORINS ON PAPER

By ROBERT THOMAS

Squibb Institute for Medical Research, New Brunswick, New Jersey

A WIDELY used technique for the detection of antibiotics on paper chromatograms and ionograms consists of placing the paper strip in contact with an agar suspension of a sensitive organism and observing the growth inhibition zones which result after a suitable incubation period. Thus, with the aid of the Gram-positive bacterium *Sarcina lutea*, as little as 10^{-2} μgm. benzylpenicillin can be readily detected. However, despite the established value of this bio-autographic procedure for work on penicillin, inherent limitations include the delay necessitated by the incubation period and also the prerequisite of bio-activity, which precludes the possibility of detecting structurally related products which may be biologically inactive. In an attempt to overcome these disadvantages, a colorimetric reagent has been devised which is based on the penicillin iodometric assay procedure of Alicino[1]. This is dependent on the finding that, although the intact penicillin molecule is inert to iodine in neutral aqueous solution, after hydrolysis of the β-lactam ring with alkali or penicillinase the resulting penicilloic acid rapidly consumes nine equivalents of iodine.

This procedure can be easily adapted to paper chromatograms as a two-stage spray consisting of dilute alkali followed by a starch–iodine solution in aqueous acetic acid. Under suitable conditions it was found that both penicillins and the related products cephalosporin *C* and cephalosporin *N* were readily detected as white zones against a dark blue background, with a sensitivity of 1–2 μgm. Thus, chromatogram *A* (Fig. 1) received a preliminary spraying with 0·5 *N* sodium hydroxide then, after an interval of 10–15 min. to allow partial drying, it was further sprayed with a reagent composed of a mixture of 1 per cent aqueous starch, glacial acetic acid and 0·1 *N* iodine in 4 per cent potassium iodide solution (50 : 3 : 1 v/v). Decolorization of the iodine reagent by the hydrolysed penicillins was fairly rapid, yielding maximum contrast after 5–10 min.

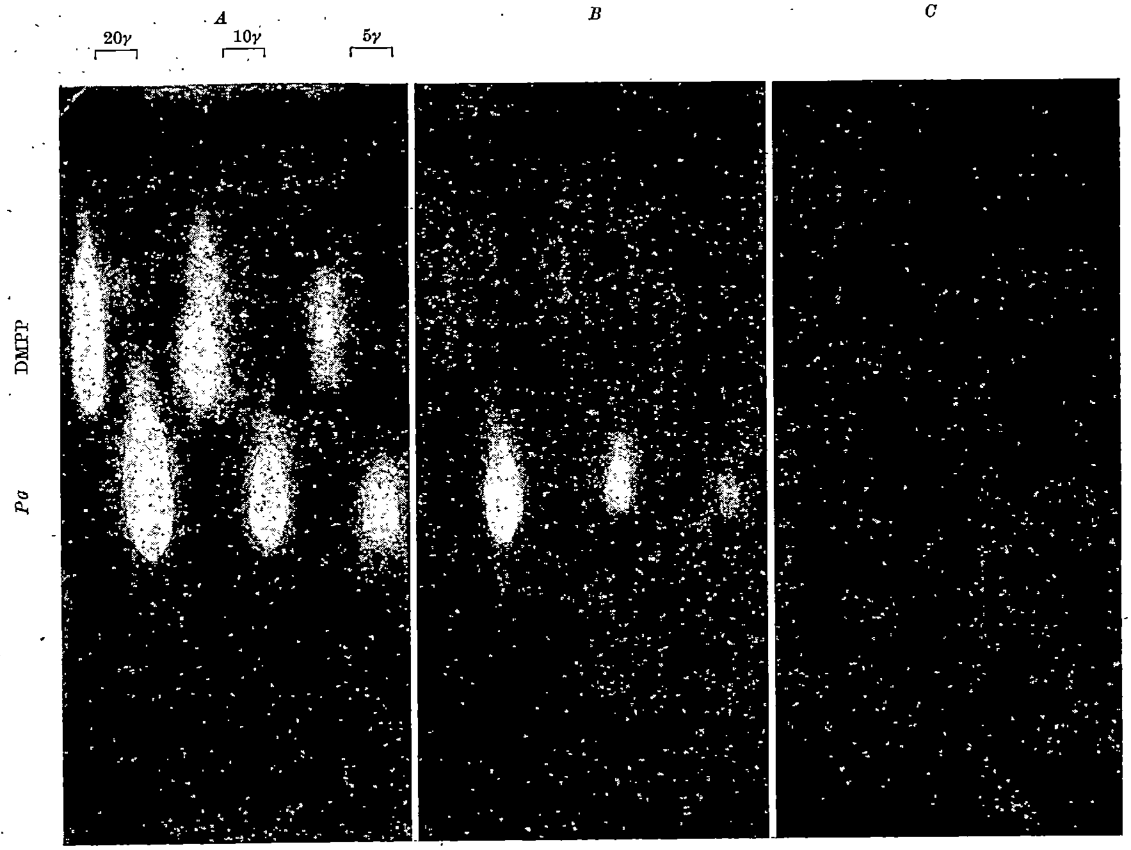


Fig. 1. Chromatograms of benzyl penicillin (*Pc*) and 2 : 6-dimethoxyphenyl penicillin (DMPP) at three concentrations, 20 μgm., 10 μgm. and 5 μgm. Solvent, water-saturated diethyl ether. Whatman No. 1 paper buffered with 10 per cent sodium citrate at *p*H 5·7. After 2 hr. the chromatograms were dried and sprayed with: *A*, 0·5 *N* sodium hydroxide, followed, after partial drying, by a mixture of 1 per cent starch, glacial acetic acid and 0·1 *N* iodine in 4 per cent potassium iodide (50 : 3 : 1); *B*, SchenLabs *B. cereus* penicillinase (10,000 units/ml.), 1 per cent starch, 0·1 *N* iodine, and *p*H 7 *M* sodium phosphate buffer (5 : 50 : 1 : 1); *C*, as in *B*, minus the penicillinase solution

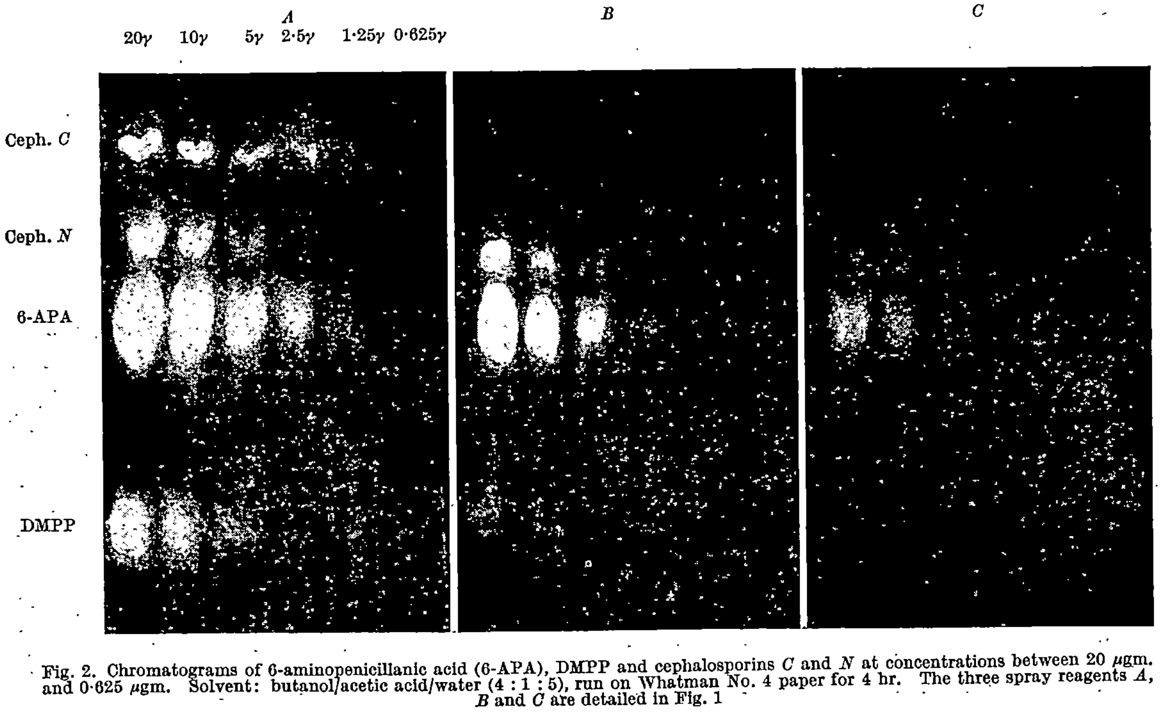


Fig. 2. Chromatograms of 6-aminopenicillanic acid (6-APA), DMPP and cephalosporins *C* and *N* at concentrations between 20 μgm. and 0·625 μgm. Solvent: butanol/acetic acid/water (4 : 1 : 5), run on Whatman No. 4 paper for 4 hr. The three spray reagents *A*, *B* and *C* are detailed in Fig. 1

An identical chromatogram (*B*) was sprayed with an alternative reagent, in which the required preliminary hydrolysis of the β-lactam ring was effected enzymatically with penicillinase. By this means it proved possible to use a single-stage spray for the detection of benzylpenicillin and other penicillinase-sensitive penicillins. The reagent contained a mixture of SchenLabs (SchenLabs Pharmaceuticals Inc., New York) purified *Bacillus cereus* penicillinase (10,000 units per ml.), 1 per cent starch, 0·1 *N* iodine and *p*H 7 *M* sodium phosphate buffer, in the ratio 5 : 50 : 1 : 1. The rate of decolorization of the spray by the penicillin substrate was found to be dependent on the penicillinase concentration and, with this mixture, development was complete at room temperature (*c*. 22°) in 10–15 min.

It is evident from the chromatogram *B* that, while benzylpenicillin was readily detected by this reagent, 2 : 6-dimethoxyphenylpenicillin was relatively insensitive. This is in keeping with the known partial resistance of this recently described semi-synthetic penicillin (trade names, 'Celbenin' 'Dimocillin' and 'Staphcillin') to the action of the penicillinases of *B. cereus* and *Staphylococcus pyogenes*[2]. The necessity for cleavage of the β-lactam ring is apparent from the negative results obtained when the penicillinase component of the second spray reagent was omitted (see chromatogram *C*).

Thus, in addition to providing a sensitive system for the colorimetric detection of penicillins, by the combined use of the alkali and penicillinase variants of the starch–iodine reagent, it appeared to be applicable to the differentiation of penicillinase-sensitive and -resistant penicillins. This aspect was further examined by a comparison of cephalosporin *C*, known to contain a penicillinase-resistant β-lactam moiety, with cephalosporin *N* and 6-aminopenicillanic acid, both of which are penicillinase sensitive. The results (Fig. 2) clearly show that cephalosporin *C* is even more resistant to penicillinase than 2 : 6-dimethoxyphenylpenicillin.

It can be seen from the third chromatogram (*C*) which, as before, was sprayed with a neutral buffered starch–iodine solution, that in contrast to the results shown in Fig. 1, the penicillinase-sensitive penicillins were still detectable, although with reduced sensitivity. However, since this second chromatographic solvent contained acetic acid then some decomposition of acid-labile penicillins would be anticipated, particularly during the final drying stage, and this could possibly account for the enhanced iodine absorption.

The blue background of the paper chromatogram, after spraying with the penicillinase reagent, faded far more slowly than that obtained with the alkali spray and in both instances the colour was more stable when stored in the dark. In addition, it was shown that the original dark blue colour of the freshly sprayed sheet could be regenerated, even after several days, by spraying again with water.

The sensitivity of the iodometric spray reagent towards certain clinically valuable penicillins is considerably less than that of the bio-autographic procedure. However, for cephalosporin *N*, their relative sensitivities are of the same order and for the detection of both 6-aminopenicillanic acid and cephalosporin *C*, the iodometric reagent is considerably superior when compared with bio-activity against either *Sarcina lutea* or the Gram-negative *Salmonella gallinarum*.

In practice, the sensitivity of these reagents has proved adequate for routine use in the direct examination of chromatograms of mould culture filtrates containing penicillins and cephalosporins. Furthermore, it was found that amino-acids and the normal constituents of corn steep media do not interfere with the detection of these antibiotics in culture broths.

Preliminary experiments have shown that a simple modification of this technique also permits the detection of penicillinase on paper by taking advantage of the ability of the enzyme to render penicillin iodine-sensitive. Thus on spraying initially with a solution of benzyl penicillin (200 units/ml.) followed, after drying, by the *p*H 7 buffered starch–iodine reagent (*C*) described above, *Bacillus cereus* penicillinase could be detected on paper as a white zone against a blue background with a sensitivity of 5–10 ShenLabs units.

An alternative colorimetric reagent for the detection of penicillins has been previously described, which is based on the well-known hydroxamic acid reaction[3]. However, this is much less sensitive than the starch–iodine reagent, which has the additional advantage of permitting the rapid differentiation of penicillinase-sensitive and -resistant penicillins.

I am indebted to Miss Evelyn Albu for her technical assistance.

[1] Alicino, J. F., *Indust. and Eng. Chem.*, **18**, 619 (1946).

[2] Robinson, G. N., Stevens, S., Batchelor, F. R., Wood, J. C., and Chain, E. B., *Lancet*, ii, 564 (1960).

[3] Baker, P. B., Dobson, F., and Martin, A. J. P., *Analyst*, **75**, 651 (1950).

ESTIMATION OF SPECIFIC PROTEINS IN SERUM AND OTHER MIXTURES

By Dr. D. A. DARCY

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital,
Fulham Road, London, S.W.3

THE purpose of this article is to report the increase in accuracy and simplicity brought about by certain modifications of a method previously described[1]. The method is based on the agar-gel immune precipitation method of Ouchterlony[2]. It consists essentially of placing the protein solution in a hole made in an agar-gel and allowing it to diffuse towards a specific antibody for the protein diffusing towards it from a nearby hole. After the two reactants meet, a band of precipitate is formed composed of both substances. The more concentrated the protein, the faster will the band migrate towards the antibody source, and this phenomenon is used to estimate the concentration of the protein. This is achieved by standardizing the conditions of the reaction and by calibrating the antiserum against a solution of the protein (antigen) which has been selected as the standard.

The details of preparation of the agar plates, the antiserum, the calibration curve, and the incubation conditions, etc., have been described in the earlier paper: it was shown there that an average error of about ± 14 per cent (95 per cent confidence limits) was obtained routinely. The modifications introduced here reduce this error to less than ± 5 per cent.

Incubation conditions. It was found after numerous experiments that a major source of the ± 14 per cent error was that the gels were not incubated on the level. When ordinary Petri dishes are stacked they invariably tilt a few degrees from the horizontal and this is sufficient to cause large errors. Furthermore, even the bottom plates of the stacks were found to give irregular readings, and this was ascribed to the uneven thickness of their bottoms, which lead to an uneven depth of agar-gel in a given plate. Both these troubles were remedied by the use of flat Petri dishes. It was also found necessary to check (with a small spirit-level) that the platform of the moist chamber on which the plates were stacked was likewise level when in place in the incubator. If a moist chamber consisting of a desiccator jar with a plate glass platform was used, it was found that wrapping the jar with household aluminium foil speeded up the band migration during the 3 days at 37° C.

Preparation of the agar plates. The agar was made up as described previously. No general advantage was found in using lower concentrations of agar than 1 per cent. Although these gave clearer gels and faster migration of precipitates, accuracy was not appreciably increased thereby. Nor, curiously, was readability appreciably increased on these weaker gels—for the precipitate formed was less dense so that the band-gel contrast was about the same as on the 1 per cent agar.

The chief modification introduced in the plates was the use of round holes (wells) in place of the square ones previously employed. The round wells had the advantage that they could be punched out by a commercially available cutter; furthermore, they could be spaced farther apart than the square wells while giving the same density of precipitate, thereby extending the range of protein concentrations that could be measured. The excellent method of Feinberg[3] was followed in making up the plates. A precision cutter and special flat 2·5-in. Petri dishes were obtained from the Shandon Scientific Co. Each dish, after silicone treatment, was filled to a depth of 3 mm. with 1 per cent agar (9 ml.) and allowed to set for a day or more before the wells were punched. The wells were 1 cm. in diameter and the distance between them and the centre (antiserum) well was also 1 cm. (Fig. 1).

Measurement of the precipitate. This was carried out, very simply, with a segment of 'Perspex' ruler enclosing a centimetre ruled in half-millimetres to which a small 'Perspex' handle was attached. The ruled centimetre was laid on the gel surface and lined up so that its limits coincided with the centimetre distance between the centre well and one of the outer wells along a line between their centres (see Fig. 1). The distance from the edge of the outer well to the sharp leading edge of the precipitate was estimated to the nearest 0·1 mm., using a × 2 magnifier and a suitable illumination source. The method is an improvement on that previously used where the rule was laid across the top of the plate, but it is still subject to some parallax error; it is nevertheless capable of a high degree of accuracy as will be seen later. A second method of measurement used in this Laboratory was more elaborate but completely ruled out parallax. It consisted of using a low-power lens (about × 1) to project the image on an ocular micrometer scale of a centimetre with 0·1 mm.-divisions where it was read by means of an eyepiece (× 7). This gave somewhat greater accuracy than the rule method and has the slight additional advantage of not disturbing the plate.

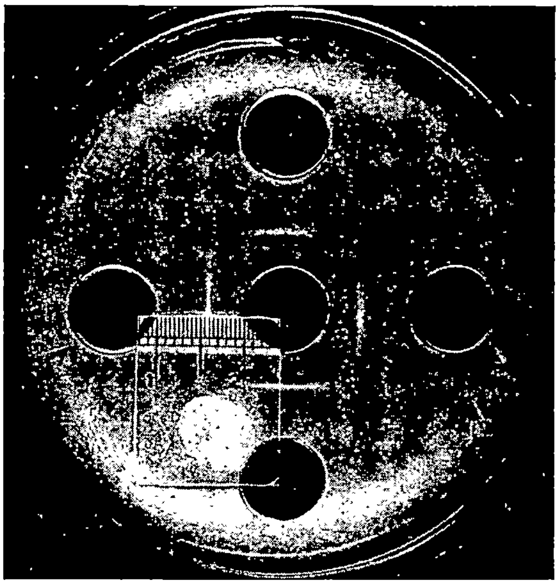


Fig. 1. Standard agar plate showing the centimetre rule lying on the surface of the gel in position for measurement

Experimental test of the method. Accuracy tests were made using rat serum and a rabbit antiserum to the α-globulin of rat serum described earlier[1]. Rat serum yielded with this antiserum one strong band (for the α-globulin) and only traces of other precipitates (Fig. 1). Tests were made with five different concentrations of the rat serum in saline; at the highest concentration the α-globulin band reached nearly to the antiserum well, with the standard conditions, and at the lowest concentration (which was barely in antigen excess) it reached a little beyond midway. Each of the five concentrations was tested against concentrations lower than itself by 5, 6 and 7 per cent. These solutions were presented as unknowns to the person carrying out the test and were indistinguishable by eye. Sixteen separate tests (equivalent to 4 plates) were made on each unknown, using the rule to measure. In every case the method gave a statistically significant discrimination between the solutions differing by 5 per cent ($P = 0{\cdot}01$ or less in every case, except one where P fell just short of 0·01). It was decided to test the method to its limit at the highest of the five concentrations of rat serum, and it was found that a statistically significant discrimination was obtained between the solutions differing by 3 per cent when the rule was used ($P = 0{\cdot}02$–$0{\cdot}01$) and between solutions differing by only 2 per cent when the more elaborate optical method was used ($P = 0{\cdot}05$).

Since, as was seen in the earlier paper[1], accuracy is proportional to the square root of the number of tests made on each unknown, then for the routine method of using only four tests for each unknown the above discriminating power will be halved, or the error doubled. The error for the routine procedure will thus be ± 5 per cent (95 per cent confidence limits) or less, and at the best ± 2 per cent. For many purposes it may be sufficient to use only a single test per unknown since the error would, on the average, be less than ± 10 per cent. Again with some sacrifice of accuracy, the incubation period could be shortened to 2 days or less if needed.

The method was found to be suitable for other serum proteins such as albumin, for enzymes such as deoxyribonuclease I, but not for xanthine oxidase (mol. wt. 300,000), except possibly if the agar concentration were to be reduced and the incubation time lengthened. The limits of sensitivity are those of the gel-diffusion method, which can at best give a visible precipitate with about 2 μgm. of protein per ml. The method is also applicable to the titration of antisera, for example, by placing a standard antigen in the centre well and measuring to the sharp edge of the precipitate, which will then be on the side facing the peripheral wells.

My thanks are due to Mr. M. S. C. Birbeck of this Institute for advice on optical matters, and to Mr. Peter Harpel of Harvard Medical School for suggesting the effect of tilt.

This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

[1] Darcy, D. A., *Immunology*, **3**, 325 (1960).
[2] Ouchterlony, Ö., *Ark. Kemi, Min., Geol.*, **10**, 228 (1948).
[3] Feinberg, J. G., *Int. Arch. Allergy*, **11**, 129 (1957).

CIRCUMSCRIBING IRRADIATION OF SURFACE STRUCTURES OF CYLINDRICAL ANIMAL AND PLANT CELLS WITH POLONIUM-210 α-PARTICLES

By R. M. BERGSTRÖM, R. F. BLÅFIELD and M. W. BRENNER
Institute of Physics, University of Helsinki, and the Institute of Physiology, University of Helsinki

A STUDY of the effects of ionizing radiation on living tissues provides a valuable supplement to investigation of the function of these tissues. The energy of the radiation may be used for complete or partial destruction of the cell material. It can, however, also be used in studying the normal physiological functions of the cells. To this end, irradiation energy can be used as a stimulus energy, which occasions alterations in many physiological processes.

Use of radiation energy for physiological research on cell functions is favoured by the possibility of reaching microscopic structures within the cell, and of partial destruction of fractions of them. As pointed out by Zirkle and Bloom[1], it complements micro-surgery.

This article is concerned with a method which makes use of a cylindrical polonium-210 α-particle source. This renders possible the destruction or the stimulation of surface structures of cylindrical animal or plant cells. The results of the irradiation of single nerve fibres and cells of some algae will be presented elsewhere. This article refers briefly to the ultrastructural changes in the cells of the algae *Nitella flexilis* obtained by this method.

The aim was to produce significant, graded destruction or stimulation of the surface layers of the living nerve and plant cells, with the view of facilitating study of the alterations in the electrical functions of the cells due to the irradiation. Thus, the choice of the ionizing radiation had to fulfil some basic requirements. The ionizing power of the radiation should be as high as possible in order to produce the structural changes. For this reason, heavy-particle radiation was to be preferred. To reduce the effects to a limited surface layer of cells with a small diameter, the depth of penetration of the radiation in organic tissue should be small. For these reasons, and because the aim was to irradiate the membrane of the cylindrical cells from all directions, polonium-210 α-radiation was considered the most satisfactory choice. The reasons for this decision were based on the high ionizing power of the heavy, charged α-particles and the low penetration in organic material.

The half-life of polonium-210 is 138·4 days and the energy of the α-particles emitted 5·298 MeV. The polonium-210 is deposited on the cylindrical inner surface of a silver ring. The nerve or plant cell is held in the centre of the ring, along the axis of the active cylindrical area (Fig. 1). The radius of the active area is 10 mm., and the height, h, is 3 mm. On beginning the irradiation, the ring must be placed rapidly in position around the material to be irradiated. This is made possible by the provision of a slot for the passage of the cell from the outside to the interior of the ring. Thus the whole periphery cannot be used for the active area since it is reduced by an amount, d, of 7 mm. The active area is 167 mm.^{2}.

The source was supplied by the Radiochemical Centre, Amersham, United Kingdom. Its strength, as stated by the manufacturer, was 1·1 mc. ± 20 per cent at the time of delivery, November 3, 1959. The measurements were carried out 11 months later, the activity then being 0·21 mc. The activity was also measured using the nuclear plate technique. The results of this measurement were consistent with the strength stated by the manufacturer. The nuclear plates were kept in a diametrical plane of

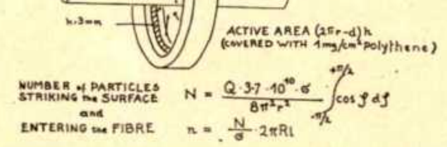

V = πϱ(2R − ϱ)l
RADIUS = R
h = 3 mm
10 mm = r
DOSE RATE:
DR = k Q/ϱ(2−ϱ/R) rep/min
AXON
ACTIVE AREA (2πr − d)h
(COVERED WITH 1 mg/cm² polythene)
NUMBER of PARTICLES STRIKING the SURFACE
N = Q·3·7·10¹⁰·σ / 8π²r² ∫ cos ϱ dϱ (−π/2 to +π/2)
and
ENTERING the FIBRE
n = N/σ · 2πRl

Fig. 1. A schematical representation of the circumscribing irradiation of a nerve fibre from a cylindrical polonium-210 α-source

the active cylindrical surface. By counting the density of the tracks along the cylindrical axis, the intensity of the α-radiation and the activity of the source could be determined.

To avoid contamination, the active area was covered with a 1 mgm./cm.^{2} film of polythene. Strong α-particle bombardment may destroy this and, consequently, it does not provide sure protection. Thus the source was enclosed in a container, from which it could be removed and put in position around the cell by means of a lever. The entire equipment is kept in a special cabinet intended for this particular experiment. Possible contamination originating from the source is detected by means of an end-window type Geiger-counter tube. The window of the tube consists of 2 mgm./cm.^{2} mica.

There are certain advantages in using a cylindrical source. The cell membrane is bombarded equally from all directions. All α-particles pass through equal distances of air and tissue. However, only those particles which enter the surface of the cell perpendicularly will penetrate radially into the cell to a distance which corresponds to the residual range, ρ, in tissue. Evidently, the effect of the radiation is concentrated in only a certain volume, V, of the cell. V is bounded by the surface of the cell and a surface defined by the largest depth of penetration. In order to calculate the dose, we thus have to evaluate the depth of penetration in tissue.

The range in air of the 5·3-MeV. α-particles from polonium-210 is 38 mm. (ref. 2). The loss of energy due to the penetration of the polythene film covering the source is 0·6 MeV. The loss of energy due to the distance covered in air between the source and the cell is 1·1 MeV. Thus the particles entering the tissue have an energy of 3·6 MeV. This value is in accordance with the length of the tracks, observed in nuclear emulsions. The corresponding range in air is 21 mm. (ref. 2) and in tissue 20μ approximately.

Assume that the organic cell is cylindrical and has radius R. Now the volume, V, reached by the particles is:

$$V = [\pi R^2 - \pi(R - \rho)^2]l = \pi\rho(2R - \rho)l \qquad (1)$$

where l is the length of the irradiated part of the cell. Each particle will lose an amount of energy 3·6 MeV. or $5{\cdot}75 \times 10^{-6}$ erg in the cell. If length l is much shorter than the radius, r, of the source, every surface element, σ, of the irradiated part is struck at the same rate of α-particles. We recognize that the height, h, of the cylindrical area is small and thus we can approximate the source by an infinitely thin closed ring. This approximation is regarded as being perfectly satisfactory. The number of particles, N, striking the surface element, σ, in a second can then be written:

$$N = \frac{3{\cdot}7 \times 10^{10}\, Q\sigma}{8\pi^2 r^2} \int_{-\pi/2}^{+\pi/2} \cos\varphi \, d\varphi \qquad (2)$$

φ is the angle between the normal of the surface element and the direction of the particle. Q is the strength of the source given in curies. The rate of α-particles, n, entering the cell is obtained by summing over the surface of the irradiated part of the cell. We get:

$$n = \frac{N}{\sigma}\, 2\pi R l \qquad (3)$$

The energy dissipation in a unit volume of tissue and in unit time is obtained by combining equations (1), (2) and (3). If we assume that the density of the tissue is 1 gm./cm.^{3}, and divide by 83 ergs, we get the dose-rate in rep/min.:

$$\text{The dose-rate} = 7{,}800\rho \frac{Q}{\left(2 - \frac{\rho}{R}\right)} \text{ rep/min.} \qquad (4)$$

If the nominal strength of the source, 1·1 mc., and the range 20μ are substituted in this equation, we get $4{,}300/(2 - \rho/R)$ rep/min. Because of the inaccuracy of the value for the activity of the source and the error in the residual range, ρ, we expect an error in the dose-rate of ± 15 per cent, as calculated by means of equation (4). It should be pointed out that the calculated dose-rate is a mean value taken over the volume reached by the α-particles. When $\rho < R$, there is a volume in the interior of the cell which is not influenced by the radiation at all. When $\rho > R$, the equation (4) will give too high values, because a considerable number of α-particles escape from the cell.

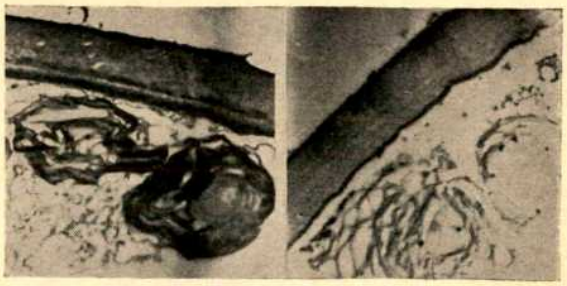


Fig. 2. An electron-micrograph of the cell wall and protoplasm of *Nitella flexilis*, irradiated with polonium-210 α-particles. A normal cell is shown on the left. Magnification: left, × 5,500; right, × 4,400

Here, only one application of the method is described, that is, some ultra-structural changes of the internodal cells of the alga *Nitella flexilis* caused by α-irradiation. The cells, cultured in sea-water from their original environment, were placed in the centre of the polonium-210 ring. Exposure of the cell surface to the α-irradiation was carried out at short intervals, after which the cell was moved to its water environment to prevent it from drying. The whole procedure was performed under a microscope.

In Fig. 2 is shown an electron-micrograph of a *Nitella* cell irradiated with 8,900 rep. The non-irradiated control is shown to the left. The protoplasmic layer of the irradiated cell is separated from the cell wall, whereas it is connected to the wall in the non-irradiated cell. Separation of the protoplasm in the irradiated cell is seen where osmiophilic grains (usually situated near the wall) are displaced to the inner part of the cell. Arrangement of the particles in a row near the wall due to the irradiation with α-particles possibly indicates the existence of a membrane normally connecting the inside of the cell wall. The large chloroplasts, with definite structure in the control, show a diffuse structure with a prominent vacuolization near the cell wall after irradiation.

This work was supported by grants from the Finnish State Committee for Natural Sciences.

We wish to thank Prof. L. Simons for advice and encouragement.

[1] Zirkle, R. E., and Bloom, W., *Science*, **117**, 487 (1953).

[2] Landolt-Börnstein, *Zahlenwerte und Funktionen*, **6**, Aufl. I, 5, edit. Joos, E., Eucken, A, and Helivege, K. H. (Berlin–Heidelberg, Springer Verlag, 1952).

MUTAGENIC EFFECT OF RADIANT HEAT SHOCKS ON PHANEROGAMOUS PLANTS

By DR. BROR PETTERSSON

Botanical Museum of the University of Helsinki

In order to prove a possible mutagenic effect of physical and chemical agencies liberated and activated by volcanic eruptions I have made a number of experiments with various phanerogamous plants during the past eight years. The plants chosen for this purpose belonged chiefly to native species, but foreign ones were also represented; these were obtained from the seeds of some Canarian endemics collected by me; there were also fresh herbarium specimens from Madeira, the Cape Verde Islands and Ethiopia. Finally, the material included a few garden plants.

The experiments with the native plants were carried out either on individuals of small island populations easily kept under observation at the place of growth, or on plants of other species settled on an island where they had not grown before. As regards the foreign plants, some were cultivated as common pot-plants and the remainder in garden beds. No particular care was extended to the experimental plants. Pollination control, namely, selfing and crossing, was performed in some cases; otherwise, the rule was free cross-pollination within the population. Only when the fruits were approaching the ripening stage were they protected from scattering by a gauze envelope.

Although the most important occurrence in the evolutionary process of organisms is the genesis of individuals which are distinguished by new characteristics quite different from those characterizing the members of the population within which they had arisen, there can be no question of a new taxon before a mutation has shown itself capable of propagating and stabilizing the new genotype in the environment of the parent population or elsewhere. Accordingly, the aim of my experiments was not only to investigate factors causing mutations in Nature, but also to study the evolutionary pattern of the mutated plants, and the time, measured in generations, necessary for an eventual establishment of new biotypes. Strictly speaking, therefore, the experiments were not exactly genetical in the orthodox sense.

The first stage of this investigation was concerned with the effect of momentary strong radiant heat (such as that caused by incandescent pyroclastic material scattered over surrounding vegetation by an active volcano) on flower buds in various stages of development. In order to obtain the necessary momentary high temperature, use was made of a common burning glass, with a magnification of × 3; by this means, the young flower buds or bud clusters were subjected to the 'burning' rays until some smoke appeared, or until there could be observed a visible change in the colour of the bud, which generally occurred within 2–15 sec.

In conjunction with radiant heat, treatment with sulphur dioxide as sulphurous acid, hydrochloric acid and boric acid was also given in some cases. A small cotton pellet, drenched with one of the solutions, was wrapped round a bud or cluster of buds, kept in this position, and heated during a period of some minutes by means of the burning glass. However, these chemical tests have been too few in number so far to permit any definite conclusion as to differences in mutagenic effect.

The seeds obtained by these rather rough treatments were generally few in number and of inferior germination capacity. The effect of radiant heat could be observed already in the seedlings, principally as teratological deformities. Depending on the severity of the injuries caused by burning, the number of such mutated seedlings varied between 2 and 40 per cent. Frequently, the cotyledons were either already deformed, or their number and position altered. The rather common occurrence of seedlings with three cotyledons was most obvious with *Aichryson dichotomum* (Fig. 1), *Celsia insularis*, *Cynoglossum lanceolatum*, *Dimorphotheca pluvialis*, *Kallanchoë glauca*, *Knautia arvensis*, *Lythrum salicaria* and *Nemophila menziesii*, or with one of them more or less forked; if they were split to the base it was often a difficult matter to distinguish them from true tricotyledons (*Aichryson dichotomum*, *Celsia insularis*, *Centaurea cyanus*, *Kallanchoë glauca*). Of less frequent occurrence were seedlings with only one cotyledon, a

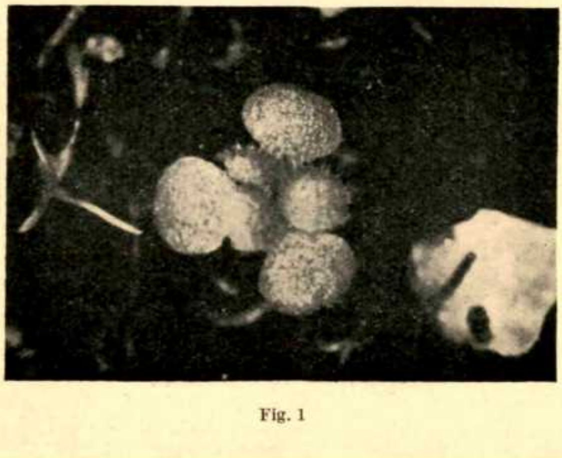


Fig. 1

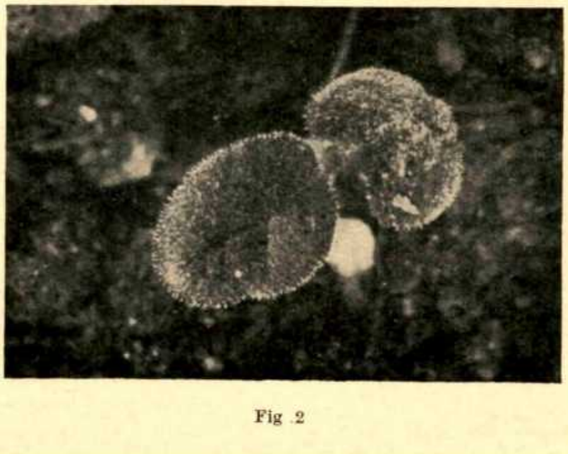


Fig 2

syncotyly clearly formed by fusion of the normal ones and appearing as a single organ, often bent in various ways, such as with *Achillaea millefolium*, *Celsia* (Fig. 2), *Solidago virgaurea*, *Spiraea ulmaria*, *Tanacetum vulgare*. Only in two species, namely, *Angelica silvestris* and *Allium schoenoprasum* did seedlings occur which were entirely lacking in chlorophyll, or were of a more or less yellow-green colour.

As a rule, the true 3-cotyledons developed into plants with a leaf position which was other than that of the parent plants; thus plants originating from parents with opposing leaves became verticillate with three leaves in the whorl (*Kallanchoë*, *Lythrum*); again, those with scattered leaves mutated to an almost pseudo-verticillate leaf position (*Dimorphotheca*, *Nemophila*, *Centaurea* and *Aichryson*) with very densely scattered leaves, sometimes growing into giant individuals.

As is known, both polycotyledonous and monocotyledonous seedlings of a number of different dicotyledonic species have on occasion been found to occur spontaneously in Nature. Among my control cultures, only *Lythrum salicaria* showed a slight tendency to produce tricotyledons spontaneously; of 400 seedlings, one was found to have three cotyledons.

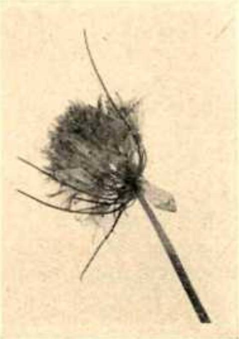


Fig. 3

However, some of the anomalous seedlings developed quite normally with no teratological characteristics, although the majority were subject to fasciations of various kinds. Most important and interesting was the occurrence of bud mutations, which sometimes appeared as early as at the seedling stage, but most commonly in more developed young plants. In the majority of cases they appeared as more or less strange fasciations with anomalous leaves and flowers. However, insufficient study has been devoted so far to the hereditary pattern of these 'thermomorphoses'. Apart from these teratological malformations in the progeny of the experimental plants, a number of other mutations also took place, both with plants and buds. In this category were included all individuals with strange characteristics which were observed neither in the parent population nor were known as existing in the species concerned elsewhere. So far as space permits, the following gives a survey of the most important cases investigated:

Seeds obtained from a burnt *Allium schoenoprasum* plant gave rise to 21 young plants, of which not a single one agreed in all its characteristics with the parent. The most marked peculiarities were discovered in seven of these plants; these displayed quite strange characteristics which had not been observed in the parent population nor had hitherto been known to occur elsewhere in any population of this species. In five of them, there developed among the flowers in the umbel bulbils which varied in number from few to many (Fig. 3), in a very similar fashion to *Allium oleraceum*; the remaining two became drooping umbels, as the single flower of *Galanthus*.

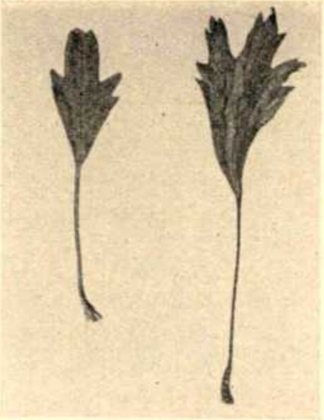


Fig. 4

The results of experiments with *Angelica sylvestris* were no less surprising. Unfortunately only a small number of healthy seeds were obtained, and these gave rise to 26 seedlings. Even as early as in the first summer, the young plants showed many peculiar characteristics which were to be observed neither in the controls nor in the natural populations, One plant in particular was remarkable: as a seedling, it showed two plumules, the second of which sprouted from the root neck, obviously as a kind of bud mutation. The normal plumule developed common trifoliate leaves, but the bud mutation gave rise to only simple cuneate leaves (Fig. 4) tapering into a petiole with a broad apex irregularly toothed or incised, and thus bearing a strong resemblance to those of the Malesian *Trachymene koebrensis* and *T. novoguinëensis*. During the following summer, the same shoot had only pinnate leaves, with two to three pairs of leaflets, broadly ovate, serrate and somewhat convex, with some resemblance to the leaves of *Sium latifolium*. The main shoot gave rise to quite normal double pinnate leaves. In the third summer, this 'Siamese' twin plant reached the flowering stage. By then, the vegetative parts seemed to belong to two different plants, with a main stem 110 cm. high, pale green in colour, with double pin-

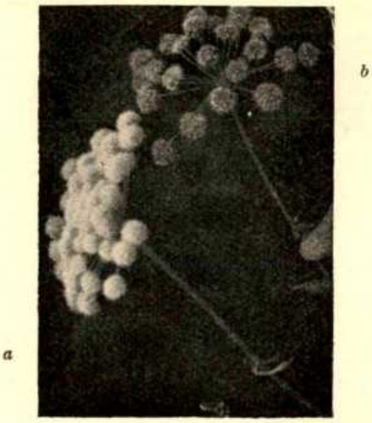


Fig. 5

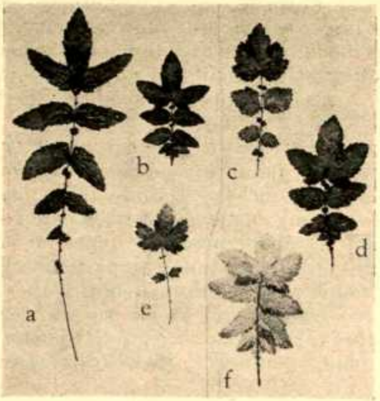


Fig. 6

nate *Angelica* leaves which were normal in every respect. The stem of the bud mutation grew only 70 cm., and was coarser, dark green in colour, with leaves which were broader and darker, with fewer and broader leaflets than those of the main stem. The umbels of the bud mutation were almost flattened, the umbelets depressed; rather few flowered, with flowers which were conspicuously larger than those of the parent population and of a milky white colour. The main stem had almost spherical umbels with dense globular umbelets and remarkably small, whitish, slightly purple-sprinkled flowers. The fruits of the main shoot were narrow lanceolate, approximately one-third longer than those of the parent, and of a vivid brownish shade. The mericarps of the bud shoot had a thick flattened corky rim instead of the normal thin membraneous one of the parent population, and had a greyish sooty colour.

These odd twins were crossed in both directions, but only the bud mutation gave viable seeds. Of 42, only 15 germinated, and these resulted in the same number of healthy young plants. Ten of these F_1 plants flowered last summer. They represented as many forms, not one of them resembling either the others or the individuals of the parent population. Some plants were strikingly different in appearance; they had not been observed before in any *Angelica silvestris* population known to me. Their mode of growth approached that of *Angelica littorialis* rather than that of the wild *Angelica*. One of them is worthy of special note: the plant itself was low, large and richly ramified, with short internodes and broad leaves, with conspicuous sheaths which were entirely milky white in colour. The lower part of the stem and the branches were also glossy white. The umbels (Fig. 5*a*) were large and very irregular in size, extremely dense, and composed of about twice as many umbelets as normally occur in any plant of the parent population (Fig. 5*b*); these were irregular and almost flat, bearing a closer resemblance to some Japanese *Angelica* species than to those in Finland. The umbelets were also conspicuously dense, hemispherical, and the flowers ivory white. Another similar plant had the white stem and leaf sheaths striped with purple.

No less surprising results were obtained from the experiments with *Spiraea ulmaria*. About 31 per cent of the young plants of the first summer were mutated in various ways; during the last, that is, the second summer, the mutations were more pronounced. Some could be termed giants (Fig. 6*a*), with the leaf attaining a length of 34 cm., and four pairs of large leaflets. Others displayed a smaller number of leaflet pairs, but had a prominent terminal leaflet, often of peculiar form, either circular or lobed, toothed, undulated or crispate (Fig. 6*b*, *c*, *d*), and in several instances with the leaves white-spotted or striated (Fig. 6*f*). One plant had a dwarfish growth, densely tufted, with numerous small delicate leaves with only one pair of small leaflets and a large terminal leaflet which strongly resembled the leaves of some *Ribes* species (Fig. 6*e*).

Generally speaking, all the species treated in this way had teratological progenies, which provided evidence for either mere malformations of different parts of the plant or more 'normal' alterations in the size of the leaves and in colour, structure and number of the various parts of the flower.

My experiments have shown that a common burning glass may have the same effect in producing mutations as the Röntgen tube and radium needle.

EFFECTIVENESS OF LIPID AND LIPIDOPHILIC SUBSTANCES AS ADJUVANTS

By D. W. DRESSER*

Department of Zoology, University of Edinburgh

FREUND[1] has stated that adjuvants of the Freund type may act in one or more of three ways: (1) by storing antigen at the site of injection and releasing it slowly; (2) by stimulating the migration of macrophages and lymphocytes to the injection site; (3) by stimulating antibody production in some way which is not understood. This preliminary article is a brief description of a method whereby the effectiveness of different substances as stimulants of antibody production can be tested. In these experiments the antigen and adjuvant have been injected by different routes so that Freund's categories (1) and (2) can be eliminated as the cause of the observed effect, for which the name 'adjuvanticity' seems to be suitable.

It has been known for some time that intravenous injection of protein solutions into rodents is an unreliable method of immunization. This has been confirmed[2] using BGG (bovine gamma globulin, Armour and Co.) in *CBA* strain mice. Intravenous injection of a freshly made solution in 0·9 per cent saline was found to immunize 5 out of 23 mice when the effectiveness of the first injection was tested a month later by the antigen-elimination technique[3] with BGG labelled with iodine-131 (BGG–^{131}I).

In some recent experiments, summarized in Table 1, it has been shown that no mice at all out of

---

* Present address: Division of Biology, California Institute of Technology, Pasadena, Calif., U.S.A.

43 tested were immunized when the BGG solution first injected into the mice was centrifuged at 30,000*g* for 15 min. A very small amount of precipitate was always found after centrifugation, and it is thought that it is this material removed by centrifugation which enabled some of the mice injected with uncentrifuged BGG solution to be immunized. Freund's adjuvant containing BGG injected subcutaneously has been shown to be an effective means of immunizing mice. In Table 1 the secondary responses obtained after challenge with BGG–^{131}I, a month after the first antigen injection, have been arbitarily divided into five categories ranging from no response at all (=0) to the degree of response obtained after previous immunization with BGG in Freund's adjuvant (=4).

It was found that an intravenous injection of centrifuged BGG was effective in conferring immunity if a separate subcutaneous injection of Freund's adjuvant, made up without the antigen, was given at the same time. Several substances have been tried in place of the Freund's adjuvant without antigen. The results of these experiments are included in the table. Of the substances tested, 'Crill 16', oleic acid and powdered mycobacteria all have a marked adjuvanticity.

There seem to be two possible mechanisms responsible for adjuvanticity: (1) the adjuvant may affect the lymphoid cells of the animal, perhaps by stimulating them to take up the antigenic protein, or (2) the adjuvant, even when injected subcutaneously, may affect the stability of the circulating antigen, causing it to precipitate, thus immunizing the mouse in a similar way to alum-precipitated BGG. The latter alternative seems unlikely when the antigen is injected intravenously and the adjuvant subcutaneously, because a large part of the injected substances, for example, 'Crill 16', can be found 2 months later in subcutaneous vesicles. Although some of the 'Tweens' are known to affect the electrophoretic mobility of gamma globulins[4], it seems unlikely that they could affect the circulating BGG in preference to the large amounts of mouse protein present. Furthermore, 'Tween 60', although soluble in water and affecting gamma globulin, does not appear to have a marked adjuvanticity, whereas oleic acid is insoluble in water but is a good adjuvant. The first alternative, that the adjuvant affects the cells of the injected animal, seems to be the more likely.

Recent work has shown that mycobacterial waxes contain oleic acid, palmitic acid, various triglycerides and related substances[5]. It has been known for some time that mycobacterial waxes can be responsible for the establishment of hypersensitivity to complex antigens and simple chemicals[6]. A suspension of mycobacteria, oleic acid and some esters of sorbitol have been found to be substances with a marked adjuvanticity as measured by the method described here. Some of these esters (non-ionic detergents) are known to have deleterious effects[7], including carcinogenic effects[4], on animal tissues. It is possible that some lipids and non-ionic detergents, either in pure form or as mixtures in mycobacterial waxes or croton oil, may owe their adjuvanticity or carcinogenicity to their ability to damage cell membranes. In the first instance, adjuvanticity may be postulated as being due to a non-specific effect on cells, which might act in either or both of two ways: first, damaged cells may be stimulated to take up antigen by phagocytosis or pinocytosis; secondly, as a result of damage, lymphoid cells might be stimulated into a hypercompensatory reaction, one aspect of which could be the production of antibodies.

It seems that the observations recorded here may have a theoretical connexion with some auto-immune phenomena. BGG solutions centrifuged free of traces of particulate matter lack adjuvanticity in mice. When the BGG is particulate, for example after precipitation by alum, it possesses adjuvanticity on its own. It is not unlikely that most of the antigens with which an animal comes in contact during its life must have sufficient adjuvanticity either in themselves or closely associated with them for the immune mechanism to receive the necessary stimulus. There may, in fact, be two mechanisms preventing an animal from becoming immunized

Table 1

First injection of antigen	Treatment, 3 days before injection of antigen	Effect of challenge with 2 mgm. BGG-iodine-131 one month after first injection of antigen Category of response, see text (No. mice responding—No. tested). (Increasing response →)				
		0	1	2	3	4
Freund's adjuvant containing 8 mgm. BGG/mouse; subcut.	Nil	—	—	—	—	25/25
5 mgm. alum-precipitated BGG; intraper.	Nil	—	—	—	—	5/5
5 mgm. BGG as sol. in 0·9 per cent saline; not centrifuged; intraven.	Nil	18/23	—	—	—	5/23
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraven.	Nil	18/18	—	—	—	—
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	Nil	20/20	—	—	—	—
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; subcut.	Nil	5/5	—	—	—	—
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	Freund's adjuvant made up with saline only; subcut.	—	—	—	—	10/10
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	2 mgm. powdered mycobacteria in saline; subcut.	—	—	—	—	5/5
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	0·4 ml. liquid paraffin; subcut.	9/10	—	—	1/10	—
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	0·4 ml. 'Crill 16'*; subcut.	—	—	—	—	13/13
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	0·2 ml. 'Tween 60'†; subcut.	5/5	—	—	—	—
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	0·2 ml. 'Span 20'‡; subcut.	1/5	—	—	1/5	3/5
5 mgm. BGG as sol. in 0·9 per cent saline; centrifuged; intraper.	0·2 ml. oleic acid; subcut.	—	—	1/5	—	4/5

* Commercial preparation of sorbitol monoleate containing other esters of sorbitol as impurities. Made by Croda, Ltd., Goole, Yorks.
† Polyoxyethylene sorbitol stearate. Made by Atlas Powder Co. (Honeywill and Stein, Ltd., Piccadilly, London).
‡ Sorbitol monolaurate. Made by Atlas Powder Co.

against its own tissues; (1) lack of antigenicity (immunological tolerance); (2) lack of adjuvanticity. An example of the latter might be spinal cord or brain tissue. Allergic encephalomyelitis may in some cases be caused by prolonged injections of dried spinal cord tissue, but a single injection of spinal cord or brain in Freund's adjuvant will always lead to this condition[8]. This effect of Freund's adjuvant is unlikely to be due to the breakdown of a previously acquired state of tolerance, as mice tolerant to BGG do not give an immune response even when challenged by an injection of BGG in Freund's adjuvant[9]. It would seem from this that mammals are not normally immunologically tolerant to brain or spinal cord, and that the lack of an immune response is due to some other factor. It has recently been shown that tolerance to brain or spinal cord may possibly be induced experimentally[8]. It is a possibility, therefore, that this and some other auto-immune phenomena may be initiated by a gain of adjuvanticity by a tissue which normally lacks adjuvanticity, but to at least one antigen of which the animal is not normally immunologically tolerant.

I am grateful to Dr. R. A. Kille for the benefit of discussion.

[1] Freund, J., *Amer. J. Clin. Path.*, **21**, 645 (1951); in *Nature and Significance of the Antibody Response*, edit. by Papenheimer, jun., A. M., 46 (Columbia Univ. Press, 1953).

[2] Dresser, D. W., *Immunology*, **3**, 289 (1960).

[3] Talmage, D. W., Dixon, F. J., Bukantz, S. C., and Dammin, G. J., *J. Immunol.*, **67**, 243 (1951).

[4] Setala, K., Holsti, P., Lundbom, S., and Setala, H., in *Biochemical Problems of Lipids*, edit. by Popjak, G., and Le Breton, E., 496 (Butterworth, London, 1956).

[5] Michel, G., *Bull. Soc. Chim. Biol.*, **41**, 1649 (1959).

[6] Raffel, S., *J. Inf. Dis.*, **82**, 267 (1948). Raffel, S., and Fourney, J. E., *J. Exp. Med.*, **88**, 485 (1948).

[7] Goldhammer, H., and McManus, W. R., *Nature*, **186**, 317 (1960).

[8] Patterson, P. Y., in *Cellular and Humoral Aspects of the Hypersensitive State*, edit. by Lawrence, H. S., 469 (Cassell, London, 1959).

[9] Dresser, D. W., *Immunology*, **4** (13) (1961).

INCREASE OF THE RATE OF RELEASE OF THYROIDAL IODINE-131 AND OF CIRCULATING THYROTROPHIC ACTIVITY AT EARLY STAGES OF PROPYLTHIOURACIL TREATMENT IN THE RAT

By Dr. F. ESCOBAR del REY, G. MORREALE de ESCOBAR, M. D. GARCÍA GARCÍA and J. MOURIZ GARCÍA

Instituto G. Marañon, Consejo Superior de Investigaciones Científicas, Velázquez, 138, Madrid

THE idea that induction of goitrogenesis by propylthiouracil (PTU) and related compounds only involves those effects of such drugs which lead to the blocking of thyroid hormone synthesis is generally accepted. The hypothesis that these goitrogens do not have an extrathyroidal effect on thyroxine metabolism which might be playing a part in thyroid–pituitary interrelationships was confirmed in the case of thiourea when Griesbach and Purves[1] showed that thyroxine requirements for the maintenance of normal pituitary cytology of thyroidectomized animals were the same for control and treated rats. It has been widely assumed that this conclusion is valid for propylthiouracil also.

Observations have, however, been reported which are not in complete agreement with such hypotheses. Rawson[2] has observed a 50 per cent increase in the mean acinar cell height in rat thyroids as soon as 24 hr. after onset of PTU treatment, at which time no differences are yet demonstrable in the plasma protein-bound iodine. This finding was interpreted[2] as the result of an enhancing effect of PTU on thyrotrophic hormone (TSH) effectiveness[3,6]. Van Middlesworth[5] found that thyroxine doses necessary for suppression of PTU-induced goitrogenesis raised the plasma protein-bound iodine of the rat well above normal.

We have reported[6], moreover, that as soon as 5 hr. after onset of PTU administration to thyroidectomized, l-thyroxine-maintained rats, a depression of the peripheral deiodination of the hormone is demonstrable, this extrathyroidal effect of the drug lasting until suppression of treatment. The possibility was suggested that the peripheral effects of PTU which result in a decreased thyroxine dehalogenation might be triggering secretion of TSH by the pituitary, and that this response might be mediated by a factor related to the alteration of intracellular metabolism of thyroxine. Observations such as those summarized above[2,5] could then be interpreted along such lines.

This article reports an attempt to investigate the early phase of PTU treatment in the rat, when blocking of hormone synthesis by the drug has not yet resulted in a decreased plasma protein-bound iodine. Young adult male rats of a giant Wistar strain were used. They were fed a medium residue low-iodine diet. 10–15 μc. iodine-131 were injected intraperitoneally and the activity over the neck plotted against time. About 70 hr. after the injection of the tracer half the experimental group received 15 mgm. PTU intraperitoneally, and the radioactivity over the thyroid was followed for 24 hr., at which time all animals were killed. Fig. 1 shows typical results: immediately after PTU administration a sharp increase in the slope of the thyroidal iodine-131 disappearance curve has been consistently observed. These results do not exclude, among several possible interpretations, that the change in rate was due exclusively to blocking of iodine-131 recirculation by PTU. This possibility was investigated in a group of rats receiving 200 mgm./rat/day potassium perchlorate in order to avoid recirculation of radioiodide and thus measure the rate of release of labelled compounds from the thyroid. Half the group received later 2 × 10 mgm. PTU intraperitoneally, all animals being killed after 24 hr. As may be seen in Fig. 2, an increase of the thyroid iodine-131 release curve is observed soon after onset of PTU treatment. Due to the fact that the potassium perchlorate dose used is more than enough to block recirculation of iodine-131, the finding is interpreted as indicating a 100 per cent increase in the rate of secretion of labelled compounds from the gland. The protein-bound iodine-131 of the plasma was the same for both experimental groups or somewhat higher for PTU-treated rats. The histological appearance

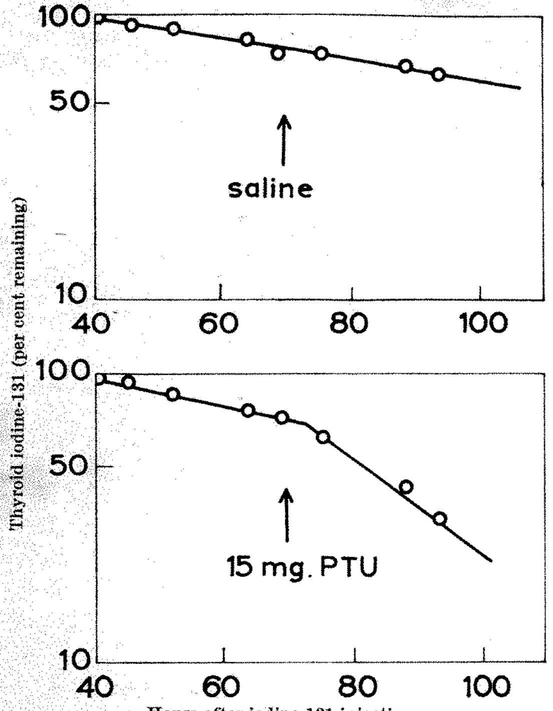


Fig. 1. Influence of 15 mgm. PTU injected intraperitoneally on the disappearance rate of iodine-131 from the thyroid, in male rats, injected with 10 μc. iodine-131. The radioactivity over the thyroid is expressed as percentage of the iodine-131 content 40 hr. after injection of the tracer; mean values of four animals in each group are given

of the thyroids of these PTU-treated animals showed clear signs of an increased activity when compared with their corresponding controls. The pooled plasmas of the animals the thyroidal data of which are shown in Fig. 2 were assayed for TSH activity by McKenzie's method[7]: the increase in blood radioactivity 2 hr. after intravenous injection of the sample is measured using mice injected with iodine-131, treated with thyroxine and thyroid powder to block endogenous TSH secretion. With 0·3 ml. of pooled plasma from the potassium perchlorate-treated controls the response was 119 ± 8 per cent (mean ± *S.E.*); with plasma from potassium perchlorate + PTU-treated animals it was 187 ± 15 per cent ($P < 0{\cdot}01$). Injection of saline gives a response of about 95 per cent.

Two pools were made with plasmas from 4 male rats and with those from another group of animals injected 24 hr. previously with 15 mgm. PTU and assayed for TSH activity: the responses were 100 ± 6 per cent and 158 ± 5 per cent for control and PTU-treated rats respectively, $P < 0{\cdot}001$. When 2 mgm. PTU were added per ml. of control plasma, the response was higher (130 ± 8 per cent) than that obtained without addition of the drug, though still significantly lower ($P = 0{\cdot}01$) than that given by the plasma of PTU-treated animals. When the acetic acid (0·25 per cent)–saline extracts of the pooled pituitaries of these animals were tested for TSH activity, it was found to be significantly lower for PTU-treated animals as compared with controls. This finding was confirmed with pituitary extracts from two other experiments carried out as just described.

The thyroid iodine-131 release curve of perchlorate-treated rats receiving daily 2 × 20 μgm. l-thyroxine to block endogenous TSH secretion was not altered by the administration of 2 × 10 mgm. PTU.

Since the hypotheses tested with the above experiments assumed that alterations in the peripheral metabolism of thyroxine induced by PTU might influence pituitary secretion of TSH, it was necessary to ascertain whether potassium perchlorate might be interfering with such extra-thyroidal effects of PTU or itself have an effect on peripheral thyroxine metabolism. On the basis of data reported by others[8] it seemed unlikely; however, the point was investigated in thyroidectomized, l-thyroxine-maintained rats, isotopically equilibrated with radiothyroxine as described elsewhere[6]. Administration of 200 mgm./rat/day of potassium perchlorate alone or together with 15 mgm. PTU showed that perchlorate neither inhibits the peripheral deiodination of thyroxine nor interferes with the depressing effect of PTU.

As referred to above, Griesbach and Purves[1] found that 0·25 per cent thiourea in drinking water did not affect thyroxine requirements of thyroidectomized rats for the maintenance of normal pituitary cytology. Using the same experimental procedure indicated above we found that such doses of thiourea did not result in peripheral alterations of l-thyroxine metabolism similar to those induced by PTU.

Some of the present findings are in good agreement with results recently reported by Mayberry and Astwood[9] while the present work was in progress: they found that the rate of disappearance of thyroid iodine-131 was greater for PTU-treated than for potassium perchlorate-treated rats. Moreover, a progressive disappearance of labelled thyroxine was observed from the hydrolysated thyroid of animals treated with PTU starting 24 hr. after injection of iodine-131. With either control, iodide- or perchlorate-treated animals, the proportion of thyroid radioactivity appearing as thyroxine remained unaltered during the seven-day observation period. This point is suggestive in view of the following hypotheses:

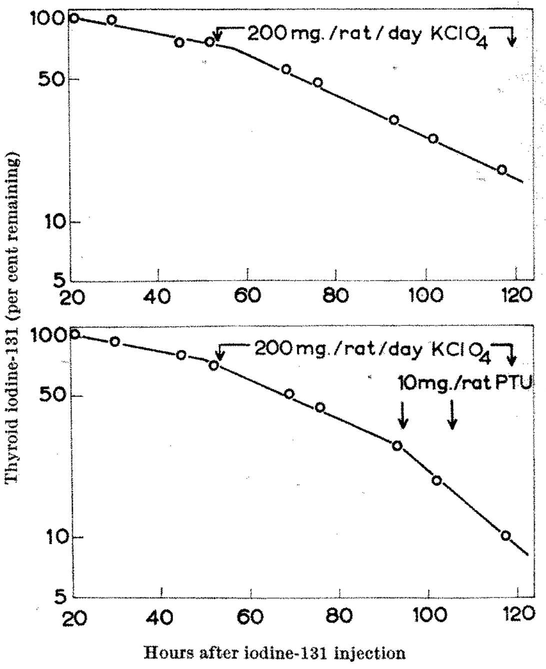


Fig. 2. Effect of PTU on the rate of release of iodine-131 labelled compounds from the thyroid of perchlorate-treated rats. Radioactivity over the thyroid is expressed as percentage of iodine-131 at 20 hr. after injection of 12 μc. Mean values of four animals in each group are given

We believe the present preliminary results may be tentatively interpreted as follows:

(*a*) At very early stages of treatment with PTU there is a considerable increase in the release of labelled compounds from the rat thyroid.

(*b*) Blocking of endogenous TSH secretion with thyroxine interferes with this effect of PTU.

(*c*) Within 24 hr. after onset of PTU treatment there is a higher thyrotrophic circulating activity than that corresponding to controls, as shown both by TSH bioassay of plasmas and thyroidal histology. The activity of pituitary TSH is lower, as shown by bioassay.

(*d*) The three points summarized above strongly suggest that PTU administration results very soon in an increased TSH secretion by the pituitary. Data reported by others[2,5,9] may be interpreted as leading to the same conclusion.

(*e*) It is further suggested that the rapid and intense alteration of the peripheral metabolism of thyroid hormone induced by PTU administration[6] plays an important part in this early response. Moreover, it might aggravate the effects of a low thyroxine pool in later stages of PTU treatment. The fact that blocking of endogenous TSH secretion abolished the effect of PTU makes a direct action of the drug on the pituitary or thyroid gland, not mediated via the thyroid hormone, rather unlikely. This finding does not disagree with the present interpretation: though PTU decreases the deiodination of such high doses of thyroxine to about half that of the corresponding controls (Escobar del Rey and Morreale de Escobar, unpublished results) the amount of thyroxine dehalogenated is still much higher than that corresponding to a physiological dose of the hormone. Neither do the findings of Griesbach and Purves[1] disagree with the present interpretation, since treatment with thiourea does not result in extrathyroidal alterations of thyroxine metabolism comparable to those exerted by PTU.

We should like to emphasize that the present interpretation is merely tentative and needs further experimental confirmation.

This work is supported by a research contract with the International Atomic Energy Agency, Vienna.

[1] Griesbach, W. E., and Purves, H. D., *Brit. J. Exp. Path.*, **26**, 13 (1945).
[2] Rawson, R. W., *Ciba Found. Coll. Endocrinol.*, **4**, 294 (1952).
[3] Albert, A., Rawson, R. W., Merrill, P., Lennon, B., and Riddell, C., *Endocrinol.*, **40**, 299 (1947).
[4] Albert, A., Rawson, R. W., Merrill, P., Lennon, B., and Riddell, C., *Endocrinol.*, **40**, 303 (1947).
[5] Van Middlesworth, L., Jagiello, G., and Vanderlaan, W. P., *Endocrinol.*, **64**, 186 (1959).
[6] Escobar del Rey, F., and Morreale de Escobar, G., *Endocrinol.* (in the press).
[7] McKenzie, J. M., *Endocrinol.*, **63**, 372 (1958).
[8] Jones, S. L., and Van Middlesworth, L., *Endocrinol.*, **67**, 855 (1960).
[9] Mayberry, W. E., and Astwood, E. B., *J. Biol. Chem.*, **235**, 2977 (1960).

TRANSFER FUNCTION OF A BIOLOGICAL PHOTORECEPTOR

By Dr. LAWRENCE STARK and Dr. HOWARD T. HERMANN

Neurology Section, Electronic Systems Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts

A FULL description of the function of a sensory organ or sensory element should include definition of the limits of signal transfer. This can be done if the stimulating energy is monitored, and, at the same time, the nervous impulses emanating from the sensory receptor are detected and studied. It is believed that the signal which represents the input stimulus and which is transmitted to other centres of the central nervous system is the average frequency of these nerve impulses. By correlating the instantaneous values of the stimulus and the nerve impulse signal, it is possible to make a rigorous statement regarding the transfer characteristics of the sensory receptor. The task then is to find the equation (generally an integro-differential equation) which characterizes the functional relationship between input and output[1–4].

To explore the problem, we have chosen to study the photosensitive tail ganglion of *Cambarus astacus*, the common crayfish[5–8]. Behaviour and reflex studies have demonstrated a physiological role for the receptor[9–10].

Through a window in the ventral abdominal exoskeleton, large electrodes were applied to the ventral nerve cord. The animal was kept in a humidity- and temperature-controlled environment (100 per cent saturation, 19° ± 0·5° C.). An electronically controlled light source was used to generate transient steps as well as sinusoidal variations of light[11]. Relatively small light fluctuations in a darkened environment permitted small signal linearization.

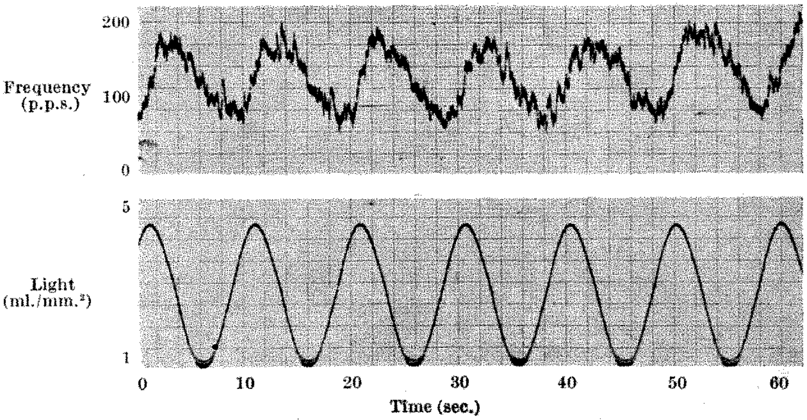


Fig. 1. Response of a photoreceptor to sinusoidal light changes. Note that the harmonic distortion is minimized by small signal approach. The 0·1 c.p.s. input produces an output with amplitude of ± 50 c.p.s., a mean of 130 p.p.s., and a phase-lag of 105°

The nerve impulses were averaged over a time period (*T.C.* = 0·24 sec.) long with respect to pulse-intervals, but short with respect to the response-frequency of the receptor. A combination of operational amplifiers, pulse-shaping circuits, and low-pass filters carried out the averaging. An amplitude window screened from the averaging process any pulses with heights greater or smaller than the medium-sized pulses which carry the light energy information[12].

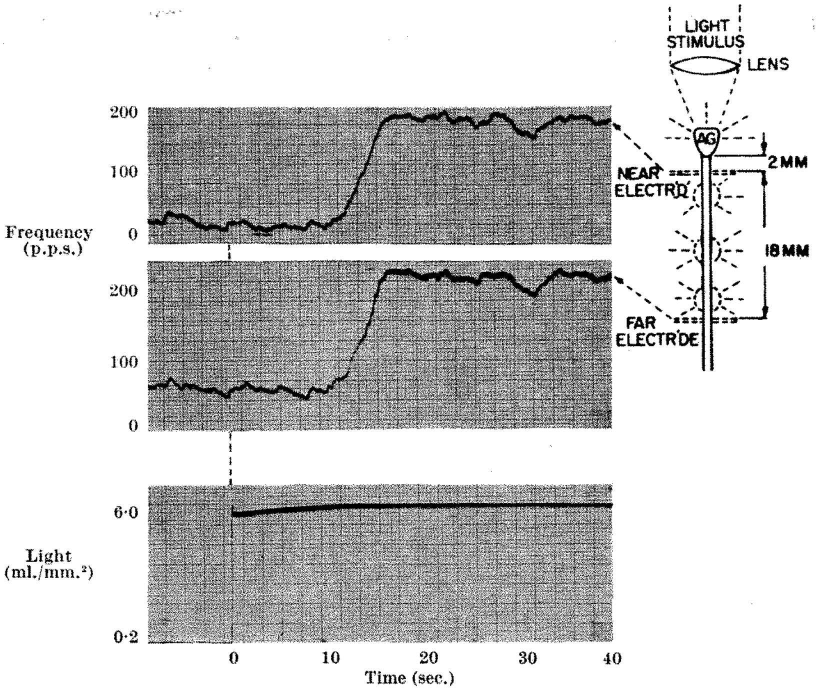


Fig. 3. Transient experiment. The lack of contribution of nerve conduction time to the non-minimum phase element (time delay) is shown. Thus, processes in the photosensitive ganglion are responsible for this operator. Conduction velocity is approximately 4 m./sec. It is interesting to note that details of the response are preserved over the 18 mm. conduction path, through three abdominal ganglia. This step of light was rather large, violating completely the theory of small perturbation analysis. Damping is inversely proportional to amplitude of forcing function

A response of the photoganglion to a sinusoidal stimulus is shown in Fig. 1. The noise seen comes in part from random groupings of pulses from different fibres. Harmonic distortion was minimized by the small-signal experimental conditions, but still is apparent here. The mean amplitude of response over a number of cycles and the mean phase relationship between stimulus and response were determined from such a recording. The parameter triplet—gain, phase and frequency—completely specifies a linear system.

The frequency response of the photosensitive ganglion was obtained by means of a series of experiments at different driving frequencies. Note in Fig. 2: the static or d.c. gain, the absence of adaptation, the break frequency, the attenuation slope, and the large phase lag, composed of both minimum and non-minimum elements. A transfer function:

$$G(s) = 32 \frac{e^{-s}}{(1 + 1{\cdot}2s)^2} \; p \text{ sec.}^{-1} \text{ mlm.}^{-1}$$

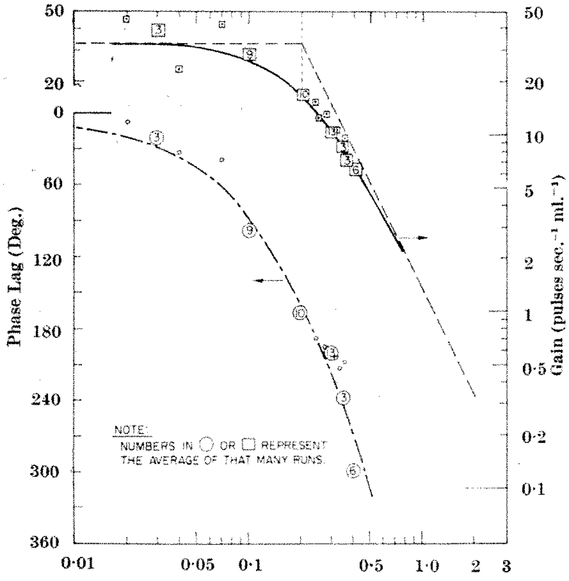


Fig. 2. Bode plot of photosensitive ganglion response. The solid curve represents the theoretical behaviour of a system described by the transfer function. The dashed lines are asymptotes showing the break frequency and attenuation slopes more clearly. The phase-lag curve is computed from the sum of minimum and non-minimum phase elements. The points are experimental

in Laplace operator ('s') notation can be quantitatively derived from this graphic display of system behaviour[3]. It is somewhat more convenient than the equivalent integro-differential equation:

$$32L(t - 1) = 1{\cdot}44 \frac{d^2P}{dt^2} + 2{\cdot}4 \frac{dP}{dt} + P$$

where L = light flux in millilumens, P = pulses per sec., t = time in sec.

The time-delay operator which accounts for the non-minimum phase lag is due, not to nerve conduction time, but rather to processes in the photosensitive ganglion, as reference to Fig. 3 demonstrates. Further investigations of non-linearities are proceeding; as can be seen in the large-signal step experiments, the law of superposition does not hold.

This research was supported by the Air Force (*AF*36(616)–7282, *AF*33(616)–7588), and in part by the Office of Naval Research (*Nonr*–609(39), *Nonr*–1841(70), the Army Chemical Corps (*DA* 18–108–405–Cml–942), and the U.S. Public Health Service (*B*–3055, *B*–3090).

[1] Pringle, J. W. S., and Wilson, V. J., *J. Exp. Biol.*, **29**, 220 (1952).
[2] Stark, L., and Sherman, P. M., *J. Neurophysiol.*, **20**, 17 (1957).
[3] Stark, L., *WADC Tech. Rep.*, 59 (August 1959).
[4] Hartline, H. K., *Amer. J. Physiol.*, **83**, 466 (1928).
[5] Prosser, C. L., *J. Cell. Comp. Physiol.*, **4**, 363 (1934).
[6] Welsh, J. H., *J. Cell. Comp. Physiol.*, **4**, 363 (1934).
[7] Kennedy, D., *Amer. J. Ophthalmol.*, **11**, 19 (1958).
[8] Wiersma, C. A. G., Ripley, S. H., and Christensen, E., *J. Cell. Comp. Physiol.*, **46**, 307 (1955).
[9] Kropp, B., and Enzmann, E. V., *J. Gen. Physiol.*, **16**, 905 (1933).
[10] Hermann, H. T., and Stark, L., *Lab. Prog. Rep. Res. Lab. Electronics, MIT*, No. 61, 230 (1961).
[11] Stark, L., *Proc. Inst. Rad. Eng.*, **47**, No. 11 (November 1959).
[12] Hermann, H. T., Stark, L., and Willis, P. A., "Instrumentation for Study of Nerve Impulse Codes", *Electronic Systems Lab., Tech. Note* (in preparation).

DISTRIBUTION OF SODIUM AND POTASSIUM IN CERTAIN CELLS AND TISSUES

By Dr. NORMAN R. JOSEPH, Dr. MILTON B. ENGEL and Dr. HUBERT R. CATCHPOLE

University of Illinois Professional Colleges, Departments of Chemistry, Orthodontics and Pathology, Chicago

IN primitive oceans, as in the waters of all other epochs, the concentration of sodium greatly exceeds that of potassium, approaching in sea-water of the present era a ratio of about 45 : 1. An excess also occurs in the blood serum of vertebrates, where the ratio is about 30 : 1. In many cells and tissues, however, the ratio is much lower, reaching states in which potassium concentration is higher than sodium concentration. One method of explaining this apparent lack of equilibrium attempts to relate concentration gradients to intracellular metabolic processes; various 'pumps' and 'ion-carriers' deriving energy from respiration have been postulated. This view seems to have been accepted by biologists for many years, and has tended to become the dominant theory[1-3].

In our work with connective tissues, in which respiration is conceded to be low, it was found that ion distributions could be described in terms of the densities of negative colloidal charge and the standard free energies of ion-exchange reactions[4]. Changes in the charge densities of tissue surfaces in relation to *p*H have been represented as titration curves of the surfaces[5-7]. Similarly, Ling, working with nerve and muscle, has supported an explanation of ionic gradients in terms of fixed charges, ionic radii, and dielectric constants[8,9].

Extension of titration studies to frog muscle showed that when respiration was lowered by cooling the tissue, there was no important change in the titration curve (hence, in ion distribution), but that application of respiratory inhibitors greatly altered the curve by lowering colloidal charge[10]. This points to a possible fallacy in all experiments where respiratory poisons have been used to support the argument that ion distribution is determined by respiration rather than by colloidal state. Respiratory inhibitors such as fluoride and dinitrophenol are nucleophilic anions which are bound to amphoteric colloids (including respiratory enzymes), changing colloidal charge and ion distribution. In cooling experiments the effects of temperature and of anions are clearly separable. In attempts to demonstrate the effect of respiration on ion distribution, the possible simultaneous effects of inhibitors on the insoluble structural colloids have been considered only rarely[10].

Changes in Free Energy in Heterogeneous Systems

Cells and tissues are heterogeneous systems[11-14]. It is assumed therefore that they are composed of a limited number of phases, including colloid-rich and water-rich phases which are heterogeneous at sub-microscopic levels, and which are in equilibrium with each other and with blood.

In the theoretical treatment to follow, the distribution of ions will be considered between blood serum, to be denoted phase 1, and a cellular or tissue phase, to be denoted phase 2. In accordance with the above definition, the properties of phase 2 should approach those of a heterogeneous aggregate rather than those of an isolable homogeneous phase. The following formulations apply to the equilibrium of sodium, potassium and chloride, but may be extended to include other mobile anions and cations. The theoretical method differs from that of Donnan[15] by introducing standard potentials of the ions in the two phases. When, for all the ions, the standard potentials are constant throughout the heterogeneous system, the resulting equilibrium conditions are those of the classical Donnan equilibrium. When the standard chemical potentials vary in different phases, the distribution ratios for sodium and potassium may be quite unequal at equilibrium. For the equilibrium of sodium and chloride in two phases:

$$\mathrm{Na}' + \mathrm{Cl}' = \mathrm{Na}'' + \mathrm{Cl}'' \tag{1}$$

Table 1. Values of Q_3 and $-\Delta F_3^\circ$ for Exchange Reactions $\mathrm{K}' + \mathrm{Na}'' = \mathrm{Na}' + \mathrm{K}''$ ($\Delta F_3^\circ = -RT \ln Q_3$)

State of aggregation	Low (or zero) metabolism (closed systems)				Moderate or high metabolism (open systems)			
	System	Q_3	$-\Delta F_3^\circ$ (kcal.)	Ref.	System	Q_3	$-\Delta F_3^\circ$ (kcal.)	Ref.
Solution	Œdema fluid *a*	0·75	−0·17	19				
	Synovial fluid *b*	0·8	−0·13	19				
	Ascitic fluid *a*	1·0	0·0	19				
	Aqueous humour *c*	1·2	0·10	20				
	Vitreous humour *b*	1·5	0·24	20				
	Peritoneal fluid *a*	0·85	−0·08	20				
	Thoracic lymph *a*	0·95	−0·03	20				
	Blood dialysate *d*	0·8	−0·13	19				
Solid or semi-solid	Zeolite		0·21	16	Peripheral nerve *c*	5·5	1·0	20
	Zeolite		1·0	17	Kidney *a*	18	1·75	20
	Dental enamel *a*	3·6	0·8	20	Heart muscle *g*	60	2·5	20
	Dentine *a*	5·1	1·0	20	(A-V bundle) *h*	17	1·7	27
	Bone *a*	2·2	0·5	20	(Rt. ventricle) *h*	57	2·5	27
	Erythrocyte *a*	210	3·2	21	Whole brain *d*	58	2·5	20
	Erythrocyte *c*	260	3·4	21	Whole brain *g*	67	2·6	20
	Erythrocyte *d*	2·2	0·5	21	Whole brain *f*	49	2·4	20
	Leucocyte *c*	48	2·4	22	Whole brain *i*	52	2·4	20
	Ascites cells *e*	81	2·7	23	Skeletal muscle *j*	111	2·9	28
	Whole skin *a*	5·0	1·0	24	Uterus, muscle *i*	29	2·1	29
	Epidermis *a*	45	2·3	25	Liver *i*	85	2·7	20
	Epidermis *e*	36	2·2	26	Giant axon *k*	90	2·7	30
	Cartilage *f*	7·5	1·25	4				
	Lens *a*	33	2·1	20				
	Lens *b*	159	3·1	20				
	Lens *f*	230	3·3	20				

a, Human; *b*, cattle; *c*, horse; *d*, dog; *e*, mouse; *f*, rabbit; *g*, guinea pig; *h*, ox; *i*, cat; *j*, rat; *k*, squid.

where Na′ and Cl′ refer to phase 1, and Na″ and Cl″ refer to phase 2. Thermodynamic equilibrium is determined by the standard free-energy change, $\Delta F_1{}^0$, of reaction 1. A similar reaction describes the equilibrium of potassium and chloride:

$$K' + Cl' = K'' + Cl'' \qquad (2)$$

Equilibrium in the system depends on six chemical potentials, one for each of the three ions, K, Na, and Cl, in each of the two phases. The chemical potential, $\bar{F}_{K'}$, of K′ in phase 1, is given by:

$$\bar{F}_{K'} = (\bar{F}_K{}^0)' + RT \ln (K') \qquad (3)$$

where $(\bar{F}_K{}^0)'$ and (K′) are, respectively, the standard potential and concentration of the ion. (If equilibrium be considered for normal physiological states, it is convenient to define the standard chemical potential of an ion in any phase by equation 3. The activity coefficient referred to some other standard state (infinite dilution in water, for example) would be included in the standard chemical potential of the ion in the given phase. For such a 'physiological standard state' activity coefficients of sodium, potassium, and chloride would be 1·0 by definition, whereas the standard chemical potentials would be defined in terms of the ion concentrations at equilibrium.) Five additional equations of the same form are required for K″, Na′, Na″, Cl′ and Cl″. These yield the following expressions for ΔF_1 and ΔF_2, the free-energy changes of reactions 1 and 2:

$$\Delta F_1 = \Delta F_1{}^0 + RT \ln \frac{(Na'') \times (Cl'')}{(Na') \times (Cl')} \qquad (4)$$

and:

$$\Delta F_2 = \Delta F_2{}^0 + RT \ln \frac{(K'') \times (Cl'')}{(K') \times (Cl')} \qquad (5)$$

where $\Delta F_1{}^0$ and $\Delta F_2{}^0$ are the standard free-energy changes. By combining equations 4 and 5, and eliminating the chloride ion:

$$\Delta F_3 = \Delta F_3{}^0 + RT \ln \frac{(K'') \times (Na')}{(K') \times (Na'')} \qquad (6)$$

where ΔF_3 and $\Delta F_3{}^0$ refer to the reaction:

$$K' + Na'' = K'' + Na' \qquad (7)$$

The free-energy changes are related as follows:

$$\Delta F_3 = \Delta F_2 - \Delta F_1 \qquad (8)$$

$$\Delta F_3{}^0 = \Delta F_2{}^0 - \Delta F_1{}^0 \qquad (9)$$

The equilibrium conditions require that ΔF_1, ΔF_2 and ΔF_3 be zero. Then:

$$\Delta F_1{}^0 = - RT \ln Q_1 \qquad (10)$$

with two additional equations of the same form relating $\Delta F_2{}^0$ to Q_2, and $\Delta F_3{}^0$ to Q_3. The mass-law constants Q_1, Q_2 and Q_3 are defined by the ion ratios of equations 4, 5 and 6. They are therefore related by the formula:

$$Q_2 = Q_1 \times Q_3$$

Sodium–potassium equilibrium is expressed by Q_3:

$$Q_3 = \frac{(K'') \times (Na')}{(K') \times (Na'')}$$

When the standard free-energy change of reaction 7 is zero, $Q_3 = 1{\cdot}0$. When $\Delta F_1{}^0$ and $\Delta F_2{}^0$ are zero, Q_1 and Q_2 are also equal to 1·0. In this situation equilibrium is described by the classical Donnan conditions[15]:

$$\frac{(K'')}{(K')} = \frac{(Na'')}{(Na')} = \frac{(Cl')}{(Cl'')}$$

Otherwise the ratios Q_1, Q_2 and Q_3 depend on the three standard free-energy changes according to equation 10. From the four equations for the chemical potentials of K′, K″, Na′ and Na″, one obtains:

$$\Delta F_3{}^0 = \Delta \bar{F}_K{}^0 - \Delta \bar{F}_{Na}{}^0$$

The distribution of ions is therefore determined by the four standard potentials of the two ions in the two phases. The distribution of chloride is determined by the values of Q_1 and Q_2 (or by $\Delta F_1{}^0$ and $\Delta F_2{}^0$). Any two values of Q_1, Q_2 or Q_3 are sufficient to determine the third. The equilibrium is then described by any two of the mass-action constants or standard free-energy changes, and by the colloidal charge density, which is usually negative[4,6]. Electrostatic equilibrium depends on the negative colloidal charge, x, which is balanced by mobile ions:

$$(Na'') + (K'') = (Cl'')\,) + x$$

where it is assumed that other mobile ions are present in only small quantities. In the following discussion, sodium–potassium equilibrium is expressed independently of other ions in terms of calculated values of Q_3 and $\Delta F_3{}^0$. These values express 'potassium selectivity' according to the mass-action law. For any values of Q_3 and either Q_1 or Q_2, the equilibrium is then determined by the colloidal charge, x. These three quantities are sufficient to determine K″, Na″, and Cl″ from K′, Na′ and Cl′. In any heterogeneous system, therefore, equilibrium between blood serum and other phases depends on the colloidal charge of the given phase and the standard free-energy changes of ion-exchange reactions[4,6].

Values of Q_3 and $\Delta F_3{}^0$ for a large number of systems are given (Table 1). These show a number of standard free-energy changes of 0·1 kcal. or less, indicating values of Q_3 near 1·0 and an approach to the ideal Donnan distribution of ions. This occurs in all systems in which the colloids are in aqueous solution. When water is the dispersion medium in both phases, $\Delta \bar{F}_K{}^0$ and $\Delta \bar{F}_{Na}{}^0$, the standard changes of chemical potential of potassium and sodium, tend to approach zero. For solid or semi-solid states of aggregation, on the other hand, $\Delta F_3{}^0$ is negative, Q_3 is greater than 1·0, and potassium ions tend to accumulate in the colloidal phase. Increasing negative values of $\Delta F_3{}^0$ indicate increasing accumulation of potassium ions in phase 2. This occurs not only in cells and tissues (Table 1) but also in solid structures such as zeolites[16,17].

Effects of Ionic Size and Hydration

In all systems the tendency of potassium to displace sodium may be at least partly explained by the relative effects of the ions on the diffuse double layer at the colloidal interface[16,18]. (The total surface area of the interface between the colloid-rich and water-rich phases of ground substance of connective tissue is of a high order of magnitude. Assuming most of the tissue water to be contained in the system of vacuoles described by Gersh *et al.*[12], the total surface area of the vacuoles would be about 60 m.^{2} per cubic ml. of tissue water[31]. This estimate is based on diameters of 1000 Å. for the vacuoles and represents only extracellular active surface. Intracellular surface areas are probably of a similar high order of magnitude.) These effects are related to the 'average effective diameters' of the ions and to the dielectric constant of the solvent. The theory of Debye and Hückel yields the following expression for the electrostatic potential ψ of a univalent cation with unit charge, ε:

$$\psi = \psi^0 - \frac{\varepsilon\varkappa}{D(1 + \varkappa a)}$$

where a is the 'average effective diameter' of the ions in an electrolyte solution; $\varkappa$ is proportional to the square root of the ionic strength, and is the reciprocal of the thickness of the ionic atmosphere in centimetres; D is the dielectric constant; ψ^0 is the potential at the surface of the ion in a solution at zero ionic strength. For a univalent ion of radius b:

$$\psi^0 = \frac{\varepsilon}{Db}$$

The standard chemical potentials of ions, $\bar{F}_K{}^0$ and $\bar{F}_{Na}{}^0$, depend on values of b, while the activity coefficients depend on a. Values of a and b for potassium and sodium in water are given in Table 2, with the free energies of hydration, which depend on b. In this treatment, ionic size terms are considered in relation to thermodynamic functions which determine equilibrium. These terms may also be related to rates of diffusion and exchange, as studied by Ling[9] and by Simon[13].

For the exchange reaction:

$$K(H_2O) + Na'' = K'' + Na(H_2O)$$

the standard free-energy change is − 16·2 kcal. (Table 2). This represents a theoretical limit for exchange reactions involving hydrated ions in an aqueous phase and a co-existent phase of low dielectric constant, in which some of the ions are not hydrated.

Table 2. FREE ENERGIES OF HYDRATION OF SODIUM AND POTASSIUM (ref. 16)

Ion	Ionic radius (Å.) (crystal.) b	Ionic diameter (Å.) (av. effective) a	$-\Delta F^0$ (hydr.) (kcal.)
Na	0·98	5·2	89·7
K	1·33	4·1	73·5
Difference	—	—	16·2

Relation of Hydration to Entropy

Because of the lower hydration energy of potassium ions, the fraction of unhydrated ions in any phase would be much greater than for sodium. For this reason it would be possible for potassium to participate more readily in short-range electrostatic interactions in media of low dielectric constant. This implies a greater number of possible states of potassium ions than of sodium ions in the colloidal phase. The entropy of physicochemical systems increases with statistical probabilities of given states; for systems in which sodium and potassium are free to exchange, the negative free-energy change, ΔF_3, occurring for any spontaneous process, would be associated with a corresponding positive entropy change, ΔS_3. Because the free energy of hydration of sodium is greater than that of potassium, the standard entropy change, $\Delta S_3{}^0$, of reaction 7 would be positive. The reaction tends toward an equilibrium which yields the maximal number of ionic states, including all the interactive forms of unhydrated ions in the colloid-rich phase. This implies for all possible distributions of sodium and potassium an equilibrium state in which free energy is minimal and entropy maximal. The temperature coefficient of the ion distribution ratio, Q_3, and the temperature coefficient of the standard free-energy change depend on $\Delta S_3{}^0$. In particular:

$$\frac{\partial \Delta F_3{}^0}{\partial T} = -\Delta S_3{}^0$$

Since in general $\Delta S_3{}^0$ would be positive, $\Delta F_3{}^0$ would become increasingly negative as the temperature increases. If Q_3 is increased the equilibrium would shift towards potassium accumulation in the colloidal phase. When this is found experimentally, the usual interpretation has been to attribute the increase of potassium to increased metabolic rates. The effect of temperature on the distribution of potassium in erythrocytes has been interpreted in this way[2]. This ignores the possibility that the effect depends on the free energies of hydration of the ions. Potassium, having the lower free energy of hydration, has a greater tendency than sodium to form unhydrated ions in a colloid-rich phase; it therefore has more freedom to neutralize fixed charges[9,13]. The presence of hydrophobic charged colloids decreases the number of hydrated ions in any system and increases the entropies of all ions of the colloid-rich phase. This effect is selective for potassium. In all systems for which this is true, $\Delta S_3{}^0$ of reaction 7 would be positive, and $\Delta F_3{}^0$ negative.

Standard Free Energy and Ion Concentration Gradients

The values of $\Delta F_3{}^0$ in Table 1 range from 0·17 kcal. to about − 3 kcal. Values near zero indicate an approach to the ideal Donnan distribution, which occurs in body fluids with colloids in solution. For solid or semi-solid states of aggregation, the values range from about − 0·2 kcal. (zeolite or femur) up to − 3 kcal. High negative values are found for erythrocytes, ascites cells, leucocytes, epidermis and lens. The most actively metabolizing tissues such as muscle and nerve show standard free-energy changes in the same range (− 2 to − 3 kcal.). Changes of sodium and potassium distribution which occur during physiological activity may depend on small transitory changes of the colloids following stimulation, as observed by light-scattering[32]. Changes of $\Delta F_3{}^0$ of a few tenths kcal. would produce rather large shifts of sodium and potassium independently of mechanisms of ion-transport. Values of $\Delta F_3{}^0$ in all systems depend on states of aggregation of cells and tissues and on the nature of the colloidal components such as proteins, glycoproteins, phospholipids and nucleoproteins.

Accordingly, it is unnecessary to invoke metabolic energy to explain steady concentration gradients in tissues. Distribution of anions and cations in steady states depends only on the colloidal charge and on the standard free energies of ion-exchange reactions[4,6]. Homeostasis within heterogeneous systems of cells and tissues depends on the maintenance of constant chemical potentials of water and electrolytes[4,14]. Examples of analogous concentration gradients at minimal free energy are very numerous, and would include the distributions of surface-active substances according to their adsorption isotherms, the distribution of gases in the gravitational field of the Earth according to molecular weights (law of atmospheres), and the distribution of ions in immiscible solvents. Concentration gradients in tissues depend on the standard potentials of the ions in different phases. These values depend on dielectric constants and on the ionic radii (b values). The presence of fixed negative colloidal charges establishes electrostatic conditions for equilibrium, requiring an excess of mobile cations over mobile anions for electrical neutrality. The ratio of potassium to sodium at any charged interface depends on the 'average effective diameters' (*a* values). The distribution of cations depends on the distribution of colloidal charge, on the dielectric constants of various phases, and on hydration energies and sizes of the ions. Redistribution of ions during metabolic changes would depend on changes of colloidal charge and states of aggregation, on changes of concentration of various metabolites (lactic acid, carbon dioxide, di- and tri-carboxylic acids), and on redistributions of water accompanying these changes[31]. According to Donnan's treatment, ionic equilibrium is determined by the condition of minimal free energy. This condition is of general validity for cells and extracellular substances, but the standard chemical potentials of mobile ions in different phases of the heterogeneous system must be included in the formulation.

This work was supported in part by grants from the U.S. Public Health Service (*A*–968–*c* and *D*–1147).

[1] Murphy, Q. R., ed., *Metabolic Aspects of Transport across Cell Membranes* (Univ. of Wisconsin, 1957).

[2] Harris, E. J., *Transport and Accumulation in Biological Systems* (Butterworth, London, 1960).

[3] Ungar, G., *J. de Physiol.*, **49**, 1235 (1957).

[4] Joseph, N. R., Engel, M. B., and Catchpole, H. R., *Amer. Med. Assoc. Arch. Path.*, **58**, 40 (1954).

[5] Joseph, N. R., and Engel, M. B., *Arch. Biochem. Biophys.*, **85**, 209 (1959).

[6] Joseph, N. R., Catchpole, H. R., Laskin, D. M., and Engel, M. B., *Arch. Biochem. Biophys.*, **84**, 224 (1959).

[7] Engel, M. B., and Joseph, N. R., *Arch. Biochem. Biophys.*, **86**, 117 (1960).

[8] Ling, G., *Amer. J. Physiol.*, **167**, 806 (1951).

[9] Ling, G., *J. Gen. Physiol.*, **43**, 149 (1960).

[10] Engel, M. B., Catchpole, H. R., and Joseph, N. R., *Science*, **132**, 669 (1960).

[11] Thompson, D'Arcy W., *On Growth and Form* (Cambridge, 1945).

[12] Gersh, I., Isenberg, I., Bondareff, W., and Stevenson, J. L., *Anat. Rec.*, **128**, 149 (1957).

[13] Simon, S., *Nature*, **184**, 1978 (1959).

[14] Engel, M. B., Joseph, N. R., Catchpole, H. R., and Laskin, D. M., *Ann. N.Y. Acad. Sci.*, **85**, 399 (1960).

[15] Donnan, F. G., *Z. Elektrochem.*, **17**, 572 (1911).

[16] Boyce, G. E., Schubert, J., and Adamson, A. W., *J. Amer. Chem. Soc.*, **69**, 2828 (1947).

[17] Jenny, H., *J. Phys. Chem.*, **36**, 2217 (1932).

[18] Gouy, G. J., *J. Phys.*, **9**, 457 (1910).

[19] Ropes, M. W., and Bauer, W., *Synovial Fluid Changes in Joint Disease* (Harvard, Cambridge, 1953).

[20] Spector, I., ed., *Handbook of Biological Data* (Saunders, Philadelphia, 1956).

[21] Bernstein, R. E., *Science*, **120**, 459 (1954).

[22] Endres, G., and Herget, L., *Z. Biol.*, **88**, 451 (1929).

[23] Hempling, H. G., *J. Gen. Physiol.*, **41**, 565 (1957).

[24] Eisele, C. W., and Eichelberger, L., *Proc. Soc. Exp. Biol. Med.*, **58**, 97 (1945).

[25] Suntzeff, V., and Carruthers, C., *J. Biol. Chem.*, **160**, 567 (1945).

[26] Carruthers, C., and Suntzeff, V., *Cancer Res.*, **3**, 744 (1943).

[27] Davies, F., Davies, R. E., Francis, E., and Whittem, R., *J. Physiol.*, **118**, 276 (1952).

[28] Conway, E. J., and Hingerty, D., *Biochem. J.*, **40**, 561 (1946).

[29] Daniel, E., and Singh, M., *Canad. J. Biochem. Physiol.*, **36**, 959 (1958).

[30] Keynes, R. D., and Lewis, P. R., *J. Physiol.*, **114**, 151 (1951).

[31] Engel, M. B., Joseph, N. R., Laskin, D. M., and Catchpole, H. R., *Amer. J. Physiol.* (in the press).

[32] Tobias, J. M., *Modern Trends in Physiology and Biochemistry*, 291 (E. S. G. Barron, Academic Press, New York, 1952).

DISTRIBUTION OF HUMAN CHROMOSOME COUNTS IN RELATION TO AGE

By PATRICIA A. JACOBS and W. M. COURT BROWN, O.B.E.

Medical Research Council Clinical Effects of Radiation Research Unit, Western General Hospital, Edinburgh

AND

DR. RICHARD DOLL

Medical Research Council Statistical Research Unit, London School of Hygiene and Tropical Medicine, Gower Street, W.C.1

AN investigation has been made of the distribution of chromosome counts in leucocytes from blood cultures, prepared by the technique of Moorhead *et al.*[1], with the final spreading of the chromosomes achieved by drying in air. Ninety-seven people (50 males and 47 females) have been examined who, on chromosome analysis, appeared to have a normal karyotype. These subjects ranged in age from 5 months to 82 years, and were drawn from volunteers, hospital patients and the parents of patients with chromosome abnormalities. No subject had received X-ray treatment, and none was known to have a malignant tumour. While it would have been preferable for the subjects to have been a sample of the general population, there is no reason for believing that the composition of the present group has introduced any serious bias into the findings.

Two other groups were also studied. One consisted of 24 phenotypic males, all with a diploid number of 47 and a complement of *XXY* sex chromosomes. These patients ranged in age from a new-born child, to a man of 68 years. The other group consisted of 42 mongol imbeciles (26 males and 16 females), ranging in age from 3 to 51 years, and all shown to be trisomic for a small acrocentric autosome, presumed to be No. 21 (Denver Classification). There was no evidence that any patient with a chromosome abnormality was a chromosome mosaic.

For the 97 subjects with a normal karyotype, the distribution of chromosome counts has been examined in eight age-groups (Table 1). For the abnormal subjects, three age-groups were studied in the *XXY* males, and four in the mongol imbeciles (Table 2). An average of 36 cells was counted for each subject with a normal karyotype, 30 for each *XXY* male, and 32 for each mongol imbecile. Less than 25 cells were scored in only 4 normals, 2 *XXY* males and 2 mongol imbeciles. Variations from subject to subject in the number of cells scored were allowed for in calculating the average age of each age-group, by weighting the age for the number of cells counted.

The chromosome preparations were scanned under low-power for dividing cells in which the chromosomes were well spread. These were then examined under an oil-immersion lens to decide whether they were suitable for counting. Three criteria had to be satisfied. First, the cell must not be obviously broken; secondly, there must be no stray chromosomes in the same optical field; thirdly, the fixation and spreading of the chromosomes must be such that there is no ambiguity in interpretation for the purposes of counting. If any one of these criteria was not

Table 1. DISTRIBUTION OF CHROMOSOME COUNTS IN CELLS OF PERSONS WITH A NORMAL KARYOTYPE

Age-group (yr.)	No. of subjects	Mean age of subjects* (yr.)	Total No. of cells counted	No. of cells with chromosome counts of:								Hypomodal counts as percentage of modal counts	Hypermodal counts as percentage of modal counts
				< 43	43	44	45	46	47	48	> 48		
0–4	8	1·49	222	1	0	2	9	209	1	0	0	5·74	0·48
5–14	8	9·78	276	0	0	0	8	265	3	0	0	3·02	1·13
15–24	26	21·53	1,087	2	2	4	36	1,036	6	1	0	4·25	0·68
25–34	16	28·91	626	0	3	2	19	595	6	1	0	4·03	1·18
35–44	14	38·23	450	0	0	1	20	420	9	0	0	5·00	2·14
45–54	6	49·09	276	1	0	0	17	254	4	0	0	7·09	1·57
55–64	9	57·77	271	0	3	0	13	251	2	2	0	6·37	1·59
65 or more	10	72·18	320	1	2	5	19	283	10	0	0	9·54	3·53
All ages	97	34·42	3,528	5	10	14	141	3,313	41	4	0	5·13	1·36

* Weighted for the number of cells counted at each age.

satisfied, the cell was discarded; once the chromosomes had been counted, however, the count was always included.

Not all cells that satisfy these criteria can be analysed precisely; in particular, full analysis is often impracticable if there is excessive contraction of the chromosomes, although an accurate count is still possible.

In all, there were 66 cells with hypermodal counts (Tables 1 and 2), including 45 from persons with a normal karyotype. Of the 66 cells, 6 could not be analysed because of excessive contraction of the chromosomes, and in 5 other cells the distribution of the chromosomes between the various sub-groups recognized in the Denver classification was grossly abnormal. Among the remaining 55 cells, there were 38 from persons with normal karyotypes, with a total of 43 additional chromosomes. After two telocentric chromosomes were excluded, it was found that there was a slight excess of chromosomes in the 13–22 range of the Denver classification (24 against 18·39 expected); but this excess was not statistically significant.

Occasionally chromatid fragments, chromosome fragments and telocentric chromosomes were found. Chromatid fragments were always in proximity to their parent chromosomes, and, therefore, were assumed to have arisen from breakage of a chromatid during the preparation of the slide. They were not scored as separate bodies. Chromosome fragments were of two types depending on whether or not the fragment lay close to the parent chromosome. In the latter instance, analysis of the cell usually revealed the chromosome from which the fragment was derived, and neither type was scored as a separate chromosome.

Telocentric chromosomes do not occur in the normal human karyotype, and their presence in a cell is taken to indicate the breakage of a chromosome transversely through its centromere. They were uncommon in our preparations, and, when present, were almost always in pairs. Analysis of such cells always showed a metacentric or a sub-metacentric chromosome to be missing, the breakage of which is presumed to have given rise to the two telocentrics. A cell with two telocentric chromosomes and a count of 47 bodies was therefore recorded as having 46 chromosomes. Very occasionally a single telocentric was present, and our present practice is to score this as a chromosome.

In Tables 1 and 2, the numbers of cells in persons in each age-group with counts (*a*) greater than the modal number, and (*b*) less than the modal number, have been expressed as percentages of the number of cells with the modal number of chromosomes. The rates of change with age are indicated by the coefficients of x in the following regression formulæ:

For the proportion of hypermodal cells in subjects with a normal karyotype:

$$y = 0{\cdot}034x + 0{\cdot}369 \qquad (1)$$

For the proportion of hypomodal cells in subjects with a normal karyotype:

$$y = 0{\cdot}066x + 3{\cdot}384 \qquad (2)$$

For the proportion of hypermodal cells in subjects with an abnormal karyotype:

$$y = 0{\cdot}057x - 0{\cdot}229 \qquad (3)$$

For the proportion of hypomodal cells in subjects with an abnormal karyotype:

$$y = 0{\cdot}097x + 3{\cdot}550 \qquad (4)$$

Three of the regression coefficients are significantly different from zero: for (1), $P < 0{\cdot}01$; (2), $P = 0{\cdot}03$; and (4), $P = 0{\cdot}01$. The remaining coefficient (3) is not significantly different from zero ($P = 0{\cdot}07$).

An analysis of variance for all the data shows that the differences between the regression coefficients, considered as a group, can reasonably be attributed to chance ($P = 0{\cdot}2$), and the same result is obtained by examining separately the differences between the regression coefficients for the hypermodal and

Table 2. DISTRIBUTION OF CHROMOSOME COUNTS AMONG THE CELLS OF PATIENTS WITH KLINEFELTER'S SYNDROME OR MONGOLISM

Diagnosis	Age-group (yr.)	No. of subjects	Mean age of subjects* (yr.)	Total No. of cells counted	No. of cells with chromosome counts of:								Hypomodal counts as percentage of modal counts	Hypermodal counts as percentage of modal counts
					< 44	44	45	46	47	48	49	> 49		
Klinefelter's syndrome	0–24	9	12·16	279	0	0	3	10	266	0	0	0	4·89	0·00
	25–34	8	28·91	237	2	0	4	9	219	3	0	0	6·85	1·36
	35 or more	7	47·59	206	0	0	3	10	187	4	1	1	6·95	3·21
	All ages	24	27·77	722	2	0	10	29	672	7	1	1	6·10	1·34
Mongolism	0–14	12	9·69	393	1	0	2	11	376	3	0	0	3·72	0·80
	15–24	13	16·75	396	2	0	6	12	374	2	0	0	5·35	0·53
	25–34	6	29·95	232	0	0	2	12	213	4	1	0	6·57	2·35
	35 or more	11	40·24	331	0	2	8	16	303	2	0	0	8·58	0·66
	All ages	42	22·74	1,352	3	2	18	51	1,266	11	1	0	5·85	0·95

* Weighted for the number of cells counted at each age.

hypomodal counts, and for the normal and abnormal karyotypes.

It may be noted, however, that the rate of increase with age is greater for the hypomodal counts than for the hypermodal, and for the abnormal karyotypes than for the normal. The present data are not numerous, and the fact that the differences between the regression coefficients are not statistically significant does not necessarily mean that they are not real.

When plotted, the regression lines for the proportion of hypermodal cells (equations 1 and 3) pass close to the origin; in neither case is the regression constant significantly different from zero, and it would appear that at birth hypermodal cells must be rare. In contrast, the constants in the regression formulæ for the proportion of hypomodal cells (equations 2 and 4) are both significantly different from zero (in each case, $P < 0{\cdot}01$). The two estimates of the proportion of hypomodal cells at 0 years of age are similar to one another (3·384 and 3·550 per cent, respectively), and it is reasonable to assume that, with the present technique, the ratio of hypomodal to modal cells at birth would be about 3·5 per cent.

It has been assumed in the above analysis that the data are best fitted by straight lines. In the present limited state of knowledge this seems reasonable; in only one case (hypomodal cells in persons with a normal karyotype) is there statistical evidence of a significantly better fit with a quadratic curve.

One explanation for these findings could be that all non-modal cells are artefacts arising during preparation. A study of the hypermodal cells, however, provides evidence that this is not so. The type of artefact which will give rise to non-modal counts is rupture of a cell with the loss of one or more chromosomes; if these lost chromosomes become included in a neighbouring cell, a hypermodal count can be obtained. Usually, however, a true hypermodal cell can be distinguished from one which has gained a chromosome from a neighbouring cell. Such a 'foreign' chromosome can be recognized, because the degree of contraction of the chromosomes in a preparation tends to differ between cells, as the cells will be in different stages of their division cycle when colchicine is added to the culture medium. A further type of error which can give rise to a count of 47 is the presence of a single telocentric chromosome. As noted, our present practice is to record such cells as having a genuine count of 47 chromosomes. This may be wrong, but the number of cells involved is very small.

The great majority of the cells with hypermodal counts are therefore regarded as the products of errors in division. One possible mechanism by which these cells could be produced is somatic non-disjunction in a normal diploid cell. There will, of course, be an equal number of cells formed with counts of 45 chromosomes, and, if viable, these will be represented among the hypomodal cells. Hypomodal cells are also likely to be produced by anaphase lagging. The analysis of hypomodal cells, however, cannot distinguish between those that result from artefacts and those that are the products of errors in division. That the latter do occur can be inferred only from the study of the hypermodal cells. It follows, in our view, that the presence of aneuploid cells cannot be accounted for wholly on the basis of artefacts, nor can they all be regarded as genuine aberrations. It is suggested that the figure of 3·5 per cent hypomodal cells at birth, calculated from the regression formulæ, may provide an approximate estimate of the proportion of aneuploid cells arising from artefacts with the present technique.

Several biological mechanisms could be postulated to account for these results. One is that there is an increase in the frequency of abnormal divisions with age. These abnormal divisions may have occurred in ancestral cells, so that the cells which divide in culture to produce abnormal daughter cells were themselves abnormal when taken from the body. Another mechanism could be that the rate of abnormal division is a constant; but that some of the abnormal daughter cells are viable and maintain themselves in equilibrium with the normal cells. Lastly, it may be that the cells were normal when taken from the body and that abnormal division is related to the conditions of culture. In either case there exists some age factor which leads to the presence in the cultures of more abnormal cells in an older subject than in a younger subject.

[1] Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Exp. Cell. Res.*, **20**, 613 (1960).

RECOMBINATION IN THE *X*-CHROMOSOME OF *DROSOPHILA MELANOGASTER*

By C. N. LAW*

Department of Agricultural Botany, University College of Wales, Aberystwyth

PREVIOUS work on *Drosophila* species has indicated that inter-chromosomal effects and genetic differences between the homologous chromosomes being studied influence recombination appreciably[1,2]. So far, few attempts have been made to determine whether or not interactions are occurring at the intra-chromosomal level. Recently, an experiment using different wild-type iso-alleles at the white-eye locus (*w*) has suggested that the presence of different alleles of major genes may influence recombination in their own vicinity[3]. In view of this, experiments have been carried out to investigate more closely the inter-relations between genes and regions within a chromosome.

Recombination was studied in an *X* chromosome, carrying the genes, yellow Hairy-wing (*yHw*), vermilion eye (*v*) and Bar-eye (*B*). The positions of these genes as indicated by Bridges are respectively, 0·0, 33·0 and 57·0. (For the purpose of this experiment, *y* and *Hw* are considered to be one gene, since they are both situated at position 0·0.) An inbred Samarkand–Bayfordbury stock was used, into which, by the use of a *CyL*/*Pm*; *Sb*/*H* technique, a single marked *X* chromosome was introduced without any appreciable alteration to the other chromosomes.

This stock was developed in such a way that all informative combinations of homozygosity and heterozygosity for two alleles at the three loci could be derived simultaneously (Fig. 1). Thus the experi-

---

* Present address: Plant Breeding Institute, Trumpington, Cambridge.

Fig. 1. Experimental design

$\frac{yHw\ v\ B}{+\ +\ +}$	$\frac{yHw\ +\ B}{+\ +\ +}$	$\frac{yHw\ v\ +}{+\ +\ +}$	$\frac{+\ v\ B}{+\ +\ +}$
$\frac{yHw\ v\ +}{+\ +\ B}$	$\frac{yHw\ +\ +}{+\ +\ B}$	$\frac{yHw\ +\ +}{+\ v\ +}$	$\frac{+\ v\ +}{+\ +\ B}$
$\frac{yHw\ +\ +}{+\ v\ B}$	$\frac{yHw\ v\ +}{+\ v\ B}$	$\frac{yHw\ +\ B}{+\ v\ B}$	$\frac{yHw\ v\ B}{yHw\ +\ +}$
$\frac{yHw\ +\ B}{+\ v\ +}$	$\frac{yHw\ v\ B}{+\ v\ +}$	$\frac{yHw\ v\ B}{+\ +\ B}$	$\frac{yHw\ v\ +}{yHw\ +\ B}$

ment permits the study of the influence of *B* and the proximal region on recombination distally, *yHw-v*; the effect of the median region containing the gene *v* on the whole marked region, *yHw-B*; and the effect of *yHw* and the distal region on the proximal segment, *v-B*. The experiment was carried out at 24° C., and recombination was estimated by the product formula.

Table 1 shows that different states of the distal region have insignificant effects on recombination in the proximal region, *v-B*, ($\chi^2_{(2)} = 0{\cdot}3414$, $P = 0{\cdot}90\text{–}0{\cdot}80$). The other two regions, however, have large influences on recombination. Thus, there are significant differences in recombination in the distal segment, *yHw-v*, when the different states of the *B* locus and the proximal region are considered ($\chi^2_{(2)} = 9{\cdot}2297$, $P < 0{\cdot}01$). Similarly, differences in the median region with the *v* locus are associated with significant effects on recombination for the whole chromosome, *yHw-B* ($\chi^2_{(2)} = 10{\cdot}7384$, $P < 0{\cdot}01$).

Table 1. PROGENY OBTAINED FROM COUPLING AND REPULSION CROSSES FOR THE NINE PARENTAL GENOTYPES

Region	Parental genotype	Progeny				Recombination (p)
		Coupling cross		Repulsion cross		
		Recombinants	Total	Recombinants	Total	
Proximal	*B*/*B*	84	324	151	513	0·2765 ± 0·0160
	B/+	489	1,827	540	2,001	0·2639 ± 0·0065
	+/+	303	1,344	256	1,003	0·2400 ± 0·0097
Median	*v*/*v*	352	912	449	1,192	0·3813 ± 0·0107
	v/+	829	2,017	774	1,811	0·4192 ± 0·0088
	+/+	350	766	464	1,144	0·4311 ± 0·0116
Distal	*yHw*/*yHw*	92	464	54	302	0·1844 ± 0·0143
	yHw/+	419	2,184	335	1,644	0·1978 ± 0·0065
	+/+	281	1,382	122	638	0·1971 ± 0·0097

The failure to detect an influence of the distal region on recombination, in contrast to the large effects produced by the other regions studied, may have a number of causes of which the following are considered here. Either the proximal and median regions are more active genetically than the distal region so far as the control of recombination is concerned, or the proximal and median regions are more active because of their position in relation to the centromere. Thus, if the recombination process commences proximally and moves towards the ends of the chromosome, and there is evidence to support this in *Drosophila*[4], direct genetic alteration of recombination in regions near the centromere must have consequences on recombination distally. On the other hand, direct genetic changes in distal recombination will have little effect proximally, because recombination will already have occurred here. Clearly, a definite distinction between these possibilities cannot be derived from the results obtained in these experiments. It seems, however, unlikely from examinations of other characters that the proximal and median regions should have a higher genetic activity than the distal region. The distributions of both genes controlling sternopleural chaetæ[5], and major genes in the *X* chromosome, are concentrated at both ends of the genetic map, so that according to such evidence the distal as well as the proximal region should show high activity. The most plausible hypothesis, therefore, appears to be the latter.

From the results it is also apparent that large variations in recombination are produced by the different genetic states used in the proximal and median regions. The possibility that some of this variation may arise as a result of the activity of the major genes used to measure recombination has, therefore, to be considered. A means of detecting such influences may be obtained from a comparison of the dominance relations expected from the normal phenotypic expressions of the genes involved and their observed level of action in recombination. Some agreement between the two may indicate that the major genes are having an effect. Evidence from the two major genes *B* and *v*, which lie in the regions producing significant effects on recombination, shows some agreement with expectation. Thus *v* acts as a recessive on eye-colour and appears to do likewise when recombination is considered, since a comparison between the homozygous recessive (*v*/*v*) and the combined homozygous and heterozygous dominant (+/+ and *v*/+), accounts for almost all the heterogeneity in this region and is highly significant ($\chi^2_{(1)} = 10{\cdot}4483$, $P < 0{\cdot}001$). Similarly, the gene *B*, which behaves as a partial dominant on eye-shape, produces analogous effects on recombination. In this case, the difference between the homozygous recessive (+/+) and the combined homozygous and heterozygous dominant (*B*/*B* and *B*/+) is also significant ($\chi^2_{(1)} = 8{\cdot}7194$, $P < 0{\cdot}01$). It appears, therefore, that some of the results could be produced by the major genes themselves and not entirely from genes closely linked to them.

The observations made in these experiments clearly demonstrate that large differences in recombination can be obtained by genetic alterations within a chromosome. Large structural changes seem to be excluded, since the combinatorial type of experimental design used here should show disturbance of this type. The lack of precision brought about by the use of large genetic map distances, which of necessity must be used in *Drosophila*, cannot allow an exact appraisal of the genetic factors responsible. Some of the differences may, however, be produced by the major genes used for estimating recombination. Further, the experiments indicate a definite relationship between regions and, moreover, one which appears to be orientated with respect to the centromere. They also illustrate the possible usefulness of such designs in recombination studies within a chromosome, particularly where small regions of a chromosome could be considered.

I thank Dr. A. Durrant for advice and guidance during the course of this work, and also acknowledge the receipt of an Agricultural Research Council studentship.

[1] Levine, R. P., and Levine, E. E., *Genetics*, **39**, 677 (1954).
[2] Levine, R. P., and Levine, E. E., *Genetics*, **40**, 399 (1955).
[3] Green, M. M., *Nature*, **184**, 294 (1959).
[4] Mather, K., *Biol. Rev.*, **13**, 258 (1938).
[5] Wigan, L. G., *Heredity*, **3**, 53 (1949).

LETTERS TO THE EDITORS

ASTROPHYSICS

Terrestrial Accretion from the Solar Wind

C. M. DE TURVILLE[1] has suggested that most of the water now present on Earth has been formed from hydrogen in the solar wind, in combination with oxygen in the Earth's atmosphere. If this is so, then the process must still be going on, forming water vapour, according to de Turville, at a rate of about 2×10^{-12} gm. cm.$^{-2}$ sec.$^{-1}$. The water is presumably being formed at very high levels (above 100 km.) where oxygen in the atomic form exists. The continuous formation of water vapour at these levels must lead to a gradient of the water vapour mixing ratio (the ratio of the mass of water vapour to the mass of air) decreasing downwards until overtaken by the gradient decreasing upwards arising from evaporation of water from the surface of the Earth. What measurements there are do suggest such gradients[2], and it is interesting to consider whether the humidities found could conceivably arise in this way.

We may assume a flux equation of the form $F = K\rho \frac{d\chi}{dt}$, where χ is the mixing ratio. If we now assume that K is invariant with height, and assume that the air density, ρ, is given by the equation $\rho = \rho_0(\exp - bz)$, then we find, inserting an appropriate value for b, and putting F equal to 2×10^{-12}:

$$\chi - \chi_0 = \frac{1{\cdot}5 \times 10^{-6}}{K}\left(\frac{1}{\rho} - \frac{1}{\rho_0}\right) \qquad (1)$$

or, if m denotes the water vapour density:

$$m = \frac{1{\cdot}5 \times 10^{-6}}{K}\left(1 - \frac{\rho}{\rho_0}\right) + \frac{\rho}{\rho_0}\, m \qquad (2)$$

It will be seen that, in any layer with a more or less constant K, the water vapour density tends rapidly to the value $\frac{1{\cdot}5 \times 10^{-6}}{K}$ gm. cm.$^{-3}$. Frost points corresponding to values of K ranging from 10^2 to 10^5 are given in Table 1.

Table 1

K	10^2	10^3	10^4	10^5
Frost points	−58	−74	−88	−101° C.
	215	199	185	172° K.

Few measurements of water vapour have been made at high levels, but Barclay *et al.*[2] obtained a value at 27 km., which corresponds to a frost point of −75° C. 'Mother-of-pearl' clouds are also occasionally seen, from Sweden, at these levels[3]. There is good reason for believing that these are water clouds, and since the temperature at these levels is known to be between −70° and −80° C., this also suggests a frost point of about −75° C. A flux of water vapour of 2×10^{-12} gm. cm.$^{-2}$ sec.$^{-1}$, and a turbulence coefficient of 10^3 would provide just such a frost point. This is rather a low value, even in this region of great stability, but it is certainly not so low that it can be dismissed as inconceivable.

There are no measurements of humidity at greater heights, but clouds—'noctilucent clouds'—are also reported, from high latitudes in summer, at levels of about 80 km. These look very much like water (ice) clouds, but the great difficulty, hitherto, in explaining how the water got there has led some meteorologists to conclude that they must be dust clouds[4]. The temperature at this level, in the regions where the clouds are seen, has never been measured, but it is probable that it is, in summer, very low—perhaps −90° C. or lower. This suggests a value for the turbulence coefficient of between 10^4 and 10^5—and this higher value is consistent with the known instability in this region (temperature maximum at about 55 km. and temperature minimum at about 85 km.). Some spectroscopic measurements in a water vapour absorption band have been made from aircraft[5]. Unfortunately, the absorption coefficients, at the wave-lengths used, are dependent on pressure, so that it is not possible to deduce the amount of water vapour above the level of the highest reading; but a rough calculation suggests that the results of Houghton and Seeley are quite consistent with the amounts suggested here.

It must be concluded that if water vapour is being added at the rate suggested by de Turville, then water (ice) clouds at about 80 km. and at about 25 km., and such measurements of water vapour at these levels as have been made, are all consistent with turbulent diffusion downwards of this water vapour, and coefficients of diffusion which it is reasonable to suppose may be found at the levels concerned.

This communication is published by permission of the Director-General of the Meteorological Office.

RONALD FRITH

Meteorological Office,
Air Ministry,
London.

[1] de Turville, C. M., *Nature*, **190**, 156 (1961).

[2] Barclay, F. R., Elliott, M. J. W., Goldsmith, P., and Jelley, J. V., *Quart. J. Roy. Meteor. Soc.*, **86**, 259 (1960).

[3] Störmer, C., *Nature*, **145**, 221 (1940).

[4] Ludlam, F. H., *Tellus*, **9**, 341 (1957).

[5] Houghton, J. T., and Seeley, J. S., *Quart. J. Roy. Meteor. Soc.*, **86**, 358 (1960).

DE TURVILLE[1] has suggested that the Earth is gaining hydrogen from the Sun at the rate of 1·5 tons a second, and points out that this rate of accretion will account for all the hydrogen in the oceans in the course of 3×10^9 years. His idea is that the geomagnetic field captures the protons of an incident solar wind.

Pirie[2] has expressed some concern at the idea of a 'hydrogen hurricane' depositing hydrogen in the atmosphere at such a high rate. For if de Turville is correct, the question is where does the oxygen come from ? The suggestion therefore creates rather than solves a problem.

The values proposed by de Turville, a density of $n = 12$ protons cm.$^{-3}$ and a velocity of $v = 4{,}300$ km. sec.$^{-1}$, have indeed transformed the solar wind into a 'hydrogen hurricane'. These values yield a particle energy flux of 10^3 ergs cm.^{2} sec.$^{-1}$ at the Earth's orbit, or one-thousandth of all the radiation flux from the Sun. Where does this immense amount of energy come from? It is assumed apparently that the particles are continuously accelerated as they travel from the Sun to the Earth, but it is not explained how this is possible. Parker, who is the principal advocate of the solar wind idea, suggests that hydromagnetic waves heat the solar corona out to a distance of possibly 8 solar radii, and therefore an isothermal corona must expand hydrodynamically away from the Sun. Beyond 8 solar radii the solar wind flows at a constant velocity, and the temperature falls off adiabatically. Applying de Turville's relation of $v \propto r^{2/3}$ out to a distance of only 8 solar radii, and not to the Earth's orbit, yields a solar wind velocity of 600 km. sec.$^{-1}$, in agreement with the more modest ideas that are prevalent.

Even if de Turville's values were correct, it is doubtful whether the hydrogen in the oceans can be accounted for in the manner he suggests. The momentum flux of this 'hydrogen hurricane' would deform and compress the geomagnetic field to within a distance of roughly 3 Earth radii, and not 12 Earth radii as stated. If the incident particles are captured by the geomagnetic field, the rate of accretion is reduced to only 5 per cent of the value proposed. However, one questions strongly whether the geomagnetic field does capture entirely the incident solar wind. In every analysis of the subject published so far, starting from the early work of Chapman and Ferraro, it is seen that the Earth's magnetic field acts as a shield, and forms a cavity inside the low-energy corpuscular streams from the Sun. The particles that penetrate, as a result of complex diffusion processes, are relatively few. If magnetic fields are embedded in the solar wind, and this is quite possible, the solar wind will stream hydrodynamically around and past the Earth, very much in the same manner that a fluid flows past a submerged object.

At the present moment it is not known definitely whether there is a solar wind or not. Should it turn out that there is a solar wind, or even a 'hydrogen hurricane', it is almost certain that the rate of accretion of hydrogen is negligible and cannot by any means account for the formation of the oceans in the life-time of the Earth.

E. R. HARRISON

Atomic Energy Research Establishment,
Harwell,
Berks.

[1] de Turville, C. M., *Nature*, **190**, 156 (1961).

[2] Pirie, N. W., *Nature*, **190**, 706 (1961).

MR. HARRISON has raised a number of important issues which can only be answered tentatively at the present time. Presumably the oxygen is liberated from terrestrial rocks by solar ultra-violet radiation, and an estimate of the mean quantum efficiency of this photochemical process should be readily obtainable in the laboratory, by irradiation of outgassed rock samples in an evacuated chamber, using a strong ultra-violet source and an ionization pressure gauge. In the absence of hydrogen accretion, this process would lead to an evolution of oxygen trending towards zero as ultra-violet absorption by atmospheric atomic and molecular oxygen and ozone increased, and the oxygen content of the atmosphere would reach asymptotically some terminal value corresponding to complete absorption of ultra-violet radiation. The effect of hydrogen accretion is to remove atomic oxygen at levels above 100 km., and the ultra-violet absorption provides a regulating mechanism which ensures a dynamic balance between the rate of liberation of oxygen from the rocks and the rate of removal of oxygen in the upper atmosphere.

Concerning the acceleration mechanism responsible for the solar wind, it must be understood that the difference between Parker's 600 km./sec. and the 4,300 km./sec. previously proposed by me is due solely to a different estimate of the range of applicability of the relation $v = Bn^{-\frac{1}{4}}$. I accept Parker's account of the origin of the solar wind in magnetohydrodynamical terms, the ultimate source of energy being the thermal energy at the solar surface, which is clearly adequate. It is known from a study of the behaviour of solar cosmic rays that the region of disordered magnetic fields begins at about 1·2 A.U. from the Sun, and it seems plausible to suppose that the régime of flow of the solar wind is preserved until this point is reached. As pointed out by Parker[1], the assumption that the isothermal corona extends to only 8–20 $R_\odot$ and that beyond this point the temperature falls off adiabatically in the absence of any further coronal heating is merely the simplest and most conservative model consistent with observations of the intensity of electron scattering of photospheric white light; the fact that no observations are available beyond 20 $R_\odot$ is due to technical difficulties and affords no evidence of the absence of a corona beyond this point. If other evidence relating to conditions at the orbit of the Earth is also taken into account, Parker's estimates of the extent of the corona and of the velocity of corpuscular radiation turn out to be excessively conservative. The simplest model consistent with all observations is surely that of a radial solar wind the character of which is unaltered out to a distance of 1·2 A.U.

Nevertheless, it must be admitted that the numerical values previously quoted are seriously in need of revision, since the flight of *Pioneer V* has revealed that the velocity of the solar wind in the vicinity of the Earth is in fact $1{\cdot}45 \times 10^8$ cm. sec.$^{-1}$. Owing to equipartition of proton and electron energies in the terrestrial magnetic field, this implies a velocity $\sqrt{2}$ times larger in free space, so that the velocity of the solar wind in free space at the orbit of the Earth is $v_1 = 2{\cdot}05 \times 10^8$ cm. sec.$^{-1}$ and the corresponding ion concentration $n_1 = (B/v_1)^4 = 232$ cm.$^{-3}$, in better agreement with Biermann's estimate than with the values quoted by Pope and Kupperian—no theory can be expected to agree simultaneously with several discordant observations. The recalculated transit time of solar protons is then 2 days, but may well be shorter during magnetic storms. The rate of emission of hydrogen by the Sun becomes $2{\cdot}24 \times 10^{14}$ gm. sec.$^{-1}$, and implies a rate of emission of deuterium of $3{\cdot}3 \times 10^{11}$ deuterium atoms/cm.^{2} sec.

The adjusted values of n_1 and v_1 are quite compatible with van Allen's data[2] concerning the electronic energy and flux in the outer radiation belt. With the aid of these new values, it becomes possible to accept Harrison's valid objection concerning the deformation of the terrestrial magnetic field and yet recover the previous value of the rate of terrestrial accretion. The equatorial dipole field of the Earth is $H = 0{\cdot}316/x^3$ gauss, where x is the geocentric distance expressed in Earth radii, and if x_0 denotes the distance at which the pressure and magnetic energy density are equal, then $H^2/8\pi = n_1 m_H {v_1}^2/3$ and hence $x_0 = (80\pi n_1 m_H {v_1}^2/3)^{-1/6} = 3{\cdot}00$, which coincides approximately with the centre of the outer radiation belt.

The mass of hydrogen accreted by the Earth in time T is then $Q = \pi x_0^2 R_{\oplus}^2 m_H n_1 v_1 T$ and using these new values of x_0, n_1 and v_1 and the more plausible figure $T = 1{\cdot}5 \times 10^{17}$ sec. for the age of the Earth, the revised rate of accretion is $1{\cdot}37 \times 10^{23}$ gm., which is not very different from the previous value. Oxidation would yield $1{\cdot}23 \times 10^{24}$ gm. of water, but this is only a rough estimate. During magnetic storms, the rate of hydrogen capture may be greatly increased, and there are other complicating factors which indicate that the present simple treatment can only be accepted as a first approximation.

In particular, the question of the efficiency of capture and retention of particles from the solar wind is bound to be mathematically very complex, as it involves not only the combination of a radial solar magnetic field and a rotating terrestrial dipole field, but also the interaction of the incoming particles with the local plasma and the dissipation of electron energy by bremsstrahlung radiation. But if we are to judge by the recent findings discussed by Dr. Frith, the observational evidence afforded by high-altitude meteorology does suggest that the capture process is in fact fairly efficient. Thus the proposed account of the origin of the oceans remains eminently defensible, though much further work will be needed before it can be considered as established.

C. M. de Turville

35 Regent Street,
Clifton,
Bristol, 8.

---

[1] Parker, *Astrophys. J.*, **132**, 821 (1960).

[2] van Allen, *Proc. Moscow Cosmic Ray Conf.*, **3**, 7 (1960).

[3] Parker, E. N., *Astrophys. J.*, **128**, 677 (1958); **132**, 821 (1960).

PHYSICS

Theory of the Red Shift and the Nature of Planck's Constant

Since the Mössbauer experiment, the definition of the frequency of light requires further attention. In the theory of General Relativity the following definitions are possible :

(1) *Based on Maxwell's Theory.* Let us call A_a (A_0, A_1, A_2, A_3) the four potential. Let us assume $A_a = a_a \rho^{i\varphi}$ as the solution of Maxwell's equations. The transition to geometrical optics follows from the assumption that $\left|\dfrac{\partial\varphi}{\partial x^a}\right| = \varphi_{/a}$ is very great. Maxwell's equations are then reduced to:

$$g^{a\beta}\varphi_{/a}\varphi_{/\beta} = 0$$

We call $\varphi/_0 = \nu_0$ the frequency, and then we can prove the following statements : (*a*) ν_0 is constant along the light ray in every static field and in every static co-ordinate system; (*b*) ν_0 depends on the gravitational field at the point of emission (red shift)[1].

(2) *Based on Quantum Theory.* We can just as well define $\nu_0 = \nu$ in a natural way as the frequency. Let us define the inertial mass μ by:

$$\mu^2\left(g_{a\beta}\,\frac{\mathrm{d}x^a}{\mathrm{d}t}\,\frac{\mathrm{d}x^\beta}{\mathrm{d}t}\right) = m^2 = \text{constant}$$

$\mu\xi/_0{}^a$ is a vector with $\xi/_0{}^0 = 1$. Einstein's fundamental assumption for light rays and de Broglie's for the waves of matter is:

$$\mu\xi/_0{}^a = h\nu\xi/_0{}^a$$

where $\xi/_0{}^a$ is the velocity of light or that of the wave packet. It is natural to identify ν with the frequency, but to do so and also in order that (*a*) and (*b*) should be valid for ν, we must assume that h is a scalar density, and a quasi-Cartesian co-ordinate system has been chosen[2].

L. Infeld

University of Warsaw and
Polish Academy of Sciences.

---

[1] Laue, M., *Die Relativitätstheorie* (Vieweg Braunschweig, 1921).

[2] Białynicki, I., *Bull. Pol. Acad. Sci.*, Cl. III, **5**, 805 (1957).

Measurements of Heats of Adsorption of Organic Vapours on Adsorbents of High Surface Area using a Continuous Flow Calorimeter

An apparatus for the measurement of heats of preferential sorption from liquid carriers by means of a continuous-flow system has already been described[1]. It was felt that this technique might also be applied to adsorption processes taking place from the vapour phase. A later model of the apparatus originally described was therefore modified to investigate the adsorption of organic vapours on to surfaces of high specific area.

Fig. 1 shows the calorimeter, in which the temperature-sensing elements comprise four thermistors, two reference thermistors situated in the body of the apparatus to ensure that they remain at constant temperature and a further pair of thermistors embedded in the surface of the adsorbent under investigation. Nitrogen is passed through the calorimeter at a rate of 50 ml./min., and a sample of gas, saturated with the vapour being used, is injected into the nitrogen stream by means of a long hypodermic needle. When the vapour reaches the adsorbent it is adsorbed and the rise in temperature measured and recorded by the thermistors, which are connected in a Wheatstone bridge network which feeds a 10-mV. potentiometric recorder via a 'Vibron' electrometer.

In order to obtain values for the heats of adsorption of the materials used the calorimeter was calibrated

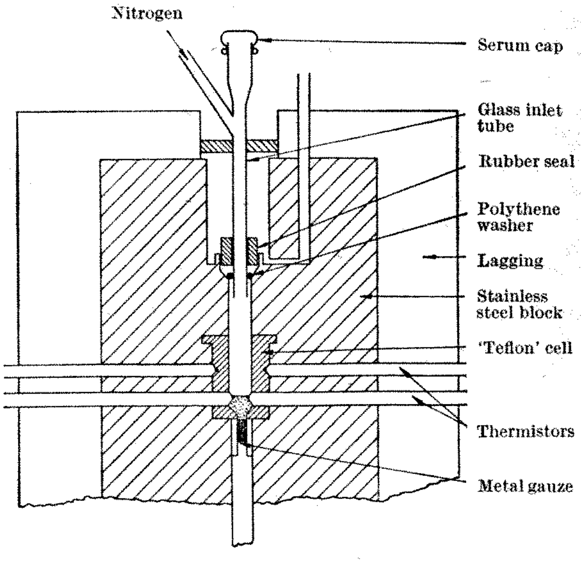


Fig. 1. Calorimeter for the determination of heats of adsorption from gases

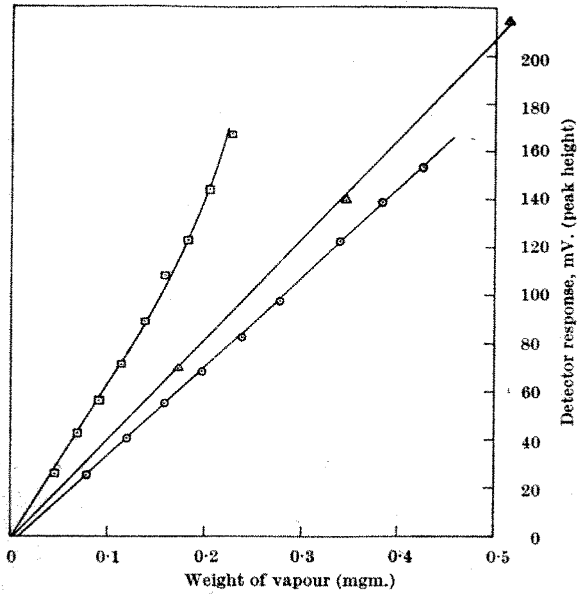


Fig. 2. Adsorption on silica gel. ○, *n*-heptane; △, benzene; ◻, isopropyl alcohol

by means of a coil of nichrome wire which was introduced into the surface of the adsorbent and through which known electric currents were passed. It was shown by this means that the response of the apparatus, in terms of peak height in mV. recorded, was linear with the quantity of heat dissipated in the adsorbent. An approximate value for the quantity of the materials contained in the samples of gas saturated with the vapour was obtained from the equation :

$$pV = \frac{w}{M} RT$$

where p is the vapour pressure of the sample at temperature $T°$ absolute, V the volume of gas in which the vapour is contained, w the weight of the vapour, M its molecular weight and R the gas constant. Vapour pressure values were obtained from the literature and from the weights thus obtained molar heats of adsorption were calculated.

Table 1. ADSORPTION ON SILICA GEL

Material	Temp. (°C.)	Response per 1·0 ml. vapour (mV.)	Response per μgm. (mV.)	Response per μmole (mV.)	Heat of adsorption (kcal./mole)
n-Heptane	22·0	71	0·33	33	12·1
Benzene	22·0	140	0·41	32	11·9
Toluene	22·0	45·5	0·38	35	12·9
Methanol	25·0	162	0·86	28	10·3
Ethanol	24·5	96	0·69	32	11·8
Isopropanol	21·0	71	0·63	38	14·0
1,2-Dichloroethane	23·5	102	0·29	28	10·3
1,2-Dibromoethane	22·0	22·5	0·18	35	12·9
Pyridine	22·0	37·5	0·63	50	18·4

Table 1 shows results obtained when 1·0-ml. samples of gas saturated with different organic vapours are adsorbed on to silica gel. It must be noted that the weights of the organic materials used are different in each case since a wide range of vapour pressures is covered; the surface coverages therefore differ widely. It was shown that for *n*-heptane the quantity of silica gel contained in the cell which takes part in the initial adsorption of the vapour was about 50 mgm., which corresponds to a surface coverage of approximately 0·1 μmol./m.², assuming that the sample is quantitatively adsorbed. The value of 12·1 kcal./mol. obtained for the adsorption of *n*-heptane is similar to values quoted in the literature for similar adsorbents[2].

When irreversible adsorption occurred it was found that on successive adsorptions of 1·0-ml. samples of vapour on to the same sample of silica gel the heat evolved decreased steadily. For example, the values obtained for benzene as shown in Table 1 are the means of a number of successive determinations. The initial response obtained for benzene adsorbed on to a fresh sample of gel was always higher than this, 155 mV. for 1·0 ml. sample of gas saturated with vapour at 22° C. This corresponds to an initial heat of adsorption of 12·9 kcal./mol., a value which might be expected in relation to 12·1 kcal./mol. for *n*-heptane[2]. For benzene and *n*-heptane the relationship between the quantity of vapour adsorbed, again assuming complete adsorption, and the response obtained, that is, the heat evolved, was found to be linear in the range of surface coverage investigated, up to about 0·2 μmol./m.² (Fig. 2).

Table 2. ADSORPTION ON ACTIVATED CARBON

Material	Temp. (°C.)	Response per 1·0 ml. vapour (mV.)	Response per μgm. (mV.)	Response per μmole (mV.)	Heat of adsorption (kcal./mole)
n-Heptane	20·0	117	0·59	59	21·7
Benzene	22·5	235	0·65	51	18·8
Toluene	20·0	86	0·78	72	26·5
1,2-Dichloroethane	19·0	167	0·50	50	18·4
1,2-Dibromoethane	20·0	30	0·27	51	18·8
Pyridine	20·0	55	0·85	67	24·6

Activated carbon has also been used as the adsorbent and results obtained are shown in Table 2. Heats of adsorption on this material can be seen to be very much higher than in the case of silica gel. It has been reported[3] that heats of adsorption of this magnitude are obtained on adsorbents of this type when the surface coverage is low. This probably explains the very high value obtained for toluene in relation to that obtained for benzene, since the amount of toluene used was much less than that of benzene.

We wish to thank the Chairman and Directors of the British Petroleum Co., Ltd., for permission to publish this communication.

G. H. BELL
A. J. GROSZEK

Petroleum Division,
BP Research Centre,
Sunbury-on-Thames,
Middlesex.

---

[1] Groszek, A. J., *Nature*, **182**, 1152 (1958).

[2] Kiselev, A. V., *Effect of Surface Dehydration and Pore Size of Silica on Heat of Adsorption* (Acad. Sci., U.S.S.R.; 1957; in English).

[3] Kiselev, A. V., Dreving, V. P., and Runov, A. R., *C.R. Acad. Sci., U.S.S.R.*, **46**, 283, b (1945), in English.

An Experimental Apparatus for Contact Microradiography at 200–500 V.

OWING to the strong absorption of soft radiation, a sealed-off X-ray tube[1] is not suitable for contact microradiography. We have, therefore, used an X-ray tube without window.

The principle of the cathode/anode part is given in Fig. 1. A departure from conventional construction is made in order to comply with the requirements of small focus, high anode current and low accelerating

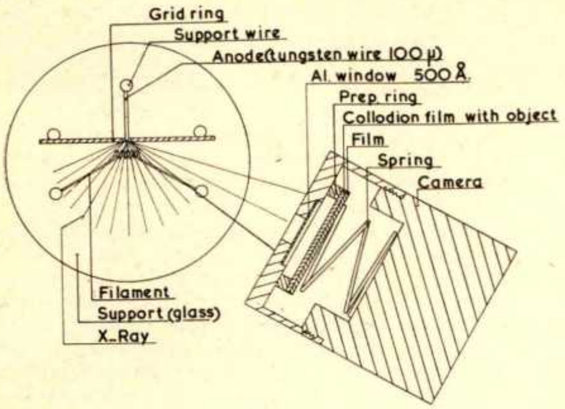


Fig. 1

voltage. The three components anode, grid, ring and filament are very simple to mount on a sintered glass bridge.

The cathode provides for the emission of the electrons which impinge mainly on the tip of the wire anode. The ring ensures that no electrons are deflected beyond the edge of the anode by either electrostatic or mechanical means; this is in order to prevent X-rays from being produced there also. A voltage of from +10 to +20 V. at the ring is sufficient. The diameter of the hole is 0·7 mm. Overload of the anode need not be feared, since the load is of the order of 0·2 W. continuous.

As can be seen from the diagram, the camera itself can be mounted in a position as favourable as possible with respect to the anode. The aperture of the camera has to be made light-proof by means of an aluminium membrane[3]. In our tests we used a shadow-casting apparatus. In principle, any evacuated vessel is suitable, provided it has three connecting wires: two for the cathode and one for the ring.

The electrical part consists of a filament-current transformer for the cathode, and a source of direct voltage for the ring and the anode, of 10 and 200–500 V. respectively.

The advantage of the microradiographic method is that the soft radiation can provide information which may be correlated with the mass and chemical composition of the microscopic objects. The resolving power is about the same as that of light microscopes[2–4].

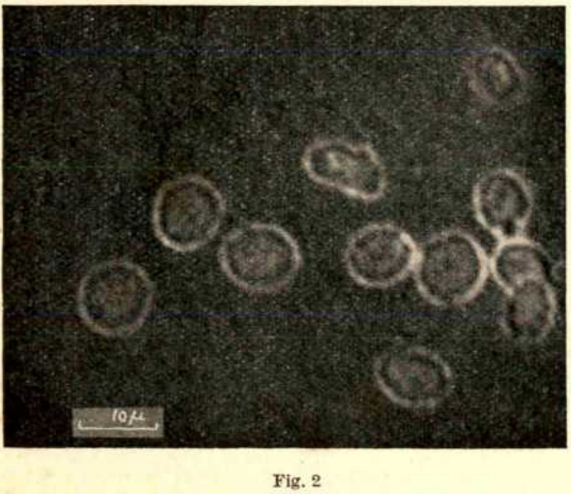


Fig. 2

The preparation techniques employed are those of Greulich and Engström[2]. An example of the results obtained is given in Fig. 2, which shows a contact microradiograph of erythrocytes (350 V., 0·4 m.amp., exposure time 5 min. Eastman Kodak Spectroscopic Emulsion 649–0. Distance from X-ray source and emulsion, 6 mm.).

A. RECOURT
G. H. F. DE VRIES

Physical Technical X-Ray Laboratory,
N.V. Philips' Gloeilampenfabrieken,
Eindhoven.

---

[1] Combée, B., and Recourt, A., *Philips Tech. Rev.*, **19**, 221 (1957/1958).

[2] Greulich, R., and Engström, A., *Exp. Cell Res.*, **10**, 251 (1956).

[3] Henke, B., *Air Force Office of Scientific Res., Tech. Rep.* No. 2 (1958).

[4] Lindström, B., thesis, Stockholm, *Acta Rad. Supp.*, 125 (1955).

Irradiated Lithium Fluoride as an Optical Filter in the Far Ultra-Violet

PHOTOCHEMICAL studies of aqueous solutions of organic compounds are being carried out at this Laboratory using the 1849 Å. radiation emitted by a low-pressure mercury resonance lamp. It has been found necessary to eliminate the contribution of the intense 2537 Å. line emitted by such a source. This has been done by using a filter of irradiated lithium fluoride. The idea of using this material as the filter for mercury resonance radiation was suggested to us by P. Yuster of this Laboratory. The absorption spectrum of X-irradiated lithium fluoride shows a sharp band with a maximum at 2420 Å. due to the formation of *F*-centres in the crystal[1]. Even with complete absorption at this wave-length, the transmission of the irradiated crystal remains high at 1849 Å.

Polished disks of lithium fluoride, 40 mm. in diameter and 0·8 mm. in depth, were prepared from a crystal obtained from the Harshaw Chemical Co. Irradiations were carried out with a high-intensity cobalt-60 source of γ-radiation at several dose-rates. Spectral measurements were made with an accurately calibrated Cary model 14 recording spectrophotometer.

The increase in absorption at 1849 Å. and 2537 Å. with radiation dose is shown in Fig. 1, where dose is plotted against T/T_0, the ratio of transmissions after and before irradiation. The absorption spectra of the irradiated filters between 1849 Å. and 4700 Å. are similar to those recently reported by Kato *et al.*[2]. The decrease in transmission at 1849 Å. is due to the tail of the *F*-band.

After irradiation the crystals were exposed to a high-intensity mercury resonance lamp for various lengths of time, and it was found that the rate of bleaching of the *F*-centres was low enough to allow the crystals to be used as filters. The experimental results for a typical filter are shown in Table 1.

Table 1. TRANSMISSION OF LITHIUM FLUORIDE FILTER AT 1849 Å. AND 2537 Å. AFTER IRRADIATION AND BLEACHING

Treatment		Percentage of transmission at 1849 Å.	Percentage of transmission at 2537 Å.
Cobalt-60 γ-irradiation	Bleaching* (2537 Å.)		
None	None	70	85
1·7 Mrad	None	40	< 0·1
1·7 Mrad	2 × 10^{20} quanta	47	< 0·1
1·7 Mrad	4 × 10^{20} quanta	49	~ 0·1
1·7 Mrad	6 × 10^{20} quanta	50	~ 1

* Lamp intensity, I (2537 Å.) ~ 10^{17} quanta sec.$^{-1}$.

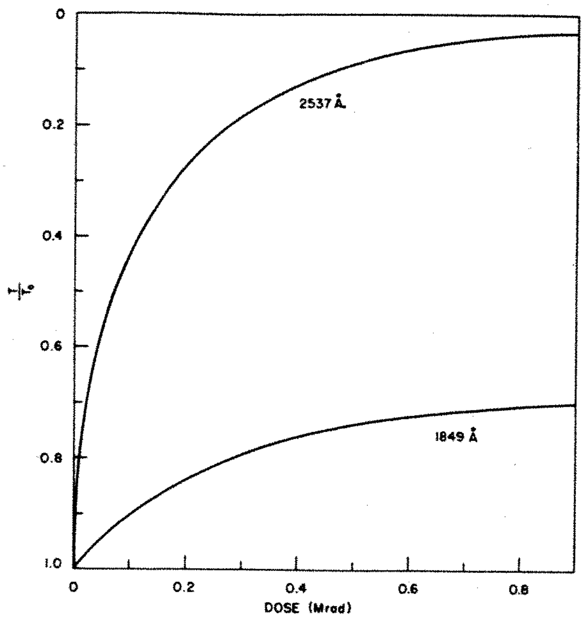


Fig. 1

This work was performed under the auspices of the United States Atomic Energy Commission.

J. L. WEEKS
S. GORDON
G. M. A. C. MEABURN

Argonne National Laboratory,
Argonne, Illinois.

[1] Pringsheim, P., and Yuster, P., *Phys. Rev.*, **78**, 293 (1950).

[2] Kato, R., Nakashima, S., Nakamura, K., and Uchida, Y., *J. Phys. Soc., Japan*, **15**, 2111 (1960).

CRYSTALLOGRAPHY

X-ray Diffraction Spikes of Diamond

THE anomalous X-ray reflexions of 'diffraction spikes' from type I diamonds have been studied by a number of authors[1-5] since they were discovered by Raman and Nilakantan[1].

Frank[4] explained the diffraction spikes, on the basis of the detailed data about the intensity of spikes by Hoerni and Wooster[3], as being due to segregated (100) planes of impurity atoms. He considered one central plane of atoms with spacing $(1+\delta)$ units of length to its neighbour planes, all interplanar spacings thereafter being 1. Thus calculating one-dimensionally, the amplitude of the spikes belonging to each reciprocal point h will be ultimately proportional to $\sin h\theta$ ($\theta = 2\pi\delta$). Frank evaluated $2\theta = 180° + 2\varphi + n \times 360°$, $2\varphi = 52° 17'$ or $67° 9'$ (n; integer) from $\pm\sqrt{(5/100)} = \sin 30/\sin\theta$, using the observed value of Hoerni and Wooster in $h = 1,3$. Also, he calculated with this θ the intensity in h = 1–5 with the results as shown in Table 1. The amplitude in the case of $h = 0$ was given separately by the equation:

$$A = B - 1 - 2\delta \qquad (1)$$

where B is the scattering weight of the impurity planes when the weight of carbon planes is assumed as 1. Considering that this observed value becomes almost zero, as well as the valency condition and size effect by using the result of the above calculation, the impurity atoms were considered to be silicon.

Table 1. INTENSITY OF DIFFRACTION SPIKES OF DIAMOND

h	Hoerni and Wooster observed value	Frank calculated value		Yoneda calculated value
		$2\varphi = 52° 17'$	$2\varphi = 67° 9'$	
0	< 5	0	0	0
1	100	100	100	58
2	75	77	122	38
3	5	5	5	7
4	30	116	70	68
5	absent	53	138	17
6	—	19	20	24

Caticha-Ellis and Cochran[5], who evaluated the impurity concentration, by their detailed calculation and experiment have criticized Frank's calculation as giving a concentration of silicon more than 80 times larger than that actually present.

I have used Frank's method[4] of calculation in its original form. It is, however, not effective, since the value 5 is too small in intensity and large in relative error to determine θ only from 100/5 without recourse to other available data. Accordingly, here θ and K have been determined by using all measured values and the method of least squares, putting $K \sin^2 h\theta = L_h$ (h = 1–5). (L_h is the intensity of spikes, K is a constant.) As the result, the most probable value obtained was $2\theta = 2\varphi + n \times 360°$, $2\varphi = \pm(132° \pm 3°)$, $K = 69 \pm 19$, which is quite different from Frank's calculation in spite of the same data being used. The result of evaluating the intensity of spikes by using this value as Frank did is shown in Table 1. This result seems to be a little nearer than Frank's to the observed value as a whole. The most probable value of θ, however, is more reliable than these calculated values of intensity. Frank has calculated the Si—C bond-length as 1·95 Å. or 1·935 Å., but the calculation of the bond-length corresponding to this by using my most probable value gives 1·45 Å. or 1·64 Å.

Kaiser and Bond[6] proved that many type I diamonds contain about 0·2 per cent of nitrogen as impurity. They also confirmed that this nitrogen is characteristic of type I diamonds.

Smith *et al.*[7] have found that there is a C—N bond in about half the diamond samples by electron-spin resonance experiments. According to them, the result of electron-spin resonance can be explained by assuming the C—N bond-length of one of the four bonds as having increased by several per cent, since the nitrogen donor electron has the tendency to take an antibonding orbit due to the Jahn–Teller effect. I believe, therefore, that 1·64 Å. should be adopted, which is 6·5 per cent larger than the 1·54 Å. for the C—C bond-length and nearly corresponds with the results obtained by Smith *et al.*

Also, assuming the impurity to be nitrogen, the spike amplitude of $h = 0$ is as weak as 0·2 from equation (1), which approaches Hoerni and Wooster's observed value[3], even if it is not zero as is calculated by Frank[4].

Evaluating the concentration of nitrogen by δ from my calculation and using the result of experiment and the equation of Caticha-Ellis and Cochran[5], about 0·8 per cent is obtained. Though wide differences in the nitrogen content of various samples have been pointed out by many authors and the nitrogen content of the samples used by Caticha-Ellis is not known, this result seems to be too large compared with the result of analysis by Kaiser and Bond[6].

I wish to thank Prof. T. Futagami for valuable discussions.

Y. YONEDA

Department of Applied Physics,
Faculty of Engineering,
Kyushu University,
Fukuoka, Japan.

[1] Raman, C. V., and Nilakantan, P., *Proc. Ind. Acad. Sci.*, A, **11**, 389 (1940).
[2] Lonsdale, K., and Smith, H., *Proc. Roy. Soc.* A, **179**, 8 (1941).
[3] Hoerni, J. A., and Wooster, W. A., *Acta Cryst.*, **8**, 187 (1955).
[4] Frank, F. C., *Proc. Roy. Soc.*, A, **237**, 168 (1956).
[5] Caticha-Ellis, S., and Cochran, W., *Acta Cryst.*, **11**, 245 (1958).
[6] Kaiser, W., and Bond, W. L., *Phys. Rev.*, **115**, 857 (1959).
[7] Smith, W. V., Sorokin, P. P., Gelles, I. L., and Lasher, G. J., *Phys. Rev.*, **115**, 1546 (1959).

THE quantity 2φ defined by Yoneda for his own calculations differs from my quantity 2φ, the definition of which he quotes earlier, by 180°. Consequently his result 132° ± 3° becomes, in my notation, 48° ± 3°, which is not very different from my 52°. Whether it is superior is very debatable. Yoneda's value increases the discrepancy from the observed spike intensities (relative to that at first order) in the second, third, and fourth orders, without gaining any satisfactory agreement at fifth order, for which he still obtains a stronger spike than at third order, instead of an absent one. I supposed another factor to be present, giving a general falling off of intensity at higher order. Yoneda's calculation does not dispose of the evidence for such a factor, but if such a factor were present, his least squares analysis is less appropriate than my method of calculation, the essential point of which is to give great weight to the location of a minimum (a zero, according to the theory) near the third order. The most remarkable result is the smallness of the change which his method of calculation brings about.

The bond-lengths 1·45 Å. or 1·64 Å. which Yoneda calculates are members of a series of bond-lengths (between carbon atoms and an assumed monolayer of impurity atoms) which would all give the same diffraction spikes except at zero order, namely :

1·399, 1·453, 1·640, 1·719, 1·955, 2·046 . . . . . .

for Yoneda's value ($2\varphi = 48°$) or

1·395, 1·457, 1·633, 1·723, 1·950, 2·053 . . . . . .

for my value ($2\varphi = 52°$)

while my less preferred value ($2\varphi = 67°$) would give bond-lengths about another 0·012 Å. alternately smaller and greater than the latter list. The associated numbers for the impurity to give minimal spike intensity at zero order are in all three cases:

2, 4, 8, 10, 14, 16 . . .

It is clear on this basis that if the impurity were to be identified as nitrogen ($Z = 7$), the third member of this series should be selected, and, as Yoneda remarks, this gives a credible bond-length, namely, 1·640 Å. according to Yoneda, or 1·633 Å. or 1·620 Å. (with preference for the former) according to me.

The basis for Yoneda's revision of Caticha-Ellis and Cochran's estimate of atomic concentration of impurity from 8×10^{-4} to 8×10^{-3} is not apparent: no acceptable change in δ should alter the result by so much. On the contrary, Caticha-Ellis and Cochran's estimate must be essentially independent of the nature of the defect, within its acceptable range, so that Kaiser and Bond's nitrogen contents are adequate to produce the diffraction spikes.

While this gives a credible interpretation of the 'spike-producing' defects, it must be borne in mind that the multiplicity of alternative magnitudes of displacement giving similar diffraction results makes the diffraction evidence alone incapable of discriminating against other possible models (such as one with nitrogen atoms in two layers). The matter can scarcely be settled before we have X-ray diffraction, infra-red absorption, and spin-resonance measurements all on the same diamond.

F. C. FRANK

H. H. Wills Physics Laboratory,
Royal Fort,
Bristol 8.

BIOPHYSICS

Proposed Use of Magnetic Fields in Electron Microscopic Radioautography

THE use of strong magnetic fields has been proposed as a possible way of increasing resolution in radioautography[1]. It was reasoned that a sufficiently strong magnetic field would bend the path of emitted β-particles into a circle of arbitrarily small radius, thereby restricting the resulting radioautographic grains to a small zone around the source. In recent reports[2,3], Harford and Hamlin, using the same argument, attempted to improve the resolution of electron microscopic radioautographs by placing the specimens in a 10-kilogauss magnetic field during exposure. They could not demonstrate such an improvement; but they attributed a considerable increase in the number of exposed grains to the effect of the field on the β-particles from tritium.

A simple calculation shows, however, that magnetic fields at present available can have little influence on the path in emulsion of low-energy β-particles. Tritium emits electrons of approximately 18-keV. maximum energy and 5·5-keV. average energy. Let us take the case of a 10-keV. electron (in the high-energy side of the spectrum, but with still a fairly high probability of occurrence). For such an electron moving perpendicularly to the lines of force of a uniform magnetic field the product $HR = 3{\cdot}386 \times 10^{2}$ gauss cm. (ref. 4) (where H = magnetic field intensity and R = radius of curvature). For a commonly available field of 10^{4} gauss $R \simeq 3{\cdot}4 \times 10^{-2}$ cm. = 340μ. The maximum range of a 10-keV. electron in material of density 1 is calculated to be of the order of 2·5μ (ref. 5). In emulsion it is probably about one half this value. On such a short path a curvature of radius 340μ would have no detectable effect. As the electron slows down, the radius of curvature will decrease, but, even for a 1-keV. electron with a calculated range in water of 0·053μ, the radius of curvature would still be about 100μ.

We may ask what fields would be needed to have a useful influence. A resolution of the order of 0·2–0·3μ can be theoretically expected (unpublished results), and experimentally demonstrated[6], when a monolayer of a fine-grained emulsion is used in electron microscopic radioautography[7]. We can ask, therefore, that the field be of sufficient strength to give a 10-keV. electron a curvature of 0·1μ radius. Taking $HR \simeq 3{\cdot}4 \times 10^{2}$ gauss. cm., we get, under these conditions, $H = 3{\cdot}4 \times 10^{7}$ gauss. This is approximately one hundred times stronger than any permanent field yet achieved. Since the change in electron velocity in going through matter is small we have used in these calculations the velocity *in vacuo*.

It might be added that, even with stronger magnetic fields, an increase in grain counts could not easily be explained on the basis of an altered path of the electrons. Assuming the field to be perpendicular to the horizontal plane of the section the vertical component of velocity of emitted electrons would be unaffected, while the horizontal component would be changed only in direction but not in magnitude. The total length of the path in emulsion would, therefore, remain the same, since the time spent by the electron in the emulsion depends only on the vertical component.

It does not seem that magnetic fields can be used effectively to modify the characteristics of radioautographs with tritium. A similar situation prevails in the cases of carbon-14 and sulphur-35. Only in the case of phosphorus-32 do the radii of curvature become of the same order as the ranges. But since in that case the entire path of the particle appears as a track there seems to be no great advantage in modifying its shape. It is therefore likely that the increase in grain counts observed by Harford and Hamlin is due to causes other than the effect of the magnetic field on the path of the β-particles.

LUCIEN G. CARO

The Rockefeller Institute,
New York 21.

[1] Platt, J. R., *Perspectives in Biology and Medicine*, 2, 24 (1958).

[2] Harford, C. G., and Hamlin, A., *Nature*, 189, 505 (1961).

[3] Harford, C. G., and Hamlin, A., *Lab. Invest.*, 10, 627 (1961).

[4] Johns, H. E., and Laughlin, J. S., in *Radiation Dosimetry*, edit. by Hine and Brownell (Academic Press, Inc., New York, 1956).

[5] Lea, D. E., *Actions of Radiations on Living Cells* (Cambridge Univ. Press, 1956).

[6] van Tubergen, R. P., *J. Biophys. Biochem. Cytol.*, 9, 219 (1961).

[7] Caro, L. G., *J. Biophys. Biochem. Cytol.*, 10, 37 (1961).

GEOLOGY

Uranium in British Surface and Underground Waters

RECENT interest in the alpha-activity of potable water[1] has prompted the following summary of the results of analyses for uranium in surface and underground waters, obtained during an assessment of uranium mineral resources in Great Britain (Table 1). Most of the samples were collected by the Atomic Energy Division of the Geological Survey of Great Britain and the uranium content determined by the National Chemical Laboratory, the Atomic Energy Research Establishment, Harwell, and the Atomic Energy Division. The analytical techniques were : the colorimetric method developed by Smith and Chandler[2], with later modifications by Hunt[3] ; the simple fluorimetric method of Milner and Barnett[4] ; and the fluorimetric method normally used in the laboratory.

Average background values based on 678 samples taken from surface waters in south-west England, north England and west Scotland are 1γ/l. or less (1γ/l. equals 10^{-6} gm./litre). Higher values in streams emerging at the limestone/shale contact in the Castleton area of Derbyshire can be related to leaching of uraniferous phosphate in the limestones[5] and to high bicarbonate ion concentration, the latter being well known to influence the solution of uranium in natural waters. Conversely, the low bicarbonate ion concentration probably accounts for the occurrence of small quantities of uranium in the Dalradian rocks of Knapdale[6] not being reflected in the analyses of water samples from that area. The value of 50γ/l. in water from the Goon Vean china clay leat is due chiefly to uranium from the artificially introduced particulate matter (mica and clay minerals) suspended in the water[2]. Filtered water from the same source contains less than 1γ/l.

As is to be expected, some underground waters contain higher concentrations of uranium than surface waters. The highest recorded uranium concentration (30–50γ/l.) is in water from a shaft at the disused South Terras uranium mine. Uranium concentration in spa waters is consistent with their origin, composition, and extended contact with crustal rocks.

Lack of consistency between the results of analyses undertaken at different times on Cornish mine waters is considered to be due to the effect of differing weather conditions immediately preceding the collection of samples, since checks have shown that comparable results are obtained with the different analytical techniques used. Measurements of the uranium content of waters in Great Britain and abroad have indicated that the amount of dissolved uranium reaches a maximum when rain follows a dry spell during which normal weathering processes in the zone of oxidation have made available abnormal quantities of uranium in a readily soluble form. The range of values recorded over a period of several weeks at St. Anne's Well, Buxton (from four samples), may also be attributable to varying ground-water circulation.

Table 1. URANIUM CONCENTRATIONS IN SOME SURFACE AND UNDERGROUND WATERS

District	Locality	Year	Uranium, γ/l.	Type of water	Technique
South-West England	St. Ives' Water Supply	1956	1·2	mine	fluorimetric and colorimetric
		1958	8·0		
	Tretheague	1958	1·0	radioactive spring	colorimetric
	Pengreep	1958	1·0–5·0	mine	colorimetric
	Wheal Speed	1958	7·0–8·0	mine	fluorimetric and colorimetric
	South Terras Shaft	1958	30–50	mine	fluorimetric and colorimetric
	Goon Vean	1958	50	china clay leat (unfiltered water)	fluorimetric and colorimetric
	Wheal James	1956	10	mine	fluorimetric
	Adit	1958	2	mine	colorimetric
	Wheal Bray	1956	10	mine	fluorimetric
	Winze	1958	2	mine	colorimetric
West England	Cheltenham Spa	1958	2·3	Spa	fluorimetric
Derbyshire	St. Anne's Well, Buxton	1958	2·5–6·0	Spa	fluorimetric and colorimetric
	Riber Mine, Matlock (western end of 220-ft. level)	1958	4	mine	fluorimetric and colorimetric
	Russel Well, Castleton	1958	2	spring	fluorimetric and colorimetric
	Spring, Grid Ref. 154837	1958	13–15	spring	fluorimetric and colorimetric
Cheshire and Shropshire	White Grit Mine	1959	2·6	mine	fluorimetric
	Eardiston Copper Mine	1959	2·3	mine	fluorimetric
Argyllshire, Knapdale	Achaglachgach	1959	1·7*	stream	fluorimetric
	Kilberry	1959	1·5*	stream	fluorimetric

* The highest uranium concentration found in the area.

This communication is published by permission of the Director of the Geological Survey of Great Britain.

J. D. Peacock

Atomic Energy Division,
Geological Survey of Great Britain,
Young Street, Kensington,
London, W.8.

[1] Turner, R. C., Radley, J. M., and Mayneord, W. V., *Nature*, **189**, 348 (1961).

[2] Smith, G. H., and Chandler, T. R. D., *Peaceful Uses of Atomic Energy*, 2, 148 (Geneva, 1958).

[3] Hunt, E., *National Chem. Lab. Sci. Rep. NCL/AE.*/538 (1958).

[4] Milner, G. W. C., and Barnett, G. A., *U.K.A.E.A. Rep. AERE C/R* 2723 (1958).

[5] Peacock, J. D., and Taylor, K., *Bull. Geol. Surv. Great Britain* (in the press).

[6] Miller, J., and Dawson, J., Geol. Surv. Great Britain, Atomic Energy Division (unpublished reports).

Continuation of the Great Glen Fault Beyond the Moray Firth

During 1944–45 I was stationed in Fair Isle, Shetland, during which time I was able to make a number of general observations on the geology of the island which tend to support Dr. Flinn's hypothesis[1] that the Great Glen Fault continues northwards, past the west coast of Fair Isle, to Shetland, where it is called the Walls Boundary Fault. Because of the inaccessibility of the Isle and its important position relative to the suggested course of the fault, it is worth while recording the observations; but it must be emphasized that they are recollections, supported by only a few photographs, specimens and notebook data.

Fair Isle is composed mainly of a barren series of shales, sandstones and conglomerate of Old Red Sandstone type, which the Geological Survey doubtfully identify as Lower Old Red Sandstone[2]. A detailed search for fish-beds was made but without success. The beds dip approximately to the south-east[3] with dips ranging from about 60° on the east side of the island (Fig. 1) to very nearly vertical on the west. On the east and north sides the rocks are unaltered, but towards the south-west corner of the island the shales and sandstones appear to pass into phyllites and low-grade schists containing quartz lenticles. Several of the pebble beaches in this part of the island were made up, almost entirely, of a dark, fine-grained, but porphyritic rock containing phenocrysts of plagioclase, indicating that igneous rocks were probably *in situ* just off the coast, if not in the inaccessible cliffs of Malcolm's Head at the south-west of Fair Isle. Farther north on the west coast, one or more dolerite dykes cut the cliff in an east–west direction.

When compared with Orkney, to the south, and Shetland, to the north, the most surprising feature is the attitude of the rocks, for in Orkney and Shetland the Old Red Sandstone has low dips of the order of 40° or less in the limbs of the gently rolling anticlines and synclines[4]. The fact that the dips become increasingly steep and approach the vertical towards the west coast of Fair Isle would accord with the hypothesis that a large disturbance lies to the west.

A further point is that erosion on the east side of the island reflects the lithology and structure of the rocks, soft bands being removed and more resistant beds standing up as headlands, the whole relief being aligned along the north-east–south-west strike. On the west side, however, erosion has been along joints and minor faults, and erosion features are parallel to the direction of dip. This tends to mask the fact that the general trend of the west coast does not coincide with the strike of the rocks but is about parallel to the trend of the Great Glen Fault extension as suggested by Dr. Flinn.

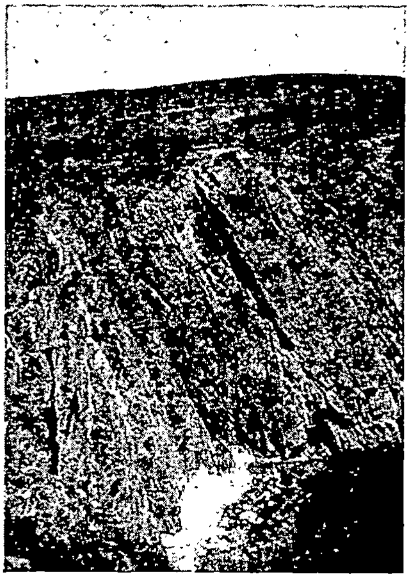


Fig. 1. Old Red Sandstone dipping to the south-east at a high angle on the east coast of Fair Isle, Shetland

Lastly, there is no large-scale shattering of the rocks in Fair Isle. This is also to be expected if the Great Glen Fault occupies the position suggested by Dr. Flinn, for in the Seli Voe area of Shetland there is shattering for distances of more than a mile from the fault, while Fair Isle lies about four miles east of the supposed position of the fault. The fault zone and the zone of shattering have been removed, as they are being rapidly removed at the present day at Seli Voe, Aith Voe and Brae.

Thus, the increase of the dip towards the west coast of Fair Isle, the trend of the west coast-line and the absence of large-scale shattering are three pieces of geological evidence which accord with Dr. Flinn's hypothesis.

Robert M. Appleby

Department of Geology,
University College,
Cardiff.

[1] Flinn, D., *Nature*, **191**, 589 (1961).

[2] Geological Survey, Geological Map of Scotland and North England. (Scale, 10 miles to the inch.) (London : H.M.S.O., 1948.)

[3] Wilson, G. V., *Summary of Prog., Geol. Surv. Great Britain for* 1933, Pt. 1, p. 73 (1934).

[4] Wilson, G. V., *et al.*, *The Geology of the Orkneys* (H.M.S.O., Edinburgh, 1935).

Lung-books in the Devonian Palæocharinidae (Arachnida)

The typical breathing organs of most of the larger recent Arachnids are lung-books. These are thought to have arisen from the segmentally arranged gills of an aquatic ancestor. The presence of lung-books in fossil Arachnids is thus of importance in any phylogenetic studies on the group. Petrunkevitch[1-3] has reviewed the known Palæozoic and Mesozoic fossils and shown the presence of paired structures on the proximal opisthosomal sternites of a few forms, particularly among the Carboniferous Anthracomartida

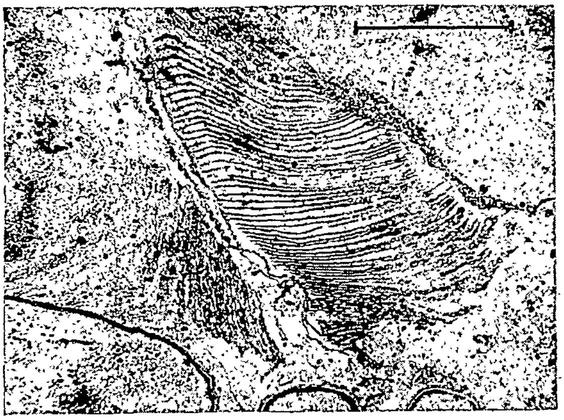


Fig. 1. Detailed structure of a single lung-book (scale 0·5 mm.)

and Trigonotarbida. These he has reasonably interpreted as representing the stigmata of lung-books. However, there seem to be no records of definite lung-book structure in any fossil.

The fine structure of lung-books may only be expected in fossils from deposits formed under exceptionally favourable conditions of preservation. Such a deposit is the Rhynie Chert (Scottish Middle Old Red Sandstone). Remarkably well preserved Arachnids attributed to the family Palæocharinidae, recently placed in the order Trigonotarbida by Petrunkevitch[1,3], have been described by Hirst[4], who was able to give full descriptions of external features but made no mention of lung-books or their stigmata.

During a re-investigation of the plants of the chert carried out by one of us (A. G. L.), numbers of these Arachnids have been encountered together with the plants among which they lie. Thin serial sections have been made by means of the palæobotanical peel method, after etching with hydrofluoric acid. Some of these sections show clear evidence of lung-book structure (Figs. 1 and 2), resembling very closely the comparable organs of modern spiders. Longitudinal sections show the presence of two pairs apparently opening by small stigmata on the sternites of the first and second opisthosomal segments (Fig. 2). With this and further sectioned material it should be possible to make detailed reconstructions and identifications.

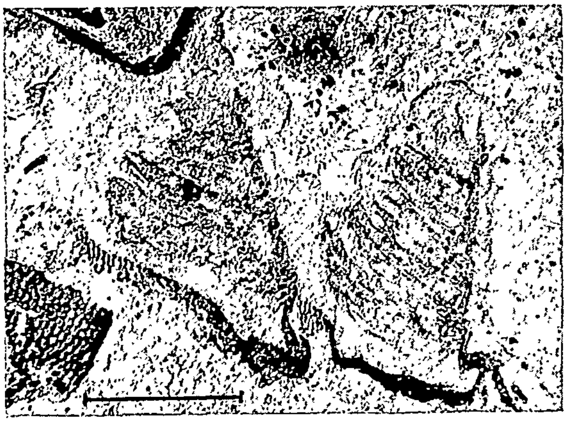


Fig. 2. Longitudinal section of successive lung-books and stigmata (scale, 0·1 mm.)

The Palæocharinidae have been recorded only from the Rhynie Chert and are of particular interest since they are among the earliest terrestrial Arachnida known with any certainty, and because they show a number of important similarities to the Araneida. To the latter can now be added the presence of two pairs of lung-books.

M. F. Claridge

Department of Zoology,

A. G. Lyon

Department of Botany,
University College,
Cardiff.

---

[1] Petrunkevitch, A., *Trans. Connecticut Acad. Arts Sci.*, **37**, 69 (1949).

[2] Petrunkevitch, A., *Mem. Geol. Soc. Amer.*, **53** (1953).

[3] Petrunkevitch, A., in *Treatise on Invertebrate Palaeontology* (edit. by Moore, R. C.), *P. Arthropoda*, 2 (Kansas, 1955).

[4] Hirst, S., *Ann. Mag. Nat. Hist.* (9), **12**, 455 (1923).

CHEMISTRY

Complex Compounds of Zero-valent Platinum

Recently, Chopoorian, Lewis and Nyholm[1] have indicated that the triphenylphosphine complexes of platinum(0) described by Malatesta and Cariello[2] are hydrides $[PtH_2(PPh_3)_n]$ (n = 2, 3 or 4), and question the existence of any platinum(0) complexes. Some years ago we prepared a compound $[Pt(Ph_2PCH_2CH_2PPh_2)_2]$. We have now examined it very carefully and conclude that it is indeed a complex of zero-valent platinum.

It is precipitated during the vigorous reaction which ensues on the addition of concentrated aqueous sodium borohydride solution to a hot ethanol/water (8 per cent) solution of $[Pt(Ph_2PCH_2CH_2PPh_2)_2]Cl_2$, and crystallizes from benzene/methanol in golden yellow plates, m.p. 252–255°, in an evacuated tube. It is a non-electrolyte of very low dipole moment, and oxidized only very slowly by air. The infra-red and nuclear magnetic resonance spectra show no sign of Pt—H bonds or hydridic hydrogen. On oxidation in benzene solution with an excess of iodine (3 mol.) and extraction with water no acidity is developed, as it would were the complex a hydrido-complex.

With Dr. H. R. Watson's assistance we have also obtained a purely aliphatic analogue $[Pt(Me_2PCH_2CH_2PMe_2)_2]$ by the reduction of the corresponding dipositive cation with sodium naphthalenide (NaC_8H_{10}) in tetrahydrofuran[3]. It is a colourless substance highly sensitive to air.

It is presumed that both these d^{10} complexes will have a tetrahedral configuration, corresponding to that of nickel carbonyl.

We thank Dr. N. Sheppard for examination of the nuclear magnetic resonance spectra.

J. Chatt
G. A. Rowe

Imperial Chemical Industries, Ltd.,
Heavy Organic Chemicals Division,
Akers Research Laboratories,
The Frythe,
Welwyn, Herts.

---

[1] Chopoorian, J. A., Lewis, J., and Nyholm, R. S., *Nature*, **190**, 528 (1961).

[2] Malatesta, L., and Cariello, Miss C., *J. Chem. Soc.*, 2323 (1958).

[3] Chatt, J., and Watson, H. R., *Nature*, **189**, 1003 (1961).

Reduction of Zirconium and Hafnium Oxides

MANY attempts have been made to reduce zirconium dioxide with hydrogen. At temperatures up to 1,500° C. no positive results have been reported[1-3]. Newbury and Pring[4] reported that at 2,500° C. and a hydrogen pressure of 150 atm. no reduction took place. Mott[5] stated that in an electric arc at 6,000° C. and at a hydrogen pressure of 22 atm. reduction was difficult but that sometimes a small amount of "yellow Zr_2O_3" was produced. In the reduction of ZrO_2 with lithium or calcium hydride it has been suggested that the active reducing agent is atomic hydrogen. Simonenko[6], using a d.c. arc between tungsten electrodes, in a hydrogen atmosphere, claimed to have produced metallic zirconium from ZrO_2 placed in the cathode cavity. He believed that the ions of molecular and atomic hydrogen were responsible for the reduction, their effectiveness being increased by the negative potential.

Atomic hydrogen produced by passing hydrogen at low pressure through an electromagnetic field of 29 Mc./s. or lower (Blackwood and McTaggart[7]) will reduce many oxides but not ZrO_2. However, it was found that if the frequency of the exciting field was increased to 2,500 Mc./s. a surface layer was formed which was black or golden. This material had a specific resistivity of the order of 1 ohm-cm. although measurements were difficult for obvious reasons. It has not been possible so far to determine the Zr:O ratio, but X-ray diffraction indicated that no change of phase occurred, the pattern remaining that of the dioxide. During treatments the temperature of the samples varied from about 200° C. to 1,200° C. depending on the power supplied (50–400 watts input to the magnetron).

Hafnium oxide, when treated in the same way, also became black and conducting. Thorium oxide remained unchanged. It would appear that a reduction took place in the case of ZrO_2 and HfO_2 similar to that occurring when TiO_2 is reduced in hydrogen at red heat. In the 2,500 Mc./s. field the energy normally possessed by hydrogen atoms is presumably increased by further excitation and ionization, as visualized by Simonenko, to a value which permits reduction of the above oxides to proceed.

F. K. McTAGGART

Commonwealth Scientific and
Industrial Research Organization,
Division of Mineral Chemistry,
Fishermen's Bend,
Victoria.

[1] Friederich, E., and Sittig, L., *Z. anorg. Chem.*, **143**, 293 (1925).

[2] Grube, G., and Ratsch, K., *Z. Electroch.*, **45**, 838 (1939).

[3] Meister, G., *Metal Progr.*, **53**, 515 (1948).

[4] Newbury, E., and Pring, J. N., *Proc. Roy. Soc.*, A, **92**, 276 (1916).

[5] Mott, W. R., *Trans. Amer. Electrochem. Soc.*, 31, 365 (1917).

[6] Simonenko, D. L., *Doklady Akad. Nauk S.S.R.* (2), **51**, 303 (1946).

[7] Blackwood, J. D., and McTaggart, F. K., *Austral. J. Chem.*, **12**, 114 (1959).

Irradiation Decomposition of Carbon Dioxide

DURING investigation of the reaction between carbon dioxide and graphite induced by radiation, we have examined the decomposition by irradiation of carbon dioxide in the absence of graphite. These irradiations have been carried out in transparent silica vessels into which the required amounts of carbon dioxide are condensed before sealing off. Particular care was taken to exclude organic contaminants by cleaning the silica vessels, wool and rods in hot chromic acid, followed by three washings in hot distilled water and firing at 1,000° C. for 2 hr. in an atmosphere enriched with oxygen. Carbon dioxide was prepared from 'Cardice', distilling three times before use, with rejection of first and last fractions. Mercury vapour was eliminated by the use of a trap at −78° C. placed immediately before the vessels to be filled. Analysis of the gas after irradiation was carried out by a gas chromatographic method[1]. The samples received a thermal neutron dose of $2\cdot3 \times 10^{18}$ n.cm.$^{-2}$ in the core of the Harwell reactor *Bepo*. From the integrated dose of thermal neutrons as measured by cobalt wires, the energy dose received by the carbon dioxide was calculated using the calorimetric data of Anderson and Waite[2].

Four general conclusions may be drawn from the results :

(1) The decomposition by irradiation of carbon dioxide in the absence of an oxygen acceptor is extremely small. (2) An increase in the surface area of the silica as effected by the introduction of silica wool into the vessels does not increase the efficiency of carbon monoxide production (the G_{CO} value). The higher G_{CO} values obtained using vessels packed with silica rods are inexplicable at present. (3) An increase in the carbon dioxide pressure up to 9·5 atmospheres at the operating temperature of 100° C. does not cause a major change in G_{CO}. (4) There is always a deficiency of oxygen compared with the carbon monoxide formed.

Table 1. RADIATION-INDUCED DECOMPOSITION OF CARBON DIOXIDE IN EMPTY AND PACKED SILICA VESSELS

Empty vessels, surface area ~ 50 cm.² filling pressure (at 0° C.) mm. mercury	G_{CO}	Rod-packed vessels, surface area ~ 200 cm.² filling pressure (at 0° C.) mm. mercury	G_{CO}	Wool-packed vessels surface area ~ 1,200 cm.² filling pressure (at 0° C.) mm. mercury	G_{CO}
42	<0·03	66	0·21	42	<0·007
81	<0·004	142	0·04	169	<0·002
158	<0·002	287	<0·009	307	<0·001
343	<0·001	596	<0·015	671	<0·001
629	<0·001				
807	<0·002				
1,761	<0·002				
2,130	<0·001				
3,237	<0·001				
3,967	<0·001				
5,327	<0·001				

The first of these conclusions is in agreement with the earlier work of Harteck and Dondes[3], but the second and third conclusions are in direct contradiction to their work. The formation of ozone in the radiolysis of carbon dioxide as postulated by Harteck and Dondes would explain the oxygen deficiency in the present work. This would also agree with the recent work of Mahan[4] on the photochemical decomposition of carbon dioxide.

It is hoped to publish more details of this work at a later date. The support and irradiation facilities of the United Kingdom Atomic Energy Authority are gratefully acknowledged.

T. B. COPESTAKE
N. S. CORNEY

General Electric Co., Ltd.,
Central Research Laboratories,
Hirst Research Centre,
Wembley, Middlesex.

[1] Konrath, Timms and Chirnside, *Analyst*, **83**, 600 (1958).

[2] Anderson and Waite, *A.E.R.E.-C/R* 2253 (1960).

[3] Harteck and Dondes, *J. Chem. Phys.*, **23**, 902 (1955); **26**, 1727 (1957).

[4] Mahan, *J. Chem. Phys.*, **33** 959 (1960.)

Mechanism of Synthesis of Adenine from Hydrogen Cyanide under Possible Primitive Earth Conditions

It has recently been shown that adenine and the purine precursors 4-aminoimidazole-5-carboxamidine and formamidine are formed spontaneously from hydrogen cyanide in water–ammonia systems under conditions assumed to have existed on the primitive Earth[1].

I wish to propose now a mechanism for the formation of adenine from hydrogen cyanide under the conditions of the above experiments which is in line with previous observations[2]. Adenine formation from hydrogen cyanide is thought to be initiated by base catalysis and to require the participation of 4-aminoimidazole-5-carboxamidine and formamidine as the key intermediates. The different steps involved in this synthesis are summarized in Fig. 1 and briefly described below. Direct experimental evidence for the last and most important step in this synthesis is also given below.

Reaction 1. In the presence of ammonium hydroxide, cyanide ion is formed which condenses with hydrogen cyanide to form iminoacetonitrile. Indirect evidence for the formation of iminoacetonitrile as the primary product of the base-catalysed hydrogen cyanide polycondensation has been obtained from kinetic studies on the formation of polymeric hydrogen cyanide[3].

Reaction 2. As soon as iminoacetonitrile is formed cyanide ion condenses with this compound to form aminomalonodinitrile. The transformation of hydrogen cyanide into a hydrogen cyanide trimer, presumed to be aminomalonodinitrile, although not completely elucidated, was claimed a long time ago by several investigators[4], and this trimer of hydrogen cyanide, of its isomer α,β-diiminopropionitrile, has also been considered as an intermediate in the formation of tetrameric hydrogen cyanide[5].

Reactions 3 *and* 4. Hydrogen cyanide as well as aminomalonodinitrile are ammonolysed into the corresponding amidines, formamidine and aminomalondiamidine. These reactions deserve no special comment, since the ammonolysis of nitriles is well known, and is in fact one of the main methods used in the preparation of amidines.

Reaction 5. Aminomalondiamidine condenses with formamidine to yield 4-aminoimidazole-5-carboxamidine. Indirect evidence for this reaction can be found in the very similar syntheses of 4-amino-5-substituted imidazoles carried out by other workers[6]. In the experiments carried out in my laboratory the appearance of 4-aminoimidazole-5-carboxamidine as well as formamidine among the reaction products was observed very early during the course of the hydrogen cyanide condensation reaction. In addition to 4-aminoimidazole-5-carboxamidine, 4-aminoimidazole-5-carboxamide was also isolated from the reaction mixture. The latter compound was observed to be formed by hydrolysis of the former, as was to be expected from the strongly basic conditions of the reaction mixture.

Reaction 6. In the final step, 4-aminoimidazole-5-carboxamidine condenses with another molar equivalent of formamidine to yield adenine. In support of this reaction, preliminary kinetic studies on the formation of formamidine and 4-aminoimidazole-5-carboxamidine have shown that these compounds make their appearance and disappearance in the reaction mixture as if they were intermediates of adenine. The cyclization of 4-aminoimidazole-5-carboxamidine with formamidine into adenine was thus considered the key step in the elucidation of the mechanism of adenine synthesis. For this reason, and because the reactants were easily available, an independent experimental demonstration of this reaction was undertaken.

This cyclization reaction was studied under different conditions of *p*H and carried out as follows: About 50 mgm. of 4-aminoimidazole-5-carboxamidine and 90 mgm. of formamidine hydrochloride were dissolved in 1 ml. of distilled water. Exactly 0·2 ml. aliquots of this solution were mixed with an equal volume of either water, 1 *M* disodium hydrogen phosphate, or 7 *N* ammonium hydroxide. The *p*H of the resulting reaction mixtures was approximately 1, 7 and 12 respectively. These three mixtures were heated in closed vessels at a temperature of 130° C. for 12 hr. At the end of the reaction time, a very small amount of residue was separated by centrifugation from each reaction product and each of the supernatants was analysed by paper chromatography and ultra-violet

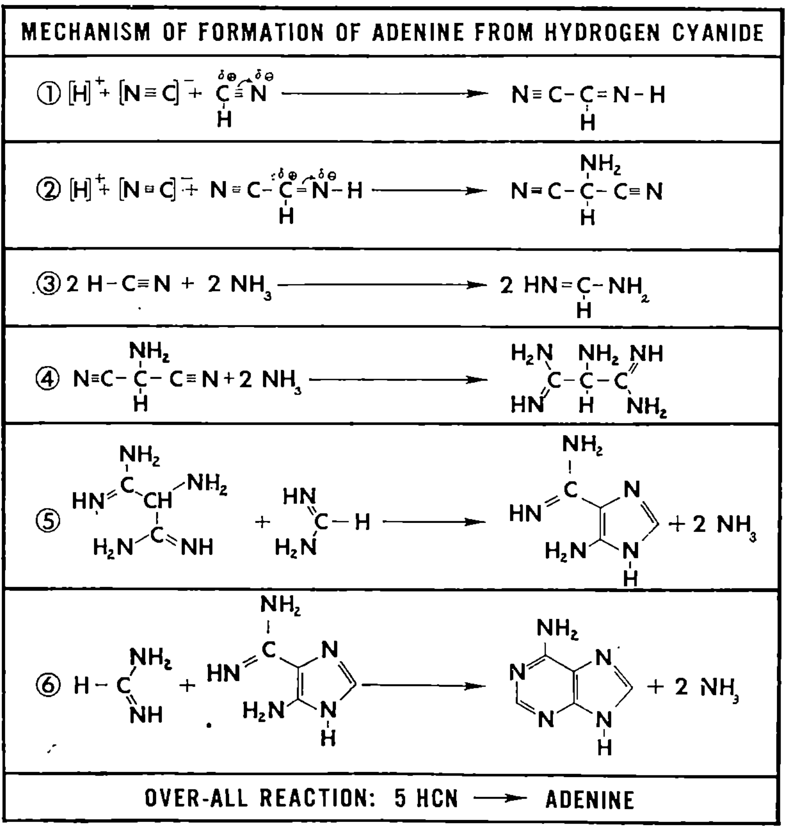

MECHANISM OF FORMATION OF ADENINE FROM HYDROGEN CYANIDE
①
②
③
④
⑤
⑥
OVER-ALL REACTION: 5 HCN → ADENINE

Fig. 1

spectrophotometry as described previously[1]. In all cases adenine was formed in high yield. Some unreacted 4-aminoimidazole-5-carboxamidine was observed in the acid and neutral reaction mixtures, but none in the basic reaction mixture. The experiment has been repeated recently at lower temperatures, and similar results have been obtained.

Although alternative mechanisms cannot yet be ruled out, the evidence obtained in my laboratory as well as recent evidence on the so-called 'one-step' synthesis of purines[7] supports the imidazole pathway outlined in Fig. 1 as a mechanism for the synthesis of adenine from hydrogen cyanide under possible primitive Earth conditions.

Work is being carried out at present on the isolation of the three-carbon compounds assumed to be the precursors of the 4-amino-5-substituted imidazoles isolated, and also on the synthesis of other purines from hydrogen cyanide.

This work was supported in part by research grant No. G-13117 from the National Science Foundation.

J. ORÓ

Chemistry Department,
University of Houston,
Houston 4,
Texas.

[1] Oró, J., *Biochem. Biophys. Research Comm.*, **2**, 407 (1960); Oró, J., *Nature*, **190**, 389 (1961); Oró, J., and Kimball, A. P., *Arch. Biochem. Biophys.* (in the press).

[2] Grossman, F., Cristopher, J., and Canavan, G., in *Proc. Nat. Sci. Found. Summer Inst. for High School Juniors*, 8 (University of Houston, 1960). Oró, J., *Fed. Proc.*, **20**, 352 (1961); *Proc. Fifth Intern. Cong. Biochem., Moscow* (in the press).

[3] Völker, T., *Angew. Chem.*, **62**, 728 (1957); *Angew. Chem.*, **72**, 379 (1960).

[4] Lange, O., *Ber.*, **6**, 99 (1873); Wippermann, R., *Ber.*, **7**, 767 (1874). See also: Long, D. A., and George, W. D., *Proc. Chem. Soc.*, 285 (1960).

[5] Bredereck, H., Schmötzer, G., and Becher, H. S., *Ann.*, **600**, 87 (1956).

[6] Richter, E., Loeffler, J. E., and Taylor, E. C., *J. Amer. Chem. Soc.*, **82**, 3144 (1960).

[7] Taylor, E. C., and Cheng, C. C., *Tetrahedron Letters*, No. 12, 9 (1959).

A New Phase formed by High-Pressure Treatment : Face-centred Cubic Molybdenum Monocarbide

THE attempted preparation and retention of polymorphs dependent on pressure is an important area of high-pressure research. Some of these phases are found in Nature; others were unknown prior to the recent development of equipment suitable for research at high pressure and high temperature.

Although high-pressure phases are thermodynamically unstable at ambient conditions, a few have been retained by quenching to room temperature under pressure. High-pressure polymorphs found in the Earth's crust[1] include several minerals, for example, the pyroxene jadeite, $NaAlSi_2O_6$ (ref. 2); kyanite, Al_2SiO_5 (ref. 3); and diamond (ref. 4). Previously unknown phases are coesite[5], a form of SiO_2 with a higher density than quartz; borazon[6], a cubic form of boron nitride; and several compounds of the garnet type[1]. A number of high-pressure polymorphs of pure metals, which unlike the above compounds cannot be retained at ambient conditions, have been reported[7].

High-pressure experiments on the molybdenum–carbon system have revealed a new form for the monocarbide phase. The new form, designated α-MoC, has been retained to atmospheric pressure. Synthesis was achieved from both an equiatomic mixture of molybdenum and carbon and an equiatomic mixture of the compound Mo_2C and carbon at pressures and temperatures in the range of 40–70 kilobars and 1,800°–2,500° C. The X-ray diffraction pattern for α-MoC showed the first ten lines for a face-centred cubic structure. No additional lines were observed. The average lattice parameter was $a_0 = 4{\cdot}27$ Å. In addition to high-pressure experiments in which the only product was α-MoC, other experiments carried out at lower temperatures and pressures afforded evidence for all the phases reported[8] for the molybdenum–carbon system at atmospheric pressure. A summary of the lattice parameters of the various phases is given in Table 1.

Table 1. COMPOSITION AND DIMENSIONS OF PHASES IN THE MOLYBDENUM–CARBON SYSTEM

Composition	Symbol	X-ray data Structure	a_0(Å.)	c_0(Å.)	c_0/a_0	Molybdenum atoms/unit cell
Mo_2C	β	hexagonal closest packing	3·002	4·724	1·574	2
MoC	γ	hexagonal	2·898	2·809	0·969	1
MoC	γ'	hexagonal	2·932	10·97	3·742	4
MoC	η	complex hexagonal closest packing	3·00	14·58	4·86	6
MoC	α	face-centred cubic (sodium chloride)	4·27	—	—	4

Further experimental detail and a thermodynamic analysis of the effect of pressure on the equiatomic region of the molybdenum–carbon system will be published elsewhere.

This work was sponsored by the Metals and Ceramics Laboratory, Materials Centre, Wright Air Development Division, Air Research and Development Command, United States Air Force.

E. V. CLOUGHERTY
K. H. LOTHROP
J. A. KAFALAS *

Research Division,
Manufacturing Laboratories,
Cambridge,
Massachusetts.

* Present address: Lincoln Laboratory, Massachusetts Institute of Technology, Lexington, Massachusetts.

[1] Clark, jun., S. P., *Conference on Very High Pressure* (J. Wiley and Sons, New York, 1961).

[2] Robertson, E. C., Birch, F., and MacDonald, G. J. F., *Amer. J. Sci.*, **255**, 115 (1957).

[3] Clark, jun., S. P., Robertson, E. C. and Birch, F., *Amer. J. Sci.*, **255**, 628 (1957).

[4] Bovenkerk, H. P., *et al.*, *Nature*, **184**, 1094 (1959).

[5] Coes, jun., L., *Science*, **118**, 131 (1953).

[6] Wentorf, jun., R. H., *J. Chem. Phys.*, **26**, 956 (1957); *Conference on Very High Pressure* (J. Wiley and Sons, New York, 1961).

[7] Bridgman, P. W., *The Physics of High Pressure* (G. Bell and Sons, Ltd., London, 1952). Kaufman, L., in *Solids under Pressure* (McGraw-Hill Book Co., New York, 1962).

[8] Pearson, W. B., *A Handbook of Lattice Spacings and Structures of Metals and Alloys* (Pergamon Press, London, 1958).

Generation of Radicals in Pairs and the Kinetics of Radical Chain Reactions in Solution

FREE radicals responsible for the propagation of chain reactions are commonly formed by the photochemical or thermal decomposition of an appropriate labile substance, and are, in such cases, inevitably produced in pairs. The restricted mobilities of radicals in solution have some special influence on the fate of these radicals, in that many pairs of radicals recombine before bringing about any reaction[1]. This effect, sometimes known as the cage effect but more recently called[2] geminate recombination, has been widely discussed. A further consequence of the production of radicals in pairs has not, however, received consideration. Even if a free radical avoids recombination with its twin radical in the primary cage, the chain carrier will in general, at least in the earlier part of its life, lie closer to its twin than to another chain carrier. There is, therefore, a finite and sometimes appreciable probability of the mutual termination of geminate chains. The proportion of mutual termination of geminate chains must depend, on one hand, on factors controlling the rate of diffusion of the radicals in the medium and, on the other, the average relative distances of a radical to its twin and other radicals, and this will depend on the rate of decomposition of the initiating molecule.

The kinetic effects of recombination of geminate chains is best demonstrated by considering the extreme case where the various pairs of chains are so isolated that termination occurs exclusively by reaction between geminate chains. The rate of propagation of the reaction is then:

$$k_p\,(A)\,2n$$

where n is the number of geminate pairs of chains (expressed in terms of moles/l.), and k_p and (A) are respectively the rate coefficient for the propagation reaction and the molar concentration of the substrate:

$$n = I\tau$$

where τ is an average lifetime of a pair of geminate chains with respect to their mutual termination and I is the rate of decomposition of the initiator molecule. The rate of propagation of the reaction in the case of long chains is:

$$2\,k_p\,I(A)\tau$$

Since τ is determined only by diffusion factors, the rate of reaction is proportional to the first power of the rate of initiation. Normally in reactions in which chains terminate in bimolecular processes the rate of propagation depends on the square root of the rate of initiation[3].

In general there will be competition between the geminate chain termination and normal bimolecular termination, in which case, assuming a stationary state in the overall concentration of radicals (R):

$$\mathrm{d}(R)/\mathrm{d}t = 0 = I - 2k_t(R)^2 - 2(R)/\tau$$

where k_t is the rate coefficient for the bimolecular reaction between radicals of non-geminate chains and τ is now the mean lifetime of chains which terminate geminately. The rate of propagation of the chain reaction is:

$$k_p(R)(A)$$

The rate of propagation will, therefore, be a complicated function of the rate of initiation, but over a restricted range it may appear to depend on a power of I that lies between one-half and one.

In most systems the exponent of the rate of initiation in the rate equation is close to one-half[3], and in these cases geminate chain termination must be unimportant. However, systems are known where the exponent is significantly higher, notably the later stages of radical polymerization[4,5]. Among effects that can formally lead to first-order dependence on I are degradative transfer[6] and trapping of radicals[7]. In the former case it is difficult to visualize an appropriate reaction that is operative only at high conversion, and this, together with the observed electron-spin resonance spectra of trapped radicals[8,9] at very high conversions, has given support to the second explanation. This explanation is itself not without difficulties as the exponent begins to rise relatively early in the polymerization when the medium is far from rigid, and small radicals, particularly primary radicals, termination by which leads to an exponent less than one-half, are still mobile. Geminate chain termination can account formally for high-conversion kinetics and at the same time offers a plausible physical model in that the diffusing apart of growing geminate radicals must become severely restricted as the gel structure develops.

P. E. M. ALLEN
C. R. PATRICK

Chemistry Department,
The University,
Birmingham, 15.

[1] Rabinowitch, E., *Trans. Farad. Soc.*, **33**, 1225 (1935).

[2] Noyes, R. M., *J. Amer. Chem. Soc.*, **77**, 2042 (1955).

[3] Walling, C., *Free Radicals in Solution* (Wiley, New York, 1958).

[4] Bengough, W. I., and Melville, H. W., *Proc. Roy. Soc.*, A, **230**, 429 (1955).

[5] Hayden, P., and Melville, Sir Harry, *J. Polymer Sci.*, **43**, 201 (1960).

[6] Allen, P. W., Merrett, F. W., and Scanlan, J., *Trans. Farad. Soc.*, **51**, 95 (1955).

[7] Burnett, G. M., and Loan, L. D., *Coll. Czech. Chem. Comm.*, **22**, 113 (1957).

[8] Bamford, C. H., Jenkins, A. D., Ingram, D. J. E., and Symons, M. C. R., *Nature*, **175**, 894 (1955).

[9] Atherton, N. M., Melville, H. W., and Whiffen, D. H., *J. Polymer Sci.*, **34**, 199 (1959).

Integration with Ionization Detectors in Gas Chromatography

THE ionization current of the hydrogen flame ionization detector consists of a standing current and a current depending on the concentration of organic components emerging from the column. The standing current i_0 is constant when carrier gas velocity and column temperature are constant throughout the experiment. The ionization current due to organic compounds separated on the chromatographic column is a variable and will be denoted by $i(t)$.

The ionization current may, to some extent, depend on constructional parameters, but the voltage

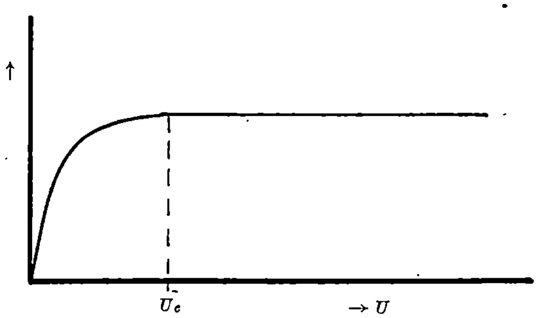


Fig. 1. Voltage-current characteristic of an ionization detector

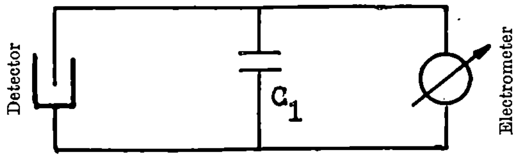


Fig. 2. Principle of the integrator

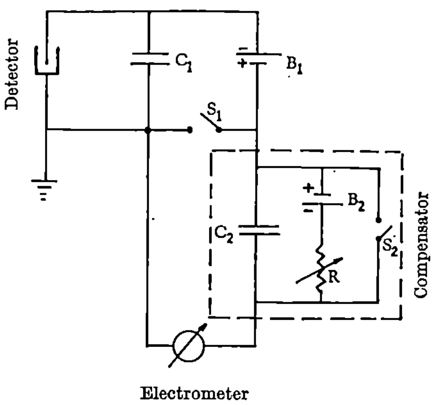


Fig. 3. Integrator with compensation. The values of the batteries, capacities and resistance given are approximate and may be varied with the operating conditions. C_1, 1,000 pF.; C_2, 100 μF.; B_1 and B_2, 90 V.; R, 100 $M\Omega$

across the electrodes has very little influence on the ionization current so long as the potential difference exceeds the value U_c in Fig. 1[1,2]. Therefore the usual constant-voltage source may be replaced by a condenser C_1 (Fig. 2).

During the experiment this condenser will be discharged and its potential drop over the time interval t_1–t_2 will be given by:

$$\Delta U = -\frac{1}{C_1}\int_{t_1}^{t_2}(i_0 + i(t))\,dt \tag{1}$$

where C_1 is the capacity of the condenser.

When the detector response is linear with concentration, $-\frac{1}{C_1}\int_{t_1}^{t_2} i(t)\,dt$ will be proportional to the amount of an organic compound leaving the chromatographic column, while $-\frac{1}{C_1}\int_{t_1}^{t_2} i_0\,dt$ will be equal to $-\frac{i_0}{C_1}\cdot(t_2 - t_1)$ if the standing current can be considered as a constant.

Leakage of detector and condenser due to imperfect insulation will cause a current which is dependent on the voltage. This leakage, however, can be made small and almost independent of the voltage if ΔU is kept small as compared with U and very good insulation is being used. The leakage is then to be considered as part of i_0.

As one is only interested in $-\frac{1}{C_1}\int_{t_1}^{t_2} i(t)\,dt$, it is essential to separate the two parts of the ionization current. This can be done by using the arrangement shown in Fig. 3, where the standing current is compensated by charging a second condenser C_2 at a rate corresponding to that at which the voltage across C_1 decreases due to the standing current.

If the signal of the electrometer is registered on a potentiometric recorder, the initial conditions can be established again by operating the switches S_1 and S_2 simultaneously through the end contact of the recorder.

The integrator may be used, in principle, with any other ionization detector having a current-voltage characteristic as shown in Fig. 1, such as the argon detector[3].

P. M. E. M. van der Grinten
A. Dijkstra

Central Laboratory,
Staatsmijnen Geleen,
The Netherlands.

[1] Onkiehong, L., *Gas Chromatography*, edit. by Scott, R. P. W., 7 (1960).

[2] Desty, D. H., Geach, C. J., and Goldup, A., *Gas Chromatography* edit. by Scott, R. P. W., 46 (1960).

[3] Lovelock, J. E., *Anal. Chem.*, 33, 161 (1961).

BIOCHEMISTRY

Non-Random Occurrence of Amino-Acid Replacements

One approach to elucidating the genetic code is to study its manifestations in the primary structure of proteins. Of particular interest in this respect are amino-acid replacement regions, that is, those few positions in a protein chain where 'identical' proteins isolated from different species differ in amino-acid composition, differences presumably arising from mutations in the underlying genetic apparatus[1]. For example, insulin *A* chains isolated from beef, rabbit, whale, etc., have identical amino-acid sequences except at positions 8–10, where certain differences in amino-acid composition occur[2].

Sufficient data are now available so that we can begin to characterize amino-acid replacements to some degree, and hopefully then begin to understand more about the nature of the underlying genetic coding. The first characteristic of amino-acid replacements to note is that replacements resemble burst errors; that is, given that one replacement (error) has occurred, there is a greatly increased probability that another replacement (error) will occur at an adjacent position in the protein chain. Examples of this are the 8–10 positions in the insulin chain already referred to and the 32–39 positions in the adrenocorticotropin molecule[3]. It is not possible to say whether the replacements in such a region all occurred simultaneously, but this seems a possibility. Recent experiments on chemically induced mutants in tobacco mosaic virus could help to elucidate this point[4]. A second characteristic of amino-acid replacements is shown in Table 1. Here amino-acids are listed in order of their abundance in the proteins which contain known replacements. From columns *A* and *B* it is evident that there is no striking tendency for certain amino-acids to be preferred over others, either immediately following replacement regions or actually involved in them. (The terms 'following' and 'preceding' are defined so that a given amino-acid 'precedes' amino-acid further than itself from the amino end of the molecule. A 'replacement region' is any contiguous section of a protein chain, one or more amino-acids in length, where replacements have been recorded at all positions within the region.) In contrast, there is a strong tendency for amino-acid replacement regions to be preceded by one of the four amino-acids glycine, proline, tyrosine, or cysteine. None of these four amino-acids is in the top 30 per cent in abundance, and only glycine is in the top 50 per cent in abundance. One would expect, on a

Table 1. Frequency of occurrence of amino-acids (*A*) in, (*B*) immediately following, and (*C*) immediately preceding replacement regions; data are those collected by Ycas (ref. 8). Amino-acids are listed according to order of abundance in the proteins which contain replacement regions. Only those replacements which do not occur at either terminal position of a protein chain are tabulated

Amino-acids in order of abundance	*A* Involved in replacement region	*B* Immediately following replacement region	*C* Immediately preceding replacement region	$\frac{C}{A+B}$
1. glu	11	3	1	0·07
2. ala	9	3	0	< 0·1
3. asp	5	1	1	0·2
4. ser	10	0	0	< 0·1
5. val	4	1	1	0·2
6. lys	7	1	0	< 0·2
7. gly	3	2	5	*1·0*
8. leu	5	1	0	< 0·2
9. thr	3	0	1	0·3
10. cys	0	2	2	*1·0*
11. pro	3	1	5	*1·3*
12. arg	3	1	0	< 0·3
13. tyr	0	1	3	*3·0*
14. phe	1	0	0	—
15. his	1	1	0	< 0·5
16. ilu	3	0	0	< 0·3
17. met	0	1	0	—
18. try	0	0	0	—

random-choice basis, one of these four amino-acids to precede replacement regions in one-fifth to one-quarter of cases, but the observed frequency is four-fifths. In summary, then, amino-acid replacements are possibly burst errors and have a high probability of being preceded by one of four other amino-acids.

If one attempts to find other genetic phenomena having characteristics similar to those of amino-acid replacements, at least two are found. One is the occurrence of genetic 'hot spots' in the saturation genetic mapping studies on T_4 bacteriophage[5]. These 'hot spots' are 'points' in a T_4 genetic map where about 100 mutants are recorded. At other points in such a map only one or at most a few mutants have been found. This phenomenon recalls the likelihood of amino-acid replacements to occur at certain regions in a protein chain. (There are about ten replacements recorded in the various examples of the insulin *A* chain, all at positions 8–10 (ref. 2).)

The second genetic phenomenon in ways resembling amino-acid replacements again arises in connexion with the T_4 genetic map. Specifically, certain mutations, termed 'deletions', occupy a considerable distance on the linear genetic map[5]. There is no evidence to our knowledge that a segment of the bacteriophage genome has actually been removed in these cases. It is evident from the above discussion that some 'deletions' could also be burst errors.

In that genetic 'hot spots' and 'deletions' both have characteristics similar to those of amino-acid replacements, we should attempt a unified explanation for all three. Recently, Golomb has suggested a coding scheme which appears to account for genetic 'hot spots'[6]. On this scheme the origin of a hot spot is in the make-up of certain code words in an error-correcting, 'biorthogonal', comma-free, nucleotide sextuplate code. In that this code is comma-free (therefore, no influence of one code word on neighbouring ones), it does not explain either of the characteristics associated with amino-acid replacements (burst error aspect or high probability of following one of four amino-acids), so it is not a unified explanation of all three phenomena.

It seems difficult in the present state of our knowledge to present an explanation of these characteristics of amino-acid replacements, but attempts to do so will force us to consider many genetic coding mechanisms now given little or no serious attention. For example, the question of whether an amino-acid template includes an adjacent amino-acid residue in addition to nucleotides is raised. The possibility that an error in protein synthesis can result in a mutation is also suggested. (Stent *et al.* have been collecting evidence that replication of deoxyribonucleic acid intimately involves very specific protein synthesis[7].) Perhaps overlapping degenerate coding schemes should be investigated. These results also indicate that genetic error-correcting mechanisms may exist in the cell.

Recent attempts by me to develop a nucleotide triplet code based on the composition of the RNA viruses have given a suggestion as to how an explanation of these phenomena might be developed, and, at the same time, become reconciled in part with existing concepts of genetic coding[9,10]. When such a nucleotide triplet code was devised, it turned out that most of the triplets could be included in a comma-free system. However, a few of them could not fit such a system; notable among the exceptions were the triplets corresponding to the amino-acids proline, tyrosine, and cysteine (that is, three of the four amino-acids which precede replacement regions with a high probability). The cardinal characteristic of a comma-free code is that 'displaced words', words formed from adjacent letters in two adjacent coding words (for example, if *ABC* and *DAB* were two adjacent coding words, then *BCD* and *CDA* are displaced words), are never coding words also. This suggests that associated with the amino-acids having a high probability of preceding replacement regions are nucleotide coding words capable of producing some displaced words which themselves are coding words. For example, overlap between *CCC* and *CXX* produces the displaced word *CCC*, which is also a coding word. Now, where the displaced words, like that in the example, are the same as one of the code words producing them, we might have a mechanism whereby displaced words used mistakenly by the cell as code words initiate burst errors, resulting in replacement regions which have a high probability of being preceded by certain amino-acids. Such a mechanism as briefly sketched here would, of course, necessitate postulating also cellular mechanisms which cope with, eventually terminate, such a burst error without interrupting synthesis of a protein chain, and a feedback mechanism whereby an error in certain protein synthesis eventually produces a genetic error (mutation). (The previously cited work of Stent *et al.* makes this suggestion not too improbable.) Though these concomitant postulates appear somewhat extreme at present, it seems as though an explanation of errors producing amino-acid replacements may force us to think in now unfamiliar terms.

C. R. Woese

General Electric Research Laboratory,
Schenectady, New York.

[1] Levinthal, C., *Rev. Mod. Phys.*, **31**, 249 (1959).
[2] Sanger, F., *Brit. Med. Bull.*, **16**, 183 (1960).
[3] Li, C., Dixon, J., and Chung, D., *J. Amer. Chem. Soc.*, **80**, 2587 (1958).
[4] Tsugita, A., and Fraenkel-Conrat, H., *Proc. U.S. Nat. Acad. Sci.*, **46**, 636 (1960).
[5] Benzer, S., *Proc. U.S. Nat. Acad. Sci.*, **41**, 344 (1955).
[6] Golomb, S., *Symp. Mathematical Problems in Biol. Sci.*, Amer. Math. Soc. Pub. (in the press).
[7] Uchida, H., and Stent, G., *J. Mol. Biol.*, **2**, 262 (1960).
[8] Ycas, M., *J. Theor. Biol.*, **2**, 244 (1961).
[9] Woese, C., *Nature*, **190**, 697 (1961).
[10] Woese, C., *Biochem. and Biophys. Res. Comm.*, **5**, 88 (1961).

A Simplified Method for separating Amino-acid Mixtures from Protein Hydrolysates on One Ion-Exchange Column during Preparation

MOST of the methods used for the separation of mixtures of amino-acids are based on the work of Moore *et al.*[1–3]. Separation is achieved either on two columns, acidic amino-acids being separated on a 'Dowex' 1 anion-exchange column and the basic and neutral amino-acids on a 'Dowex' 50 cation-exchange column or by chromatography on one column on 'Dowex' 50 in the H-cycle, elution being carried out with citrate phosphate buffer solutions. Hamilton and Anderson[4] recently described a semi-automatic method for these separations containing several improvements of the original process. These methods ensure good separation of amino-acids; the eluates, however, contain either the hydrochlorides of the amino-acid and hydrochloric acid or considerable amounts of salts, which are difficult to remove. An efficient method for the separation of amino-acids should permit separation in a small volume of the elution reagent in a short time; the buffers used should be removable by a simple method. Wilcox *et al.*[5] used volatile buffers for the separation of aspartic and glutamic acid on an anion-exchange column. Vaněček *et al.*[6] and Tomášek[7], used volatile buffers successfully for the separation of peptides on 'Dowex' 50.

In connexion with our work[8,9] on the preparation of amino-acids-^{14}C from proteins containing carbon-14 obtained from a photosynthesizing organism, we elaborated a method fulfilling the requirements mentioned here.

Separation of the amino-acids from the protein hydrolysate was carried out on one 'Dowex' 50 4 × 200–400 mesh column. A column of 2·0 × 150 cm. was operated at 37° C. Amounts of amino-acids of 600 mgm. were separated on this column. The ion-exchange column was prepared according to Tomášek[7], that is, from the H-cycle transferred to the pyridine cycle and equilibrated with a 0·1 *M* pyridine formate buffer solution of *p*H 3·1. Elution was carried out by the gradient system. The buffers used are listed in Table 1. The effluent was collected by an automatic fraction collector in 5–20 min. intervals and at an effluent-rate of 7·5 ml./10 min. The fractions were identified by chromatography and autoradiography and the radioactivity of the suitably combined fractions determined. A typical result of the separation is given in Fig. 1. The amino-acids were obtained in about 1,200 ml. of the eluate, the total volume being 2·5–3 l. All the amino-acids were obtained in pure form except serine–proline and alanine–glycine, which were eluted in pairs and have to be separated chromatographically. The pure amino-acids were isolated in the free form by repeated evaporation of the aqueous solution *in vacuo*. The yield of pure amino-acids amounted to 75 per cent of the radioactivity of the hydrolysate. The method is easily reproducible.

Table 1. COMPOSITION OF THE BUFFER SOLUTION

Buffer	Pyridine (mole)	*p*H	*p*H adjusted with
1	0·1	3·1	formic acid
2	0·2	3·1	formic acid
3	0·2	4·4	acetic acid
4	1·0	5·1	acetic acid
5	2·0	5·1	acetic acid

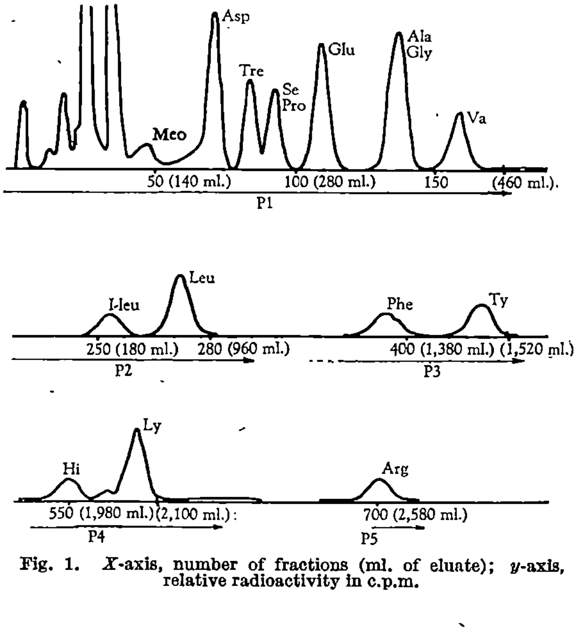


Fig. 1. *X*-axis, number of fractions (ml. of eluate); *y*-axis, relative radioactivity in c.p.m.

As this method is simple to perform and gives good separation results, it should be generally applicable to the separation of amino-acids from protein hydrolysates.

J. LIEBSTER
K. KOPOLDOVÁ
M. DOBIÁŠOVÁ

Radioisotope Laboratories,
Biological Institute,
Czechoslovak Academy of Sciences,
Na Cvičišti 2, Prague, 6.

[1] Moore, S., and Stein, W. H., *J. Biol. Chem.*, 192, 666 (1951).

[2] Hirs, C. H. W., Moore, S., and Stein, W. H., *J. Amer. Chem. Soc.*, **76**, 6063 (1954).

[3] Moore, S., and Stein, W. H., *J. Biol. Chem.*, **211**, 893 (1954).

[4] Hamilton, P. B., and Anderson, R. A., *Anal. Chem.*, **31**, 1505 (1959).

[5] Wilcox, P. E., Cohen, E., and Wen Tan, *J. Biol Chem.*, **228**, 999 (1957).

[6] Vaněček, J., Meloun, B., Kostka, V., Keil, B., and Šorm, F., *Collection*, **25**, 2358 (1960).

[7] Tomášek, V., Holejšovský, V., Mikeš, O., and Šorm, F., *Collection* (in the press).

[8] Liebster, J., Kopoldová, J., Dobiášová, M., and Kozel, J., I, II, III, *Collection*, **26** (1961).

[9] Liebster, J., Dobiášova, M., and Kopoldová, J., Czech. Patent App. *PV* 4476-60.

Oxidative Demethylation and Carcinogenicity of 4-N-Dimethylaminoazobenzene Derivatives

RECENT work on the hepatocarcinogenic amino-azo compounds has shown that the presence of at least one N-methyl group and the formation of a firmly bound compound between metabolites of the azo compound and rat-liver proteins are probably necessary for the strong activity[1]. The biochemical behaviour of N-methyl groups with respect to the protein-bound polar dye formation has not yet been elucidated satisfactorily, because of the structural complexity and very little amounts of polar dye obtained. We showed that the N-^{14}C-methyl group of 4-N-^{14}C-methylaminoazobenzene was incorporated almost quantitatively into the polar dye[2]. The N-methyl group of 4-N-methylaminoazobenzene (MAB), being one of the most reactive sites and rapidly demethylated to split formaldehyde, appears to be responsible for the polar dye formation in an active metabolic intermediate.

N-Demethylation, which is one of the major metabolic pathways of 4-N-dimethylaminoazobenzene

(DAB), MAB and their derivatives[3], is carried out by liver microsomes and requires both reduced triphosphopyridine nucleotide and free oxygen[4], though a clear picture of its precise mechanism is not yet established. Unpublished investigations in this laboratory have shown that the system consisting of the iron chelate of ethylenediaminetetraacetic acid (EDTA), ascorbic acid and free oxygen, which was established as a hydroxylase model by Udenfriend[5], is also suitable for the demethylase model. The present communication demonstrates that this system demethylates ring-methylated derivatives of DAB to their MAB analogues, and the rate of reaction is correlated with the π electron density[6] at N-dimethylamino nitrogen.

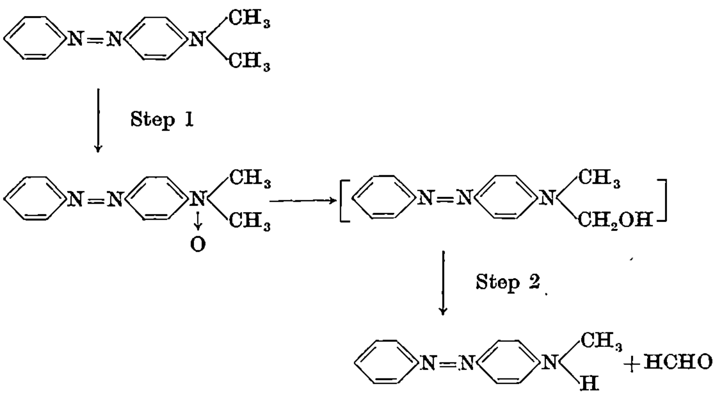


Fig. 2. Proposed mechanism of oxidative demethylation of DAB

The reaction system given in Table 1 was incubated in the metabolic shaker, designed for the purpose, for 4 hr. at 40° in an atmosphere of oxygen (Po_2 = 785 mm. mercury). The reaction was terminated by adding 50 ml. of 0·5 per cent sodium bicarbonate solution and the dyes were extracted three times with chloroform. Chloroform was evaporated under reduced pressure, and the residue dissolved in light petroleum (boiling point 60°–80°) was subjected to chromatography using an alumina column, in order to separate the demethylated products from the substrate. Demethylation, as measured by the amounts of MAB derivatives produced, depended on pH and temperature. As the reaction did not occur in the absence of oxygen, the system may function as an oxygenase.

Table 1. DEMETHYLATION OF DAB DERIVATIVES

Compound	Rate of demethylation (10^{-6} moles/4 hr.)	Carcinogenicity (ref. 3)
4′-Me-DAB	1·42 ± 0·11	1
3′-Me-DAB	1·12 ± 0·08	10 ~ 12
2′-Me-DAB	1·41 ± 0·08	2 ~ 3
DAB	1·18 ± 0·08	6
2-Me-DAB	1·36 ± 0·04	0
3-Me-DAB	0·843 ± 0·043	0

The system contained $1·5 \times 10^{-5}$ moles of azo dyes, $1·5 \times 10^{-6}$ moles ferrous ion as ferrous ammonium sulphate, $3·0 \times 10^{-5}$ moles EDTA, $3·0 \times 10^{-4}$ moles ascorbic acid, and $10 \times 1·0^{-3}$ moles phosphate buffer, pH 7·3, in a total volume of 50 ml. (30 per cent acetone solution).

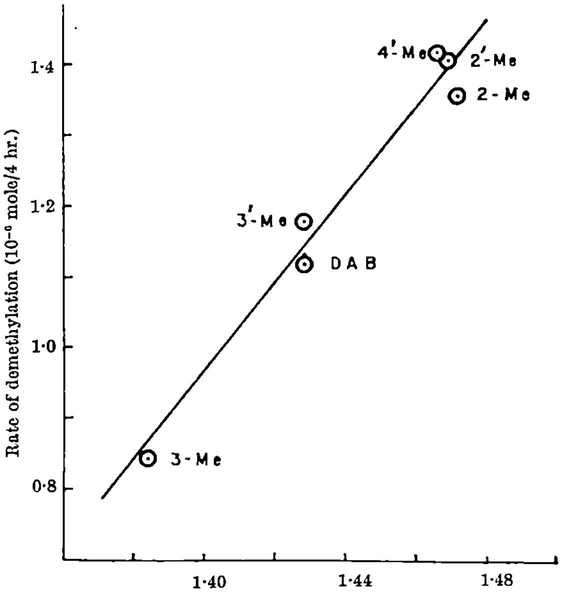


Fig. 1. Relation between the rate of demethylation and total π electron density at the dimethylamino nitrogen of DAB derivatives

The rate of demethylation was shown to be satisfactorily correlated with the π electron density at N-dimethylamino nitrogen, but not with the carcinogenicity (Fig. 1 and Table 1). However, it is interesting to see that strong carcinogens such as DAB and 3′-methyl DAB show moderate values of the reaction-rate. 4-N-Dimethylaminoazoxybenzene amino-oxide, which was assumed to be an analogue of the active intermediate, could be demethylated quickly to MAB in the presence of ferrous ion alone, and formaldehyde was detected. With the same conditions, 4-N-dimethylaminoazoxybenzene was resistant to the oxidative demethylation. The active oxygen thus appears to attack first the nitrogen atom of the dimethylamino group (electronegative centre), and forms the unstable amino-oxide, which seems to be rearranged in turn to split off formaldehyde (Fig. 2). According to the relation shown in Fig. 1, step 1 in Fig. 2 may determine the rate.

We thank Prof. H. Terayama, University of Tokyo, for his advice and interest in our work.

MORIZO ISHIDATE
AKIRA HANAKI*

Faculty of Pharmaceutical Science,
University of Tokyo.

---

* Present address: National Institute of Radiological Sciences, Chiba, Japan.

[1] Miller, E. C., and Miller, J. A., *Cancer Res.*, **7**, 468 (1947).

[2] Terayama, H., Hanaki, A., and Ishidate, M., *Gann*, **51**, No. 4 (in the press).

[3] Miller, E. C., and Miller, J. A., *Adv. in Cancer Res.*, **1**, 339 (1953).

[4] Conney, A. H., Brown, R. R., Miller, J. A., and Miller, E. C., *Cancer Res.*, **17**, 628 (1957).

[5] Udenfriend, S., Clark, C. T., Axelrod, J., and Brodie, B. B., *J. Biol. Chem.*, **208**, 731 (1954).

[6] Fukui, K., Nagata, C., Yonezawa, T., Itano, Y., and Imamura, A., *Gann*, **51**, 67 (1960).

Chromatographic Fractionation of Hog Thyroglobulin

DERRIEN *et al.*[1,2] have purified thyroglobulin, the main protein component of thyroid, in a native state, and stated that their preparation was ultracentrifugally as well as electrophoretically homogeneous. However, since the iodine content of thyroglobulin varies with preparations, especially with

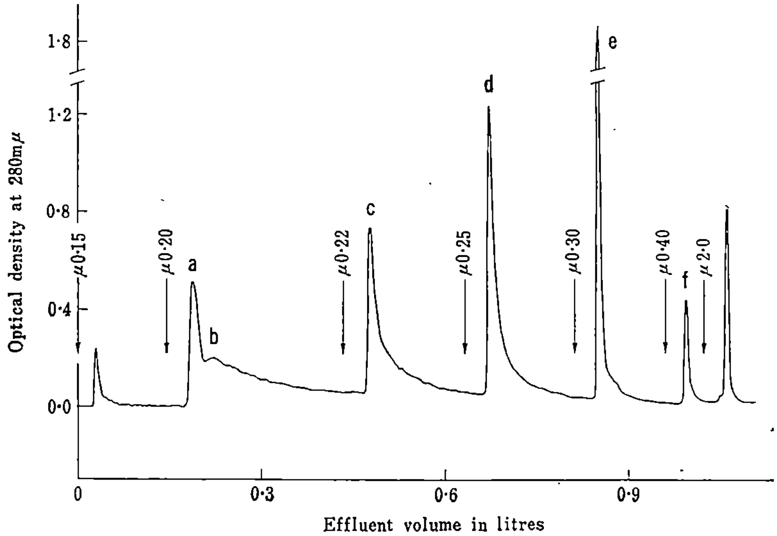


Fig. 1. Chromatographic diagram of hog thyroglobulin on *DEAE*-cellulose. Vertical arrows indicate the point of application of eluants. 1/*N* for the peaks are: *a*, 25; *b*, 27; *c*, 30,; *d*, 34; *e*, 39; *f*, 35

different physiological states of animals from which it is prepared, it is reasonable to assume that thyroglobulin is not a single protein, but consists of proteins iodinated to different degrees.

The following evidence, obtained in our laboratory (Ui, N., and Tarutani, O., work to be published), also indicates inhomogeneity of thyroglobulin. Ultracentrifugal analyses showed, in agreement with the findings of other investigators[3], the presence of a greater or lesser amount of a faster sedimenting component ($S^0_{20,w}$ = 28 S.) in the preparations. This component, tentatively named *F*-component, could not be distinguished from the main component ($S^0_{20,w}$ = 19 S.) with respect to solubility characteristics. In moving-boundary electrophoresis, although the preparation always showed a single boundary, the heterogeneity was indicated by the 'reversible boundary-spreading' technique[4], even with a preparation essentially free from the *F*-component which was occasionally obtained.

In view of these findings, we have attempted to fractionate thyroglobulin. The present communication describes the result of cellulose ion-exchange chromatography and some properties of the fractions obtained.

Thyroglobulin used in this work was prepared from hog thyroid glands according to a modified method of Derrien *et al.*[1], in which fractional salting-out between 1·55 and 1·75 *M* ammonium sulphate was performed at a low temperature instead of the 20° C. in the original method.

In Fig. 1 is shown a typical chromatographic diagram. In this experiment, about 130 mgm. of thyroglobulin dissolved in a phosphate-sodium chloride buffer (NaCl: 0·05 *M*) of *p*H 6·5 at ionic strength (μ) of 0·15 was applied to a column of *DEAE*-cellulose[5] (1·5 cm. × 30 cm.) equilibrated with the same buffer. The column was operated at a rate of approximately 20 ml./hr. The effluent was collected in 3-ml. fractions; and the optical density at 280 mμ was determined.

When stepwise elution was carried out according to the elution schedule indicated by arrows in Fig. 1, there appeared several thyroglobulin peaks (fractions $a - f$) with an increase in the concentration of sodium chloride in the buffer. Other small peaks, one which emerged on washing the column and one with elution at ionic strength 2·0, were due to minor impurities; the latter component was found by means of its ultra-violet absorption spectrum and chemical analysis to be ribonucleic acid. The total apparent recovery was 87 per cent as determined by ultra-violet absorption at 280 mμ. Re-chromatography was almost satisfactory, but fractions eluted in lower ionic strengths had a tendency to emerge on re-chromatography at a higher ionic strength than in the initial chromatography if the ratio of the load of protein to the amount of *DEAE*-cellulose was lower on re-chromatography. In a gradient elution experiment, too, heterogeneity of thyrogobulin was indicated, but the separation was poorer, as reported by Ingbar *et al.*[6].

Chemical analyses of these chromatographic fractions showed that the ratio of iodine to nitrogen increased steadily from fraction *a* to fraction *e* with an increase in the ionic strength of the eluant. The ratio expressed as μgm. iodine/mgm. nitrogen for each peak is given in the legend of Fig. 1. In an ultracentrifuge (Fig. 2), fractions *a*, *b* and *c* showed a single boundary corresponding to the main component of the original preparation, while *F*-component was found to be concentrated in fractions *d*, *e* and *f*, especially in fraction *e* (about 30 per cent). Fraction *e* contained, besides ordinary *F*-component (F_α), an additional component with a higher sedimentation coefficient (F_β).

Attempts to obtain pure *F*-component have been, so far, unsuccessful, the purity being no more than 62 per cent after repeated chromatography. However, it was considered from iodine analysis of these preparations that *F*-component would be iodo-protein(s). Further work is necessary to confirm this.

Although these experiments were carried out on a preparation derived from a number of thyroid glands, a similar chromatographic diagram was obtained with a preparation obtained from a single gland.

On the basis of these observations it is suggested that hog thyroid contains a large number of iodoproteins, which differ in the degree of iodination

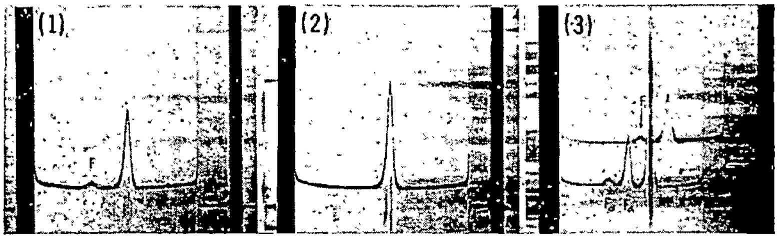


Fig. 2. Ultracentrifugal patterns of thyroglobulin fractions. (1) Original thyroglobulin preparation; (2) fraction *a*; (3) upper, fraction *d*; lower, fraction *e*. Sedimentation proceeds from right to left

and in other properties. It should also be noted that, in spite of this heterogeneity, no iodine-free component was found in thyroglobulin.

Further work is now in progress, and the results will be published elsewhere together with details of the present experiments.

NOBUO UI
OSAMU TARUTANI
YOICHI KONDO
HIROKO TAMURA

Institute of Endocrinology,
Gunma University,
Maebashi,
Japan.

[1] Derrien, Y., Michel, R., and Roche, J., *Biochim. Biophys. Acta*, **2**, 454 (1948).

[2] Derrien, Y., Michel, R., Pedersen, K. O., and Roche, J., *Biochim. Biophys. Acta*, **3**, 436 (1949).

[3] O'Donnell, I. J., Baldwin, R. L., and Williams, J. W., *Biochim. Biophys. Acta*, **28**, 294 (1958). Edelhoch, H., *J. Biol. Chem.*, **235**, 1326 (1960).

[4] Alberty, R. A., *J. Amer. Chem. Soc.*, **70**, 1675 (1948).

[5] Peterson, E. A., and Sober, H. A., *J. Amer. Chem. Soc.*, **78**, 751 (1956).

[6] Ingbar, S. H., Askonas, B. A., and Work, T. S., *Endocrinol.*, **64**, 110 (1959).

Selenomethionine as an Antioxidant

EVIDENCE has accumulated indicating that: (1) vitamin E functions, at least in part, as a lipid antioxidant; (2) unrelated substances having antioxidant activity can alleviate or prevent some of the symptoms of vitamin E deficiency[1]; (3) selenium compounds also can alleviate or prevent some of the symptoms of vitamin E deficiency[2]; (4) the ingestion of selenium oxide renders tissues less susceptible to oxidative attack[3]; (5) some seleno-amino-acids have antioxidant activity in aqueous lipid emulsions[4]. The experiments reported here were undertaken to determine whether seleno-amino-acids could act as antioxidants in anhydrous fats and oils.

Denison and Condit[5] had first shown that organic selenium compounds were powerful antioxidants in lubricating oils. Woodbridge[6] has summarized the literature dealing with selenium compounds as anti-oxidants in rubber and lubricating oils and contributed information on their effectiveness in squalene.

Most of the experiments were run with selenomethionine, one sample of which was obtained from E. Painter[7], and a second from Y. Natori. The latter sample was pure by paper chromatography. Similar results were observed for both samples. Triolein was obtained from the Hormel Foundation. Lard was a commercial sample containing no added antioxidants. Menhaden oil was a purified sample obtained from the Bureau of Commercial Fisheries.

Antioxidant activity was estimated by the gain-in-weight method described previously[8]. Covered beakers containing the oil samples were incubated in constant-temperature draught ovens. Once daily they were removed from the oven, tested for rancidity by odour, allowed to cool to room temperature, weighed and returned to the oven. The end of the induction period was indicated by a gain in weight and also by the development of rancidity.

Peroxides were determined by iodimetry, essentially according to the A.O.C.S. method Cd 8-53 but adapted for a sample size of 200 mgm. The containers were kept filled with nitrogen during the one minute reaction period with potassium iodide.

Table 1. EFFECT OF SELENOMETHIONINE ON INDUCTION PERIOD OF TRIOLEIN, LARD, MENHADEN OIL*

Additive	Amount (μM/gm.)	Induction period (days)
Triolein (60°):		
None	—	5, 5
Selenomethionine	1	5
	2·5	9
	5	57
	10	31
	25	> 355
Methionine	10	8
	25	9
Lard (60°):		
None	—	1, 2
Selenomethionine	10	6, 6
	25	> 240, > 240
	50	> 240, > 240
Methionine	10	1, 1
	25	1, 1
Menhaden oil (50°):		
None	—	1, 1
Selenomethionine	10	1, 1
	25	1
	50	1

* Fat samples which were held for months at 60° without becoming rancid did, however, develop a garlic odour, and gradually became more viscous.

As shown in Table 1, selenomethionine was ineffective in the various substrates at levels below 5 μM/gm.; at higher concentrations it gave remarkable stabilizing effects with lard and triolein, but was ineffective in menhaden oil (a highly unsaturated fish oil, iodine value 160). The protection afforded the more saturated fats was not uniformly demonstrable, and it was noted in most instances that oxidation, as measured by gain in weight, proceeded for a few days to 0·25–0·75 per cent of the total weight, and then came to an abrupt stop. No changes then occurred for several months. Parallel runs with methionine did not show similar behaviour. Other seleno-amino-acids, namely, selenocysteine, phenyl selenoglycine, selenohomocysteine, and phenyl selenomethionine, also acted as antioxidants when added in relatively large amounts, but they were not as exten-

Table 2. PEROXIDE CHANGES IN LARD INCUBATED AT 60° WITH AND WITHOUT ADDED SELENOMETHIONINE AND HYDROPEROXIDE

	Additive	Amount (μM/gm.)	Original peroxide value	Time (hr.)	Final peroxide value
Run 1*					
	None	—	100	44	435
	Se-methionine	10	0	44	104
				138	77
	Se-methionine	10	100	74	122
				138	76
	Se-methionine	25	0	74	80
				138	41
	Se-methionine	25	100	74	44
				138	15
Run 2†	None	—	0	24	305
				48	380
	None	—	200	24	445
				48	370
	Se-methionine	25	0	24	71
				48	63
				72	50
	Se-methionine	25	200	24	91
				48	69
				72	54
	Methionine	25	0	24	250
				48	245
				72	293
	Methionine	25	200	24	420
				48	485
				72	370

* Hydroperoxide added as oxidized safflower oil methyl esters.

† Hydroperoxide added as menthane hydroperoxide.

sively studied. Quantitation with the selenoamino-acids was rendered difficult by the heterogeneity of the system; none was completely in solution in the substrates used.

Changes in peroxide value were followed in lard samples with and without added selenomethionine and also with and without added peroxides. The results, shown in Table 2, indicate that in the presence of selenomethionine the peroxide values did not rise so high and tended to decrease with time, more so than when either methionine or no additional additive was present. In this sense selenomethionine appeared to be able to decompose peroxides.

The results with selenomethionine differed from those observed with other antioxidants in: (1) they appeared to have no effectiveness in low concentrations; (2) the protection even in large amounts could not be uniformly demonstrated; (3) no protection was afforded to a highly unsaturated oil; (4) some oxidation appeared to occur before the antioxidant effect appeared. These observations may tentatively be explained by assuming that selenomethionine is not itself an antioxidant but that it is oxidized to a derivative which is. Such systems have been described for thio- and dithio-compounds by Barnard *et al.*[9], and for seleno-compounds by Woodbridge[6]. In the former case the active antioxidants are thought to be the derived sulphoxides, and thiosulphinates.

It is reasonable to suppose that the reactions which lead to the formation of a sufficient amount of a powerful antioxidant so that oxidation stops would be of a complexity such that only at times could this consummation be reached. Thus, if a highly unsaturated oil formed peroxides rapidly, the intermediate antioxidant might be further oxidized before it could stabilize the reaction.

These results give further suggestive evidence that some of the tocopherol-like physiological properties of selenium compounds may be mediated through an antioxidant action[4]. Perhaps the lack of parallelism in their physiological effects is due not only to the differences of availability to different cells of tocopherols compared with seleno-compounds but also to the requirement that specific selenium derivatives, which occur or are synthesized in ways not yet understood, are required to achieve the antioxidant state.

H. S. Olcott
W. Duane Brown
J. Van der Veen

Institute of Marine Resources,
Department of Nutritional Sciences,
University of California,
Berkeley.

[1] Dam, H., *Pharmacol. Rev.*, **9**, 1 (1957).

[2] Schultze, M. O., in *Ann. Rev. Biochem.*, **29**, 391 (1960).

[3] Bieri, J. G., Dam, H., Prange, I., and Søndergaard, E., *Acta physiol. Scand.*, **52**, 36 (1961).

[4] Zalkin, H., Tappel, A. L., and Jordan, J. P., *Arch. Biochem. and Biophys.*, **91**, 117 (1960).

[5] Denison, jun., G. H., and Condit, P. C., *Indust. Eng. Chem.*, **41**, 944 (1949).

[6] Woodbridge, D. T., M.Sc. Thesis, University of London (1959).

[7] Painter, E., *Chem. Rev.*, **28**, 179 (1941).

[8] Olcott, H. S., and Einset, E., *J. Amer. Oil Chemists Soc.*, **35**, 161 (1958).

[9] Barnard, D., Bateman, L., Cole, E. R., and Cunneen, J. R., *Chem. and Indust.*, 918 (1958).

Occurrence of Mevalonic Acid in Carrots

Although tracer studies in a number of carotenogenic systems[1,2] have conclusively proved that mevalonic acid is an effective precursor of carotenes, the presence of mevalonic acid has not been demonstrated in any of such systems. In this laboratory an attempt has been made to isolate mevalonic acid from carrots. Previous work[3,4] has shown that cell-free extracts of carrots can synthesize carotenes from acetate and mevalonic acid.

100 gm. (wet weight) of freshly collected carrots were minced and placed in 100 ml. phosphate buffer (0·1 *M*, *p*H 5·8) for about 2 min. in a Waring blender to prepare the homogenate. From the filtered homogenate the proteins were removed by treatment with trichloracetic acid and the supernatant, after neutralization, was concentrated *in vacuo* to about 60 ml. For isolation of mevalonic acid the concentrated material was acidified with 8 ml. of 10 *N* sulphuric acid, then extracted with ethyl ether for 8 hr. and the extract treated according to the method of Lynen and Grassl[5]; finally mevalonic acid was determined by reaction with hydroxylamine at *p*H 7·0 and subsequent colorimetric measurement of the resultant hydroxamic acid by formation of a complex with ferric salts. The quantitative range of mevalonic acid present in carrots varied from 2 to 4 μmoles/100 gm. of carrots (wet).

The dibenzylethylenediamine (DBED) salt of isolated mevalonic acid was prepared according to the procedure described by Hoffman *et al.*[6]; a crystalline salt was obtained which melted at 115° (114–119°).

For further confirmation the mevalonic acid DBED salt was dissolved in minimum amount of water, the *p*H was adjusted to 10 and the free amine was extracted with ethyl ether. The alkaline solution was readjusted to *p*H 6·5–7, and then, after concentration, portions were chromatographed according to the method of Yokoyama *et al.*[2] and the spot of mevalonic acid was identified by spraying with bromophenol blue.

The compound isolated was also confirmed as mevalonic acid by identifying the hydroxamate derivative which was chromatographed by using butanol saturated with water as the solvent system as reported by Knauss *et al.*[7]. The spots were made visible by spraying with 5 per cent $FeCl_3.6H_2O$ in 90 per cent ethanol and 0·1 *N* hydrochloric acid.

In view of the recent findings of Braithwaite and Goodwin[8] that mevalonate is more efficiently incorporated than acetate into carotenes of carrots, the occurrence of mevalonic acid in carrots makes the carrot system a valuable tool for further work.

V. V. Modi
D. K. Patwa

Biochemistry Department,
Faculty of Science,
M.S. University of Baroda,
India.

[1] Goodwin, T. W., *Advances in Enzymol.*, **21**, 295 (1959).

[2] Yokoyama, H., Chichester, C. O., and Mackinney, G., *Nature*, **185**, 687 (1960).

[3] Modi, V. V., and Patwa, D. K., *Nature*, **184**, 983 (1959).

[4] Modi, V. V., and Patwa, D. K., *Enzymologia*, **23**, 27 (1961).

[5] Lynen, F., and Grassl, M., *Z. physiol. Chem.*, **313**, 291 (1958).

[6] Wolf, D. E., Hoffman, C. H., Aldrich, P. E., Skeggs, H. R., Wright, L. D., and Folkers, K., *J. Amer. Chem. Soc.*, **78**, 4496 (1956).

[7] Knauss, H. J., Porter, J. W., and Wasson, G., *J. Biol. Chem.*, **234**, 2835 (1959).

[8] Braithwaite, G. D., and Goodwin, T. W., *Biochem. J.*, **76**, 194 (1960).

HISTOCHEMISTRY

Tissue Dilution Artefact; a Re-interpretation of Variations in Levels of Succinic Dehydrogenase during Chemical Carcinogenesis

RECENT biochemical studies on succinic dehydrogenase in livers of rats fed with the liver carcinogen *p*-dimethylaminoazobenzene (DAB) for periods of up to 150 days have confirmed the well-established phenomenon that the level of this enzyme generally falls as feeding progresses (Table 1). Samples of tissue from each liver lobe were taken for histological and histochemical investigations, and the remainder was homogenized. Succinic dehydrogenase was estimated to within an accuracy of ± 10 per cent by the anaerobic methylene blue technique, basing the Q_{MB} values on dry weight. For histochemical purposes the tissue was frozen[1], stored for not longer than a week, and cut at $-20°$ C. in a refrigerated microtome. Succinic dehydrogenase was detected histochemically by incubating frozen sections in a neotetrazolium–succinate buffer system[2]; in addition, the periodic acid–Schiff method[3] was used to stain glycogen, while phospholipid was stained by the acid hæmatin method[4].

The histochemical results indicated that the succinic dehydrogenase activity of the liver cells was high in liver from rats fed on a complete diet or on a low-protein methionine-supplemented diet[5] with or without DAB, and that in the course of feeding this activity per cell did not alter detectably. In contrast, normal bile duct epithelium had a low activity, while connective tissue gave no apparent reaction ; as the period of feeding increased, the proportion of these less or non-reactive components became greater, and the gradual fall in Q_{MB} was qualitatively matched by a reduction in the proportion of liver cells, which histochemically still reacted in a manner comparable with normal liver cells. Sections have also been stained with hæmatoxylin and eosin, while the periodic acid–Schiff and acid hæmatin stains have provided useful information in distinguishing cell types. The latter, in particular, differentiates the weakly staining cells of bile duct and connective tissue in contrast to the strong reaction of liver cells.

When rats were allowed to remain on diet for 200 days, the Q_{MB} values, even of tissue samples selected from the same lobe, showed considerable variation. High Q_{MB} values, about 75–90 per cent of control values, were given by areas containing a high proportion of liver cells, though this proportion was not as high as in normal liver. Low values of Q_{MB}, 20–50 per cent of control values, were obtained from areas of liver composed principally of bile duct cells and fibrous tissue.

Daoust and Cantero[6], who studied the livers of Wistar rats, showed that as the period of feeding the carcinogen DAB was extended, so the relative proportion of liver cells decreased, while the proportions of connective tissue and bile duct cells increased. In these studies, rats of the August strain have been used, but the histology of these livers was qualitatively similar to that reported by Daoust and Cantero. Thus the proportion of liver cells containing a high level of succinic dehydrogenase fell, while the amount of much less active bile duct cells and inactive connective tissue increased ; the overall fall in succinic dehydrogenase was due, not to any change, malignant or otherwise, in any particular cell type, but rather to an alteration in the relative proportions of cell types. It is suggested that the term 'tissue dilution artefact' be used to describe this phenomenon.

Table 1. VARIATIONS IN VALUES OF Q_{MB} IN THE RIGHT ANTERIOR LOBES OF MALE RATS (AUGUST STRAIN) FED WITH THE LIVER CARCINOGEN DAB ON A LOW PROTEIN DIET

Period of feeding	Q_{MB} in right lobe (DAB)	Q_{MB} in right lobe (control)	DAB Q_{MB} as percentage of control values
No DAB		25 ± 4	
30 days	23	25	92
60 days	20	24	83
90 days	13	22	59
120 days	15	29	52
150 days	10	22	45

It is claimed then that this artefact may occur generally in biochemical studies of a tissue containing two or more cell types which may vary in proportion during the course of the experiment. A notable example of such investigations is that on livers of animals treated with liver carcinogens. In this kind of work the onus lies with the experimenter to demonstrate that biochemical comparisons reflect a true alteration in cell metabolism, and are not due to a change in the proportion of cell types of different metabolic activities. Rigid histochemical and histological checks become of the utmost importance in interpreting such biochemical results if invalid conclusions based solely on such data are to be avoided.

We are grateful to the British Empire Cancer Campaign for supporting this work.

G. R. N. JONES
L. BITENSKY
J. CHAYEN
G. J. CUNNINGHAM

Department of Pathology,
Royal College of Surgeons,
Lincoln's Inn Fields,
London, W.C.2.

---

[1] Cunningham, G. J., Bitensky, L., Chayen, J., and Silcox, A. A., *First Intern. Congr. Histochem. and Cytochem.*, Abstracts, 5 (Pergamon Press, 1960).

[2] Bitensky, L., Baldwin, R. W., and Chayen, J., *Brit. J. Cancer*, **14**, 690 (1960).

[3] Hotchkiss, R. D., *Arch. Bioch.*, **16**, 131 (1948).

[4] Baker, J. R., *Quart. J. Microscop. Sci.*, **87**, 441 (1946).

[5] Elson, L. A., *Brit. J. Cancer*, **6**, 392 (1952).

[6] Daoust, R., and Cantero, A., *Cancer Res.*, **19**, 757 (1959).

PHYSIOLOGY

Sub-fractionation of Human Serum Leucine Aminopeptidase

OUR earlier starch-gel electrophoretic zymograms[1], obtained with 1-leucyl-β-naphthylamide (LBNA) substrate, revealed a single leucine aminopeptidase (LAP) zone for human serum. The substitution of alanyl-β-naphthylamide (ABNA) substrate now consistently reveals, within the diffuse trail behind the primary zone, an additional less-intense zone (Fig. 1*a*), detectable also for some sera by LBNA. Occasional sera give three or more zones; and one chronic pancreatitis serum has given a remarkable zymogram with five zones which, when observed through a suitable blue filter (Dupont No. 9 plastic 'Varigam' filter), can be distinguished as eight zones (Fig. 1*b*).

Whereas some zymograms are distinctive because of this multiplicity of zones, others are distinctive because of a single zone. In 21 determinations, ABNA substrate on 18 serum samples from 8 varied patients (run on 4 different days over a 3½-month period), the usual two zones were resolved in every instance. Conversely, in each of 5 determinations on 5 serum samples from one other patient (run on 3 different days along with the other patients' samples), only the one primary zone could be found. Although no correlation with diagnosis is at present justified in this case, the serum of this particular patient seems clearly characterized by a marked deficiency or absence of at least one common peptidase sub-fraction.

The electrophoretic sub-fractionation is accomplished (as previously described[1,2]) in 15 per cent starch gel, prepared in 0·03 M, pH 9·0, borate buffer (final gel pH 8·5); and a 6 V./cm. gradient for 6 hr. The gel strip (2·0 × 0·65 × 25 cm.) is then cut horizontally into two thin slices and incubated at room temperature in a substrate solution[3], prepared by mixing and dissolving: (*a*) 8·0 ml. of a 1 per cent solution of ABNA (or LBNA) in water; (*b*) 40·0 ml. of 0·2 M *tris*(hydroxymethyl)aminomethane buffer that has been adjusted to pH 7·1 with hydrochloric acid; (*c*) 160 ml. of water; (*d*) 120 mgm. of diazotized *o*-aminoazotoluene. The zymogram of orange red zones against an orange yellow background usually begins to develop within 15 min., although 2 hr. are allowed for full development. The gels are finally rinsed thoroughly with water and saturated overnight with glycerol, which prevents drying and preserves the zymograms.

Special clinical interest attaches to this new evidence that LAP is heterogeneous. An early optimism that increased LAP activity in serum may be diagnostic of pancreatic cancer[4] has been questioned by similar findings for non-malignant diseases[5,6]. (Similarly, while Burstone[7] has associated abnormal LAP increases in certain tissues with invasive cancer, Monis *et al.*[8] attribute such increases to non-specific inflammatory and proliferative processes.) But beyond the controversial significance of total serum LAP activity, it is now apparent that a zymogram or distribution pattern of this activity among several different LAP sub-fractions can be much more informative, perhaps because each sub-fraction independently reflects the condition of its own particular source tissues. This situation has been demonstrated for other enzymes, tissues and diseases, most recently by Wroblewski[9] for heart lactic dehydrogenase, which selectively increases in serum after myocardial infarct. On this background our present work on LAP zymograms will be extended with emphasis on pancreatic cancer, other pancreatic and liver diseases, pregnancy and other conditions showing unusual serum LAP activity.

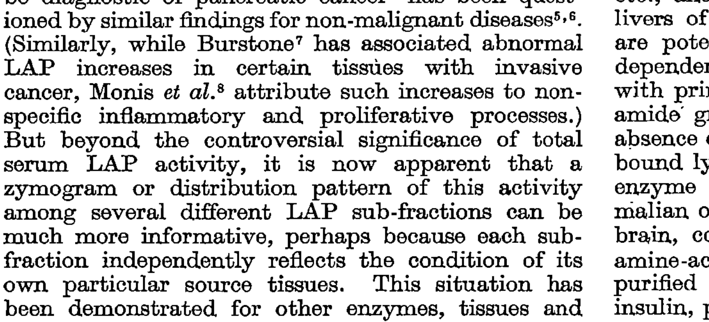


Fig. 1. Leucine aminopeptidase zymograms (ABNA substrate) of human serum (schematic). *a*, Typical pattern; *b*, a chronic pancreatitis pattern (observed through blue filter). The relative locations of the major protein zones (Smithies nomenclature) are noted below the zymograms

This work was aided by grant No. *T*-77 from the American Cancer Society and by a gift from Mollie E. Swinney. We also wish to acknowledge laboratory facilities and other valuable support provided by Dr. Samuel H. Bassett.

Working independently of us, Sanford H. Lawrence, Perry J. Melnick and Harry E. Weiner (private communication) have also found two or more LAP zones for human serum.

CLYDE A. DUBBS
Veterans Administration Center and
University of California Medical Center,
CHARLOTTE VIVONIA
JEAN M. HILBURN
University of California Medical Center,
Los Angeles, California.

[1] Dubbs, C. A., Vivonia, C., and Hilburn, J. M., *Science*, **131**, 1529 (1960).
[2] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[3] Burstone, M. S., and Folk, J. E., *J. Histochem. and Cytochem.*, **4**, 217 (1956).
[4] Rutenburg, A. M., Goldbarg, J. A., and Pineda, E. P., *New England J. Med.*, **259**, 469 (1958).
[5] Arst, H. E., Manning, R. T., and Delp, M., *Amer. J. Med. Sci.*, **238**, 598 (1959).
[6] Pineda, E. P., Goldbarg, J. A., Banks, B. M., and Rutenburg, A. M., *Gastroenterol.*, **38**, 698 (1960).
[7] Burstone, M. S., *J. Nat. Cancer Inst.*, **16**, 1149 (1956).
[8] Monis, B., Nachlas, M. M., and Seligman, A. M., *Cancer*, **12**, 601 (1959).
[9] Wroblewski, F., Gregory, K., and Ross, C., *Clin. Res.*, **8**, 195 (1960).

Incorporation of Histamine into Liver Protein *in vivo*

RECENTLY we have described an enzyme system which catalyses the exchange of the amide group of protein-bound glutamine with primary amines, such as methylamine, putrescine, histamine, serotonin, etc., and also hydroxylamine and ammonia[1]. The livers of rodents, and in particular of guinea pigs, are potent sources of the enzyme. This calcium-dependent enzyme not only catalyses the exchange with primary amines but also the hydrolysis of the amide group of protein-bound glutamine[2]. In the absence of added amine the ε-amino group of protein-bound lysine may act as the replacing agent[3]. The enzyme was named 'transglutaminase'. All mammalian organs examined, and in particular liver and brain, contain proteins which may act *in vitro* as amine-accepting substrates, as are also a number of purified proteins, such as casein, lactoglobulin, insulin, pepsin, etc.

Whereas there is no difficulty in demonstrating transglutaminase action *in vitro*, in early experiments, attempts to show fixation of administered amines in the liver proteins of mice were unsuccessful even after experimental liver damage (carbon tetrachloride) or after nephrectomy. The possibility was considered that proteins modified by incorporated amines may have antigenic properties and could be involved in auto-immune reactions. Since *H. pertussis* vaccine enhances anaphylactic sensitization its effect and that of a lipopolysaccharide from *S. typhosa* on liver transglutaminase[4] and histamine incorporation into liver proteins was studied. In mice, 16 hr. after a single injection of lipopolysaccharide, the transglutaminase-level in liver was increased up to three-fold. The enzyme was assayed in the 100,000g supernatant fluid of 0·25 molar sucrose liver homogenates with a tryptic digest of casein as substrate and hydroxylamine as replacing amine[2]. *H. pertussis*

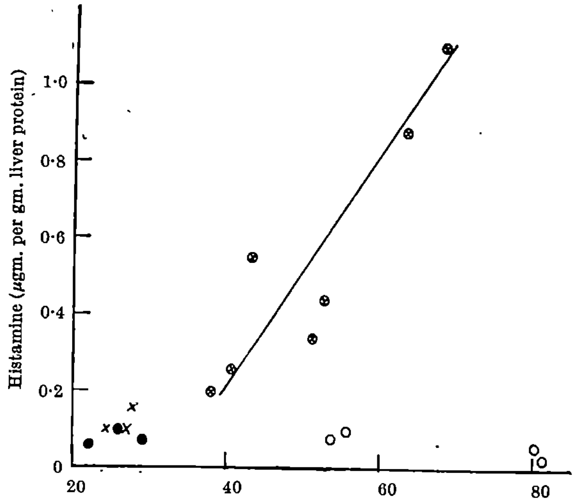


Fig. 1. Transglutamine activity and concentration of protein-bound histamine in mouse livers. Transglutaminase unit = optical density (525 mμ) × 1,000 of the Fe^{+++} complex of hydroxamic acid formed after 10 min. incubation at 37° C. Normal mice (●); normal mice + 37·5 mgm./kgm. of histamine (×); mice treated with 5 mgm./kgm. of *S. typhosa* lipopolysaccharide (○); mice treated with lipopolysaccharide, and 37·5 mgm./kgm. of histamine (⊗)

vaccine acted similarly, but was less effective in raising the enzyme-level. Histamine (37·5 mgm./kgm.) was administered intraperitoneally to groups (6–10) of normal and lipopolysaccharide-treated mice which were killed 30 min. later. Small portions of each liver were homogenized together for enzyme determination; the pooled remainder of the livers was precipitated by trichloracetic acid, the protein purified and traces of adsorbed histamine removed by thioglycollate treatment. Histamine was extracted from the ammonia-freed acid hydrolysates of the purified liver proteins using a modification of the method described by Shore *et al.*[5] and assayed on the superfused guinea pig ileum. The results of enzyme and histamine determinations are shown in Fig. 1. The histamine content of the liver proteins from normal or lipopolysaccharide-treated mice with or without histamine injection was less than could be detected and the points shown indicate the limits of the sensitivity of the assay. The liver proteins of lipopolysaccharide-treated animals after histamine injection contain significant amounts of histamine, the quantity of which correlated with the transglutaminase-level ($r = 0·9$; $P < 0·001$). The validity of the above results was further confirmed in experiments in which histamine labelled with carbon-14 was administered.

It should be emphasized that the increase of enzyme activity and protein-bound histamine does not necessarily represent a causal relationship, nor do the results permit the conclusion that the histamine is incorporated by exchange with the amide group of glutamine bound in liver protein. In order to demonstrate increased histamine fixation in liver protein of lipopolysaccharide-treated mice, it was necessary to administer large doses of histamine. This raises the question whether histamine is the natural replacing amine and experiments are now in progress to study the conditions of *in vivo* incorporation of other amines. Experiments testing the antigenic properties of proteins into which histamine was incorporated *in vitro* by transglutaminase have up to now given equivocal results.

This work was supported in part by grants from the National Institute of Neurological Diseases and Blindness (grant *B*–226), from the Ford Foundation, and by a contract between the Air Force Office of Scientific Research and the Research Foundation for Mental Hygiene, Inc. One of us (M. G.) is indebted to the Wellcome Trust for a travel grant. We are grateful to Miss Ethel Danielson for the performance of most of the enzyme assays and to Mrs. Paula Mela for her assistance.

ISABEL WAJDA
MICHAEL GINSBURG*
HEINRICH WAELSCH

New York Psychiatric Institute
and College of Physicians and
Surgeons,
Columbia University,
New York.

---

* Permanent address, Department of Pharmacology, University of Bristol.

[1] Clarke, D. D., Neidle, A., Sarkar, N. K., and Waelsch, H., *Arch. Biochem. Biophys.*, **71**, 277 (1957). Clarke, D. D., Mycek, M. J., Neidle, A., and Waelsch, H., *ibid.*, **79**, 338 (1959). Mycek, M. J., Clarke, D. D., Neidle, A., and Waelsch, H., *ibid.*, **84**, 528 (1959).

[2] Mycek, M. J., and Waelsch, H., *J. Biol. Chem.*, **235**, 3513 (1960).

[3] Neidle, A., Mycek, M. J., Clarke, D. D., and Waelsch, H., *Arch. Biochem. Biophys.*, **77**, 227 (1958).

[4] The increase of histidine decarboxylase in the skin and lung of rodents after administration of *B. pertussis* vaccine and under various stress situations has been reported (cf. Schayer, R. W., *Science*, **131**, 226 (1960)).

[5] Shore, P. A., Burkhalter, A., and Cohn, jun., V. H., *J. Pharmacol. Exp. Ther.*, **127**, 182 (1959).

Comparison of Short-Circuit Current and Net Water Movement in Single Perfused Proximal Tubules of Rat Kidneys

THE major portion of renal salt and water reabsorption occurs within the proximal convoluted tubule, amounting in mammalian kidneys to about 80 per cent of the quantities present in the glomerular filtrate. Based on indirect evidence, proximal sodium transport has been postulated to be active. Recently, however, results obtained in stop-flow experiments were interpreted in such a way as to indicate passive bulk movement of tubular fluid as the predominant mode of proximal tubular salt transfer. Micropuncture work on rats has demonstrated unequivocally the existence of active sodium transport across proximal epithelium[1], but did not provide information as to the relative contribution of active sodium transport to the overall net movement of this ion species. The present work represents an attempt to clarify this point by correlating active transport of ions as measured by the short-circuit method with measurements of net movement of water under comparable conditions. Assuming that proximal fluid reabsorption is isosmotic—an assumption in agreement with all known measurements of osmotic pressure of proximal tubular fluid—net water movement can be used as an index of net transport of sodium. Thus short-circuit current can be correlated with net movement of sodium.

Experiments were performed on rat kidneys *in vivo*. Short-circuit current was measured by means of the original short-circuit method as described by Ussing and Zerahn[2], using double-barrelled electrodes[3], or by a modification as described by Karger *et al.*[4]. By definition, short-circuit conditions occur when the trans-membrane potential difference is reduced to zero. Under such conditions the current generated by the epithelium passes through an outer circuit of zero effective resistance. Applied to the renal tubule, the short-circuit current is that current which, applied from an external battery, renders the electrical resistance of a probing micro-electrode within the tubular lumen identical to that of the same electrode when it is outside the lumen. Thus the resistance of the membrane is effectively reduced to zero. Since electrolyte concentrations are nearly identical in proximal tubular fluid and interstitial fluid, the current measured under these conditions must be generated by active ion transport.

All measurements of short-circuit current were performed on tubular fluid segments one diameter in length, isolated from the rest of the tubular fluid by means of columns of mineral oil. The reason for this choice of distance between the bordering oil droplets arises from conclusions derived by cable analysis. In this type of analysis the renal tubule is treated as an electrically leaky cable, and a quantitative measure of its resistance to passive ion flow can be obtained. Use was made of equations derived by Hodgkin and Rushton for the giant axon of the squid[5]. Assessment of the length constant was made possible by passing a known current through one micro-electrode into the tubular lumen and recording voltage displacements at known varying distances within the same tubule by means of a second micro-electrode. With anodic stimulating currents of 10^{-6} amp., voltage amounted to 14 mV. (2 observations, range 8–17 mV.) at 15μ, 9·6 mV. (6 obs., range 6–14 mV.) at 30μ, 5 mV. (2 obs., range 4–6 mV.) at 60μ, and 3·3 mV. (2 obs., range 2·5–4·0 mV.) at an interelectrode distance of 90μ. These results indicate that a current applied through the stimulating micro-electrode leads to a decline in voltage amounting to 63 per cent of its original value at a distance of about 55μ. An identical decline of current intensity can be predicted provided that the tubular epithelium behaves as an ohmic resistance. Therefore, experiments were performed in which the voltage-current relationship was directly examined. With varying anodic and cathodic d.c. currents (up to 3×10^{-6} amp.), proportional displacements of voltage were recorded. Thus proximal tubular epithelium behaves as a linear, non-rectifying circuit element, and the decline of a locally applied current can be predicted from the value of 55μ obtained for the length constant. From these findings it can be concluded that a distance of one tubular diameter (30μ) between the isolating oil droplets is adequate to assure constancy of current density and effective short-circuiting. The average short-circuit current, as measured in a series of 10 experiments by means of the method of Karger *et al.*[4], amounted to 100×10^{-10} amp./$2{\cdot}8 \times 10^{-5}$ cm.^{2} (*S.E.* ± 20 per cent). Using the approach of Ussing and Zerahn[2], in which double-barrelled electrodes were applied, the average short-circuit current obtained in 6 experiments amounted to 101×10^{-10} amp./$2{\cdot}8 \times 10^{-5}$ cm.^{2} (*S.E.* ± 7 per cent). The pooled data of these two series of short-circuit current measurements correspond to a net ion transport of 34×10^{-10} equiv./cm.^{2}/sec.

The measurements of short-circuit current were compared with the net movement of sodium ions as calculated from water movement observed in perfusion experiments on single proximal tubules of the rat kidney. In this technique coloured mineral oil is injected into the tubular lumen and split by a small amount of perfusion fluid. The position of the isolated column of perfusion fluid is then secured by injecting mineral oil through a second pipette into the most distal surface loop of the proximal tubule. The perfusion fluid is left within the lumen for a short period, usually about 30 sec., and is then collected through the second, distally located pipette, while mineral oil is injected through the proximal micropipette.

Tubules were perfused with fluid containing 145, 100, and 10 m.equiv./l. of sodium. All perfusion fluids were kept isosmotic to plasma by addition of adequate amounts of mannitol. ^{14}C-inulin concentration ratios of collected and injected perfusion fluid were taken as an index of net water movement. At an intratubular sodium concentration of 145 m.equiv./l., water movement amounted to an average value of 59 per cent of the injected volume (4 obs., range 50–67 per cent), at 100 m.equiv./l., to 31 per cent (2 obs., 30 and 31 per cent), whereas at 10 m.equiv./l. the direction of net water movement was reversed and intratubular fluid volume increased by 46 per cent (3 obs., range 41–50 per cent). It should be noted that water reabsorption was reduced to a mean of 5 per cent of the injected volume (4 obs., range −6 to +17 per cent) when sodium cyanide at a concentration of 4×10^{-3} *M*/l. was added to the perfusion fluid containing 145 m.equiv./l. of sodium. It may be concluded from these results that a decrease in intratubular sodium concentration results in a decrease in water reabsorption. However, the net water movement may not be ascribed solely to the effect of the intratubular concentration of sodium chloride, since mannitol also was present in the perfusion fluid. To correct the above results for water movement secondary to mannitol flux, a second series of 9 experiments was performed under the same experimental conditions, except that ^{14}C-mannitol was measured rather than ^{14}C-inulin. The ratios of concentrations of ^{14}C-mannitol between collected perfusate and injected perfusion fluid averaged 0·31 (4 obs., range 0·17–0·49) at 10 m.equiv./l. intratubular sodium, and 0·84 (5 obs., range 0·63–0·94) with perfusion fluid containing sodium at a concentration of 100 m.equiv./l. Calculation of the net water movement which may be ascribed to transport of sodium chloride, after correction for mannitol-induced transfer[6], shows that net movement of sodium chloride out of the tubule can take place against a considerable concentration gradient. Water movement ceases between a solution containing 88 m.equiv./l. sodium and one containing sodium at a concentration of 145 m.equiv./l. When the concentration gradient exceeds 57 m.equiv./l., the direction of water movement, ascribed to sodium transfer, is reversed and water enters the tubule. The magnitude of this critical gradient is somewhat greater than that found during free flow conditions in micropuncture studies on rats during osmotic diuresis[1].

Net movement of water observed in experiments in which tubules were perfused with fluid containing 145 m.equiv./l. of sodium could be expressed as net movement of sodium without correction for mannitol flux. In this calculation a mean tubular radius of 15μ was used in estimating the exposed tubular surface area. Furthermore, the assumption was made that proximal fluid absorption is isosmotic and that tubular fluid volume changes as a linear function of time. The calculated mean trans-tubular net flux of sodium, thus obtained, amounts to 30×10^{-10} equiv./cm.^{2}/sec.

The results obtained in this work indicate that the critical sodium concentration gradient at which net movement of sodium across proximal tubular epithelium stops is 57 m.equiv./l. Comparison of measurements of short-circuit current and calculated sodium reabsorption obtained from data on perfusion experiments indicates that the major portion, possibly all, the proximal sodium reabsorption in the rat kidney is active in nature.

This work was supported by grants from the National Science Foundation and the American Heart Association.

ERICH E. WINDHAGER*
GERHARD GIEBISCH†

Department of Physiology,
Cornell University Medical College,
New York City.

---

* Supported in part by a grant from the John Polachek Foundation.

† Established Investigator of the American Heart Association.

[1] Windhager, E. E., and Giebisch, G., *Amer. J. Physiol.*, **200**, 581 (1961).

[2] Ussing, H. H., and Zerahn, K., *Acta physiol. Scand.*, **223**, 110 (1951).

[3] Coombs, J. S., Eccles, J. C., and Fatt, P., *J. Physiol.* (Lond.), **130**, 291 (1955).

[4] Karger, W., Eigler, F. W., and Hampel, A., *Pfluegers Arch. ges. Physiol.*, **272**, 187 (1960).

[5] Hodgkin, A. L., and Rushton, W. A. H., *Proc. Roy. Soc.*, B, **133**, 444 (1946).

[6] Windhager, E. E., Whittembury, G., Oken, D. E., Schatzmann, H. J., and Solomon, A. K., *Amer. J. Physiol.*, **197**, 313 (1959).

CYTOLOGY

Cytology of Some Genera of Polypodiaceae in Eastern India

POLYPODIACEAE (*sensu* Copeland[1]), a part of the once comprehensive family Polypodiaceae (*sensu lato*), comprises 65 genera, occurring mostly as epiphytes in the tropical and subtropical evergreen and semi-evergreen forests. The genera are characterized by simple, pinnatifid or rarely pinnate fronds with round and rarely linear or acrostichoid typically exindusiate sori. A thorough analysis of the literature together with the study of herbarium specimens available in the Central National Herbarium, Sibpore, West Bengal, and Eastern Regional Herbarium of the Botanical Survey of India, Shillong, Assam, suggests that the family is represented in India by 26 genera and 104 species[2], of which 91 species are distributed in various parts of eastern India, which incidentally appears to be richest in the fern flora in the whole country.

Although Manton[3] has worked out the cytology of 40 species, mostly from Ceylon and Malaya, none of the Indian plants belonging to the family Polypodiaceae (*sensu* Copeland, 1947) has yet been studied cytologically (the first report of the basic number in *Paraleptochilus decurrens* (Bl.) Copel. from Darjeeling has been published only recently by S. S. Bir in *The Nucleus*, **3**, 121; 1960) despite much work on the cytotaxonomy of the other families of ferns. As a part of the larger scheme of undertaking a cytotaxonomic revision of the predominantly epiphytic family Polypodiaceae, the East Indian species are being investigated. The present communication records the chromosome numbers unequivocally established for the species listed in Table 1.

Table 1

1. *Arthromeris wallichiana* (Sprg.) Ching	Shillong	$n=37$	Diploid
2. *Colysis elliptica* (Thbg.) Ching var. *pothifolia* (Don) Ching	Cherrapunji	$n=36$	,,
3. *Crypsinus crenatopinnatus* (Clarke) Copel	Shillong	$n=36$	,,
4. *Crypsinus oxylobus* (Wallex-Kunze) Sledge	,,	$n=35$	,,
5. *Lepisorus excavatus* (Bory) Ching	,,	$n=35$	,,
6. *Loxogramme avenia* (Bl.) Pr.	Jowai	$n=35$	,,
7. *Loxogramme involuta* (Don) Pr.	Shillong	$n=70$	Tetraploid
8. *Loxogramme lanceolata* (Sw.) Pr.	,,	$n=35$	Diploid
9. *Loxogramme scolopendrina* (Bory) Pr.	,,	$n=35$	,,
10. *Microsorium membranaceum* (Don) Ching	Cherrapunji and Pynursla	$n=36$	,,
11. *Microsorium pteropus* (Bl.) Copel.	Cherrapunji	$n=36$	,,
12. *Microsorium superficiale* (Bl.) Ching	Shillong	$n=36$	,,
13. *Pseudodrynaria coronans* (Wall.) Ching	Kameng F. D.	$n=36$	,,
14. *Pyrrosia flocculosa* (Don) Ching	Jowai	$n=36$	,,
15. *Pyrrosia mannii* (Gies.) Ching	Shillong	$n=37$	,,
16. *Pyrrosia subfurfuracea* (Hk.) Ching	Assam	$n=37$	,,
17. *Lepisorus pseudonudus* Ching	,,	$2n=39$	Auto-triploid
18. *Lepisorus* sp. nov.	Jowai	$n=22$	Diploid

While the present findings confirm the basic chromosome numbers for the genera *Lepisorus*, *Pyrrosia*, *Microsorium*, and *Crypsinus* reported earlier by Manton and Sledge[3], new records of basic chromosome numbers for four genera are now established, namely, *Arthromeris* with $n = 37$ (Fig. 1), *Colysis* with $n = 36$ (Fig. 2), *Loxogramme* with $x = n = 35$ (Figs. 3 and 4), and *Pseudodrynaria* with $n = 36$ (Fig. 5). Although Manton reported three different basic numbers, namely, $x = 35$, 36 and 37, in three species of *Lepisorus* from Ceylon, two species studied by us from eastern India each show $n = 35$ only, whereas two other species show the lowest basic chromosome numbers, namely, $x = 11$ and 13, discovered so far in the family. While the genus *Pyrrosia* appears to be characterized by $n = 37$,

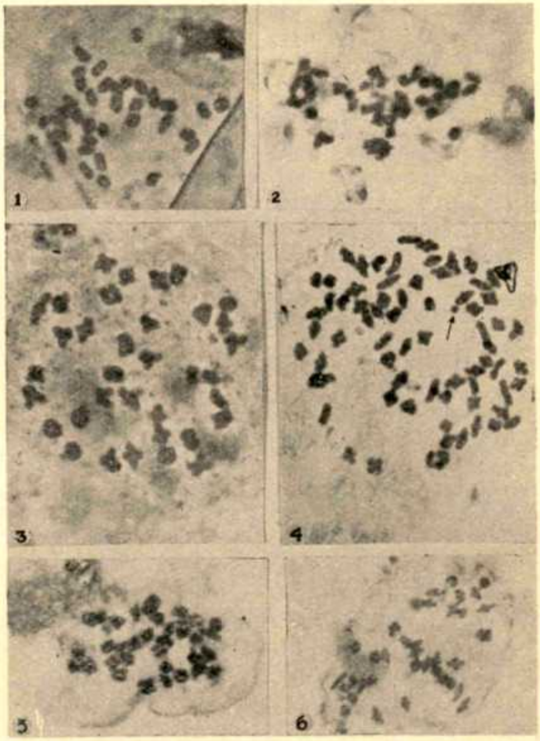


Fig. 1. Samples of meiotic behaviour in wild plants of Polypodiaceae of eastern India (all × 575) from permanent acetocarmine squashes prepared by S. N. Patnaik and photographed by C. C. Mukerjee: (1) *Arthromeris wallichiana*, a diploid ($n=37$); (2) *Colysis elliptica* var. *pothifolia*, a diploid ($n=36$); (3) *Loxogramme lanceolata*, a diploid ($n=35$); (4) *Loxogramme involuta*, a tetraploid ($n=70$, arrow indicating an extremely small bivalent); (5) *Pseudodrynaria coronans*, a diploid ($n=36$); (6) *Pyrrosia flocculosa*, a diploid ($n=36$)

Pyrrosia flocculosa alone shows a deviation with $n = 36$ (Fig. 6). The finding of $n = 36$ in *Pseudodrynaria coronans* (Wall.) Ching as distinct from $n = 37$ in three other species of *Drynaria* studied (Manton and Sledge[3]) lends support to the separation of *Drynaria coronans* (Wall.) J. Sm. for the construction of the monotypic genus *Pseudodrynaria* C. Chr. Of the four species of *Loxogramme* cytologically investigated, three species are diploids each with $n = 35$, whereas *L. involuta* is a tetraploid species showing unmistakably $n = 70$, with a single conspicuously small bivalent at diakinesis. Thus *Loxogramme* is characterized by the basic number $x = 35$ and not 36 (ref. 4). Hence, Manton's report of the chromosome number of *L. involuta* from Ceylon as $n = 70$ to 72 at meiosis and as "probably of the order of 140 in root tip" and her suspicion of the basic number as "probably 36" need revision in view of the unequivocal finding of $x = 35$ in four species of *Loxogramme* in eastern India. Manton (personal communication, 1961) agrees with this finding on the basis of her recent studies of *L. avenia* from Asian tropics.

Copeland's suggestion for merging *Arthromeris* with its parent genus *Crypsinus* to form a natural group is discounted by our discovery of $n = 37$ in *A. wallichiana* from Shillong as against $n = 36, 35, 33$ in *Crypsinus* reported from Eastern India, Ceylon and Malaya. On the other hand, the cytological findings of discordant basic numbers in *Crypsinus* corroborate Copeland's observations regarding the unnaturalness of the genus.

The discovery of the same basic chromosome number, namely, $n = 36$, in the genera *Microsorium*, *Colysis*, and *Leptochilus*, shows close affinity between them and may afford cytological evidence in favour of Holttum's contention of the origin of *Leptochilus* from *Microsorium* through *Colysis*-like ancestors.

Our thanks are due to Dr. J. C. Sen Gupta, chief botanist, Botanical Survey of India, Calcutta, for encouragement, and to the authorities of the Council of Scientific and Industrial Research, New Delhi, for the award of a research fellowship to the junior author and other financial assistance.

G. PANIGRAHI
S. N. PATNAIK

Botanical Survey of India,
Eastern Circle,
Shillong.

[1] Copeland, E. B., *Genera Filicum* (Waltham, Mass., 1947).
[2] Panigrahi, G., and Patnaik, S. N., *Indian Forester*, **87** (4), 242 (1961).
[3] Manton, I., *Problems of Cytology and Evolution in the Pteridophyta* (Cambridge, 1950); Appendix in Holttum, R. E., *Flora of Malaya*, **2** (Singapore, 1954). Manton, I., and Sledge, W. A., *Phil. Trans. Roy. Soc.*, **238**, 127 (1954).
[4] Pichi-Sermolli, R. E. G., *Vistas in Botany* (London, 1959).

HÆMATOLOGY

Association of Hæmoglobin *N* and Microcythæmia in a Sardinian Family

IN the course of a survey of the frequency of microcythæmic syndromes conducted in the summer of 1960 in Sardinia[1], we encountered a family in which were present both an abnormal hæmoglobin, faster than Hb *A*, and the microcythæmic (or thalassæmic) character.

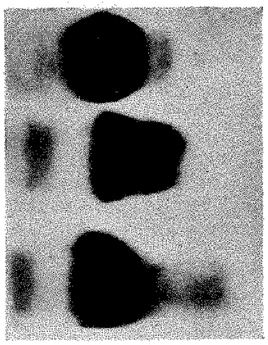


Fig. 1. Paper electrophoresis of Hb Bart's in cord blood (above), Hb *N* (at centre) and Hb *H* (below). Glycine buffer *p*H 8·8, ionic strength 0·04. Staining with bromophenol blue. Mobility of Hb *N* is similar with that of Hb Bart's; but less than that of Hb *H in toto*

The father (Table 1) was found to be a carrier of abnormal hæmoglobin; the mother was a carrier of microcythæmia; the first son, Gaet., was completely normal; the other two sons, Aless. and Franc., twins, were those that brought the family under our observation, because it was thought that they were affected by Cooley's disease. Indeed, both showed paleness of the skin and of the mucous membranes, skull slightly increased in volume, spleen enlarged to three fingerbreadths below the costal margin, and liver palpable 2 cm. below the right costal margin.

It has not been possible to conduct a hæmatological examination of Aless.: the blood smears, however, showed very intense erythrocytic alterations, quite similar to those of Cooley's disease. The other twin brother, Franc., presented an evident anæmia with hæmatological characters of microcythæmia (hypochromia, microcytosis, diminished cell fragility) and intense alterations of the morphology. Furthermore, both twins were carriers of fast hæmoglobin, while the proportion of Hb A_2 was less than the normal. The abnormal hæmoglobin presented the following characters: (1) on paper and starch electrophoresis at alkaline *p*H (Fig. 1) the hæmoglobin had a mobility identical with that of Hb Bart's, but lower than that of Hb *H in toto*; (2) on electrophoresis at acid *p*H (Fig. 2), the abnormal hæmoglobin tends to separate from Hb *A*, forming a thicker hæmoglobinic spot; (3) on agar-gel electrophoresis at acid *p*H, this abnormal hæmoglobin cannot be distinguished from Hb *A*; (4) on 'Amberlite *IRC*-50' chromatography at acid *p*H, the abnormal hæmoglobin precedes Hb *A* and separates from it (Fig. 3), but it is slower than Hb *F*.

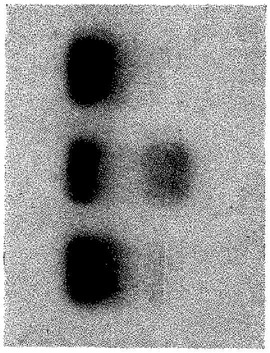


Fig. 2. Starch-gel electrophoresis of Hb *N* (above and below) and Hb *H* (centre). Phosphate buffer *p*H 6, ionic strength 0·12. Staining with amidoschwarz. The Hb *N* tends to separate from Hb *A* and forms a thicker spot than Hb *H*

Table 1. HÆMATOLOGICAL AND HÆMOGLOBINIC FINDINGS

Name and age (yr.)	Hb (gm./100 ml.)	Red blood cells (millions per c.mm.)	Mean corpuscular volume (μ^3)	Mean concentration of corpuscular hæmoglobin (per cent)	Hæmolysis in 0·4 sol. of Simmel's method	Blood film	Hæmoglobin types*			Percentage alkali-resistant Hb of total Hb
							Hb A_2	Hb A_1	Hb *N*	
Father, 36	15·2	4·7	95	33·7	97·9	Normal	1·35	62·2	36·4	1·06
Mother, 39	11·5	6·0	65	29·3	88·4	Anisocytosis, poikilocytosis, target cells, oval cells, moderate hypochromia	4·58	95·4	abs.	2·05
Gaet., 4	13·2	4·2	91	34·3	97·7	Normal	2·61	97·4	abs.	1·25
Aless., 1½						Marked anisocytosis and poikilocytosis, numerous target cells, oval cells and microcytes, marked hypochromia	1·14	62·0	36·9	1·98
Franc., 1½	6·8	3·6	67	27·8	94·5	Marked anisocytosis, poikilocytosis, microcytosis, numerous target cells and oval cells, marked hypochromia	0·78	63·5	35·7	2·36

* Percentages by starch-block electrophoresis, according to Kunkel *et al.*

In all the subjects (Table 1), the proportion of fast hæmoglobin was about 36 per cent, and that of Hb *a-r* was normal. In the ultra-violet region, the fast fraction isolated has a normal absorption spectrum; the resistance to alkali of this fraction is normal.

It is evident that the above characters correspond to those of Hb Liberia I of Robinson *et al.*[2] or Hb *N* of Ager and Lehmann[3]. Therefore, the present hæmoglobin may be classified as Hb *N*. The behaviour of Hb *N* on agar-gel electrophoresis at acid *p*H is not different from that of Hb *A*.

The present observation is, so far as we know, the first of Hb *N* identified in the Italian population. Association with the microcythæmic trait is not surprising, as the family is Sardinian and microcythæmia has an average frequency of 23 per cent on the Island[1]. It is interesting to visualize the presence of an anæmic-splenomegalic syndrome in the twin brothers, who are carriers of abnormal hæmoglobin. True Cooley's disease cannot be involved, since the microcythæmia is absent in the father, and illegitimacy is ruled out by the presence of the abnormal hæmoglobin in the father and sons. However, it is quite probable that both sons are carriers of the microcythæmia inherited from the mother, and that their illness is due to the association of microcythæmia and Hb *N*. Therefore, this would be another example of illness due to the association of two different hæmatological anomalies, the first example of which is the microdrepanocytic disease which we have identified[4-5].

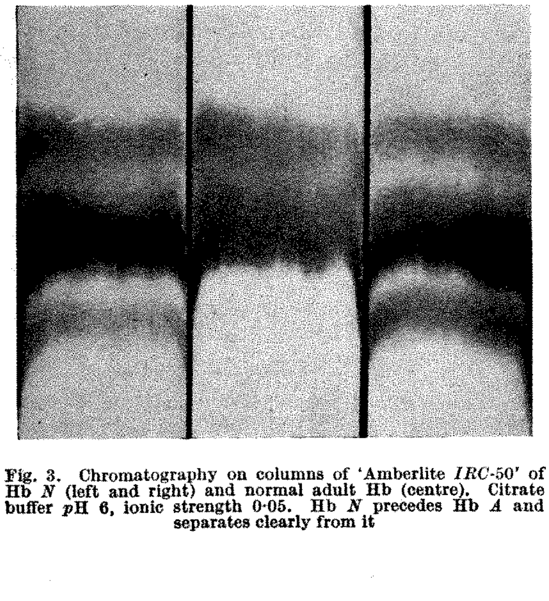


Fig. 3. Chromatography on columns of 'Amberlite *IRC*-50' of Hb *N* (left and right) and normal adult Hb (centre). Citrate buffer *p*H 6, ionic strength 0·05. Hb *N* precedes Hb *A* and separates clearly from it

The proportion of Hb A_2, which is strangely low in the twins, suggests the consideration that the presence of an abnormal hæmoglobin, faster than Hb *A*, may represent the determining factor for the reduced proportion of Hb A_2, as we see also in the carriers of Hb *H*.

This investigation was supported by a grant from the Department of Public Health.

E. SILVESTRONI
I. BIANCO

Istituto d'Igiene, Università di Roma,
Centro di Studi della Microcitemia
e delle Anemie Microcitemiche,
Roma.

[1] Silvestroni, E., and Bianco, I., *Nuovi Ann. Ig. Micr.*, **11**, 339 (1960).

[2] Robinson, A. R., Zuelzer, W. W., Neel, J. V., Livingstone, F. B., and Miller, M. J., *Blood*, **11**, 902 (1956).

[3] Ager, J. A. M., and Lehmann, H., *Brit. Med. J.*, i, 929 (1958).

[4] Silvestroni, E., and Bianco, I., *Boll. Atti Accad. Med. Roma*, **70**, 347 (1944–45).

[5] Silvestroni, E., and Bianco, I., *Haematologica*, **29**, 445 (1946).

Anti-Leucocyte Cytoplasm, Anti-Leucocyte Nuclei and Anti-Platelets in Disseminated Lupus Erythematosis

THE indirect antiglobulin consumption test[1,2] has been carried out on human leucocyte cytoplasm, leucocyte nuclei and platelets, using sera containing anti-leucocyte and/or platelet antibodies from non-transfused or minimally transfused patients: six disseminated lupus erythematosis (DLE), five idiopathic leuco-thrombopenias (ILT), four idiopathic thrombocytopenic purpuras (ITP). The auto-antibody nature of these substances was confirmed by the positive direct antiglobulin consumption test performed on the patients' own leucocytes and/or platelets. Three sera containing iso-leucocyte and platelet antibodies from three multiple transfused patients were also tested.

Leucocyte nuclei were isolated from liquefied cytoplasm[3]. Cytoplasmic proteins were obtained from the supernatant by ethanol precipitation (Colombani, J., unpublished work). Four absorptions were performed systematically and total absorption was always controlled. Elution was performed by heating the sensitized deposit for 30 min. at 56° C. in hypertonic saline. Sera, before and after absorption, and eluates were respectively tested against the three antigens (Table 1).

Table 1. ANTI-CYTOPLASM: ANTI-NUCLEUS AND ANTI-PLATELET ANTIBODIES STUDIED BY THE INDIRECT ANTIGLOBULIN CONSUMPTION TEST

		Trans-fusions	Pregnancies	Direct anti-globulin con-sumption test		Before absorp-tion*			After absorption on leucocyte nucleus*						After absorption on leucocyte cytoplasm*						After absorption on platelet*					
									Absorbate			Eluate			Absorbate			Eluate			Absorbate			Eluate		
				L	*P*	*N*	*C*	*P*	*N*	*C*	*P*	*N*	*C*	*P*	*N*	*C*	*P*	*N*	*C*	*P*	*N*	*C*	*P*	*N*	*C*	*P*
DLE	ALI.	0	0	+	+	8	7	8	0	6	8	3	1	0	7	0	8	0	4	0	8	7	0	0	0	3
	PAG.	1	0	+	+	7	6	8	0	6	8	2	0	0	6	0	8	0	4	0	5	6	0	0	0	8
	SOT.	3	4	−	(+)	6	6	8	0	5	8	3	0	0	7	0	8	0	3	0	7	5	0	0	0	3
	BAS.	0	0	+	+	4	6	7	0	6	7	1	0	0	5	0	7	0	3	1	5	5	0	0	0	2
	LAP.	0	0	+	+	5	5	7	0	4	6	1	0	0	5	0	7	0	2	0	5	4	0	0	0	3
	HUB.	0	1	+	+	8	8	8	0	8	8	1	1	0	7	0	8	0	5	1	8	8	0	0	0	3
ILT	BUR.	0	0	+	+	0	8	7	0	8	3	0	0	0	0	0	8	0	4	0	0	8	0	0	0	3
	D'AM.	0	2	+	+	0	6	5	0	6	4	0	0	0	0	0	5	0	3	0	0	6	0	0	0	2
	CHA.	1	1	+	+	0	8	7	0	7	6	0	0	0	0	0	6	0	4	0	0	7	0	0	0	3
	DEY.	3	1	+	+	0	8	8	0	8	8	0	0	0	0	0	8	0	6	0	0	8	0	0	0	3
	FAU.	0	4	+	+	0	5	4	0	5	4	0	0	0	0	0	4	1	4	0	0	5	0	0	0	1
ITP	CAR.	0	4	−	+	0	0	6	0	0	5	0	0	0	0	1	5	0	0	0	1	1	0	0	0	3
	CARL.	2	2	−	+	0	0	5	0	1	4	0	0	0	0	1	4	0	0	0	0	0	0	0	0	2
	MAL.	2	1	−	+	0	0	6	0	0	6	0	0	0	0	0	5	0	0	1	0	0	0	0	0	2
	MAC.	0	0			0	0	5	0	0	6	0	0	0	0	0	6	0	0	0	0	0	0	0	0	1
Iso-anti-bodies	CARP.	122	3	−	−	0	8	8	0	8	8	0	0	0	0	0	8	0	4	0	0	6	0	0	0	4
	RIC.	147	1	−	−	0	7	6	0	5	5	0	0	0	0	0	6	0	4	0	0	6	0	0	0	3
	ROG.	27	2	−	−	0	7	6	0	6	5	0	0	0	0	0	5	0	3	0	0	6	0	0	0	3

L, Leucocytes; *C*, leucocyte cytoplasm; *N*, leucocyte nucleus; *P*, platelet.

* The figures are the number of geometric dilution lost by the antiglobulin titre (titrated against *O* Rh + RBC sensitized by incomplete anti-*D*) after contact with the antigens sensitized by the auto- or iso-antibody.

DLE sera contained three distinct antibodies against cytoplasm, nuclei and platelets. ILT sera contained two antibodies against cytoplasm and platelets. ITP sera contained only one antibody against platelets. The sera of patients with multiple transfusions behave like ILT sera.

Table 2. DIFFERENTIATION BETWEEN THE ANTI-LEUCOCYTE AND ANTI-PLATELET ANTIBODIES DETECTED BY THE INDIRECT ANTI-GLOBULIN CONSUMPTION TEST

	Action on animal leuco-cyte or platelet	Agglutinating power on leuco-cyte and/or platelet	Antibodies not detected by some antiglobulin sera
Anti-leucocyte antibodies			
DLE	+ +	±	−
ILT	−	−	−
Iso-antibody	−	+ +	−
Anti-platelet			
DLE	+ +	±	−
ILT	−	−	−
ITP	−	−	+ +
Iso-antibody	−	+ +	−

The three anti-cytoplasm antibodies are not identical. DLE anti-cytoplasm reacts with the leucocyte cytoplasm of animal origin (ox, sheep, pig, horse), though the others do not react. ILT sera are not leuco-agglutinating, though iso-leuco-antibodies used in this work are strongly agglutinating.

The four anti-platelet antibodies are not identical. DLE anti-platelet reacts with animal platelets, though the others do not. ILT and ITP sera are not thrombo-agglutinating, though iso-thrombo-antibodies used in this work are agglutinating.

Table 3. ANTIGLOBULIN CONSUMPTION TEST ON LEUCOCYTE NUCLEUS AND CYTOPLASM AND ON PLATELETS FROM VARIOUS ANIMAL SPECIES

Antibodies from	Name	Ox			Sheep			Pig			Horse		
		N	*C*	*P*	*N*	*C*	*P*	*N*	*C*	*P*	*N*	*C*	*P*
DLE	ALI.	8	7	3	5	7	3	6	7	6	6	6	5
	BAS.	7	6	2	7	6	2	8	6	4	6	5	5
	SOT.	6	7	2	8	6	2	7	5	3	6	7	5
	HUB.	7	5	2	6	7	3	7	4	4	6	6	4
ITP	CARL.	0	0	0	0	0	0	0	0	0	0	0	0
	CART.	0	0	0	0	0	0	0	0	0	0	0	0
ILT	BUR.	0	0	0	0	0	0	0	0	0	0	0	0
	DAM.	0	0	0	0	0	0	0	0	0	0	0	0
Iso-antibodies	CARP.	0	0	0	0	0	0	0	0	0	0	0	0
	RIC.	0	0	0	0	0	0	0	0	0	0	0	0
Control		0	0	0	0	0	0	0	0	0	0	0	0

ITP antiplatelet antibodies react with a component of the antiglobulin sera which is different from the component reacting with the three other anti-platelet antibodies[4] (Tables 2 and 3).

These findings show that DLE sera possess an anti-cytoplasm factor at least as strong as the anti-nucleus factor when using the antiglobulin consumption test, although with fluorescent antiglobulin only the nucleus seems to be sensitized. Anti-cytoplasm antibodies in DLE were already suspected[5,6] but had not been directly demonstrated in human material.

J. DAUSSET
J. COLOMBANI
M. COLOMBANI

Centre National de Transfusion Sanguine,
Paris, XVe.

[1] Steffen, C., and Schindler, H., *Münch. Med. Wchschr.*, **15**, 469 (1955).
[2] Miescher, P., *Vox Sang.*, **5**, 116 (1955).
[3] Nathan, D. J., and Snapper, I., *Blood*, **13**, 883 (1958).
[4] Dausset, J., Colombani, J., and Colin, M., *Nature*, **188**, 865 (1960).
[5] Asherson, G. L., *Brit. J. Exp. Path.*, **40**, 209 (1959).
[6] Seligmann, M., *Acta Hæmat.*, **24**, 81 (1960).

IMMUNOLOGY

Elimination of Bovine Serum Albumin in Acute Experimental Uræmia

In patients suffering from acute or chronic uræmia the production of classical antibodies has been shown to be within normal limits[1-4]. The Mantoux test may be positive in chronically uræmic humans, implying that the formation of certain intra-cellular antibodies is also maintained. However, skin homografts survive for prolonged periods in the uræmic human[1,2], indicating that the onset of transplantation immunity is delayed. Medawar[5] has stated that the instruments of transplantation immunity are antibodies bound to cells of the lymphoid series and not serum antibodies. Skin homografts also survive for extended periods in uræmic rabbits[6], and this has been shown to be due to a delay in the onset of the immune responses in the draining lymph nodes (ref. 6 and unpublished work by Barwell, R. G., *et al.*). Having demonstrated the effect of uræmia on the production of the cell-bound antibodies of transplantation immunity, we decided to investigate (*a*) the elimination of a hetero-

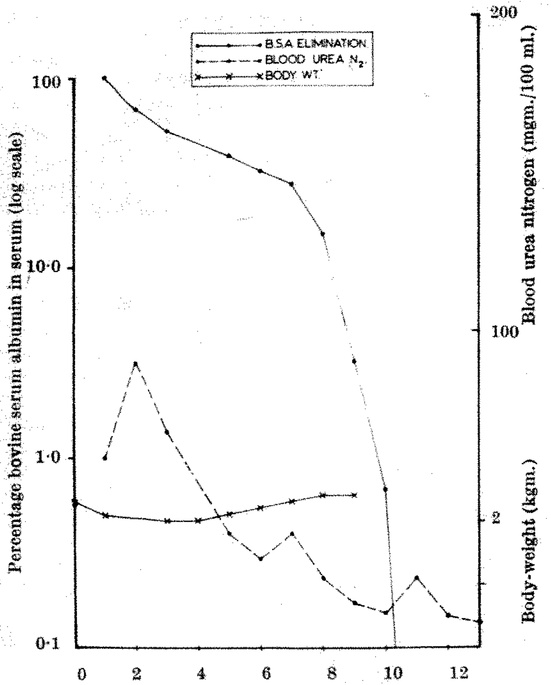


Fig. 1. Graph to show elimination of bovine serum albumin, levels of blood urea nitrogen and body-weight in a mildly uræmic rabbit

logous serum protein and (*b*) the production of serum antibodies in the uræmic rabbit.

Rabbits of Flemish giant strain each weighing 2·5–3·5 kgm. were used. The animals were anæsthetized by intravenous sodium pentobarbitone. The left kidney was removed from each animal and the right renal vascular pedicle occluded for 1 hr. by a non-crushing arterial clamp. After release of the clamp, the kidney was seen to flush and was then replaced within the abdominal cavity and the wound closed. Immediately after removal of the clamp, each rabbit received 5 ml. of a 5 per cent solution of bovine serum albumin (Armour) intravenously. Specimens of blood were taken for immunological examination, and blood urea nitrogen was determined at 24 hr. after the injection of bovine serum albumin and at daily intervals for a further twelve days. The blood urea nitrogen was estimated by the method of Archibald[7] and each animal was weighed daily.

The samples of blood obtained for immunological analysis were allowed to clot, and serum separated by centrifugation and stored in sterile rubber-stoppered glass tubes at 4° C. for at least 7 days to allow precipitation to occur. The sera were then recentrifuged and the bovine serum albumin contents were determined by the constant-antibody precipitation technique. The level of serum bovine serum albumin 24 hr. after injection was taken as 100 per cent, and readings obtained thereafter for each animal were expressed as a percentage of the first reading.

The effect of mild uræmia on the elimination of bovine serum albumin and on the body-weight is shown in Fig. 1. In this animal the blood urea nitrogen rose to 90 mgm. per 100 ml. 48 hr. after operation and then fell, at first rapidly, and then more slowly towards normal levels. Loss of weight occurred but only during the first three days of observation. The elimination of bovine serum albumin in this animal proceeded in a manner which could not be distinguished from normal. After equilibration, a logarithmic phase of elimination occurred lasting four days, followed by a period of rapid 'immune' elimination for the next two or three days before the bovine serum albumin became undetectable. This pattern of response was found in four rabbits.

The results obtained from an animal with severe uræmia are shown in Fig. 2. By the fifth day, the blood urea nitrogen had risen to 190 mgm. per 100 ml. and, after a transient fall, it again rose before death occurred in severe uræmia on the thirteenth post-operative day. A progressive loss of weight occurred during the course of the experiment. After the equilibration phase, the bovine serum albumin was eliminated logarithmically from the blood over the remaining period of observation. Its half-life during the logarithmic phase was 4 days, which is similar to the half-life in the logarithmic phase of normal rabbits. The half-life of endogenous albumin in normal rabbits is 6–8 days; hence despite the absence of the immune phase of elimination in the severely uræmic rabbit, the bovine serum albumin is still recognized as a foreign protein. Two of the three severely uræmic rabbits which showed this pattern of response and survived the experiment had formed serum antibodies when tested on the thirty-fourth post-operative day.

These experiments demonstrate a marked delay in the elimination of bovine serum albumin from the sera of severely uræmic rabbits. It seems likely that the persistence of detectable antigen for longer periods than in the mildly uræmic or normal animal is associated with the impaired production of serum antibodies, but how this occurs is unknown. It has been shown that the elimination of heterologous proteins from the blood stream is also prolonged after

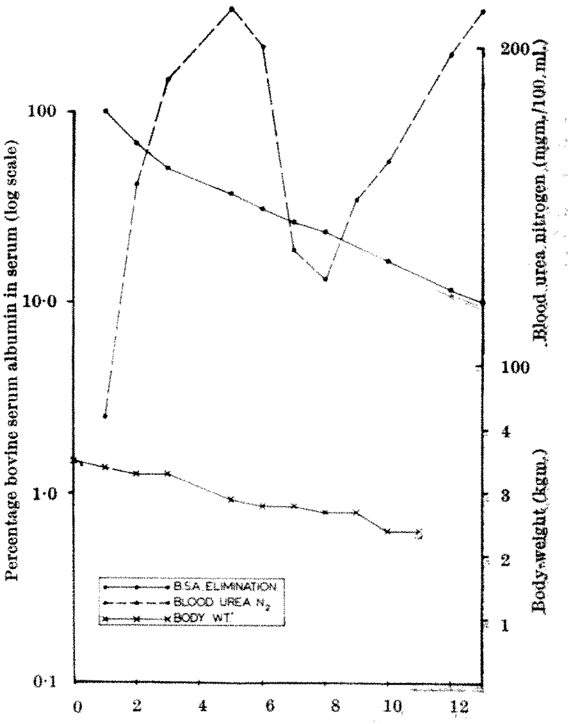


Fig. 2. Graph to show elimination of bovine serum albumin, levels of blood urea nitrogen and body-weight in a severely uræmic rabbit

the administration of the purine metabolic analogue, 6-mercaptopurine[8]. This drug also prolongs the survival of skin homografts in rabbits[10] and renal homotransplants in dogs[9].

A more detailed account of our findings, together with further work on the effect of experimental uræmia on classical immunological responses, will be published later.

G. GOWLAND*
F. G. SMIDDY
R. G. BURWELL
F. M. PARSONS

Departments of Bacteriology,
Surgery, Anatomy and the
Medical Research Council
Unit on Metabolic Disturbances in Surgery,
University of Leeds.

* Present address: Department of Zoology, University College, London, W.C.1.

[1] Couch, N. P., *et al.*, *J. Clin. Invest.*, **34**, 324 (1957).

[2] Dammin, G. J., *et al.*, *Ann. N.Y. Acad. Sci.*, **64**, 967 (1957).

[3] Stoloff, J. L., *et al.*, *New England J. Med.*, **259**, 320 (1958).

[4] Balch, H. H., *Ann. Surg.*, **142**, 145 (1955).

[5] Medawar, P. B., in *Cellular and Humoral Aspects of the Hypersensitive States*, edit. by Lawrence, H. Sherwood (Cassell, London, 1959).

[6] Smiddy, F. G., *et al.*, *Brit. J. Surg.* (in the press).

[7] Archibald, R. M., *J. Biol. Chem.*, **157**, 507 (1945).

[8] Schwartz, R., *et al.*, *Proc. Soc. Exp. Biol. Med.*, **99**, 164 (1958). *J. Clin. Invest.*, **38**, 1394 (1959). Schwartz, R., and Dameshek, W., *Nature*, **188**, 1692 (1959).

[9] Meeker, W. R., *et al.*, *Proc. Soc. Exp. Biol. Med.*, **102**, 459 (1959); *Ann. N.Y. Acad. Sci.*, **87**, 203 (1960). Robinson, J. L., and Christian, C. L., *Nature*, **187**, 796 (1960). Schwartz, R., *et al.*, *J. Clin. Invest.*, **39**, 952 (1960).

[10] Calne, R. Y., *Lancet*, i, 417 (1960).

Demonstration by Paper Electrophoresis of an Auto-immunological Precipitin possibly due to Fibrinolysins

WE have examined recently blood containing fibrinolysins in high titre. The blood was taken from a man who was later diagnosed as suffering from prostatic malignancy. The presence of high-titre fibrinolysins, raised formal acid phosphatase, and greatly reduced fibrinogen content, were all carried out by routine, accepted biochemical methods, and were fully diagnostic. It was then considered that there could be present a circulating auto-immunological factor which we might be able to demonstrate.

The method chosen was what we have called joint paper electrophoresis, and is essentially that of Flynn and Mayo[1], the only addition being that we offer a suitable source of fibrinogen to the serum containing fibrinolysins. We do this by applying plasma from another subject a short distance behind the serum start line, the result being, naturally, two protein separations, one behind the other. Our findings show that it is possible to produce a thin band typical of auto-immunological precipitins. This band appears in the region of the serum γ-globulin separation and we believe it to be an auto-immunological fibrinolysate.

Procedures taken to discover the optimal source of fibrinogen and to qualify our belief have been fully embodied in a paper which we hope to publish in the near future.

MARION I. COOPER
J. R. SYKES

Worthing Hospital,
Worthing, Sussex.

[1] Flynn, F. V., and Mayo, P., *Lancet*, ii, 235 (1951).

PATHOLOGY

Immunological Observations in Experimental Silicosis

EVIDENCE which suggests that an immunological mechanism may participate in the pathogenesis of human silicosis has been summarized by Vigliani and Pernis[1]. We have attempted to identify such a mechanism in the experimental disease by applying the fluorescent antibody technique of Coons and Kaplan to rats with silicosis at different stages of development, while observing at the same time the γ-globulin- and conglutinin-levels in their sera.

Silicosis was induced in rats by the intratracheal injection of a quartz suspension under ether anæsthesia, the rats being killed approximately 1–12 months later. Serum was obtained from the rats by cardiac puncture just before death and analysed for protein composition (paper electrophoresis) and for conglutinin titre[2]. Sera were prepared in rabbits against rat whole-serum and against rat γ-globulin obtained by ethanol fractionation[3]. Antibody protein was conjugated with lissamine rhodamine *B* 200[4] and applied to sections fixed in ethanol after preparation from freshly frozen silicotic lung in a cryostat. The customary control procedures were employed.

The serum globulin and conglutinin (Table 1) tended to show changes similar in type to those found by Ceppellini and Pernis[5] in human silicosis. In rats with silicosis more than 6 months the serum globulin was sometimes raised, but there was no elevation in three long survivors. When an increase occurred it mainly affected γ-globulin, though in some animals there was also a slight elevation of β-globulin. Silicotic rats often had a considerable augmentation of conglutinating activity in their sera, but this rise was related neither to the duration of their disease nor to the occurrence of hyperglobulinæmia.

Table 1. SERUM FINDINGS IN EXPERIMENTAL SILICOSIS

Duration of disease (months)	Conglutinin titre	Hyper-globulinæmia
1	1 in 32	Absent
1	8	Absent
1	64	Absent
3½	8	Absent
3½	256	Absent
6½	8	Present
6½	128	Present
6½	4	Present
11	64	Present
11	32	Absent
11	128	Absent
12	4	Absent
12	32	Present

By immuno-electrophoresis[6], it was established that the rabbit antiserum to rat whole-serum produced precipitin bands to all components of rat serum proteins, whereas the rabbit antiserum to rat γ-globulin showed the characteristic bands in the appropriate zone with only very slight β-globulin activity. Although many of the rat silicotic nodules showed hyalinization, no fluorescence of their connective tissue was observed in any of the sections treated with conjugated antibody to rat whole-serum or to rat γ-globulin. Some fluorescence did, however, occur within the larger blood vessels and in exudate lining alveoli, and was, presumably, a reflexion of the presence of serum proteins. In view of this negative finding in the fibrous tissue of the nodules it was necessary to provide a positive control to our technique. Sections from a human rheumatoid nodule were therefore treated with a conjugated antiserum to human globulin. Vivid specific fluorescence of the central hyaline material was obtained, thereby confirming the findings of Vazquez and Dixon[7].

Chemical analysis of the hyalinized connective tissue from human silicotic nodules showed that it consisted of 80 per cent protein, but only about 40 per cent of this was thought to be collagen, the remainder resembling serum β- and γ-globulins[1]. The immunological work of Ceppellini and Pernis[5] supported this view. Using a different technique and experimentally induced silicosis, we have been unable in mature nodules to provide evidence of the presence of globulins similar to those of the serum. Moreover, the conglutinin titre and the occurrence of hyperglobulinæmia bore no consistent relationship to the stage of maturity of the lesions. Our essentially negative results may mean that the induced lesions did not contain globulins or that, if present, our technique was not sufficiently sensitive to detect them. Further chemical and immunological investigations are evidently needed to decide whether or not the pathogenesis of the experimental disease is essentially different from that of human silicosis.

Dr. Nagelschmidt kindly provided the sized quartz dust.

J. H. JONES

Department of Pathology,
University of Wales,
Cardiff.

A. G. HEPPLESTON *

Department of Pathology,
University of Durham.

* Formerly University of Wales.

[1] Vigliani, E. C., and Pernis, B., *J. Occup. Med.*, **1**, 319 (1959).
[2] Coombs, A. M., and Coombs, R. R. A., *J. Hyg.*, **51**, 509 (1953).
[3] Nichol, J. C., and Deutsch, H. F., *J. Amer. Chem. Soc.*, **70**, 80 (1948).
[4] Chadwick, C. S., McEntegart, M. G., and Nairn, R. C., *Lancet*, i, 412 (1958).
[5] Ceppellini, R., and Pernis, B., *Nature*, **181**, 55 (1958).
[6] Grabar, P., and Williams, jun., C. A., *Biophys. Biochim. Acta*, **17**, 67 (1955).
[7] Vazquez, J. J., and Dixon, F. J., *Lab. Invest.*, **6**, 205 (1957).

Transplantation of Murine Leukæmia by Unconventional Routes

A LYMPHOCYTIC type of leukæmia which arose spontaneously in a mouse of strain *CBA* has already been described[1]. Investigation of transmission of this leukæmia between mice of the strain of origin revealed the following features: transmission could not be effected by the injection of cell-free homogenates of leukæmic organs into mice either before or after birth; transplantation bioassays of single-cell suspensions of morphologically intact leukæmic cells showed that an average intraperitoneal inoculum of only two cells would consistently achieve successful transplantation to 50 per cent of injected mice (that is, $TD_{50} = 2$ cells); similar very low TD_{50} values were obtained from assays performed by the subcutaneous or intravenous routes; no solid tumours appeared at sites of subcutaneous injection, and no trace of ascites appeared after intraperitoneal injection of the cells.

With the foregoing features of this strain of leukæmia in mind, I was encouraged to attempt transplantation of the cells by several unconventional routes: pervaginally, pernasally, via the conjunctival sac, percutaneously, and by limited surgical incision of the skin using instruments contaminated with viable cells. In each case, the inoculum consisted of a single-cell suspension of viable leukæmia cells in Tyrode solution containing 5 per cent of *CBA* mouse serum; the total number of cells available in each inoculum was within the range 10^4–10^5.

For pervaginal transplantation, a drop of suspension was placed in the vagina with a smooth-tipped Pasteur pipette; pernasal transplantation was effected by placing a drop of suspension on the noses of inverted lightly anæsthetized mice; application to the eyes was made simply by depositing a drop of suspension on the globe of each eye of anæsthetized mice. All the above applications were made with meticulous avoidance of trauma to the mucosal surfaces. For percutaneous transplantation, the fur was plucked from one flank and the skin was scarified with a needle sufficiently lightly to penetrate the keratin layer without drawing blood; a drop of suspension was rubbed into the scarified surface with a glass rod. To test for transplantation of cells from contaminated instruments, scissors which had been dipped in cell suspension were used to remove a small V-shaped segment from the periphery of the auricles of mice, a minor surgical procedure commonly employed to differentiate mice that are to be caged together.

The incidence of successful transplantation by these various routes, together with the survival-times of affected mice following treatment with cells, are given in Table 1.

Table 1. INCIDENCE OF LEUKÆMIA, AND SURVIVAL TIME OF AFFECTED MICE, FOLLOWING IMPLANTATION OF LEUKÆMIA CELLS AT UNUSUAL SITES

Site	Proportion of treated mice developing leukæmia	Survival-times (days)
Vagina	1/4	20
Nasal cavity	11/12	15–17
Conjunctiva	0/22	—
Scarified skin	4/4	24–27
Incised skin	6/6	18–37

It is probable that the success of intravaginal transplantation is influenced by œstrus; an effect of œstrus at the time of painting the cervix with benzopyrene has been demonstrated for the chemical induction of cervical neoplasia[2].

Since most of the cells deposited on the globe of the eye are likely to accompany the tear stream into the naso-lacrymal duct, it may appear surprising that this transplantation route was far less effective than the direct instillation of cells into the nose. On the other hand, the method used to introduce cells into the nose would be expected to result in cells reaching the ethmoid region or being inhaled into the bronchi, where they would come to lie on non-stratified epithelium; cells entering the nasal cavity from the naso-lacrymal duct would be unlikely to reach these regions.

About ten days after application of cells to the scarified skin, temporary enlargement of the regional lymph nodes was observed, suggesting that leukæmia cells may have entered the lymphatics of the skin.

In no case was a local tumour seen at any of the sites of implantation used.

The results of these experiments seem to justify use of the term 'contagion' to describe the means of cellular transplantation reported, and it is clear that a strictly 'aseptic' routine must be followed if mixing of cell strains is to be confidently avoided in laboratories where several different leukæmia strains indigenous to the same mouse strain are maintained by serial transplantation. It is not inconceivable that occasional accidental transplantation could occur from cannibalization of dead leukæmic mice by isologous cage-mates.

Although a considerably longer latent period usually distinguishes the development of virus-induced leukæmia from that of transplanted leukæmia, the present findings indicate that stringent criteria for cell exclusion must be observed in all experiments which purport to demonstrate subcellular transmission of leukæmia.

All mice dying of leukæmia following the introduction of cells by the unusual routes described showed the typical pattern of generalized visceral infiltration, with no signs referable to the point of entry of the cells. This finding seems to emphasize that evidence for a multifocal origin of spontaneous leukæmia, commonly mooted in text-books of general pathology, cannot be got from histopathological or morbid anatomical study of the leukæmic individual.

Harold B. Hewitt

John Burford Carlill Laboratories,
Westminster School of Medicine,
London, S.W.1.

[1] Hewitt, H. B., *Brit. J. Cancer*, **12**, 378 (1959).
[2] Stein-Werblowsky, R., *Brit. J. Cancer*, **14**, 300 (1960).

BIOLOGY

Effect of Thyroxine Plus Thiourea on the Early Development of the Chum Salmon (*Oncorhynchus keta*)

Available evidence indicates that the effect of the thyroid hormone in fishes is morphogenetic rather than metabolic. Thyroid treatment stimulates growth differentially during development, so that proportions of fins, body-wall, weight and length are altered. Deposition of guanine in the skin of the salmonids[2] and the rate of formation of scales in sturgeons (genus *Acipenser*) are accelerated[3]. Working with the chum salmon, Dales and Hoar[4] found that thiourea slowed down growth and morphogenetic processes. They found that, while the body-wall started growing over the yolk sac very early in the case of alevins treated with thyroxine, this process was considerably retarded in the forms treated with thiourea. The experiment, the results of which are presented here, was undertaken in order to ascertain the effect of a mixture of thyroxine and thiourea on the early development of the chum salmon. It was of interest to find out whether, in a mixture, the effect of thyroxine would be dominant over that of thiourea or vice versa.

Chum salmon eggs were collected by stripping fish migrating into Cultus Lake, B.C., on November 6, 1954. They were fertilized immediately and brought to the University hatchery. The eggs were transferred to 4 troughs (5,000 in each). The capacity of each trough was 10 litres. They were placed in a running tap-water bath; the first trough was the control and contained tap water; the second contained a thyroxine solution (1 : 12,000,000); the third one contained thiourea (0·05 per cent) and the fourth contained a mixture of thyroxine (1: 12,000,000) and thiourea (0·05 per cent). The solutions were aerated at all times, and their temperatures ranged from 5° to 11° C. during the 92 days of the experiment. Temperature was recorded daily and the solutions changed weekly. Dead eggs were removed once a week.

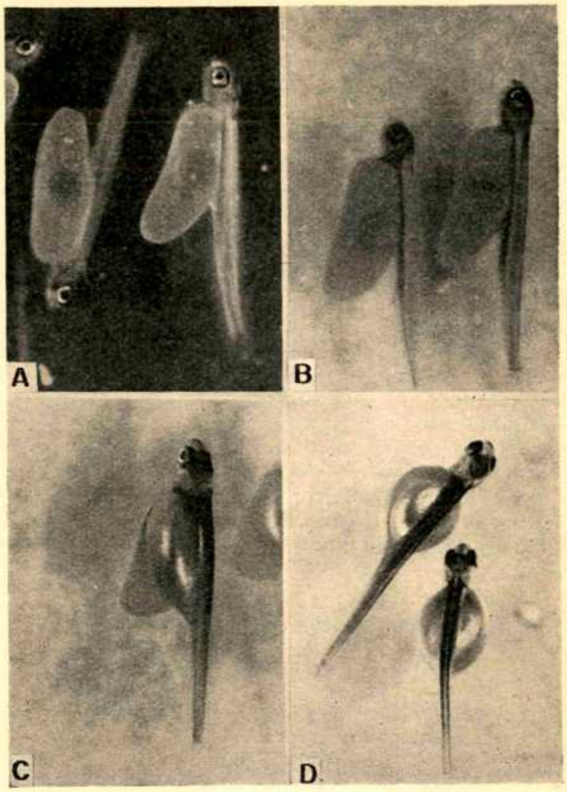


Fig. 1. Alevins soon after hatching. *A*, Control; *B*, treated with thiourea; *C*, treated with thyroxine; *D*, treated with thyroxine + thiourea. Sizes range from 20 to 26 mm. Note the growth of the body-wall and deposition of guanine in *C* and *D*

As a result of heavy mortalities from fungal infection, the experiment was terminated 92 days after it commenced. It is of interest to point out that, while the eggs in the other three tanks suffered heavy fungal attack, none of the eggs in the thiourea tank was infected. Since the eggs in the thyroxine and thiourea mixtures were infected, it may be speculated that the infecting organisms found it possible to thrive in the presence of thyroxine even though thiourea was also present, although they did not survive in a pure thiourea solution.

Eggs in the thyroxine and thiourea mixture hatched first (56 days after fertilization), followed by those treated with thyroxine (60 days), control (64 days) and those treated with thiourea (67 days).

The early development of the chum salmon has already been studied[5]. Development of the embryos prior to hatching, their morphological characteristics before and after hatching, rate of yolk absorption, pigmentation, guanine deposition, rate of heart-beats and mouth and gill movements were investigated. Observations made in the case of control, thyroxine and thiourea groups were similar to those made by Dales and Hoar[4] and will not be repeated here.

Careful examination showed that the development of eggs incubated in a mixture of thyroxine and thiourea solutions is very similar to that of eggs treated with thyroxine alone and the observations of Dales and Hoar[4]. Small variations do occur but are not of any significance. The accompanying photographs (Fig. 1) show the alevins from the four tanks soon after hatching. In these photographs, the similarity between the alevins treated with thyroxine and those treated with thyroxine + thiourea, and the dissimilarity between the alevins treated with the mixture and those treated with thiourea as well as the control alevins are obvious.

From these results it would appear that during the early development of the chum salmon the influence of thyroxine prevails over that of thiourea. While thiourea exerts some influence in the absence of thyroxine, in a mixed solution the effects of thyroxine are dominant and completely suppress the effect of thiourea, even though the former is present only in a much more dilute form than the latter.

M. A. Ali

Département de Biologie,
Université de Montréal,
Montréal, P.Q.

[1] Hoar, W. S., in *The Physiology of Fishes*, **1**, 259, edit. by Brown, Margaret E. (Academic Press, New York).

[2] Fontaine, M., *J. Cons. Perm. Int. Explor. Mer.* **19**, 23 (1953).

[3] Gerbilsky, N. L., and Saks, M. G., *Doklady Akad. Nauk S.S.S.R.*, (N.S.), **55**, 663 (1947).

[4] Dales, S., and Hoar, W. S., *Canad. J. Zool.*, **32**, 244 (1954).

[5] Mahon, E. F., and Hoar, W. S., *J. Morphol.*, **98**, 1 (1956).

Oocyte Numbers as a Diagnostic Character for the Identification of Ammocœte Species

THE nature of the relationship between the European brook lamprey *L. planeri* and the closely related parasitic form, *L. fluviatilis*, has been the subject of considerable discussion, especially since the classical studies of Weissenberg[1-3] showed that differences between these species are based on biological rather than morphological criteria. The difficulty of finding taxonomic characters for the identification of their larval stages has been a matter of great inconvenience to workers in this field, especially since the two species often occur together in the same river system.

In view of the great difference in fecundity between the adult brook and river lampreys which produce about 1,000–2,000 and 4,000–40,000 eggs respectively[4-6], the suggestion was made by Weissenberg[3] that there would be corresponding differences in the number of oocytes at the ammocoete stage. This point has now been examined, using ammocoetes from the R. Yeo (Somerset) and R. Nevern (Pembrokeshire), which are known to consist of *L. planeri* only, and others from the Rivers Teifi and Usk, where both species occur.

Although the larval ovary extends almost throughout the entire length of the body cavity, the oocytes tend to occur in greatest number in the region of the mesonephric kidney anterior to the first dorsal fin. Counts have been made of the number of oocytes in random transverse sections of this part of the body for a large number of animals and the results plotted as a frequency histogram (Fig. 1). Since practically all male ammocoetes develop a few oocytes during the earlier stages of larval life, all the groups examined show a fairly high proportion with small oocyte numbers (less than 20). In *planeri* ammocoetes from the R. Yeo only one case has been recorded of more than 45 oocytes per section, but for both the Teifi and the Usk no less than 32 per cent have exceeded this figure, and at the upper limit numbers of more than 100 have occurred. The frequency distributions for the Teifi and Usk also appear to indicate the presence of two groups with smaller and larger oocyte numbers, the latter with a frequency peak at 50–55 oocytes/section. As it seemed probable that the animals with the larger oocyte numbers represented *fluviatilis* ammocoetes, estimates were made of the total numbers of oocytes in the whole ovary in animals selected from the upper and lower ranges of oocyte numbers (Table 1). This was done by the laborious method of taking sample serial sections through the whole ovary at intervals of 1–2 mm. using the microtome to measure these intervals. Measurements of the average oocyte diameters for each animal enable the total number of oocytes to be estimated. For *planeri* ammocoetes (R. Yeo and R. Nevern) these estimates have varied from 4,900 to 10,600 for animals with 25–50 oocytes/section. For material from the Teifi and Usk, ammocoetes with 60 or more oocytes/section have given total counts of 8,300–26,000. Bearing in mind the reported range of egg production in *L. fluviatilis*, it seems safe to refer the higher counts to this form, and, while there may be some overlapping of oocyte numbers in the two species, counts in excess of 14,000 or 65/section may be taken as indicating river lamprey ammocoetes. Although *P, marinus* is known to spawn in the Usk, the possi-

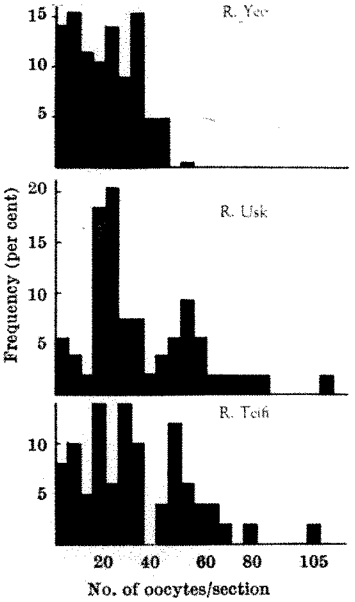


Fig. 1. Frequency histograms for oocyte numbers in ammocoetes from three different rivers

Table 1

	Total length (mm.)	Maximum No. oocytes/section	Total oocytes	Mean oocyte diameter (μ)
River Yeo	53	33	7,200	36
	62	45	8,100	57
	63	50	10,600	47
	68	32	6,100	64
	68	25	5,500	61
	84	27	5,800	75
	125	32	7,400	100
River Nevern	48	29	6,300	48
	48	37	6,500	42
	53	38	9,300	42
	54	28	4,900	38
River Teifi	61	58	11,700	41
	77	110	26,000	40
	78	63	8,300	92
	83	79	15,000	77
	110	35	6,351	103
River Usk	79	108	21,500	59
	83	65	14,600	73
	99	70	18,000	81
	99	81	22,800	70

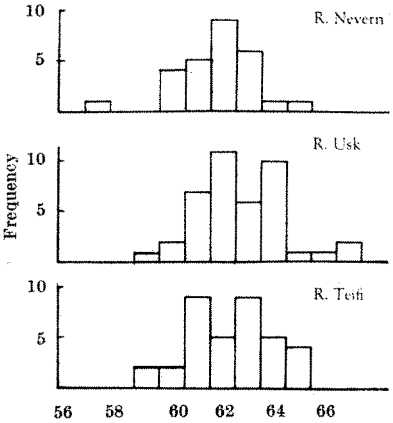


Fig. 2. Frequency distribution of myotome numbers in ammocoetes from three Welsh rivers

bility that ammocoetes with the largest oocyte counts might belong to this species can be excluded, as a number of sea lamprey larvæ which have been examined have given total counts of more than 100,000.

Myotome counts have been made on 104 ammocoetes from the Rivers Nevern, Teifi and Usk and, while the Nevern population gave a unimodal distribution with the maximum frequency at 62, histograms for the Teifi and Usk were bimodal with frequency maxima at 61, 63 and 62, 64 respectively (Fig. 2). Although previous workers have reported no significant differences in myotome numbers for the adults of *L. planeri* and *L. fluviatilis*[7-10], it was thought possible that the bimodal distributions might be due to small differences in the myotome numbers of the ammocoetes. An examination was therefore made of the gonads in two groups of ammocoetes from the Teifi and Usk, falling within the upper and lower ranges of myotome numbers. Of eleven female ammocoetes with 64–66 trunk myotomes three were of the *fluviatilis* type with more than 65 oocytes/section; the other group of twelve animals with 59–63 myotomes contained one ammocoete of this species with 110 oocytes/section. Since *planeri* ammocoetes were found over the whole range of myotome numbers (59–66) there is no evidence for differences in the distribution of myotome numbers in the two species.

Although the method of identification based on oocyte numbers has obvious limitations, it is quite possible that in older ammocoetes the males also could be differentiated by the number of spermatogonial follicles and the relative size of the testis. In any event the ability to identify at least the female ammocoetes provides the necessary basis for further attempts to discover other more convenient diagnostic characters.

M. W. HARDISTY

College of Science and Technology,
Bristol.

[1] Weissenberg, R., *Zool. Anz.*, **63**, 293 (1925).
[2] Weissenberg, R., *Z. Mikr.-anat. Forsch.*, **5**, 153 (1926).
[3] Weissenberg, R., *Z. Mikr.-anat. Forsch.*, **8**, 193 (1927).
[4] Ivanova-Berg, M. M., *Bull. Inst. Freshwater Fish.*, **14**, 5 (1932).
[5] Lanzing, W. J. R., *Studies on the River Lamprey* (Utrecht, 1959).
[6] Zanandrea, G., *Boll. Pesc. Pisc. e. Idrobid.*, **6**, 1 (1951).
[7] Cotronei, G., *Rend. Acc. Naz. Lincei*, **6**, 3–4, 371 (1927).
[8] Cotronei, G., *Pubbl. Staz. Zool. Nap.*, **8**, 3–4, 371 (1927).
[9] Vladykov, V., *Copeia*, **3**, 215 (1955).
[10] Zanandrea, G., *Arch. Zool. Ital.*, **42**, 249 (1957).

Warm-water Species in the Plankton off Newfoundland during Winter Months

SINCE September 1959 plankton samples have been taken, at monthly intervals whenever possible, with the Continuous Plankton Recorder[1] between Iceland and Newfoundland. The Recorder is towed by commercial ships and obtains a continuous sample of plankton from a depth of 10 m. This sampling is part of a regular survey of the plankton of the North Atlantic Ocean and North Sea conducted from this Laboratory.

The western portions of the Newfoundland Recorder routes during the winter months of 1959–60 are shown in Fig. 1. In this sea area, boreal species, particularly the copepod *Calanus finmarchicus* and the euphausiid *Thysanoessa longicaudata*, usually constitute almost the whole of the zooplankton sampled by the Recorder. During December 1959, however, many *Calanus* present in the region off the Belle Isle Strait agreed with the description by Jaschnov[2] of the stage V copepodites of *Calanus glacialis*, an arctic form. In February 1960, the sampling followed a course towards Cape Race, and a clearly defined patch of warm-water species was found off the north-eastern slope of the Grand Banks. These included the copepods *Nannocalanus minor*, *Clausocalanus arcuicornis*, *Lucicutia flavicornis*, *Pleuromamma gracilis*, *Pleuromamma borealis* and *Heterorhabdus spinifrons*, together with the euphausiids *Thysanoessa gregaria* and *Euphausia krohnii*. The record for March followed closely a similar course to that taken during February; no warm-water species were observed but two species normally associated with cold-water conditions, the copepod *Metridia longa* and the chætognath *Sagitta maxima*, appeared in the same area. In April the Recorder was towed along a more southerly course and again no more warm-water species were encountered, but two patches of cold-water copepods, *Metridia longa* and *Calanus hyperboreus*, were found.

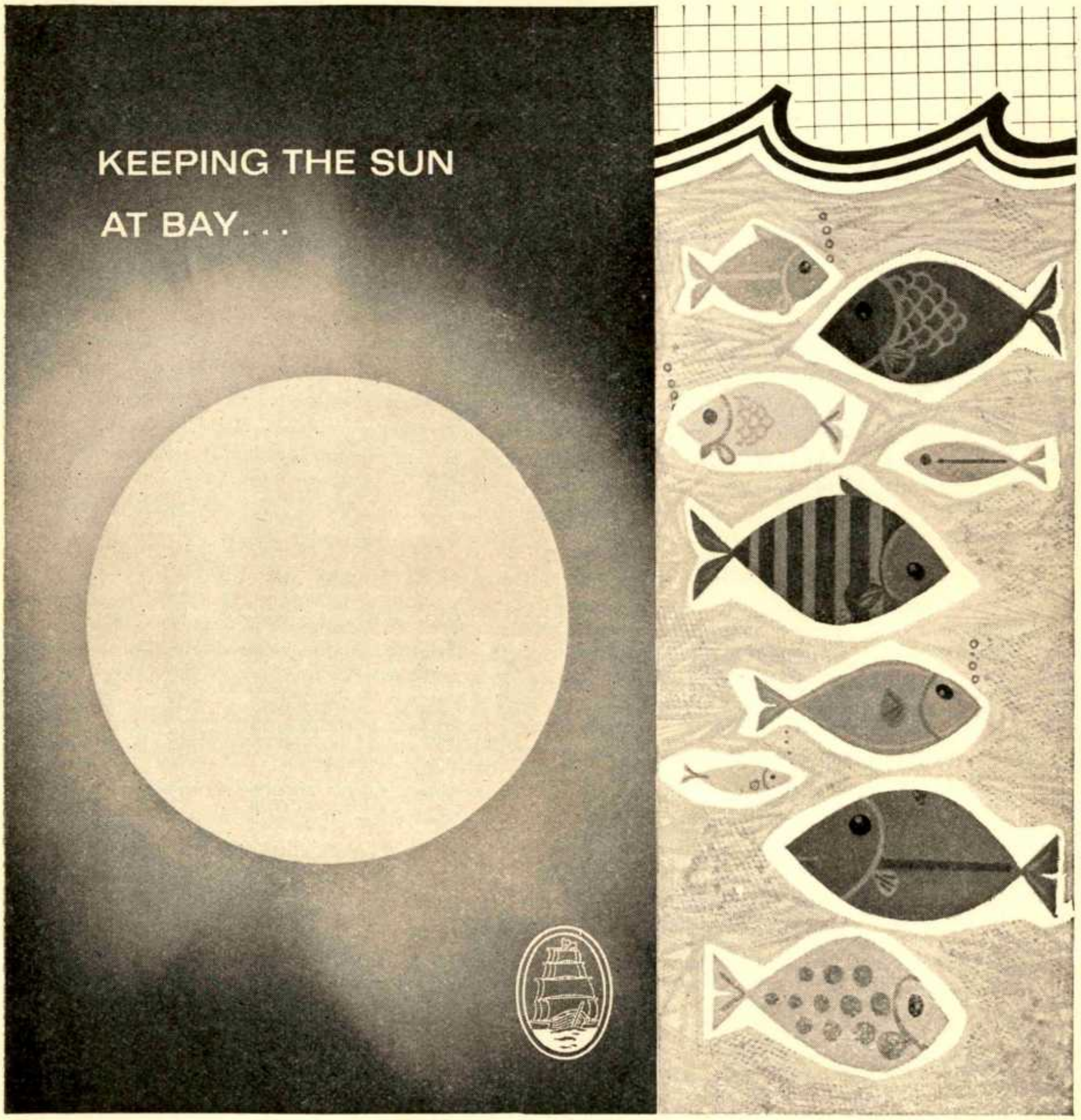
During early March 1961, a patch of warm-water species was again found, on this occasion between 52° 00′ N., 42° 25′ W. and 50° 20′ N., 43° 25′ W., that is, between 90 and 140 miles east of the patch observed during February 1960.

The occurrence of warm-water species in February and March, when surface temperatures in the region are usually close to the annual minimum[3], is related presumably to the changing pattern of water movement.

The area east of Newfoundland is influenced, from the north, by the cold water of the Labrador Current and, from the south, by the warmer waters of the North Atlantic Current, once thought to be formed by a branching of the Gulf Stream System[4]. Fuglister[5,6] has suggested that there is no real branching; but that the Gulf Stream System in this region consists of a number of disconnected filaments of current the pattern of which is variable.

The presence of warm-water species to the north-east of the Grand Banks, therefore, may indicate a temporary extension of the Gulf Stream System. It is not known whether such events occur at regular or irregular intervals, but it is interesting to note that Kielhorn[7] found several warm-water species of zooplankton in January and February at Weather Station *B* in the Labrador Sea during a survey throughout 1950.

Stommel[8] has directed attention to the lack of knowledge about fluctuations in ocean currents,



THE SUN'S THIRST is remorseless and insatiable. In the world's arid spaces, loss of water by evaporation puts both life and industry in jeopardy. Techniques developed by Price's for spreading a monomolecular film of Cetyl Alcohol blends on the water surface have successfully reduced evaporative loss by as much as 35%. Government departments and divisions of industry concerned with the conservation of water are employing Price's materials and techniques throughout the world.

A PRICE'S TECHNICAL PUBLICATION, "Fatty Alcohols for Water Conservation", tells the whole story—and may well show how our resources, and resourcefulness, can be of service to you. You are invited to take full advantage of Price's wide experience and unique facilities at our Bromborough Pool plant for creative oleochemical research, pilot-scale development, and our specialist technical sales service.

PRICE'S (BROMBOROUGH) LTD

OLEOCHEMICALS IN THE SERVICE OF INDUSTRY

PRICE'S (BROMBOROUGH) LTD., BROMBOROUGH POOL, BEBINGTON, WIRRAL, CHESHIRE. Telephone: Rock Ferry 2020

PS 76-7049-100

ACADEMIC PRESS New York and London

A Major New Book . . .

Comparative Neuropathology

By J. R. M. Innes, *Brookhaven National Laboratory, and* L. Z. Saunders, *Smith, Kline & French Laboratories, and University of Pennsylvania*

With contributions by
Ludo van Bogaert, *Institut Bunge, Antwerp*
R. Fankhauser, *University of Berne*
W. J. Hadlow, *U.S. Department of Agriculture and Agricultural Research Council Field Station, Compton, Berks, England*
K. V. Jubb, *Ontario Veterinary College, Guelph*

October, 1961, about 825 pp. (8 in. × 10 in.), approximately 215s.

COMPARATIVE NEUROPATHOLOGY is a combination atlas, text, and reference work. The coverage of neurological diseases is world-wide in scope, with a correspondingly international bibliography of approximately four thousand references. Of the about six hundred selected illustrations, a substantial majority are photographs, including two colour plates. The unusually large format of the book was chosen to permit the reproduction of the photographs in complete detail; many full-page plates are included. All aspects of cerebrospinal fluid examination are discussed comprehensively. Wherever possible the authors have made analogies between animal and human neuropathology.

Contents:

Neuropathology
Neuroanatomy
The Cerebrospinal Fluid
  By R. Fankhauser
Neurologic Diseases of Apes and Monkeys
  By Ludo van Bogaert *and* J. R. M. Innes
    Clinical Observations of the Nervous System of Apes and Monkeys
      By Ludo van Bogaert
Diseases of Skeletal Muscle
  By W. J. Hadlow
The Hypophysis
  By K. V. Jubb
Inherited Diseases and Congenital Anomalies
Viral and Rickettsial Encephalomyelitides
Protozoan Infections
Bacterial Infections
Mycotic Infections
Insect Larval Infections
Helminthic Infections
Demyelinating or Myelinoclastic Diseases
Encephalomalacia and Myelomalacia
Encephalitis: Post-Infective, Post-Vaccinal, and Experimental Allergic
Transplacental Viral Complications
Meningitis
Pigmentations and Depositions
Deficiency Diseases
Intoxications
Neoplastic Diseases
The Spinal Cord
Spinal Ganglia, Nerve Roots, and Peripheral Nerves
Miscellaneous Neurologic Disorders
APPENDIX: Herman Dexler (1866–1931)
SUBJECT INDEX.

Telephone: WHItehall 6631

111 FIFTH AVENUE, NEW YORK 3 17 OLD QUEEN STREET, LONDON, S.W. 1

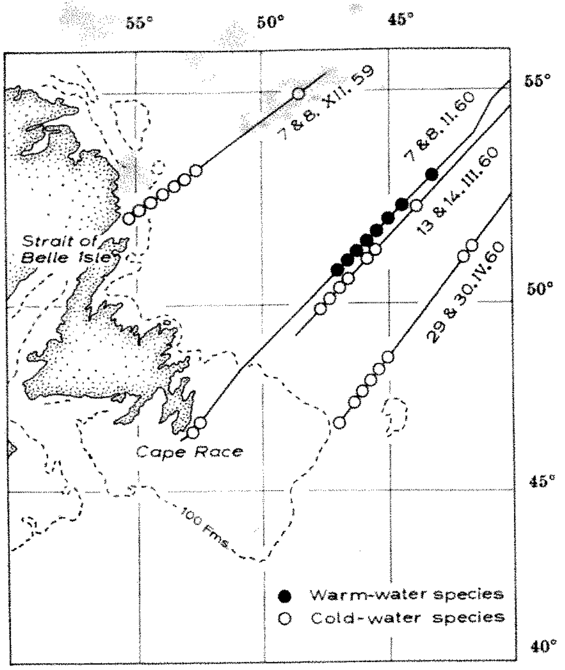


Fig. 1. Recorder routes in the region of the Newfoundland Grand Banks, during December 1959–April 1960

an omission which can only be remedied by long-term oceanographic surveys. Regular sampling with the Continuous Plankton Recorder between Iceland and Newfoundland may help to provide information on the variations and irregularities in the current system of the western North Atlantic.

I am most grateful to the staff of the Icelandic Fisheries Laboratory and to the ships' crews of the Icelandic Steamship Co. for their help in obtaining the samples.

V. Bainbridge

Oceanographic Laboratory,
Edinburgh.

[1] Hardy, A. C., *Hull Bull. Mar. Ecol.*, **1**, 1 (1939).

[2] Jaschnov, W. A., *Zool. Zh.*, **34**, 1201 (1955).

[3] Krauss, W., *Wiss. Ergebn. deutsch. atlant. Exped. 'Meteor'*, **5**, 251 (1958).

[4] Sverdrup, H. U., Johnson, W., and Fleming, R. H., *The Oceans* (Prentice Hall, New York, 1942).

[5] Fuglister, F. C., *Tellus*, **3**, 230 (1951).

[6] Fuglister, F. C., *Deep-Sea Res.*, **2**, 213 (1955).

[7] Kielhorn, W. V., *J. Fish. Res. Bd. Canad.*, **9**, 223 (1952).

[8] Stommel, H., *The Gulf Stream* (University of California Press, 1958).

Effect of Ferric Dimethyldithiocarbamate on the Rooting of Cuttings of *Hevea brasiliensis*

A METHOD involving the technique of mist-propagation which has led to the successful rooting of leafy clonal cuttings of the Para rubber tree, *Hevea brasiliensis*, has been reported earlier[1,2]. A routine application of 'Fermate' dust (ferric dimethyldithiocarbamate, kindly supplied by Messrs. E. I. Dupont de Nemours and Co., Inc.) to the base of the cuttings was mentioned in these publications and elsewhere by Levandowsky[3] also working with *Hevea* cuttings.

This compound was used as a fungicide to protect the trimmed base of the cutting which might be subject to fungal attack; but later observations showed that rotting of the stem was not usually severe, even on untreated cuttings under mist-propagation conditions, and poor rooting was often due to the deterioration of the leaves, which resulted from a long period under the spray. Improved rooting where this compound was used could not therefore be attributed entirely to its fungicidal effect and recent work has established its importance in stimulating initiation of roots in *Hevea* cuttings. Some of the evidence is presented here.

After trimming, the cuttings were stood in water for a few minutes to coagulate the latex, which is then peeled off; the bases were then dipped into the dithiocarbamate powder (diluted in talc to give 35 per cent of active ingredient) to a depth of 1 in. and immediately planted into the rooting beds containing a 1 : 1 mixture of sawdust and sand. Talc was used as the control and treatments replicated at least three times. After forty days under mist, periodical inspections for rooting were made; dead cuttings or those in poor condition due to deterioration of the leaves were discarded. Table 1 shows the rooting records from some of these experiments.

Table 1 shows that clones *RRIM* 501, 605 and 632, *AVROS* 157 and *PB* 5/63 show little or no success where untreated, but application of 'Fermate' improves rooting significantly at all stages. With *GT* 1 (two experiments) and *PB* 86 there is good final success with both treatments, but rooting is markedly accelerated where 'Fermate' is applied.

Three concentrations of 'Fermate' and the talc control were compared in two experiments with clones *PB* 86 and *RRIM* 501; the rooting records are shown graphically in Figs. 1 and 2.

Fig. 1 shows that all three concentrations of 'Fermate' accelerated the rate of rooting of *PB* 86 cuttings relative to the talc control. This is most marked with the 75 per cent powder treatment, which achieves 100 per cent success after 75 days. After 98 days, this treatment is significantly better than the control, and the 17·5 per cent concentration.

Fig. 2 illustrates the rooting of *RRIM* 501 cuttings treated similarly. Again, the 75 per cent concentration results in greatest final success and induces earlier rooting. After 114 days, all three levels of 'Fermate' show significantly greater success than the control, which has given only 10 per cent final success.

'Manzate', 'Zerlate' and 'Parzate', manganese ethylenebisdithiocarbamate, zinc dimethyldithiocarbamate and zinc ethylenebisdithiocarbamate respectively, all at 40 per cent concentration, were used in comparison with 40 per cent 'Fermate' and talc in one experiment. All four compounds led to significant increases in rooting success relative to the talc control. In final success the superiority of 'Fermate' over 'Manzate' and 'Parzate' was not established, although it was significantly better than 'Zerlate'.

Preliminary experiments using growth substances including indole-3-butyric acid and naphthalene acetic acid have proved unsuccessful in promoting root initiation in leafy clonal *Hevea* cuttings, but more experiments with these compounds and with other dithiocarbamates are in progress and will be reported in detail elsewhere.

Van der Kerk *et al.*[5] first reported that the compound *S*-carboxy-methyl dimethyldithiocarbamate showed growth-regulating activity. Ng and Carr[4] also showed that this compound at an optimal concen-

Table 1. Rooting of Seven *Hevea brasiliensis* Clones with and without 'Fermate' Treatment

Clone	Treatment	No. of cuttings	No. rooted. Cumulative totals						
			Days after insertion						
			40	50	60	70	80	98	114
RRIM 501	Talc	40	—	0	0	2	3	3	4 (10%)
	Fermate	40	—	3	3	12	14	15	18 (45%)
RRIM 605	Talc	21	0	0	0	—	—	—	—
	Fermate	21	10	13	14 (67%)	—	—	—	
RRIM 632	Talc	21	0	0	0	1	1 (5%)	—	
	Fermate	21	10	13	14	15	17 (81%)	—	
AVROS 157	Talc	21	0	0	0	1	2 (10%)	—	
	Fermate	21	6	13	16	16	19 (90%)	—	
PB 5/63	Talc	21	0	0	0	—	—	—	
	Fermate	21	5	7	7	9	10 (48%)	—	
GT 1	Talc	21	1	9	18	20 (95%)	—	—	
	Fermate	21	11	21 (100%)	—	—	—	—	
GT 1	Talc	27	2	8	17	20	21 (78%)	—	
	Fermate	27	8	17	24	24	25 (93%)	—	
PB 86	Talc	36	1	8	15	22	26	29 (82%)	
	Fermate	36	12	24	28	30	30	31 (88%)	

tration of 20 mgm./l. effected a stimulation of growth of wheat coleoptile segments and had an activity of the same order as indole-3-acetic acid.

In our experiments, related dithiocarbamate compounds have been used at extremely high concentrations and have shown a marked effect on root initiation which cannot be completely explained by their fungicidal action.

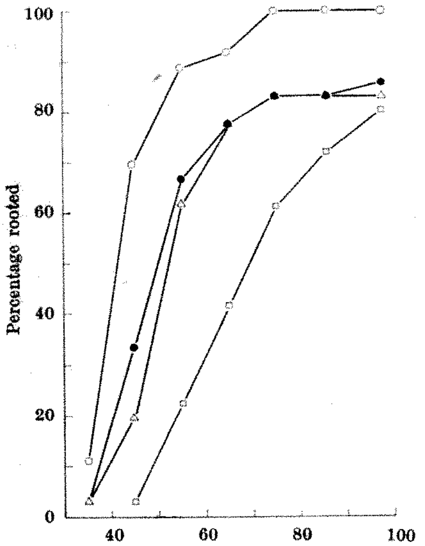


Fig. 1. Effect of three concentrations of 'Fermate' on the rooting of *PB* 86 cuttings (3 × 12 cuttings per treatment)

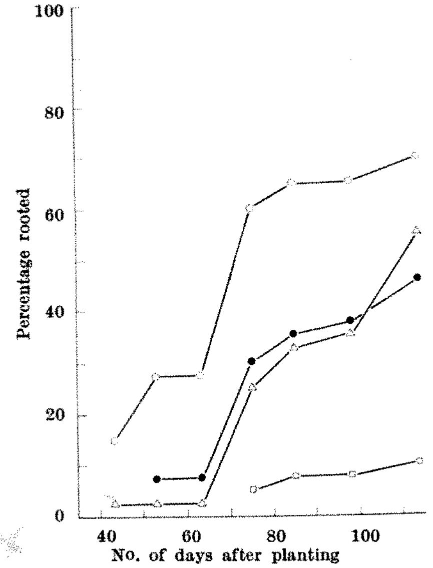


Fig. 2. Effect of three concentrations of 'Fermate' on the rooting of *BRIM* 501 cuttings (4 × 10 cuttings per treatment). Talc control: 17·5, 35 and 75 per cent 'Fermate'

G. H. Tinley

Rubber Research Institute of Malaya,
Kuala Lumpur,
Federation of Malaya.

[1] Tinley, G. H., and Garner, R. J., *Nature*, **186**, 407 (1960).
[2] Tinley, G. H., *Proc. Nat. Rubb. Res. Conf.*, Kuala Lumpur (1960).
[3] Levandowsky, D. W., *Trop. Agric.*, **36**, 247 (1959).
[4] Ng, E. K., and Carr, D. J., *Physiol. Plant.*, **12**, 275 (1959).
[5] Van der Kerk, G. J. M., van Raalte, M. H., Kaars Sijpesteijn, A., and van der Veen, R., *Nature*, **176**, 308 (1955).

Possibilities of Vegetative Propagation in the Coconut (*Cocos nucifera* L.)

The coconut is a perennial palm which takes 6–8 years to begin bearing, but later continues to bear right through the year and all through its life, extending during a period of 60 years or more depending on local conditions. It is highly cross-pollinated in Nature, particularly the tall variety, and is propagated only through seed. These features, namely, the long interval between generations and the highly heterogeneous nature of the plant population, are proving to be great handicaps in making quick headway in coconut breeding. The perennial nature of the palm, on the other hand, is of advantage, in that the benefit from any improvement can be enjoyed over a very long period.

Vegetative propagation is the one sure method of propagating lines true to type, and it could be very valuable in coconut research, as has been recently pointed out (refs. 1–3). In the coconut, which is a monocotyledonous plant devoid of cambium, the prospect of vegetative propagation is at present remote, but there are indications that the problem is worth following up. In Nature, tendencies on the part of the palm to produce aerial roots under adverse conditions in the environment and/or diseased conditions of the palms have been noticed[4]. Rare cases of the palms producing suckers have also been reported[5,6]. If these could be produced at will, some definite progress in vegetative propagation of coconut

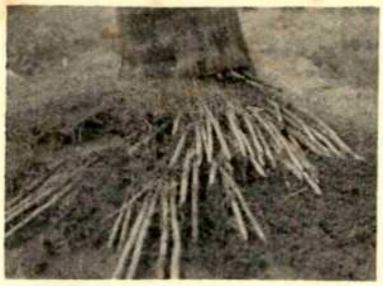


Fig. 1. Roots formed at the girdled portion on the coconut stem above ground-level where the growth substances were applied

would have been made. That rooting can be artificially induced in the stem of the coconut palm above ground-level through marcottage, has been reported[4]. After the formation of large numbers of roots, the crown can be separated by severing the palm just below the rooted region and planting elsewhere. This procedure, of course, does not permit the multiplication of desirable palms, but may help to rejuvenate and to maintain them for a much longer period than otherwise.

It is now well established that use of appropriate growth regulators in optimum concentrations can induce rooting and other meristematic activity in a variety of plants. In order to investigate how far their use in coconut will be helpful in inducing meristematic activity leading to rooting and probably suckering too, trials were carried out at this Research Station with mixtures of selected growth regulators, namely, α-naphthalene acetic acid, indole-3-butyric acid and certain purine compounds such as adenine, adenosine, alloxanthine and uric acid, in different concentrations and proportions. The growth regulators were applied as an aqueous spray or in lanolin paste after girdling or debarking the stem near ground-level. The points of application were then covered with soil which was kept moist during the rainless period by artificial watering. Initiation of roots was observed in about 1 yr. in the case of the following formulations only and roots were profuse (Fig. 1) in about eighteen months: 1 per cent indole-3-butyric acid in lanolin; 0·05 per cent naphthalene acetic acid in lanolin; and a mixture of 20 ml. of 80 p.p.m. adenosine, 5 ml. of 20 p.p.m. naphthalene acetic acid and 15 ml. of water applied as spray. There was no sign of suckering seen in any of the treatments so far.

These results thus indicate that inducing meristematic activity in the coconut is possible. Further systematic trials with a larger number of important growth regulators alone and in combinations and mixed in different proportions are under way to assess their relative merits.

Our thanks are due to Mr. A. K. Kamalakaran for his assistance.

S. R. GANGOLLY
K. M. PANDALAI

Central Coconut Research Station,
Kasaragod, S. India.

[1] Holttum, R. E., *Phytomorphology*, **5**, 339 (1955).
[2] Harland, S. C., *Bulletin* 15, Coconut Research Institute, Ceylon (1957).
[3] Haldane, J. B. S., *Indian Coconut Journal*, **12**, 1 (1958).
[4] Menon, K. P. V., *et al.*, *Indian Coconut Journal*, **8**, 79 (1955).
[5] Menon, K. P. V., and Pandalai, K. M., *The Coconut Palm—A Monograph*, 305 (Indian Central Coconut Committee, Ernakulam, 1958).
[6] Davis, T. A., *Bulletin*, Indian Central Coconut Committee, **9**, 179 (1956); **12**, 224 (1958).

Effect of Red and Far-red Irradiation on the Germination Process in Seeds of *Pinus sylvestris* L.

SINCE the first work on the red – far-red reversible mechanism controlling the germination of lettuce seeds[1], many investigations have shown the same type of light-controlled germination in seeds of herbaceous plants as well as in tree seeds[2]. In the genus *Pinus* it has been reported for *P. virginiana* Mill.[3], *P. taeda* L.[4] and earlier by me for *P. sylvestris* L.[5]. Results from further work on the red – far-red controlled germination in seeds of *Pinus sylvestris* L. are described here.

The germination experiments were carried out in Jacobsen apparatuses at +25° C. during 20 days. For the irradiations the following equipment was used: red irradiation from red fluorescent tubes (Philips *TL*/15, emission range between 5800 and 7200 Å. with a sharp maximum at 6600 Å.), far-red irradiation from a 500-W. incandescent lamp with a 10-cm. water filter and double layers of blue and red 'Cellophane' (transmission from 6800 Å. and upwards together with some transmission in blue). The intensity of both sources was $3\cdot5 \times 10^3$ ergs/cm.^{2} sec. The time of irradiation, the same for all the experiments here reported, was 30 min. The irradiations were started after 6 hr. imbibition. The respiration measurements were made by the Warburg direct method. Cell divisions in the seeds were examined by aceto-orcein staining of embryo smears[6]. All handling of the seed materials was performed in green safe-light[7].

As in lettuce seeds, the light-requirement for germination can be satisfied by a single irradiation. Maximum sensitivity is obtained after 6–12 hr. imbibition. As reported earlier[5,8], seeds which have not imbibed water can also be influenced by irradiation. From the action spectrum it is evident that red light around 6600 Å. is most effective in stimulating germination. Stimulation can be nullified by far-red irradiation immediately afterwards (see Fig. 1). With a time-lapse of 48 hr. between a red and a far-red irradiation, the latter became completely ineffective. At that time, all seed embryos investigated showed mitoses. The seed temperature between the red and far-red light impulses was of importance, but not the temperature during the irradiations. The light-requirement of the seeds for germination depends on seed coat being intact. After a small puncture or complete removal of the seed coats, germination in the dark became high. It was then impossible to inhibit germination by far-red irradiation. The same results were obtained after stratification of the intact seeds (storage on wet cotton pads in Petri dishes at +2–4° C. in the dark for 2 weeks).

In order to establish whether the red – far-red irradiations influence any phases of the germination process earlier than the visible germination, work on seed respiration and on the occurrence of mitoses in seed embryos was carried out during the first three days of the germination period. Some of the results are presented in Fig. 1.

The respiration measurements shown in Fig. 1 were started after 48 hr. imbibition, that is, 42 hr. after the start of irradiation, and similarly for the work on mitoses. The values for respiration are based on 3 × 50 seeds per experiment and expressed in μlitres oxygen/50 seeds and ¼ hr. The values for mitoses are the percentages of embryos investigated with mitoses determined on 2 × 10 embryos per

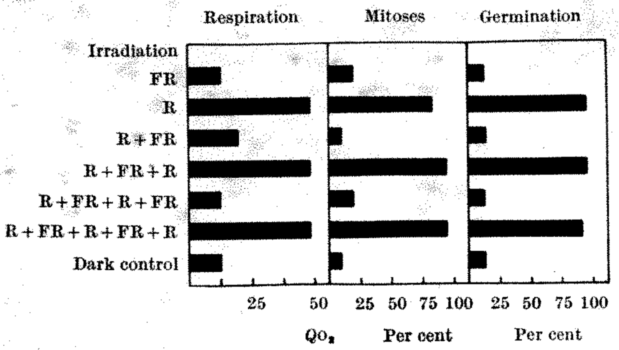
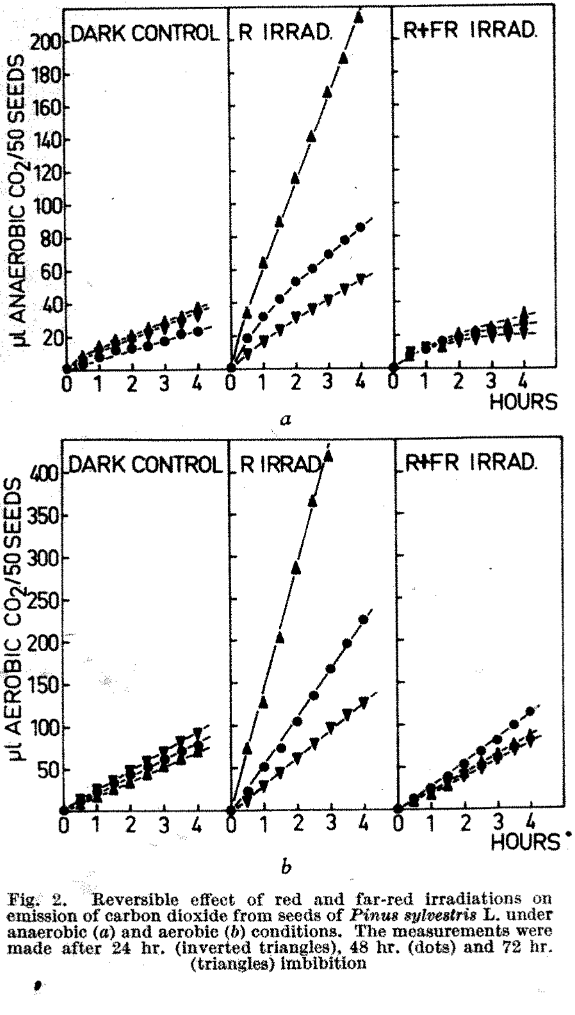


Fig. 1. Reversible effects of red (R) and far-red (FR) irradiations on the respiration-rate, the occurrence of mitoses in embryos and the percentage of germination in seeds of *Pinus sylvestris* L.

experiment. The germination values are based on 2 × (100–10) seeds per experiment (after 20 days). The stimulating effect of red irradiation is significant (0·001 > P) in all the three groups of experiments. From these results it is evident that the effect of red and far-red irradiations on the germination can be traced quite early in the germination process. Red light stimulates, and far-red light inhibits, respiration as well as mitotic activity in the embryos. A significant increase in respiratory activity induced by red light can be observed after imbibition for 24 hr. and stimulation of mitotic activity after 36 hr. The visible signs of germination (protruding rootlets) appear first after more than 48 hr. imbibition. In seed samples irradiated with red + far-red the inhibited visible germination can be traced in an inhibition of respiration and mitotic activity. In recently published work on lettuce seeds[9] it has been stressed that the occurrence of mitoses in the embryos is independent of the red – far-red irradiation, as is also the respiration of the seeds before the appearance of the first visible signs of germination. This is evidently not the case for the seeds investigated here, which in many other respects show the same responses as lettuce seeds.



a

b

Fig. 2. Reversible effect of red and far-red irradiations on emission of carbon dioxide from seeds of *Pinus sylvestris* L. under anaerobic (*a*) and aerobic (*b*) conditions. The measurements were made after 24 hr. (inverted triangles), 48 hr. (dots) and 72 hr. (triangles) imbibition

In other respiration measurements the effects of red and far-red irradiations on the emission of carbon dioxide under aerobic and anaerobic conditions have been investigated after different times of imbibition. The results are given in Fig. 2.

For both aerobic and anaerobic conditions, the rate of emission of carbon dioxide is already higher after 24 hr. in the red-irradiated seeds as compared with the dark control and the red + far-red irradiated seeds (0·001 > P). There is thus an effect of irradiation, not only on the aerobic but also on the anaerobic phase of the seed respiration. The effect on the anaerobic phase can perhaps be combined with the red – far-red effect in the synthesis of the TPN-linked triosephosphate dehydrogenase[10].

The results will be published in detail elsewhere. This work has been supported by a grant from the Swedish Forest Research Foundation.

Bengt Nyman

Institute of Forest Botany,
Royal School of Forestry,
Stockholm.

[1] Borthwick, H. A., Hendricks, S. B., Parker, M. W., Toole, E. H., and Toole, V. K., *Proc. U.S. Nat. Acad. Sci.*, **38**, 662 (1952). Borthwick, H. A., Hendricks, S. B., Toole, E. H., and Toole, V. K., *Bot. Gaz.*, **115**, 205 (1954).

[2] Toole, E. H., in *Photoperiodism and Related Phenomena in Plants and Animals*, edit. by Withrow, R. B., 89 (1959).

[3] Toole, E. H., Snow, jun., A. G., Toole, V. K., and Borthwick, H. A., *Plant Physiol.*, **31** (Supp.), xxxvi (1956).

[4] Toole, V. K., Borthwick, H. A., and Toole, E. H., *Plant Physiol.*, **33** (Supp.), xxiii (1958).

[5] Nyman, B., *Rep. First Swedish Conf. Cell Res.*, *Ark. f. Zool.*, Ser. 2, **11**, 122 (1957).

[6] Sass, J. E., *Botanical Microtechnique*, third ed. (1958).

[7] Withrow, R. B., and Price, L., *Plant Physiol.*, **32**, 244 (1957).

[8] Nordström, L., *Bull. Roy. School Forest. Sweden*, **17**, 91 (1953).

[9] Haber, A. H., and Luippold, H. J., *Plant Physiol.*, **35**, 486 (1960).

[10] Marcus, A., *Plant Physiol.*, **35**, 126 (1960).

MICROBIOLOGY

Numbers and Types of Bacteria from the Rhizosphere of Pasture Plants : Possible Occurrence of *L*-forms

During work on micro-organisms in meadow and pasture soils[1] large numbers and high activity were found in the surface soil layer of 0–5 cm. I decided to investigate the plant roots which form a dense mat in this zone since these might influence micro-organisms. Microscopical counts and estimations of the quantity of fungal and actinomycete mycelia in the soil between the roots and in soil washed from the roots were carried out using stained agar films as Jones and Mollison did, but with phase-contrast observation[2,3]. Agar plate counts were used as a second method, yielding various results, and an attempt to explain this is the purpose of this communication.

Table 1. NUMBERS OF BACTERIA IN THE RHIZOSPHERE OF *Holcus lanatus* AND SURFACE SOIL

		No. $\times 10^9$			
		Sandy loam, dry		Clay, moist	
Date	Log dilution	*Holcus lanatus*	soil	*Holcus lanatus*	soil
12.11.1957	7	170	1·50	156	1·25
	8	—	—	616	
20.1.1958	7	565	0·25	190	0·37
	8	4,430		1,260	
23.4.1958	7	367	1·24	155	0·99
	8	2,810		866	
3.6.1958	7	1,980	1·09	420	0·68
	8	2,610		2,490	
16.9.1958	7	563	1·22	1,080	1·36
	8	1,490		8,360	

Samples were collected from two locations. The suspensions from 0–5 cm. surface soil and roots obtained by vigorous shaking for 5 min. with sterilized tap-water were diluted by successive steps of 1:10 to 1:10^7 or 1:10^8 and plated with soil extract agar cooled to 45° C. When collected the day before, the samples were kept at $+2$–4° C. in a refrigerator to avoid multiplication of the bacteria.

Table 1 shows the numbers of bacteria (average values from five replicate plates) in the rhizosphere of *Holcus lanatus* on five dates as an example for twelve species of plants investigated. These were: *Dactylis glomerata, Helictotrichon pubescens, Poa pratensis, Trifolium repens, Plantago lanceolata, Achillea millefolium, Galium mollugo, Rumex acetosa*, in a meadow on sandy loam, and *Alopecurus pratensis. Festuca pratensis*, and *Ranunculus acris* in a moister meadow on clay soil. *Holcus lanatus* was collected from both locations. The magnitude of bacterial numbers is surprising and has not been reported before. It reached 8×10^{12}/gm. and depended largely on the logarithm of the dilution. In contrast the values for the soils are much lower, between 1 and 2×10^9/gm. The maximum number of rhizospheres in sandy loam soil occurred in winter when the soil population was a minimum whereas in the clay soil rhizosphere maximum was observed in early autumn when soil bacteria also reached their greatest number.

These results exclude the assumption of normally developed bacteria occurring in such large numbers. Assuming a minimum size for a small coccoid soil form of $0{\cdot}125\mu^3$, 8×10^{12} of these would fill completely 1 cm.^{3} without leaving any space between them. Even if the sample consisted entirely of bacteria of this size it could contain only about 4×10^{12}/gm. Experimental error, on the other hand, could be excluded because the control plates showed no colony growth in any case. Plates inoculated with diluted rhizosphere suspension showed even with logarithm of dilution 8 up to 2,000 colonies per plate, which could be counted with reasonable precision using a binocular microscope. Agar films gave maximal numbers between 300 and 400×10^9/gm. in the rhizosphere of *Holcus lanatus*, which had the highest numbers among all plants investigated. This is in any event considerably lower than the plate counts, which normally yield lower numbers than direct counts.

Table 2. PERCENTAGE COMPOSITION OF BACTERIAL POPULATION ISOLATED FROM SOIL AND RHIZOSPHERE DILUTION PLATES

Date	Source	No. of isolates	*Arthrobacter*	*Mycobacterium*	Gram-positive rods	Gram-negative rods	Cocci	Motile bacteria
12.11.1957	*Trifolium repens*	100	74	17	—	1	8	
12.11.1957	*Ranunculus acris*	40	91	6	2	1	—	
12.11.1957	*Alopecurus pratensis*	50	88	—	4	2	6	
3.6.1958	Sandy loam, dry	51	48	4	16	32	—	18
3.6.1958	*Trifolium repens*	59	35	3	5	57	—	33
3.6.1958	Clay, moist	53	57	2	8	24	9	11
3.6.1958	*Ranunculus acris*	54	48	5	15	32	—	37
3.6.1958	*Alopecurus pratensis*	52	75	—	12	13	—	19

These high numbers could only be caused by very small propagation units known as '*L*-forms' of 0·1–0·2μ diameter. Küster[4] obtained them from soil bacteria after treatment with penicillin or glycine and Vilas[5] identified such *L*-forms in soil. The occurrence of filterable *L*-forms in the rhizosphere of the plants investigated may be postulated. It may be favoured by effects originating from the dense population of microbes occurring in a naturally grown grass sward.

The main group of bacteria obtained belonged to *Arthrobacter*, a genus of Conn and Dimmick, 1931, autochthonous coryneform soil bacteria. They are Gram-positive to Gram-variable rods of different length, which form either smaller ovoid cells of 0·5–0·8μ diameter or larger cells in terminal swellings of 1·0–1·2μ diameter, the so-called cystites, within their cell walls. These may grow and form rods again[6,7]. Their proportion in soil and rhizosphere decreases more or less during the summer in favour of Gram-positive and Gram-negative rods. The latter are pseudomonas-like, motile short rods, some of them fluorescent and long, thin rods of 10–20μ length, which usually show a slowly creeping movement. These long rods reached the high proportion of 26 per cent only in the rhizosphere of *Trifolium repens*. In accordance with the results of Lochhead[8], the rhizosphere generally contained relatively more motile and Gram-negative bacteria than the soil. This selective rhizosphere effect was most marked with *Trifolium repens* and least with *Alopecurus pratensis*. Probably the most numerous *Arthrobacter* forms and the Gram-negative rods would provide the *L*-forms causing the high counts.

G. JAGNOW *

Institut für Grünlandwirtschaft und Futterbau,
J. Liebig-University,
Giessen.

---

* Present address: Gezira Research Farm, Wad Medani, Sudan.

[1] Jagnow, G., *Z. Pflanzenernähr., Düng., Bodenkunde*, **82**, 50 (1958).

[2] Jagnow, G., *Naturwiss.*, **46**, 269 (1959).

[3] Jagnow, G., *Zentralblatt für Bakteriol.* (in preparation).

[4] Küster, E., *Landbauforsch. Völkenrode*, **5**, 79 (1955).

[5] Vilas, L., Tejerina, G., and Rubio, M., *Fifth Internat. Congr. of Soil Sci., Leopoldville, III*, **26**, 141.

[6] Lochhead, A. G., and Burton, M. O., *Canad. J. Bot.*, **31**, 7 (1953).

[7] Chaplin, C. E., *Canad. J. Microbiol.*, **3**, 103 (1957).

[8] Lochhead, A. G., *J. Bact.*, **55**, 579 (1948).

Morphological Variation in *Ferrobacillus ferrooxidans* related to the Rate of Iron Oxidation

WHILE investigating the removal of pyritic sulphur from coal by a microbiological method, we propagated large numbers of the chemoautotrophic iron-oxidizing bacterium *Ferrobacillus ferrooxidans*. During mass culture of the bacterium in an inorganic iron salts medium (9*K*)[1], it was observed that alterations in cellular morphology occurred in response to excessively vigorous aeration. Normal cells measure 1·0–1·7μ by 0·5μ (Fig. 1*A*), while the abnormal (morphologically altered) cells differed by having a diameter of 1·0μ, so that many enlarged, coccoidal forms were present (Fig. 1*B*). The abnormal strain, on large-scale sub-culture using less vigorous aeration, or in shake-culture flasks, showed virtually complete reversion to the normal morphology after the second transfer (Fig. 1*C*, *D*).

Normal (Fig. 1*A*), abnormal (Fig. 1*B*) and reverted (Fig. 1*D*) cells were collected as previously described[1] and the rates at which they oxidized ferrous to ferric iron were determined by standard manometric techniques. Each Warburg vessel contained a total of 1·7 ml. liquid consisting of 500 μ*M* Fe^{++} (as $FeSO_4.7H_2O$ dissolved in distilled water acidified to *p*H 2·6 with sulphuric acid) in the main compartment, 0·5 ml. of cell suspension in the side arm, and 0·2 ml. of 20 per cent potassium hydroxide in the centre well. The gas phase was air. All suspensions were tipped in at zero time and the uptake of oxygen at 30° C. was measured at 10-min. intervals over a period of 70–120 min. Cellular nitrogen was determined by a micro-Kjeldahl procedure.

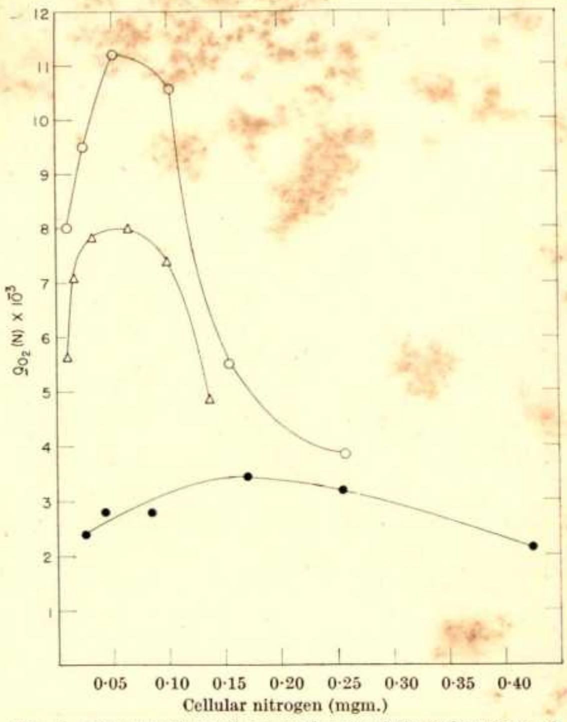


Fig. 2. Rate of uptake of oxygen by morphological variants of *F. ferrooxidans* as a function of cellular nitrogen. ○, Reverted cells; △, normal cells; ●, abnormal cells

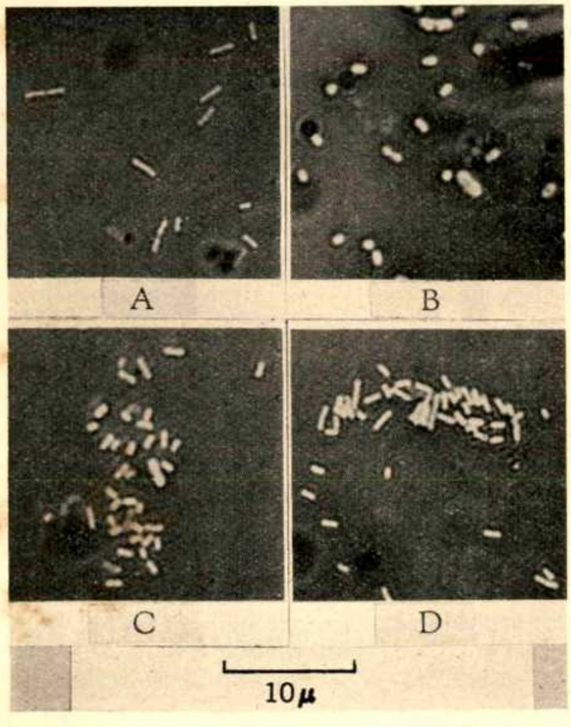


Fig. 1. Different morphological variants of *Ferrobacillus ferrooxidans*. *A*, Normal cells; *B*, abnormal cells; *C*, intermediate stage in reversion to normal morphology; *D*, reverted cells (final stage in reversion to normal morphology). Negative stain with congo red. (*c*. × 1,800)

The results indicated that abnormal cells were consistently less active than their normal or reverted counterparts. However, Qo_2(N) values (μl. oxygen uptake/mgm. cellular nitrogen/hr.) varied considerably with cell concentration within each of the resting cell suspensions. A more systematic investigation was undertaken where the rate of iron oxidation by normal, abnormal and reverted cells was examined as a function of cellular nitrogen. The results are shown in Fig. 2. The rate of oxidation of the three cell suspensions for any given concentration of cellular nitrogen followed the order—reverted > normal > abnormal. Maximum and minimum Qo_2(N) values ranged from 11,209 (0·051 mgm. nitrogen) to 3,840 (0·257 mgm. nitrogen) for reverted cells, 8,012 (0·065 mgm. nitrogen) to 4,784 (0·162 mgm. nitrogen) for normal cells, and 3,480 (0·170 mgm. nitrogen) to 1,932 (0·425 mgm. nitrogen) for abnormal cells.

Beck[2] reported that an iron-oxidizing bacterium, isolated from the acid water of Bingham Canyon, Utah, exhibited Qo_2(N) values that decreased with increasing concentrations of cells. He attributed this to the possible failure of oxygen equilibrium between the fluid and gaseous phases during rapid oxygen uptake. Our findings might be explained on this assumption up to the maximum Qo_2(N) values, but the decrease in Qo_2(N), on further dilution of the cells, argues against oxygen solubility as the sole factor in determining the curves illustrated in Fig. 2.

On the assumption that the nitrogenous constituents of the larger, more spherical, abnormal cells increased in proportion to the increased cell volume, it is interesting to speculate as to the site(s) of iron oxidation in this bacterium. The smaller surface-to-volume ratio of abnormal cells, coupled with the lower rate of oxidation, seems to imply that the cell surface may play an active part in the oxidation of iron, and that at least one, and possibly more, of the unknown steps in the transfer of electrons from ferrous iron to oxygen may occur at this site.

M. P. SILVERMAN
M. H. ROGOFF

Bureau of Mines,
4800 Forbes Avenue,
Pittsburgh 13, Penn.

[1] Silverman, M. P., and Lundgren, D. G., *J. Bact.*, **77**, 642 (1959).

[2] Beck, J. V., *J. Bact.*, **79**, 502 (1960).

Simple, Quick Methods for Detection of 6-Amino-penicillanic Acid

ONE of the greatest discoveries of the past years in penicillin research was the discovery, isolation[1] and preparation of 6-amino-penicillanic acid (6-APA) forming the common 'nucleus' of natural penicillins[2,3], and the successful introduction into therapy of semi-synthetic penicillins produced from it[4,5]. The new semi-synthetic penicillin preparations have already made great progress in the fight against resistant bacteria. The possibility is now laid open for the preparation of penicillins possessing novel effectiveness[6]. The basis for efforts of this kind is 6-APA research, and its quick identification in fermentation liquor and other reaction mixtures.

The biological methods hitherto used (such as paper-chromatography; the chemical transformation of 6-APA into some familiar natural penicillin, or bioassay) even if not complicated, are comparatively lengthy. The following two simple methods have been devised for quick detection of 6-APA:

(1) The essence of the paper-slice method is the cutting of 1 cm.-wide slices from Whatman No. 1 filter-paper in appropriate lengths and washing them first in a solution of 3 per cent sodium bicarbonate, then, after drying, in a solution of 1 per cent phenylacetylchloride in acetone. After drying in air, a solution containing 6-APA is added, drop-wise, to the impregnated paper. If there is 6-APA in the solution, it will be converted to *G*-penicillin on the paper and yield an inhibition zone. This method is appropriate for qualitative determination.

(2) The 6-APA content of fermentation liquid or other solutions is demonstrable also by means of the hole plate method. 6-APA is transformed directly into a natural penicillin within holes in an agar plate inoculated with *B. subtilis* spores. In this manner, the time and effort needed to perform this reaction in a separate test-tube are saved. Fig. 1 illustrates such a demonstration. Each fermentation liquid is measured into two holes. Into one hole (upper row in the figure) a single drop of 3 per cent $NaHCO_3$ solution is added, while into the other hole (lower row in the figure) one drop of 1 per cent phenylacetylchloride in acetone is also added. The plate is then incubated for 5 hr. at 37° C. If there is 6-APA in the fermentation liquid, it is converted to *G*-penicillin and yields an inhibition zone, as can be seen around the holes 1–4 of the lower row in the figure. Around the holes of the upper row is the inhibition zone due to the penicillin remaining in the fermentation fluid after extraction. The fifth hole, where distilled water was introduced, the reagents in the quantity applied do not produce an inhibition zone. This method is applicable for the quantitative, quick detection even of a tenth unit of 6-APA.

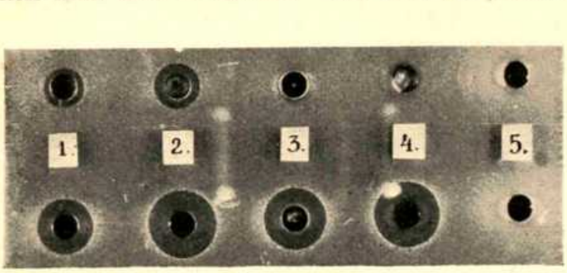


Fig. 1. Detection of 6-APA content of fermentation liquids by use of the hole plate method.

Test micro-organism: *B. subtilis*, 0·1 ml. of the liquid containing 6-APA (1, 0·04 unit; 2, 0·2 unit; 3, 0·06 unit; 4, 0·2 unit; 5, dist.H_2O) measured into holes. One drop of 3 per cent $NaHCO_3$, and, on the lower row, an additional drop of 1 per cent phenylacetylchloride in acetone is measured. In the upper row: inhibition zone of remaining natural penicillins. In the lower one: inhibition zone of *G*-penicillin deriving from 6-APA

J. URI*

Department of Pharmacology,
University Medical School,

F. SZTARICSKAI

'Biogal' Pharmaceutic Factory,
Debrecen, Hungary.

[*] Present address: Department of Biochemistry, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1.

[1] Batchelor, F. R., Doyle, F. P., Nayler, J. H. C., and Rolinson, G. N., *Nature*, **183**, 257 (1959).

[2] Huang, H. T., English, A. R., Seto, T. A., Shull, G. M., and Sobin, B. A., *J. Amer. Chem. Soc.*, **82**, 3790 (1960).

[3] Sheehan, J. C., and Henery-Logan, K. R., *J. Amer. Chem. Soc.*, **81**, 3089, 5838 (1959).

[4] Perron, Y. G., Crast, L. B., Gottstein, W. I., Minor, W. F., and Cheney, L. C., *Antib. Ann.*, **107** (1959).

[5] Rolinson, G. N., Stevens, S., Batchelor, F. R., Cameron Wood, J., and Chain, E. B., *Lancet*, ii, 564 (1960).

[6] Rolinson, G. N., and Stevens, S., *Brit. Med. J.*, ii, 191 (1961).

ENTOMOLOGY

Exchanges of Sodium Ions in the Central Nervous System of an Insect (*Periplaneta americana* L.)

IN earlier work it was shown that the exchanges of sodium and potassium ions between the hæmolymph and the central nervous system of the cockroach occurred relatively rapidly[1]. It was concluded from these results that the connective tissue layer surrounding the nervous system did not function as a significant diffusion barrier and that in such a dynamic system any selective permeability must be attributed to the underlying cellular layer of the nerve sheath or some associated elements.

An attempt has recently been made to throw some further light on these processes by studying the efflux of sodium ions from isolated abdominal nerve cords and single connectives and ganglia of *Periplaneta americana* L. In these experiments the rate of loss of sodium-24 was measured by determining the decline in radioactivity of isolated preparations maintained in a flowing physiological solution. The greater part of the efflux of sodium-24 measured in this way was found to approximate to a simple exponential function, both for the whole nerve cord and for the isolated fragments. The presence of 2:4-dinitrophenol and cyanide in low concentrations was found to reduce substantially the rate of efflux of sodium measured in these experiments (Fig. 1). Similarly, an absence of potassium ions from the external solution resulted in a slowing down of loss of sodium, although sodium-free solutions appeared to have no appreciable effects on the measured efflux from the nerve cord. Thus it seems that, as for some other cells and tissues[2], sodium ions are extruded from the central nervous system by a metabolically maintained secretory mechanism which is also associated with the uptake of potassium ions.

In another group of experiments the effect of removing the cellular and fibrous nerve sheath on the rate of sodium extrusion was investigated. It was found that the rate of efflux of sodium-24 from the terminal abdominal ganglion was not appreciably affected by the removal of substantial portions of the perilemma (Fig. 2). It must be concluded, then, that the rate-limiting process measured in these experiments was not the transfer of the ions across

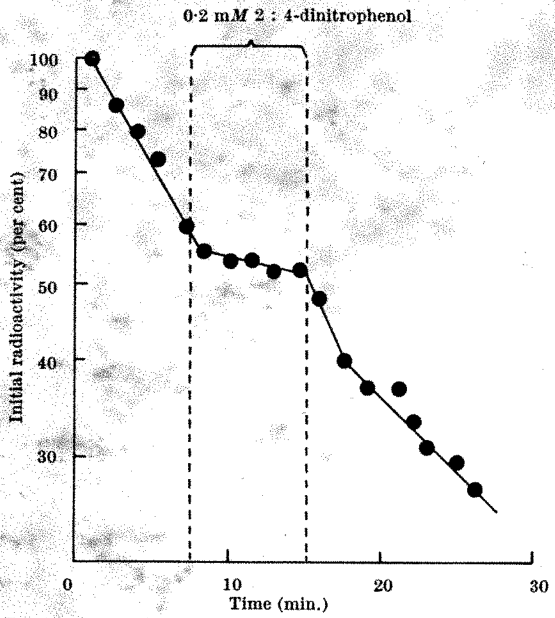


Fig. 1. Effect of 0·2 mM 2 : 4-dinitrophenol on the rate of loss of sodium-24 from a single, ligatured, terminal abdominal ganglion washed in inactive physiological solution

the cellular perineurium, but the exchanges associated with some underlying cellular components of the central nervous system. The relatively rapid effects of the poison molecules and the potassium-free solutions on the measured effluxes from the intact abdominal nerve cord thus imply that the changes in the chemical composition of the external solution were quickly transmitted to the deeper layers of the central nervous system.

The ionic fluxes calculated in the previous account[1], using conventional equations, involved the assumption that the rate-limiting process was the transfer across the superficial boundary of the central nervous system and that the movements within the underlying tissues occurred relatively rapidly so that the labelled ions were effectively well mixed. The results here show that this represents an over-simplification, and consequently the calculated values have little significance. It is hoped that in a future investigation, in which any relatively rapid movements can be measured, a quantitative analysis can be given for this more complex system.

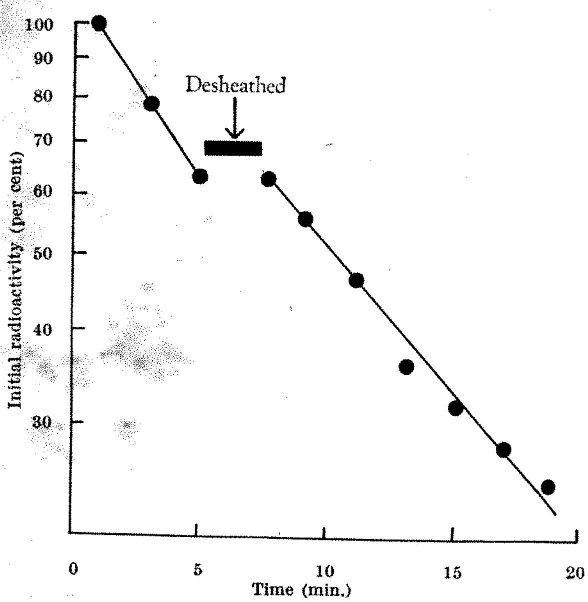


Fig. 2. The effect of removing the nerve sheath from the dorsal surface of the ganglion on the rate of loss of sodium-24

J. E. TREHERNE

Agricultural Research Council
Unit of Insect Physiology,
Department of Zoology,
University of Cambridge.

---

[1] Treherne, J. E., *J. Exp. Biol.*, **38**, 315 (1961).

[2] Hodgkin, A. L., *Proc. Roy. Soc.*, B, **148**, 1 (1958).

An Acid Mucopolysaccharide in Cockroach Ganglia

IN a recent histochemical investigation of the neurones and connective tissues of the meso- and meta-thoracic ganglia of the cockroach, *Periplaneta americana*[1], it was noticed that an area surrounding the central mass of nerve fibres stained metachromatically with toluidine blue. The region separates the neurones and connective tissue sheath from the main mass of nerve fibres. There is no comparable region in the interganglionic connectives or peripheral nerves. This seems at present to be a structure peculiar to *Periplaneta* and other cockroaches; a similar region has been seen after staining with toluidine blue in *Blaberus craniifer*, *Blatta orientalis*, *Blattella germanica* and *Diploptera punctata*. No such region is found in insects of other orders which have been studied histochemically, for example, *Ephestia kühniella* (Richards, A. G., personal communication), *Locusta migratoria* (unpublished observations) and sucking lice[2].

The metachromatic substance in *Periplaneta* has no apparent structure and appears to be extra-cellular; the inner and outer borders are delimited by the glial cells which send slender processes across it, and, in addition, occasional glial cell bodies are seen. This is the appearance in sections of ganglia fixed in Zenker's fluid (Fig. 1). If, however, a section from a ganglion fixed in formalin is stained with toluidine blue, the metachromatic substance is displaced inwards; the region is seen to be disrupted and the substance now lies on the surface of the broken glial processes around the central nerve fibre mass. This appearance is described by Hess[3], who stained sections of these ganglia with cresyl violet, after fixation in either Bodian's or Bouin's fluids. He describes the metachromatic cytoplasm as forming a 'reticulated net' and identifies it as glial cell cytoplasm. A similar appearance is described by Pipa, Cook and Richards[4] in Bodian's protargol preparations of these ganglia. In preparations made by the osmium and ethyl gallate technique, Wigglesworth[5] describes a "glial lacunar system", in which the glial cells lie in extensive cavities, the contents of which are not stained in the preparations. He finds that comparable regions are found in all the thoracic and abdominal ganglia of *Periplaneta*. It appears, then, that this region in the ganglia is very sensitive to the action of fixatives.

The only group of substances which can be identified histochemically in this area are acid mucopolysaccharides. The reactions for proteins (coupling reaction, mercury nitrite and Sakaguchi tests) and lipids (sudan black) give negative results, and so does the periodic acid–Schiff test. This latter result is in agreement with the finding of Leblond, Glegg and Eidinger[6] that acid mucopolysaccharides are negative

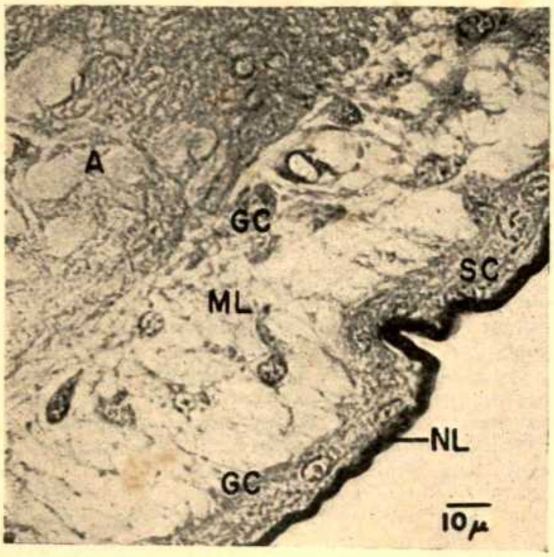


Fig. 1. Section through a thoracic ganglion of *Periplaneta americana* to show the region containing acid mucopolysaccharides. *A*, axons; *GC*, glial cells; *ML*, metachromatic layer; *NL*, neural lamella; *SC*, sheath cells

to the periodic acid–Schiff test. The metachromasy with toluidine blue is removed by incubation of the sections in hyaluronidase ('Hyalase'), but as this enzyme is not specific for hyaluronic acid, this does not give any information as to the nature of the acid mucopolysaccharide. An attempt is being made to characterize it biochemically.

The acid mucopolysaccharide forms a ground substance which resembles that of vertebrate connective tissue in its histochemical reactions, but the degree of chemical similarity remains to be determined. The function of this layer of acid mucopolysaccharide is not apparent. There are suggestions that acid mucopolysaccharides can act as ionic barriers allowing cations to pass through, but restricting the passage of anions[7]. But it seems unlikely that this acid mucopolysaccharide forms an ionic barrier, both because it is restricted to the ganglia and because removal of the connective tissue sheath from either the ganglia or connectives results in the blockage of nerve conduction[8]. Wigglesworth[5] suggests that the region might serve as a pool for nutrients, or as a means of maintaining the outer shape of the ganglion as changes in the state of nutrition alter the size of the cellular components of the ganglion. But, until more is known of the chemistry of this substance, it will be difficult to ascertain its function.

I am very grateful to Dr. A. G. Richards for his criticism of this communication.

DOREEN E. ASHHURST *
Department of Zoology,
The University, Manchester, 13.

* Present address: Department of Entomology and Economic Zoology, University of Minnesota, St. Paul, 1, Minnesota, U.S.A.

[1] Ashhurst, D. E., *Quart. J. Micr. Sci.* (in the press).
[2] Pipa, R. L., and Cook, E. F., *J. Morph.*, **103**, 353 (1958).
[3] Hess, A., *J. Biophys. Biochem. Cytol.*, **4**, 731 (1958).
[4] Pipa, R. L., Cook, E. F., and Richards, A. G., *J. Comp. Neurol.*, **113**, 401 (1959).
[5] Wigglesworth, V. B., *J. Exp. Biol.*, **37**, 500 (1960).
[6] Leblond, C. P., Glegg, R. E., and Eidinger, D., *J. Histochem. Cytochem.*, **5**, 445 (1957).
[7] Kantor, T. G., and Schubert, M., *J. Histochem. Cytochem.*, **5**, 28 (1957).
[8] Twarog, B. M., and Roeder, K. D., *Biol. Bull. Woods Hole*, **111**, 278 (1956).

Experimental Transmission of *Borrelia anserina* (Sakharoff 1891) by *Aedes aegypti*

THE causal organism of spirochætosis, *Borrelia anserina*, was first reported from geese in the Caucasus, and has since been isolated from the blood of infected geese, turkeys, ducks, fowls, partridges, crows and sparrows from all parts of Africa, Australia, Austria, Bulgaria, Brazil, Egypt, East Indies, Germany, Greece, Hungary, India, the U.S.S.R., Rumania and Turkey.

The known common vectors of spirochætosis are various species of *Argas* and *Ornithodoros*.

Recent outbreaks in the Kaduna area of Northern Nigeria gave indications that the disease could be transmitted by other blood-sucking insects, and it was therefore decided to attempt experimental transmission of spirochætosis with *Aedes aegypti* as this was readily available and was known to feed well on experimental birds. Laboratory-bred females were placed in plastic tubes with mosquito netting fixed at each end. This tube was held firmly on a bird (Rhode Island Red) that showed spirochætes in a stained thin blood film. After approximately 45 min. the tube was removed and the fully fed *A. aegypti* were separated from the unfed. After starving for several days they were then fed on clean birds. These birds were transferred to separate cages and thin blood films were taken daily. Control birds of the same age and breed were maintained under the same conditions and thin blood films were also taken daily from these birds.

The birds on which the *A. aegypti* had been fed developed a fever, became anæmic and showed spirochætes in their blood.

The control birds were normal throughout the experiment. Similar experiments are being carried out with *Culex fatigans*.

J. A. ROBERTS
Investigation and Training Centre,
Ministry of Animal Health and Forestry,
Kaduna,
Northern Nigeria.

PSYCHOLOGY

Cross-modal Transfer in Man

LITTLE is known about transfer between different sensory systems during learning of complex material. Positive cross-modal transfer implies that learning to discriminate between a pair of stimuli in one modality leads to accelerated discrimination between the same stimuli presented in a second modality. Previous experiments showed no evidence for transfer between visual and tactile[1], or visual and auditory[2], discrimination habits in monkeys, although one investigation has been reported recently in which transfer between auditory and visual habits was obtained[3]. In certain conditions, human subjects are able to match geometrically equivalent contours successively presented for visual and tactile comparison[4]. Auditory 'flutter' can similarly be matched with visual 'flicker' by subjects specifically instructed to equate rates of intermittance of sounds and lights[5]. Such cross-modal comparisons, however, are performed more poorly than intra-modal comparisons. A general theoretical argument against the immediate equivalence of perception in differing sense modalities has been presented by Hebb[6].

Table 1. DIFFERENT LEARNING SEQUENCES USED WITH FOUR GROUPS OF SUBJECTS

Group I (*N* = 6)	Group II (*N* = 6)	Group III (*N* = 12)	Group IV (*N* = 12)
VISUAL ● – – vs. – – ● (+) (–)	Auditory ······ vs. · · · (+) (–)	Alternating VISUAL and auditory ● – – vs. – – ● (+) (–)	Matching auditory vs. visual ● – – vs. – – ● ● – – vs. ● – – – – ● vs. – – ● – – ● vs. ● – –
followed by	followed by		
auditory ● – – vs. – – ● (+) (–)	VISUAL ● – – vs. – – ● (+) (–)		

In the rhythm discriminations temporal parameters were as follows: Duration of short components, 148 msec.; duration of long components, 504 msec.; duration of intervals between components, 110 msec.; duration of intervals between rhythm cycles, 990 msec. In the rate discrimination, the sound was interrupted 10 times per sec. for the positive stimulus, and 4 times per sec. for the negative stimulus.

In the present work cross-modal transfer of rhythm discrimination by human subjects was investigated. We wished to determine, first, whether learning of a rhythm discrimination in one modality (audition) leads to more rapid learning of the same rhythm discrimination in the second modality (vision); secondly, we investigated the ability to match auditory and visual rhythms.

The subject sat facing a pair of boxes, each of which contained a filament lamp and loudspeaker. Luminance of the lamp was 230 ft. lamberts. The basic sound source was a 400-c.p.s. tone with a small component at 2,500 c.p.s. Its intensity was judged to be about 60 db. An electro-mechanical timing device interrupted the lights and sounds to produce the rates and rhythms described in Table 1. On each trial, stimuli were successively presented on the left and right; two cycles of the rhythms were always presented, whereas the intermittent stimuli were presented for 5 sec. The position of the positive stimulus, to left or right, was determined by reference to the Gellerman schedule. Testing continued, in all conditions, until the subject made no errors in 10 consecutive trials. By convention, the final 10 trials are not included in the scores reported here.

Thirty-six adult subjects were divided into four groups and tested according to the design shown in Table 1. A pilot experiment indicated that the rhythm discrimination was more difficult in the visual than in the auditory form. For this reason it was decided to study cross-modal transfer only from audition to vision. Group 1 (*N* = 6) received no auditory training and group II (*N* = 6) learned an irrelevant discrimination of the auditory rate before both groups learned the visual rhythm discrimination. Group III (*N* = 12) learned the auditory and visual forms of the rhythm discrimination concurrently, the same pair of rhythms being given in auditory and visual form on alternate trials. None of the subjects in the first three groups was informed of the relationship between the auditory and visual rhythms. By contrast, the subjects in group IV (*N* = 12) were specifically instructed to determine, on each trial, whether a visual rhythm was identical with an auditory rhythm which immediately preceded it.

The scores of subjects in groups I and II for the visual rhythm discrimination were combined (*N* = 12) and then compared with the scores for the same discrimination of subjects in group III and also with the auditory-visual matching performance of subjects in group IV. Subjects in the first two groups required an average of 13·5 trials (range 0–57 trials) to learn the visual rhythm discrimination in the absence of associated auditory learning. Subjects in group III required an average of 20·4 trials to reach criterion on the visual rhythm discrimination (range 1–120 trials). Half the trials for these subjects, it must be noted, consisted of presentation of the auditory form of the rhythm discrimination. If these auditory trials are excluded, the average for group III becomes 10·3 trials (range 0–60 trials). Comparison of these test scores by means of the Mann-Whitney '*U*' test failed to yield any significant differences between the groups, regardless of whether the auditory trials of group III were combined with the visual trials or not. Further analysis, using a less stringent performance criterion (90 per cent, instead of 100 per cent of 10 trials), again failed to reveal significant differences between the various groups. Finally, successful matching of the auditory and visual rhythms by subjects in group IV required an average of 8·4 trials (range 0–34 trials). This value does not differ significantly from the learning scores of the first three groups.

Another measure of the degree to which learning of one form of the discrimination influences learning of the other may be obtained by determining the difference in number of trials required by subjects in group III to learn the two forms of the discrimination. The average difference between auditory and visual learning scores of these subjects was 7·3 trials (range 1–56 trials). Three subjects showed such rapid learning of both auditory and visual forms of the discrimination (that is, fewer than five trials of each) that we would attribute these low difference scores to equally rapid concurrent learning in the two modalities. The visual learning scores given by 3 of the remaining 4 subjects with low difference scores, when calculated on the basis of combined auditory and visual trials, exceed the average visual learning score of control subjects. The other five subjects in group III showed large difference scores, suggesting little, if any, transfer between modalities.

With our conditions of testing, therefore, we have found no evidence for transfer from auditory to visual learning of rhythms in man. This result agrees with the finding of relatively poor cross-modal matching performance in human subjects[5]. If confirmed and extended, results such as ours might imply that discrimination learning of complex material is largely modality-specific.

The apparatus used in this experiment was designed and constructed by Mr. H. B. Morton.

M. COLE *
S. L. CHOROVER †
G. ETTLINGER

Psychological Laboratory,
Institute of Neurology,
Queen Square,
London, W.C.1.

* Fellow of the National Multiple Sclerosis Society.
† Fellow of the National Foundation.

[1] Ettlinger, G., *Behaviour*, **16**, 56 (1960).
[2] Burton, D., and Ettlinger, G., *Nature*, **186**, 1071 (1960).
[3] Stepien, L. S., and Cordeau, J. P., *Amer. J. Psychol.*, **73**, 388 (1960).
[4] Krauthamer, G., unpublished Ph.D. thesis, New York University (1959).
[5] Gebhard, J. W., and Mowbray, G. H., *Amer. J. Psychol.*, **72**, 521 (1959).
[6] Hebb, D. O., *The Organization of Behaviour* (J. Wiley, New York, 1949).

FORTHCOMING EVENTS

Wednesday, September 20

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Dr. W. Domzalski: "Airborne Techniques in Petroleum Exploration".

Thursday, September 21—Friday, September 22

Royal Entomological Society of London (at the Imperial College of Science and Technology, South Kensington, London, S.W.7)—Symposium on "Insect Polymorphism".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Mycologist (with an honours degree in botany, with specialization in plant pathology) at the States' Experimental Station (Department of Agriculture)—The Establishment Officer, States' Greffe, Jersey, C.I. (September 23).

Research Demonstrator (graduate in botany) to engage in research, preferably in plant physiology and biochemistry, plant pathology and ecology; and a Research Assistant (graduate in botany or graduating this year), to assist the Professor in the establishment and maintenance of a collection of plant organ and tissue cultures and in research involving these cultures—The Registrar, University College, Singleton Park, Swansea (September 23).

Lecturer in the Department of Electrical Engineering—The Registrar, The University, Leeds (September 25).

Professors of Crop Husbandry and Horticulture; Animal Husbandry and Health; and Agricultural Economy at the University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (September 25).

Scientific Officer or Senior Scientific Officer (physicist, chemical or mechanical engineer), to join a group studying the growth and spread of fire in and between buildings—The Director, Fire Research Station (D.S.I.R.), Borehamwood, Elstree, Herts (September 25).

Lecturer (with at least an honours degree in geology, together with special qualifications to teach geomorphology to the fourth year (honours) level in the Department of Geography) in Geography and Geology at the University of Natal, Pietermaritzburg—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, September 30).

Research Assistant (graduate or postdoctoral), to work on rapid high temperature reaction kinetics, fundamental flame properties, flame spectroscopy and allied subjects—Dr. G. Dixon-Lewis, Houldsworth School of Applied Science, The University, Leeds 2 (September 30).

Research Assistant (with a degree in botany or equivalent qualifications) in the Department of Botany—Dr. A. G. Smith, Department of Botany, The Queen's University of Belfast, Belfast 7 (September 30).

Scientific Officer or Senior Scientific Officer (with research experience in enzymology and/or protein chemistry, and some knowledge of, and interest in, fundamental genetic problems), initially to assist in current research on the effects of mutation on the structure of specific proteins in the mould *Neurospora crassa*—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (September 30).

Vice-Principal (with a good university degree and preferably higher qualifications and Associate Membership of one of the engineering institutions, and a sound technical background and experience in a similar capacity) of the Khartoum Technical Institute, Sudan—The Appointments Section, Sudan Embassy, 3 Cleveland Row, St. James's, London, S.W.1 (September 30).

Vokes Research Fellow (preferably with a special interest in fluids or elastic theory) in the Department of Mechanical Engineering—The Secretary and Registrar, The University, Southampton (September 30).

Lecturer or Assistant Lecturer (preferably with special qualifications and interests in cytology and genetics, or in protozoology, or in the ecology of terrestrial animals) in the Department of Zoology, University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (October 2).

Lecturer in Botany and Assistant Curator of the Bolus Herbarium, University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, October 4).

Lecturer in Philosophy; and a Lecturer in Psychology at the University of Kong Hong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, October 13).

Chair of Chemistry in the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

Lecturer (with a science degree specializing in geology and research experience to Ph.D. or comparable level, and well trained in modern mineralogical techniques) in Geology at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 15).

Reader in Human Genetics in the Department of Zoology, University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 15).

Lecturer (with a veterinary qualification) in Animal Husbandry in the School of Veterinary Medicine—The Registrar, Trinity College, Dublin, Republic of Ireland (October 16).

Reader in Microbiology at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, October 16).

Senior Lecturer in Physics at the Australian National University, Canberra—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 21).

Senior Lecturer (preferably with research experience in low temperature physics) in Physics at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 21).

Scientific Officer (with a first- or second-class honours degree in physics), to work on transmission of sound through panels and solid structures—The Director, Building Research Station (D.S.I.R.), Garston, near Watford, Herts, quoting Ref. BRS.32Y (October 24).

Teaching Fellows (with an appropriate honours degree) in the Department of Biochemistry (two appointments); and in the Department of Botany (one appointment), University of New South Wales, Australia—The Agent-General for New South Wales, 56 Strand, London, W.C.2, and the Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (October 31).

Lecturer in Education in the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, University of Otago (London and New Zealand, November 20).

Assistant Lecturer (graduate in chemistry or agricultural chemistry) in Soil Science—The Registrar, Massey Agricultural College (University of New Zealand), Palmerston North, New Zealand (November 22).

Lecturer or Senior Lecturer in Physiology at Monash University, Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, Monash University (Australia and London, December 4).

Agronomist (Soils) (male, between 26–40, with an honours degree in agricultural or horticultural science with agricultural chemistry as the main subject, experience in field experimental work on soil fertility, and a knowledge of tropical soils and crops) in the Department of Agriculture, Sarawak, to design and conduct field experiments with tropical crops on selected soils to assess the potentiality of the soils—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting RC.213/128/01/T.

Assistant Lecturer, Grade B (well qualified in physics and an interest in the development of some branch of applied physics) in Physics—The Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex.

Assistant (preferably with previous training), for work in cytogenetics—The Registrar (BX), Queen Mary College (University of London), Mile End Road, London, E.1.

Physical Chemist, Senior Scientific Officer grade (at least 26, with a degree or Dip. Tech. with first- or second-class honours degree in chemistry or equivalent qualification, and at least three years postgraduate research experience) at the Rocket Propulsion Establishment, Westcott, Bucks, to lead a small team investigating properties of materials at very high temperature with special emphasis on high temperature-resistant ceramics—The Ministry of Labour, Technical and Scientific Register (K), 28 King Street, London, S.W.1, quoting Ref. F.531/iA.

Postdoctoral Fellow in Organic Chemistry—Dr. J. I. G. Cadogan, Department of Chemistry, King's College (University of London), Strand, London, W.C.2.

Professor of Analytical Chemistry at Peshawar University—The Department of Technical Co-operation, 26/28 King Street, London, S.W.1, quoting TCS/PAK/529.

Reader in Applied Mathematics; a Senior Lecturer in Mathematical Statistics; and a Senior Lecturer or Lecturer in any branch of Pure or Applied Mathematics at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, University of Adelaide.

Research Assistant (science or engineering graduate), to work on problems related to food processing or food engineering, and to undertake some teaching—The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

Research Technician (with some experience in a biochemical research laboratory) in the Department of Chemistry and Food Technology, for work on the isolation and chemical studies of lipoproteins from cereals and oilseeds—The Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

Senior Research Assistant and a Research Assistant in the Chemistry Department, to work on optical rotatory dispersion—Prof. W. Klyne, Westfield College (University of London), Hampstead, London, N.W.3.

Technicians and Junior Technicians (young men or women with an interest in synthetic organic chemistry or in biochemistry)—The Secretary (LA), Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Royal Observatory Bulletins. No. 34: Design and Tests of a Mirror Transit Circle Axis. By R. d'E. Atkinson. Pp. A51–A70. (London: H.M. Stationery Office, 1961.) 4*s*. net. [127

Memoirs of the Royal Astronomical Society. Vol. 68, Part 3: Classification and Radial Velocities of Bright Southern Galaxies. By Gerard de Vaucouleurs and Antoinette de Vaucouleurs. Analysis of Limb Darkening Observations. By A. Keith Pierce and John. H. Waddell. Pp. 69–112. (London: Royal Astronomical Society, 1961.) 20*s.*; 3 dollars. [127

United Kingdom Atomic Energy Authority. Seventh Annual Report for the period 1st April, 1960—31st March, 1961. Pp. viii+80+4 plates. (London: H.M. Stationery Office, 1961.) 5*s.* net. [127

Scottish Plant Breeding Station. Report, 1961. Pp. 78+1 plate. Pentlandfield, Roslin: Scottish Plant Breeding Station, 1961.) [137

General Register Office. The Registrar General's Statistical Review of England and Wales for the two years 1957–1958. Supplement on Mental Health. Pp. ix+102. (London: H.M. Stationery Office, 1961.) 7*s.* 6*d.* net. [137

The British Coke Research Association. Seventeenth Annual Report for the year 1960. Pp. 33. (Chesterfield: The British Coke Research Association, 1961.) [147

Durham University Appointments Board. Fifteenth Annual Report. Pp. 9. (Durham: Durham University Appointments Board, 1961.) [177

Food for the Chemist. By Prof. S. H. Harper. (An Inaugural Lecture given in the University College of Rhodesia and Nyasaland on 28 October 1960.) Pp. 16. (London: Oxford University Press, 1961.) 4*s.* net. [177

Regional Advisory Council for Technological Education: London and Home Counties. Regional Development Plan for Courses for the Diploma in Technology. Pp. 16. (London: Regional Advisory Council for Technological Education: London and Home Counties, 1961.) [277

Department of Scientific and Industrial Research. Report of the Warren Spring Laboratory 1960:—Report of the Warren Spring Laboratory Steering Committee and the Report of the Director of the Warren Spring Laboratory for 1960. Pp. vi+25+4 plates. (London: H.M. Stationery Office, 1961.) 3*s.* net. [277

B.B.C. Engineering Monograph No. 37: An Instrument for Measuring Television Signal-to-Noise Ratio. By S. M. Edwardson. Pp. 23. (London: H.M. Stationery Office, 1961.) 5*s.* [277

Horticultural Marketing Council. Fifth Annual Report and Statement of Accounts for the period 9th May, 1960 to 31st March, 1961. Pp. 16+4 plates. (London: Horticultural Marketing Council, 1961.) [277

Society of Chemical Industry. Proceedings of the Chemical Engineering Group, Vol. 39, 1958/9. Pp. viii+111. (London: Chemical Engineering Group, Society of Chemical Industry, 1961.) 42*s.* [277

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 705, Vol. 243 (20 July 1961): Different Types of Cirral Activity of Barnacles. By D. J. Crisp and A. J. Southward. Pp. 271–308+ plate 66. 15*s.* No. 706, Vol. 244 (27 July 1961): Studies on Triassic Fossil Plants from Argentina. 1: *Rhexoxylon* from the Ischigualasto Formation. By S. Archangelsky and D. W. Brett. Pp. 1–19+plates 1 and 2. 9*s.* 6*d.* No. 707, Vol. 244 (27 July 1961): The Layered Ultrabasic Rocks of South-West Rhum, Inner Hebrides. By W. J. Wadsworth. Pp. 21–64+plates 3–7. 22*s.* 6*d.* (London: Royal Society, 1961.) [277

Problems of Psychology, Numbers 1 and 2 (1960). Published quarterly, containing selected papers translated from *Voprosy Psikhologii*. Four issues of approximately 400 pages per annum. Annual subscription: (A) For libraries £15 (40 dollars); (B) For private subscribers £5 (15 dollars). Single issues £4 (12 dollars). (London and New York: Pergamon Press, 1961.) [277

Glamorgan Forests. Edited by H. L. Edlin. (Forestry Commission Guide.) Pp. vi+99+20 plates. (London: H.M. Stationery Office, 1961.) 5*s.* net. [277

Department of Scientific and Industrial Research. Proceedings of Conference on Ergonomics in Industry, 27–29 September 1960. Pp. x+190. (London: H.M. Stationery Office, 1961.) 12*s.* 6*d.* net. [277

Northampton College of Advanced Technology. Part-time Day and Evening Courses, Session 1961–62. Pp. 34. (London: Northampton College of Advanced Technology, 1961.) [277

General Register Office. The Registrar General's quarterly return for England and Wales—Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, No. 449, 1st Quarter 1961. Pp. 28. (London: H.M. Stationery Office, 1961.) 2*s.* 6*d.* net. [277

Freshwater Biological Association. Twenty-ninth Annual Report for the year ended 31 March 1961. Pp. 88. (Ambleside: Freshwater Biological Association, 1961.) 4*s.* [277

The British Ceramic Research Association. Thirteenth Annual Report, 1960. Pp. 70. Special Publication No. 27: Symposium on Dust Control in the Pottery Industry, 8th April, 1960. Pp. 95. (Penkhull, Stoke-on-Trent, 1961.) [277

United Kingdom Atomic Energy Authority. Atom 1961: an Illustrated Summary of the Seventh Annual Report from 1st April 1960 to 31st March 1961. Pp. 36. (London: H.M. Stationery Office, 1961.) 2*s.* 6*d.* net. [277

United Kingdom Atomic Energy Authority. Dounreay Experimental Reactor Establishment, Thurso, Caithness—Information Booklet. Pp. 59. (London: United Kingdom Atomic Energy Authority.) [277

Government of Northern Ireland: Ministry of Commerce. Memoirs of the Geological Survey. Geology of the Country around Dungannon, (One-inch Geological Sheet 35.) By A. Fowler and J. A. Robbie. With an Appendix on Geophysical Investigations by Dr. W. Bullerwell. Second edition of Explanatory Memoir by E. T. Hardman. Pp. xii+274+14 plates. (Belfast and London: H.M. Stationery Office, 1961.) 30*s.* net. [277

Other Countries

Iceland. The University Research Institute—Department of Fisheries. Rit Fiskideildar. Vol. 2, Nr. 9: The Salinity at the Shores of Southwest Iceland. By U. Stefansson. B. Lindal, J. Jakobsson, and I. Jonsson. Pp. 26. Vol. 2, Nr. 10: Icelandic Driftnet Herring Tagging Experiments. By J. Jakobsson. Pp. 16. (Reykjavik: University Research Institute—Department of Fisheries, 1961.) [277

Académie Royale de Belgique. Classe des Sciences. Mémoires. Tome 32, Fascicule 6: Les Deux Flores d'Afrique Tropicale. Par J. Lebrun. Pp. 81. (Bruxelles: Académie Royale de Belgique, 1961.) 70 francs. [277

Canada: Department of Northern Affairs and National Resources. Bulletin No. 172 (Biological Series, No. 65): Contributions to Zoology, 1959. Pp. iv+135. 1 dollar. Natural History Papers. No. 9 (April 13, 1961): Bird and Mammal Observations on Ellef Ringnes Island in 1960. By D. B. O. Savile. Pp. 6. No. 10 (April 13, 1961): Noteworthy Records of Marine Molluscs from the Bay of Fundy. By E. L. Bousfield. Pp. 3. (Ottawa: Queen's Printer, 1961.) [277

Thirteenth Annual Report of the Pacific Marine Fisheries Commission for the year 1960. Pp. 60. (Portland, Oregon: Pacific Marine Fisheries Commission, 1961.) [277

East Africa High Commission. East African Virus Research Institute Report, July 1959–June 1960. Pp. i+57. (Entebbe: East African Virus Research Institute, 1960.) [277

Hungary. Year-Book of the Institute of Nutrition, Budapest, for 1960. Pp. iv+88. (Budapest: Institute of Nutrition, 1961.) [277

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Map 42–1960: Drift-Thickness Contours—Montreal Area, Quebec. Map 1089A: Perth, Lanark and Leeds Counties, Ontario. (Ottawa: Geological Survey of Canada, 1960 and 1961.) [277

Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel Exercise 1960. Pp. 255. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1961.) [277

International Atomic Energy Agency, Vienna. IAEA—What It Is and What It Does. Pp. 16. (Vienna: International Atomic Energy Agency, 1961.) [277

Commonwealth of Australia: Department of External Affairs. Australian National Antarctic Research Expeditions. ANARE Reports, Series B, Vol. 3: Oceanography, Hydrology. Introductory Studies: Hydrology and Plankton, Mawson, June 1956—February 1957. By J. S. Bunt. Pp. 135 (9 plates). (Melbourne: Antarctic Division, Department of External Affairs, 1960.) [277

Commonwealth of Australia. Research Review of the Commonwealth Scientific and Industrial Research Organization for the year ended 30th June, 1960. Pp. iv+418. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1961.) [277

Geological Survey of British Guiana. Quarter Degree Sheet 0660 S.E.: Apaiqua S.E. Edited by E. Williams. (Georgetown: Geological Survey, 1961.) 1 dollar. [277

Académie Royale de Belgique. Classe des Sciences. Mémoires, Tome 32, Fascicule 7: Hydrogénations Électrochimiques et Catalyse. Par Xavier de Hemptinne. Pp. 44. (Bruxelles: Académie Royale de Belgique, 1961.) 70 francs. [277

New Zealand Forest Service: Forest Research Institute. Technical Paper No. 33: Measurement of Stand Basal Area with Optical Wedges. By W. G. Warren. Pp. 12. (Wellington: Goverment Printer, 1960.) [277

Federation of Malaya. Report on Forest Administration for the year 1959. By Mohd. Alwy bin Haji Suleiman. Pp. iv+117+2 plates. (Kuala Lumpur: Goverment Printer, 1961.) 2.50 Malayan dollars. [277

United States Department of Agriculture. Publication Pa–436: The Khapra Beetle—a Pest of Stored Grains and Cereal Products. Pp. 8. (Washington, D.C.: Government Printing Office, 1961.) [277

Report and Accounts of the National Botanic Gardens of South Africa, for the financial year ended 31 March, 1961. (Kirstenbosch, Newlands, Cape; Karoo Garden, Worcester; and Harold Porter Botanic Reserve, Betty's Bay.) Pp. 27. (Kirstenbosch, Newlands, Cape: National Botanic Gardens of South Africa, 1961.) [277

Records of the Australian Museum. Vol. 24, No. 13 (9th March, 1959): Cave Art of the Conjola District, New South Wales. By Frederick D. McCarthy. Pp. 191–202+plate 22. Vol. 24, No. 14 (9th March, 1959): Rock Engravings of the Sydney-Hawkesbury District. Part 2: Some Important Tirual Groups of the County of Cumberland. By Frederick D. McCarthy. Pp. 203–216+plates 23–26. Vol. 25, No. 1 (9th March, 1959): Some Australian Polyclads (Turbellaria). By Libbie H. Hyman. Pp. 1–17. Vol. 25, No. 2 (1st September, 1959): Serpulidae (Polychaeta) from Australia. By Barbara Dew. Pp. 19–56. Vol. 25, No. 3 (26th November, 1959): The Peloridiidae of Lord Howe Island (Homoptera, Coleorrhyncha). By J. W. Evans. Pp. 57–62. (Sydney: The Australian Museum, 1959.) [277

Durban Museum Novitates. Vol. 6, Part 8 (30th June, 1961): Miscellaneous Taxonomic Notes on African Birds, XVII. By P. A. Clancey. Pp. 105–118. 30 cents net. Vol. 6, Part 9 (30th June, 1961): Revisionary Notes on the Southern and Eastern Races of the Yellow-Breasted Apalis *Apalis flavida* (Strickland) of Africa. By Walter J. Lawson. Pp. 118–126. 20 cents net. (Durban: Durban Museum, 1961.) [277

Annals of the South African Museum. Vol. 46, Part 5: A New Cichlid Fish of the Limpopo Basin. By E. Trewavas. Pp. 53–56. (Cape Town: South African Museum, 1961.) 10 cents. [277

East African High Commission. Annual Report of the East African Freshwater Fisheries Research Organization for 1960. Pp. iv+48. (Jinja, Uganda: East African Fisheries Research Organization, 1960.) Shs. 4. [277

Report of the South African Museum for the year ended 31st March, 1960. Pp. ii+19+4 plates. (Cape Town: South African Museum, 1961.) [277

South Australia. Report of the Museum Board, 1st July, 1959 to 30th June, 1960. Pp. 16. (Adelaide: Government Printer, 1961.) [277

Svenska Aeroplan Aktiebolaget (Swedish Aircraft Co. Ltd.). Annual Report, 1960. Pp. 15. (Linköping: Svenska Aeroplan Aktiebolaget, 1961.) [277

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office

THE HUMANITY OF MAN

"A SCIENTIST is a person, before and after becoming a scientist. Whatever your interest, your field of research—whether you are an astronomer or geologist, physicist or botanist—you cannot climb outside the humanity through which you live and have your being."

Such was the main thesis, presented and expanded by Dr. Launcelot Fleming, Bishop of Norwich, in his sermon at the Civic Service in Norwich Cathedral and attended by civic dignitaries and officers and members of the British Association for the Advancement of Science on Sunday, September 3, during the recent annual meeting of the Association.

It was a compelling address given by a man who is not only one of the leaders in the Christian Church but is also himself a trained scientist. Dr. Fleming was a member of scientific expeditions to Iceland and Spitsbergen in 1932 and 1933 and chaplain and geologist to the British Graham Land Expedition during 1934–37. Later, though still continuing to become recognized as a distinguished theologian, he was director of the Scott Polar Research Institute, Cambridge, during 1947–49. The crowded congregation in Norwich Cathedral on that day therefore had the rare privilege of listening to a sermon by an outstanding priest and a philosophical discourse by a scientist at one and the same time.

Dr. Fleming pointed out that though scientists could not be grouped, and most of them would not wish to be grouped, together as a "separate species of the human race", set apart from the rest of humanity, yet they now have almost come to be so regarded. The reasons for this are manifold. At one time, the man of science was looked upon as an individual living practically in a world of his own ; one to be kindly tolerated or merely ignored or openly feared and despised according to the attitude of the society around him. But to-day many scientists have collaborated and science has quickly developed, gradually forming laboratory teams and then spreading more widely on national and international scales to such an extent that to-day man's very existence largely depends on it. Industrialists have recognized this to their own and to the world's material advantage and, in many cases, to the benefit of science itself ; even leaders in national and world politics must now at least acknowledge that science is firmly entrenched, though it is up to them to decide to what extent it shall be used for the advancement of mankind or, on the other hand, to its detriment and possibly eventual destruction.

In this sense, scientists cannot be a race apart, for they are now welcome in industry and, willy-nilly, have become absorbed into the body politic. It is still the so-called humanitarians—men of letters and other leaders of our society—who persist in ignoring scientists or at any rate turning deaf ears and presenting sightless eyes to scientists and their work, especially in so far as results of the latter are now exerting a profound influence on society. Sir Charles Petrie has recently complained that certain leaders of society show little interest in, or sympathy with, men of letters; this protest can be echoed by the scientists.

As a member of human society, the average scientist seems a tolerant individual. Yet, instead of waiting for the time to come—as come it must if the world is to survive, let alone progress in peace and prosperity—when all members of society recognize and encourage science, it would be better if the man of science periodically paused to examine more closely the basic unit of humanity, namely, himself.

This was the wise advice offered by Dr. Fleming at Norwich. We are each urged to get to know ourselves ; to ask : Who am I ? How can I be myself ? Dr. Fleming went far in aiding us to discover the answers to these fundamental questions ; for, as he claimed, we should recognize that each of us comprises several different selves.

"If I am a scientist, there is the scientific self—student, teacher, research worker—absorbed in lab. or classroom or museum, in some particular area of knowledge—using a particular method of enquiry, empirical, analytical—selecting only such categories as will serve the purpose best. Then there is another and a different self who plans out the course of lectures or the progress of research, who signs the cheques, makes up the diary, and arranges meetings. One might call this my administrative self, and here rather different qualities come into play than those I need in the lab. And finally there is a third, the social self, involved in relationships of many kinds—relationships at work, relationships outside work, relationships at home, and here I am in so many ways a different kind of person—more emotional, though not necessarily on that account less reasonable, than the scientific or administrative self.

"And these three selves, although I know perfectly well that each of them has its own proper place, appear so often to be pulling in opposite directions. It is not only that their interests may clash. That can, of course, happen. A man may become so absorbed in his work that his home life suffers ; or so taken up with his home that his work suffers : or the ambition for administrative control, for the sake of advancement or for money, may affect his relationship with fellow scientists or even prejudice the integrity of his work."

But the tension does not end there, at least so far as one's scientific self is concerned, for new scientific facts are continually being discovered, and so some conclusions have to be modified or cast aside. Therefore, as Dr. Fleming pointed out, the scientist for the purpose of his work must be detached and dispassionate, and his conclusions provisional. All sensible scientists now recognize this and act accordingly ; but many of even the best of us might ponder Dr.

Fleming's emphasis on our social relationships, for here, as he said, the values and forms of truth which relate to this side of our lives apply very differently. As a member of society, not even the scientist can remain detached, and he cannot, indeed he must not, remain dispassionate. "He must be prepared to stand committed, for better or for worse."

"To resolve the tension between one's three selves poses the question whether there are valid conceptions of truth to which all three sides of my life can be related, and by which they can be reconciled and governed. Is there such a thing as ultimate truth—however limited my understanding of it may be—a value outside myself to which every side of my personality may be related ?

"Either we think that our lives are purposive—and sustained by a power greater than ourselves—or we believe that life is a chance arrangement in the sense that it is without purposive significance, and we are left to make what we can of the existence with which we have been landed.

"The way one lives requires an affirmation or a denial concerning the very nature of Creation. Christian teaching on this subject has been the occasion for extraordinary misrepresentations, as past records of the British Association only too clearly demonstrate—not so much as to what is believed as to the arguments which have been adduced to support belief. The trouble started, perhaps, in the non-scientific and rather static age, when scholastic proofs for the existence of God—such as the argument from Design and the argument from a First Cause were produced. This had a doubly unfortunate result. In the first place, later generations, finding these arguments unsatisfactory, tended to assume that the conclusions must also be false—a natural, but quite illogical, assumption to make. But in addition, these arguments tended to associate Christian belief about Creation with the thought of a single decisive act of initiation, on the analogy of a divine architect. If you want an analogy, it were better to think rather of the singer who both makes the song and sustains it. The trouble has largely centred around the earliest Biblical story of Creation in the first Chapter of Genesis, as if it were to be understood in historical terms, whereas its intention is different.

"It is when I have recognized within myself that I have part and place in a purposive order of Creation—and this is implied in Christian teaching—that I can use this perception to understand the way the stars are moved—and, in addition, I can see more clearly how the different strands of my life and experience can be reconciled and unified.

"The Christian belief about Creation does not involve any necessary ground of contradiction between the scientist and the Christian in regard to the observable facts. [There is] no argument, for instance, between the Christian and the Doctor as to how a baby is conceived and born. The question which remains is the interpretation that we set upon these facts : 'What is the meaning and the purpose of the life thus born ?' If the Doctor should also happen to be the father of the child, then this is precisely the kind of question he will be disposed to ask. For, as a father, he has a special interest in the child, to whose care and welfare he is committed—committed in a way which includes and transcends his scientific interest for the child's good. Furthermore, his caring for the child must be the regulating factor if the father's relationship is to be adequately exercised, and if the child's interests are to be adequately secured.

"To give precedence to caring—to maintain that other factors must be subservient to it—is, in fact, to illustrate a vital affirmation concerning the end and purpose of Creation. [Sir Wilfrid le Gros Clark, president of the British Association] . . . particularly stressed the significance of altruism in the individual and in society alike, and the necessity for co-operation in man's development."

No matter which of the world faiths one professes (if any), there can be no argument against the fact that the Mosaic Laws or Commandments do, if conscientiously practised, at least make possible the communal life of man. These Commandments enable man to live in communion with himself and also with his fellow-men. "Thou shalt not kill." "Thou shalt not bear false witness." "Thou shalt not steal."

Then much later, Jesus Christ—to the Christian, the greatest sociologist in history—extended the policy pervading the Mosaic Commandments by preaching His second Commandment : "Thou shalt love thy neighbour as thyself". This aims at making the social life of man not only possible but also more purposive, peaceful and contented. Dr. Fleming made much of this doctrine, first of all by carefully emphasizing how to-day the word 'love' has become "sentimentalized and debased".

"In the sense of caring for, and caring about the world and its life—caring in particular about other people for their own sake, love is a creative and positive force. Caring involves and even demands an openness of mind as well as of heart to the spirit of enquiry. There is repeated insistence on this in the teachings of Jesus. Love is also a unifying power within man—for once given its place, the different sides of our personality at last fit purposively together. And love is a unifying power between men, enabling them to sacrifice their personal interests for the good of others ; a requirement of which, in the face of world events to-day, we are being made acutely conscious."

Man's nature and nurture are not inherited or acquired and are not evolving solely according to the broad biological principles generally accepted to-day ; for if so the law of the jungle would still be universally prevalent. A physically healthy body governed by an acute brain is not enough. The weakest of men need not, indeed must not, go to the wall. They need the help of the strongest, and it is difficult to conceive of the latter recognizing, and acting on, such profound responsibility to his fellow-men unless he is supported by, and imbued with, a spirit of humility, righteousness and even loving-kindness which can come to him only if he acknowledges some purpose in life. Science, among other disciplines, can give him strength ; but the spirit of purpose must come from within himself. All the great world faiths teach this in some form or other ; in fact, it is the very basis of the Christian faith.

Many people associate love too closely with sex : they must have a very superficial knowledge of the biological sciences. Yet this is and has been exemplified in literature and drama throughout the ages. Then there are others who, with a smattering of psychology or a tendency to use psychological terms which they do not understand, look upon love as being purely 'emotional'—a very overworked term these days. Even now, in all the disciplines of life (and that includes science), there are those who, though they may have made brilliant contributions to knowledge, are content to let the world go by provided they themselves are not unduly disturbed. Such men are morally indolent and slovenly. Then at the other end of the scale we have those, perhaps good in their chosen profession, who are never really happy unless they are metaphorically perched on a rostrum expounding social, economic and political theories about which they usually understand very little. Such men are bores, perhaps even dangerous. If the concept of more than one 'culture' be incorporated in our future school and university education, then the former might be stirred out of their selfish apathy ; the latter can, and must, be challenged, for, despite their sometimes good academic achievements (and, maybe, arguments), beneath it all frequently lurk a meanness of spirit and self-seeking, unkind, unscrupulous attitudes. They pass from pillar to post telling others how to live, boring those who prefer to think and act for themselves, yet, alas, persuading many who are willing to let others do their thinking for them. Perhaps the best retort to such people might be : "Physician, heal thyself" ; for they are just deceiving themselves with self-satisfaction and false pride, misleading their weaker brethren with rhetoric or monotonous verbosity, yet withal unable to justify their arguments (frequently one-sided) by either precept or example—destructive criticism scarcely followed by constructive suggestion.

Here, therefore, is much food for thought, for the Bishop of Norwich has given men of science a great objective which can render their work not only of rational and practical value but also of greater social significance. The problem is to reconcile collective progress and ultimate fate with individual will. These must not be divided aims, but should be joined together having one objective—the advancement and betterment of world civilization. The connecting links are kindness and consideration for others, or, as the Bishop put it, love. Love must be construed as indicating consideration and care for all those who need it, thus getting outside oneself (selflessness) and sometimes even outside one's own family circle (avoiding nepotism). Neither sentiment nor sentimentality is involved in this philosophy. It is plain common sense, though it may involve some sacrifices which all too few seem ready to make. Moreover, the more kindly and tolerant among us might do better for society by sometimes becoming militant.

There are those among us, scientist and non-scientist alike, who have, perhaps too nonchalantly, cast aside their faiths (whatever they may have been), mainly because, in their so-called scientific reasoning, such faiths cannot be sustained by observable and measurable facts. They then risk ignoring the other selves which Dr. Fleming emphasized. Yet many facets of man's social life are intangible, though none the less real, and the scientist especially might ignore these at his peril—certainly to the detriment of social satisfaction and even world peace. Knowing and recognizing man's social history, surely we have reached a point in our evolution when there need be no more melodramatic religious revivals or insurrections ; nevertheless, caring and loving in their truest sense could be practised much more than they are to-day ; and this with the help of science, which might well go hand in hand with belief in a purpose in communal life. Thus man may find direction, purpose, integration and quality in everything he does.

There are many indications to-day that this is just what people need, and, more important, realize that they need it : otherwise, for example, can there be any valid reasons why the Cathedral Church of Norwich was packed to capacity on September 3 ? It is doubtful if any member of that congregation went there to scoff at what he imagined to be outmoded philosophies ; some have been and still are agnostics ; a few may have appeared either just to see or be seen ; but it is most likely that the majority attended that Service to listen to, and afterwards ponder, the words of one of Britain's leaders in thought —at once a sociologist, a geologist and a theologian.

PROGRESS REPORTS AND TUMOUR RESEARCH

Progress in Experimental Tumor Research

Vol. 1. Edited by F. Homburger. Pp. xv + 476. (Basel and New York: S. Karger, 1960.) 83 Swiss francs.

It is not unlikely that in a twenty-first century history of science and medicine, the second half of the twentieth century will be called a period of high fashion for reviews, advances and progress reports. The future reviewer of these reviews will say probably that there was a pressing need for these to help the student, the research worker, the team leader, and the spectator on the touch-line to digest the enormous bulk of publications ; but he will also discover that the number of specific publishers, without giving due consideration to avoiding duplication, brought out, if not identical but very similar, collections of articles almost simultaneously. These, in addition to symposia reports, congress minutes, etc., so the reviewer of reviews probably will write in A.D 2061, were rather overwhelming in numbers and a burden to the budget of libraries.

Consequently, when a new series of progress reports appear in the crowded market, they have to be very good indeed to justify their existence even in a field like cancer research, with its fronts quickly changing. Of course, if the names of the authors of subsections or chapters are identical with those of the leading workers in special branches then most authoritative accounts can be expected. This is the case with the contributions in the present volume dealing with virology, including oncolytic viruses, carcinogenesis and tumour biochemistry. However, frequently one cannot escape the impression of having read similar reports, sometimes by the same authors, in other collections. Consequently, the reader is torn between his desire to go back to the original papers and his inclination to skim superficially over the texts of the reviews. Another criticism which can be raised against this specific volume is that, under the heading "Progress in Tumour Inhibition", a review of cytotoxic agents of the purine and the steroid group is presented which refers only to compounds synthesized by Lettré and his colleagues and mainly tested on cell cultures. The article by Sokoloff on oncolytic antibiotics is fascinating from the point of view of combination therapy with reticulo-endothelial system activators, such as hæmatoporphyrin and congo red, but it deals solely with four mould products which, so far as one knows, do not belong to the same chemical family.

There is no desire to throw doubt on the value, which is considerable, of this progress report. However, and this question can be raised with a large number of others of a similar type and of high quality, are these progress reports "really necessary ?". It is suggested that the answer lies mainly with the review authors, who in future should have the strength of mind to turn down requests for reviews and only accept the task of writing a few inside a period of three to five years. It is not certain which of us would have this strength.

F. BERGEL

ILLUSTRATIONS OF HUMAN ANATOMY

Atlas of Human Anatomy

Edited by Prof. Ferenc Kiss and Prof. János Szentágothai. Contributor: István Munkácsi. Vol. 1: Osteology—Syndesmology—Myology. Pp. 297. Vol. 2: Splanchnology—Endocrine Glands—Heart. Pp. 214. Vol. 3: Nervous System—Vascular System—Lymphatic System—Sense Organs. Pp. 289. Eighth edition. (Budapest: Publishing House of the Hungarian Academy of Sciences—Publishing House Medicina; London: Collet's Holdings Ltd. 1960.) 180*s*. the three volumes.

THE pioneer work in the field of modern anatomical illustration we owe to Andreas Vesalius, who published his *De Corporis Humani Fabrica* in 1547. It is true that Leonardo da Vinci produced a considerable number of magnificent anatomical drawings —with the legend in his characteristic mirror writing —but for various reasons these never attained the wide circulation and popularity of the drawings prepared by Vesalius.

The Vesalian illustrations were in black-and-white. Aselli's work, *De Lactibus Sive Lacteis Venis*, published in Milan in 1627, contained the first anatomical illustrations in colour. It is a far cry from Aselli's few simple woodcuts to our modern profusely illustrated anatomical atlases, all of which have followed Aselli's lead in making liberal use of colour.

It is generally assumed that the art of anatomical illustration has improved greatly since the time of Vesalius; but it is questionable whether this is entirely true. One of the striking differences between the Vesalian drawings and those found in modern atlases, such as that now before us, is the way in which Vesalius repeatedly depicted the body as a whole, more especially in relation to the skeletal and muscular systems. Though to do this adequately requires plates which are too large for a volume of average size, it possesses very considerable advantages.

Again, it is not clear what purpose is served by the indiscriminate use of colour. Where the colours used resemble those in the original, the illustration will be of value in recalling the dissection which it is intended to portray. Where, however, the colours are purely conventional and bear little or no resemblance to the original, their value becomes very problematical. They may be magnificent, but they are not anatomy. It is far easier with the eye of the mind to superimpose the colours of the original on a black-and-white diagram than on a diagram with colours so greatly at variance with the original that it becomes impossible to substitute the latter for the former.

The *Atlas* now before us is on the whole well done. The illustrations frequently tend to be schematic, but that is a feature common to most modern atlases. The authors have commendably allowed the illustrations to explain themselves, and have refrained from burdening the reader with superfluous legend. Vol. 1 depicts bones, joints, muscles and fasciæ. Vol. 2 deals with viscera, endocrine glands, and heart; and Vol. 3 is devoted to the nervous, vascular and lymphatic systems, concluding with the sense organs.

It would appear from the preface that this *Atlas* has attained great popularity in countries behind the Iron Curtain, and that a Chinese edition has been prepared by Prof. Yü-Ch'üang Tsang.

J. M. YOFFEY

RUSSIAN WORK IN ORGANO-PHOSPHORUS CHEMISTRY

Soviet Research on Organo-Phosphorus Compounds, 1949–1956

In English Translation. Part 1: Pp. viii+448. 50 dollars. Part 2: Pp. vii+449–890. 50 dollars. Part 3: Pp. iv+891–1208. 30 dollars. Complete collection, 120 dollars. (Chemistry Collection Series.) (New York: Consultants Bureau, Inc., 1960.)

DURING the past two decades, there has been a great resurgence of interest in the chemistry of organo-phosphorus compounds, both on the purely scientific side and on the technical side: the academic chemist is attracted by the many novel reactions of these compounds, and the technical chemist by their potential application for a variety of purposes. This resurgence of interest has been strongly marked in chemical studies in the United States and the U.S.S.R.; but in Britain, apart from certain manufacturing processes, the interest has appeared to be very much less active, possibly because the new work has tended to be in highly specialized fields.

The Russian contributions to organo-phosphorus chemistry have naturally been published almost entirely in Russian, and consequently these contributions, although very considerable, have not been easily and rapidly available to English-speaking chemists. The latter are now deeply in debt both to Prof. Alfred Burger of the University of Virginia, who has selected about 250 of the chief Russian papers on this subject which appeared during 1949-56, and classified them in three main divisions, and to the publishers, who have arranged for complete translations of these papers to appear in these three volumes. It should be emphasized that the translations are literally complete with diagrams and full experimental sections.

This has necessarily been a costly undertaking, and as a guide to the prospective purchaser the contents of the three parts (or volumes) are briefly indicated. Part 1. "Derivatives of Organic Phosphorus Acids containing (*A*) the C-O-P Linkage, (*B*) the C-O-P-S and C-S-P Linkages ; (*C*) Effects of Inorganic Phosphorus Compounds on Organic Compounds or Reactions." Part 2. "Derivatives of Organic Phosphorus Acids containing (*A*) the C-P-O Bond, (*B*) the C-P-S Bond." Part 3. "(*A*) Inorganic Complexes of Organic Compounds of Phosphorus ; (*B*) Phosphines, Phosphine Oxides, Phosphonium Compounds and their Se, N, S and Halogen Derivatives ; (*C*) Nitrogen-containing Derivatives of Organic Phosphorus Compounds."

In the table of contents in each part, the title of each paper is accompanied by a simple code symbol which indicates the name of the journal in which the paper originally appeared, the year of publication, and the page number both in the journal and in the earlier English translations which were prepared by the present publishers and from which the papers in the present collection have been reprinted.

The papers in these three parts cover a very wide range of subjects, and will be almost indispensable to chemists who are actually working in the phosphorus field, and who therefore require greater detail than any abstracts can possibly supply. My only (and very minor) criticism of this publication is that, although the majority of the papers are printed very clearly, an occasional paper has formulæ which have become rather blurred in the printing process employed.

It is pleasant to see in this collection a number of papers by Prof. A. E. Arbuzov (who celebrated his eightieth birthday in 1957), and also by his son, Prof. B. A. Arbuzov, and their co-workers.

F G. MANN

TRANSITION-METAL CHEMISTRY

An Introduction to Transition-Metal Chemistry

Ligand-Field Theory. By Leslie E. Orgel. Pp. 180. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1960.) 25*s*. net.

An Introduction to Co-ordination Chemistry

By Dr. D. P. Graddon. (International Series of Monographs on Inorganic Chemistry, Vol. 3.) Pp. vii+111. (London and New York: Pergamon Press, 1961.) 25*s*. net.

The Theory of Transition-Metal Ions

By Prof. J. S. Griffith. Pp. x+455. (Cambridge: At the University Press, 1961.) 95*s*. net.

IT is a sign of the astonishing development of inorganic chemistry during the past fifteen years that no less than three books come to be reviewed simultaneously. Furthermore, all three are so distinct in their emphasis that not one of them is included in the others.

The briefest possible description would be to say that Dr. Orgel's book gives the most pictorial account yet provided of the reasons why transition metals form such interesting complexes; Dr. Graddon's book gives an account of the many different varieties of co-ordination compounds recently developed, and increasingly used in various industrial processes; Prof. Griffith's monumental tome lays down the basic theory in a form that is definitive and impressive.

Dr. Orgel has set out to write a book which displays the pattern of transition-metal chemistry in the simplest, and most pictorial, fashion. This aspect of chemistry is really bound up with the behaviour of atomic *d*-electrons. Orgel is able to show, with a series of most effective and elementary diagrams, how the energies of these *d*-orbitals are affected in the presence of a given set of ligands, having tetrahedral or octahedral symmetry. The one impression that stands out from reading this admirable volume is the simplicity and order that have been brought into this previously complicated field, by quite elementary notions of wave mechanics. At the elementary level to which this book aspires, it would be very difficult to improve on it. I find it hard to believe that any undergraduate reading for a degree in chemistry could not understand it, and profit greatly at the same time.

Dr. Graddon's emphasis is on the experimental results. He begins with a history of co-ordination chemistry and shows that this may be traced back as long as 1753, when Macquer obtained potassium ferrocyanide. It is good to be reminded that the famous series of cobaltammines dates back to Tassaert in 1798. Subsequent chapters deal with polymerization, stability constants, oxidation potentials, carbonyls and π-complexes, solvent extraction and sequestration. It will be clear from this that Dr. Graddon provides a good deal of experimental information not covered by Orgel. I wish I could speak equally favourably of his theoretical treatment. This, alas, is marred by several bad mistakes. To mention a few: the combination of d_{z^2} and $d_{x^2-y^2}$ atomic orbitals is not spherically symmetrical; ligand-field theory is not an electrostatic theory (though crystal-field theory much more nearly is so); two *d*-electrons do not necessarily lead to a state with angular momentum 3; the molecular orbital for ferrocene which Dr. Graddon refers to as Σ is not "analogous to an atomic *s*-orbital". These are serious errors, which much reduce the value of the book, and make it dangerous in the hands of a student.

Prof. Griffith has written a large and impressive account. It is hard going, and no one should be tempted to accept the beginning disclaimer, that scarcely any quantum theory is needed. Perhaps no one is very likely to do so, when he sees that preliminary reading includes chunks of Dirac's *Quantum Theory*, and the language of bra's and ket's is used without apology. But for those who will normally be theoretical physicists, who can cope with the mathematics, this is a book with no rival in its field. In the earlier chapters Griffith revises the theory of atomic structure, and recapitulates much of the corresponding work in Condon and Shortley's well-known monograph in the same "Cambridge-Blue" series. This is followed by a valuable account of group theory, including spinors, and the application in one-electron and many-electron ions. The Jahn–Teller effect, paramagnetic effects, optical spectra, the relation between holes in nearly filled shells and a similar number of particles, the effective Spin–Hamiltonian, electron-spin-resonance are all dealt with. The account is clear, and—if it were not for a few silly footnotes in praise of discussions around a coffee table—set-out with dignity, precision and completeness. My only comment of an unfavourable kind concerns the distinction between crystal-field theory and ligand-field theory. Here Orgel seems to be more explicit and helpful. It is true that the distinction (without which one cannot adequately deal with the successes and failures of Pauling's early theory of hybrids) is implicit in Chapter 7. But it would have helped very much to have had it developed more explicitly. Then the role of the linear combination of atomic orbitals approximation would have become more apparent, and it would not have been relegated to an appendix. It would also have been easier to relate nuclear hyperfine and electron-spin measurements to the degree of delocalization of the various molecular orbitals. I could also have wished that the author had distinguished more clearly between what chemists call orbitals and spin-orbitals.

But these are small points. This is a really important book, and will at once take its pride of place as the most definitive full-scale study of this field.

Inorganic chemistry has moved a long way from the situation in which the poor unhappy chemist had to learn long lists of formulæ and reactions by heart. It now has a shape and a coherence. Wave mechanics has provided much of this understanding: and in so far as these books—particularly those by the Cambridge School—are able to show this pattern, they are to be welcomed most warmly.

C. A. COULSON

TERPENE CHEMISTRY

The Chemistry of the Terpenes

By Dr. A. R. Pinder. Pp. vii+223. (London: Chapman and Hall, Ltd., 1960.) 50*s*. net.

THIS book is intended to provide honours undergraduates with a source of information on terpenes which is intermediate between the general text-books and the comprehensive monographs. There is no obvious reason why terpenes should be singled out for special treatment in this way, and it would be impracticable to require undergraduates to delve into a large number of works of this type each dealing at considerable length with one particular aspect of chemistry. It is, of course, impossible to cover everything in lectures to undergraduates, and there is no denying that it is desirable each year to deal with a few subjects in some detail. The topics selected for such treatment should, if possible, serve to illustrate important general principles or general methods of degradation and synthesis, and there can be no doubt that the terpenes are among those which are ideally suited to this purpose. However, it is questionable whether the students should be provided with an extra text-book to accompany all lectures giving special attention to a particular topic. That such a book might provide a hard-pressed lecturer with considerable assistance in planning a series of lectures is another matter. The present book is intended for students, and it must therefore be considered primarily in this light.

All the main classes of terpenes are covered, including the carotenoids, which, strangely, are omitted in the otherwise admirable reference works by Simonsen and his co-authors and in the excellent two volumes provided recently by de Mayo. Following a short chapter devoted to the determination of structure by both chemical and physical methods, the various classes of terpenes are discussed in a conventional way giving rather greater attention to the mono-, sesqui-, and di-terpenes than to the more complex compounds. More examples of each class are often given than are necessary to illustrate many general points, and details on occurrence, isolation, and physical properties are included which can be of little use to the undergraduate. The text itself is always clear, but at times could with advantage be more critical, particularly when reproducing claims from the earlier literature and for which the evidence would not now be considered conclusive. I should also have liked to see more attention given to the mechanisms of the various reactions described. No explanation is offered for such simple processes as the cyclization of geraniol and the hydrolysis of citral; the direction of additions to carbon-carbon double bonds is explained in terms of Markownikow's rule; and the opportunity of introducing such concepts as non-classical ions and concerted processes is missed. At the end of each chapter references are given, though none was noticed that was later than 1957. Some sections are inevitably a little out of date; thus mevalonic acid receives very little mention in the chapter on biogenesis. However, those who require a brief survey of the terpene field will find it in this book.

B. C. L. WEEDON

ALGOLOGICAL STUDY ON THE EASTERN COASTS OF THE AMERICAS

Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas

By Prof. William Randolph Taylor. (University of Michigan Studies—Scientific Series, Vol. 21.) Pp. ix+870. (Ann Arbor: University of Michigan Press, 1960. Distributed in the United Kingdom by the Cresset Press, Ltd.) 19.50 dollars; 140*s*.

WR. TAYLOR'S major work is the first complete account of the algae of the eastern coasts of the Americas, from Bermuda and from North Carolina (respecting the more southern species) to southern Brazil.

A historical survey, with bibliography later in the book, is of particular importance because such early records as exist are mainly scattered through general works or hidden in rare volumes. Brief reference is made to geographical distribution, though seasonal changes are much reduced in such warm waters where major currents tend to spread tropical conditions beyond their normal limits.

Preliminary discussion of algal habitats outlines the effect of fluctuating conditions in the aquatic environment. The author stresses the suitability of the region for algal study because of the possibility of a continuous working season with minimal tidal amplitude, although species are restricted by relatively stable water-temperature and insolation. Ecological habitats, intertidal and at greater depth, appear to be comparable in some cases with those in sub-tropical waters off parts of the Australian coasts, especially where terraces clothed with coralline and other algae extend into the sea and the roots of *Avicennia* and *Rhizophora* provide a habitat around the sun-lit margins of the groves. In the sub-littoral, Chlorophyceae, particularly members of the Siphonales, together with members of the Rhodophyceae, have been found at a depth of 90 metres. The author finds no evidence that the pelagic forms of *Sargassum*, which form the bulk of the floating algae in the Sargasso Sea, need to be constantly replenished from a benthonic source.

This section of the book concludes with a helpful chapter on the preservation and collection of specimens, a subject on which the author's painstaking work is well known.

The main text of some 600 pages is a descriptive catalogue of 750 species and 140 varieties, with keys for families, genera and species, bibliographical references, location and ecological notes. Uncertain records are listed at the end of the section on each genus. The book is illustrated by 74 beautifully executed line drawings and 20 black-and-white photographs.

The whole volume is the result of thirty years of patient and skilled endeavour by W. R. Taylor, his wife and a group of young workers. It has added greatly to algological knowledge, and at the same time it will be a foundation and indeed an inspiration, for further taxonomic and ecological work in an area for which no algal Flora had formerly been published. It is a pity that a map has not been included, particularly as locations are cited at the ends of descriptions of species. LILY NEWTON

STEROIDS AND CANCER

Biological Activities of Steroids in Relation to Cancer

Proceedings of a Conference sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, U.S. Department of Health, Education and Welfare. Edited by Gregory Pincus and Erwin P. Vollmer. Pp. xvi+530. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 15 dollars.

THIS book is the record of a conference of American endocrinologists, held in October 1959, to review the progress of a cancer therapy programme set up in 1955 by the U.S. National Cancer Institute. This record, complete with verbatim reports of the uninhibited discussions, reflects the high standard of present-day endocrinology in the United States, and the close alliance, if not identity, between clinical investigators and animal experimentalists. However, although the programme has clearly been intelligently organized and has already given grounds for cautious optimism, "Difficulty has been experienced in making use of information derived from animal studies.... The possibility exists that we may well become quite proficient at treating mouse and rat cancers, but not those found in man" (H. Brendler). Even with animal tumours there are of course numerous difficulties; for example, the admixture of cells dependent on hormones and cells independent of hormones in rat mammary tumours induced by a technique which C. Huggins describes. His paper is notable for the international flavour of its list of references.

The papers on steroid chemistry (J. Fried), on the prostate as a target organ for steroids (W. W. Scott), and on steroid metabolism in human endocrine tumours (R. I. Dorfman) are informative in themselves and indicative of the range of subjects dealt with. V. A. Drill lucidly discusses the physiology and pharmacology of steroids in relation to the growth of experimental tumours. He also deals with the pituitary gland in a short section, which opens with the over-terse phrase: "Estrogens and androgens can inhibit the pituitary gland . . ."; in fact, only some of the functions of the pituitary are known to be thus inhibited. R. Vandewiele and S. Lieberman discuss the mysterious 'adrenal androgen' dehydroisoandrosterone—a major secretory product, the output being 15–25 mgm. a day in man. Among other papers on basic work, G. C. Mueller's is notable for shrewd experiments on possible points of action of œstrogens, ethanolamine phospholipids being a new candidate for consideration. Hydrogen transport may be another candidate in the view of P. Talalay and collaborators, but this view has been challenged.

While the book will be indispensable for clinical investigators, it also offers much of value to workers in basic disciplines, including chemistry. It contains not only an index but also a glossary. E. REID

JUNGIAN PSYCHOLOGY

The Structure and Dynamics of the Psyche

By C. G. Jung. Translated by R. F. C. Hull. (The Collected Works of C. G. Jung, Vol. 8.) Pp. x+596. (London: Routledge and Kegan Paul, Ltd., 1960.) 42*s*. net.

THIS volume of Jung's collected works is not a book devoted to one developed theme but a collection of essays: some written many years ago and worked over more recently before publication. They are a witness to his incredible industry, which seems to have remained undiminished up to the very last years of his long life.

In a way it is a pity that he re-wrote such a great deal since one would like to have observed the evolution of his ideas. Some of the essays do show this, and the one "On Psychic Energy", for example, is really a defence of the stand he took after his book, *Psychology and the Unconscious*, caused such a violent opposition that he resigned his presidency of the International Psychoanalytical Society in 1913. In it he discusses symbol formation, myths and his views on the nature of the libido.

This book shows clearly both Jung's virtues and faults. There is no doubting his tremendous application, his great erudition, and originality. However, this is offset by his vagueness and his tendency to interpret unknowns by unknowns. In spite of this, those interested in Jung's psychology should certainly read these essays. They will find considerable space devoted to his theories of complexes, archetypes and the structure of the psyche. The breadth of his reading is frightening, particularly when one considers that not only has he read very widely the literature of psychology from most countries but also he has made a deep study of mythology as well.

The last essay will not be acceptable to many psychologists—it is on "Synchronicity: an Acausal Connecting Principle" and deals with extra-sensory perception.

Those interested in Jung's thought should certainly read this series. It is beautifully produced in a cloth binding with gold lettering, and clear print. The bibliography takes up seventeen pages and the index thirty-three. The translation by R. F. C. Hull is faultless. C. ALLEN

Rapid Statistical Calculations

A Collection of Distribution-free and Easy Methods of Estimation and Testing. By M. H. Quenouille. Pp. xv + 81. (London: Charles Griffin and Co., Ltd., 1959). 10s.

ORTHODOX statistical techniques usually involve a fair amount of computation. Particularly when data are cheap, there is a need for rapid methods that can be applied with no more than pencil and paper. Some of these methods have the added advantage of being 'robust'—they remain valid (at least approximately) over a wider range of assumptions than do the more usual techniques.

The advantage of speed is lost if the user of a rapid method has to spend time looking up the details in a journal. Mr. Quenouille has accordingly collected together a selection of the most useful techniques and has had them published in a genuine pocket-book, measuring only 16 cm. × 11 cm. Each method is presented with comments and an example on a pair of facing pages. The topics covered include the estimation of means, standard deviations and regression coefficients and tests for inequality of means, differences between means of groups, differences between proportions and association. In the index, the methods are marked "faster than usual", "quick" or "very quick", and tests that are distribution-free are indicated. The list of references shows that several of the tests are new.

Rapid Statistical Calculations is well produced at a reasonable price; those for whom the ordinary statistical techniques are too cumbersome should find that it meets their requirements.

M. J. R. Healy

Theory of Detonation

By Ia. B. Zeldovich and A. S. Kompaneets. Pp. i + 284. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 10 dollars.

THIS book, based mainly on papers written at the Chemical Physics Institute of the Academy of Sciences of the U.S.S.R., gives a systematic account of the theory of detonation. The first chapter considers the relevant fundamental concepts of gas dynamics, and includes a discussion of shock waves, the flow equations, and 'characteristics', for one-dimensional and centrally symmetric flow.

Most of the book is concerned with gas explosions, and throughout the authors are at pains to explain the physical and chemical concepts underlying their reasoning and not merely to manipulate mathematical symbols. For example, in the hydrodynamic theory of detonation, the complex argument required to show that the Chapman–Jouguet plane coincides with the end of the reaction zone in a plane, stable, lossless wave is carefully detailed. Deflagration is discussed, and there is a useful contribution on non-detonative explosions. The effect of thermal and fluid-mechanical losses in smooth pipes, and spin detonation are treated, and so also are detonation in roughened pipes and the transition from combustion to detonation.

Less space is devoted to detonation in condensed explosives. The authors point out that, generally, the density of the gaseous products is so high that the full hydrodynamic theory is inapplicable; they obtain useful results by combining the appropriate hydrodynamic equations with observed empirical relations. The final chapter discusses the motion of the detonation products.

It would have been helpful if reference had been made to a wider range of original papers, but, nevertheless, this is a valuable text-book which should be widely appreciated. The English translation reads well.

W. L. Murray

Thermodynamics with Quantum Statistical Illustrations

By Prof. P. T. Landsberg. (Monographs in Statistical Physics and Thermodynamics, Vol. 2.) Pp. x + 499. (New York: Interscience Publishers, Inc.; London; Interscience Publishers, Ltd., 1961.) 109*s*.

IN an appendix to this book Prof. Landsberg tells us that a common misconception about thermodynamics is that, since the subject is mathematically simple, it is also intrinsically simple. That this is indeed a misconception is demonstrated by the hidden, and sometimes unnecessary, assumptions made in the usual treatments of the subject. In order to avoid these, the author has used some mathematical tools which, sadly, will be unknown to many physicists. However, the reader is carefully introduced to them, and the usual claim, that no previous knowledge of these subjects is necessary, is probably in this case justified.

The first three chapters consist of a discussion, couched in the language of elementary topology, of the three laws of thermodynamics, and the fourth chapter of some rather formal applications. The fifth and final chapter, probably the best in the book, deals with statistical mechanics from the point of view of probability distributions rather than ensembles. Finally, there are seven useful appendixes and a rather brief bibliography.

The book is written throughout in a most readable style. Prof. Landsberg is particularly to be praised for his excellent and unusual selection of examples, and for his references to many little-known parts of the literature. It would be a mistake to use this book as a standard text at any level, but it can be recommended to anyone who has a serious interest in thermodynamics, or indeed, who wants such an interest stimulated.

W. E. Parry

Techniques in Tropical Pathology

By Prof. B. G. Maegraith, Prof. W. E. Kershaw and D. Dagnall. Pp. xi + 165. (Edinburgh and London: Oliver and Boyd, Ltd., 1961.) 25*s*. net.

THIS simple book, written by three workers deeply experienced in the subject, will prove of value to medical men and others who live in the more remote parts of the tropics. Perhaps a better title would have been "Tropical Parasitology" instead of *Tropical Pathology*, because very little pathology in the classical sense is included, and scarcely any histological staining methods. Likewise, the bacteriological side is a little weak, and there is no mention of an incubator in the list of apparatus given in the appendix. With these reservations, the book can be thoroughly recommended; it is divided into two main portions: (1) the technique of examination of different material (blood, urine, fæces, sputum, cerebrospinal fluid, pus, and biopsy specimens); and (2) the diagnosis of specific infections, in which a very short account of epidemiology is followed by a good description of the individual agents of disease.

Three coloured plates (one of which by its presumably unintentional 'fuzziness" gives a life-like impression of a thick blood film stained by Field's method), three photomicrographs, and four text figures illustrate the text.

P. C. C. Garnham

LONG-RANGE RADIO COMMUNICATION BY SATELLITE MICROWAVE DIPOLES

THE WEST FORD PROJECT

IN the autumn of 1959, the Space Science Board in the United States was informed of the proposal by the Lincoln Laboratory of the Massachusetts Institute of Technology to erect a belt of thin microwave dipoles in a polar orbit about the Earth at an altitude of a few thousand kilometres to obtain experimental verification of such a system for reliable long-range radio communications. This experiment involves the establishment of a belt consisting of some 35 kgm. of dipoles, resonant at about 8,000 Mc./s., and therefore serving to reflect radio waves at this frequency. Each dipole is 1·77 cm. long with a diameter of 0·00286 cm. $3·5 \times 10^8$ dipoles are involved, distributed uniformly along the orbit and afterwards dispersing until, at 60 days after launch, a belt 8 km. wide by 40 km. thick is formed. At this time the density will be about 21 dipoles per $km.^3$. The orbital altitude for the experiment has been chosen to ensure that effects of solar radiation pressure will act to produce an ever-increasing eccentricity in the orbit, thus bringing perigee into the atmosphere within a few years with consequent destruction of the belt.

Extensive theoretical and laboratory studies of the properties of orbiting dipole belts have been carried out at the Lincoln Laboratory. In addition, at the request of the Director of the Laboratory, the Space Science Board undertook to examine the effects of the proposed experiment to ascertain whether the project would have any noticeable effects on activity in any field of research.

The Board, on December 24, 1959, appointed an *ad hoc* committee of scientists under the chairmanship of Dr. O. G. Villard, jun. (Stanford University), to examine the consequences of the proposed experiment. Astronomers on this committee included G. M. Clemence (U.S. Naval Observatory), C. H. Mayer (Naval Research Laboratory), and F. L. Whipple (Smithsonian Astrophysical Observatory). The committee held several meetings during the first half of 1960, and invoked the assistance of some twenty additional scientists, including optical and radio astronomers and other specialists.

Acting on the findings of this committee, the Space Science Board in June 1960 reached a number of conclusions and adopted several recommendations. (i) This exploratory test would not have an adverse effect on any branch of science. (ii) There is justifiable concern for the interference which an operational system might entail for optical and radio astronomical observations: any planning for such a system must protect the interests of astronomical research and of science in general. (iii) Full information on the scientific and operational aspects of the initial experiment should be published as soon as possible. (iv) In view of the possible interference to radio astronomy, which might result not only from extensive dipole belts but also from active and even passive communication satellites, frequency bands for radio astronomy should be provided on the basis of world-wide agreements.

The Board also decided that continued study of the subject was worth while, and in this the Lincoln Laboratory concurred. Accordingly, the Board established a special committee on July 29, 1960. The present composition of this committee is as follows: John Findlay (National Radio Astronomy Observatory) (*chairman*), F. T. Haddock (University of Michigan), William Liller (Harvard University), A. E. Lilley (Harvard University), W. A. Hiltner (Yerkes Observatory), and Allan Sandage (Mount Wilson and Palomar Observatories). Leo Goldberg (Harvard University), chairman of the Astromony Committee of the Space Science Board, meets with the group. The committee was asked by the Board to examine carefully all calculations of the effects of the West Ford experiment on astronomy, and to recommend additional necessary research. The committee has maintained continuous liaison with the project throughout the past year and has formally convened with the directors of the West Ford project of Lincoln Laboratory on three occasions. At each of these sessions the committee has received a thorough review of the project and full information on all aspects of the experiment.

The Space Science Board has felt that a discussion of the proposed experiment should not be limited to committee consideration, but should be given critical analysis by the scientific community as a whole. Accordingly, as a result of the foregoing recommendation (iii) of the Space Science Board and following almost immediately the initial discussions of its Astronomy Committee with Lincoln Laboratory scientists (August 29, 1960), the Laboratory agreed to publish its work on orbiting dipole belt properties. Dr. W. E. Morrow, director of the West Ford project, presented a paper on the topic before the general assembly of the International Scientific Radio Union (URSI) in London in September 1960.

The Board has also recommended that the theoretical and technical aspects of the West Ford project should be published in the astronomical literature so that astronomers throughout the world could examine for themselves the calculations on which the project is based. For this purpose, four articles were published in the *Astronomical Journal*, Vol. 66, No. 3, pp. 105–118, April 1961. Some 1,400 copies of these papers have been distributed by the Board to the scientific community, more than 1,100 of them to members of the International Astronomical Union, including 800 astronomers abroad.

During the course of its considerations, the Committee also established the desirability of securing the widest possible participation of observational astronomers to study the characteristics of the West Ford project to determine its performance under actual conditions. In March 1961 the Committee distributed a circular letter to a large number of optical astronomers throughout the world (i) providing a technical description of the experiment, (ii) containing recommendations for an observational programme which might be undertaken, and (iii) inviting the co-operation of individual astronomers in obtaining observations and measurements.

The Space Science Board's recommendations on the West Ford project have been made available to the U.S. Government as they were developed. In response to these recommendations, made by the Board on behalf of the scientific community, the Government has issued appropriate policies. These, as transmitted by the Special Assistant to the President for Science and Technology to the chairman of the Space Science Board, state: "The United States Government, in conducting the West Ford Project, will be guided as follows:

"1. No further launches of orbiting dipoles will be planned until after the results of the West Ford experiment have been analysed and evaluated. The findings and conclusions of foreign and domestic scientists (including the liaison committee of astronomers established by the Space Science Board of the National Academy of Sciences) should be carefully considered in such analysis and evaluation.

"2. Any decision to place additional quantities of dipoles in orbit, subsequent to the West Ford experiment, will be contingent on the results of the analysis and evaluation and the development of necessary safeguards against harmful interference with space activities or with any branch of science.

"3. Optical and radio astronomers throughout the world should be invited to co-operate in the West Ford experiment to ascertain the effects of the experimental belt in both the optical and radio parts of the spectrum. To assist in such co-operation, they should be given appropriate information on a timely basis. Scientific data derived from the experiment should be made available to the public as promptly as feasible after the launching."

In the light of its investigations and of U.S. Government policy, the Space Science Board concludes:

"(1) The Project West Ford experiment will constitute no interference to optical or radio astronomy. As a matter of fact, the belt will be barely detectable, even by astronomers with advance information and on the taking of special efforts for detection. It is true that a belt or belts could be erected which could cause interference to astronomical observations; however, the United States Government policy provides that no further launches of orbiting dipoles will be planned (i) until the West Ford results have been analysed and evaluated and, further, (ii) will be contingent on the development of necessary safeguards.

"(2) The Board will continue its studies of this area of experimentation on behalf of the scientific community. In these studies it will depend on objective and quantitative assessments that constitute the foundation for scientific discussions, recommendations and decisions. These assessments can only be achieved through a carefully controlled, harmless test, and Project West Ford provides a clear opportunity for scientists of all nations to co-operate in making observations to form the basis for an objective understanding of the behaviour of an orbiting dipole belt, both in terms of its astronomical properties and of its possibilities for communication.

"(3) The Board will continue to keep the scientific community everywhere informed and it invites the co-operation and assistance of scientists everywhere who have interest and specialized knowledge in this area. The Board acknowledges with gratitude the assistance of many scientists—both at home and abroad—who have already contributed to its studies."

BIOCHEMISTRY AT THE UNIVERSITY OF MELBOURNE

By Prof. V. M. TRIKOJUS

In April of this year the Russell Grimwade School of Biochemistry at the University of Melbourne was officially opened by Sir John Eccles, president of the Australian Academy of Science. During his opening address Sir John made a strong plea for increased support for the biological sciences and stressed the unifying role of biochemistry in this regard. Fifty years hence we would be judged as having been far too concerned with the sciences of inorganic matter, such as nuclear physics, and the probing of space, and with giving too little attention to the potentialities of biological research for the welfare of mankind. Much the same view had been expressed by the late Sir Russell Grimwade when, in 1944, he made a gift of £50,000 to the University towards a new home for biochemistry. Himself a graduate in chemistry, Russell Grimwade played a leading part in the development of chemical industry in Victoria and elsewhere in Australia. A man of wide vision and interests, skilled moreover in several of the crafts, he exercised a profound influence on the cultural progress of his native city and beyond. He also found time to serve the University for many years as a member of Council and as deputy chancellor.

A start with the new School was delayed for a long period by building restrictions and then inflation, but through the generosity of Lady Grimwade and grants from the University's Building Appeal Fund and from the State and Commonwealth Governments sufficient finance became available over the past six years to bring construction to the final stage.

Erected in two sections, the first, mainly for teaching, being opened by Lady Grimwade in 1958, the completed building, of about 66,000 sq. ft., is by far the largest in Australia devoted to biochemical science. Of modern construction the building consists of a main laboratory wing, running east–west, of five floors, each 200 ft. long and of approximately 10,000 sq. ft., plus a penthouse area. A north extension of three floors at one end houses student facilities, an electrical sub-station, the main entrance and foyer, a lecture theatre and departmental library. The total cost, including furnishings, amounted to approximately £710,000.

The ground-floor of the main structure contains the stores, a large-scale laboratory with associated cold- and hot-room assemblies, an area for physical biochemistry and a mechanical plant section. At this level a bulk solvent store connects with, but is external to, the main building framework.

There are three teaching laboratories situated on the first and part of the second floors, capable of

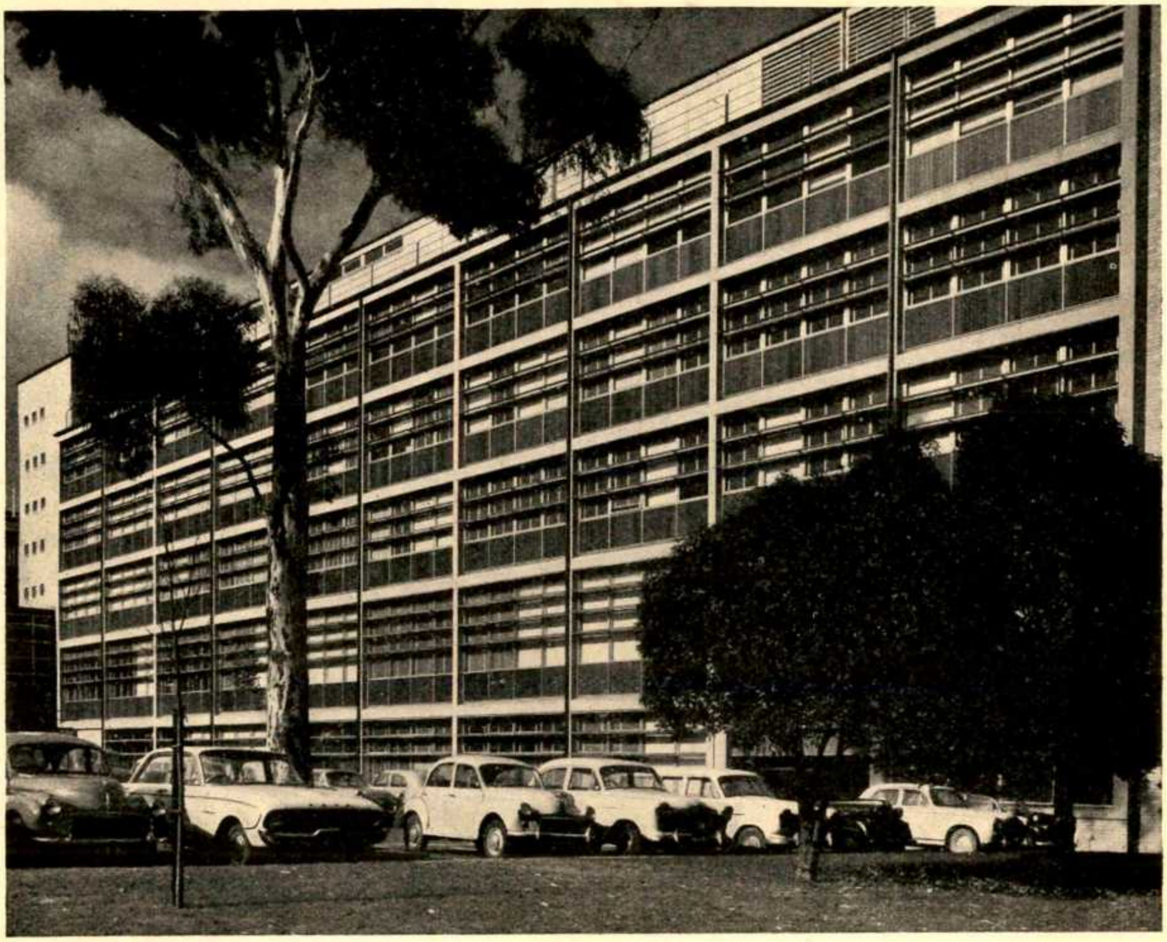


Fig. 1. The Russell Grimwade School of Biochemistry, University of Melbourne (viewed from north-west and showing the main laboratory wing only)

handling up to 180 students at one time. Undergraduate instruction to some 550 students is spread over four faculties: medicine (two years), science (two years), agricultural science (two years) and dental science (one year).

The remainder of the building comprises mainly offices and laboratories for staff and research students and rooms for specialized research facilities, such as air-conditioned areas for physical equipment and chromatography and a section for isotopes. There are cold rooms on each floor. On part of the fourth floor are the Nicholas Nutrition Laboratories for teaching and research, which include the section for the breeding and housing of small laboratory animals and an adjoining incinerator room. The penthouse area, which runs the full length of the building, comprises a second mechanical plant room, a toxic laboratory and a general laboratory section which will house for a limited period the Sugar Research Unit of the Commonwealth Scientific and Industrial Research Organization.

The structure is steel framed with light-weight floors of prefabricated metal units. Steel beams are protected with suspended fire-resistant ceilings. The rectilinear pattern of the steel columns and beams on the elevations of the laboratory wing has been finished in cast stone. Walling is in light-toned brickwork. Window frames are aluminium and along the north side are fitted with horizontal hoods to protect the glass from the summer sun. Spandrels below the windows are faced with aluminium, ribbed and anodized grey.

The general planning of the laboratories permits a wide degree of flexibility to follow changing techniques and methods, and the positions of laboratory benches and the location of partitions are adjustable to revised requirements. The modular system adopted for the purpose provides a series of working bays each equipped with its set of service installations.

The pressure services—compressed air, gas, hot and cold water, filtered and demineralized water, and the heating system—are run in horizontal ducts below the window sills and connect to distribution ducts along the centres of the laboratory benches. The fitments, which have been designed in standard units to reduce manufacturing costs, are protected with a plastic acid-resistant finish.

The drainage system is run in corrosion-resisting polyvinyl chloride piping through polythene traps. Wastes are concealed in ceiling ducts and connected to a series of vertical ducts spaced along the centre of the building; thus all pipes and wastes are readily accessible for maintenance. Various parts of the building are mechanically ventilated, including the larger teaching laboratories, by connexion to a system of positive pressure. Plastic tube vents from the fume cupboards rise in the vertical ducts and terminate with individual exhaust fans grouped at 40-ft.

intervals above the penthouse-level. Automatic fire alarms and extinguishers give protection against the spread of fire and a warning system indicates any failure in the mechanical plant. A passenger lift located in the eastern tower and an electrical hoist from the stores in the centre of the building provide additional services.

The landscaped development of the grounds adjacent to the building includes two levels of lawn and gardens and a parking area for forty cars.

Biochemistry gained its independence at the University of Melbourne in 1938 when the late Dr. W. J. Young—co-discoverer of the Harden–Young ester—was appointed as the first professor and he was succeeded in 1943 by Dr. V. M. Trikojus. From small beginnings the school has now a full-time teaching, research and technical staff of about seventy. A broad pattern of research is in progress, and all members of the staff look forward to a period of vigorous development in their new environment.

PLANT EXPLORATION AND INTRODUCTION

"THE key to our understanding of the evolution of living organisms is, as Darwin discovered, the study of what happens to them in the hands of man. During the past ten thousand years man has transformed the plants with which he has most concerned himself. The origin of cultivated plants, therefore, in some respects proved to be, as Darwin supposed, a model of the origin of species" (C. D. Darlington, *Chromosome Botany*, 1956).

In the years which have elapsed since N. I. Vavilov and his colleagues in the Institute of Plant Industry, Leningrad, carried out their pioneer and fundamental work on the centres of origin of economic plants, considerable progress has been made in the general understanding and application of the principles and techniques involved. Many collecting expeditions have visited what have now come to be called the centres of greatest diversity, simple, multiple or shifting, and studies have been made on the origins and peregrinations of many cultivated plants.

During the past five or six years, the Food and Agriculture Organization of the United Nations has been giving increased attention to the subject, because the assembly and study of wild species and primitive forms appeared to be an activity that called for international action and support. In recent years also, there has been an increasing realization that many important crop plants, particularly in the more developed countries, have a dangerously narrow genetical basis, and at the same time the world gene resources in and around the centres of diversity are being destroyed through intensive and unwise use of land and the advance of 'improved' cultivars into regions of primitive agriculture.

This work recently culminated in the convening at the headquarters of the Organization in Rome of a technical meeting on plant exploration and introduction, held during July 10–20. Seventy delegates and observers from some thirty countries and other international organizations took part. The chairman was Mr. William Hartley (Australia), and the vice-chairmen were Dr. E. Åberg (Sweden), Prof. F. G. Brieger (Brazil) and Mr. A. Marzocca (Argentina).

The programme was designed to permit the presentation of papers and full discussions, first on present-day actions and future requirements with regard to specific major and minor crops (cereals, legumes, industrial crops, tropical perennial crops, fruits, vegetables and potatoes, and the herbage and fodder plants). This was followed by a series of seven papers on the genecological bases of exploration and collection, with contributions by William Hartley on the phytogeographical basis of pasture plant introduction, Prof. H. R. Oppenheimer (Israel) on the origin of cultivated wheat, and Dr. H. Ross (Germany) on the potato gene centre and its importance with respect to the origin and breeding of the cultivated potato. The potato also featured in the plea by Prof. J. G. Hawkes (University of Birmingham) for greater international collaboration in collecting expeditions and the subsequent distribution of the material, and in the report of the British Commonwealth Potato Collection presented by Dr. P. S. Hudson (Commonwealth Agricultural Bureaux).

Prof. J. W. Purseglove (Trinidad) directed attention to the work of I. H. Burkill, and to the fact that not all cultivated plants have necessarily arisen in the eight independent centres of origin recognized by Vavilov. Furthermore, some crops may have died out of the areas where they formerly flourished, and have found secondary centres of diversity in favourable areas where conditions for evolution are different. The generalization that the centre of origin of a taxon is the area in which the most advanced species are found should, according to Prof. Purseglove, also be treated with caution. New cultivars are evolved at the periphery of migration or when encountering unfavourable conditions. Plants rarely occupy anything like the whole of their potential geographical range. Although the Vavilov dispersal centres may show the greatest qualitative variability, they are not necessarily the centres of maximum expression of a character in a new direction.

Although the value and significance of comparative climatic investigations have not yet been generally accepted, there is no doubt that they may ultimately be expected to make a great contribution to the better understanding of the geographical basis of the subject. G. Perrin de Brichambaut (Food and Agriculture Organization), who is at present engaged with C. C. Wallén (Sweden) on a comparative study of agro-climates in the winter-rainfall regions, involving the collaboration of the Food and Agriculture Organization, Unesco and World Meteorological Organization, presented a review on these relations. It must in the first place be emphasized that, in underdeveloped countries and remote areas, microclimatology cannot be taken as a basis for plant introduction and that only regional climatology sometimes permits useful comparisons to be made. Distinctions may be drawn on the following basis:

(*a*) The plant explorer who collects wild species and primitive cultivated forms generally goes to primary and secondary centres which are often located in

Announcing

International Edition

ANGEWANDTE CHEMIE

– in English –

Volume I, Number I, January 1962

The *International Edition* of ANGEWANDTE CHEMIE is under the editorial supervision of Dr. W. Foerst, who is also chief editor of the German-language ANGEWANDTE CHEMIE, founded in 1888. This journal has achieved great distinction in chemical literature and has often been chosen as the medium of publication by distinguished scientists, including many Nobel Laureates.

Each number of ANGEWANDTE CHEMIE/*International Edition* contains

- review articles
  from all fields of chemistry written by authorities—
  concise, easy to read, well documented
- selected papers
  from the affiliated journal "Chemie-Ingenieur-Technik"
- communications
  from all fields of chemical research, carefully selected to assure highest standards
- conference reports
  abstracts of lectures given at the most important European chemical meetings, covering unpublished results
- selected abstracts
  reporting the highlights from international chemical literature—
  fast, reliable
- book reviews

chosen from the semimonthly issues of the German edition.

Volume 1, about 600 pages (¾ of the German edition), will consist of 12 issues, to be released monthly.

Subscription price: 107*s*. 6*d*. (*plus postage*)

Please place your order with your subscription agent or with one of the publishers.

A limited supply of sample issues, representing a complete translation of Volume 72, No. 22, November 21, 1960, has been prepared. A detailed brochure is available from one of the publishers:

ACADEMIC PRESS

111 Fifth Avenue
New York 3, New York
U.S.A.

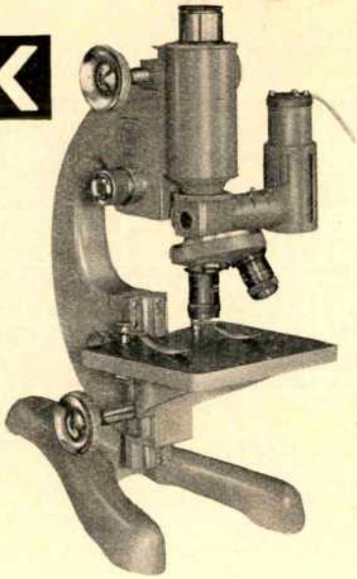
17 Old Queen Street
London, S.W.1
England

VERLAG CHEMIE, G.m.b.H.

Pappelallee 3
Weinheim/Bergstr.
Germany

BECK

Metallurgical Microscope
Model No. 3227



Designed specifically to fulfil the requirements of Technical Colleges.

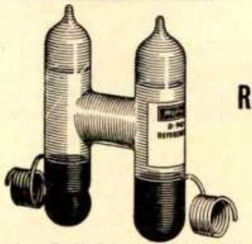
For details of this instrument and others in our range of metallurgical equipment, write for literature (Publication No. C255) to:—

R. & J. BECK, LTD.
69/71 Mortimer St., London, W.1.

STABILITY WITH TIME

For long-term stable D.C. Reference Voltages
use
MUIRHEAD

STANDARD CELLS



REFERENCE CELLS

They are Stable, Reliable, Robust, Repeatable—and the price is right. Saturated or Unsaturated types for industrial or academic purposes
WRITE FOR PUBLICATIONS 425 & 430

MUIRHEAD
Precision Electrical Instruments

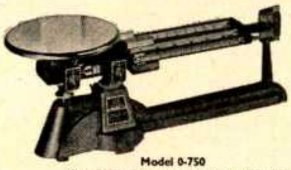
488

MUIRHEAD & CO. LIMITED · BECKENHAM · KENT · ENGLAND
Phone: BEC 4888
MUIRHEAD INSTRUMENTS LIMITED · STRATFORD · ONTARIO · CANADA
Phone: 271-3880
MUIRHEAD INSTRUMENTS INC · 441 LEXINGTON AVE · N.Y.17 · U.S.A.
Phone: Murray Hill 2-8131

WEIGHT on your Mind?

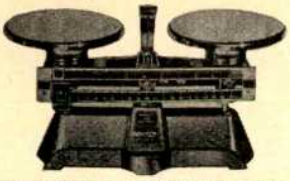
Cent-O-Gram
Cap: 311g.
Sensitivity 0·01g.
Price: £16.16.0d.

OHAUS



Model 0-750
Cap: 2610g. Sensitivity 0·1g. Price: £10 5.0d

Model 0-1119
Cap: 2270g. Sensitivity 1g. Price: £55.10.0d



Model 0-1550 Cap: 2kg. Sensitivity 0·1g. Price £10.17.6d.

Model 0-1910 Cap: 5kg. Sensitivity 0·5g. Price: £49.15.0d.

These world-famous balances, made by the OHAUS Scale Corporation of America, are justly renowned for their outstanding value. They incorporate special, built-in high-quality features, listed below, making them more durable, more accurate and easier to operate.
1. Self-aligning, semi-rigid agate bearings.
2. Dust-proof, self-locking bearing covers.
3. Patented scale-plates with threaded hubs.
4. Elliptical check rods and spindles. 5. Relief-etched stainless steel beams. 6. Hollow-ground steel knife-edges.

Dust-proof, self-locking bearing covers, designed to protect the vital agate bearings from dirt and dust, help to preserve accuracy and sensitivity.

No. 2 of a series of advertisements highlighting special features of Ohaus balances.

For full details write your name and address in the margin of this advertisement and send it to—

SHANDON **SHANDON SCIENTIFIC COMPANY LTD.**
6, Cromwell Place, London, S.W.7. Tel. KNIghtsbridge 1131

GD795

highly dissected topography, creating a mosaic of contrasting environments and thus geographical and genetic isolation in Vavilov's mountain-and-valley regions nearest the equator, but in which microclimatic work is neither feasible nor of value. It is, however, always desirable to have information on the regional climate which determines a number of biological characters.

(*b*) When land-races composed of many related forms are to be introduced, general information on the climate of the region in which they are grown should suffice, since these varieties are generally adapted to a fairly wide range of conditions.

(*c*) Climatic comparisons appear to be promising for introduction of improved cultivars and of ecotypes, and should make it possible to avoid certain errors. A good knowledge of the climate of the site and region where a cultivar is to be found and a study of the response of that variety to certain climatic extremes should indicate where it is likely to be climatically adapted. Introduction nurseries are, however, still essential in the different climatic zones of the importing country.

(*d*) The introduction of highly bred cultivars cannot be based exclusively on the comparability of regional climates. Only microclimatic investigations combined with data on soil and agronomics can, according to Mr. Perrin de Brichambaut, indicate the areas of adaptation of these cultivars.

The general climatic classifications now in use do not make it possible to delimit the regions to which a plant might be climatically adapted. For the interchange of plant material, it is therefore necessary to study all climatic factors in relation to the growing season and climatic thresholds of crop plants, extreme values, and so on. The more progress is made in climatology, the more useful will climatic comparisons become for the purposes of plant introduction.

One of the outstanding conclusions of the meeting was that the precious gene resources of the world, represented more particularly in the wild species and primitive forms in and around and between the centres of diversity, are in great and increasing peril. At a time when the breeders of many crops are suffering from what Prof. P. N. Zhukovsky of Leningrad has called 'gene starvation', the centres of maximum diversity are in many cases being destroyed at an increasing rate, by continuing misuse of land or by modern 'development'. This is more serious in the Middle Eastern centres of diversity, where the natural vegetation has suffered from intensive pressure of human and livestock populations for millenia, than in the South American centres, for example. Yet in both of these and in other important centres of diversity there is an urgent need for the establishment of protected areas of natural vegetation in genetically strategic sites, and Governments in and around centres of diversity are to be requested to take the necessary action in that respect. These protected areas would have to be managed in a special way to prevent desirable species from becoming eliminated through ecological progression. Their establishment was strongly supported by a number of participants, including Dr. P. S. Hudson, speaking on behalf of the Nature Conservancy.

There is also an urgent need to conserve the primitive cultivated forms and local varieties or agro-ecotypes in and around regions of greatest diversity. Old cultivated crops have developed a great number of varieties that are well adapted to local conditions but are often restricted in their area of cultivation; they are well fitted to primitive agriculture, but often do not respond to improved conditions of cultivation. Although they are of great value as a basis for breeding, they tend to disappear with the rapid dissemination of the improved varieties, which often have a very narrow genetical basis, for example, wheat in Australia and Sweden, maize in the United States, *Digitaria decumbens* or Pangola grass in the West Indies. What should and can be done to save these valuable gene resources, to assemble and maintain collections, to examine them and make the material available to breeders everywhere?

The main outcome of the meeting, apart from sixteen recommendations directed to the Food and Agriculture Organization or its member countries, was the preparation of a blueprint for further action, with regard to (*a*) wild species, (*b*) primitive cultivated forms and land races, and (*c*) cultivars improved by selection, hybridization or other breeding methods. For their collection, distribution, maintenance, further study and interchange, it was agreed that the following structure would be a desirable long-term objective:

Plant Exploration Stations. The need to establish outstations of this type in, or reasonably near to, the centres of greatest diversity has been stressed for some years by Dr. R. O. Whyte (F.A.O.) and Prof. W. Rudorf (Germany). It is visualized that these would be national or international stations, and that priority would be given to those proposed at the meeting, namely, a Mediterranean and Middle Eastern station at Izmir, Turkey, a South American station in northern Argentina with possible sub-stations in the high Andes, and a station in Peshawar, West Pakistan, for the Hindu Kush and Western Himalayan region. The scientific staff of these stations would need to have a thorough knowledge of everything from the flora and vegetation to the travelling facilities in their regions, and would make collecting expeditions on behalf of inquirers or as members of special expeditions. The work would be linked to and would largely depend on a network of areas of conserved and appropriately managed natural vegetation, and on the availability within the region of adequate and continuing resources of primitive cultivated forms for collection, study and initial multiplication. The fact that this would be done in or near the centres of greatest diversity would ensure that the collections express the complete range of variability before being distributed to appropriate plant introduction stations with environments that might restrict that expression.

Plant Introduction Stations. These would be established in receiving countries, either on a regional basis where expedient or on a national basis, and would be responsible for groups of plants or individual crop plants. They would undertake the receipt, study and maintenance (and application of quarantine standards) of material from the plant exploration stations and independent expeditions, the assembly and maintenance of local collections of wild species and primitive cultivated forms, and the interchange of cultivars. They might also be responsible for maintenance of discarded but still potentially useful breeders' lines.

Maintenance of Living Collections. This would be the responsibility not only of the plant introduction stations but also of research institutes, botanic gardens, research foundations and similar centres.

Research and Education. This would involve the establishment of a research department in a university, and also the provision of practical training at plant introduction stations or similar centres.

Co-ordinating Bureau. The Food and Agriculture Organization was asked to consider the establishment of such a Bureau, which might be staffed by at least three specialist officers responsible for co-ordination and development with regard to all important food, horticultural, pasture and fodder, and industrial crops, in temperate and tropical latitudes. It was recommended that the publication of the *Plant Introduction Newsletter* should continue as a method of reporting on current developments.

The mimeographed report of this meeting will be available in English, French and Spanish in about three months' time, and it is hoped to publish the complete Proceedings, comprising some forty-five technical papers, in a special issue of the Italian journal, *Genetica Agraria*. R. O. WHYTE

X-RAY DIFFRACTION STUDIES OF BACTERIAL VIRUSES

By DR. A. C. T. NORTH and PROF. ALEXANDER RICH

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

BACTERIAL viruses are widely used for genetic studies, and a great deal is known about their composition and gross structure. They have polyhedral heads which consist of a protein coat enclosing a volume which contains deoxyribonucleic acid (DNA). Attached to the head is a tail structure which varies considerably in size among different viruses. Electron microscopic studies have yielded much information about the external morphology of these viruses, but not very much is known of their internal structure, especially the mode of organization of the DNA. This has prompted us to carry out X-ray diffraction work in an attempt to learn something about the internal configuration of the virus.

We have chosen for our initial investigation two morphologically extreme types, the *T*2 virus which has a very substantial tail structure, and the *T*7 virus which has a very small tail. From the *T*2 virus we have been able to obtain oriented X-ray diffraction fibre patterns, and we have been successful in crystallizing the *T*7 virus and have initiated an X-ray diffraction study of the crystalline preparations.

In the *T*2 bacteriophage, the polyhedral head has a length of approximately 1000 Å. and a hexagonal cross-section with a width of 700 Å. Its tail, of length 1100 Å., carries a number of fibres attached to the end. This bacteriophage is known to consist of about 50 per cent protein and 50 per cent DNA. Recent work has shown that all the DNA in *T*2 phage is contained in a single continuous molecule[1,2], which would have a length of approximately 60μ if it were stretched out, or 600 times the length of the virus head; thus it is apparent that the DNA is considerably folded. DNA from *T*2 contains hydroxymethylcytosine instead of the cytosine that is normally found in DNA, and glucose molecules are attached to almost 70 per cent of the hydroxymethylcytosine bases. Polyamines have been found in the virus head[3], probably attached to the DNA. In contrast to *T*2, the *T*7 virus has a smaller polyhedral head measuring about 550 Å in width and somewhat less in length. The tail is extremely short, about 140 Å. Its DNA contains cytosine and no glucose.

Recently a report has appeared by Bendet, Goldstein and Lauffer[4], who have presented birefringence evidence which suggests that some of, or perhaps all, the DNA in the *T*2 virus is organized with its molecular axis parallel to the viral tail, since the virus preparations have a negative birefringence on streaking or in flow birefringence experiments. Although the head of the virus is highly symmetrical, the possibility of orienting the preparations in these experiments arises presumably from the great asymmetry introduced by the elongated tail structure.

Oriented Virus Preparations

We have attempted to obtain oriented *T*2 phage specimens for X-ray diffraction studies by two methods. First, material from a centrifuge pellet was slowly sucked into thin-walled capillary tubes in the hope that the velocity gradient would be sufficient to orient the particles and thereby form an ordered gel in a fashion analogous to that found in the case of the rod-shaped tobacco mosaic virus[5].

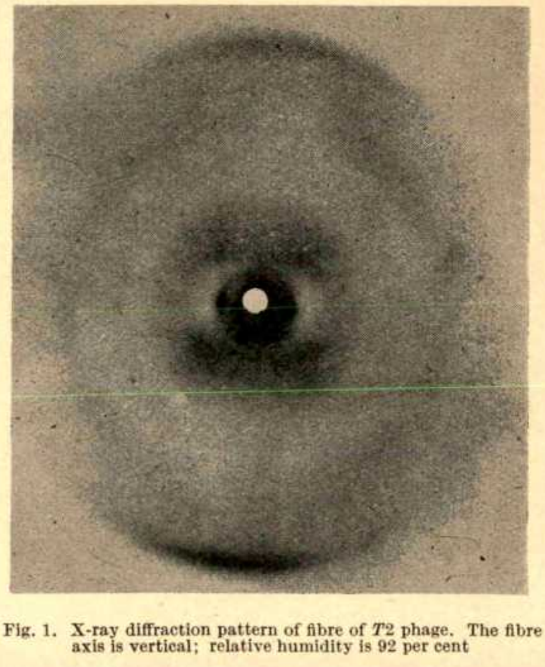


Fig. 1. X-ray diffraction pattern of fibre of *T*2 phage. The fibre axis is vertical; relative humidity is 92 per cent

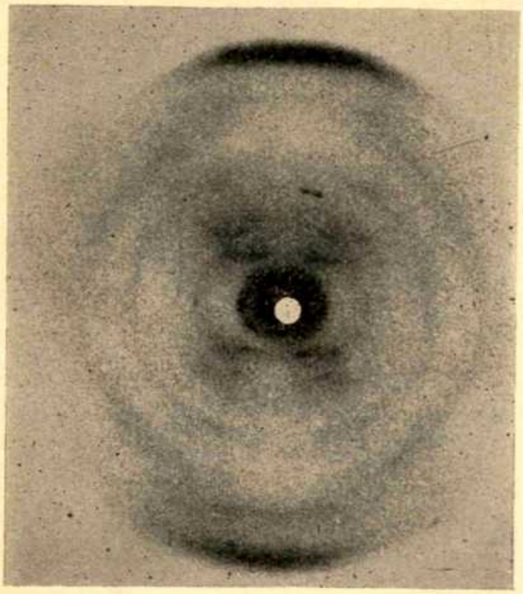


Fig. 2. X-ray diffraction pattern of DNA extracted from $T2$ phage. The fibre axis is vertical; relative humidity is 75 per cent

However, although birefringent, these preparations have not had sufficiently high orientation to produce an oriented X-ray diffraction pattern. We therefore tried to obtain oriented specimens by drawing fibres from a gel of the $T2$ phage.

There is an important difficulty in these experiments, since it is very easy to break open the phage head and liberate the DNA. Great care was taken to avoid this. Fibres were prepared by dipping a fine needle or glass rod into the virus gel, and slowly withdrawing it. It was found comparatively difficult to draw fibres from fresh centrifuge pellets and very much easier to prepare them from pellets which had been manipulated extensively. This was taken to indicate that DNA is released on working the material and, conversely, that the fresh pellets which did not yield fibres readily probably did not contain much free DNA. Therefore, we used only fibres prepared from fresh pellets in our experiments. Deoxyribonuclease was routinely added to all preparations so that DNA released from any ruptured phage would be digested during the centrifugation; however, the deoxyribonuclease could not be expected to digest DNA released in the course of drawing the fibres from the gel. To be more certain that we were actually photographing phage rather than liberated DNA, the effects on the fibres of variations in relative humidity was investigated, as described here.

The virus fibres were negatively birefringent, although the birefringence was not as strongly negative as that of $T2$ DNA alone. Fibres of diameter 50μ were mounted in a microcamera and photographed on a Hilger microfocus X-ray tube using copper-$K\alpha$ radiation. A typical X-ray diffraction pattern is shown in Fig. 1. It can be seen that the pattern is very similar to that of imperfectly oriented DNA. There is a strong 3·4-Å. meridional reflexion and a series of layer lines the spacings of which are sub-multiples of 33·8 Å. The cross-like distribution of diffracted intensity through the centre of the pattern is characteristic of a helical molecule[6]. The first layer line is quite weak, which is in marked contrast to the results obtained from DNA-protamine complexes, in which the protein chain fills the narrow groove of the DNA molecule[7]. The second and third layer lines are strong, while the fourth is absent. On the equator is a strong reflexion near the centre, which arises from the hexagonal packing of the DNA molecules.

For comparative purposes, DNA was prepared from $T2$ phage by osmotic shock. No dialysis was used in the preparation in order to minimize the risk of detaching the polyamines from the DNA. Fig. 2 shows a diffraction pattern given by a fibre prepared from this material. This pattern is similar to that reported earlier by Wilkins and his collaborators for DNA from $T2$ (ref. 8). It is difficult to compare the pattern from DNA with that from the phage preparation, since the orientation in the latter is not so good. On the whole, the two diagrams are similar, which suggests that the protein component represents only a small part of the X-ray scattering power of the virus. Careful scrutiny of the two photographs suggests that there may be some difference in the distribution of intensity along the second and third layer lines; in the pattern of the isolated DNA, the maximum intensity along the layer lines is nearer the meridian than in the phage pattern. This could be due to slight differences in packing of the DNA molecules inside the phage head as compared with the reconstituted assembly of free DNA. However, it is difficult to assess this in view of the difference in degree of orientation in the two fibres.

It is of interest that the phage fibres, as well as the DNA from $T2$ (ref. 8), yield only a B type diffraction pattern[9] at all relative humidities. This is in marked contrast to most preparations of DNA, and the difference may be associated with the attached glucose residues.

We have examined the change in the position of the equatorial reflexion as a function of relative humidity in both the DNA from $T2$ and in the phage fibre. This was done as a means of demonstrating that we were not photographing DNA liberated from broken phage heads. The relative humidity was stabilized by filling the camera with helium gas, which had been bubbled through washing columns filled with a saturated salt solution. The specimen was usually allowed to equilibrate for several hours before the X-rays were turned on. The results are presented in Fig. 3 for both DNA from $T2$ and the phage fibres. The two curves are similar until the relative humidity is raised to more than 90 per cent. Above this the

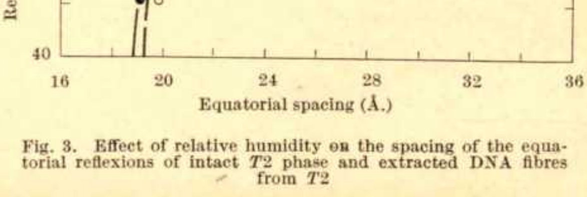


Fig. 3. Effect of relative humidity on the spacing of the equatorial reflexions of intact $T2$ phase and extracted DNA fibres from $T2$

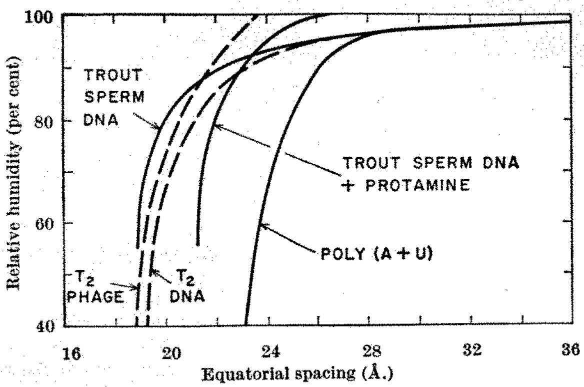


Fig. 4. Effect of relative humidity on the spacing of the equatorial reflexions in various nucleic acids. Poly (A + U) is polyadenylic acid plus polyuridylic acid

DNA molecules move further apart as more water molecules surround them and, approaching 100 per cent relative humidity, the free DNA molecules are very far apart in solution. In contrast to this, in the phage head at relative humidities greater than 90 per cent, the DNA molecules stay close together, constrained presumably by the protein coat of the head, although the polyamines also may play a part in holding the DNA together. At 100 per cent relative humidity the molecules are confined so that they produce a reflexion at 23·8 Å.

Similar work on the effect of changes in relative humidity have been reported for trout sperm DNA and DNA-protamine complexes[7], and for a two-stranded helical synthetic polyribonucleotide, polyadenylic acid plus polyuridylic acid[10]. All these curves are shown in Fig. 4, where it is clearly seen that the phage and the DNA-protamine curves differ markedly from the others. Neither the phage nor the protamine complex goes into solution in water (100 per cent relative humidity); however, the DNA molecules are further apart in the protamine complex than in the phage, presumably because of the interposed protein. The isolated DNA molecules from *T*2 may be slightly further apart than the trout sperm DNA molecules because of the glucose attached to the hydroxymethylcytosine bases. The synthetic polyribonucleotide curve in Fig. 4 is displaced to a greater spacing relative to the DNA curves probably because the additional hydroxyl group in the backbone makes the helical chains assume a slightly larger radius of curvature.

These experiments suggest, but do not prove, that the phage fibre actually contains intact virus rather than ruptured heads and released DNA. It is very difficult, however, to be certain that we have not disrupted the virus head in preparing the specimens. It is clear that virus particles can be oriented in a gel; this has been demonstrated in electron microscope studies of replicas from oriented gel preparations[4]. But since the virus diffraction pattern is similar to that obtained from isolated DNA, we have to be cautious in the interpretation and say that our evidence for orientation of the DNA in the virus head is suggestive but not conclusive. In particular, since X-ray diffraction is enhanced from regions of regularity, we cannot say what proportion of the DNA is oriented parallel to the tail. It is obvious that all the DNA cannot be oriented this way since it must be folded to be contained within the head.

Unoriented Virus Gels

The intense X-ray reflexion on the equator of the oriented virus pattern has its origin in the lateral hexagonal packing of DNA molecules. If it were not for this regular packing, we should see a streak along the equator, which would represent the continuous Fourier transform of the molecule. This reflexion can also be seen in X-ray photographs of unoriented gel specimens, where it appears as a Debye–Scherrer powder ring at 24 Å. The presence of this ring indicates that the DNA molecules are packed parallel to each other inside the virus. We have observed this powder ring not only in *T*2 gel preparations but also in specimens of *T*7 and λ bacteriophages, which are not sufficiently asymmetrical to be oriented in fibres in the same way as *T*2. These observations have been made with the phage in suspension in the presence of DNase, so that there can be no liberated DNA present. We have also taken X-ray photographs of gel preparations of DNA molecules extracted from *T*2 phage; the sharp 24-Å. ring is not observed, which shows that the DNA molecules are dispersed in this case.

We can estimate the average lateral extent of the regions of regular packing of DNA molecules by measuring the breadth of the 24-Å. diffraction line in the unoriented gel preparations. In order to make careful measurements of X-ray line broadening, it is desirable to have an experimental arrangement in which the instrumental broadening is minimized. We have accomplished this through the use of a focusing monochromator, in which the X-ray beam converges to a line focus after reflexion from a curved quartz crystal. This produces a particularly clean X-ray diffraction pattern through the elimination of wave-lengths other than the copper-$K\alpha_1$. The intensity distribution in the diffraction pattern was measured by tracing the blackening on the photographic film with a Joyce Loebl

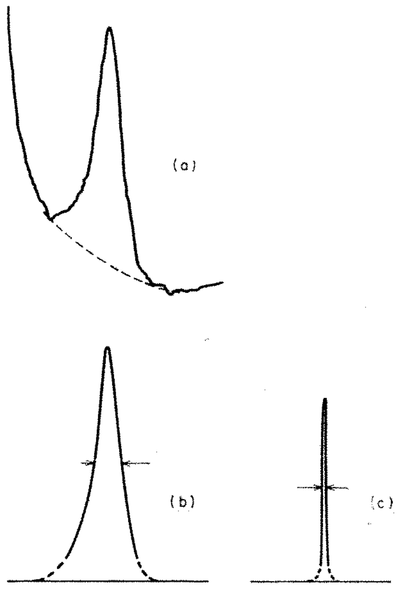


Fig. 5. (*a*) Densitometer tracing of the profile of the 24-Å. ring of *T*2 bacteriophage. Intensity is plotted vertically, the horizontal axis is a radius of the X-ray diffraction pattern. (*b*) Profile after subtraction of the background (dashed line in (*a*)). The arrows indicate the angular width at half maximum (β_0). (*c*) Profile of the direct beam to the same scale. The arrows indicate β_1

recording microdensitometer. A typical curve is shown in Fig. 5, where the peak (*5a*) due to the ring is superimposed on a varying background. The line profile (*5b*) was then constructed by subtracting the estimated background value (shown dashed) from the corresponding peak value. The instrumental broadening is shown in Fig. *5c*, which contains a tracing of the direct beam. Theory shows that the breadth of the angular diffraction line, β_d, is related to the appropriate linear dimension D of the diffracting crystallite by the expression $\beta_d = k\lambda/D \cos\theta$, where θ is the Bragg angle and k is a numerical constant of the order of 0·9 (ref. 11). β_d is defined as the angular width at half peak-height. The observed line-breadth, β_0, in practice arises not only from the diffraction line-breadth but also from instrumental broadening, β_i, the magnitude of which depends on the geometry of the X-ray apparatus. For the monochromator, the use of a converging beam makes β_i very small, and it can be measured by a tracing of the direct beam. Although there are several methods for obtaining β_d from β_0 and β_i, probably the most valid in this case is $\beta_0^2 = \beta_i^2 + \beta_d^2$, which is based on the approximation that the diffraction broadening and instrumental broadening curves are both Gaussian[12]. We think this is a reasonable assumption in the present case (Fig. 5).

Measurements were carried out both for *T*2 and for *T*7 phage. The size of the crystalline domain for *T*2 was 143 ± 15 Å. as measured on several different preparations. In *T*7 the size of domain of the DNA was 119 ± 10 Å.

These sizes of domain are considerably smaller than the diameters of the corresponding phage heads. There are two possible explanations for this finding: one is that the virus head consists of a number of sub-units, each containing 25–30 sections of the DNA chain in a highly ordered array; the second is that the dimensions do not indicate the sizes of separate domains, but that the line-broadening arises from imperfections in the regularity of a single array which fills the entire head. For example, such irregularity might arise from a non-random distribution of polyamines or other substances within the phage head. However, the interpretation of line-broadening in terms of domains is well known in both crystals and fibres, and we believe it is the most probable interpretation here. The difference in size of domain between *T*2 and *T*7 is sufficiently great for us to inquire whether it depends on the size of the viral head. In order to search for such a dependence, further investigations are under way involving a wide variety of bacterial viruses.

At this early stage of the investigation, there are many unanswered questions concerning the organization of the DNA. If the domains are separate units, are they held together by the polyamines which are associated with the phage DNA ? Could the association of these ordered domains give rise to the hexagonal cross-section which is so characteristic of viral heads ? Further: Is this sub-structure of organization important to the folding process which the DNA molecule must undergo to form the virus head when it is made inside bacteria ? We cannot answer these questions at the present time.

Crystallization of Bacteriophage

In the course of taking X-ray photographs with the monochromator camera, we observed a crystalline diffraction pattern from the *T*7 virus. The sample had been prepared by gently sucking the virus gel into a thin-walled quartz capillary, which was left unsealed for a considerable period, during which time the sample evaporated to about half its original volume. There was a change in the birefringence observed in the capillary during this process. The capillary was then sealed, and photographed in the monochromator. The diffraction photograph showed a series of crystalline reflexions which were superimposed on the continuous powder pattern produced by the unoriented gel. An array of well-resolved reflexions could be seen around the beam stop with a prominent repeat distance of 570 ± 20 Å. in one direction, and 470 ± 30 Å. at right angles to it. The resolution was best along the 570-Å. axis because this lay perpendicular to the focal line of the converging X-ray beam. In this direction about 15 orders were visible. In the region of the 24-Å. ring, a series of striations could be seen with the 570-Å. spacing, showing that the Fourier transform had been sampled in that region by a larger lattice.

Up to the present time we have not observed such crystallization with the *T*2 virus. It is of interest that in contrast to *T*2, the *T*7 virus has an extremely short tail and is therefore much closer in shape to the 'spherical' viruses which crystallize with relative ease. Furthermore, one of the observed spacings is quite close to the diameter of the *T*7 virus as observed in the electron microscope, so it is possible that the crystalline organization is a simple one. We are now preparing other crystalline specimens in an attempt to get three-dimensional information about the packing of the *T*7 virus and its enclosed DNA.

Conclusions

These experiments suggest that some of the DNA inside the bacteriophage *T*2 virus head is oriented parallel to the tail. Furthermore, the evidence suggests that the DNA may be organized into crystalline domains about 140 Å. across in *T*2 and 120 Å. in *T*7. The *T*7 virus can be crystallized, and this should prove to be very useful in furthering the study of the internal organization of the bacteriophage.

We wish to thank Drs. P. F. Davison, D. Freifelder and R. Hamers for assistance in obtaining virus preparations. This investigation was aided by grants from the National Institutes of Health, Bethesda, Maryland, and the U.S. National Science Foundation.

[1] Davison, P. F., Freifelder, D., Hede, R., and Levinthal, C., *Proc. U.S. Nat. Acad. Sci.* (in the press).

[2] Rubenstein, I., Thomas, C. A., and Hershey, A. D., *Proc. U.S. Nat. Acad. Sci.* (in the press).

[3] Ames, B. N., Dubin, D. T., and Rosenthal, S. M., *Science*, **127**, 814 (1958).

[4] Bendet, I. J., Goldstein, D. A., and Lauffer, M. A., *Nature*, **187**, 781 (1960).

[5] Bernal, J. D., and Faukuchen, I., *J. Gen. Physiol.*, **25**, 111 (1941).

[6] Cochran, W., Crick, F. H. C., and Vand, V., *Acta Cryst.*, **5**, 581 (1952).

[7] Feughelman, M., Langridge, R., Seeds, W. E., Stokes, A. R., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F., Barclay, R. K., and Hamilton, L. D., *Nature*, **175**, 843 (1955).

[8] Hamilton, L. D., Barclay, R. K., Wilkins, M. H. F., Brown, G. L., Wilson, H. R., Marvin, D. A., Ephrussi-Taylor, H., and Simmons, N. S., *J. Biophys. Biochem. Cytol.*, **5**, 397 (1959).

[9] Franklin, R. E., and Gosling, R. G., *Nature*, **171**, 740 (1953).

[10] Rich, A., in *The Chemical Basis of Heredity*, edit. by McElroy, W. D., and Glass, B., 557 (Johns Hopkins Press, Baltimore, 1957).

[11] See, for example, *X-ray Diffraction Procedures*, by Klug, H. P., and Alexander, L. E., chap. 9 (Wiley and Sons, New York, 1954).

[12] Warren, B. E., *J. App. Phys.*, **12**, 375 (1941).

OBITUARIES

L'Abbé H. Breuil

The Abbé Henri Breuil died on August 14 at the age of eighty-four. I first met Breuil in 1912 in Cambridge. At that time, except for the interest of a few amateurs and geologists, the subject of prehistory scarcely existed in Britain. It is true that in 1859 Prestwich and Evans had been sent over to France by the Royal Society to study the work of Boucher de Perthes in the gravels near Abbeville, and as a result had accepted the fact of the great antiquity of man on the Earth. But whereas in France interest in prehistory was abounding, there was only a handful of people in Britain who were keen about it. Breuil invited me to go abroad for a year or two and, after assisting at the famous cave excavation at Castillo in northern Spain under his colleague and great friend, Hugo Obermaier, to work with him in Spain. Thus, after a 'campaign' with Obermaier—another Roman Catholic priest, by the way, though few people seem to have known it—I joined Breuil and with him studied and traced the eastern Spanish and the Copper Age rock-shelter paintings of the Peninsula.

The Abbé was at this time thirty-six years of age and then, as always, of an electric character. Travelling for months together, often 'sleeping rough', makes either for enduring friendship or the opposite: my admiration for Breuil both as a prehistorian and as a man never flagged. He had only one really useful eye, but with it he saw far more clearly than did other more normally equipped persons, and his intuitions were brilliant. The working combination of Obermaier and Breuil was perfect. Both had by now become professors of the Institute of Human Palæontology founded by the then Prince of Monaco in Paris. Breuil would see the answer to a problem in a flash; Obermaier, a Bavarian born, built up his solution with careful Teutonic thoroughness and documentation; but almost always they both came to the same conclusions. If you told Breuil a fact he would always remember it; Obermaier would note it down on a special card, of which he kept a supply in his pocket, and would later place it in his card index. Breuil was an artist, but a scientifically accurate one. It has been sometimes suggested that he used too much imagination when copying the cave art, but it is not always realized that there is a close similarity between examples of paintings belonging to the same style, and long experience had enabled him to interpret correctly what were sometimes apparently only faint indications. I can vouch for the meticulous accuracy of the tracings he made during the times I was his assistant.

At the beginning of the First World War we were together in the French Red Cross at a hospital near Bordeaux run by the late Dr. Lalanne. At that time there was not much 'war' work to do, and we spent most of our energies in typing and arranging the immense collections of stone implements which had been excavated by Dr. Lalanne at Laussel, and one could not fail to admire his scientific quickness of eye and his tirelessness in tackling this problem.

After the War, Breuil came often to England and frequently stayed with us at Cambridge, and the University awarded him an honorary doctorate of science. It must have been in about 1921 that I asked him to come over and look at some new tertiary finds of *soi disant* flint tools which the late Reid Moir had unearthed from the Red Crag near Ipswich. He came over, and spent the afternoon somewhat silently examining the finds. In the evening, as I drove him back to our hotel, he turned suddenly towards me and said: "My dear friend to-day has greatly aged humanity". It was a magic moment and a characteristic remark.

From what has been already said, it will be clear that his prehistoric interests were by no means confined to problems of palæolithic art, nor only to the upper palæolithic periods in general. Of course, *Les subdivisions du paléolithique supérieur et leur signification*, published in 1912, remains a classic, as do the series of great cave-art monographs published under the auspices of the Prince of Monaco, but so too does his work on the gravels of the Somme Valley of lower and middle palæolithic ages.

Between the two Wars the Abbé was elected to a professorship at the Collège de France—an honour in which he took a very great pride.

During the Second World War, Breuil did important work on river terraces in Portugal before he went to South Africa. He had stayed in France as long as he could, but both his age and his eyesight precluded him from much active war-work—he did, however, discover a secret underground enemy aeroplane factory and wrote to me on a postcard some lines in Latin about strange subterranean winged birds, purporting to come from a newly discovered MS. of Ovid and labelled "Quelque vers pour vos latinistes". It was duly forwarded to the proper authority in London and, later, I had an acknowledgment asking for more such verses.

The bare facts of Breuil's life have been well set out in the obituary in *The Times* of August 22. But his impact on the study of prehistory throughout the world can scarcely be exaggerated from the time long ago when he started with a study of the Bronze Age in the Paris basin to his later work, much of it in Africa. He inspired, instigated or personally undertook research in an immense number of parts of the Old World and correlated and assimilated the results—you had to go to the chilly cellars of the "Institute" to study the finds from the *Sinanthropus* site of Choukoutien. China, Abyssinia and South and South-West Africa were some of the regions included in his own travels. Thus, his knowledge of world prehistory became immense, and when he spoke at conferences there was little more to be said. He was, it is true, inclined to be a little pontifical—and, after all, after Obermaier's death in the Second World War, there were fewer people equipped to dispute with him—and when he had pronounced about something he had indeed pronounced, and nothing would make him change his mind. As he got older and perhaps less sure of touch in his intuitions—his opinions about Rouffignac and the 'white lady' of South-West Africa were decidedly open to question—he did not become less pontifical and it became more personally difficult to disagree with him. He knew that in the past he had so often been right. He was indeed one of the world's few really great prehistorians, and it is the world, as well as France, that has suffered a loss by his death.

MILES BURKITT

Prof. Enzo Boeri

Prof. Enzo Boeri died on October 28, 1960, at the age of forty-six. He was associated with the Institute of Physiology of the University of Milan in various capacities from 1938 until 1955 and was appointed professor and chairman of the Department of Human Physiology of the Medical School, University of Ferrara, in 1955. In 1958 he also held the post of director of a group in the Biology Division of the Italian Nuclear Research Centre.

During the War his initial studies were on underwater physiology and he was active as a 'frogman'. His more important role was as a leader in the Resistance against the Nazis during 1944–45. He served bravely in great personal danger and under most difficult conditions : he was on one occasion saved from the firing squad by a two-hour margin. His radio contacts with the Allies were most valuable and were recognized by military decorations, including a Bronze Star from the United States.

Even though he had open to him a clear pathway to leadership in the post-war era, his love of science took him back to Naples and to the study of the vanadium compounds of *Phallusia mamillata*. During 1951–53 he undertook biochemical studies with Prof. H. Theorell at Stockholm. There he carried out experiments on the magnetic susceptibility of the vanadium compounds and studied enzymic properties of cytochrome *c* and catalase, particularly the reaction of the latter with thiol compounds. The next topic of research was cytochrome b_2, the flavohæmoprotein prepared from yeast. He made an intensive study of the method of preparing this enzyme from the kinetic point of view. He made important contributions to the mechanism of its action, particularly the stoichiometry of the lactate-flavin reaction and the function of the cytochrome portion in electron transfer from lactate to ferricyanide or cytochrome *c*. A portion of the latter work was carried out as an associate at the Johnson Research Foundation, University of Pennsylvania. Most recently he studied the properties of hæmerythrin.

Boeri's scientific work was characterized by high regard for precision, an open mind in the interpretation of his results and an understanding of the physiological importance of the biochemical data. He had soon established an international reputation as a physical biochemist. As a man, he was endowed with the rich gifts of his people. He was brave and unselfish, having a keen sense of fairness and a great interest in the problems of others. He was articulate —an irresistible conversationalist, and was forthright and often outspoken in his defence of what he believed right—in some cases in opposition to contemporary science in Italy. Therefore, he often embraced unpopular objectives and was a particularly strong crusader in the task of injecting new interests and life into an Italian science that he found to be resting too much on a glorious historical tradition.

He saw the urgent need for the re-introduction of experiments into the physiology course, in reviving the possibilities for foreigners and apostate priests to be eligible for professorial appointments in Italy and in providing adequate funds for the support of departmental chairmen and their research.

Prof. Boeri's example as a man and as a scientist serves as a landmark in his humble yet fearless dedication to high and worthy principles. His early passing leaves many goals unattained, but his ideas and his example clearly survive him.

Britton Chance

NEWS and VIEWS

Statistics in the University of London : Prof. James Durbin

Mr. James Durbin has been appointed to the University chair of statistics at the London School of Economics and Political Science, in succession to Prof. M. G. Kendall (see *Nature*, 190, 1060; 1961). Prof. Durbin read mathematics at St. John's College, Cambridge, and after a period of military service during the War with the Army Operational Research Group, returned to Cambridge, where he took the postgraduate diploma in mathematical statistics, with a special field of application in economics. After a short period with the British Boot, Shoe and Allied Trades Research Association and as a junior research officer in the Department of Applied Economics at Cambridge, he joined the staff of the London School of Economics in January 1950, being appointed a reader in 1953. Prof. Durbin has worked in both theoretical and applied statistics. His major contributions in the former cover time series analysis, regression analysis and social survey methodology. In the practical field he has made equally notable contributions to econometrics, social survey research and model building. He has also been closely associated with editorial work in the University of London, as a member of various committees of the Royal Statistical Society, as an associate editor of *Biometrika*, and as a Fellow of the International Statistical Institute. He has lectured in a number of foreign centres, including Calcutta, Beirut, North Carolina, Stanford University, Uppsala and Copenhagen.

Royal Society of Victoria Research Medal : Dr. George Baker

The first award of the Research Medal of the Royal Society of Victoria has been made to Dr. George Baker of the Commonwealth Scientific and Industrial Research Organization. The citation accompanying the award states that Dr. Baker "has published approximately a hundred original research papers on geological and mineralogical topics, including: (1) the geology of the Port Campbell coast; (2) phytoliths, the minute opaline bodies in plants; (3) the nature and origin of the small glassy meteorites called australites. Dr. Baker originated the theory of aerodynamic control of the shaping and sculpturing of the primary form of australites. Experiments by the American National Aeronautics and Space Administration have proved this theory correct. Australites throw light on the nature of re-entry phenomena of ballistic missiles, and indicate an excellent material for heat shields for manned spacecraft".

Verco Medal of the Royal Society of South Australia : Dr. R. L. Specht

The Verco Medal of the Royal Society of South Australia has been presented to Dr. R. L. Specht, who is distinguished for his contributions in the field of plant ecology, particularly in Arnhem Land and in South Australia. The citation continues: "In the first-named area his work has provided essentially the first critical study of an important area of Australia. In South Australia he has contributed especially to the study of the detailed edaphic and microclimatic factors which determine the nature of the plant associations and the distribution of the dominant species. He has taken full advantage of the recent knowledge gained in geological surveys, in soil surveys and in rainfall studies, particularly in the Mount Lofty Ranges, to establish detailed edaphic and climatic correlations with native vegetation and so has built soundly on the early pioneering work of Osborn and of Wood".

New National Nature Reserves:

Shapwick Heath

THE Nature Conservancy recently declared Shapwick Heath as a National Nature Reserve. This Reserve contains some of the last remnants of the extensive raised bogs, about 15 ft. above sea-level, which once stretched from Glastonbury to within a few miles of the coast. These raised bogs were similar to those now covering a large part of the Central Irish Plain and were gently domed in shape and dominated by communities of *Sphagnum* moss, heather and cotton grass. The Reserve is set up under Nature Reserve Agreements with the Eclipse Peat Co. and the Eclipse Land Co. and covers 484 acres. Extensive peat-cutting has turned the area into a complex patchwork of marsh and scrub woodland. The peat is used mainly for horticultural purposes, and under the Nature Reserve Agreement the Eclipse Peat Co. will continue to extract it. There is a wide range of flora and fauna because of the diverse habitats produced by peat-cutting and because of variations of acidity. For example, heather and bog asphodel are characteristic of one part of the area and a species of sedge, *Cladium mariscus*, of another. The rare royal fern is also present. Peat diggings have uncovered many archæological remains of neolithic, bronze age, iron age and Romano-British date, and Shapwick Heath has been the centre of these discoveries. The area was crossed by a number of timber trackways built towards the end of the bronze age to form a passage between the Polden Hills and the 'islands' in the valley when the climate grew wetter. Blade marks, the size and shape of which are typical of those made by late bronze age axes, can be seen on the larger timbers in several trackways. Within a mile or two of the Reserve neolithic trackways have likewise been identified. Access to the Reserve is by permit only. Applications to visit and for permission to collect specimens of animals and plants should be made to the Regional Officer for the South-West, the Nature Conservancy, Furzebrook Research Station, Wareham, Dorset.

Wye and Crundale Downs

Wye and Crundale Downs have also been declared as a National Nature Reserve. This Reserve forms part of the escarpment of the North Downs between Ashford and Canterbury. It is the first area of chalk downland to be declared as a National Nature Reserve in Kent and adds to the incomplete series stretching from Wiltshire and Hampshire to Bedfordshire and Sussex. To-day the chalk downlands are particularly vulnerable to destruction because of increased mechanization of farming and it is urgent that the Nature Conservancy should acquire further chalk areas to preserve them for study. The present acquisition consists of 123 acres of chalk downland, scrub and mature woodland. The downland is basically a product of sheep-grazing which was practised as early as the Roman occupation, and became such an important feature in the English economy during the fourteenth and fifteenth centuries. The scrub and woodland are the result, at least in part, of the natural colonization of the downland by woody species (such as hawthorn and beech) and clearly demonstrate the necessity for grazing in order to maintain the downland character. The Reserve has a rich flora including the lady orchis, man orchis and fly orchis. One of the many notable insects is the feathered ear moth. The Devil's Kneading Trough, a natural steep-sided valley, cuts into the Downs on the Reserve, forming one of its most conspicuous features. On the crest of the Downs are several tumuli which indicate the antiquity of human influence in this type of country. Access to the Reserve along the public footpath is unchanged; elsewhere it is by permit only. Applications to visit and for permission to collect specimens of animals and plants should be made to the Regional Officer for the South-East, the Nature Conservancy, 19 Belgrave Square, London, S.W.1.

Rehabilitating Lepers

OF an estimated world total of 10 million people suffering from leprosy, probably not more than 20 per cent are receiving treatment of any kind for the severe disabilities that the disease often causes. The World Health Organization scientific meeting on rehabilitation in leprosy, through an on-the-spot examination of leprosy patients with typical varieties of deformity at a research sanatorium in India, set out to determine the extent of the problem of disability, to advise on the best application of available knowledge in prevention, treatment, and rehabilitation of disabled leprosy patients, and to recommend further research (Scientific Meeting on *Rehabilitation in Leprosy*. Report. World Health Organization: Technical Report Series, 1961. No. 221; Pp. 37. Price 3*s*. 6*d*. Published also in French and Spanish).

Teaching Psychiatry and Mental Health

THE need for reform of the medical curriculum in training doctors has been the subject of much controversy, and the place that psychiatry should occupy in the curriculum has often been at the centre of the controversy. The reason for this is twofold. Psychiatry as a medical speciality has now developed to the stage at which it can claim a place of its own in the company of other branches of medicine; it is considered by many to be a leaven permeating medicine as a whole and re-orientating medical practice and medical teaching from its present tendency to separate into compartments towards a better awareness of the human being. A publication (*Teaching of Psychiatry and Mental Health*. Various authors. World Health Organization. Public Health Papers No. 9, 1961. Pp. 186. Price 10*s*. Also published in French. Available through H.M.S.O.) brings together a group of papers by teachers of psychiatry from various parts of the world, describing how the teaching of psychiatry and mental health care can be organized, what problems of content and method are likely to arise, and in what way the teaching should be integrated into the medical curriculum. The authors demonstrate the relationship of psychiatry and mental health to clinical medicine and the sciences of man. They examine the role of neurological investigations in the teaching of psychiatry, the teaching of medical psychology, and the social character of mental disorder. There is also a description of how psychiatry is taught at present in different countries and of the principles being adopted in guiding its future development. The publication presents the basic argument in favour of more psychiatry in the medical curriculum and shows how this can be achieved.

Ageing and the Semi-Skilled

A REPORT by the Medical Research Council's Unit for research on occupational aspects of ageing presents the results of an inquiry into prevailing attitudes and policies towards the older semi-skilled worker in some of the larger manufacturing firms on Merseyside (*Ageing and the Semi-Skilled: a Survey in Manufacturing Industry on Merseyside*, by Alistair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine Cunningham. Medical Research Council Memorandum No. 40. Price 5*s*. H.M.S.O., London). Managers and foremen were agreed that, of the changes which accompany increasing age, some are for the better ; for example, the older worker is more 'steady' and more responsible, while others, such as his slower movement and his slower rate of learning, often tend to impair his working efficiency. The survey also showed that, in the eyes of his superiors, the worker's skill, born of years of experience, often compensates for the 'slowing-up' that occurs with age; in the view of the authors an ever-increasing strain is placed on the older man if he continues to do exacting work.

The findings of the inquiry suggest that, while it is generally accepted in industry that middle-aged and older men should be transferred from physically heavy work, there is little recognition of the fact that work making a constant demand for speed or for close concentration is probably also unsuitable. Moreover, it appears that the possibilities of change of jobs are frequently limited; often the only alternative that can be offered involves both loss of status and loss of pay. An increasing rate of technical innovation in industry may mean that a single group of limited skills will no longer last a man throughout his working life. Both this possibility and the present problems faced by the older worker point to the need for re-training, and the authors suggest that industry should examine methods of training and re-training in relation to the special needs of the semi-skilled worker in the second half of his working life.

Sources of Ionizing Radiation at Public Exhibitions

THE recognition of the need for rigorous control of the use of sources of ionizing radiations has been made evident by the appearance in recent years of codes of practice and regulations concerned with the protection of workers in, for example, hospitals, university laboratories and factories. The latest such publication is a *Code of Practice for the Display of Sources of Ionizing Radiations at Exhibitions* (H.M.S.O., 1961. Pp. 6. Price 6*d*.), and this briefly but clearly sets out regulations aimed at safeguarding both staff and visitors at exhibitions where these sources are used. Thus, maximum permissible dose-rates are specified both in respect of the accessible parts of the shielding and also at positions where visitors may stand. The maximum activities of various radioactive isotopes which may be used unsealed are listed and an example is given of a warning notice to be carried by exhibition stands where radioactive sources are on display.

The Fitzwilliam Museum, Cambridge

THE annual report of the Fitzwilliam Museum for 1960 records the completion of the corridor linking the Museum with Grove Lodge. One of the most important acquisitions was a painted limestone relief purchased from the Elderton Fund. This was from the wall of an eighteenth-dynasty Theban tomb and shows the head and shoulders of a man. An important group of coins forming the Lakenheath Hoard was purchased from the S.G. Perceval Fund. The Hoard included three gold coins, 63 Roman silver coins and 397 silver coins of the Iceni. The fifty-second annual report of the Friends of the Fitzwilliam Museum, also for 1960, indicates important gifts to the Museum including the terra-cotta relief by Clodion, and a double trencher salt of Sèvres white soft porcelain. The total membership at the end of the year was 361.

Control of Soil-transmitted Helminths

AMONG all the species of worms parasitic in man the soil-transmitted intestinal nematodes—the roundworm, the whipworm, and the hookworm—hold the distinction of exerting the greatest influence on individual health and group productivity throughout the world, their prevalence in different communities serving indeed as an index of social and economic standing. Although they cause anæmia, reduce physical and mental capacity, weaken resistance to other diseases, and at times affect health directly and severely, attempts at their control have met with disappointing results. As a consequence they present a health problem that is apt to be given perfunctory attention or even neglected. Prof. Beaver discusses the factors involved in the control of these soil-transmitted helminths, the control methods that have been used and should be used, and the research which problems of control raise (*Control of Soil-transmitted Helminths*. Paul C. Beaver. World Health Organization Public Health Papers, No. 10, 1961. Pp. 44. Price 3*s*. 6*d*. Also published in French. Available through H.M.S.O.). A section is devoted to other miscellaneous helminths the role of which in parasitic infections is still somewhat obscure. An annex deals with diagnostic methods.

British Iron and Steel Research Association : Systems Evaluation Section

IN recognition of the steady growth in the scope of its activities over recent years, the Computer Applications Section of the Operational Research Department of the British Iron and Steel Research Association has now been renamed the Systems Evaluation Section. Mr. D. H. Kelley will be in charge of the new section, which carries out operational research investigations into the automation of large-scale systems. The immediate programme will be to study the automatic production, planning and scheduling of various kinds of steelworks, and to investigate the information requirements of steelworks management. The services to members which were previously supplied by the Computer Applications Section and which included operation of the Association's *Pegasus* computer, advice on the uses of digital computers in steelworks offices and the training of *Pegasus* programmers will be continued unchanged.

Animal Health Trust Research Awards

The Animal Health Trust has announced the following awards under its education assistance scheme which is designed to increase the flow of skilled personnel into the veterinary, medical and scientific fields : *Research Training Scholarships :* W. J. Herbert, for a course in microbiology and a Ph.D. course in virology at the Department of Bacteriology, University of Edinburgh, under Prof. R. Cruickshank; R. C. Chubb, to investigate the use of the gel-diffusion precipitin technique for the diagnosis of infectious bronchitis and pathogenic pleuropneumonia-like organisms. Mr. Chubb will work at the Houghton Poultry Research Station under Dr. R. F. Gordon. *Wellcome Research Fellowships :* B. A. Matson, to make a study of the life-cycle of *Piroplasmosis* under Dr. E. J. L. Soulsby in the Department of Animal Pathology, University of Cambridge; I. E. Lush, to enable him to extend his work on biochemical polymorphism in birds, and its possible connexion with hybrid vigour, at the Agricultural Research Council's Poultry Research Station, Edinburgh, under Dr. A. W. Greenwood.

Unesco Science Study Fellowships

Unesco is offering six study fellowships to Member States to enable specialists to undertake research abroad in either mechanical translation or reduction of geophysical data. The fellowships will be tenable for a period of six months. Travel to and from the country in which the research is to be carried out, and living expenses, will be paid by Unesco. Further information can be obtained from the Unesco National Commission for the United Kingdom, Ministry of Education, Curzon Street, London, W.1.

Transfer of Calcium and Strontium across Biological Membranes

A conference on transfer of calcium and strontium across biological membranes will be held during May 13–16, 1962, at Cornell University, Ithaca, N.Y., under the joint sponsorship of the United States Atomic Energy Commission, National Institutes of Health, National Science Foundation and the New York State Veterinary College. Members of the steering committee are: Dr. R. H. Barnes and Dr. C. L. Comar, Cornell University; Dr. D. Harold Copp, University of British Columbia, Vancouver; Dr. H. E. Harrison, Johns Hopkins University, Baltimore, Maryland; and Dr. R. H. Wasserman, Cornell University. The purpose of the meeting is to discuss present knowledge of basic aspects of movement of calcium and strontium ions across various animal membranes and to present information on physiological, biochemical and nutritional factors which influence this movement. Twenty-two papers are to be presented. Inquiries should be addressed to Dr. R. H. Wasserman, Department of Physical Biology, New York State Veterinary College, Cornell University, Ithaca, N.Y.

Announcements

Dr. F. T. Last, of Rothamsted Experimental Station, has been appointed as head of the Department of Mycology and Bacteriology at the Glasshouse Crops Research Institute in succession to Mr. P. H. Williams, who is retiring after 38 years service, first at the Experimental and Research Station at Cheshunt and latterly at the Institute.

The Nutrition Society's symposium on "Agricultural Problems in Developing Countries" will be held at the National Institute for Research in Dairying, Shinfield, Reading, at 10.45 a.m. on October 13, 1961. Further particulars can be obtained from Miss D. F. Hollingsworth, c/o Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, S.W.1.

A lecture entitled "A National Spectra Index: Plans and Problems" is being organized at the University of Reading on September 26 and will be given by P. S. Davison, of the Scientific Documentation Centre, Halbeath House, nr. Dunfermline, Fife, immediately following the meeting of the Infra-red Discussion Group. The lecture is open to all, admission free.

A conference on "Electrically Exploded Wires", sponsored by the Thermal Radiation Laboratory of the Geophysics Research Directorate of the Air Force Cambridge Research Laboratories, is to be held at the Kenmore Hotel, Boston, Massachusetts, during November 13–14. Further information can be obtained from W. G. Chace, Thermal Radiation Laboratory, CRZCM, Geophysics Research Directorate, Air Force Cambridge Research Laboratories, Bedford, Massachusetts.

An interscience conference on "Antimicrobial Agents and Chemotherapy" is to be held at the Hotel Commodore, New York City, during October 31–November 2. The first day will be devoted to "Infectious Diseases" and the second and third to "Various Aspects of Antimicrobial Agents, including their Clinical Evaluation". The conference is sponsored by the American Society for Microbiology, 19875 Mack Avenue, Detroit 36, Michigan, from which further information can be obtained.

Nearly 1,000 members and delegates will be attending the twenty-eighth conference of the National Society for Clean Air, to be held at Brighton during October 4–6. A special exhibition preview for the Press and others will be held at the Brighton Corn Exchange on Tuesday, October 3, from 3.30 p.m. to 5.30 p.m. Coach transport will be arranged if demand is adequate, and will leave Victoria at 1.15 p.m. Full particulars may be obtained from the National Society for Clean Air, at Field House, Breams Buildings, London, E.C.4.

The Winter Meeting of the Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals will be held in the Hoare Memorial Hall, Church House, London, S.W.1, during December 7–8, 1961. The main discussion of the Winter Meeting will be a symposium on "Sintered High Temperature Compounds" on December 8, between 10 a.m. and 5 p.m. The papers comprising the symposium will be published in *Powder Metallurgy*, No. 8. Further details of the Joint Group and its meetings can be obtained from the Secretary of the Powder Metallurgy Joint Group, 17 Belgrave Square, London, S.W.1.

Erratum. In the leading article entitled "Purpose in Publication" in *Nature* of August 5, it was stated that there is no journal which deals exclusively with biological or biochemical research published more frequently than once a month. We regret this is an error, because *Biochimica et Biophysica Acta* (Elsevier Publishing Co.) has appeared fortnightly since January 1, 1960.

MANAGEMENT STUDIES IN BRITAIN

THE frequency with which reference has been made in recent debates in Parliament to the quality of management and the desirability of training for management gives special interest to two recent broadcast talks on management and the universities. It was particularly noticeable in the debate in the House of Commons on July 26 on the economic situation, although the Chancellor of the Exchequer himself, in opening the debate, merely referred to a re-arrangement of priorities in favour of scientific and technical education and expressed his dissatisfaction with the progress being made in training skilled labour. Mr. P. Walker, however, urged that more attention should be given to commercial education; it is not enough, he said, to increase the supply of scientists and technologists if this is not balanced by sufficient skilled administrators to ensure efficient industrial management; this plea was strongly supported by Mr. J. Diamond and Mr. R. Gunter, both in this context and in its bearing on improved industrial relations. Indeed, both Mr. G. Brown and Mr. D. Howell attributed part of our present difficulties directly to bad management. Less was made of this point in the debates on Britain and the Common Market in both Houses of Parliament on August 2 and 3, but whether or not Britain enters the European Common Market, considerable changes in the industrial life of Britain are inevitable, and these will make large demands on management. Lord Poole indeed was confident that in any event most of British industry would be able to deal effectively with competition from Europe or elsewhere; but, while Lord Plowden also believed that large parts of British industry are highly efficient and competitive, he also thought that it is doubtful whether, in certain industries at least, the United Kingdom offers, on its own, a market large enough to support units of the most economic size.

It is certainly true that, if Britain is to take advantage of the opportunities which entry into the Common Market would offer, industrial management must be competent to meet the most searching demands for efficiency. Nor is management likely to be adequate to meet the situation alone, although, as Mr. D. F. Hutchison pointed out in a broadcast talk on authority and the individual in industry (*Listener*, Feb. 2, 1961), the management function is essentially an autocratic one which should be clearly differentiated from the function of a trade union. It is indeed this conflict between management and the unions that retards the vital function of taking decisions, and although Mr. Hutchison sees such conflict as inherent in the industrial situation, he insists that it should be constructive conflict. He does not suggest that the managerial function should be weakened—that indeed would be detrimental to the trade union members themselves in the long run—but it follows that in his view it is a vital test of the competency of management that it should, while in no way yielding its power to take decisions, fully safeguard the rights and personality of individuals and secure, so far as possible, the understanding and co-operation of all concerned.

Mr. Hutchison was concerned both that management should be effective and dynamic, and that the trade unions also should exercise their proper function and regain the ground they have lost with the community. His talk thus provides an interesting introduction to the two talks some months later on management and the universities, in that he emphasized practical points that lie rather outside the scope of either Dr. V. L. Allen or Mr. J. H. Smith's talk (*Listener*, July 13). Mr. Allen, like Mr. Hutchison, recognizes the inherent conflict of interests in industry, and because social scientists have tended to ignore this question of power and conflict, he is inclined to reject the whole idea of management training or studies as at present practised in the universities. Management, he argues, is not a self-contained activity; it is a single layer of control in a hierarchical structure involving power relations in industry and power and status relations in the community at large, and nothing makes sense unless due account is taken of these relations. It might be interpolated that some status relations in industry also need to be recognized. Furthermore, Mr. Allen rejects, perhaps too precipitately, the idea that any body of knowledge about the subject is being built up; little serious research has been done and less has involved structural or comparative analysis.

If Mr. Allen is right here, it is indeed difficult to maintain that management meets the requirements for an academic discipline sufficiently to justify its being taught in universities. He dismisses, too, the idea that the study of management is vocational, and he is highly critical of all three main methods of teaching management which are in use. In so far as managers can be trained, he suggests that they should be trained through other firms and outside agencies, such as technical colleges, and he would alter both the substance and direction of existing management courses in the universities. Recognizing that industrial behaviour as a whole must be studied if any part of it is to be properly understood, he would give priority to large-scale comparative studies, which should yield results of value both to management and to trade unions, and from which, ultimately, a sociology of industry would emerge, standing in its own right as an academic discipline, and which could be taught openly and honestly and usefully in our universities.

Mr. J. H. Smith (*Listener*, July 20) takes the opposite view. Management studies in British universities, he maintains, deserve much more prominence than they have had so far, both on academic grounds and in terms of what universities could usefully do to raise standards of management in British industry. The most urgently needed development is a strong postgraduate business school, mixing graduates from different disciplines and focusing attention on a common core of subjects which illumine the principles and practice of management. Such a school should be part of a university already strong in the social sciences, but it could be closely linked with other postgraduate administrative studies, notably in public and social administration, which are already well established in some universities.

Mr. Smith points out that it is now widely recognized that the social sciences can be regarded as having the same bearing on the practice of management as the natural sciences have on the practice of medicine, and agrees that research in industrial behaviour is an indispensable condition of the growth of management studies. He thinks Dr. Allen is too pessimistic as to the amount and extent of fundamental research in this field. He does not believe that the universities are pushing ahead too quickly and too uncritically. On the contrary, he considers that the new developments give an impression of caution and of conscious experiment: published plans seem prudently tentative, taking different starting points, and ready to try new combinations of disciplines and unfamiliar disciplines.

By and large, the picture of the present position which Mr. D. Freeth, Parliamentary Secretary for Science, gave in the debate in the House of Commons on August 4 on the need for a social sciences research council seems to support Mr. Smith's view rather than that of Dr. Allen. Moreover, Mr. Smith suggests that much of the impetus towards education for management derives from industry's wish to broaden the intellectual outlook of its managers, to take an outside view of the industrial system, particularly in its social and international context. He challenges Dr. Allen's assertion that the universities are simply seeking to meet the desire of the managers to know how to manipulate a labour force under full employment.

The demand for higher education bearing on management is a direct consequence of the growth of large organizations in industry and commerce, and in public administration. The complexities of these organizations pose problems of a high intellectual order: how to control and co-ordinate individuals responsible for diverse activities, how to direct and re-shape these activities in response to technical, economic and social change, how to deal with an increasingly literate and independent labour force. The administration and organization of scientific research and the problems of Government and science are a particular example where there is need for constructive and creative thought and experiment. Mr. Smith points out that if the universities do not take up this challenge, industry must almost certainly attempt to do so alone, and there will then be little chance either that management studies will be liberated from the framework of discussion fixed by industry itself or that the whole subject will be studied with the freedom and objectivity it deserves.

Accordingly, he takes the view that the universities should move much faster; that they are already in a position to develop management studies much more vigorously if they wish; and that there is a core of subjects now generally agreed to be relevant, and a growing body of research findings to provide, *inter alia*, material for a critical understanding of the industrial system in general, and the management function in particular. He sees nothing in the programmes of existing courses in Britain or in the attitude of those teaching them, which suggests a departure from the high standards claimed for the universities, nor does he believe that real weight is now attached to social harmony rather than conflict, or that social scientists are eager to help managers to manipulate behaviour.

Dr. Allen's objection that management education is directed towards producing an *élite* is treated as more substantial. Many of those receiving it will come to occupy positions of considerable authority and exercise power in large measure over their fellow human beings. The view they take of their responsibilities is a matter of the utmost concern to society, and the role of the universities in shaping that view is highly important. Mr. Smith is undoubtedly right in suggesting that this is no reason for suggesting that the universities should move still more cautiously in this field: rather it is a reason for them coming to terms with management education, and quickly. The real question is not whether there will be a management *élite* but what sort of *élite* it will be. If, as seems certain, this *élite* is increasingly educated in the universities and in the colleges of technology, it follows that those institutions must pay more attention, through research and teaching in the social sciences, and through education for management at the postgraduate-level, to the world in which this increasing proportion of their graduates will spend their working lives. A recent statement issued by the Manchester College of Science and Technology, in connexion with Mr. R. B. Dew's appointment as first visiting professor in industrial administration from October 1, is worth noting here. The statement indicates that the College hopes to expand the work of the Department under Prof. R. W. Revans and affirms a strong belief in the value of management studies. The College believes that a university can make three distinct contributions. First, by its own research, the study of the problems of management and analysis of the consultant's case-book to extract basic teachable ideas; secondly, by communicating these ideas to students so as to encourage them to seek the underlying structure within which the problems of management may be more fully identified and progress more readily controlled; and thirdly, to study the actual processes of management. That statement is both in accord with the general sense of the debate in Parliament on August 4 and with Mr. Smith's argument rather than that of Dr. Allen.

INDUSTRIAL RESEARCH IN BRITAIN

RESEARCH FOR INDUSTRY 1960, follows the same pattern as the past three reports on the industrial research associations in the Government scheme*. The report of the Industrial Grants Committee is followed by a review of the achievements of the 10 research associations, the terms of grant of which came up for review during the year, and two chapters in which Dr. A. H. Sully reviews the contributions of the research associations to health and safety, and Dr. T. Moran the position of food research in the United Kingdom, including the role of the research associations. The remainder of the report (roughly two-thirds) comprises a list of the associations, their officers and publications during the year with a brief indication of the scope of their work.

---

* Department of Scientific and Industrial Research. Research for Industry 1960: A Report on Work done by Industrial Research Associations in the Government Scheme. Pp. iv+148+12 plates. (London: H.M.S.O., 1961.) 8s. net.

The total income of the associations has now risen to £7·9 million and the Department's grant to £1·8 million, while the number of organizations is 52. The Research Council has accepted the Industrial Grants Committee's recommendation regarding admission of non-Commonwealth firms to membership: such firms may now be admitted, subject to certain safeguards, but no grant will be payable on income received from such firms. The Committee has also been concerned with the rising cost of research and the minimum level of income needed if the level of research is to be maintained in real terms, particularly in associations serving small industries. The Cutlery Research Council, which first received a grant-in-aid in 1951, is instanced as serving one such industry which has faced this problem and placed the Council on a sounder financial basis by arranging for a statutory levy. The two new associations coming into the scheme during the year are the British Industrial Biological Research Association, incorporated in October 1960, chiefly to examine the effects on health of chemicals likely to be ingested with food, and the British Brush Manufacturers' Research Association, formed in 1946. Stress is laid on the co-ordination of research effort between the associations, and following the formation of the Cotton, Silk and Man-made Fibres Research Association, the Research Association of British Rubber Manufacturers was re-organized to serve the plastics industry also, as from January 1, 1961, under the title "Rubber and Plastics Research Association of Great Britain". Stress is also laid on the application of the results of research, and it appears that the scheme under which the Research Council made available special grants to accelerate applications appears to have had some effect.

Reviewing the work of the Cutlery Research Council, the report notes that the adoption of a higher carbon stainless steel which keeps an edge far longer than traditional non-stainless steel saves at least 75 per cent of the time a butcher normally spends in whetting his knives. A special investigation undertaken to determine the acceptance in the industry of new ideas propagated by the Council revealed the danger of distortion through inaccurate verbal transmission of information, and indicated generally the conditions under which innovations might become acceptable to manufacturers. The British Glass Industry Research Association's work on instruments for controlling quality has led to the production of an improved strain viewer, to more efficient means of calibrating the subsidiary standards known as strain disks, and to the development of an automatic apparatus which will plot a thermal-expansion curve, and other work has indicated that great increases in the strength of glass articles can be produced under laboratory conditions. A new and permanent fire-proofing process for jute cloth, which gives a high degree of protection against fire and afterglow and is unaffected by prolonged immersion in water, has been developed by the British Jute Trade Research Association, which has also found that by careful choice of cloth and by applying a special chemical finish, jute-resin laminates of a wide range of types can be fabricated from jute fabric impregnated with polyester or epoxy resins.

Work by the Linen Industry Research Association has led to the virtually complete change-over in the industry from bleaching linen yarns in the hank to bleaching in the more economical package form, and very encouraging results have been obtained by blending flax with a proportion of an inherently non-inflammable fibre to give a blend which is itself non-inflammable. A simple method of severe cold-rolling of highly stressed areas of heavily loaded components of motor-cars, such as the fillets of stab-axles and crank-shafts has been shown by the Motor Industry Research Association to increase the fatigue strength by more than 100 per cent, while the Printing, Packaging and Allied Trades Research Association has developed an instrument which measures the resistance to abrasion of two printing surfaces rubbed against each other. Work on cutting and creasing cartons has enabled an important cause of spoilage to be eliminated almost completely, and also increased the speed at which boxes will run on automatic filling machinery, while information acquired in a study of journey hazards in road, rail or mixed goods transport and of the choice of suitable cushioning material is being utilized in the design of packages. The British Scientific Instrument Research Association, besides an ultra-sonic flow-meter, which should be on the market in 1961, has developed a position-sensitive photocell, with 3 or 5 electrodes, which easily detects a movement of 0·01 millionth of an inch, while, besides pioneering work in industrial roofing, the Research and Development Committee of the Timber Development Association designed a prefabricated portable plywood building for the Himalayan Scientific and Mountaineering Expedition, led by Sir Edmund Hilary in 1960, and has used the ultra-sonic pulse technique to trace the extent of decay and insect attack in structural timbers in old buildings. The improved dryers resulting from the Wool Industries Research Association's work on the rate of diffusion of water into fibres are used now by the entire industry, and productivity continues to be improved by the Raper autoleveller and the Raper drawing system. In the study of the structure of keratin the emphasis is now placed on the chemistry of the more reactive amino-acids such as cystine and tryptophan, and fundamental research on the diffusion of dyestuff anions into wool fibre has been linked with the discoloration and variable dyeing properties of wool after storage.

In the debate on science in the House of Commons on July 10, Mr. Aubrey Jones and some other speakers were highly critical of the work of the research associations in general, criticizing the scale of their effort, which was regarded as too small to be effective, and the concentration on fundamental research. This view was not generally accepted in the debate, the preoccupation with fundamental research being flatly denied by Mr. Denzil Freeth, while Mr. J. H. Osborn and others wished to see their activities extended, the use of operational research being specially mentioned. *Research for Industry 1960* is not sufficiently detailed to go far in resolving any doubts expressed in the debate, but it does display something of the range of problems in which co-operative attack promises effective results, and the extent to which the Council for Scientific and Industrial Research is concerned both to see that research effort between different industries, as well as in particular industries, is effectively co-ordinated, and to encourage as much as possible the early utilization of research results in industry. On the educational functions of the associations and the question of research-mindedness in industry, by and large, the report is again silent.

THE ONTARIO RESEARCH FOUNDATION

THE annual report of the Ontario Research Foundation for 1960 contains interesting accounts of sponsored research in its various departments*. In biochemistry, for example, work has been carried out on such projects as the tanning of leather with spruce bark from northern Ontario, the improvement of household sanitation units, the utilization of rice-hull ash, and the properties of pozzolans and other cementitious materials. Besides many existing investigations, four new industrial projects were initiated in the Chemistry Department in 1960. Work for the Reichhold Chemical Co. was started and contracts negotiated with the Brick and Tile Institute of Ontario, Toronto; L'Air Liquide, Montreal; and the National Starch and Chemical Co., Plainfield, New Jersey.

Of particular interest in this Department was work carried out under the Moore Business Forms, Ltd., Fellowship. The investigators designed and constructed instruments to measure manifolding characteristics and developed analytical methods for carbon paper coatings based on new physical methods of analysis. The instruments developed permit a study of the density and clarity of carbon copies, the formation of 'haloes' in high-speed printing, the pressure-time relationship for various impacts of type on multilayers of paper and carbon paper, and provide a visual demonstration of the clarity of carbon copies. The analytical methods permit analysis of very small specimens of carbon ink such as may be recovered from carbon copies and thus yield basic information on transfer and other properties.

In the Engineering and Metallurgy Department, service work increased by about 25 per cent and accounted for about 70 per cent of the external revenue, and more than 50 per cent of the total work load of the Department. The total number of investigations in the Ore Dressing Division increased from 45 in 1959, to 56 in 1960, 55 different clients being served. The Engineering Division provides engineering services for a number of industries, and operates the Gas Appliance Testing Laboratory for the Canadian Gas Association.

Another department, Industrial Research Services, helps industries in the Province by providing a free technical information service coupled with a field engineering service. The latter delineates technical problems and recommends means for their solution. Any industry in Ontario may use this confidential service without obligation. This work is carried out under contract for the Ontario Department of Commerce and Development and for the Technical Information Service of the National Research Council.

This Department also keeps a record of the Foundation's activities. In 1960, more than 4,300 separate contacts with manufacturing and service industries, individuals, associations and departments of Government were recorded. Nearly 1,400 investigations were carried out in the scientific laboratories of the Foundation, ranging from short-term evaluations of a special nature through minor research and development projects, field engineering studies, consultation, project management and technical surveys to major research and development fellowships. Answers to nearly 1,900 technical problems were provided by Industrial Research Services and more than 400 problems requiring laboratory work were co-ordinated by the Department either at the Foundation or in some other appropriate and competent agency.

For many years the Department of Parasitology has been building up a body of knowledge concerning the parasites occurring in fish, birds and mammals of Ontario. Thirteen scientific papers were published in 1960, an outstanding contribution from a small group which is also responsible for a teaching and research programme in parasitology at the University of Toronto.

Service work undertaken by the Department of Physics included work on back-scattering of electrons from surfaces, a test-jig for an extensometer, tests of insulation tape, and consultation regarding electronic instruments, optical measuring systems, light meters and radioactive tracers.

* Ontario Research Foundation Annual Report, 1960. Pp. 46. (Toronto: Ontario Research Foundation, 1961.)

THE COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, NEW DELHI

THE annual technical report, 1959–60, of the Council of Scientific and Industrial Research, New Delhi, covers the work of twenty national laboratories, including the National Botanic Gardens, Lucknow, and the Birla Industrial and Technological Museum, Calcutta, as well as sponsored research in progress or completed at the Rain and Cloud-Physics Research Unit of the National Physical Laboratory, New Delhi, and at the three Essential Oils Research Centres at Kanpur, Bangalore and Ootacamund*. There are also notes on the work in progress under some fifteen research committees and under fellowships, as well as on completed projects. Lists of 379 research schemes in progress in 106 centres in 1959–60, of grants-in-aid, of 306 fellowships, of 738 research papers published during the year, and of patent applications filed are appended. Besides the Central Mechanical Engineering Research Institute and the Central Indian Medicinal Plants Organization, which are still being planned, the Council has approved the establishment of a National Aeronautical Laboratory at Bangalore, a Regional Research Laboratory at Jorhat (Assam), a Central Scientific Instruments Organization and a Centre for Research, Training and Documentation in Petroleum and Natural Gas.

* Council of Scientific and Industrial Research, New Delhi. Annual Technical Report, 1959–60. Pp. iv+440. (New Delhi: Council of Scientific and Industrial Research, 1961.)

The research schemes covered a wide range of problems in biology and public health, engineering, essential and vegetable oils, fuel, leather and food technology, metallurgy, pharmaceuticals and drugs, and physics, while a research school in earthquake engineering was established at Roorkee University.

It is impossible here to do more than refer very briefly to a few features of the work of some of the National Laboratories and Institutes to illustrate the range of the Council's activities. The National Physical Laboratory, New Delhi, was responsible for theoretical investigations on the conduction of heat in solids, and an evaluation of the convolution integral with particular reference to its application in heat conduction when the excitation function is an arbitrarily plotted function of time. Broadcast transmission of time signals over a standard carrier of 10 Mc./s. continued for 5 hr. daily on an experimental basis, and also investigations on the determination of the minimum amount of costly materials which suffices for the correct measurement of their melting points which are fixed points of temperature, and the internal energies of tantalum, platinum and titanium have been calculated. The National Chemical Laboratory, Poona, has developed a new complexing eluant, sodium triphosphate, particularly useful in preparing pure samaria from a 30 per cent samarium concentrate, and has also developed a general theory of super-exchange interaction mechanism and satisfactory processes for production of theophylline, sodium warfarin, *N*-methyltaurine and methylamine and for recovering quinine and quinidine from cinchona febrifuge, as well as a new route to naturally occurring anthraquinone carbinols using diborane.

The Central Drug Research Institute, Lucknow, has found, in indigenous medicinal plants screened for pharmacological activity, a good cardiovascular drug in ruvoside, from *Thevetia neriifolia* (Kaner) and a drug with musculotrophic properties in *Daemia extensor* (Utranjutaka), and obtained evidence of amœbicidal activity in deoxyhexahydroquinine, while immunochemical work on *Vibrio cholerae* has shown that neutral and acid polysaccharide fractions possess high serological activity. The Central Electroclinical Research Institute, Karaikudi, has developed a new technique for preparing 2 : 4-diaminophenol by electrolytic reduction of *m*-dinitrobenzene, and has used a rotating-cathode technique in the preparation of benzidine and substituted benzidines, while a new cathodic technique has been developed for the study of cathode protection based on the use of the Hull cell and a polarographic technique used for the determination of cystine. The Regional Research Laboratory, Hyderabad, has prepared short-, medium- and long-oil varnishes from maleic and modified phenolic resins, using dehydrated castor oil, bodied dehydrated castor oil and linseed stand oil, and established optimum conditions for the preparation of terpeneol from carene, 8-hydroxyquinoline from *o*-aminophenol, phenylacetic acid from benzyl chloride and phenylacetamide from benzyl cyanide.

The Indian Institute for Biochemistry and Experimental Medicine, Calcutta, has gained a clearer understanding of the biological activity of human chorionic gonadotrophin which accelerates the transformation of germ cells in the male toad, and has also obtained a comprehensive idea of the pattern of metabolism supporting growth and activity in *Leishmania donovani*.

RADIO RESEARCH 1960

THE Radio Research Board of the Department of Scientific and Industrial Research was established in 1920, and has in the intervening forty years continuously advised the Department on its programme of research in the radio field. For many years the work was centred on the National Physical Laboratory at Teddington, although facilities for field experiments were long ago made available in the grounds of the Admiralty Compass Observatory, at Ditton Park, near Slough. Dr. R. L. Smith-Rose has been associated with this research programme from its inception, and in 1947 he was appointed as the first director of radio research of the Department of Scientific and Industrial Research. Following this appointment, the Radio Research Station at Slough, with its new laboratories erected in 1956, became the centre for the radio research work of the Department and independent of the National Physical Laboratory.

The report of the Radio Research Board for 1960, published recently*, records that Dr. R. L. Smith-Rose retired from his post of director of radio research on September 30, when he was succeeded by Mr. J. A. Ratcliffe from the Cavendish Laboratory, University of Cambridge.

During the year, the work of the Station was re-oriented so as to apply about 50 per cent of its effort to some space research problems, which are now being actively investigated. To assist in this work, a major development during the year under review was the installation of a 'Minitrack' equipment for tracking artificial satellites of the Earth, and for communication with, and reception of, telemetry signals from such satellites. The equipment has been provided on loan by the National Aeronautics and Space Administration (N.A.S.A.) of the United States, and is operated by staff of the Station. Data obtained from it are available for use by both organizations.

Predictions, on an increased scale, have continued to be issued during the year, giving the positions of all satellites of interest to observers in the United Kingdom. Signals have been received from a number of satellites at Winkfield, near Slough, and at the Radio Research sub-station at Singapore; and measurements have been made of signal strength, Doppler frequency-shift, and Faraday fading effects. The results are of particular interest in work on the ionosphere both in temperate and equatorial regions.

For many years the Radio Research Station has been very actively engaged in exploring the ionosphere by radio waves sent up from the surface of the Earth. During the past year, collaboration with the corresponding Canadian and United States laboratories has continued in the development of a technique to use satellites for sounding the ionosphere from above—'top-side sounding' as it is called. The station at Port Stanley in the Falkland Islands, one of the Department's ionospheric observatories for many years past, will also be engaged in receiving signals from these and other satellites.

---

* Department of Scientific and Industrial Research. Radio Research 1960: The Report of the Radio Research Board and the Report of the Director of Radio Research. Pp. iv+23+4 plates (London: H.M.S.O., 1961.) 2*s*. 6*d*. net.

The ionospheric forecasting service provided by the Radio Research Station has always been used by civilian and defence organizations in the planning of their long-distance high-frequency communication circuits. With the knowledge of ionospheric behaviour that has been accumulated at the Station, consideration is now being given to the production of a reference book containing details of all ionospheric conditions likely to be encountered throughout the world. A single figure or index derived from observations at a few ionospheric sounding stations would be used as a guide to find out the conditions prevailing at any time and place.

During the period under review, the analysis of the atmospheric radio noise data obtained during the International Geophysical Year has been continued. The main experimental work during the year has been the recording of single atmospherics from storms at known short distances; and the evaluation of the peak power and total radiated energy from lightning discharges. At frequencies greater than 30 Mc./s., interference from atmospheric noise is not of major importance for ground receiving stations. But the need to find a 'quiet' site for the Station's projected radio telescope has indicated that man-made noise, due to electrical machinery and the like, may cause serious disturbance. Accordingly, measurements are being made in the higher radio frequency bands to determine more precisely the levels of such man-made noise.

There are still many problems awaiting solution in connexion with the transmission of radio waves over both long and short distances through the troposphere. A detailed knowledge of the propagation conditions for very-high and ultra-high frequencies is of particular importance in connexion with the future development and planning of broadcasting, including television, services throughout the world. Staff of the Radio Research Station collaborate with the Post Office and the British Broadcasting Corporation in experimental investigations in Britain. But, on a wider scale, they also participate in the work of the International Radio Consultative Committee (C.C.I.R.), concerning the planning, over a wide geographical area, of transmitters using the same or adjacent frequency channels, in such a way as to avoid mutual interference.

Closely associated with this work is the direct experimental study of the refractive index structure of the troposphere by two techniques: one of these uses an airborne micro-wave refractometer for direct measurements at various heights; the other comprises radar sounding of the tropospheric layers at vertical incidence. Preliminary investigations have been made by both methods, and improved equipment has been under development during the year.

While the work on the electrical properties of components and materials, such as transistors and ferrites, has been terminated during the year, the past experience gained in this type of investigation is proving valuable in the development of techniques and equipment used in other parts of the research programme. Apart from this, however, all aspects of the programme demand facilities for the accurate measurement of various parameters; it is a continuing policy of the Station to conduct research on the appropriate measurement techniques.

As in the case of former published reports, that for 1960 contains three appendixes. The first gives the references to 36 scientific papers published by the staff during the year. The second is a list of staff of the Radio Research Organization on January 1, 1961; while the third gives the terms of reference and constitution of committees of the Radio Research Board. These committees provide the expert advice on which the Board frames its recommendations for the present and future programmes of research.

BIOLOGICAL CONTROL OF CONCEPTION*

By Dr. A. S. PARKES, C.B.E., F.R.S.

National Institute for Medical Research, Mill Hill, London, N.W.7

THE Oliver Bird Lectures and the Oliver Bird Prize were instituted with the idea of reviewing and stimulating work bearing on the control of conception in man. Such review and stimulation were badly needed. In many countries of the world, population is increasing at an unprecedented and accelerating rate. The increase is not due to any sudden increase in human fertility; it is due to the work of medical science in decreasing mortality and increasing the expectation of life at birth in many parts of the world where the birth-rate is still high. Total world population is now around 3,000 million, just about double the number thought to have existed at the beginning of this century. Population growth cannot go on indefinitely at this rate. Something will happen to stop it, and the human race must decide whether that something is to be pleasant or unpleasant.

Different countries are contributing in very different degrees to this population explosion. In the United Kingdom the small natural increase in population now occurring is not in itself causing any new problems. But the practical, æsthetic and eugenic requirements of the individual in relation to the control of conception are far from satisfied. In countries faced with a crippling increase in population it is vitally necessary that a simple and effective method of controlling fertility shall be evolved. It is not surprising, therefore, that intensive, though belated, work is now in progress with the view of applying existing knowledge of the physiology and biochemistry of the processes leading to conception to provide better methods of preventing it, and to extend our fundamental knowledge of the processes so as to broaden the basis of possible applications. In general, therefore, the trend of thought is towards what may be called biological means of fertility control, involving systemic treatment, and away from local action of a mechanical or chemical nature. Systemic treatment is, of course, most easily made by mouth, in other words by 'the pill'.

---

* Substance of the fifth Oliver Bird Lecture delivered on June 20.

Biological control involves one or other of three things: (*a*) the prevention of formation or release of germ cells in male or female; (*b*) the prevention of fertilization; (*c*) the prevention of implantation of the fertilized egg. The first of these involves either preventing the release of ova from the ovary or the formation of spermatozoa in the testis. Spectacular progress has been made in the past few years towards the use of orally active progestagens (the 'Pincus pill') to depress the ovary-stimulating activity of the anterior pituitary body and thus to prevent release of the ovum. The Pincus pill has been the subject of extended trials, and is likely to come into routine use to at least a limited extent. The outstanding problem of the Pincus pill is not as to whether it works —it certainly works—but as to the extent of uncomfortable side-effects and of possible hazards arising from the long-term inhibition of a vital gland and the prolonged induction of an artificial rhythm in the uterus.

Work on the inhibition of spermatogenesis by oral medication is not so far advanced. Obvious disadvantages attend the depression of ovary or testis activity by the use of substances, particularly steroid hormones, reducing pituitary gonadotrophic activity; but encouraging results on the direct inhibition of spermatogenesis without interference with the androgenic interstitial tissue have been obtained by the use of various non-steroidal compounds. In any event, there is little doubt that a pill for males will be forthcoming in the future. When this happens the obvious course will be for husband and wife to take, say, yearly shifts in consuming pills, so as to minimize the risk of adverse effects arising in either sex through indefinitely prolonged treatment.

So far, little progress has been made with the problem of preventing fertilization by biological means in circumstances where both male and female are producing viable germ cells, that is to say, in preventing effective contact between egg and sperm. It is possible, though not certain, that the most hopeful line of work here is towards immunizing the female against spermatozoa and thence against pregnancy, a possibility which arises from the fact that spermatozoa are antigenic when administered parenterally to animals of a different species from the donor, and that the antibodies raised are organ- rather than species-specific. A serious difficulty, however, lies in obtaining an effective concentration of antibody at the necessary site of action within the female reproductive tract, and only recently, by the use of adjuvants, has it been possible to obtain any serious indications of a positive result. However, other immunological approaches to fertility control exist.

Work on the prevention of implantation raises the important question as to what constitutes conception. Biologically there can be little doubt that implantation of the fertilized egg in the uterus, not fertilization of the egg, constitutes conception, and that contraception can therefore properly be exercised up to the time of implantation. This is important because much interesting work is now being carried out on inactivating the fertilized egg or preventing the changes in the endometrium necessary for implantation. A method of fertility control based on such work would have the great advantage of requiring retrospective action rather than action anticipating the uncertain contingency of exposure to the risk of pregnancy. Scope for inactivating the fertilized egg is perhaps limited, but there are many possibilities of disturbing the hormonal, metabolic, pharmacological, or neurohumoral relationships necessary for the development of progestational changes in the uterus. A particularly interesting line of thought in this field arises from the observation that newly mated female mice fail to become pregnant if exposed to the smell of alien males from a different strain. The effect is exerted first through nervous and then through hormonal pathways, and the observation is evidently of great interest and potentiality; the attractive idea of a contraceptive perfume is no longer quite incredible.

In conclusion, it must be remembered that the best methods will be of little value in countries faced with an explosive growth of population unless people can be persuaded to use them, and this may not be easy in countries conditioned to the idea of a high birth-rate and a high death-rate, and in which public health measures have reduced the death-rate so rapidly that the social background has had little chance to adjust itself. But at least we should ensure that demographic and personal aspects of human reproduction are as widely understood as possible, and that simple and reliable methods of fertility control are available for those who wish to use them. In this we have far to go. Established methods of fertility control, being archaic in principle, are a disgrace to science in this age of spectacular achievement. In general, I am in favour of science pushing on in every possible direction, but I sometimes think that the further exploration of inner man would be more immediately useful than the exploration of outer space.

SAFE HANDLING OF RADIOACTIVE SUBSTANCES

In 1949 a printed version of the booklet *Introductory Manual on the Control of Health Hazards from Radioactive Materials*, prepared by the Atomic Energy Research Establishment, was issued by the Medical Research Council. This manual has been in great demand by those working with or just beginning to work with radioactivity, but, as it has not been available for some years, a new edition*, commissioned by the Medical Research Council's Committee on Protection against Ionizing Radiations, is timely and welcome. It is intended to give guidance on the principles of safe working to those handling radioactive substances in laboratories and elsewhere, and contains a valuable summary of the recommendations on maximum permissible doses and the state of legislation in the United Kingdom at the end of 1960.

---

* Privy Council. Medical Research Council Memorandum No. 39: *Introductory Manual on the Control of Health Hazards from Radioactive Materials*. (Committee on Protection against Ionizing Radiations.) Pp. vi+21. (London: H.M.S.O., 1961.) 1*s*. 9*d*. net.

In Section 3, which follows a brief introductory section and a descriptive section on the general nature of the hazards, are given the present recommendations on maximum permissible levels of radiation for those occupationally exposed to radiation and for those working nearby; these are based on the Medical Research Council's evaluation of the recommendations of the International Commission on Radiological Protection. In addition to listing the levels for external radiation, examples are given of actual values of maximum permissible body burdens and maximum permissible concentrations in air and water.

There is a brief outline of the measurement of radiation dose and dose-rate in Section 4; monitoring for neutrons is outside the scope of the manual. Due consideration is given to the use and value of film dosimeters, but there is no mention of whether any film-badge service is generally available. In this section reference is made to the selection of radiological safety officers to ensure that protection measures in a particular department are carried out; and to the appointment of an appropriately qualified physicist as 'radiological protection adviser' to advise the head of each department. These officers are akin respectively to the departmental radiation supervisor and university radiation protection officer recommended in the recently published *Code of Practice for the Protection of Persons exposed to Ionising Radiations in University Laboratories* (The Association of Universities of the British Commonwealth, London, 1961). It is also pointed out in the manual that the radiological protection adviser should work in close association with a qualified medical adviser. These officers can also be compared with the "competent persons" in the Factories Acts Regulations whose duties are to "exercise special supervision with regard to the requirements of these Regulations and to assist in enforcing the observance of them". (*Factories, The Ionising Radiations (Sealed Sources) Regulations*, 1961 (London, H.M.S.O., 1961); Ministry of Labour, Factories Acts, 1937–1959, *Unsealed Sources Regulations, Preliminary Draft of Regulations* (London, H.M.S.O., 1961).)

Sections 5 and 6, on "Acceptable and Hazardous Amounts of Radioactive Material", contain a table classifying isotopes according to their relative radiotoxicity. This table is in line with the table in the first schedule of the Unsealed Sources Regulations. Laboratories are graded as *A*, *B* or *C* according to the quantity of radioactivity involved and its radiotoxicity; in addition, account is taken of the nature and complexity of the processes involved. This method of classification is the one that is most often given in codes of practice for safe handling of radioactive materials; however, it is not the only one, and practical experience has shown that other systems of classification of radiation and contamination give effective control of exposure to radiation.

The remaining sections of the manual cover protective clothing and devices; exhortations on 'do's' and 'don'ts' when handling radioisotopes; eating, smoking and washing; storage; a valuable section on disposal of radioactive wastes which takes account of what the situation will be when the Radioactive Substances Act, 1960, comes into force; and a final short section giving advice on where to apply for particulars concerning transport of radioisotopes.

The bibliography covers publications issued up to the end of 1960. In the field of radiological safety so much coding and regulation is in progress that there have been at least three major documents issued since the manual was published. With this accretion of regulations and advice the manual is invaluable for all those concerned with the use of radioactive substances and the control of health hazards as a handy summary of authoritative advice at a given point in time.

NEUTRON CAPTURE THERAPY

NEUTRON capture therapy is an experimental radiotherapeutic procedure involving new concepts in the treatment of cancer. This treatment is a means of achieving selective irradiation of a tumour tissue by inducing throughout that tissue radioactivity of high biological efficiency. The radioactivity is induced *in situ* by means of the capture of thermal neutrons by a suitable target element. To develop the most efficient system, a target element is chosen which, on thermal neutron capture, decays immediately through emission of energetic heavy particles such as α-particles. Since the range of heavy particles of 2–4-MeV. energy in tissue is very short, of the order of less than 15μ, the resulting radiation effects are limited to the region of origin. Because radioactive decay is practically instantaneous and results in a stable element, physiological dispersion from the origin is prevented. Only those elements which have a high thermal-neutron capture cross-section, such as boron-10, lithium-6, and uranium-235, are useful. This suggestion was first put forward in 1936 by Locher. The utilization of thermal neutron capture by boron-10 for the treatment of human-brain tumours, chiefly glioblastoma multiforme, has been under investigation at the Medical Research Center of Brookhaven National Laboratory, New York, since 1949.

An account of the biological effectiveness of thermal neutrons and of heavy particles from the $^{10}B(n,\alpha)^{7}Li$ reaction for the rabbit's ear and its utilization for neutron capture therapy has been given by Yaskazu Lucas Yamamoto (*Yokohama Medical Bulletin*, 12, No. 1, February 1961).

Throughout this series of cases, the pathological effects of thermal neutrons and of the heavy particles from the $^{10}B(n,\alpha)^{7}Li$ reaction on human skin occurring at the point of neutron entry have been known as one of the most vexing side-effects of neutron-capture therapy. Among other side-effects such as boron toxicity, radiation parotitis, and radiation conjunctivitis, it has particularly attracted the attention of investigators, and an effort has been made to prevent or reduce the complication. In reviewing the brain tumour cases treated by neutron-capture therapy at the Medical Research Center, Yamamoto and his co-workers have encountered varying degrees of skin reactions in a large percentage of the cases ranging from erythema to severe radiation dermatitis.

In a previous series of experiments on human brain tumours, attempts were made to decrease skin radiation by decreasing the boron concentration in the skin while maintaining a maximum concentration in the neoplastic tissue in the brain. The methods used were those of scalp pressure bandages, temporary bilateral ligation of external carotid arteries, and boron-10 infusion into the internal carotid artery on the same side as the tumour. These methods were not adequate in preventing severe reaction since a large neutron dose was required to treat a deep-lying tumour because the thermal neutrons attenuate rapidly in tissue.

A research project has therefore been undertaken to determine the minimal dose of thermal neutrons and of the heavy particles from ^{10}B(n,α)^{7}Li reaction to produce radiodermatitis on the ear of the rabbits, and to investigate the details and characteristics of skin reaction and their prevention, especially by the employment of neutron-shielding materials for medical use.

The findings show that the safe skin dose (no more than grade-2 skin reaction) of thermal neutrons and of the heavy particles from the ^{10}B(n,α)^{7}Li reaction on rabbits' ears are established to be less than 10×10^{12} n/cm.^{2} with thermal neutrons exposure alone and $1{\cdot}5 \times 10^{12}$ n/cm.^{2} with 35 mgm. ^{10}B per kgm. body-weight. A review of clinical data shows the maximum safe skin dose to be roughly $0{\cdot}5 \times 10^{12}$ n/cm.^{2} with 25–50 mgm. ^{10}B per kgm. body-weight. These clinical data have been correlated with work on the differences of radiosensitivity between human beings and rabbits' ears by Ellinger.

The medical uses of neutron-shielding materials from both physical and biological points of view have been considered, and the utilization of the above data to avoid skin complications in neutron-capture therapy for human brain tumours is indicated by Yamamoto. The use of the temporary skin flap and lithium metal shielding has been successful in avoiding skin complications. The value of relative biological effectiveness is calculated to be 3·9 from thermal neutron exposure alone and to be 1·87 from neutron-capture therapy from the ^{10}B(n,α)^{7}Li reaction, with 35 mgm. per kgm. body-weight.

LAND-USE SURVEY: NICARAGUA

It is now well recognized that the world's agriculture must become more intensive if it is to keep pace with the increasing population of this planet. Earlier warnings by Lord Boyd-Orr can now only be accepted as fundamentally real, and have been placed in perspective by Dr. Norman Wright, deputy director of the Food and Agriculture Organization of the United Nations. He estimates that if agriculture makes use of all the technical assistance available, there is no reason why it cannot keep pace with the increasing demands for food, at least to the turn of this century. One of the first technical contributions to agricultural intensification of a country is a survey of the optimum uses of land, and the first parts of such a survey in Nicaragua have been published recently.

Nicaraguan agriculture is at present not very intensively developed. The latest returns (for 1952, in the Food and Agriculture Organization's *Yearbook 13*, 1959) show it to have a total area of 14,800,000 hectares of which more than 26 per cent is built on or otherwise unusable. Of the productive area the largest proportion is forest (57·5 per cent). Pastures account for nearly 6 per cent and arable crops nearly 8 per cent, while 29 per cent is potentially productive, though not used at present. This should be a most rewarding setting for a land-use survey, shows particular foresight of the originators, and Dr. Taylor has indeed produced a report which demonstrates how Nicaragua could not only feed more than her own rapidly expanding population for some time to come but also maintain and increase the forestry assets*.

Grassland improvement is perhaps the most basic, for stock depends on it more than on arable crops. Enhanced grazing value of the savannah is possible by mowing or controlled burning, and on more fertile ground, the wasteful bush fallow should be replaced by a period of from four to six years of rotation pasture with controlled grazing by stock. There is, however, urgent need to introduce more productive grasses and legumes.

Perhaps the greatest need for the survey is shown in forestry organization. In the Puerto Cabezas–Rio Coco area, the pine forests are only sufficient for a further seven years supply, yet the industry could be built up to have a perpetual annual income of at least 23,000,000 dollars (U.S.), and measures to effect the transition are discussed. On the other hand, in an area to the north of Santo Domingo, standing timber valued at approximately 100,000,000 dollars (U.S.) is likely to be destroyed in the gradual clearing of land for agriculture and grazing. A project to utilize it is described. It is recommended that a small forest service concentrating on fire control be established, pasture and plant introduction officers be appointed and that there should be a small service for crop protection, for diseases and pests are severe in the condition of high rainfall.

Climate and soils in Nicaragua are very suitable for a considerable expansion of cash crops. Large areas would, for example, be suitable for growing rice, with or without low-lift irrigation, which could cover immense areas at low cost. The survey indeed leads to the conclusion that Nicaragua might well export rice, meat, milk, cheese, sugar, citrus products, peanuts, beans and cacao. This raises one of the main difficulties of expanding world food production, namely, of adjusting increased production to new markets. Unplanned flooding of world markets would destroy the whole economics of production, and Dr. Taylor very rightly considers this aspect in addition to the detailed ecology of his valuable report. The population of Nicaragua, however, has increased from 765,000 in 1937 to 1,378,000 in 1958, so if this very rapid increase persists, the internal market alone will demand considerable expansion of food supply and resources.

The classic instances of Israel and Taiwan show what can be achieved by the planned use of land, and, in complimenting Nicaragua and the Food and Agriculture Organization on this progress, we look forward to the remaining parts of the survey and to the implementation of its recommendations.

John Grainger

---

* Republica de Nicaragua, C.A. Ministerio de Economia, Instituto de Fomento Nacional, y Organizacion de las Naciones Unidas para la Agricultura y Alimentacion. Estudios Ecologicos, Volumen No. 1 (1959). *Estudios Ecologicos para el Aprovechamiento de la Tierra en Nicaragua* (Ecological Land Use Surveys in Nicaragua). Pp. xv+338. By Dr. B. W. Taylor. (Managua, D.N.: Ministerio de Economia, Instituto de Fomento Nacional y Organizacion de las Naciones Unidas para la Agricultura y Alimentacion (F.A.O.), 1959.)

FOREST INSECT PESTS AND DISEASES IN CANADA

IN 1935 a Forest Insect Survey was begun in Canada with a small organization located at Ottawa and Fredericton, mainly concerned with European spruce sawfly. It has now expanded to the extent of having 11 laboratories and a permanent staff of 100 employees. In 1957 a Forest Disease Survey was initiated concerning itself with diseases due to organisms and agencies other than insects. Late in 1960, with the formation of a Department of Forestry which absorbed the Forest Biology Division of the Department of Agriculture, a combined survey of all forest diseases became possible.

The size of the annual report for 1960 of the combined survey indicates the extent of the work now being undertaken.* In it each province reports separately, and a rough glance over the index shows that almost 400 individual organisms living on Canadian trees are the subject of record and observation. Compared with tropical rain-forest the number of Canadian tree-species is not large, yet they seem to harbour a considerable number of organisms injurious to them. Not all these are of primary importance, however, and the early spring and hot summer of 1960, except in British Columbia, were not favourable to fungous diseases. A northward spread of Dutch elm disease in Ontario and New Brunswick was recorded. The major insect marauders are the spruce bud-worm, the larch sawfly and the forest tent caterpillar on aspen, reported from all regions. The bud-worm has severely defoliated spruce and silver fir over an area of nearly 8 million acres in British Columbia.

The survey makes considerable use of aeroplanes for locating certain diseases the distribution and spread of which are shown on appropriate maps. Aerial spraying is used in trying to control the spruce bud-worm and other insects. The introduction of various insect predators to combat pests like the balsam woolly aphid is on trial, and in Newfoundland a shrew has been introduced, apparently with some success, as a predator on larvæ of the larch sawfly. Quite a number of diseases have been imported from Europe, but not the dwarf mistletoe which infests lodgepole pine in Alberta, and spruce in the Maritime provinces. A damaging ice-storm occurred in Quebec province and frost and storm damage occurred elsewhere.

The minute size of certain organisms is often in inverse proportion to the length of their names. Little did the unsuspecting Indian of a bygone era realize that he was surrounded by such formidable creatures as *Pseudexentera improbana oregonana* Wlshm. or *Lambdina fiscellaria fiscellaria* (Guen.). The general value of this annual report would be immensely enhanced if the survey dealt first with the individual organisms in order, using the regions as a secondary basis of reporting and not as the primary one.

M. L. ANDERSON

---

* Canada: Department of Forestry. Forest Entomology and Pathology Branch. Annual Report of the Forest Insect and Disease Survey, 1960. Pp. 121. (Ottawa: Queen's Printer, 1961.)

HEALTH IN YUGOSLAVIA

IN 1957 the School of Public Health, Zagreb, Yugoslavia, conducted an inquiry into the health, living conditions and social and economic problems of the 880 inhabitants of eleven mountainous villages in the Velebit highlands above the Adriatic coast. The report of the inquiry has now been published by the World Health Organization (*WHO Chronicle*, 15, No. 7; July 1961). These villagers have no roads, no electricity, no radio and no newspapers; the region in which they are has no developed agriculture or cattle breeding, no industry, or mining. Life is just what it was fifty years ago—simple and hard, perhaps harder, since goat-raising is now forbidden, and the trade in wood, wool and cattle that used to be carried on along the coast has fallen into decay. The young people no longer emigrate to the United States as they used to, but try to get away to other parts of Yugoslavia where conditions are easier.

This situation has left a deep mark on the villages. Since 1900 the population has gone down. In 1900 there were 94 women to 100 men; in 1953 the figure was 112. The average age of the population has gone up, the percentage of people aged 65 and more increasing from 4·4 per cent in 1900 to 11·1 per cent in 1953, the percentage of children aged 14 decreasing from 43·1 per cent to 32·6 per cent in the same period.

Infectious diseases accounted for 46·5 per cent of deaths at the end of the last century. Now they have almost ceased to be a cause of death, the chief causes for 1953–57 being: from infant diseases 35·9 per cent, from old age 33·3 per cent and from pneumonia 10·3 per cent. Infant mortality has always been a serious problem. Cancer and cardiovascular diseases have been responsible for very few deaths.

The majority of cases of respiratory disease found in the investigation lay on the borderline between pathological and the physiological. Diseases of the digestive tract were strikingly low, perhaps because of a diet containing much milk. The nutritional status of the villagers was good. The intestinal parasites found were similar to those of neighbouring districts. Syphilis was diagnosed in only two villagers. Investigation for antibodies to the polioviruses showed that contact with the viruses and immunity to them must have occurred in early childhood. Antibodies against *Q*-fever were found in 26·4 per cent of the population—not surprisingly, as the villagers live in close contact with sheep.

This health study was carried out by teachers and students of the School of Public Health, and, apart from the knowledge it gave of conditions in a remote area, brought the students into contact with the life of the villages, taught them public health techniques, such as analytical methods and sampling, and afforded them opportunity to develop into research-minded workers. It showed that collaboration between university institutions and public health services can be of benefit to both. It also showed that much valuable information can be gleaned in a short time by work such as this and used by the public health authorities as a basis for their health programmes.

DRINKING-WATER STANDARDS

IT is frequently not realized that in Great Britain there are no legal standards of quality to which water supplies must conform; nevertheless the quality of our public supplies is such that it is automatically assumed that water from the tap is safe. This is not the case in many parts of the world, and in 1958 the World Health Organization published the minimum standards which it considered should be required everywhere in the world[1]. It was always hoped that these standards would be surpassed in more highly developed areas, and a second publication has now appeared which recommends higher standards which should be attainable in all European countries[2].

The recommendations specify the minimum bacteriological and chemical qualities that should be expected of piped supplies of water for domestic use. In the section on bacteriological examination, methods of sampling are described, the organisms which should be regarded as indicators of pollution are discussed, and tests for coliform organisms, fæcal *Streptococci*, and anaerobic spore-forming organisms are outlined, before specific recommendations for bacteriological standards are made. It is considered that, in any water entering the distribution system, coliform organisms should be absent in 95 per cent of the 100-ml. samples taken each year, and that at least 100 such samples should be examined at regular intervals during the year.

In the section on physical and chemical examination the main requirements limit the concentrations of toxic substances (lead, arsenic, selenium, chromium, cadmium, cyanide and radioactivity) and give the methods of analysis which should be used to estimate them. In addition, limits are suggested for certain constituents of water which may give rise to trouble if present in excess (for example, fluoride, nitrate and zinc), and of others, such as free carbon dioxide, and hardness, which should preferably be controlled although they do not affect the hygienic quality of the water. NORA H. JOHNSON

---

[1] *Nature*, **183**, 867 (1959).

[2] World Health Organization. *European Standards for Drinking Water*: Pp. 52. (Geneva: World Health Organization; London. H.M.S.O., 1961.) 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars.

SPECIFIC HEAT AND EXPANSION COEFFICIENT OF LIQUID HELIUM-3 UNDER PRESSURE BELOW 0·1° K.

By DR. D. F. BREWER and J. R. G. KEYSTON
The Clarendon Laboratory, Oxford

RECENT measurements of the specific heat of liquid helium-3 near its saturation pressure have shown that the second-order phase transition predicted by theory[1–4] does not occur above 0·008° K. Anderson, Salinger, Steyert and Wheatley[5] found a linear variation of specific heat with temperature between 0·008° K. and 0·04° K., and Strongin, Zimmerman and Fairbank showed that no anomaly occurred between 0·054° K. and 0·085° K.[6], the latter being the lowest temperature to which previous measurements had been made[7]. Unpublished measurements by us, also near the vapour pressure, between 0·03° K. and 0·1° K., covered the intermediate region. According to the theory of Emery and Sessler, the transition temperature, T_c, is given by the equation:

$$kT_c = 1{\cdot}09\,\frac{\hbar k^2_F}{m}\,\frac{m}{m^*}\,\exp-\left(\frac{\pi}{2}\,\frac{m}{m^*}\,\frac{1}{t}\right) \qquad (1)$$

(provided that the Fermi liquid model holds above the transition temperature), where $\hbar k_F$ is the Fermi momentum and m^*/m (the ratio of the effective mass at the Fermi surface to the true mass of the helium-3 atom) is equal to the ratio, as $T \to 0$, of the observed specific heat and the specific heat of a perfect Fermi gas with the same particle mass and number density as liquid helium-3. The quantity $1/t$ takes the values 5·56 for the Yntema–Schneider potential, and 5·46 for the de Boer–Michel potential between helium-3 atoms, yielding transition temperatures at the vapour pressure which are only slightly below 0·1° K. (later modifications of the theory by Anderson and Morel (*Phys. Rev.*, to be published) and by Bardasis and Schrieffer[8] reduce the transition temperature to about 0·01° K. and 8×10^{-4} ° K., respectively. Details of this work are not yet available, but according to Emery (private communication), the functional dependence of T_c on m^* would not be drastically changed from that given by equation (1)). Specific heat measurements by Brewer, Daunt and Sreedhar[7] allowed a provisional computation[9] of m^*/m at high pressures, and showed it to be increasing quite rapidly, in general agreement with the Fermi liquid model[10]. It follows from equation (1) that T_c will also increase with pressure, but the variation cannot be calculated with certainty since at the lowest temperature reached by Brewer *et al.* (0·12° K.) the specific heat was not linear in the temperature, and their values of m^*/m were not considered completely reliable.

We have now carried out measurements of the specific heat at constant pressure, C_p, at several different pressures between 15 cm. mercury and 27 atmospheres, and between the temperature limits 0·04° K. and 0·35° K. Again, there is no indication of any anomaly in the specific heat, which decreases smoothly with decreasing temperature, tending to a linear dependence at the lowest temperatures. We have also measured the change in temperature on adiabatic expansion and compression of the liquid near the melting pressure, and find that the mean expansion coefficient (over a range of pressure and temperature) is always negative between 0·04° K. and 0·32° K.

The calorimeter was a hollow copper cylinder (wall thickness 0·3 mm., length 1·5 cm., internal diameter 1·0 cm., approximately) of total capacity about 1·2 cm.[3]. It was packed with powdered cerium magnesium nitrate serving as a thermometer, occupying roughly 70 per cent of its capacity, and the end closed off and sealed, using cold-setting 'Araldite' type *X* 83/11. A manganin wire heater was packed evenly with the cerium salt, one end being shorted to earth and the other spot-welded to a niobium lead which was brought out of the calorimeter through a 0·5-mm. diameter german silver tube filled with the same type of 'Araldite'. Helium-3 (containing less than 1 part in 10,000 of helium-4) was condensed into the calorimeter through a 0·5-mm. diameter german silver tube. The calorimeter was connected through an indium thermal switch (actuated by a superconducting solenoid) to the refrigerant salt, about 50 gm. of potassium chrome alum, which also cooled a copper thermal shield surrounding the calorimeter. The whole assembly was enclosed in a vacuum jacket surrounded by a bath of liquid helium-4 just below 1° K., then by another vacuum jacket and a 4° K. bath of liquid helium. The chrome alum was demagnetized over a period of about 5 hr. from 25 k.gauss and about 0·5° K., the latter temperature being achieved by means of a small copper helium-3 cryostat incorporated into the refrigerant salt, which also cooled the high-pressure helium-3 through the thermal switch. The thermal switch was then closed and final temperatures of about 0·04° K. achieved. Two-stage demagnetization was not successful, probably because of eddy current heating in the calorimeter.

Temperatures of the helium-3 were determined from the ballistically measured susceptibility of the cerium salt. Background heating-rates varied from about 0·1 m.deg./min. at the lowest temperatures to effectively zero above 0·1° K., and were observed for times varying between 5 min. and 12 min. before and after the heating periods. The heater current was put on for times of 0·5–1·5 min., to give rises in temperature of about 5 m.deg. below 0·1° K. and 10–50 m.deg. at the higher temperatures.

A plot of C_p as a function of temperature at pressures of 15 cm. mercury, 11·5 atm. and 26·6 atm. is shown in Fig. 1. The calculations of Brewer *et al.*[7] show that the difference between C_p and C_v is negligible and the difference between C_p (or C_v) at 15 cm. mercury and C_{sat} is also very small. (C_{sat} is the specific heat at the saturated vapour pressure.) Since the volume of helium-3 in the calorimeter has not yet been determined with great accuracy, we have derived absolute values by normalizing the specific heat at 18 atm. to the previously determined values[7] between 0·12° K. and 0·3° K. This pressure was chosen because the present results at 18 atm. show the least scatter (less than ± 2 per cent). When the same normalizing factor is applied to our measurements at the other pressures, the results agree within ± 2 per cent with the earlier determinations, as shown by the crosses in Fig. 1, representing the values of Brewer *et al.* Near the vapour pressure, however (15 cm. mercury), the new values lie rather higher than the linear extrapolation of Brewer *et al.*, who pointed out the possibility of a residual curvature below their lowest temperature of measurement. This will bring the value of effective mass at the vapour pressure into closer agreement with the more recent experiments[5,6], and reduce the discrepancy found between the earlier entropies and those of Abraham, Osborne and Weinstock[11].

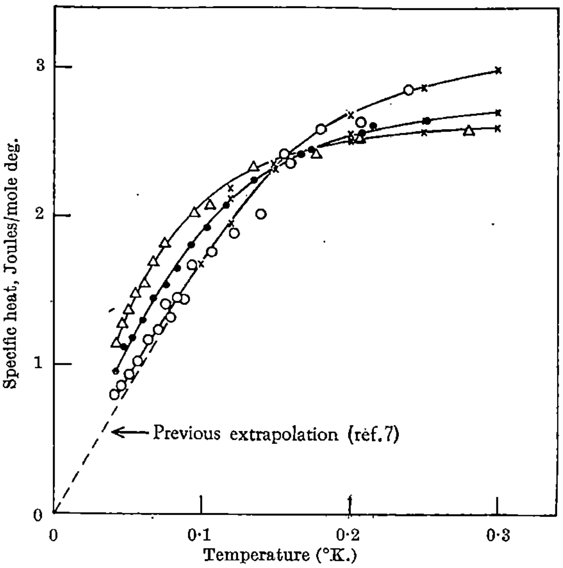


Fig. 1. Specific heat at constant pressure as a function of temperature
○, pressure p = 15 cm. mercury; ●, p = 11·5 atm.; Δ, p = 26·6 atm.; ×, previous measurements (ref. 7). The broken curve gives the linear extrapolation used in ref. 7

The reversal in sign of the pressure dependence of the specific heat[7] at about 0·16° K. is confirmed in these experiments, and positive values of $(\partial C_p/\partial p)_T$ are found down to 0·04° K. Even at this low temperature, it is still not clear that the specific heat has become linear with temperature. However, entropies (S_p) derived by integration of these curves, and using the previous entropy determinations under pressure down to 0·12° K., are more nearly proportional to temperature[9], and we have used them to derive new values of m^* under pressure. At 26·6 atm., the effective mass is found to be 1·78 times greater than at the vapour pressure. It may be calculated from equation (1) that the predicted transition temperature at our highest pressure should be about 3·2 times greater than T_c at the vapour pressure, if we use for $1/t$ the average given by the de Boer–Michel and Yntema–Schneider potentials. Taking an upper limit of 0·008° K. for T_c at the vapour pressure[5], we obtain a maximum value of 0·029° K. for T_c at 26·6 atm., which may be expected to be lower still if the specific heat is not linear at these low temperatures. We wish to emphasize that since the specific heat is still not linear at our lowest temperature, this result is still subject to revision.

As discussed by Brewer and Daunt[9], values of the expansion coefficient at constant pressure, α_p, can be determined from the change of temperature on adiabatic expansion and compression of the liquid, with the help of the equation:

$$\alpha_p = \frac{C_p}{VT}\left(\frac{\partial T}{\partial p}\right)_s \qquad (2)$$

where V is the molar volume. We have determined mean values, $\bar{\alpha}_p$, between 0·045° K. and 0·32° K., over changes in pressure of 4·7 atm. and 1·4 atm., and at average pressures of 25·2 and 26·9 atm. Fig. 2 shows the observed values of $\Delta T/\Delta p$, which are reasonably consistent with the previous observations at higher temperatures and lower pressures[9].

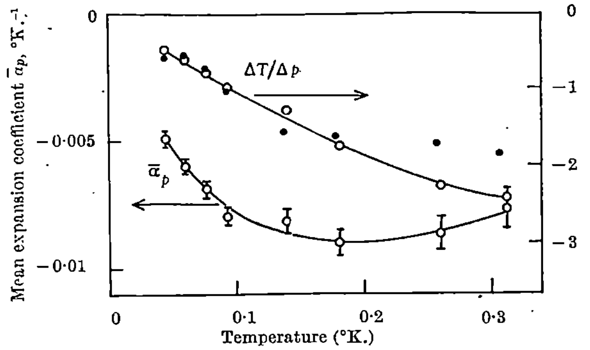


Fig. 2. The change in temperature on adiabatic compression and expansion, $\Delta T/\Delta p$ (in m.deg. atm.$^{-1}$), and the mean expansion coefficient, $\bar{\alpha}_p$ (°K.$^{-1}$), as a function of temperature

○, $\Delta_p = 4·7$ atm., average pressure = 25·2 atm.; ●, $\Delta_p = 1·4$ atm., average pressure = 26·9 atm.

Since the changes in temperature on expansion or compression through 1·4 atm. are very small, the results at the average pressure 26·9 atm. may have an error of up to 50 per cent. Values of $\bar{\alpha}_p$ shown in Fig. 2 have been calculated only for the larger changes in pressure, but in all cases the mean expansion coefficient is found to be negative, as is the case at higher temperatures[9,12,13]. This observation does not fit in readily with the result of Mills, Grilly and Sydoriak[14] (based on measurements of the molar volume near the melting curve), who conclude that $\alpha_p = 0$ at the minimum in the melting curve (0·32–0·33° K. and about 29 atm.[13–15]).

Although results for $\bar{\alpha}_p$ obtained by the present method are inherently not very accurate, it is difficult to find any qualitative error, since expansion always leads to a warming and compression to a cooling effect, which, within experimental accuracy, are of the same size. It therefore appears that there may be some discrepancy in the experimental observations of α_p, unless the behaviour of the liquid changes quite sharply between our average pressure of 26·9 atm. and the melting curve.

We are grateful to Dr. N. Kurti for several helpful discussions and suggestions. We also wish to thank Dr. V. J. Emery for discussions during the writing of this article, Mr. R. March for assistance with experiments and computer programming, and Miss K. Merrick for help with data processing on the computer.

[1] Cooper, L. N., Mills, R. L., and Sessler, A. M., *Phys. Rev.*, **114**, 1377 (1959).

[2] Pitaevski, L. P., *J. Exp. Theor. Phys.* (U.S.S.R.), **37**, 1749 (1959) (translation: *Soviet Phys. J.E.T.P.*, **10**, 267 (1960.)

[3] Brueckner, K. A., Soda, T., Anderson, P. W., and Morel, P., *Phys. Rev.*, **118**, 1442 (1960).

[4] Emery, V. J., and Sessler, A. M., *Phys. Rev.*, **119**, 43 (1960).

[5] Anderson, A. C., Salinger, G. L., Steyert, W. A., and Wheatley, J. C., *Phys. Rev. Letters*, **6**, 331 (1961).

[6] Strongin, M., Zimmerman, G. O., and Fairbank, H. A., *Phys. Rev. Letters*, **6**, 404 (1961).

[7] Brewer, D. F., Daunt, J. G., and Sreedhar, A. K., *Phys. Rev.*, **115**, 836 (1959).

[8] Bardasis, A., and Schrieffer, J. R., *Phys. Rev. Letters*, **7**, 79 (1961).

[9] Brewer, D. F., and Daunt, J. G., *Phys. Rev.*, **115**, 843 (1959).

[10] Brueckner, K. A., and Gammel, J. L., *Phys. Rev.*, **109**, 1040 (1958).

[11] Abraham, B. M., Osborne, D. W., and Weinstock, B., *Phys. Rev.*, **98**, 551 (1955); *Physica*, **24**, 132 (1958). Weinstock, B., Abraham B. M., and Osborne, D. W., *Supp. Nuovo Cimento*, **9**, 310 (1958); and private communication.

[12] Kerr, E. C., and Taylor, R. D., *Seventh Intern. Conf. Low Temp. Phys.*, 333 (University of Toronto Press, 1960). Sydoriak, S. G., Mills, R. L., and Grilly, E. R., *Phys. Rev. Letters*, **4**, 495 (1960).

[13] Lee, D. M., Fairbank, H. A., and Walker, E. J., *Phys. Rev.*, **121**, 1258 (1961).

[14] Mills, R. L., Grilly, E. R., and Sydoriak, S. G., *Ann. Phys.* (U.S.A.), **12**, 41 (1960).

[15] Baum, J. L., Brewer, D. F., Daunt, J. G., and Edwards, D. O., *Phys. Rev. Letters*, **3**, 127 (1959). Edwards, D. O., Baum. J. L., Brewer, D. F., Daunt, J. G., and McWilliams, A. S., *Seventh Intern. Conf. Low Temp. Phys.*, 328 (University of Toronto Press, 1960).

INTRACELLULAR FREEZING AND FROST RESISTANCE IN EGG-CELLS OF THE SEA URCHIN

By E. ASAHINA

Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan

Intracellular Freezing as a Cause of Frost-killing in Relatively Rapidly Frozen Cell Suspensions

It is well known that in a variety of living cells, when they were subjected to freezing, a considerable number of them are very liable to be damaged within the first very short period of time unless the cooling is very slow. Luyet and Menz demonstrated in freezing blood cells that even at near-zero temperatures the degree of hæmolysis increased markedly within the first twenty minutes of freezing[1]. Because of the negligible increase in hæmolysis before the frozen blood is cooled to temperatures below −3° C., they seem to favour the view that injury due to dehydration (or concentration) is a main cause of frost-killing. However, the result of their experiment did not, as they suggested, exclude other interpretations. After the frozen blood was cooled to a temperature near that of the cooling bath, there was found only a very slight increase in hæmolysis. This is by no means explained by salt-injury due to concentration at the time of freezing. Since the cooling-rate of freezing blood was shown to be rapid only within the first twenty minutes, the rate of cooling may perhaps be responsible for frost-killing. Also in freezing sea-urchin egg-cells suspended in sea-water nearly the same result as mentioned above was found (Fig. 1).

Unfertilized eggs of *Strongyrocentrotus nudus* were used as material. Four test-tubes containing 0·5 ml. of egg suspension were cooled simultaneously in a cooling bath of ice–salt mixture at − 16° C. In each test-tube, the egg suspension was seeded with small ice-crystals when it was cooled to about −2° C. As the change of temperature of the egg suspension measured by a small thermistor (1 mm. in length) was very similar in each of the four test-tubes, only one typical freezing-curve is shown in Fig. 1. After 2, 5, 15 and 30 min. from the time of ice-seeding, these test-tubes were removed singly and each of them was re-warmed rapidly in water at 20° C. Egg-cells frozen and thawed in this way were observed under a microscope for counting the intact cells. The percent-

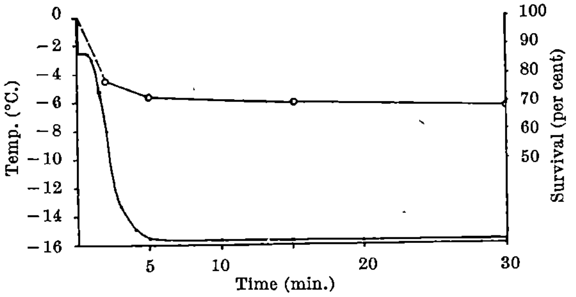


Fig. 1. A freezing-curve of cell suspension (●), and the percentage survival in cells frozen for various lengths of time (○)

age survival in Fig. 1 is based on the number of intact cells counted. At the same time, microscopic observations of the freezing process of sea-urchin eggs were made many times at nearly the same cooling-rate in a special cold room. A small amount of egg suspension was cooled under the microscope in a silicone-oil hanging drop in which the tip of a fine thermocouple was inserted. When the egg suspension was cooled to -4° C. it was seeded by means of an ice-tipped pipette. Soon after they were surrounded by frozen sea-water the egg-cells began to freeze intracellularly. At a cooling-rate of 5° C. per min., the type of intracellular freezing in a sea-urchin egg was a typical 'flashing', a very rapid darkening due to an almost instantaneous formation of a large number of minute ice-crystals throughout the cell[2]. 'Flashing' most frequently took place in egg-cells within about 30 sec. from the time of seeding, but afterwards its occurrence was reduced rapidly. After a few minutes some of the egg-cells, in which some degree of dehydration by extracellular freezing had already been found, still froze intracellularly, but the freezing process in these cells was not 'flashing'. Ice formed from a few centres of crystallization at the cell surface and gradually grew in the cytoplasm within some twenty seconds as 'irregular rosette'-type crystals[3] (Fig. 2). The amount of intracellular ice crystal varied; ice usually never occupied the whole of a frozen cell. After 4 min. or more of freezing, no additional egg-cells were found to freeze intracellularly; instead, they underwent a marked contraction. On thawing most of those cells recovered their normal size and shape.

The egg-cells of the sea-urchin can, as a rule, resist extracellular freezing without any protective additives[2]. Unfertilized eggs of *S. nudus* survived freezing at -10° C. for a full day and even at -20° C. at least for 30 min. provided they were cooled very slowly to that temperature (Asahina, unpublished results). Also in the present experiment, insemination after rewarming was followed by normal cell division in nearly all the intact cells. Intracellular freezing, on the other hand, always resulted in apparent cytolysis or a fatal coagulation of cytoplasm regardless of the amount of ice formed in the cell. It seems reasonable to assume, therefore, that under the present experimental conditions the main cause of frost-killing is intracellular freezing, and that after a cell is in contact with ice the rate of cooling may be responsible for the initiation of intracellular freezing. In freezing plant tissues, too, it has long been known that a rapid rate of cooling is highly effective in causing intracellular freezing[4,5].

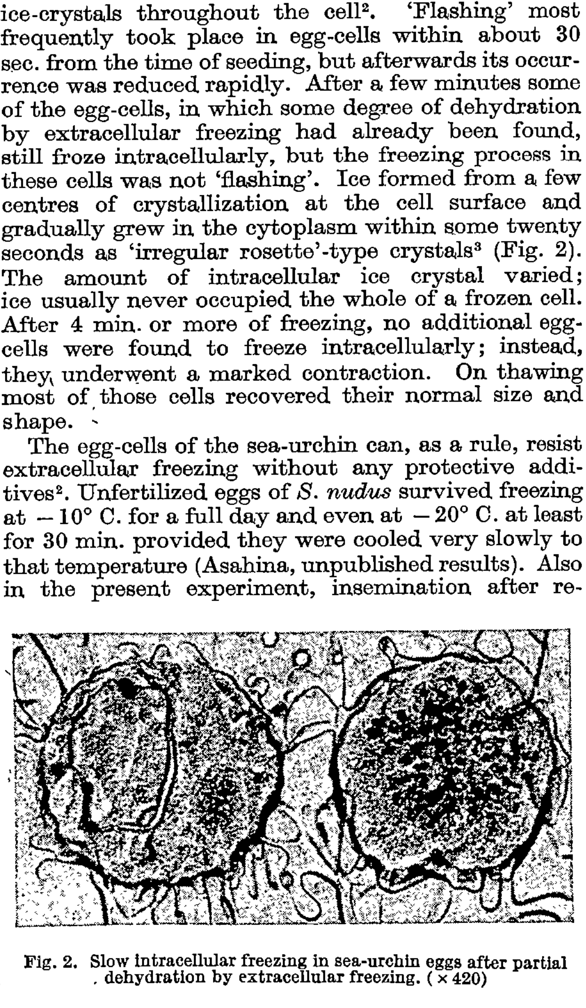


Fig. 2. Slow intracellular freezing in sea-urchin eggs after partial dehydration by extracellular freezing. (×420)

A Mechanism to prevent the Seeding of Intracellular Ice in Freezing Living Cells

The experiment reported in the previous communication clearly demonstrated that the main cause of frost-killing in relatively rapidly cooled cell suspensions is intracellular freezing, and that a high rate of cooling is very effective in causing intracellular freezing in a living cell which has been in contact with ice. A careful microscopic observation revealed that egg-cells of sea-urchin, suspended in silicone oil, never froze spontaneously even when they were cooled to a temperature as low as -15° C. (Asahina, unpublished results), but in these egg-cells, ice seeding on to the surrounding sea-water film could cause intracellular freezing even at a temperature of -8° C. (ref. 2). These results can be explained as due to the initiation of freezing within the egg-cell as a result of inoculation from the outside with ice crystals at least at moderately low temperatures. If this hypothesis is valid, an elevation of temperature at the interface between the cell and the surrounding ice-mass will be effective in preventing the ice seeding into the cell. The present article deals with evidence on this problem.

Egg-cells of a sea-urchin (*Strongyrocentrotus nudus*), both fertilized and unfertilized, were used as material. The apparatus and the method in the present experiment were almost the same as those described in the previous investigation. In the case of fertilized eggs, they were subjected to freezing after having been kept at 15° C. for 30 min. after fertilization. Four test-tubes containing 0·5-ml. aliquots of suspensions of egg in sea-water and a small thermistor for temperature measurement were cooled simultaneously in a cooling bath at -20° C. In each test-tube, egg suspension was seeded with small ice crystals when it was cooled to about -3° C. To achieve slow cooling, a double-walled test tube with adequate distance between the two walls was used. After preliminary freezing tests, two rates of cooling were applied for the experiment. One of these, rapid cooling, had a cooling-rate of about 9° C. per min., while the other, slow cooling, had a rate of about 1° C. per min. These cooling-rates were measured between -7° C. and -13° C., because, when cooled at these cooling rates, intracellular freezing in suspended cells was most frequently observed under the microscope within this temperature-range. In the case of rapid cooling the test-tubes were immersed in the cooling bath for only 5 min., while in the slow cooling they were kept there for 30 min. Egg-cells frozen in this way were thawed rapidly by immersion of the test tubes in water at 20° C.; then they were observed under the microscope for counting intact cells.

The result of this experiment showed that most of the fertilized eggs were very resistant to both rapid and slow freezing, while practically all the unfertilized eggs

Table 1. PERCENTAGE SURVIVAL AFTER FREEZING IN THE EGG-CELLS OF SEA-URCHIN

	Mean values of 6 experiments		
	Cooling rate	Fertilized eggs	Unfertilized eggs
Rapid cooling	9° C./min.	84·1 ± 7·6	2·2 ± 2·1
Slow cooling	1° C./min.	90·3 ± 5·0	74·0 ± 5·9

could not withstand rapid freezing although they, too, could survive slow freezing (Table 1). After thawing, a characteristic pattern[2] of coagulated protoplasm, which resulted from intracellular freezing, was found in most of the killed cells. The intact cells, on the other hand, could generally resume their development. Even the very few survivors in rapidly frozen unfertilized eggs could cleave normally after insemination. The high frost-resistance[8] noted in fertilized eggs was certainly not due to the existence of a fertilization membrane which is formed on fertilization, because a fertilized egg from which the fertilization membrane was removed artificially was found to be as resistant as intact fertilized eggs to rapid freezing.

A reasonable explanation of these observed results may be that in egg-cell suspensions, after the sea-water has begun to freeze at near zero temperatures, the frozen mass is promptly or gradually cooled. As the cooling proceeds, the ice which is now in contact with the cell surfaces withdraws water from the cell interior because of the difference in chemical potential between the ice and supercooled water within the cell. The water withdrawn in this way successively crystallizes at the interface between the cell and surrounding ice until most of the easily freezable water at a given temperature is lost from the cell ; after this time ice-formation within the cell is scarcely achieved even with intracellular ice seeding[2]. In these processes, if the cell surface is promptly cooled far below the freezing point, the water molecules within the living protoplasmic system can be caused to arrange themselves as an ice crystal by seeding from outside ice. But if the temperature at the cell surface is kept only a few degrees below the freezing point, the water within the protoplasm cannot crystallize and only the outside ice-crystal grows, withdrawing water from the cell interior. This can be performed effectively in the case of slow cooling since the release of latent heat of ice-crystallization at the cell surface is supposed to be sufficient to keep the temperature not much below freezing-point.

It has been amply demonstrated by many authors that, in the egg-cells of various kinds of sea-urchins, the rate of entrance or exit of water into or from the cell increases remarkably after fertilization. In an egg of the *Arbacia* the permeability of the plasmic membrane to water was found to be four times as great in the fertilized as in the unfertilized egg[6]. In the cooling of egg-cells which have been in contact with ice, this character may certainly cause a larger decrease in cooling-rate at the surface of fertilized than of unfertilized cells. The reason is that in a fertilized egg the release of latent heat per unit period of time at the cell surface is larger than in an unfertilized one, because of the higher rate of extracellular ice formation in the former unless the cooling is sufficiently slow. Even if such decrease in cooling-rate is only temporary, the cell protoplasm may be sufficiently dehydrated, within the first short period of freezing, to prevent intracellular freezing. It was reasonable therefore that in the freezing of egg suspensions cooled at an adequate rate, say 9° C. per min., intracellular freezing occurred much more rarely in fertilized than in unfertilized eggs.

In freezing plant cells, almost the same mechanism to prevent the intercellular freezing has already been suggested by me[4]. By using freezing yeast cells, Mazur recently came to a somewhat similar conclusion[7].

[1] Luyet, B. J., and Menz, L. J., *Biodynamica*, **7**, 25 (1951).
[2] Asahina, E., *Low Temp. Sci.*, **10**, 81 (1953).
[3] Luyet, B. J., and Rapatz, G., *Biodynamica*, **8**, 1 (1958).
[4] Asahina, E., *Contr. Inst. Low Temp. Sci.*, **10**, 83 (1956).
[5] Levitt, J., *The Hardiness of Plant* (Acad. Press, N.Y., 1956).
[6] Lillie, R. S., *Amer. J. Physiol.*, **40**, 249 (1916).
[7] Mazur, P., *Biophys. J.*, **1**, 247 (1961).

ELECTRICAL ACTIVITY OF A GIANT NERVE CELL UNDER ABNORMAL CONDITIONS

By DR. YUTAKA OOMURA, SACHIO OZAKI and TAKASHI MAÉNO
Department of Physiology, Faculty of Medicine, Kagoshima University

RECENTLY it was found that a node of Ranvier of the toad's nerve could generate action potentials in sodium-free isotonic choline chloride, calcium chloride, potassium nitrate or even in a sucrose solution, when an adequate intensity of hyperpolarizing current was applied to the nodal membrane[1]. Some results from experiments on the spinal ganglion cells of frogs also have shown that the electrical activity was maintained in a sodium-free solution of nitrogenous compounds[2].

On the giant nerve cells (150–400μ in diameter) of the supracœsophageal ganglion of a marine pulmonate mollusc, *Onchidium verruculatum*, observations basically similar to those of the node of Ranvier were made by us. These cells were able to generate the action potential in the 'all or none' manner in various sodium-free solutions, when the normal concentration of calcium ions was maintained.

Two intracellular micro-electrodes filled with 3 *M* potassium chloride were inserted into the single cells, one for recording potential, and the other for passing current through the cell membrane (to direct stimulation of the cell membrane). For synaptic and antidromic excitations, two groups of the roots of the ganglia, placed on two pairs of electrodes, were stimulated. The experiments were carried out at a temperature of 15–25° C.

As reported in ref. 3, the resting potentials of the giant nerve cells in normal sea-water (sodium chloride 459·6 m*M*, potassium chloride 13·7 m*M*, calcium chloride 10·4 m*M*, magnesium chloride 48·5 m*M*) were 40–60 mV., the inside being negative. The

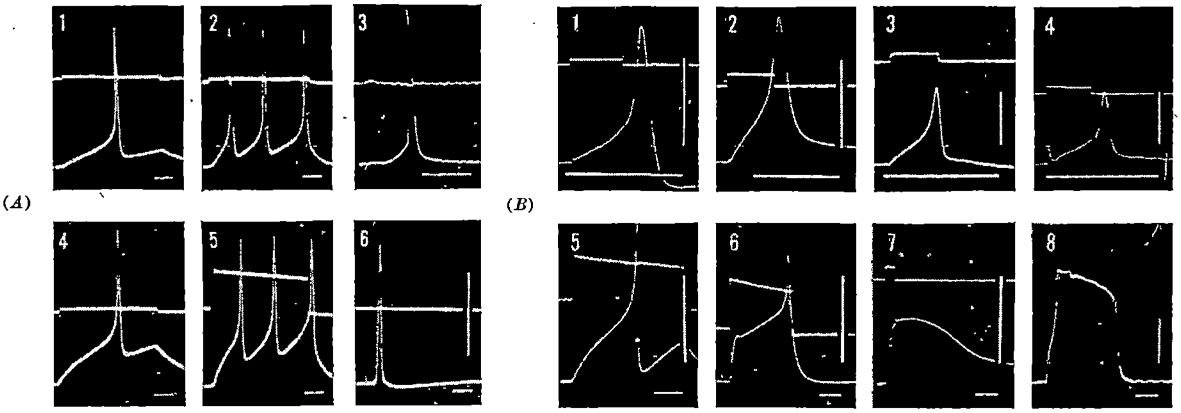


Fig. 1. *A*: Action potentials in normal sea-water (upper) and in sodium-free choline sea-water (lower) at 21° C. 1, 2, 4 and 5, action potentials generated by directly applied transmembrane current of long duration; 3 and 6, by stimulations of the roots. The resting potential increased by about 12 mV. in choline sea-water. *B*: Action potentials in various sodium-free solutions. Sodium ions in the external solution were replaced with sucrose (1), choline (2), inositol (3), ethylene glycol (4), calcium (5), magnesium (6), potassium (7) and barium (8). 20° C. Upper trace, transmembrane current; lower trace, membrane potential. Vertical and horizontal scales, 50 mV. and 100 msec., respectively

average membrane resistance and capacity were 6·8 MΩ and 0·027 μF. The amplitude of the action potentials produced by both synaptic and indirect stimulations were 75–95 mV., and if a supra-threshold polarizing current were prolonged, similar spike potentials appeared repetitively (Fig. 1*A*). With increase of hyperpolarizations in the membrane, in most cells, the spike potential produced by the direct stimulation increased the value in the critical threshold potential and failed to grow to a full-sized spike. Since such abortive pre-potential appeared by antidromic stimulations under hyperpolarization, it was concluded that the pre-potential represents an electrotonic spread of the spike in the axon to the soma membrane due to the small safety factor at the soma-axonal junction.

When the perfusing external solution was changed to choline sea-water in which total sodium chloride was replaced with isomolar choline chloride and the other components unchanged, the resting potential was hyperpolarized by about 12 mV., and full-sized action potentials without appreciable reduction in the height of the spikes were recorded for more than 3 hr. in this perfusing solution by the direct, synaptic and antidromic stimulations. The membrane resistance was not altered. In some cells only the abortive prepotentials appeared by direct stimulations, due to the hyperpolarization caused by choline. Thus the full-sized action potential was produced by the restoration of the resting potential towards the normal level by an application of a depolarizing current. Similar results were obtained with the replacement of sodium chloride in sea-water with sucrose, inositol and ethylene glycol, though the spike-height decreased by about 5 mV. in these solutions (Fig. 1*B*).

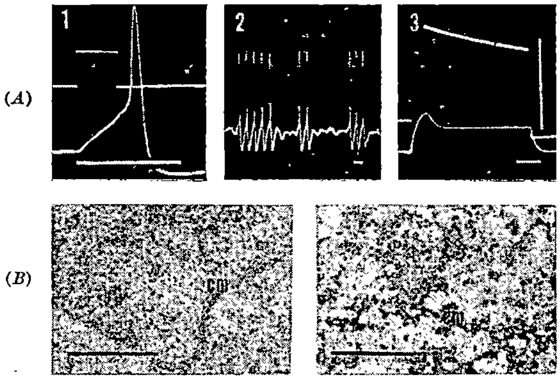


Fig. 2. *A* : Changes in electrical activity in calcium-free solutions. 1, action potential in normal sea-water; 2, increased spontaneous firing in the early stage in calcium-free sea-water; 3, graded response in calcium-free isotonic choline solution at 20° C. The resting potential decreased by 10 mV. in this medium, and no action potential could be generated. Note the decrease in the membrane resistance. Vertical scale, 50 mV. for 1 and 3, and 20 mV. for 2; horizontal scale, 100 msec. *B*: electronmicrographs of nerve cell membrane in normal sea-water (left) and calcium-free sea-water (right) showing deteriorations in the membrane structures. c.m., cell membrane; scale, 2μ

The presence of a diffusional barrier around the cell membrane for producing the action potential in a solution deficient in sodium is inconceivable, because the resting potential was depolarized abruptly when the perfusing external solution was changed to sea-water containing potassium. Even in sea-water free from sodium but containing potassium, action potential could be generated if the resting potential were hyperpolarized beyond the normal level, similar to the case of the node of Ranvier[1].

In ethylene glycol sea-water, however, the electrical activity was maintained for about 30 min. and the membrane had a tendency to deteriorate, especially when the external solution was changed to normal solution after long exposure in ethylene glycol sea-water. The deteriorative effect was attributable to the relatively high permeability of ethylene glycol into the cell membrane and to the reduction of the membrane resistance.

The action potentials also could be produced in isotonic sea-water containing magnesium chloride (magnesium chloride 370·4 m*M*, potassium chloride 13·7 m*M*, calcium chloride 10·4 m*M*) with a higher level of critical threshold depolarization than that in normal sea-water.

It is well known that marked electrophysiological disturbances occur in excitable tissues in calcium-free Ringer solutions[2,4]. Similar observations were made in the sodium-free solutions.

The action potentials in the various solutions also could be abolished by the withdrawal of external calcium and magnesium ions from the perfusing solutions. During 30 min. in this solution, the resting potential depolarized gradually by several millivolts, the value of the membrane resistance was gradually, then abruptly, reduced to one-third. The cell membrane could not produce the action potential by direct, synaptic or antidromic stimulations. Only in the very earlier stages of the calcium-free media could the membrane properties be restored by application of the calcium ions or by application of a few millivolts of anodal polarization to the cell membrane. In both cases an accompanying increase of the membrane resistance resulted. In later stages the anodal polarization did not affect the loss of excitability of the cell membrane. This is contrary to the case of the Ranvier node[1]. The effect of magnesium ions on the membrane properties was similar to that of calcium, but they were less effective.

Light-microscopic and electronmicroscopic observations revealed a considerable deterioration and myelolysis in cell soma and nerve fibre, and a thinning and decomposing of the membrane structure of the cell and of mitochondria after 30 min. immersion in calcium-free sea-water (Fig. 2*B*). This histological evidence accords with the reduction of membrane resistance.

We conclude that the electrical activity of the nerve cell membrane of *Onchidium* depends only on the calcium ions, and that keeping the membrane resistance at a normal value, that is, a normal configuration of the cell membrane by the calcium ions, is most important for excitation.

This investigation was supported by a grant from the Ministry of Education and from the Muscular Dystrophy Associations of America, Inc.

---

[1] Oomura, Y., Maruhashi, J., and Tomita, T., *J. Physiol. Soc. Japan*, **21**, 961 (1959).

[2] Koketsu, K., and Nishi, S., *J. Neurophysiol.*, **22**, 693 (1959).

[3] Hagiwara, S., and Saito, N., *J. Physiol.*, **148**, 161 (1959).

[4] Frankenhaeuser, B., and Hodgkin, A. L., *J. Physiol.*, **137**, 218 (1957).

BLOOD-TYPING COMPACT HUMAN BONE TISSUE

By H. E. EZRA-COHN and PROF. S. F. COOK

Department of Physiology, University of California, Berkeley

IN a series of papers after 1935, Candela[1] reported that he was able to secure positive blood group reactions in human skeletal material from archæological sources. He was of the opinion that his method yielded results of adequate reliability. Schiff and Boyd[2] also argued that blood-typing procedures could be adapted for work with bone as well as mummified muscle. More recently, Thieme and Otten[3] have questioned the validity of such tests when applied to archæological material. They cite various sources of error: bacterial enzyme destruction of blood-group antigens, non-specific absorption of contaminants, and even the presence of adventitious antigenic elements which cannot be distinguished from blood-group substances. They say: "In conclusion, results obtained in the typing of aged bone or stains by the inhibition test do not appear reliable under some conditions". It is to be noted that Thieme and Otten did not report in this paper any work done with archæological specimens, but rather with synthetically aged specimens. Their bone samples, mostly lumbar vertebræ, were taken at autopsy, ". . . were buried in stone jars in sandy soil, and aged from 2 to 3 years". It is thus apparent that the physical and chemical conditions to which the bones were exposed resemble very little those governing the history of skeletons preserved in caves or buried directly in the ground for centuries.

There is one consistent feature of the research carried on in the recent past by archæologists with bone blood grouping, and it is carefully pointed out by Smith[4], who surveys the present status of palæoserology. This feature is that in all reported cases the bone tissue used for testing has been cancellous. Wiener[5] pointed this out in his discussion of Candela's work, and said: "The success in the grouping of skeletal remains by the absorption technique probably depends on the presence of dried marrow and blood on the lining of the cancellous bone used for examination". At the same time it seems altogether reasonable that the organic substances present in the relatively large quantities of blood stored in the cavities of the spongy bone would be exposed particularly to the action of chemical changes and micro-organisms. Organic material locked in the interior of solid, compact bone such as the diaphysis of the humerus or femur, would be much less accessible to destructive or modifying influences.

Very recently new light was shed on the entire problem by Weinberg and Makin[6]. These workers made a careful comparison of the presence of *A* and *B* antigens in fresh human bone. They made three distinct preparations: (1), clean, compact, cortical bone; (2) cancellous bone which was taken, without modification, directly from the cadaver; (3) cancellous bone which had been subjected to very thorough washing. They found a great deal of antigen in the untreated cancellous bone, much smaller, but still perceptible amounts, in the cleaned cancellous bone, and no detectable quantity in the cortical bone. The conclusion is therefore admissible that the appearance of antigen is associated with the body fluids (blood, for example) in contact with the bone at the time of death. The findings of Weinberg and Makin render very doubtful the presence of antigens in compact bone tissue itself. Hence, the appearance of positive typing in archæological compact bone tissue strongly implies an external or post-mortem origin of the antigen-like substance.

With these considerations in mind, it seems worth while to describe a series of tests made in this laboratory on samples of compact bone which had been exposed to weathering in the soil for periods of 1,000 years and more.

Our tests were made on 21 samples of compact bone from various sites (see Table 1). Triplicate tests were run on each sample. For comparison we also ran tests on 14 compact bones from fresh autopsy material. Only the *ABO* antigens were tested. The archæological material consisted of 14 samples from White Mound, Arizona, an open site of the Gila Pueblo culture, dating A.D. 700–850, four specimens from four different open sites in Marin County, California, and two from Piedra Gorda, Baja, California.

The natural (not immune) *A* and *B* sera were prepared in suitable dilution. Merthiolate 1 : 5,000

Table 1

Specimen	Inferred blood group
14 bones from different white adult autopsies	*O*
21 fossil compact bones	
1 White Mount, Ariz.	*O*
2 ,,	*A*
3 ,,	*O*
4 ,,	*B*
5 ,,	*O*
6 ,,	*B*
7 ,,	*AB*
8 ,,	*O*
9 ,,	*B*
10 ,,	*O*
11 ,,	*O*
12 ,,	*A*
13 ,,	*A*
14 ,,	*O*
15 Mar-266, Cal.	*B*
16 Mar-265*A*, Cal.	*O*
17 Mar-242, Cal.	*AB*
18 ,,	*A*
19 Mar-232*B*, Cal.	*B*
20 B.C.-144, Baja, California	*O*
21 ,, ,,	*AB*

was added to the typing sera as preservative. The samples of bone were moistened with normal saline, homogenized and dried at 37° C. for three days.

To each of a series of tubes was added 100 mgm. of sample and 0·5 ml. of *A* and *B* serum. These were mixed thoroughly, incubated in the cold for 48 hr. with shaking at 12-hr. intervals, and centrifuged at 1,500 r.p.m. The supernatant fluid was tested by the Moss–Lee–Vincent rapid slide method for residual anti-*A* and anti-*B* agglutinins. (We use these terms in the senses indicated: agglutinogen, agglutinable substance in blood corpuscles; agglutinin, agglutinating substance in blood serum; antigen, general term referring to any agglutinable factor; antigen *A*, a substance which, with respect to agglutination, behaves chemically as agglutinogen *A*; antigen *B*, analogous to antigen *A*; blood group substance, antigen found in some tissues and fluids of the body other than blood, which conforms in chemical reaction to agglutinin in the manner of the agglutinogens of that body.)

From Table 1 we see that the compact bones from the 14 autopsies (the blood types of which were known) gave no antigenic reaction. These results would, therefore, tend to confirm those of Weinberg and Makin. On the other hand, of the 21 samples of compact bone from the Indian burials, 9 were type *O*, 4 were *A*, 5 were *B* and 3 were *AB*.

The findings with ancient bone would at first sight seem to contradict the thesis that compact bone cannot be typed, for it is clear that a relatively large proportion of the specimens tested showed an *A*, a *B* or an *AB* reaction. Nevertheless, we believe that the evidence is sufficient to warrant the conclusion that the observed *A*, *B* and *AB* reactions are not referable to blood group substances contained in the bone at the time of death. The following considerations support such a theory.

(1) Weinberg and Makin were unable to detect any blood group substances in numerous samples of fresh bone from tibia, ribs, sacrum and phalanges. Likewise, we were unable to find any antigen-like substances in 14 different compact bone samples from 14 different autopsies. In contrast, we observed positive reactions in 12 out of 21 cases of archæologically preserved bone. The statistical disparity is definite and striking.

(2) Since the prehistoric samples were all derived from native American Indians the high incidence of the *B* group is peculiar, for this blood group has been demonstrated repeatedly to be very nearly absent among North American aborigines.

(3) The climatic conditions to which the 21 bones were exposed were widely different. Fourteen were derived from open burial sites in the south-western United States, and two were from Lower California. Both these areas have been characterized by unusually hot, dry climates since the probable date of interment. The five bones from Marin County, California, had been exposed to the moderate climate of the Pacific coast. The former group showed 8 out of 16 *A*, *B* or *AB* reactions, whereas the latter group showed 4 out of 5 such reactions. There is not enough difference in the relative proportion of antigenic reactions to warrant ascription to climatic factors. This is noteworthy because in a climate characterized by relatively warm summers and cool, rainy winters there was adequate opportunity for bacterial action and chemical alteration to have occurred in the compact tissues so as to result in a product which could conceivably have an antigenic nature. This explanation, however, cannot be used to account for the high occurrence of *A*, *B* and *AB* types in samples which had been exposed to a very dry, warm environment.

(4) It is very probable that in clean, compact bone the blood present in the capillaries is not sufficient in quantity to give a positive blood group test. It would be expected, therefore, that archæological bone material would likewise be unreactive. The fact that it is reactive may be considered to be due to the effect of soil solutes and organisms. The organic matrix of the bone undergoes decomposition into products which are capable of generating antigenic reactions, or such substances are formed by biological (enzyme) action and penetrate the bone. Full consideration of these influences will be found in the papers of Iseki[7] and Salazar Mallen[8]. Hence it is likely that the 14 positive reactions which we observed in the 21 excavated bones were artefacts and bore no necessary relation to the true blood group of the individuals from whom the bone was derived. It would appear that serological techniques can yield no results of validity when applied to compact bone from burial sites.

(5) Similar considerations apply to cancellous bone. It is of course true that cancellous bone may give valid blood group reactions by virtue of retention of traces of blood. Here the test would actually be on the agglutinogens, or their products, which were derived from the blood, not on the bone tissue itself. The use of cancellous bone, however, is also complicated by the same factors which produce spurious positive tests in compact bone, that is, the possible presence of either decomposition products of the organic matrix of the bone (and/or residual body fluids) or of antigenic compounds formed by factors from the soil. Unless these factors can be eliminated positive blood group tests with ancient or archæological cancellous bone must be accepted only with serious reservation.

This investigation was supported in part by National Science Foundation grant *G*–5990. We acknowledge this support with thanks.

[1] Candela, P. B., *Amer. J. Phys. Anthrop.*, **27**, 367 (1940).

[2] Schiff, F., and Boyd, W. C., *Blood Grouping Technic* (Interscience Publishers, New York, 1942).

[3] Thieme, F. P., and Otten, C. M., *Amer. J. Phys. Anthrop.*, N.S., **15**, 387 (1957).

[4] Smith, M., *Science*, **131**, 699 (1960).

[5] Wiener, A. S., *Blood Groups and Transfusion*, third ed. (C. C. Thomas, Springfield, Ill., and Baltimore, Md., 1943).

[6] Weinberg, H., and Makin, M., *Bone and Joint Surgery*, **41B**, 151 (1959).

[7] Iseki, S., and Ikeda, T., *Proc. Jap. Acad.*, **32**, 201 (1956).

[8] Salazar Mallen, M., *Gaceta Medica de Mexico*, **81**, 122 (1951).

CHROMATOGRAPHY OF PROTEINS ON DIETHYL-AMINO-ETHYL (DEAE) CELLULOSE PAPER

By C. C. CURTAIN
Baker Medical Research Institute, Alfred Hospital, Prahran, Melbourne

CHROMATOGRAPHY of proteins on columns of DEAE cellulose powder[1] has proved a valuable preparative tool, but it is unsuitable for analytical work on very small samples, or large-scale surveys of serum proteins or other fluids.

This article outlines a system for the quantitative chromatography of proteins on Whatman DEAE cellulose paper, *DE*20. The paper (supplied by Messrs. Reeve, Angel, London) has an exchange capacity of approximately 0·4 m.equiv. per gm. or 0·003 m.equiv. per cm.^{2}.

As with cellulose ion exchangers in columns, the R_F value of most proteins on the paper is either 0 or 1, and except over a very narrow range of *p*H and ionic strength, virtually specific to each protein. Separation of mixtures must therefore be achieved by *p*H and ionic strength-gradient elution. Moreover these gradients must be controlled so that the entire pattern is developed within the length of the paper strip. This can be achieved either by making the gradient generated in a conventional mixing chamber pass through the trough in which the upper end of the paper is dipped or by using a generator the rate of flow of which can be matched accurately to that of the paper.

I used the second method, producing the gradient with a photoelectrically controlled gradient generator. A block diagram of this device is shown in Fig. 1. It consists of a photoelectric scanner which translated the light and dark areas in a graph of the gradient curve into varying currents in two electrolysis vessels, and hence into varying gas pressures in two bottles containing the respective gradient limits. Tubes pass from these bottles to a micro-mixing chamber which feeds the top of the paper strip. Detailed circuits of the generator and particulars of its application to column chromatography will be published later.

Before chromatography, the DEAE cellulose paper is cut into strips 5 cm. wide by 50 cm. long, which are placed in the chromatography tank and washed by a descending, successive flow of the following: 0·05 *N* hydrochloric acid, distilled water, the buffer representing the initial gradient limit. The paper is then dried in a current of cool air. 0·05–0·15 ml. of the protein mixture, which has been dialysed against the starting buffer is pipetted in a line 3–5 cm. from the top of the strip, which is inserted into a slit in a piece of 3-mm. polyvinyl chloride cannula and the edge sealed with silicone grease. This cannula, which is sealed at its outer end, is fitted to the outlet of the mixing chamber. The cannula between the chamber and the strip is kept as short as possible (< 5 cm.). The mixing chamber and strip are then inserted into a chromatography tank, which is kept in a room at 4° C. The bottom of the tank is filled to a depth of 1 cm. with water to saturate the atmosphere with water vapour. The gradient generator is set to run at the flow-rate of the strip. At the conclusion of the run the strip is removed from the tank and dried for 30 min. in a stream of air at an average distance of 20 cm. from a 500-W. infra-red lamp. After drying, the strip is immersed for 5 min. in a bath consisting of 60 parts of aqueous 0·075 *M*, *p*H 9·0 borate buffer and 40 parts of methanol. It is then transferred to a bath containing 500 mgm. of victoria blue *B* in 100 ml. of the same solvent. After staining for 10 min., the strip is decolorized by washing for 4 hr. in hourly changes of a bath consisting of 65 parts of 0·075 *M* *p*H 9·0 borate buffer and 35 parts of methanol. The strip is then dried in air.

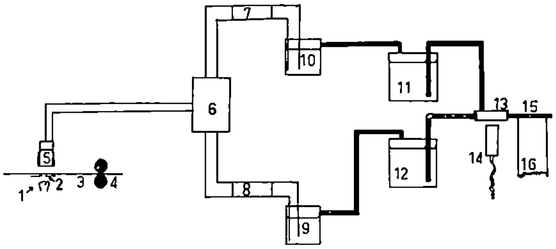


Fig. 1. Block diagram of gradient generator. 1, lamp; 2, slit; 3, 35-mm. film on which is photographed a blocked-in graph of the gradient function; 4, clock motor; 5, photocell; 6, phase inverter; 7, 'dark' d.c. amplifier; 8, 'light' d.c. amplifier; 9, 10, electrolysis vessels; 11, 12, buffer bottles; 13, mixing chamber; 14, 50-cycle electromagnet (to stir 13); 15, polyvinyl chloride cannula; 16, paper strip

Because of the basic nature of the paper, the anionic dyes used in paper electrophoresis give heavy background staining. Of the basic dyes

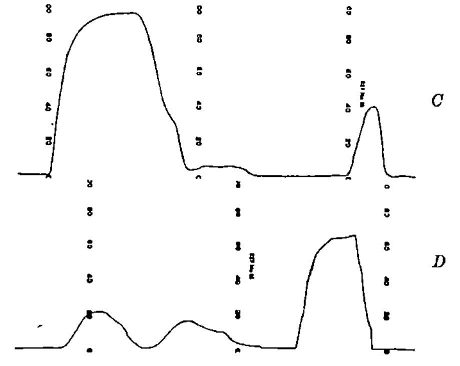


Fig. 2. *A*, separation of human γ-globulin and albumin. The point of application is at the extreme left-hand edge of the strip. The arrows represent the gradient limits, computed from the rate of flow along the strip. (1) *p*H 7·0, *M* = 0·005 phosphate; (2) *p*H 6·3, *M* = 0·010 phosphate. *B*, sub-fractionation of human γ-globulin. (1) *p*H 8·0, *M* = 0·005 phosphate; (2) *p*H 8·0, *M* = 0·350 phosphate. *C*, scanner recording of strip *A*. *D*, scanner recording of strip *B*

tested, malachite green, basic fuchsin and victoria blue *B*, the last was found to give the most intense staining of the protein bands with the least background on the paper. Care must be taken not to dry the paper at too high a temperature, particularly when it is impregnated with acid buffer salts. Under these conditions victoria blue gives a violet background which cannot be washed out.

Some typical separations are shown in Fig. 2, *A*, *B*. It is seen that the apparent position of the initial gradient limit is a considerable distance in front of the paper. Measurements of *p*H and conductivity made on the paper show that this limit is actually just at the end of the strip. As in the case of columns[2], the buffering action of the paper has a marked influence on the gradients. This buffering action places a limit on the degree of resolution attainable with short strips. With a 50-cm. strip this is only sufficient to separate serum into albumin, β- and γ-globulin. The sub-fractions of purified γ-globulin are, however, well resolved and chromatography on DEAE paper could be of great value in a large-scale survey of hyper-γ-globulinæmic sera.

The protein on the strips may be determined quantitatively by colorimetry of the dye eluted with 100 per cent methanol from each fraction. Alternatively, the strip may be scanned intact with a photoelectric electrophoresis strip scanner. In this case the height of the scanning slit should be equal to the width of the strip to allow for the irregularity of the bands.

Recordings of patterns obtained with a transistorized scanner[3] are shown in Fig. 2, *C*, *D*. I have found that the staining with uncleared paper obeys Beer's law over the range of protein concentrations encountered in the chromatograms.

I am investigating at present differences in dye uptake between the different serum protein fractions.

Stains for specific protein components may also be used, provided they are not affected by the ion-exchange properties of the paper. For example, it is possible to detect cæruloplasmin by Uriel's[4] technique and to demonstrate its heterogeneity (Fig. 3).

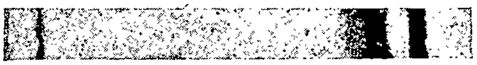


Fig. 3. Chromatogram of cæruloplasmin. 0·5 ml. of human serum in *p*H 6·4, M = 0·05 phosphate was applied to the top of the strip. The chromatogram was developed by a gradient to *p*H 6·4, M = 0·05 phosphate; M = 0·33 sodium chloride. The initial gradient limit was taken to twice the length of the strip

Further sub-fractionation of the cæruloplasmin can be carried out by cutting the bands out of unstained strips and inserting them in starch-gels[5] made in *p*H 5·1, M 0·025 acetate buffer and submitting them to electrophoresis. This technique may be of considerable value in the large-scale surveys necessary for the study of the racial and genetic variations on the individual cæruloplasmin heterogeneity reported in ref. 6.

Part of the expense of this work was defrayed by the National Health and Medical Research Council, Canberra. A.C.T.

[1] Peterson, E. A., and Sober, H. A., *J. Amer. Chem. Soc.*, **78**, 751 (1956).
[2] Sober, H. A., Gutter, F. J., Wyckoff, M. M., and Peterson, E. A., *J. Amer. Chem. Soc.*, **78**, 756 (1956).
[3] Curtain, C. C., *Austral. J. Sci.*, **23**, 193 (1960).
[4] Uriel, J., *Bull. Soc. Chim. Biol.*, **39**, 105 (1958).
[5] Smithies, O., *Biochem. J.*, **71**, 585 (1959).
[6] Morell, A. G., and Scheinberg, I. H., *Science*, **131**, 931 (1960).

PHOTO-POLAROGRAPHY WITH A FLASH-LAMP

By DR. H. BERG and H. SCHWEISS

German Academy of Sciences, Institute of Microbiology and Experimental Therapy, Jena

PHOTO-POLAROGRAPHY, originating from the synthesis of photo-chemistry and polarography, records photokinetic-currents caused by photo-depolarizers, as well as their dark reactions. Photo-depolarizers in a restricted sense can be defined as follows. They originate from a photo-reaction, for example:

$$A \xrightarrow[h_\nu]{k_\nu} B \qquad (1)$$

(with visible or ultra-violet light), reach a steady-state, and disappear in a following dark reaction ('Durchtritts'-reaction, combination, disproportion, etc.):

$$B \xrightarrow{k} C \qquad (2)$$

Hitherto, the photo-depolarizers (B), ketyl radicals[1] as well as hydroquinones[2] caused by photokinetic-currents have been demonstrated by means of continuous irradiation.

Photokinetic-currents are caused by intense irradiation of mercury electrodes during electron exchange. In analogy to classical polarography there are three principal groups:

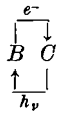
(*a*) Photokinetic-currents preceding the 'Durchtritts'-reaction may occur because of a modification of the 'Nachlieferungs'-velocity in the dark (from k' to k_ν) of phototropic equilibria produced by irradiation.

$$A + B \underset{k}{\overset{k',k_\nu}{\rightleftarrows}} C + D \qquad (3)$$

(Examples for equation (3): A = leucomethylene blue; B = benzenediazo-sulphonate; C = methylene blue; D = phenylhydrazine sulphonate).

(*b*) Photokinetic currents in opposite direction to the 'Durchtritts'-reaction (4*a*) are brought about by the regeneration (4*b*) of the photo-depolarizer according to the following scheme:

(4*a*)



(4*b*)

(Examples for equation (4) are found in ref. 2: B = anthrahydroquinone-2-sulphonic acid or other stable hydroquinones; C = anthraquinone-2-sulphonic acid or other quinones easily photo-reducible in alcoholic solution).

(*c*) Photo-reaction, following the 'Durchtritts'-reaction, frequently occurs in connexion with (*a*) and (*b*):

$$B \xrightarrow{e^-} C \xrightarrow{h_\nu} D \qquad (5)$$

(Examples for equation (5): B = anthraquinone; C = anthrahydroquinone; D = anthrone → 9,10-dihydroanthranol). For purposes of calculation, we have inserted the equations for the photo-reaction v_v, adapted to the reaction-layer into the differential equations of Koutecky:

$$\frac{dB}{dt} = v_D + v_v \qquad (6)$$

and have obtained in case (*b*), for example, good conformity between the velocity constant for (4*b*) calculated with equation (6) and the direct photo-reduction (k_v = 0·63–1·4 sec.$^{-1}$) of 4 × 10^{-4} M.C. in the solution (40 per cent *iso*propanol, phosphate buffer, *p*H 7), observed by means of current-time curves[3].

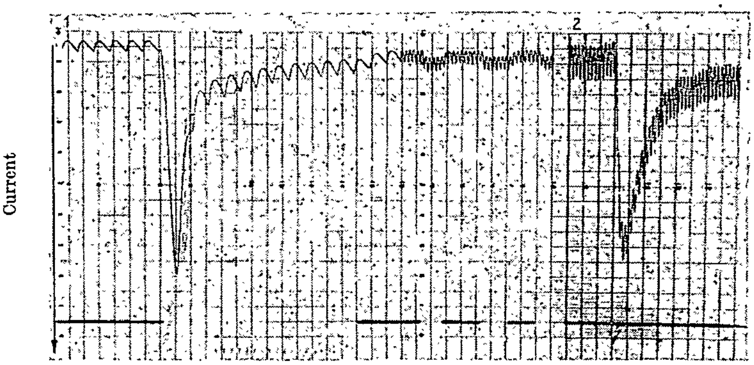
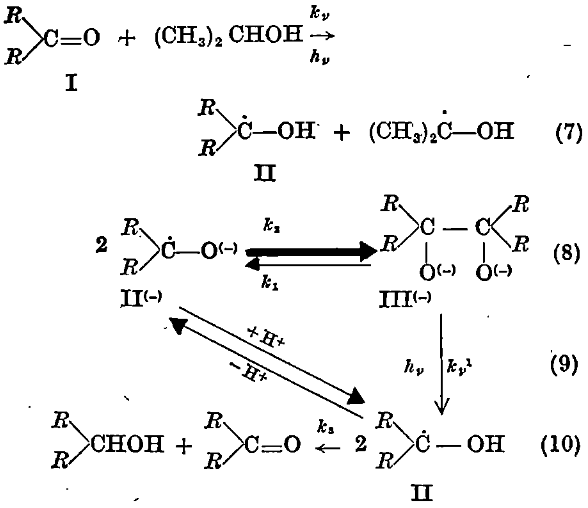


Fig. 1. Continuous irradiation (curve 1) and flash irradiation (curve 2) of 4 × 10^{-4} *M* benzophenone in 40 per cent *iso*propanol with 0·1 *N* sodium hydroxide, recorded with the selector polarograph (Atlas-Werke, Bremen); recording width, 20 cm. Curve 1: formation and decrease of II^- according to equations (7), (8) and (9). Towards the end of the reactions intermittent irradiation was used (dark period ——); sensitivity, $S = 2·5 \times 10^{-8}$ amp. cm.$^{-1}$; dropping time, τ = 2·2 sec.; time-scale, 1 cm. represents 1·95 sec., and in the last part, 1 cm. represents 7·5 sec. Curve 2: flash exposure ↙ and course of the dark reaction of the radical according to equation (8); sensitivity, $S = 3·75 \times 10^{-9}$ amp. cm.$^{-1}$; dropping-time, τ = 0·47 sec.; time-scale, 1 cm. represents 1·95 sec.

In our endeavour to clarify the photo-reduction mechanisms (equations 1 and 2) particularly of ketones, by means of continuous intense irradiation with a high-pressure mercury arc *U*-500 (this process requires a great deal of sensitivity and a high recording velocity), we noticed other consecutive reactions in addition to the reactions as in (*b*) and (*c*); this is demonstrated in the case of benzophenone. On irradiation of 4 × 10^{-4} *M* benzophenone in a solution of 0·1 *N* sodium hydroxide in 40 per cent *iso*propanol, the following gross reactions are recorded by means of the dropping electrode:



I causes a reduction wave ($\pi_{1/2}$ = − 1·52 V.), II or anion II(−) causes a reversible reduction-oxidation wave, and III(−) an irreversible oxidation wave ($\pi_{1/2}$ = − 0·65 V.). Apart from III(−) being photolysed in accordance with equation (9), the recording of II or II(−) respectively (Fig. 1, curve 1) must be superimposed by a photokinetic current in the opposite direction (as indicated by scheme *b*):

$$II^{(-)} \rightharpoonup I + 1e^- \qquad (11)$$
$$\underset{h_v}{\uparrow\; k_v \;|} \qquad (7).$$

the half-life period of the pseudo-monomolecular reaction (7) being only 0·6 sec. (Fig. 1 in ref 3).

Another photokinetic-current of opposite direction is indicated by the current-time curves ($\bar{\alpha}$ = 0·64) of the oxidation wave of III(−) according to scheme (4):

$$III^{(-)} \rightharpoonup 2I + 2e^- \qquad (12)$$
$$\underset{h_v}{\uparrow\; k_v' \;|} \qquad (7)+(8)$$

Considering an equilibrium (8) the sequence of reactions:

$$2I \xrightarrow{k_v} 2II^{(-)} \underset{k_1}{\overset{k_2}{\rightleftarrows}} III^{(-)} \qquad (13$$

is of special interest. (Protonization equilibria have been omitted in equations (11), (12) and (13).)

Although in equations (7) to (10) the detailed reaction scheme of Schenck[4] has not yet been taken into consideration, an exact calculation of equation (13) meets with greater difficulties because of the photokinetic currents (11) and (12) in the opposite direction.

For these reasons we have passed from continuous irradiation to the flash-light technique. As the excitation time of a flash is only about 10^{-4} sec., it becomes possible to record the combination of the radical photo-depolarizer $II^{(-)}$ separately as a dark reaction, and, moreover, to avoid the photokinetic current (11).

A flash from our unit (argon lamp, capacitor 64 μF., 4 kV.) produces the radical ($[II^{(-)}]_0 \approx 1·6 \times 10^{-5}$ *M*), which, according to curve 2, combines in a reaction of second order. Principally, the calculation of k_2 would be possible from the following equation:

$$k_2 = 2·303 \quad \log \frac{[\overline{II^{(-)}}]([II^{(-)}]_0 - [II^{(-)}][\overline{II^{(-)}}])}{([\overline{II^{(-)}}] - [II^{(-)}])[II^{(-)}]_0^2} \cdot \frac{[\overline{II^{(-)}}]}{t([II^{(-)}]_0^2 - [II^{(-)}]^2)} \qquad (14)$$

wherein $[II(-)]_0$ is the initial concentration of the radical, $[II(-)]$ the actual concentration of the radical and $[\overline{II^{(-)}}]$ the concentration of the radical at equilibrium. There are, however, two reasons causing the determination of the concentration at equilibrium to become faulty: (1) The concentration of $[\overline{II^{(-)}}]$ is at the limit of sensitivity of average polarographs, and will be obscured by oscillations of the photo-residual-current during irradiation. (2) From equation

(8) one might expect the limiting current of $[\mathrm{II}^{(-)}]$ already to be superimposed by a preceding kinetic dark current (k_1) and possibly by the oxidation current of a stabilized radical adduct[4].

For these reasons we have only evaluated the beginning of the reaction (8) with the simple equation of second order, as Porter *et al.*[5] did in the case of semiquinones, and the result is $k_2 = 13 \times 10^4$ l.mole^{-1} sec.$^{-1}$ (curve 2).

Further recording trials with benzophenone and other ketones[6], using the cathode-ray oscillograph, indicate clearly that after introduction of the flash-light technique into photo-polarography it will be necessary to increase further the measuring sensitivity of the polarographic method; only thus will it become possible to demonstrate photo-depolarizers in the triplet state with similar success as Porter was able to do by spectrographic analysis. On the other hand, the flash-method represents a new possibility for clarifying electrode processes.

[1] Berg, H., and Schweiss, H., *Naturwiss.*, **47**, 513 (1960).
[2] Berg, H., *Naturwiss.*, **47**, 320 (1960).
[3] Berg, H., and Kapulla, H., *Z. Elektrochem. Ber. Bunsenges. Physik. Chem.*, **4**, 44 (1960).
[4] Schenck, G.O., Meder, W., and Pape, M., *Proc. Second United Nations Intern. Conf. on the Peaceful Uses of Atomic Energy*, **29**, 352 (1958).
[5] Bridge, K., and Porter, G., *Proc. Roy. Soc.*, A, **244**, 259, 276 (1958).
[6] Berg, H., *Fifth Intern. Symp. Free Radicals, Preprint* 8 (Uppsala, 1961).

INDUCED ENZYME SYNTHESIS IN AQUEOUS SUSPENSIONS OF STARVED STATIONARY PHASE *AEROBACTER AEROGENES*

By R. E. STRANGE

Microbiological Research Establishment, Porton, Near Salisbury, Wiltshire

THERE is evidence that the survival of *Aerobacter aerogenes* held in aqueous suspension without added nutriment involves the utilization of cell polymers such as protein, ribonucleic acid (RNA) and glycogen, fragments of which are excreted[1]. Several workers have reported losses of substantial amounts of RNA from suspensions of starved bacteria and yeasts which remained viable[2,3]. There is also evidence that in the absence of an exogenous nitrogen source yeasts and bacteria make adaptive enzymes, for example, maltozymase by yeast in response to α-methyl-glucoside[4] and β-galactosidase by *Escherichia coli* in response to lactose[5] or methyl-β-D-thiogalactopyranoside (TMG)[6]. Apparently, such enzyme synthesis occurs at the expense of the endogenous amino-acid pool[4–6] and depends on protein turnover[6,7]. Ability to make adaptive enzymes in the absence of an exogenous nitrogen source is probably important for bacterial survival, but such ability might be expected to decrease if cell polymers were utilized when starvation is prolonged. Investigation of β-galactosidase synthesis by starved *A. aerogenes* in nitrogen-free medium indicates that this is so.

A. aerogenes, grown in various media as described in ref. 1, was collected after being in the stationary phase for 0·5 hr. and washed twice with buffered saline by centrifugation. Suspensions of washed bacteria (4–4·5 mgm. dry weight/ml.) in buffered saline, *p*H 6·5, were incubated in gas wash-bottles at 37° with aeration and samples were removed at intervals for viable bacterial counts, determination of per cent viability by the microscopic plate method of Postgate *et al.*[8], estimation of protein[9] and RNA[1], and for induction experiments. The latter samples (20 ml.) were centrifuged and the bacteria re-suspended in a sterile solution (50 ml.) containing TMG (synthesized according to the method of Helferich *et al.*[10]) or lactose. Bacterial suspensions (1·5–2·0 mgm. dry weight/ml.) containing inducer were incubated in small gas wash-bottles at 37° with aeration and samples were taken at intervals for measurements of viability and β-galactosidase activity. Before enzyme assay, bacteria were washed, re-suspended in water and shaken with benzene[3]. Measured volumes of benzene-treated samples were incubated with *O*-nitrophenyl-galactoside (ONPG)[11], and activity is expressed as µmoles ONPG hydrolysed/hr./mgm. dry weight bacteria at 37° in sodium phosphate buffer (0·14 *N*–Na$^+$, *p*H 7·2). *A. aerogenes* β-galactoside appeared to be less stable than the enzyme from *E. coli*[3] since activity was lost rapidly when dilute suspensions of benzene-treated bacteria were held at 20–37°. Benzene-treated suspensions containing 1–2 mgm. dry weight/ml. retained activity for at least 24 hr. at 2°.

The changes which occurred during storage of stationary phase *A. aerogenes* gathered from three different growth media are shown in Table 1. Losses of protein and RNA from viable bacteria were paralleled by a reduction in the ability of the organisms to synthesize β-galactosidase in nitrogen-free medium containing lactose. Results for β-galactosidase synthesis using TMG, which is an inducer but not a substrate[12], again showed that stored bacteria formed less enzyme than freshly gathered organisms (Fig. 1).

Harrison[14] has directed attention to the fact that in buffered suspensions of *A. aerogenes* held at 37° with

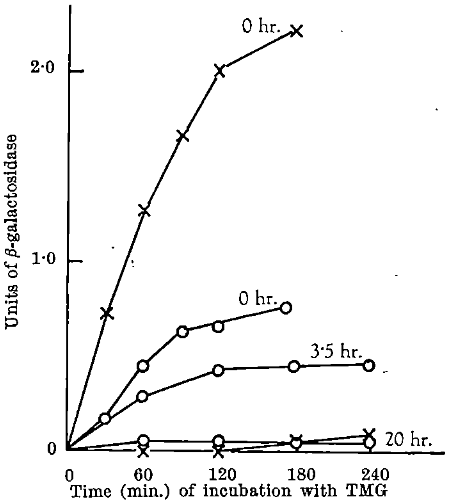


Fig. 1. Synthesis of β-galactosidase by washed stationary phase *A. aerogenes* gathered from a carbon-limiting, defined medium[1] (○) and tryptic meat broth (×), before and during storage in buffered saline, *p*H 6·5, at 37° with aeration. Bacteria were induced in buffered saline, *p*H 7·0, containing TMG (10^{-3} *M*) and metal ions. Figures on the curves refer to storage period before induction

Table 1. CHANGES IN VIABILITY, PROTEIN, RNA AND INDUCED β-GALACTOSIDASE SYNTHESIS IN STATIONARY PHASE *Aerobacter aerogenes* HELD IN BUFFERED SUSPENSION AT 37° WITH AERATION

Bacteria were stored in buffered saline at *p*H 6·5 and induced at *p*H 7·2 in a solution containing lactose (1 per cent, w/v), metal ions, citric acid (10^{-3} *M*) and phosphate buffer (0·2 *N* sodium). Enzyme synthesis was measured after induction for 3 hr.

Growth medium	Storage time (hr.)	Viability (per cent)	Loss of protein (per cent)	Loss of RNA (per cent)	β-galactosidase (units)
Defined carbon-limiting (ref. 1)	0	99	0	0	1·3
	4	97	3	8·5	—
	21	97	16	20·5	0·5
	41	97	19·5	33·0	0·07
	69	88	—	—	0
Tryptone-glucose* (ref. 13)	0	95	0	0	3·0
	24	94	6·5	7·5	0·8
	41	96	10·0	7·5	0·16
	48	94	11·0	—	—
	65	94	13·6	16·5	0·06
Tryptic meat broth	0	99·5	0	0	7·2
	3	99·0	6·0	3·0	—
	18	99·0	16·7	18·3	0·06
	24	95·0	19·4	24·0	0·05
	41	95·5	20·0	24·0	—
	67	91·0	25·5	33·4	—

* Bacteria freshly collected from tryptone-glucose medium contained about 15 per cent dry weight of glycogen (ref. 1).

aeration, surviving organisms may maintain viability at the expense of material derived from dead bacteria. Therefore, in investigations of induced enzyme synthesis in nitrogen-free medium, it is important that the percentage of dead bacteria present in suspensions should be low. In the present experiments, the initial viability of freshly collected bacterial suspensions was usually 98 per cent or more, and, immediately before induction, the bacteria were separated from the suspending medium. It appears unlikely that the small number of non-viable organisms present supplied sufficient nitrogen-containing material to account for the enzyme synthesis observed. Neither the percentage viability of suspensions nor the total number of viable bacteria present, altered significantly during the induction period in the experiments described here.

In starved bacteria, depletion of cell components and/or available energy may account for a fall-off in adaptive enzyme synthesis (ref. 5). Since adenosine triphosphate (ATP) is concerned in energy supply and transfer reactions, the concentration of this substance in *A. aerogenes* was measured during storage and induction. Cold perchloric acid extracts of washed bacteria were neutralized with potassium hydroxide, freed from precipitated salt and assayed for ATP with the fire-fly luminescence technique[15] and a scintillation counter. The results (Table 2) showed that bacteria held for 19 hr. at 37° contained rather more ATP than freshly collected organisms and that the level fell only slightly during the induction period. Thus, reduced enzyme synthesis in these starved bacteria was apparently not due to a deficiency of ATP.

Storage of buffered stationary phase *A. aerogenes* suspensions at 37° with aeration for up to 68 hr. did not reduce the ability of the bacteria to form β-galactosidase in the presence of inducer and a suitable nitrogen source such as peptone (Fig. 2). In fact, under these conditions, the initial rate of enzyme formation was often more rapid with stored than with freshly collected organisms. Evidently, starvation did not affect the mechanism for enzyme formation, but depleted potential substrates.

Table 2. CHANGES IN ENDOGENOUS ATP AND INDUCED β-GALACTOSIDASE SYNTHESIS IN STATIONARY PHASE *Aerobacter aerogenes* COLLECTED FROM CARBON-LIMITING DEFINED MEDIUM AND HELD IN BUFFERED SUSPENSION AT 37° WITH AERATION

Bacteria were stored at *p*H 6·5 and induced at *p*H 7·0 in buffered saline containing TMG (2 × 10^{-3} *M*) and metal ions

	Storage period (hr.), 0		Storage period (hr.), 19	
Induction period (min.)	ATP (μgm./ml. suspension)	Galactosidase (units)	ATP (μgm./ml. suspension)	Galactosidase (units)
0	0·67	0	0·86	0
60	0·65	0·26	—	0·07
90	—	0·41	0·85	0·09
120	0·84	0·47	—	0·08
150	1·04	0·60	—	—
180	—	—	0·80	0·08
240	—	—	0·79	0·10

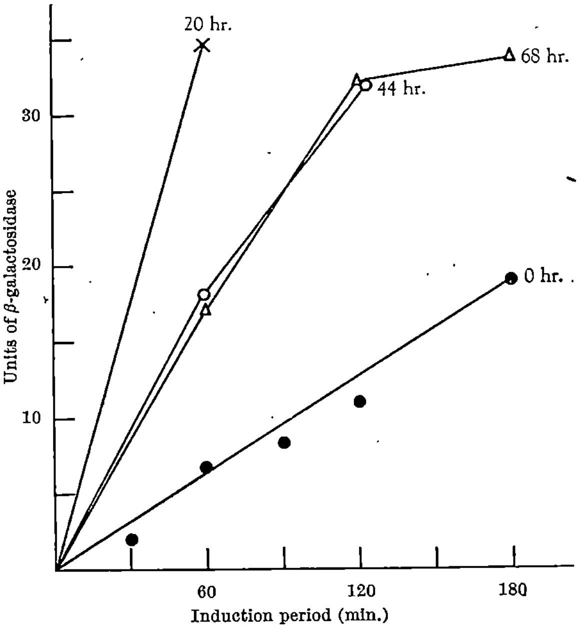


Fig. 2. Synthesis of β-galactosidase by washed stationary phase *A. aerogenes* gathered from tryptic meat broth, before and during storage in buffered saline, *p*H 6·5, at 37° with aeration. Bacteria were induced in peptone water containing TMG (10^{-3} *M*). Figures on the curves refer to storage period before induction

It is concluded from these results that utilization of cell polymers (protein, RNA and, if present, glycogen) by starving stationary phase *A. aerogenes* held at 37° in buffered suspension with aeration so depletes the reserves of these materials that, while viability may not be affected, the ability of the bacteria to make adaptive enzymes in nitrogen-free medium is reduced markedly.

I am grateful to Dr. J. R. Postgate for suggestions, Mr. J. H. R. Slade for synthesizing thiomethylgalactoside and Mr. S. Lovett for the use of a scintillation counter.

[1] Strange, R. E., Dark, F. A., and Ness, A. G., *J. Gen. Microbiol.*, **25**, 61 (1961).
[2] Borek, E., Ryan, A., and Rockenbach, J., *J. Bact.*, **69**, 460 (1955); Higuchi, M., and Uemura, T., *Nature*, **184**, 1381 (1959).
[3] Rotman, B., *J. Bact.*, **76**, 1 (1958).
[4] Halvorson, H. O., and Spiegelman, S., *J. Bact.*, **65**, 601 (1953).
[5] Løvtrup, S., *Biochim. Biophys. Acta*, **19**, 247 (1956).
[6] Mandelstam, J., *Nature*, **179**, 1179 (1957).
[7] Mandelstam, J., *Biochem. J.*, **69**, 110 (1958); Mandelstam, J., and Halvorson, H., *Biochim. Biophys. Acta*, **40**, 43 (1960).
[8] Postgate, J. R., Crumpton, J. E., and Hunter, J. R., *J. Gen. Microbiol.*, **24**, 15 (1961).
[9] Stickland, L. H., *J. Gen. Microbiol.*, **5**, 698 (1951).
[10] Helferich, B., Grunewald, H., and Langenhoff, F., *Chem. Ber.*, **86**, 873 (1953).
[11] Lederberg, J., *J. Bact.*, **60**, 381 (1950).
[12] Hogness, D. S., Cohn, M., and Monod, J., *Biochim. Biophys. Acta*, **16**, 99 (1955).
[13] Wade, H. E., *Biochem. J.*, **78**, 475 (1961).
[14] Harrison, A. P., *Proc. Roy. Soc.*, B, **152**, 418 (1960); *Bact. Proc.*, 53 (1961).
[15] McElroy, W. D., *Proc. U.S. Nat. Acad. Sci.*, **33**, 342 (1947); Strehler, B. L., and Totter, J. R., *Arch. Biochem. and Biophys.*, **40**, 28 (1952).

COMPARISON OF REDUCED ALKYLATED DERIVATIVES OF SOME MYELOMA GLOBULINS AND BENCE-JONES PROTEINS

By Dr. M. D. POULIK
American National Red Cross, Washington, 6, D.C.
AND
Dr. G. M. EDELMAN
The Rockefeller Institute, New York, 21

MYELOMA globulins are abnormal serum proteins distinguished from normal γ-globulin by their relative homogeneity and their chemical individuality. A number of comparative chemical and immunological studies[1-3] have been aimed at elucidating further their relations to normal γ-globulins. The demonstration[4] that normal and abnormal proteins of the γ-globulin family dissociate into separable sub-units after cleavage of disulphide bonds has made it possible to compare myeloma proteins with each other and with normal γ-globulins at the level of their sub-units[5,6]. This article presents additional data on 10 human myeloma globulins and some correlative experiments on myeloma proteins and Bence-Jones proteins derived from the same patient.

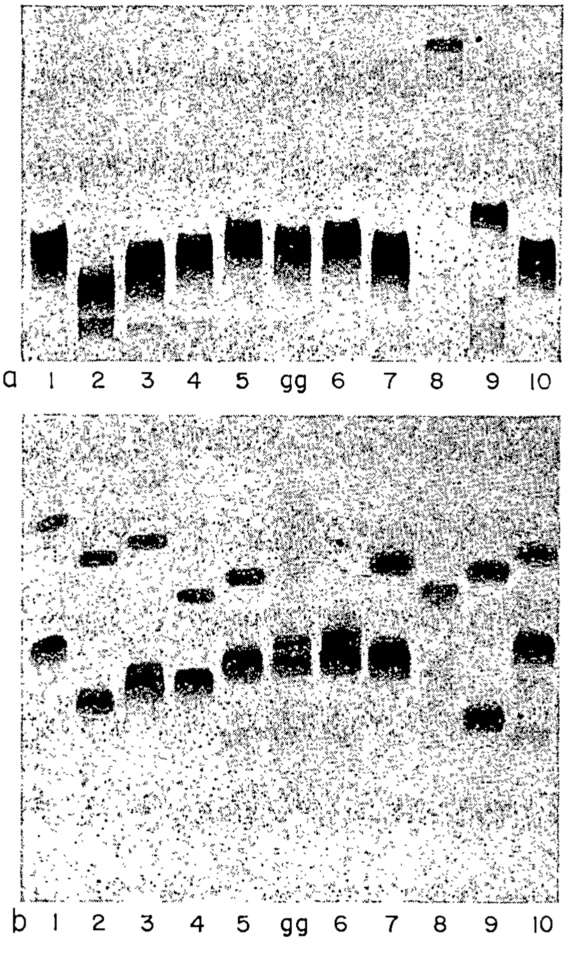


Fig. 1. Comparison of myeloma globulins and normal human γ-globulin. *a*, Before reduction and alkylation; *b*, after reduction and alkylation. 1-10 Different myeloma globulins; *gg*, normal human γ-globulin

Ten myeloma proteins of γ-mobility were isolated from individual sera of patients with multiple myeloma by means of starch-zone electrophoresis[7] and stored in lyophilized form. Bence-Jones proteins were isolated from the urine of two of the patients by precipitation at *p*H 5·0 after addition of an equal volume of saturated ammonium sulphate solution at 4° C., and one sample was further purified by starch-zone electrophoresis. Pooled normal human fraction II γ-globulin was obtained from Lederle Laboratories. Reduction and alkylation were carried out using procedures previously described[6]. In certain experiments, samples were reduced with 0·1 *M* β-mercaptoethanol in 8 *M* urea under pre-purified nitrogen for 16 hr. at room temperature. Starch-gel electrophoresis in 8 *M* urea was done following the procedure of Smithies[8,9] with modifications previously described[6,10,11]. Chromatography on carboxymethyl cellulose in acetate buffer made with 6 *M* urea was also performed as described[6] except that the apparent *p*H was 5·6 at 25° C.

In Fig. 1*a* are shown the electrophoretic patterns in the urea–starch gel of the ten myeloma globulins and of human fraction II γ-globulin. Sample No. 8 showed, in addition to the slowly migrating myeloma protein, a sharp band of higher mobility which possibly represents Bence-Jones protein in the serum[12]. Faint bands of similar type could be seen in some of the other patterns, although in reproduction they do not appear. After simultaneous reduction with mercaptoethanol and alkylation with iodoacetamide, the samples were again compared (Fig. 1*b*). The pattern of reduced alkylated γ-globulin showed a diffuse band moving ahead of two narrower bands. Each of the treated myeloma proteins differed in number and position of the bands. A striking feature was the sharpness of the fast-moving bands obtained after reduction and alkylation. The mobilities of these bands were distributed within the range of mobility defined by the diffuse fast-moving band of normal γ-globulin. Moreover, when nineteen isolated myeloma globulins were mixed in varying proportions and then reduced and alkylated, starch-gel patterns were obtained which were similar to that of reduced alkylated normal γ-globulin. In some cases, the slower-moving bands of reduced alkylated myeloma proteins had mobilities similar to those of the slower bands of normal γ-globulin.

By chromatography on carboxymethyl cellulose in 6 *M* urea the reduction products of one myeloma protein (No. 5) could be partially separated. This is shown in Fig. 2 in which the patterns of the reduced alkylated protein and the material from the first and second peak of the chromatogram are compared. Separation was effected although it should be noted that the overall yield from the chromatogram was only 32 per cent.

Previous experiments[6] indicated that, in most cases, complete reduction of all the disulphide bonds of

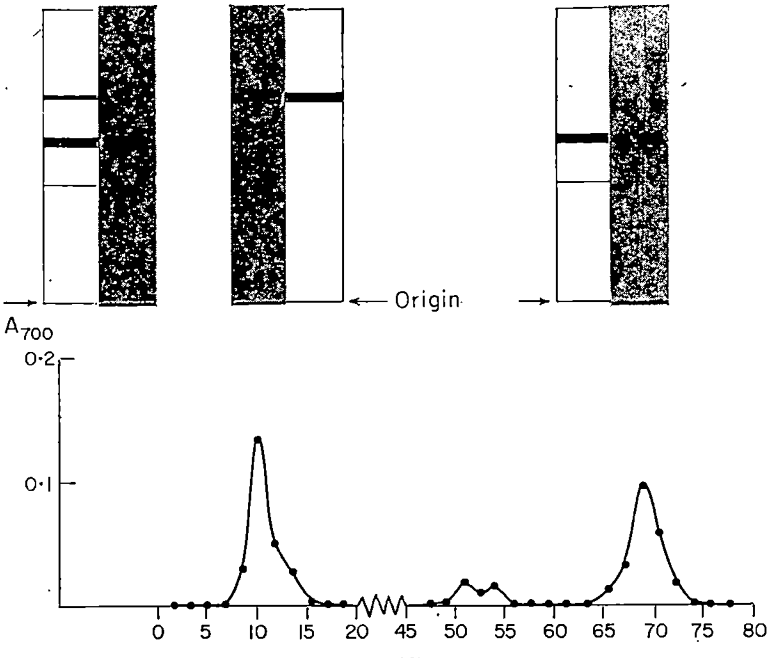


Fig. 2. Chromatographic and electrophoretic separation of myeloma globulins. Above each peak of the chromatogram is placed the pattern obtained by starch-gel electrophoresis of material from that peak. At the left is the starch-gel pattern of reduced alkylated myeloma protein (No. 5, see Fig. 1*b*) before chromatography. Total column load, 20 mgm.; A_{700} = optical density of Folin reaction at 700 mμ

γ-globulin and myeloma proteins was not achieved. The question therefore arose as to whether new bands would appear on the starch-gel as reduction proceeded to a greater extent. Three myeloma proteins were reduced with 0·01 *M* mercaptoethanol in 8 *M* urea under pre-purified nitrogen for 16 hr., before alkylation. The products were then compared on the same starch-gel. In addition to the characteristic patterns resulting from reduction, the less-completely reduced samples showed the presence of a band corresponding to untreated myeloma protein. The more extensively reduced samples showed much less of this material. Regardless of the extent of reduction, the faster-moving sharp bands for each sample were unchanged in mobility and width, and no evidence of additional bands appeared after long times of reduction. Thus, although extensive reduction led to dissociation of more of the starting material, no new sub-units were revealed by this procedure.

A large proportion of patients with multiple myeloma excrete Bence-Jones proteins in the urine. These proteins appear to be precursors of myeloma globulins or abortive by-products of their synthesis[1], although no uniform structural relation between these proteins and myeloma proteins has been established[13,14]. Antigenic similarities between Bence-Jones and myeloma proteins prompted a search for structural similarities using the methods described above. In Figs. 3*a* and 3*b* is shown the comparison, in two different instances, of a myeloma protein and a Bence-Jones protein from the same patient both before and after reduction and alkylation. Although the mobility of the Bence-Jones protein (Mul) before treatment was not greatly different from that of the myeloma protein, the band of the Bence-Jones protein was sharper and narrower. After simultaneous reduction and alkylation of the two proteins, similar patterns were obtained, each composed of two sharp bands of different mobility. The pattern of the reduced myeloma protein had, in addition, two faint bands of lower mobility (Fig. 3*a*). Bence-Jones protein (Bar) showed multiple bands in the urea–formate gel, and no similarity could be seen between this pattern and that of the myeloma protein from the same patient. After reduction and alkylation, however, two bands of the Bence-Jones protein derivative corresponded in mobility with two of the fast-moving bands of the myeloma protein derivative (Fig. 3*b*).

The findings discussed here suggest that dissociation of myeloma globulins into their constituent polypeptide chains occurs in a fashion similar to that of normal γ-globulin. Starch-gel electrophoresis of the derivatives following reduction of myeloma proteins yielded distinctive and sharp band patterns. Up to the present, nineteen myeloma proteins have been compared; the pattern obtained in each case was reproducible and unique. In several instances, bands of common mobility were found among the myeloma globulins.

Recently, it has been shown that the myeloma proteins of the mouse, although much less heterogeneous than normal γ-globulin, may nevertheless consist of several molecular species[15]. Such hetero-

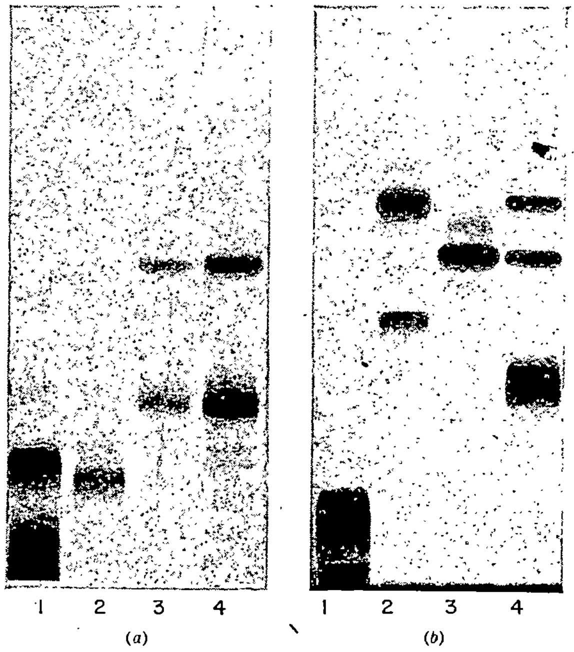


Fig. 3. Comparison of myeloma globulins and Bence-Jones proteins before and after reduction and alkylation. *a*, Patient Mul. 1, Myeloma protein (No. 9 of Fig. 1); 2, Bence-Jones protein; 3, reduced alkylated Bence-Jones protein; 4, reduced alkylated myeloma protein. *b*, Patient Bar. 1, Myeloma protein (No. 3 of Fig. 1); 2, Bence-Jones protein; 3, reduced alkylated Bence-Jones protein; 4, reduced alkylated myeloma protein

geneity may be reflected in the band patterns of certain reduced human myeloma globulins. The presence of multiple bands after reduction is not merely the result of heterogeneity, however, since γ-myeloma proteins appear to be dissociated by reduction in 8 *M* urea[6].

In both instances examined here, reduced alkylated Bence-Jones proteins and myeloma globulins from the same patient possessed certain bands of common mobility in the starch gel. Unreduced Bence-Jones protein (Bar) showed multiple bands in the urea–formate gel. This finding is in line with an earlier report of dissociation of Bence-Jones proteins at acid *p*H (ref. 16), and raises the possibility that urea may help to dissociate these proteins. The results obtained with reduced alkylated Bence-Jones proteins are consistent with the previously suggested hypothesis[6] that Bence-Jones proteins may be composed at least in part of chains of the same type as those present in the myeloma protein from the same patient.

We wish to thank Dr. R. E. Engle, jun., Dr. C. Zarofonetis and Dr. M. Bryant for the generous provision of serum and urine samples.

[1] Putnam, F. W., *Physiol. Rev.*, **38**, 512 (1958).

[2] Slater, R. J., Ward, S. M., and Kunkel, H. G., *J. Exp. Med.*, **101**, 85 (1955).

[3] Korngold, L., and Lipari, R., *Cancer*, **9**, 183 (1956).

[4] Edelman, G. M., *J. Amer. Chem. Soc.*, **81**, 3155 (1959).

[5] Edelman, G. M., and Poulik, M. D., *Fed. Proc.*, **20**, 387 (1961).

[6] Edelman, G. M., and Poulik, M. D., *J. Exp. Med.*, **113**, 861 (1961).

[7] Kunkel, H. G., *Methods and Biochem. Anal.*, **1**, 141 (1954).

[8] Smithies, O., *Biochem. J.*, **71**, 585 (1959).

[9] Smithies, O., First Conf. Genet., Genetic Inform. and Control Prot. Struct. and Func., Princeton, New Jersey, 1959, *Trans. Josiah Macy, Jun., Found., New York*, 133 (1960).

[10] Poulik, M. D., *Biochim. Biophys. Acta*, **44**, 390 (1960).

[11] Poulik, M. D., and Edelman, G. M., presented at the Ninth Colloquium, Brugge, May, 1961 (to be published in *Protides of the Biological Fluids*, Elsevier Publishing Co.).

[12] Morton, J. J., and Deutsch, H. F., *Proc. Soc. Exp. Med. Biol.*, **93**, 402 (1956).

[13] Fried, M., and Putnam, F. W., *J. Biol. Chem.*, **235**, 3472 (1960).

[14] Fahey, J. L., and Askonas, B. A., *Fed. Proc.*, **20**, 382 (1961).

[15] Askonas, B. A., *Biochem. J.*, **79**, 33 (1961).

[16] Putnam, F. W., and Stelos, P., *J. Biol. Chem.*, **203**, 347 (1953).

MULTIPLE INHIBITIONS OF CELL METABOLISM BY A SINGLE SUBSTANCE

By M. HARRIS and Dr. G. A. MORRISON

Department of Chemistry, University College of Wales, Aberystwyth

ANTIBIOTICS frequently inhibit more than one part of metabolism, and the mode of action may be different for different organisms and/or different environments[1]. Thus any method which indicates the nature of a number of underlying causes of bacteriostatic action in one set of connected experiments should be valuable, and particularly so if it selects those which operate at clinically possible concentrations of the antibiotic.

Consideration of the functioning of enzymes in a multi-enzyme system indicates that measurements of the rates of growth of bacterial cultures in the presence of a series of concentrations of any substance which combines with and inactivates enzymes could be used to demonstrate the existence of a number of such modes of action of the substance.

In a bacterial cell, series of enzymically controlled reactions are closely co-ordinated to form a chemical mechanism which converts foodstuffs into new cells and waste materials, and in a pure culture of a single strain the mechanisms of the individual cells are connected by their sharing of those intermediates which diffuse into the medium. In the phase of logarithmic growth the whole culture must operate as a single system in a steady state, and the rate of its expansion is controlled by the particular reaction which is the slowest.

The equation[2] for the rate of an individual effectively irreversible reaction in such a mechanism is a complex function C (less than unity), of the concentration(s) of the substrate(s) of the reaction multiplied by the concentration, e, of the enzyme, En; that is, $dp/dt = Ce$ where p is the concentration of a product, P. In the logarithmic phase, a steady state, C becomes constant and the rate of utilization of P must equal the rate of its production. If P is required for the production of new cells then $dp/dt = \alpha\, dn/dt$ where α is a constant and n is the concentration of cells. Hence $dn/dt = Ce/\alpha$. But $dn/dt = kn$ and $km = \ln 2$, where k is a reaction velocity constant and m is the mean generation time of the culture, and thus:

$$m = \alpha n(\ln 2)/Ce = (\alpha'/C).(n/e) \qquad \text{(i)}$$

and will be constant so long as the enzymic composition of the cells does not alter, that is, n/e remains constant.

In the presence of a substance D', concentration D, which combines with the enzyme non-competitively and renders it inactive (that is, $D' + En \rightleftharpoons D'En$, dissociation constant $= K_D$), the concentration of uncombined enzyme is $K_D e/(K_D + D)$ and this concentration must replace e in the above equation. There are, however, two possibilities for the effect of D' on C. If the materials, the concentrations of which are involved in the function C, are being produced and/or utilized by other parts of the metabolism which are not affected directly, their concentrations may be stabilized and C may not change significantly. In the absence of such stabilization these concentrations must increase and C must increase until it becomes equal to the reaction velocity constant C' for the reaction. Thus in this case a smaller concentration of active enzyme is compensated-for by a greater efficiency in its use until this is maximal.

In the first case, after a small or negligible threshold concentration of the inhibitor, and in the second case after a considerable threshold concentration, the mean generation time should give a linear plot against concentration of inhibitor.

If the substance is a competitive inhibitor, similar equations can be derived to relate m and D: for example, for a single substrate reaction $m = \beta(K_S + S + K_S D/K_D)/S$ where β is a proportionality constant, S the concentration of the substrate and K_S the dissociation constant for the enzyme-substrate complex. With these equations increases in S in response to increases in D theoretically can compensate infinitely, but when other parts of the metabolism stabilize S the equations assume the form:

$$m = \beta(A + BD) \qquad \text{(ii)}$$

and the linear plot should hold after a threshold concentration of inhibitor.

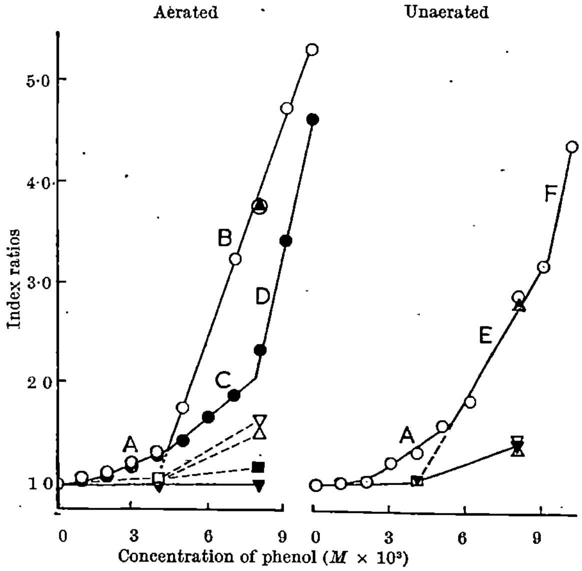


Fig. 1. ○, Control: ●, plus brilliant cresyl blue; ▽, plus phenylalanine; ▲, plus glutamate or tryptophan; △, plus casein hydrolysate; ▼, plus bacteriological peptone; □, plus glutamate, tryptophan, phenylalanine or casein hydrolysate; ■, plus brilliant cresyl blue and phenylalanine

If D_0 is the threshold concentration of inhibitor then $m = m_0$, the mean generation time of the uninhibited culture, and $m_0 = \alpha''(K_D + D_0)/C_0K_D$ from (i), or $= \beta(A_0 + B_0D_0)$ from (ii). Hence the ratio of the mean generation times of the inhibited and uninhibited cultures, which we term the index ratio, I, is given by:

$$I = m/m_0 = C_0(K_D + D)/C(K_D + D_0) = \gamma + \delta D$$

$$\text{or} = (A + BD)/(A_0 + B_0D_0) = \gamma' + \delta' D$$

If the inhibitor affects more than one enzyme then, in different ranges of concentration of the inhibitor, the rate of growth may be controlled by different enzymes and each range will have its appropriate equation with different values of γ and δ. In the figure a number of such modes due to phenol (which as an inhibitor follows $D' + En \rightleftharpoons D'En$ since dilution decreases inhibition) have been shown up.

Adding to the medium the product of such a reaction, provided it can penetrate the cells readily, must eliminate control by that reaction; similarly adding some substance from which the cells can derive the product in an alternative way must also eliminate its control, unless the alternative is not adequate to replace the lost production, then the degree of inhibition is decreased, that is, δ is made smaller. Thus trial-and-error additions to the medium may indicate the part of the metabolism affected by each mode, and as modes are eliminated, uncover others which in their turn become the rate controlling reactions.

These phenomena for phenol inhibiting *Klebsiella cloaca* in glucose-mineral salt media are shown in Fig. 1. For the purposes of demonstration phenol was chosen since the literature suggests that it affects more than one part of the metabolism. The bacterial strain must be acclimatized thoroughly to the conditions and the parent culture grown in glucose-deficient medium so that the cells do not encounter harmful conditions nor the inocula contain inhibition-removing materials. Mean generation times can be determined to within 3 per cent and multiple determinations increase the accuracy. The concentrations used for the results shown are the minimum ones to give the maximum effects.

Aerated cultures show two modes, *A* and *B*, and unaerated three modes *A*, *E* and *F*. Adding an unnatural hydrogen-carrier causes *B* to be replaced by *C* and *D*. *A* is eliminated by the addition of glutamate, tryptophan, phenylalanine, casein hydrolysate or peptone. *C* and *E* are diminished or replaced when casein hydrolysate, phenylalanine or peptone is added and may be the same. Additions of leucine had similar but less-effective actions as additions of phenylalanine, whereas no other single amino- or carboxylic-acid had any effect.

The method has been used also with tetracyclines to direct subsequent work into profitable channels. It shows up inhibitions caused by bacteriostatic concentrations, and determinations of γ and δ under defined conditions may be useful for comparison of the various members of this group of antibiotics.

---

[1] *Antibiotics: Their Chemistry and Non-medical Uses*, edit. by Goldberg, H. S., 23 (D. Van Nostrand Co. Inc., New York, 1959).

[2] *Respiratory Enzymes*, edit. by Lardy, H. A., 16 (Burgess Publishing Co., Minneapolis, 1959).

DIMER FORMATION AND GLUCOSE TRANSFER ACROSS THE MEMBRANE OF THE RED BLOOD CELL

By Dr. W. D. STEIN*

Department of Colloid Science, University of Cambridge

GLUCOSE and other sugars appear to cross the human red blood cell membrane by making use of a specific facilitated diffusion system present in the membrane[1-3]. The molecular mechanism of this and other membrane transport systems is obscure, but a number of models has been suggested consistent with the available experimental data. These models[4] involve the presence of 'carriers', 'pores' or enzymes in the membrane, to speed up the transfer of the permeant. No unambiguous criteria exist for distinguishing between these mechanisms, and there is no firm knowledge as to how these postulated mechanisms indeed act. The present article presents a new mechanism for glucose transfer and new experimental results in support of it.

I have recently shown that in the case of glycerol transfer, also across the human red blood cell membrane, there is evidence that the carrier for glycerol is a second glycerol molecule[5]. It appears that the observed facilitation of glycerol transfer occurs as a result of dimer formation between pairs of glycerol molecules, bound at the active centre of a specific catalytic component of the membrane, possibly enzymic in nature. As a result of such dimer forma-

---

* Beit Memorial Research Fellow. Present address: Department of Biological Chemistry, University Michigan, Ann Arbor, U.S.A.

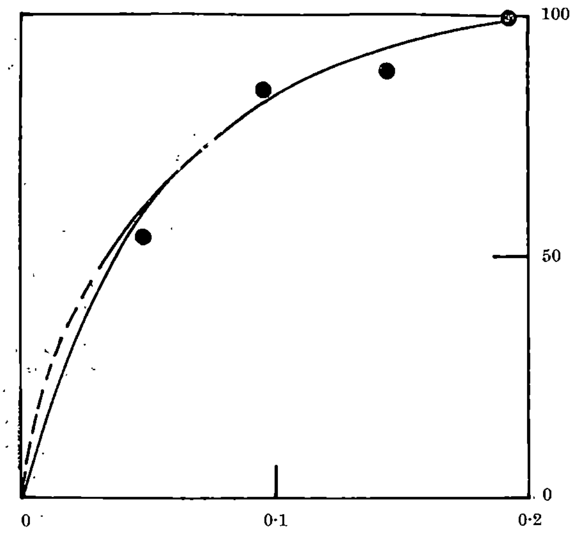


Fig. 1. Glucose penetration into the human red blood cell. Ordinate, initial velocity of glucose penetration relative to velocity at 0·19 *M*; abscissa, glucose concentration in moles per litre. Solid circles, experimental points; dashed line, prediction on monomer theory; solid line, prediction on dimer theory K = 0·05 *M*; β = 0·02 *M*

tion there is a reduction in the total number of the free hydrophilic groups of the pair of glycerol molecules, resulting in a more rapid rate of transfer across the cell membrane. I should now like to present evidence which suggests that glucose transfer, too, must occur by a similar mechanism.

It will be convenient, first, to describe more fully the model I propose for facilitated diffusion. We shall postulate the existence as part of the cell membrane, but exterior to the lipid component of the membrane, of a novel type of enzyme—a 'dimerase'. Such an enzyme has, in common with other enzymes, the property of binding its substrate by non-covalent bonds to form the classical Michaelis–Menten complex. But a dimerase differs from most other enzymes in two ways. First, rather than binding single substrate molecules, it binds pairs of these, that is, it is divalent towards its substrate. Second, the enzyme does not bring about any covalent modification in the substrate; instead, it enables the pair of bound substrate molecules to interact with one another by hydrogen bonds to form dimers. Such dimers will have a reduced number of hydrophilic groups available to form bonds with the water of the aqueous phase and can thus readily enter and cross the lipid regions of the membrane. The dimer is spontaneously dissociated again into monomers when it re-enters the aqueous phase. The 'membrane carrier' is thus the second substrate molecule and the function of the dimerase is simply to concentrate the substrate molecules and to orient these in such a way as to induce dimer formation.

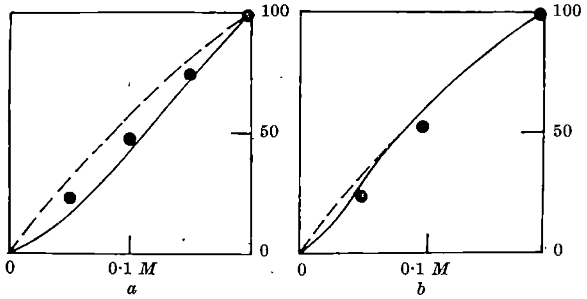


Fig. 2. (*a*) Sorbose penetration into the human red blood cell; (*b*) fructose penetration into the human red blood cell. Units and symbols as in Fig. 1, but with:

K_{sorbose} = 0·5 *M* K_{fructose} = 0·5 *M*

β_{sorbose} = 0·2 *M* β_{fructose} = 0·08 *M*

The evidence that glycerol transfer occurs by such a system has been presented in a previous paper[5]. The method most convincingly demonstrating dimer formation in the case of glycerol transfer fails to distinguish between the dimer and monomer models in the case of glucose, as we shall first discuss. But it will be seen that such a distinction can be made by a new method. (Full details of these experimental methods and the theoretical treatment will be presented elsewhere.)

Fig. 1 presents results for the initial velocity of glucose penetration into the red cell (measured by the photometric method[6]), as a function of glucose concentration (at 24° C. and *p*H 7·4). As a number of authors have demonstrated[4], such results are adequately accounted for on the basis of the classical Michaelis–Menten kinetics, so successfully applied in enzymology. The dashed curve in Fig. 1 (which merges with the solid line above 0·05 *M*) is drawn with these kinetics and a Michaelis constant K = 0·05 *M*. The solid line is computed from the 'dimerase' model, which requires that two substrate molecules be bound at the active centre and that these interact with one another to form the penetrating complex. The relevant constant analogous to the Michaelis constant and referring to the affinity of each of the pair of binding sites with single substrate molecules is β = 0·02 *M*. Clearly no distinction between these two models is possible on the data here. Similarly in Figs. 2*a* and *b* the results for the penetration of sorbose and fructose (which also use the glucose system[1]), at 24° C. and *p*H 7·4 are presented. Again the dashed line is drawn on monovalent theory with, in both cases, a Michaelis constant of 0·5 *M* (chosen arbitrarily to be ten times greater than the constant for glucose). The solid lines are drawn on divalent theory with the constants β = 0·2 *M* for sorbose and

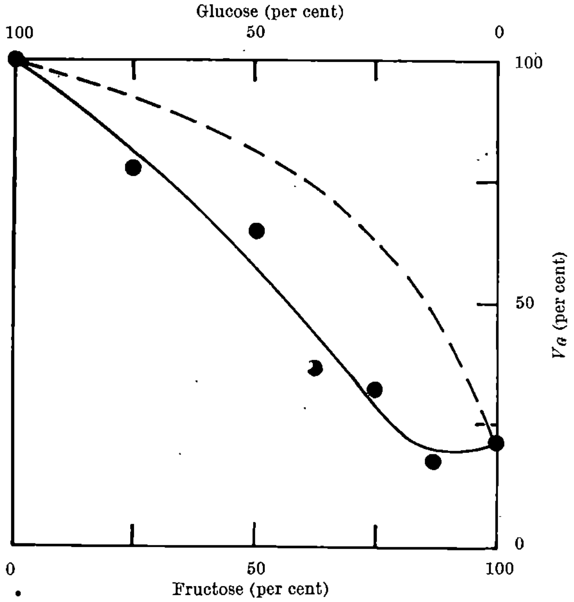


Fig. 3. Entrance of sugar into the human red blood cell from a mixture of glucose and fructose. Total sugar concentration, 0·19 *M*. Ordinate, initial velocity relative to that in 0·19 *M* glucose alone; abscissa, percentage fructose concentration increasing from left to right. Symbols as in Fig. 1. K and β as in Figs. 1 and 2

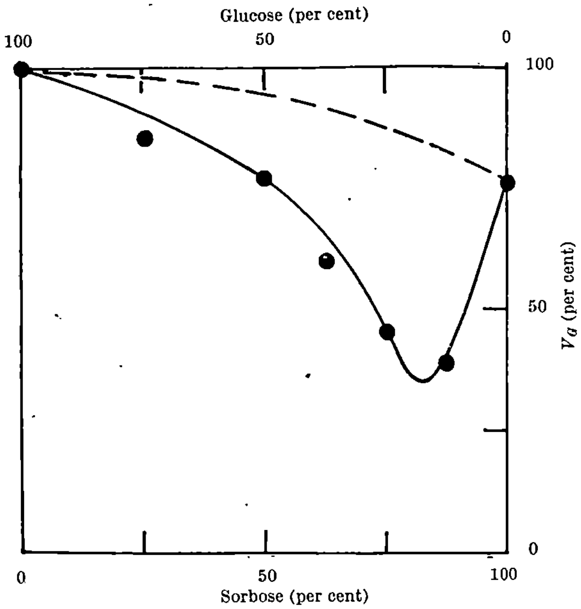


Fig. 4. Entrance of sugar into the human red blood cell from a mixture of glucose and sorbose. Units and symbols as in Fig. 1, but abscissa is 'percentage sorbose concentration'. K and β as in Figs. 1 and 2

0·08 M for fructose (these values were chosen for reasons to be described here). The divalent theory is perhaps marginally better with these values for K and β, but if K is chosen to be very large (in fact, infinite, that is, when the initial velocity is directly proportional to concentration), monovalent theory is as successful as divalent theory, within the experimental error of the results. Clearly such results do not allow us to distinguish between the models suggested but do demonstrate that divalent theory can adequately account for kinetic data of this type. We can, however, devise an unambiguous criterion to distinguish the monomer and dimer models as follows:

Let us investigate how the velocity of transfer varies in a mixture of two sugars (A and B) as the proportion of each sugar is altered. We keep the total concentration of sugar ($A + B$) constant (in the present investigation at 0·19 M) and alter the relative amount of A from 0 to 100 per cent. The experimental results for the pairs glucose/fructose glucose/sorbose and fructose/sorbose are given in Figs. 3, 4 and 5 respectively.

As we alter the relative amounts of A and B, there will always be a particular ratio of A to B at which the amount of A bound to the binding site will be exactly equal to the amount of B similarly bound. This ratio of A to B is given by the ratio of the Michaelis constants K_A and K_B (or β_A and β_B on divalent theory). On monomer theory this equivalence point is of no great theoretical interest; but such is not the case with the dimer theory. For if half the sites binding substrate are binding A, and half are binding B, molecules, the ratios of the dimers formed by any subsequent interaction are $\frac{1}{4}$ AA, $\frac{1}{4}$ BB and $\frac{1}{2}$ AB. In the mixture of A and B, therefore, we have a new species: AEB (E standing for the enzymic binding-site) present in neither of the pure components. Now it may be that such an AEB complex, by virtue of peculiar structural features of the molecules A and B, cannot form the true dimer AB, or that if such a dimer is formed it cannot penetrate the membrane. Then it will follow that at the equivalence point where A and B are equally bound to the binding sites, 50 per cent of the complexes formed will be AEB complexes (heterocomplexes) and will fail to form effective dimers. We would predict, therefore, that in such circumstances, the observed initial velocity of transfer in the mixture would be lower than would be the case for either of the components present alone. Monomer theory predicts no such minimum velocity, the predicted initial velocity never falling below the velocity found for the slower component present alone. For on monomer theory, if A inhibits the binding of B, it itself forms an effective complex with the binding site and a proportion of these complexes must be carried through the membrane to contribute to the observed velocity of transfer. The proportion of these A complexes which penetrate must be independent of the presence of B, if a ternary AEB complex is not postulated.

In Figs. 3 and 4 we have computed the predictions of monomer theory (dashed line) and dimer theory (solid line) for the glucose/fructose and glucose/sorbose systems. K for glucose has been taken as 0·05 M, the value which best fits the data on pure glucose (Fig. 1), while the corresponding constants for fructose and sorbose have been arbitrarily taken as ten times this amount. Clearly from Figs. 2 a and b, the true value of K is a good deal greater than this, but, whatever value is chosen above 0·5 M, only an infinitesimal difference to the prediction on monomer theory will result. The value of the corresponding constant β for glucose of 0·02 M is again taken from the data on pure glucose (Fig. 1). With this value, integral values between 3 and 10 of the ratios of β_{glucose} to β_{fructose} or β_{sorbose} were taken and the prediction on dimer theory computed. The best fit (and hence the lines drawn in Figs. 3 and 4) demand

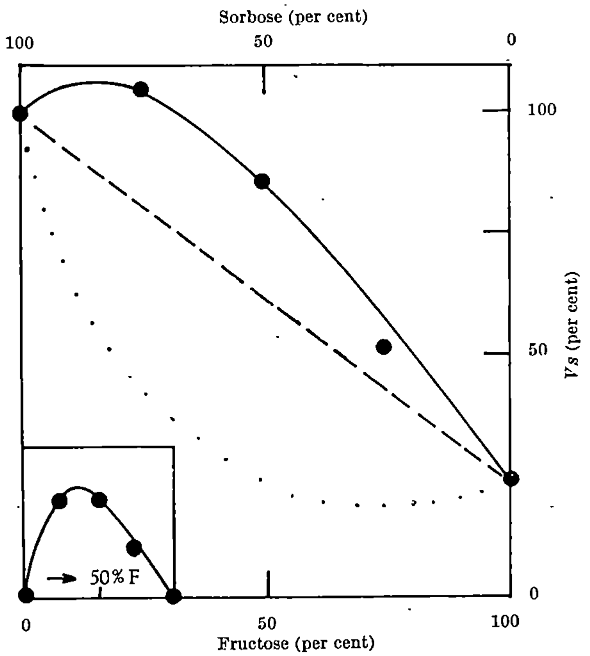


Fig. 5. Entrance of sugar into the human red blood cell from a mixture of sorbose and fructose. Ordinate, initial velocity relative to that in 0·19 M sorbose alone; abscissa, percentage fructose concentration. Symbols as in Fig. 1. K and β as in Figs. 1 and 2, but dotted line is 'prediction on dimer theory where heterocomplexes do not yield effective dimers'; solid line is 'prediction on dimer theory where heterocomplexes do form effective dimers'. Inset: difference between prediction on simple dimer theory and experiment

a ratio of β_{fructose} to $\beta_{\text{glucose}} = 4$ and a ratio of β_{sorbose} to $\beta_{\text{glucose}} = 10$, giving $\beta_{\text{fructose}} = 0{\cdot}08\ M$ and $\beta_{\text{sorbose}} = 0{\cdot}2\ M$. These values for β were then used to compute the solid lines in Figs. 2*a* and *b*. Clearly monomer theory is not consistent with both the experimental procedures of Figs. 1 and 2 on one hand, and 3 and 4 on the other; but dimer theory can very adequately account for all these results.

We have so far considered only the case in which the *AEB* complex fails to form effective dimers. But it is possible that the heterocomplex *AEB* may lead more readily to fruitful interaction than the homocomplexes *AEA* and *BEB*. It is clearly quite possible that *A* and *B* may have a complementary structure such that the dimer *AB* is more readily formed than either *AA* or *BB*. Such a consideration is forced on us by the data of Fig. 5 for the sorbose/fructose system. The dashed line in the figure is the prediction of monomer theory with $K = 0{\cdot}5\ M$ for each sugar. This line is simply the straight line between the points representing the velocities of the pure components. Variations of K above the value $0{\cdot}5\ M$ would lead to only a very small change in this prediction. The dotted line in the figure is drawn on the assumption that the heterocomplex SEF (sorbose–enzyme–fructose) fails to lead to the formation of SF dimers. Clearly this theory is even less effective than monomer theory in accounting for the data. On the inset to Fig. 5 are plotted as solid points the difference between the experimental points of the upper figure and the predicted points of simple dimer theory. The solid line drawn in this inset gives the concentration of the SEF heterocomplex computed with values for β_{sorbose} and β_{fructose} derived from Figs. 3 and 4, on a scale chosen so that the curve passes through the point on the difference curve at 50 per cent concentration. Then the solid line clearly also passes through the remaining points on the difference curve. It is thus the species SEF that must be contributing to the transfer across the membrane. It is possible to derive from the inset curve the interesting result that the species SEF is 1·76 times as effective as SES in leading to dimer formation and subsequent transfer. With this value the upper solid curve of Fig. 5 can be constructed, a curve which adequately accounts for the experimental data. In contrast to the glucose/fructose and glucose/sorbose systems, it would appear, therefore, that fructose and sorbose are in some way complementary structures and, when bound at the 'dimerase', interact with one another more effectively than they would interact with themselves.

It appears difficult to account for these data on monomer theory, but clearly on dimer theory a consistent explanation of all the above experimental data can be presented, with the values for the constants β and $V_{\text{max.}}$ (the maximum velocity of transfer) as given in Table 1 ($V_{\text{max.}}$ refers to the penetration of the dimer, β to the binding of the monomers by the separate sites of the dimerase).

Table 1

Sugars	Affinity constant β (moles/l.)	Maximum velocity (fructose = 1)
Fructose–fructose	0·08	1
Glucose–glucose	0·02	3
Sorbose–sorbose	0·2	8
Fructose–sorbose	—	14
Glucose–sorbose	—	0
Glucose–fructose	—	0

There is other evidence to suggest that the binding site for sugar may bind pairs of substrate molecules. Thus, Lefevre has shown[7] that certain inhibitors of glucose transfer (analogues of phloridzin), possessing two hydroxyl groups, appear to bind in pairs at the glucose binding site, while only one molecule of phloridzin itself is so bound. This evidence is now readily interpretable. (A similar phenomenon is found in the glycerol system[8], where the binding site is monovalent towards the competitive inhibitor 1,3-dihydroxypropane, but divalent towards isopropanol. The 1,3-dihydroxypropane apparently binds across the active centre of the dimerase, a hydroxyl interacting with each of the pair of binding sites for glycerol, while the alcohol binds independently at each of the two binding sites.) Bowyer and Widdas have shown[9], in work on the kinetics of dinitrofluorbenzene inhibition of glucose transfer in the red cell, that the rate of this reaction was second order with respect to both the inhibitor and the membrane sites. This result is readily interpretable if the binding site for glucose is divalent, as the dimerase model requires.

It is perhaps surprising that molecules as large and complicated as glucose can form dimers to any extent. It is possible, however, with both Catalin and skeleton-type molecular models, to make at least one possible dimer between pairs of glucose molecules without conflicting any stereochemical principle. But it is too early to view these artefacts seriously while it is yet uncertain as to how many hydroxyl groups must be involved in the formation of the penetrating dimer.

Such dimerases may have a wider application than simply to the red blood cell facilitated diffusion systems. In particular, the kinetics of the outward transport of arabinose from perfused rat hearts, under conditions of anoxia inhibition[10], may well follow dimer theory, the increase in velocity with concentration being faster than can be accounted for on monomer theory. Also in the case of uptake of amino-acid by ascites cells, a phenomenon similar to that of Fig. 5 has been discovered, the initial rate of uptake of L-tryptophan being increased by pre-incubation with azaserine[11].

Further analysis of these and similar systems may show that dimerases, if they exist at all, are even more widely distributed.

I wish to thank Prof. F. J. W. Roughton for his hospitality in providing me with facilities for this work, and Dr. J. C. Kernohan and Dr. D. A. Haydon for their invaluable help in the development of these ideas.

Note added in proof. It has been pointed out to me that the term 'dimerase' might suggest that the catalytic component involved in facilitated diffusion acts by forming and breaking covalent bonds, as does a true enzyme. There is no reason, however, to think that anything other than hydrogen bond interactions are concerned. I propose, therefore, to refer to such a membrane transfer mechanism as a 'dimerizer'.

[1] Lefevre, P. G., *Symp. Soc. Exp. Biol.*, **8**, 118 (1954).
[2] Wilbrandt, W., *Symp. Soc. Exp. Biol.*, **8**, 136 (1954).
[3] Widdas, W. F., *J. Physiol.*, **125**, 163 (1954).
[4] Bowyer, F., *Intern. Rev. Cytol.*, **6**, 469 (1957).
[5] Stein, W. D., *Nature*, **191**, 352 (1961).
[6] Ørskov, S. L., *Biochem. Z.*, **279**, 241 (1935).
[7] Lefevre, P. G., *Pharmacol. Rev.*, **13**, 39 (1961).
[8] Stein, W. D. (to be published).
[9] Bowyer, F., and Widdas, W. F., *J. Physiol.*, **141**, 219 (1958).
[10] Morgan, H. E., Cadenas, E., Regen, D. M., and Park, C. R., *J. Biol. Chem.*, **236**, 262 (1961).
[11] Jacquez, J. A., *Proc. U.S. Nat. Acad. Sci.*, **47**, 154 (1961).

EXTRACTION OF HEAT-LABILE ALKALOIDS FROM PLANTS

By Dr. A. R. MATTOCKS

Toxicology Research Unit, Medical Research Council Laboratories,
Woodmansterne Road, Carshalton, Surrey

THE method to be described here has been applied to the separation and subsequent purification of pyrrolizidine alkaloids from plants. It should be generally applicable to any heat-labile compound which is cationic in neutral or weakly-acid solution.

The apparatus is shown in Fig. 1. *A* and *B* are 800-ml. aspirators fitted with rubber bungs and glass connectors as indicated. They rest on a spring balance, *C*, with support from a light stand (not shown) also attached to the balance. The balance is modified by the addition of a spring, *D*, to compensate for the weight of the apparatus. A small mercury switch, *M*, is fitted in place of the pointer. The upper aspirator, *A*, is connected via flexible plastic tubing, T_1, and a T-piece to a water-pump and to an electrically operated air valve, V_1. The latter (Fig. 1, left) consists of a piece of narrow gauge, thin-walled rubber tubing which is normally closed by pressure from a spring-loaded iron plunger. When a current is applied to the solenoid, *S*, the plunger is withdrawn into the solenoid and hence the valve is opened. (This device can be constructed from a discarded motor car trafficator.) The lower aspirator, *B*, contains a triple 'Bunsen' valve, V_2, made by cutting three parallel slits, about 7 cm. long, in a length of thick-walled rubber tubing of approximately 8 mm. outer diameter. The exit tube, *E*, in the form of a syphon, leads into the extraction vessel, *F*, containing the ground plant, *P*, supported on a layer of cotton-wool, *W*. This vessel is either a large inverted bell jar (diameter 20 cm.) holding up to 3 kgm. of plant, or an inverted bottle (Winchester) with the bottom removed, for smaller quantities. It is connected via a column, *G*, containing cation exchange resin, a reservoir, *H*, and a plastic return tube, T_2, to the upper aspirator, *A*. The resin I have used is 'Dowex 50' (200–400 mesh, cross-linkage 8 or 12) in the acid form.

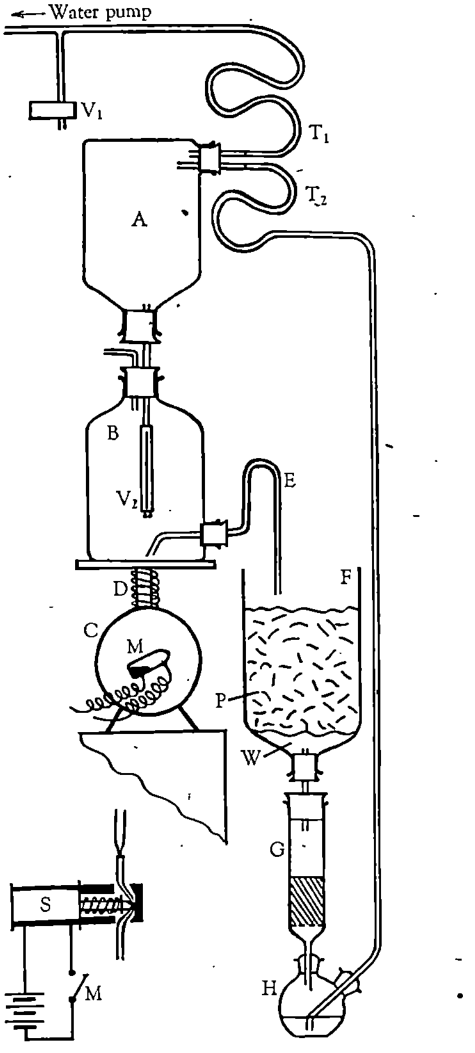


Fig. 1. Right, apparatus for continuous cold extraction (not to scale); left, details of the valve, V_1

For operation, the mercury switch, *M*, is connected via a battery to V_1, and its angle is adjusted so that it reaches the 'on' position when *A* is about three-quarters filled with solvent. The plant, *P*, is soaked in solvent, usually alcohol, and then covered to a depth of a few inches. The water pump is then turned on so as to apply a gentle suction to *A*. The apparatus functions as follows: solvent is drawn through the plant and *G* into *H* and thence into *A*, by the applied suction, which also keeps V_2 closed (it is helpful to moisten V_2 with solvent initially). When *A* is sufficiently filled, V_1 is actuated by the switch *M*, air is admitted through T_1 and the solvent flows into *B* via V_2. *B* is then emptied through *E* into *F*, and, as the weight decreases, *M* is opened, V_1 is closed, and the cycle is repeated. By this discontinuous process, ethanol can be circulated indefinitely at rates between 9 and 14 l./hr.

Alkaloids are removed from the solvent by the resin, but chlorophyll and non-basic substances remain dissolved. The resin is washed briefly with solvent and with water, and the alkaloids are eluted by dilute aqueous ammonia, reduced with zinc and acid to convert N-oxides to the tertiary bases, and extracted from alkaline solution with chloroform. (Though the alkaloids often occur as the weakly basic N-oxides, these are apparently held by the resin as readily as the free bases themselves.) Alternatively, the column can be made basic by passing a solution of ammonia in ethanol. In contrast to previous work with other alkaloids[1], this does not elute the alkaloids, which can then be obtained in more concentrated solution by eluting with water.

Table 1. Extraction of Alkaloids with Ethanol using various Procedures

Plant	Approximate weight of plant extracted (kgm.)	Alkaloid: extraction	Method of isolation	Yield of crude alkaloid (per cent wt.)	Condition of product
Crotalaria retusa	7	Soxhlet (hot)	Conventional (*D*)	0·380	Crystalline with very considerable dark brown gum
Crotalaria retusa	1·1	2 percolations (*A*)	Conventional (*E*)	0·177	Crystalline with moderate brown impurities
Crotalaria retusa	1·4	3 percolations (*A*)	Conventional (*E*)	0·192	Crystalline with moderate brown impurities
Crotalaria retusa	1·1	2 percolations (*A*)	Dowex (*F*)	0·158	Very slightly brown crystals
Crotalaria retusa	1·3	3 percolations (*A*)	Dowex (*F*)	0·150	Very slightly brown crystals
Crotalaria retusa	1·3	3 percolations (*A*)	Dowex (*G*)	0·135	Almost colourless crystals
Crotalaria retusa	0·7	Percolation (*B*)	Dowex (*B*)	0·350	Slightly brown crystals
Heliotropium indicum	1·75	2 cold soakings (4 days) (*C*)	Conventional (*E*)	0·163	Pale brown gum
Heliotropium indicum	5·2	3 soakings (5 days) (*C*)	Conventional (*E*)	0·190	Pale brown gum
Heliotropium indicum	11	2 soakings (5 days) (*C*)	Dowex (*F*)	0·140	Colourless gum
Heliotropium indicum	2	Percolation (*B*)	Dowex (*B*)	0·390	Pale brown gum
Senecio jacobaea	1·8	Percolation (*A*)	Conventional (*E*)	0·065	Dark brown, semi-crystalline
Senecio jacobaea	1·8	Percolation (*A*)	Dowex (*F*)	0·063	Very light brown crystals

A, cold ethanol continuously circulated for several hours; *B*, ethanol circulated continuously through plant and then resin; *C*, plant soaked in cold ethanol for several days with occasional stirring; *D*, the extract concentrated by distilling at atmospheric pressure; the chlorophyll extracted; reduced to convert *N*-oxides to bases; made alkaline with ammonia and extracted with chloroform; *E*, as *D*, but extract concentrated under reduced pressure; *F*, extract passed once through bed of 'Dowex 50'; *G*, extract acidified with conc. sulphuric acid (1 per cent by volume), then passed through resin

An alternative procedure is desirable if the plant already contains decomposition products, as discussed later. In this case the column, *G*, is omitted, and several batches of solvent are circulated through the plant for a few hours each. The combined extracts are then passed once through the resin column.

The dimensions of the resin column are not critical. For extractions of up to 2·5 kgm. of plant we have used a column of 5 cm. diameter, containing about 120 gm. (damp weight) of resin (depth 10 cm.). As a preliminary trial a column of 'Dowex 50' (30 gm. damp weight) was washed with 4 *N* hydrochloric acid, water and ethanol. A solution of the pure alkaloid monocrotaline (1 gm.) in ethanol (1 litre) was passed through the column: the eluate contained no alkaloid. After washing with ethanol and water, elution with a mixture of 10 ml. ammonia (sp. gr. 0·880) and 200 ml. water, and extraction of this with chloroform, gave 100 per cent recovery of crystalline alkaloid.

The details of a series of extractions are given in Table 1. For each species, plants from a single batch were used for all experiments.

The amount of resin needed to extract all the alkaloid from a given lot of plants depends on several factors; some of the resin will react with inorganic cations (for example, *Heliotropium indicum* contains potassium nitrate), and the efficiency may be reduced by the adsorption of dark impurities and some chlorophyll. Some extracts darken the resin more than others. These seem to be from plant materials which have been stored for a long time or dried by heating, and may hence contain basic decomposition products of high molecular weight. In a trial with *Heliotropium indicum*, 40 gm. of fresh resin were treated with an excess of ethanolic plant extract until alkaloid ceased to be absorbed; 4·4 gm. of crude alkaloid (mol. wt. about 300) was then recovered from the resin.

Since inorganic salts are converted to acid by the resin, an experiment was performed with an ethanolic extract from *Crotalaria retusa* to which acid had been added (Table 1). Under these conditions the resin absorbed less alkaloid, but it was almost free from dark impurities. However, much more acid was used than would normally be formed in a continuous extraction cycle.

The resin can be regenerated by washing with 4 *N* hydrochloric acid, but brown and green impurities are best eluted with ethanolic hydrochloric acid. Trials indicate that each use with a crude extract decreases the resin's capacity to absorb alkaloids by up to 25 per cent.

The extraction of pyrrolizidine alkaloids from powdered plant materials by hot ethanol in a Soxhlet-type extractor is unsatisfactory, because much alkaloid may decompose through heating and the products are always contaminated with gummy, dark brown impurities. The concentration of such extracts and removal of chlorophyll is a tedious procedure. Repeated soaking of the plant in large volumes of cold alcohol gives a better quality product, though in lower yield, but very large volumes of alcohol must then be concentrated under reduced pressure.

Continuous percolation for 9–10 hr. is a more efficient method of extraction than several days' soaking in stationary solvent. Subsequent isolation of the alkaloids by means of cation exchange resin has additional advantages. Effectively large volumes of alcohol can be used for extraction, while subsequent concentration is not necessary, and the extraction of chlorophyll is avoided. The alkaloids are not subjected to heat, and brown, gummy impurities are almost absent from the product. When a resin column is included in the continuous cycle, the alkaloids are progressively removed from the plant as in a Soxhlet extraction, with which it is comparable in efficiency.

The main drawback of this method is that the resin is rendered unusable after a few regenerations, and must be discarded.

The use of cation-exchange resin has previously been reported for the isolation of alkaloids for analytical and medicinal purposes[1], and anion exchange resin has recently been used for the large-scale extraction of insulin[2]. The usefulness of the present method is illustrated by the fact that only after extraction in this way could the new alkaloid, indicine, from *Heliotropium indicum*, be readily obtained in crystalline form[3].

Thanks are due to the Tropical Products Council, London, for its help in obtaining supplies of *Heliotropium indicum* and *Crotalaria retusa*.

---

[1] See, for example, Büchi, J., *J. Pharm. Pharmacol.*, **8**, 378 (1956) and Schultz, O. E., *ibid.*, 387.

[2] Jorpes, E., Mutt, V., and Rastgeldi, S., *Acta Chem. Scand.*, **14**, 1777 (1960).

[3] Mattocks, A. R., Schoental, R., Crowley, H. C., and Culvenor, C. C. J. (to be published).

ROLE OF POLYSACCHARIDES IN STABILIZATION OF NATURAL SOIL AGGREGATES

By Dr. D. J. GREENLAND, G. R. LINDSTROM and Dr. J. P. QUIRK
Department of Agricultural Chemistry, Waite Agricultural Research Institute, University of Adelaide

IT is well known that polysaccharide and polyuronide materials are generally present in soils[1-3], and that in sufficient concentration they are particularly effective in causing aggregation[4,5]. The amounts normally present in soils are small[6]. They are, however, more strongly adsorbed by clay than the humic and fulvic acids as a whole[7]. Furthermore, the humic and fulvic acids are much less effective in stabilizing aggregates than the polysaccharides[3]. It therefore appeared reasonable to conclude that the soil polysaccharides normally played a significant part in the cementing of aggregates. However, a recent examination of the effects of periodate treatment on soil aggregates[8] indicated that in the soils tested the polysaccharides–polyuronides were either not contributing at all to the aggregation, or if they were, then their contribution was complementary to, and dependent on, the effect of other materials. Mehta *et al.*[8] considered that the periodate-borate treatment given to the aggregates would disrupt the polysaccharide–polyuronide molecules, so that if these were the essential cement in the aggregates, then the treatment would cause their breakdown. In fact the periodate treatment did not disrupt the aggregates tested. Aggregates prepared in the laboratory by treatment of soils with various polysaccharides were broken down by the treatment, showing the effectiveness of the technique for the purpose intended.

Although the earlier evidence which suggested that the polysaccharides and polyuronides played an important part in soil aggregation was only presumptive, it appeared desirable to examine the effect of periodate treatment on a further range of soils. It also seemed desirable to examine aggregates from soils that had been under short-term pastures, as well as those that had been under pasture for many years, since it has been shown[9] that long-term pastures produce a much more durable aggregation than short-term ones. It seemed possible that the less-durable aggregation could be due to the polysaccharides–polyuronides. Mehta *et al.*[8] showed only that aggregates from a braunerde under old forest and various other (unspecified) Swiss soils, including two rendzinas, were stable to the periodate treatment.

In the investigation reported here, soils were taken from the permanent rotation trial at the Waite Institute, and from similar red-brown earths and solonized brown (mallisols) and related soils from the lower north of South Australia. The textures of the soils varied from sandy loam to clay loam, their histories from permanent pasture to continuous wheat-fallow cultivation, and their carbon contents 3·6–0·8 per cent. Aggregates (1·0–2·0 mm.) were separated from surface samples (0–3 in.) by gentle hand sieving. These aggregates were carefully made wet on a sintered-glass funnel at 100 cm. water suction to prevent disruption on flooding[11], and transferred to a permeameter similar to that described by Dettmann and Emerson[10]. It was considered that a permeability test would be more likely to reveal differences in aggregate stability than the somewhat drastic 'Yoder' wet sieving technique used by Mehta *et al.*

After the aggregates were transferred to the permeameter they were further wetted at 8 cm. suction. They were then sodium-saturated by passing 3 litres of 0·02 or 0·05 *N* sodium chloride through the permeameter under a head of 3 cm. of solution. Treatment with 0·02 *N* sodium periodate was carried out in the permeameter, followed by 0·10 *N* sodium borate according to the procedure described by Mehta *et al.*[8]. The controls were treated with 0·01 *N* sodium chloride instead of periodate, but otherwise were subjected to the same reagents. Collapse of the aggregates leads to a reduction in permeability, and this was tested by measuring the rate at which 0·01 *N* sodium chloride passed through the beds of treated aggregates. The effect of the periodate treatment was assessed by comparing the permeabilities of the treated and untreated soils at different times (Fig. 1). The results are plotted in terms of the ratio of the rate of flow of solution (P_t) at a certain time after the introduction of periodate (or corresponding salt) to the rate of flow (P_i), immediately prior to periodate treatments.

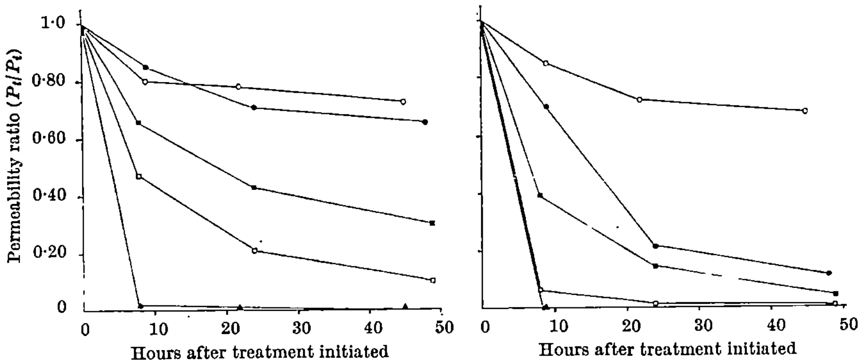


Fig. 1. Change with time in the permeability to 0·01 *N* sodium chloride of beds of aggregates from Urrbræ loam surface soil: left, control aggregates; right, periodate-treated aggregates. Histories of the soils from which the aggregates were taken are: ○, permanent pasture, 3·6 per cent C; ●, fourth year of pasture of 4 year pasture, 2 year wheat rotation, 1·92 per cent C; ■, second year of pasture of 2 year pasture, 1 year wheat rotation, 1·80 per cent C; □, continuous wheat, 1·60 per cent C; ▲, fallow of wheat-fallow rotation, 1·14 per cent C

Only the aggregates from old pasture soils high in organic matter were stable to the treatment. The soils which had been cultivated and then put down to pasture for two or four years showed a very marked decline in permeability on periodate treatment. The leachate from these aggregates contained a noticeably higher content of peptized organic material and clay. The aggregates from the continuously cultivated soils disintegrated considerably even without periodate treatment, so that it was difficult to be certain with these soils whether or not significant changes were produced. Only the results for the soils of precisely known history from the Waite Institute are given in Fig. 1; but the other soils collected from County Victoria fully confirmed these results.

It is concluded that molecules containing *cis*-hydroxyls are most probably involved in stabilizing aggregates in soils from young pastures. It is recognized, however, that periodate may react with *cis*-hydroxyls of molecules other than polysaccharides. However, the polysaccharides are most probably the constituents of soil organic matter involved.

It is possible that the periodate-resistant materials in old pasture soils are fungal hyphæ and mycelia, since Bond[12] has recently shown that in the soils from the permanent rotation trials at the Waite Institute very little of the fungal material is present in the young pastures, but it is very well developed in the older pastures.

[1] Forsyth, W. G. C., *Biochem. J.*, **41**, 176 (1947).

[2] Lynch, D. L., Olney, H. O., and Wright, L. M., *J. Sci. Food Agric.*, **9**, 56 (1958).

[3] Whistler, R. L., and Kirby, K. W., *J. Amer. Chem. Soc.*, **78**, 1755 (1956).

[4] Martin, J. P., *Soil. Sci.*, **61**, 157 (1946).

[5] Geoghegan, M. J., and Brian, R. C., *Biochem. J.*, **43**, 5 (1948).

[6] Dubach, P., and Lynch, D. L., *Soil Sci.*, **87**, 273 (1959).

[7] Greenland, D. J., *J. Soil Sci.*, **7**, 329 (1956).

[8] Mehta, N. C., Streuli, H., Muller, M., and Deuel, H., *J. Sci. Food Agric.*, **11**, 40 (1960).

[9] Low, A. J., *J. Soil Sci.*, **6**, 179 (1955).

[10] Dettmann, M. G., and Emerson, W. W., *J. Soil Sci.*, **10**, 215 (1959).

[11] Panabokke, C. R., and Quirk, J. P., *Soil Sci.*, **83**, 185 (1957).

[12] Bond, R. D., C.S.I.R.O. Division of Soils, Div. Rep., 10/60 (1960).

NON-RANDOMNESS IN AMINO-ACID 'ALLELES'

By RICHARD V. ECK

Laboratory of Biology, National Cancer Institute, National Institutes of Health, Bethesda 14, Maryland

VARIOUS proposals, general and specific, have been made in the past few years concerning a 'code' by which genetic information might be transferred from the hereditary material (presumably nucleic acids, but possibly nucleoproteins) to proteins such as hormones and enzymes. This relationship is sometimes referred to as a cryptogram, the nucleic acid chain being a 'signal' and the protein chain a coded 'message'. It is frequently assumed that it has another characteristic of cryptograms—a point-to-point relationship of some sort. Particular attention has centred on the discrepancy between the number of different nucleotide links in nucleic acids (four) and the number of different amino-acid links in proteins (twenty). If a nucleic acid sequence occurred 'at random' (with no limitation on which nucleotide could occur at any particular place), it would contain exactly two bits of information per link ($2^2 = 4$). Similarly, a completely unconstrained protein chain would contain 4·3 bits per link ($2^{4\cdot3} = 20$). Since these numbers are incommensurable, it is obvious that a point-by-point relationship of any sort must require that one or both of the chains contain redundancy or constraint.

An 'overlapping' code was first proposed by Gamow[1] in which each link in the protein chain would be determined by three adjacent links of the nucleic acid chain, the next protein link being determined by the last two of these three and the one following, etc. This code effectively reduced the amount of information in the protein chain to two bits per link. Any particular amino-acid would be determined partly by one corresponding nucleotide, and partly by the context of the immediately adjacent links. This would 'permit' a maximum of $4^5 = 1,024$ tripeptides, and 'prohibit' the occurrence of the rest of the $20^3 = 8,000$ mathematically possible tripeptides. This situation was disproved by Brenner[2], who showed that more published information already existed than could be fitted into such a limited pattern.

Since then, various proposals have been made concerning 'non-overlapping' codes, in which any protein sequence would be free to occur. One of these would have three adjacent nucleotide links to determine each amino-acid link independently of the next three. The nucleotide chain would be three times as long as the protein chain, and each amino-acid link would have six bits of information with which to be encoded. Considerable mathematical ingenuity has been exerted to develop patterns which would, in effect, dispose of the excess information, so that any random sequence of nucleotides would determine one unique sequence of amino-acids[3,4]. Another proposal would have some of the information come from a single nucleotide link, and the rest from some other unknown source[5,6]. I suggest that the unknown source might simply be the nearby links. (Brenner's analysis applies only to overlapping triplets. It would be very difficult and probably inconclusive to use his method to disprove longer overlapping codes, given the limited amount of data at present available.) However, Yčas failed to find statistical constraints on the frequency of occurrence of dipeptides. He considers this to be good evidence against an overlapping code and suggests that, in the case of a virus, the additional source may be the viral host[5].

If detailed chemical evidence were available for the link-by-link structure of some moderately long nucleic acid chain, and of the specific protein determined by the same chain, one might try to solve the cryptogram in a simple, direct way. There seems some reason to expect that such data will eventually become available, for example, from some simple virus. At present, however, there is very little evidence concerning the details of nucleic acid chains.

It is obvious that if a non-overlapping code of any sort (in which one amino-acid link contains no information concerning other links) were the basic mechanism, the code could not be solved (in fact, no information about it can be derived) by a study of the protein sequence data alone. An overlapping code, however, could, in principle, be completely solved in terms of four code numbers representing the four nucleotide bases. Chemical evidence from nucleic acids would then be needed to determine which nucleotide corresponds to each number. A code which is partly overlapping and partly determined by some other source of information might be solved to some limited extent.

I consider that the overlapping hypothesis has not been definitely disproved, and that it has several attractive features—one of which is the theoretical possibility of solving it, if it exists.

Spurious statistical constraints might be imposed on the published protein sequences by a variety of possible causes. Conversely, if constraints fundamentally exist, apparent randomness might be superimposed on them. However, if one assumes (1) that such confusion will not completely obscure and existing underlying pattern, and (2) that constraints will involve adjacent linkages (whatever else they may also involve), a general approach to the cryptogram is possible.

The published data on amino-acid sequences can be sorted, tabulated and arranged in a great variety of ways. (The simplest of these is to count and tabulate the dipeptides.) Any such manipulation will produce some sort of pattern. Tests can then be made to determine whether these patterns are more regular or more extensive than could reasonably be expected by chance. If one finds such evidence of non-randomness, it would always be possible to suggest some 'just so' explanation not requiring a code. Even so, an accumulation of several independent types of non-randomness, all of them predicted by the overlapping code, and not readily explained otherwise, would strengthen the hypothesis. Eventually, this procedure should make possible the development of the detailed structure of the code, when enough sequence information is available.

The statistical evidence of non-randomness presented here does not use the entire mass of amino-acid sequence data, but only that part which has been reported as alleles[7] or which seems to be confidently interpretable as alleles.

Examples of 'alleles' as used here are the various hæmoglobins, in which one amino-acid difference has been found to correspond to a difference in a single gene[7]. Also included are all apparently homologous proteins which differ only at particular links, such as the insulins from various species[8,9]. Allele pairs are included only if they are flanked on both sides by identical amino-acids. This eliminates many cases for which there might be presumptive justification, but no strong evidence, such as has been suggested for matching the two chains of insulin, etc.[6]. Two different amino-acids occurring at one locus are counted as one pair of alleles. Three at one locus produce three pairs, four produce six pairs, etc. A tabulation of all available cases yields 74 allele pairs. One of these (alanine : serine) occurs eight separate times, two occur four times, six thrice, five twice and 30 others occur only once. Thus, of the 74 there are 44 different pairs.

The question whether this distribution could occur by chance is approached as if the sample of 74 pairs had been drawn by lottery from the theoretical population of $\frac{20 \times (20-1)}{2} = 190$ possible pairs. One must first take into account the obvious inequality in frequency of the various amino-acids. Some are much more common than others. If they were all of equal frequency, the probability is 1/190 that any two pairs drawn at random would be duplicates. Using the reported frequency of the various amino-acids, this probability was computed to be 1/121.

In the 74 pairs, there are $\frac{74 \times 73}{2} = 2{,}701$ possible comparisons, each one of which (with trivial exceptions) has an equal chance to be a duplication. The number of duplications expected by chance is therefore: $\frac{2{,}701}{121} = 22$. Using this same method, every possible comparison being a countable instance, the duplications in the tabulated data are counted. An allele pair which occurs twice represents one duplication. A pair which occurs three times represents three duplications, etc. The total number of duplications in the data is 63. Since the 2,701 opportunities for duplication would not influence one another if the 'lottery' were truly random, the distribution would be binomial (within practical limits). The probability is infinitesimal that 63 cases could occur by chance in a binomial distribution where 22 were expected ($t = 6{\cdot}5$).

Various tests were done, in which certain of the original data were discarded on grounds that they might possibly introduce some bias. When the above calculations were repeated on these reduced bodies of data, the results were essentially unchanged.

The above calculation can be inverted, to find the effective size of the population of pairs from which the sample of 74 must have been drawn to give 63 duplications. Instead of the 190 expected by chance, this is computed to be 67, with 95 per cent fiducial limits between 52 and 87.

We conclude that these reported amino-acid alleles are not a random sample of the amino-acids found in all protein sequences reported. The same pairs are found repeatedly, far beyond their expected chance frequency. It is conceivable that some unknown cause may have biased the sample, but what this may have been is not obvious. On the other hand, this is exactly what would be expected from an overlapping code; given a particular context of surrounding links, not all possible amino-acids are 'permitted' to occur.

A full account of the mathematical calculations, and a more extensive discussion of the general approach, are being prepared for publication elsewhere.

I wish to thank Dr. Norman Shapiro, of the Computation and Data Processing Branch, U.S. National Institutes of Health, for his helpful criticism of the mathematical treatment.

[1] Gamow, G., *Nature*, **173**, 318 (1954).
[2] Brenner, S., *Proc. U.S. Nat. Acad. Sci.*, **43**, 687 (1957).
[3] Crick, F. H. C., Griffith, J. S., and Orgel, L. E., *Proc. U.S. Nat. Acad. Sci.*, **43**, 416 (1957).
[4] Gamow, G., and Yčas, M., *Proc. U.S. Nat. Acad. Sci.*, **41**, 1011 (1955).
[5] Yčas, M., *Nature*, **188**, 209 (1960).
[6] Yčas, M., *J. Theor. Biol.*, **1**, 244 (1961).
[7] Hunt, J. A., and Ingram, V. M., *Symp. Prot. Struct.*, 148 (1957).
[8] Harris, J. I., Sanger, F., and Naughton, M. A., *Arch. Biochem. Biophys.*, **65**, 427 (1956).
[9] Ishihara, Y., Saito, T., Ito, Y., and Fujino, M., *Nature*, **181**, 1468 (1958).

LETTERS TO THE EDITORS

RADIOPHYSICS

Effect of External Conditions on the Phase of Radio Signals

THE emissions at 16 kc./s. from the Post Office *GBR* transmitter at Rugby are in the form of interrupted continuous waves and are used for purposes which include the international co-ordination of standards of frequency and time and studies of propagation[1-3] by comparing the phase of the received signals with that of a local reference source. The relative observed phase of the carrier wave will change, due to changes in the rates of the controlling reference oscillators, to varying radio propagation and to phase changes in the transmitter. Some investigations have been made of the latter by comparing the phases of the aerial current and the carrier source.

We have found that phase aberrations can be caused by the following:

(1) Detuning of the low-decrement aerial by changes of weather conditions and by aerial sway. Throughout November and December 1960, phase shift was recorded during typical weather including gales and icing conditions. Slow changes of phase of 20° or more were observed on several occasions, and once a change of up to 60° in a 2-hr. period would have occurred but for manual re-tuning, which was carried out when the phase had changed some 20°. Aerial sway produced fluctuations of 12° with periods of 1–2 min. Throughout the period of observation the aerial circuit was slightly out of tune (more than 7° shift in phase) for 32 per cent of the time and noticeably out of tune (more than 13° shift) for 2·2 per cent.

(2) Fluctuations in the inductance of the aerial tuning inductors caused by mechanical forces between coupled turns set up by the current. The natural period of the fluctuations is about 3 c./s., but their magnitude is largely determined by the nature of the keyed signals. During the mark signals of normal telegraph traffic, we have observed a random fluctuation of about 7°. The seconds pulses of the time signals cause only a small phase variation from pulse because the off/on ratio has a constant value of 9. A gradually increasing current up to the maximum of 800 amp. under conditions of continuous mark produces a change of 8° corresponding to a change of inductance of 0·07 per cent.

(3) Phase changes in the amplifiers during a mark. The transient electrical and thermal effects in the class *C* amplifiers produced a phase shift of about 30° during the build up of the mark, which takes about 20 msec. to reach 90 per cent amplitude in the aerial. At the end of the mark the aerial, being unloaded by the amplifier, oscillates freely and the phase of the aerial current changes by more than 360° during the decay.

With methods of measurement at present in use, only phase changes under heading (1) would be likely to be observed at a distance. Accurate frequency comparisons are made after integrations of phase during multiples of 24 hr. to remove the diurnal propagation effect[3], but this could include a phase error due to changes of aerial tuning which might amount to 60°, equivalent at 16 kc./s. to 10 μsec. or a little more than 1×10^{-10} in 24 hr. Automatic aerial tuning and other phase-correcting devices now being actively considered, besides keeping power more constant, would reduce this possible error. It would also hold more constant the over-all response of the transmitter to transients and enable more accurate determinations to be made of epoch from time-signal transmissions.

D. E. WATT-CARTER

Overseas Radio Branch,
Post Office Engineering Department,
2–12 Gresham Street, London, E.C.2.

R. L. CORKE

Research Branch,
Post Office Engineering Department,
Dollis Hill, London, N.W.2.

[1] *Nature*, **174**, 922 (1954).
[2] *Nature*, **177**, 178 (1956).
[3] *Nature*, **187**, 914 (1960).

GEOPHYSICS

Age Measurements on Antarctic Rocks (Queen Maud Land)

WE wish to report here the results of absolute age measurements carried out on rocks from the Sör-Rondane Mountains. These mountains, extending from 23° E. to 28° E. in Queen Maud Land, were explored in 1958 in the course of the Belgian Antarctic Expedition led by Gaston de Gerlache[1]. Preliminary accounts of the geology and of the petrography of this region have been published[2,3] already.

We have measured rubidium–strontium ages on biotites obtained from various types of rocks : 'intrusive' granite and diorite, gneiss and migmatites, granitic and pegmatitic veins. The procedures for the determination of rubidium-87 and strontium-87 were similar to those described by Aldrich *et al.*[4]. The isotopic ratios were measured with a 33-cm. radius of curvature mass-spectrometer equipped with an electron multiplier. The analytical error of the concentrations is between 1 and 3 per cent, resulting in an uncertainty of ± 15 million years in the calculated ages. The analytical data and the resulting age appear in Table 1. The geographical names refer to the map of the Sör-Rondane Mountains, published by the Norsk Polarinstitutt, during 1957.

The results indicate that the biotites are all the same age, ~ 475 million years, which corresponds in the stratigraphic scale to a Lower or Middle Ordovician age.

Ravitch and Krylov[5] carried out argon–potassium measurements on rocks occurring in the mountains situated west of our region. Their data yield ages ranging from 410 to 500 million years by using the values: $\lambda_\beta = 4 \cdot 72 \times 10^{-10}$ yr.$^{-1}$ and $\lambda_e = 5 \cdot 85 \times 10^{-11}$ yr.$^{-1}$ for the decay constants of potassium-40.

On the other hand, Nicolaysen *et al.*[6] report rubidium–strontium ages of ~ 515 million years for biotites from pegmatites and charnockitic gneiss occurring near Lützow-Holm Bay, on the eastern limit of Queen Maud Land.

Our data around 475 million years refer clearly to the last major metamorphic episode in Queen Maud

Table 1. RUBIDIUM–STRONTIUM AGES OF BIOTITES

Location	Source rock	Rubidium (p.p.m.)	Radiogenic ^{87}Sr / Total ^{87}Sr	Age (m. yr.)
Romnoesfjellet	Porphyroblastic granite	563	0·42	478
Romnoesfjellet	Pegmatite vein in granite	1,108	0·91	465
Småhausane, 1,180	Quartz diorite	139	0·56	458
Småhausane, 1,180	Diorite	328	0·39	457
Nordtoppen, 1,100	Biotite gneiss (xenolith in diorite)			
	Fine fraction	1,477	0·92	476
	Coarse fraction	1,459	0·89	481
Nordtoppen, 1,100	Coarse biotite crystals from xenolith in diorite	786	0·59	493
Strandrufjellet	Biotite gneiss	545	0·56	455
Strandrufjellet	Fine-grained granite (anatectic ?)	1,329	0·90	483
Bautaen	Fine-grained granite (anatectic ?)	1,282	0·93	506
Trillingane	Amphibole biotite gneiss	371	0·50	471
Trillingane	Leucocratic pegmatite	842	0·60	474

λ rubidium-87 = $1·39 \times 10^{-11}$ yr.$^{-1}$, rubidium-87 = 0·283 gm./gm. rubidium

Land. They confirm the very large extension of this episode, which has been recorded from 165° E. (ref. 7) to 10° E. along the Antarctic coast.

Biotites are known to be very sensitive to metamorphism and to keep record only of the last metamorphism, therefore very little can be said about the age of the intrusive bodies, the migmatites and the pegmatites occurring in Queen Maud Land, in relation to the $\sim$500 million years old metamorphic episode.

In order to solve these problems, more measurements should be carried out not only on biotites but also on minerals like feldspars, muscovites or zircons, which preserve pre-metamorphic ages better than biotites[8,9].

We wish to thank Miss E. Jäger (Mineralogisches Institut, Berne), who kindly made available to us her calibrated strontium and rubidium spikes.

Financial support from the Belgian Institut Interuniversitaire des Sciences Nucléaires and Centre National des Recherches Polaires is acknowledged.

SARAH DEUTSCH
E. E. PICCIOTTO
M. REINHARZ

Laboratoire de Physique Nucléaire,
Université Libre de Bruxelles.

[1] Picciotto, E., *Ciel et Terre*, **77**, 126 (1961).

[2] Picciotto, E., *Nature*, **186**, 740 (1960).

[3] Picciotto, E., Michot, J., and Michot, P., *Bull. Soc. belge Géol.*, **69**, fasc. 2 (1960).

[4] Aldrich, L. T., Davis, G. L., Tilton, G. R., and Wetherill, G. W., *J. Geophys. Res.*, **61**, 215 (1956).

[5] Ravitch, H. G., and Krylov, A., *Inform. Bull. Soviet Antarct. Exp.*, **20**, 15 (1960).

[6] Nicolaysen, L. O., Burger, A. J., Tatsumi, T., and Ahrens, L. H., *Geochim. Cosmochim. Acta*, **22**, 94 (1961).

[7] Goldich, J. S., Nier, A. O., and Washburn, A. L., *Trans. Amer. Geophys. Union*, **39**, 956 (1958).

[8] Wetherill, G. W., Davis, G. L., and Tilton, G. R., *J. Geophys. Res.*, **65**, 2461 (1960).

[9] Tilton, G. R., Wetherill, G. W., Davis, G. L., and Hopson, C. A., *Bull. Geol. Soc. Amer.*, **60**, 1469 (1958).

GEOCHEMISTRY

Microstructural and Chemical Identity of the Bear Creek and Narraburra Iron Meteorites

METALLOGRAPHIC examination of small specimens taken from the Bear Creek (*Nin.* 352·1γ) and Narraburra (*B.M.* 1927, 1264) iron meteorites reveals a remarkable similarity of microstructural detail. This similarity of structure is matched by the analytical results of Brown *et al.*[1], who place both bodies in the same gallium–germanium group and report the following detailed analyses for Bear Creek and Narraburra respectively: nickel, 10·14 and 10·22; cobalt, 0·51 and 0·59; chromium, 1·2 and <1; copper, 154 and 157; gallium, 15 and 8·2; germanium, 25 and 29. The values for nickel and cobalt are percentages, the other values are p.p.m.

The object of the present communication is to propose that the two meteorites are fragments of the same original mass and have been subject to similar histories.

By comparison with the majority of finest octahedrites both specimens show the following anomalous structural features: acicular structure in both Widmanstätten and plessitic kamacite; bainitic structure in the larger areas of taenite; absence of a marked and continuous taenite border along the interface of coarse plessite fields, coupled with a tendency for small rounded schreibersite crystals to be present as satellite bodies in the kamacite around taenite regions instead of actually in the kamacite – taenite interface. These features are well shown in the Bear Creek photomicrograph published, as Fig. 2, Plate 37, of Perry's monograph[2].

Though it is not, as yet, possible to give a comprehensive account of the thermal history which produced this anomalous structure, the metallographic indications are of a heat treatment or series of heat treatments applied to a normal fine octahedrite. Laboratory experiments involving a single heating cycle have not so far reproduced this type of structure, but speculation may reasonably be directed to the possibility of a series of heating and cooling cycles, such as might arise by frictional heating of a body repeatedly in non-circular orbit about the Earth. This mechanism of heating is to some extent supported by the fact that the 500-lb. Bear Creek meteorite was found in the United States while the 71-lb. Narraburra was found in Australia. Such a wide geographical separation of chemically identical material, when considered in relation to the anomalous details of the microstructure, suggest the fragmentation of a captured satellite meteorite as a more plausible explanation than two separate and isolated falls. Published photomicrographs of Smith's Mountain in Perry's monograph[2] (Fig. 1, Plate 38; Fig. 2, Plate 41) suggest that this may be a third member of the same group, but detailed analytical values for Smith's Mountain are not available.

Thanks are due to Dr. Max Hey for the loan of specimens from the British Museum.

H. J. AXON

Metallurgy Department,
The University,
Manchester.

[1] Lovering, J. F., Nichiporuk, W., Chodos, A., and Brown, H., *Geochim. Cosmochim. Acta*, **11**, 263 (1957).

[2] Perry, S. H., *U.S. Nat. Mus. Bull.* 184 (1944).

PHYSICS

Recoil-Atom Reactions at Low Temperature

CHEMICAL effects attending transformations of the nuclei of atoms in solids have been observed in a large number of systems[1]. Such effects are attributed to nuclear recoil, inner shell ionization followed by an Auger cascade, or nuclear charge change, for example in β-decay. Atoms which have transformed may be found in their original chemical form[2] or in altered

Table 1. RETENTION OF RECOIL CHROMIUM-51 ATOMS IN $NA_2CRO_4.4H_2O$ IRRADIATED WITH NEUTRONS

Irradiation		Storage		Dissolution		Medium	Retention
Temp. (° C.)	Time (min.)	Temp. (° C.)	Time	Temp. (° C.)	Time		(per cent)
+ 55	3	room	1 week	+ 2	30 sec.	H_2O *	89·3 ± 0·6
+ 55	3	room	1 week	− 55	3 hr.	LiCl †	88·6 ± 1 §
c. + 30	0·5 ‡	− 78	3 days	− 55	3 hr.	LiCl	54·8 ± 0·5
− 78	3	− 78	5 days	− 55	3 hr.	LiCl	39·8 ± 1·0
− 78	3	− 78	5 days	+ 2	30 sec.	H_2O	47·3 ± 1 §
− 78	3	− 78	5 days				
and then		+ 20	3 min.	+ 2	30 sec.	H_2O	58·9 ± 1 §

* The dissolution medium used in previous work (ref. 5).

† LiCl-H_2O eutectic mixture (see text).

‡ Sample transferred from the reactor following irradiation to dry ce in less than 30 sec.

§ Single determination.

chemical states. The percentage of radioactive atoms found in the original chemical form has been called the 'retention' by Libby[2]. A recoil atom of the latter type may be considered a defect in the crystalline lattice, and in analogy with thermal annealing reactions of defects, for example interstitial atoms, produced by heavy particles of γ-ray bombardment[3], the application of heat to crystals containing recoil atoms frequently changes the observed distribution of chemical species[1]. In general, annealing reactions of recoil atoms tend to restore those atoms to their original ('parent') chemical form. Recoil reactions following slow neutron capture are known as Szilard–Chalmers reactions, after the discoverers of this effect[4].

In work on radiation damage in solid-state physics, it has been found desirable to provoke the damage, and to measure it, at very low temperatures, in order to preserve the original distribution so far as possible. Annealing reactions may take place even below liquid-air temperature, indicating activation energies of only a few tenths of an electron volt. The corresponding situation in chemical, for example Szilard–Chalmers, systems is experimentally more difficult, since in general one would wish to carry out some sort of chemical analysis, perhaps involving dissolution of the crystal containing the recoil atom, at low temperatures.

We wish to report here experiments in which we have done this. For example, we have analysed neutron-irradiated alkali chromates by dissolution in aqueous solutions at −55° C., and sodium bromacetate in methanol at −60° C. The aqueous solutions were eutectic mixtures of 33·7 gm. of lithium chloride to 100 gm. of water, freezing at about − 70° C. The lithium salt was found to be inert in affecting the distribution of chromium recoil atoms between the tri- and hexa-valent oxidation states. We have thus been able to irradiate crystals with neutrons at the temperature of dry ice (−78° C.) and analyse them without allowing them to warm up. Complete experimental details will be published elsewhere.

In Tables 1 and 2 are presented retention[2] data obtained by these low-temperature techniques, including the complete thermal history of the specimens. Each figure is the average of two or more independent determinations, unless otherwise noted.

Table 2. RETENTION OF RECOIL BROMINE-80M and 82 ATOMS in $BRCH_2COONA$ IRRADIATED WITH NEUTRONS

Irradiation		Storage		Dissolution		Retention (per cent)	
Temp. (° C.)	Time	Temp. (° C.)	Time	Temp. (° C.)	Time (min.)	Bromine-80m	Bromine-82
+ 55	3 min.	+ 25	3 hr.	− 60	15	33·5	26·2
+ 25	10 sec.	+ 25	3 hr.	− 60	15	30·5	25·4
+ 55	3 min.*	− 78	3 hr.	− 60	15	30·0	19·5
c. + 25	10 sec.*	− 78	3 hr.	− 60	15	26·6	18·3
− 78	3 min.	− 78	3 hr.	− 60	15	25·5	18·1
− 195	3 hr.	− 195	3 hr.	− 60	15	25·0	18·0
− 78	3 min.	− 78	3 hr.				
and then		+ 45	3·5 min.	− 60	15	33·1	27·6

* Sample transferred from the reactor following irradiation to dry ice in less than 30 sec.

Similar experiments have been carried out with potassium and ammonium chromates; here, too, lower retentions are observed for low-temperature conditions. For example, potassium chromate, which has a room-temperature retention of 60·5 per cent (ref. 5), shows a retention of about 47 per cent when irradiated and stored at dry-ice temperature, and dissolved at − 55° C. These experiments will also be more fully reported elsewhere.

It is surprising to note that, despite annealing effects which are important at + 55° C., one can obtain almost the same retention in sodium bromacetate by irradiating at room temperature (+ 25°) and rapidly quenching in dry ice, as by irradiating at dry-ice temperature. This demonstrates that there is little effect of the ambient temperature on the recoil-chemistry, for this compound. Similar effects have been found for bromine-80m and 82 recoil atoms in potassium bromate, and for bromine-80m formed by (*n*, 2*n*) reactions in sodium bromacetate.

It is evident from the results in Tables 1 and 2 that, in general, retention values are only significant in terms of the thermal history of the irradiated specimens. One might even say that it is probably impossible to measure 'intrinsic' retentions as meaningful quantities, since one cannot carry out chemical analyses at indefinitely low temperatures, as would be necessary. One always will observe the 'intrinsic' retention increased by a variable amount which depends on the temperature and time of irradiation, storage and analysis. Uncertainties in these unknown increments thus make dangerous any attempt to correlate retention data with recoil energies derived from spectra of neutron-capture γ-rays or with chemical compositions or to use mechanical ('billiard ball') models to explain retention values observed in crystals.

This work was carried out under the auspices of the U.S. Atomic Energy Commission.

SLOBODANKA VELJKOVIĆ *
GARMAN HARBOTTLE

Department of Chemistry,
Brookhaven National Laboratory,
Upton, Long Island,
New York.

* On leave from the University of Belgrade, Yugoslavia.

[1] Harbottle, G., and Sutin, N., *Adv. Inorg. Chem. and Radiochem.*, 1, 267 (Acad. Press, New York, 1959).

[2] Libby, W. F., *J. Amer. Chem. Soc.*, 62, 1930 (1940).

[3] Dienes, G. J., and Vineyard, G. H., *Radiation Effects in Solids* (Intersci. Pub., New York, 1959).

[4] Szilard, L., and Chalmers, T. A., *Nature*, 134, 462 (1934).

[5] Harbottle, G., *J. Chem. Phys.*, 22, 1083 (1954).

Melting and Polymorphism of Indium Antimonide at High Pressures

THE change of electrical resistivity with pressure of indium antimonide has been studied by Keyes[1] up to 12,000 atmospheres and recently by Gebbie *et al.*[2], who extended measurements to 70,000 atmospheres. The resistivity measured at room temperature, according to them, shows an initial increase with pressure and drops several orders of magnitude at 30,000 atmospheres. This sharp drop in resistance was attributed by Gebbie *et al.* to melting, with the indium antimonide changing from a state of semiconduction in the crystal to metallic conduction in the liquid phase. A melting curve, based on the pressures giving a drop in resistivity at temperatures of 150°–800° K., was presented by them. The plotted points exhibit a wide scatter and the authors mention that the latent heat of melting calculated from the melting curve slope, namely 27 cal./gm., compares unfavourably with the experimentally determined value of 47·2 cal./gm. Hence it appeared to us that the melting curve of indium antimonide should be investigated again.

A piston-cylinder apparatus was used for generating high pressures. In this device, a tungsten carbide piston advances into a 'Carboloy' high-pressure chamber 0·5 in. in diameter and 2 in. long. The sample was sealed in a length of platinum tubing, ½ in. × ⅛ in., which was placed inside the talc-sheathed graphite furnace, which serves both for heating the sample and transmitting pressure to it. The absorption of latent heat accompanying melting was detected by differential thermal analysis. The sample temperature and the differential temperature were recorded simultaneously on a strip-chart recorder. The record of differential temperature analysis shows the latent heat accompanying melting as a break in the slope, or reversal of trend. Thus the melting point can be determined accurately. The high-pressure apparatus and the experimental techniques have been described in detail by Kennedy and Newton[3]. Semiconductor grade polycrystalline indium antimonide supplied by the Indium Corporation of America was used. Kennedy and Newton used platinum containers for the melting-point determination of both indium and antimony and have found platinum an inert container for both metals.

Volume-change of transition was used to locate the lowest five points of Fig. 1, because of the small heat of transition and the advantage in accuracy in intersecting a phase-boundary at a high angle. Powdered indium antimonide was intimately mixed with silver chloride and the mix was compressed into a pellet of the shape and dimensions needed to fill the high-pressure chamber. A heating tape was wound on the steel supporting-ring of the 'Carboloy' core. In this way, a temperature of nearly 200° C. at the sample site could be reached. Ram pressure and piston displacement were recorded on an x–y recorder. Piston friction was eliminated by a piston rotation method[4] and wall-friction was reduced by a 0·001 in. lead-foil wrapping. A change of state is revealed by a break in the pressure versus displacement curve.

Fig. 1 shows the melting curve of indium antimonide obtained in the present investigation. It will be seen that the melting point goes down until a pressure of 19·4 kb. is reached and rises thereafter with increasing pressure. By inserting the initial value of the slope of this melting curve in the Clausius–Clapeyron equation and using the known volume contraction on melting ($\Delta V/V_{\text{solid}} = 0{\cdot}13$), the latent heat is calculated to be 49·0 cal./gm., which is in good agreement with the experimentally determined value of 47·2 cal./gm.. To make doubly certain of the existence of melting above 300° C. at 40 kb., a special run was made in which a pure sample was compressed to 40 kb. at room temperature, and then heated. A thermal rest was encountered at 380° C., exactly as in the run on a sample of complicated pressure-temperature history.

Below 335° C., application of pressure transforms indium antimonide to another solid phase. We designate this new high-pressure form indium antimonide II, the normal form indium antimonide I.

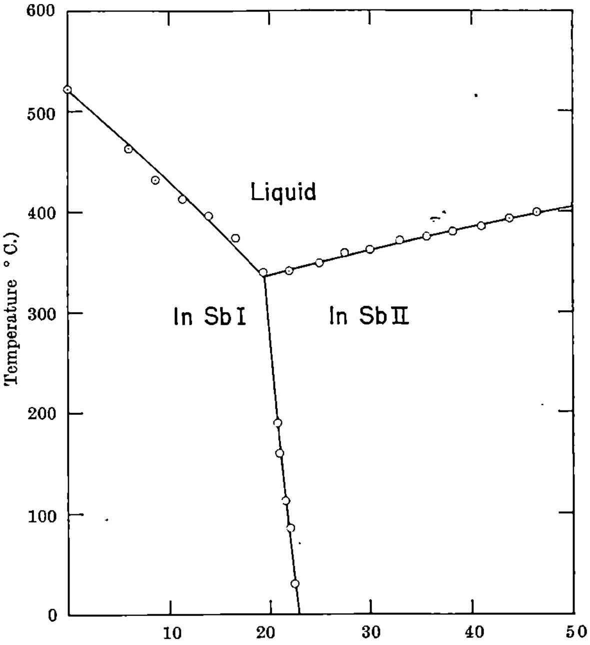


Fig. 1. Phase diagram of indium antimonide

The solid–solid boundary was located by using the volumetric method briefly mentioned earlier. This boundary intersects the melting curve at 335° C. and 19·4 kb. The solid–solid transition has a large change in volume associated with it, and an estimate from measured displacement in transition gives $\Delta V/V \approx 20$ per cent, which is probably a minimum. It was found that a rise in temperature of 150° produced a great increase in the sharpness of the transition as recorded volumetrically. This is almost certainly due to higher transition velocity at higher temperature. The transition was so sluggish at room temperature that the pressure of transition could not be determined with nearly the precision at 80° C. This effect probably accounts for the very high-pressure values for drop in resistivity obtained by Gebbie *et al.* at 150° K.

It is well known that the intermetallic compounds formed among group III and V elements have properties resembling the corresponding members of the group IV elements. Indium and antimony occupy positions in the III and V group, sandwiching tin, which is the corresponding member of the fourth group IV. We may therefore expect indium antimonide to exhibit properties analogous to tin. Tin has two forms, grey tin and white tin, designated respectively α and β. White tin transforms to grey tin below 13° C. The latter has diamond structure and is a semiconductor while the former has a body-centred tetragonal lattice exhibiting metallic properties. The change-over from grey to white tin is accompanied by a large reduction in volume, amounting to 27 per cent. The transition in indium antimonide is strongly reminiscent of transformation from grey to white tin both in regard to the change from a state of semiconduction to metallic conduction and the large reduction in volume accompanying it. We believe from these considerations that the transformation which takes place in indium antimonide at high pressure is similar to the grey-white tin transition: indium antimonide I having zinc-blende structure corresponding to grey tin transforms to indium antimonide II having the structure of white tin.

A. JAYARAMAN
R. C. NEWTON
G. C. KENNEDY

Institute of Geophysics,
University of California,
Los Angeles.

[1] Keyes, R. W., *Phys. Rev.*, **99**, 490 (1955).

[2] Gebbie, H. A., Smith, P. L., Austin, I. G., and King, J. H., *Nature*, **188**, 1095 (1960).

[3] Kennedy, G. C., and Newton, R. C., *Solids under Pressure*, edit. by Paul and Warshauer (McGraw-Hill, 1961).

[4] Kennedy, G. C., and La Mori, P. N. (to be published).

A New Photometric Technique using a Variable Shutter Device

THE present standard of accuracy in spectrophotometric measurement is unsatisfactory, partly because of the limitations of conventional techniques. In the measurement of spectral transmission of coloured filters, for example, the precision obtainable from commercially available instruments is limited to ± $1/10^3$ of full-scale reading, while the accuracy depends, *inter alia*, on the linearity of a detector-amplifier system or an attentuator-comb. This is found to be inadequate and an alternative approach to the problem is suggested.

In our proposed technique the quantity measured is, effectively, time, while the detector is used as a null indicator. The steady light flux passing through the filter to be measured is compared with the mean flux contained in the same beam when the filter is replaced by a repetitive shutter, the 'open to shut' time-ratio of which can be varied. This ratio is adjusted to make the mean flux reaching the detector the same in the two cases. The transmission factor of the filter, τ, is then obtained by measuring, r, the 'open to shut' time-ratio of the shutter $\left(\tau = \dfrac{r}{1+r}\right)$.

Three major considerations are: (1) The photocell system adopted must average the pulsed flux correctly, in other words, it must obey Talbot's Law[1]; it need not, however, exhibit d.c. linearity. (2) Since variable shutters of the type required are not available, one must be devised. (3) The timing measurement must be capable of very high accuracy and precision.

As a result of recent work we have been able to show that Talbot's law is obeyed closely by a vacuum photocell, provided the rate of repetition of the pulses is high enough. This is because the 'fatigue' processes producing non-linearity in photocells have a relatively long time constant. This result was foreshadowed by the work of Carruthers and Harrison[2] and Stiles[3], who showed that some cells which were markedly non-linear when used under steady-state conditions did in fact obey Talbot's Law to their limit of accuracy when the light was interrupted at a frequency of a few cycles a second. Preston and Gordon-Smith[4] have suggested that 'fatigue' effects may be caused by the accumulation of a quasi-static electron charge on insulating surfaces inside the photocell: such a process might have a time constant of hundreds of seconds.

As yet the ideal variable shutter does not exist, although some commercial types of high-speed shutter have operating times shorter than 10^{-8} sec. so that the prospect of developing a suitable type for our purpose appears hopeful. Since the shutter ratio could be measured for each transmission reading, it need not retain its calibration for long periods, nor is absolute constancy in operation essential, since the time-ratio measuring technique gives a mean value.

In order to test the possibility of making an accurate time-ratio measurement, we have successfully used a rotating sector disk interrupting a light beam at a very small image to 'imitate' a shutter. Although this 'shutter' was non-ideal, in that the occulting of the image by the sector edge took an appreciable time compared with the repetition period, it was possible to measure it with the same precision as with the most precise d.c. photometry.

Two sectors, having transmissions of 1·5 per cent and 3 per cent respectively, were measured (*a*) by a high-precision laboratory method of d.c. photometry, using a photometer similar to that described by Jones and Sanders[5], and (*b*) by using a simple timing technique developed this year. The transmission values obtained by these methods agreed to within the experimental tolerances (± $1/10^5$ of full scale reading), and these values were found to correspond closely with the results of direct measurement of the sector angles.

We conclude, therefore, that the new method is already capable of the same accuracy as the best conventional photometry and that, suitably developed, its potential precision and accuracy are much higher.

O. C. JONES
F. J. J. CLARKE

National Physical Laboratory,
Teddington, Middlesex.

[1] Walsh, J. W. T., *Photometry*, third ed., 98 (Constable, London, 1958).

[2] Carruthers, G. H., and Harrison, T. H., *Phil. Mag.*, **7**, 792 (1929).

[3] Stiles, W. S., *Phil. Mag.*, **7**, 812 (1929).

[4] Preston, J. S., and Gordon-Smith, G. W., *Brit. J. App. Phys.*, **6**, 329 (1955).

[5] Jones, O. C., and Sanders, C. L., *J. Opt. Soc. Amer.*, **51**, 105 (1961).

CHEMISTRY

Determination of Uranium as Pyrophosphate

WITH reference to the communication in *Nature* of June 24, p. 1188, on this subject, the following extract from Soddy's *Chemistry of the Radio-Elements*, published 1911, p. 33, may be of interest.

"This pouring of a solution of uranium containing a complex mixture of other constituents into excess of an alkaline carbonate is always a valuable first step in the separation of the uranium, and when the latter element is the only one being separated or estimated, it affords a ready means of obtaining the whole of the uranium in the filtrate in a state of approximate purity. For analytical estimations it is almost always an advantage to add some ammonium sulphide to complete the precipitation of the iron. Any suitable method can then be employed for the separation and estimation of the uranium in the filtrate. One of the most commonly employed is, after acidification and removal of the carbon dioxide, to add microcosmic salt (sodium ammonium hydrogen phosphate), to neutralize as nearly as possible with ammonia without causing a permanent precipitate to form, and then to add a considerable excess of sodium thiosulphate. Boiling for ten minutes brings down the whole of the uranium as phosphate with a bulky sulphur precipitate which settles and filters excellently. After washing, the precipitate is ignited at a dull red-heat and weighed as the 'green phosphate', a body which has been found empirically to correspond with 68·55 per cent of uranium, and which is far less hygroscopic than the pyrophosphate. When pure it dissolves completely in concentrated nitric acid, but when impure the solution is more difficult. Evaporation of this solution and ignition leaves the yellow pyrophosphate $(UO_2)_2P_2O_7$, which contains 66·81 per cent of uranium. The conversion of the 'green phosphate' in the crucible into the yellow pyrophosphate affords a check on the estimation, but the latter body is excessively hygroscopic and difficult to weigh. (For further details, Brearley's *Analytical Chemistry of Uranium* should be consulted.)"

The slight variations in percentage of uranium quoted may probably be due to difference in the atomic weights used. It would appear from this that the "green phosphate" coincides closely with the precipitate obtained by Wright, Hayes and Ryan.

F. M. Brewer

Inorganic Chemistry Laboratory,
South Parks Road,
Oxford.

We thank Dr. Brewer for directing our attention to the reference in Soddy's publication. The passage quoted would seem to be a recapitulation of Brearley's work, published in 1903, with which we are acquainted.

It would appear also that Soddy has erroneously quoted the "green phosphate" as being formed at "dull red-heat" whereas Brearley states that the "pure yellow form" is obtained at "low redness". He attributes the formula $(UO_2)_2P_2O_7$ to the yellow residue. If the ignition is carried out at a higher temperature (at no time does he quote a precise temperature) then the green residue is obtained. Brearley does not assign a formula to the green phosphate, but states that it contains 68·55 per cent uranium.

We have confirmed that treatment with nitric acid yields the yellow form, which is hygroscopic. Thermogravimetric data would lead us to believe at present that stoichiometric $(UO_2)_2P_2O_7$ (66·67 per cent uranium) is never obtained by direct ignition. A yellow-green non-hygroscopic form containing 67·2 per cent uranium has been obtained by ignition at temperatures between 500° C. and 800° C. So far, only the green phosphate (68·21 per cent uranium), obtained by ignition at temperatures in excess of 950° C., has been studied in any detail. The original communication was intended to direct attention to the presence of both tetravalent and hexavalent uranium in the green product.

Differences in atomic weights between 1903 and the present day would not explain the apparent differences in uranium content.

J. S. Wright
T. J. Hayes
J. A. Ryan

Chemical and Metallurgical Services Department,
United Kingdom Atomic Energy Authority,
Springfields Works, Salwick,
Preston.

Role of Pressure Gradients in obtaining Minimum Time in Gas Chromatography

Although a great deal of attention[1-5] has been focused on obtaining separations in the least possible time, the theory of minimum-time operation has not been developed for inlet/outlet pressure ratios greatly exceeding unity. Such a theory does not immediately seem widely applicable since most rapid separations made with an outlet pressure of one atmosphere do not require an inlet pressure greatly exceeding one atmosphere. This conclusion is incorrect, however, since a search for the optimum values of all parameters reveals that the optimum outlet pressure is very close to zero in all circumstances. Furthermore, the difference in minimum retention times found with an outlet pressure of one atmosphere and zero is large enough in many cases to be significant in obtaining more rapid analyses.

The apparent or measured plate height, $\hat{H}$, is related to the inlet/outlet pressure ratio, P, as follows[6]:

$$\hat{H} = \frac{9}{8} H_0 \frac{(P^4-1)(P^2-1)}{(P^3-1)^2} + \frac{3}{2} C_l v_i \frac{P(P^2-1)}{(P^3-1)} \qquad (1)$$

where C_l is the mass transfer term for the liquid, v_i the inlet gas velocity, and H_0 is the sum of all plate height terms remaining constant throughout the column. Although the theory is applicable to both packed and capillary columns, the equations below are derived specifically for capillary columns in which $H_0 = B/pv + C_g pv$.

Following the method of reference 5, modified by the inclusion of the appropriate P terms, the time required for the elution of the first peak of a pair is :

$$t = \frac{N}{R}\left[\frac{3}{4} \frac{H_0}{v_i} \frac{(P^4-1)}{P(P^3-1)} + C_l\right] \qquad (2)$$

where N is the number of plates required for separation and R is the ratio of zone to gas velocity. This equation can be used to optimize a wide spectrum of variables. Its use is somewhat complicated by the dependence of v_i on P with N fixed. It can be proved, however, that with any fixed inlet pressure, p_i (even if this is the optimum value for atmospheric outlet), a separation can be accomplished in the least possible time if the outlet pressure, p_0, is zero (in practice, near zero). The corresponding separation time, t, and column-length, L, may be written :

$$t = (N/R)(3H_0/4v_i + C_l) \qquad (3)$$

$$L = N(9H_0/8 + 3C_l v_i/2) \qquad (4)$$

Following this, the inlet pressure can be optimized. Its best value is $p_i = \rho p_c$, where the critical pressure, $p_c = (6/r_0)(\eta D_{g1} N)^{1/2}$, is the smallest inlet pressure capable of giving a separation. The quantity ρ is obtained from a graph of the function, $\rho^2 = 3 + 8s/3\rho$, where $s = C_l/C_g p_c$. The capillary radius has been written as r_0, the viscosity as η, and the diffusion coefficient of solute at unit pressure as D_{g1}. The inlet velocity may be obtained, once p_i is known, from the equation:

$$v_i = \left[\frac{2D_{g1}(\rho^2-1)}{C_g p_i^2 + 4C_l p_i/3}\right]^{1/2} \qquad (5)$$

If one elects to maintain an outlet pressure of one atmosphere, the time t becomes:

$$t = \frac{NC_l}{R}\left[\frac{S_1(\rho S_1/s + 1)}{(S_2\rho^2 - S_1)} + \rho S_1/s + 1\right] \qquad (6)$$

where $S_1 = 3(P^4-1)/4P(P^3-1)$ and $S_2 = 3\ (P^3-1)/4P^3$. The minimum value of t is obtained numerically by substituting various values of P into (6).

A comparison of minimum times can now be made between atmospheric and vacuum outlet. Typical capillary parameters are chosen as follows: $C_g/C_l = 10^{-6}$, $D_{g1} = 10^5$, $\eta = 10^{-4}$, $r_0 = 10^{-2}$, all in C.G.S. units. First, assume that easy separation requires 10^3 plates. The least time obtainable with $p_0 = 10^6$ dynes/cm.² (very close to one atmosphere) has been obtained from (6) as $t = 2{\cdot}08NC_l/R$. The corresponding P is approximately 1·08. With a vacuum outlet, the minimum $t = 1{\cdot}26NC_l/R$ and $p_i = 2{\cdot}30 \times 10^5$ dynes/cm.² ($\simeq 0{\cdot}25$ atm.). Thus the time required with atmospheric outlet is 1·66 times larger than the vacuum outlet time.

Assume that a more difficult separation requires $N = 10^5$. With $p_0 = 10^6$ dynes/cm.², the best parameters are $t = 2{\cdot}90NC_l/R$ and $P = 1{\cdot}8$. With $p_0 = 0$, the best parameters are $t = 2{\cdot}51NC_l/R$ and $p_i = 1{\cdot}34 \times 10^6$ dynes/cm.² ($\simeq 1{\cdot}3$ atm.). The time ratio in this case is 1·15.

The above examples show that time for analysis can be reduced by operating the outlet at very low pressures. The reduction in time depends on a number of factors. As shown above, it is least for very large plate requirements. This is true since the inlet pressures approach one another for very large plate requirements, and there is very little difference in the average performance of the columns. The reduction in time increases, also, with s, a measure of the relative mass transfer resistance in the gas phase compared with the liquid phase. In the examples above, the two terms are assumed equal ($s = 10^{-6}$) at atmospheric pressure.

This work was supported by the U.S. Atomic Energy Commission under contract No. AT(11–1)–748.

J. Calvin Giddings

Department of Chemistry,
University of Utah,
Salt Lake City, 12.

[1] Purnell, J. H., *Nature*, **184**, 2009 (1959).

[2] Purnell, J. H., and Quinn, C. P., *Third Intern. Symp. Gas Chromatography, Edinburgh* (1960).

[3] DeFord, D. D., Ayers, B. O., and Loyd, R. J., *Anal. Chem.*, **32**, 1711 (1960).

[4] Scott, R. P. W., and Hazeldean, G. S. F., *Third Intern. Symp. Gas Chromatography, Edinburgh* (1960).

[5] Giddings, J. C., *Anal. Chem.*, **32**, 1707 (1960).

[6] Stewart, G. H., Seager, S. L., and Giddings, J. C., *Anal. Chem.*, **31**, 1738 (1959); **32**, 867 (1960).

Inhibition of Ultrasonic Decolorization of Rhodamine *B* in Presence of Organic Substances

Ultrasonic waves obtained from Mullard's high-frequency ultrasonic generator type *E*-7562 with a barium titanate transducer having a frequency of 1 Mc./sec. were passed into the aqueous solution of rhodamine *B* and it was found that the solution was decolorized after some time. These waves were found to decolorize aqueous solutions of congo red, chrysoidine, aniline blue and brilliant green also[1]. 20 ml. of an aqueous solution (0·0064 gm./l.) of rhodamine *B* and 5 ml. distilled water in a Jena glass bottle were held vertically above the transducer and the position so adjusted as to produce maximum fountain inside the vessel on passing the ultrasound. When, however, 20 ml. of the same solution and 4 ml. of distilled water were taken and 1 ml. of methyl alcohol, ethyl alcohol, *n*-propyl alcohol, *n*-butyl alcohol, diacetone alcohol, acetone, dioxan or ether were added separately, and the mixtures exposed to ultrasonic radiation under the same conditions as before, very little change in colour was observed, even when the time of exposure of the solutions containing these substances was almost doubled (Table 1). The colorimetric readings were recorded on a Klett Summerson photoelectric colorimeter using a No. 54 filter, which covers a range of 520–580 mμ. Readings for the dye solution exposed without adding any organic substance are shown in the second column (blank).

Table 1

		Colorimetric readings in presence of:							
Exposure (min.)	Blank	Methyl alcohol	ethyl alcohol	*n*-propyl alcohol	*n*-butyl alcohol	Diacetone alcohol	Acetone	Dioxan	Ether
0	338	338	338	337	338	337	338	338	337
5	126	313	330	335	337	330	335	337	334
10	65	310	327	333	335	328	333	334	333
15	44	292	323	332	334	325	332	327	—
20	33	—	312	332	331	323	331	323	331
25	—	252	307	331	331	320	330	313	331
30	21	—	—	—	—	—	—	—	—
50	—	216	290	327	329	310	325	306	330

Virtanen and Ellfolk[2] noted that the ultrasonic oxidations caused by cavitation may be inhibited by substances with a high vapour pressure. Oxidative nitrogen fixation in an ultrasonic field is inhibited by more than 50 per cent by volatile substances such as aliphatic fatty acids, aldehydes, alcohols and aromatic hydrocarbons. Bresler[3] observed that by the addition of substances of high vapour pressure, such as ether or ammonia, the breakdown of the cavitation bubbles is prevented, and the luminescence of luminol otherwise produced by ultrasonic vibrations is prevented. Mellaerts[4] has reported that water treated with ultrasonic waves exhibits a non-uniform luminescence. This luminescence can be suppressed by the presence of some substances with high vapour pressure, for example, ether and acetone. In our case also, the substances added are of high vapour pressure and they prevent the breaking of the cavities and hence the inhibition of the decolorization of rhodamine *B* solution.

We thank the Ministry of Scientific Research and Cultural Affairs, India, for a scholarship to one of us (Sheo Prakash).

Satya Prakash
Sheo Prakash

Chemical Laboratories,
University of Allahabad,
Allahabad.

[1] Prakash, S., and Prakash, S., *J. Acoust. Soc. Amer.*, **32**, 138 (1960); *J. Ind. Chem. Soc.*, **36**, 601 (1959); *Vijnana Parishad Anusandhan Patrika*, **3**, 31 (1960).

[2] Virtanen, A. I., and Ellfolk, N., *Acta Chem. Scand.*, **6**, 660 (1952).

[3] Bresler, S. E., *Physicochim.*, *U.R.S.S.*, **12**, 323 (1940).

[4] Mellaerts, J. F., *Tech. eau*, **4**, 15 (1950).

Viscosity-Temperature Relationships for Dilute Solutions of High Polymers

Investigations of the viscosities of dilute solutions of cellulose derivatives[1,2] over a range of temperature have shown that the pre-exponential term A and the apparent activation energy of viscous flow Q in the viscosity-temperature relationship:

$$\eta = Ae^{Q/RT} \qquad (1)$$

where η is the viscosity, depend on both the concentration c of the polymer and its molecular weight M. $(Q - Q_0)$, where Q_0 is the value for the solvent, is generally a linear function of c in the range 0·1–0·4 gm./dl. and is a function of M. The value of A

Table 1. VALUES OF $(Q-Q_0)$ AND $(A-A_0)$ FOR 1 PER CENT SOLUTIONS OF POLYMERS

Polymer	$\bar{M}_n$	Solvent	$(Q-Q_0)$ (k.cal.)	$(A-A_0)$ ($\times 10^5$)
Polyvinyl acetate	148,000	acetone	0·3	2·6
		chloroform	0·3	9·6
		dioxan	0·2	2·0
Polymethyl methacrylate	121,000	acetone	−0·16	11·0
		toluene	−0·1	9·6
		benzene	nil	4·9
Polyisobutylene	90,000	cyclohexane	0·16	3·0
Ethyl cellulose, 48·4 per cent ethoxyl content	119,000	ethyl methyl ketone	3·7	−10·9
		butyl acetate	3·6	− 8·3
		benzene	3·8	− 7·8
Cellulose nitrate, 12·2 per cent nitrogen content	132,000	acetone	1·4	3·3
		butyl acetate	4·25	− 8·0
Cellulose nitrate, 13·8 per cent nitrogen content	130,000	acetone	4·0	−21·5
Cellulose triacetate	107,000	chloroform	1·4	− 9·6

generally decreases with increase of both *c* and *M* and $-\log A$ is a linear function of *c* and a more complex one of *M*.

Extension of such work to dilute solutions of other polymers shows that the variations in *A* and *Q* obtained when flexible, less polar polymers are used differ markedly from those obtained for comparable solutions of cellulose derivatives. Table 1 gives values of $(A - A_0)$ and $(Q - Q_0)$ for 1 per cent (w/v) solutions of fractions, of similar number average molecular weight, of polyvinyl acetate, polymethyl methacrylate and polyisobutylene. Estimated values for 1 per cent solutions of fractions of ethyl cellulose[1], a cellulose nitrate with 12 per cent nitrogen, a cellulose nitrate with 13·8 per cent nitrogen and cellulose triacetate are also given. Values of $(Q - Q_0)$ for the cellulose derivatives are all positive and about ten or more times greater than those for the other polymers, which may be positive, zero or negative. Values of $(A - A_0)$ for the other polymers are all positive, in contrast to the negative values associated with all the cellulose derivative systems with the exception of the 12·2 per cent nitrogen nitrate in acetone.

Reasons for such differences are not clear, but they may be connected with differences in chain flexibility and extension. Chains of cellulose derivatives are stiff, adopting extended forms in solution, even in thermodynamically poor solvents[3]. Chains of the other polymers are flexible and adopt much more coiled forms in solutions. Stiff, extended chains, which are likely to overlap at the concentrations considered, might be expected to cause greater interference with flow, leading to larger values of $(Q - Q_0)$ than less-extended flexible chains. The reasons for negative values of $(Q - Q_0)$ for polymethyl methacrylate in acetone and toluene are also not clear, but such negative values might be related to solvent power. Intrinsic viscosities for the fraction of polymethyl methacrylate in acetone and toluene at 25° C. are 0·26 and 0·29 respectively, that for the fraction in benzene being 0·39. Acetone and toluene may be regarded as poor solvents in which the chains are tightly coiled, and such coiling may, in some way, lead to a reduction in *Q* as *c* increases.

In terms of rate theory[4]:

$$\eta = (Nh/V)e^{-\Delta S/R}e^{Q/RT} \qquad (2)$$

where *N* and *h* are the Avogadro number and Planck's constant, *V* the molar volume of the liquid and ΔS the entropy of activation of viscous flow. From equations (1) and (2):

$$A = (Nh/V)e^{-\Delta S/R} \qquad (3)$$

In the dilute solutions considered, *V* will differ little from the value for the solvent and the value of *A* should depend largely on that of ΔS. If this were so, flexible chains presumably would cause a decrease and stiff chains an increase in ΔS. From equation (3):

$$-\log A = -\log (Nh/V) + \Delta S/R \qquad (4)$$

With cellulose derivatives[1,2] $-\log A$ is generally a linear function of *c*, so that ΔS may increase linearly with concentration.

Although the reasons for such marked differences between the two types of polymers are not clear, the view that they are connected with chain flexibility and extension is supported by the differences between the values of $(Q - Q_0)$ and $(A - A_0)$ for the two cellulose nitrates in acetone. Chain stiffness and extension of cellulose nitrate have been shown to increase with increasing degree of substitution[5]. The exponent *a* in the Mark–Houwinck equation:

$$[\eta] = KM^a \qquad (5)$$

is 0·80 at 25° C. for the 12·2 per cent nitrogen nitrate in acetone[2]. This value is the upper limit for flexible chains[6], suggesting some degree of flexibility and coiling. The value of *a* at 25° C. for the 13·8 per cent nitrogen nitrate in acetone is 0·92 (ref. 5), implying much more extended and stiffer chains. The value of $(A - A_0)$ for the nitrate with 12·2 per cent nitrogen is positive, like those for the flexible chain polymers, and the value of $(Q - Q_0)$, although comparatively large, is considerably less than that for the 13·8 per cent nitrogen. The value of the exponent *a* at 25° C. for the 12·2 per cent nitrate with 12·2 per cent nitrogen in butyl acetate is 0·91, and the value of $(Q - Q_0)$ is much larger than that for the same nitrate in acetone, while that of $(A - A_0)$ is negative.

W. R. MOORE

Polymer Research Laboratories,
Department of Chemical Technology,
Bradford Institute of Technology,
Bradford.

[1] Moore, W. R., and Brown, A. M., *J. Colloid Sci.*, **14**, 1 (1959).
[2] Moore, W. R., and Edge, G. D., *J. Polymer Sci.*, **47**, 469 (1960).
[3] Immergut, E. H., and Eirich, F. R., *Indust. Eng. Chem.*, **45**, 2500 (1953).
[4] Eyring, H., *J. Chem. Phys.*, **4**, 283 (1936).
[5] Moore, W. R., and Pearson, G. P., *Polymer*, **1**, 144 (1960).
[6] Flory, P. J., *Principles of Polymer Chemistry*, 622 (Cornell Univ. Press, Ithaca, New York, 1953).

BIOCHEMISTRY

A New Two-Stage Method for Cross-linking Proteins

THE combination of two different proteins with each other via covalent bonds is useful for certain immunological work[1-4]. This type of chemical reaction requires a bifunctional compound which, by stepwise coupling first with one and then with the other protein molecule, will form a bridge between them. Among the compounds used for this purpose have been *bis*-diazotized benzidine[1] and difluorodinitrobenzene[2]. Howard and Wild[3] developed a method consisting of three steps: one protein was coupled with *bis*-diazotized benzidine at acid *p*H, the second protein was coupled with a derivative of

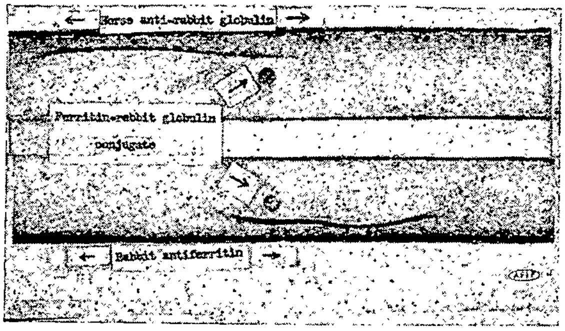

Horse anti-rabbit globulin
Ferritin-rabbit globulin conjugate
Rabbit antiferritin

Fig. 1. Immunoelectrophoresis of a crude ferritin–rabbit globulin conjugate prepared by the use of BDD. (Precipitin arcs were stained with amido black)

pyrrole at basic *p*H, and then the products of both additions were coupled with each other.

The recently introduced method of labelling antibodies with electron-dense protein ferritin[4] created a need for a coupling reagent that would be efficient and also mild enough to preserve the antibody activity. Singer[4] used for this purpose *m*-xylylene di*iso*cyanate, but he had to take precautions to avoid inactivation of antibodies by this reagent. The use of toluene 2,4-di*iso*cyanate (TDI) improved the coupling efficiency[5], but did not remove the risk of adversely affecting the antibody. The use of diazonium coupling would be preferable, since this is a mild reaction that has relatively little effect on antibody activity[6].

In my laboratory the attempted use of *bis*-diazotized benzidine for the above purpose was not successful, possibly because its reaction with ferritin proceeded too fast, leaving an inadequate number of free functional groups for subsequent coupling with immune globulin[7]. The use of *bis*-diazotized 3,3′-dimethoxy derivative, dianisidine (BDD), however, gave the desired result. The *o*-methoxy group is known to slow down the diazonium coupling ten-fold[8], whereas Pearse[9] showed that BDD reacts at acid *p*H with only one end, while at alkaline *p*H both diazonium groups react.

A solution of 160 mgm. ($2{\cdot}8 \times 10^{-4}$ m.mole) ferritin and 80 mgm. (5×10^{-4} m.mole) rabbit globulin in 7 ml. 0·1 *M* citrate buffer, *p*H 5·0, was treated at 4° with 1 ml. aliquot of a solution of 6·7 mgm. ($2{\cdot}8 \times 10^{-2}$ m.mole) dianisidine and 3·8 mgm. ($5{\cdot}5 \times 10^{-2}$ m.mole) sodium nitrite in 10 ml. of 0·017 *N* hydrochloric acid. The solution was stirred in the cold for 2 hr., and then dialysed overnight against 0·08 *M* borate, *p*H 9·4, and finally against neutral saline. The crude product gave the immunoelectrophoretic pattern shown in Fig. 1. The component of intermediate mobility, which reacted with both antiferritin and anti-rabbit globulin, is the conjugate; the slow component is the uncoupled globulin, and the fast component the uncoupled ferritin. The three components were separated by means of continuous-flow electrophoresis[7]. The middle fraction was then concentrated (by dialysis against 30 per cent polyvinylpyrrolidinone) and dialysed against neutral saline.

A conjugate prepared by the use of BDD from globulin containing antibody against tobacco mosaic virus was more homogeneous when tested by 'Millipore' filtration and viewed under the electron microscope, and it had a higher antibody activity than a similar conjugate prepared with TDI (Hartman, R. E., personal communication). Another conjugate, prepared by means of BDD from globulin containing Forssman antibody, had a hæmolysin titre twice as high as did one obtained by the use of TDI. The use of BDD avoids any loss of protein by precipitation during and after the reaction; by making the medium monophasic, it also facilitates the control of the degree of coupling.

FELIX BOREK

Immunochemistry Branch,
Armed Forces Institute of Pathology,
Washington 25, D.C.

[1] Coombs, R. R. A., Howard, A. N., and Wild, F., *Brit. J. Exp. Path.*, 33, 390 (1952).
[2] Ling, N. R., *Immunol.*, 4, 49 (1961).
[3] Howard, A. N., and Wild, F., *Brit. J. Exp. Path.*, 38, 640 (1958).
[4] Singer, S. J., *Nature*, 183, 1523 (1959).
[5] Singer, S. J., and Schick, A. F., *J. Biophys. Biochem. Cytol.*, 9, 519 (1961).
[6] Borek, F., and Silverstein, A. M., *Arch. Biochem.*, 87, 293 (1960).
[7] Borek, F., and Silverstein, A. M., *J. Immunol.* (in the press).
[8] Hammett, L. P., *Physical Organic Chemistry*, 314 (McGraw-Hill, New York, 1940).
[9] Pearse, A. G. E., *Histochemistry*, 255 (J. and A. Churchill, Ltd., London, 1954).

A Biochemical Effect of Deuterium

AMONG the naturally occurring steroid hormones and metabolites the androgens are unique in that 3-hydroxy-5α-androstanes exhibit high hormonal activity, the 3α-hydroxy (axial) compounds being highly active while the 3β-hydroxy (equatorial) compounds are much weaker androgens. It is known from both chemical and microbiological work[1] that the 3α-alcohols are oxidized more readily to the corresponding 3-ketone than are the 3β-alcohols, in accordance with the thermodynamic prediction. It is further known that enzymic oxidation of 3-hydroxy-5α-androstanes occurs in mammalian tissue, in particular in the liver. This suggests the possibility that *in vivo* these compounds are oxidized in various degrees to the even more potent androgens, the 3-keto-5α-androstanes, and that, in fact, the 3-alcohols may be androgenically inactive *per se*.

To test this hypothesis, 3α-deutero-17α-methyl-5α-androstane-3β,17β-diol (I) (m.p. 213–214°, α_D −13°), prepared by the lithium aluminium deuteride reduction of 17α-methyl-5α-androstan-17β-ol-3-one (III), was assayed in the castrated-rat in parallel with the corresponding 3α-hydrogen-17α-methyl-5α-androstane-3β,17β-diol (II) (m.p. 213–214°, α_D −14°). Should the activity of these compounds depend on oxidation to the 3-ketone with loss of the 3α-hydrogen or 3α-deuterium as the rate-determining step, then, in accord with the primary deuterium isotope effect[2], the 3α-deutero compound (I) should be oxidized more slowly and consequently would be expected to exhibit lower androgenic activity than the 3α-hydrogen compound (II).

Male albino rats were castrated at 22–24 days of age, and, beginning on the day of surgery, the compounds were injected subcutaneously once daily for seven consecutive days. The vehicle was an aqueous suspending medium consisting of sodium chloride (0·9 per cent), polysorbate 80 (0·4 per cent), carboxymethylcellulose (0·5 per cent) and benzyl alcohol (0·9 per cent), and the daily dose was contained in 0·5 ml. Twenty-four hours after the last injection the rats were killed with ether and the ventral

Table 1. Influence of $(3\alpha\text{-}^{3}\text{H})17\alpha$-Methylandrostane-$3\beta,17\beta$-Diol (I). 17α-Methylandrostane-$3\beta,17\beta$-Diol (II), and 17α-Methylandrostan-3-one (III) on the Prostate, Seminal Vesicles and Levator Ani of Castrated Rats

Material administered	Total dose (mgm.)	No. of rats	Mean tissue ratio* ± *S.E.*		
			Prostate	Seminal vesicles	Levator ani
0	0	19	0·17 ± 0·017	0·11 ± 0·006	0·24 ± 0·010
I	1·5	12	0·44 ± 0·020	0·14 ± 0·008	0·26 ± 0·012
	3·0	19	0·44 ± 0·028	0·16 ± 0·009	0·25 ± 0·009
	6·0	19	0·54 ± 0·043	0·21 ± 0·013	0·27 ± 0·015
	12·0	17	0·56 ± 0·039	0·25 ± 0·017	0·31 ± 0·019
	24·0	6	0·74 ± 0·063	0·32 ± 0·056	0·27 ± 0·025
II	1·5	13	0·54 ± 0·049	0·23 ± 0·019	0·30 ± 0·014
	3·0	18	0·63 ± 0·060	0·30 ± 0·024	0·26 ± 0·015
	6·0	17	0·61 ± 0·042	0·29 ± 0·024	0·28 ± 0·020
	12·0	18	0·75 ± 0·052	0·40 ± 0·027	0·29 ± 0·017
	24·0	6	0·83 ± 0·055	0·43 ± 0·045	0·35 ± 0·015
III	0·5	7	0·60 ± 0·036	0·28 ± 0·022	0·30 ± 0·023
	1·0	7	1·02 ± 0·086	0·43 ± 0·036	0·42 ± 0·026
	2·0	7	1·31 ± 0·168	0·71 ± 0·103	0·56 ± 0·050
	4·0	5	1·57 ± 0·053	0·92 ± 0·066	0·68 ± 0·082

$$* \text{ Tissue ratio} = \frac{\text{mgm. of tissue}}{\text{gm. of body-weight}}$$

prostate, seminal vesicles, and levator ani were removed and weighed wet after blotting.

Compound II was 3·36 (+1·64; −1·10 ($P = 0{\cdot}95$)) times more active than the deutero compound I on the basis of the ventral prostate and 4·65 (+1·67; −1·25 ($P = 0{\cdot}95$)) times more active on the basis of response of the seminal vesicles (Table 1), demonstrating that in the rat oxidation to the 3-ketone is an important, if not the sole, factor contributing to androgenicity of 3β-hydroxyandrostanes.

In the chicks' comb assay, by local inunction[3], no significant difference could be established between the activities of I, II and III. When the potency of compound II was assigned a value of 1, the relative potency of compound I was 1·03 (+0·51; −0·33 ($P = 0{\cdot}95$)) and that of compound III was 1·04 (+0·46; −0·32 ($P = 0{\cdot}95$)). This indicates that in this species either the 3β-hydroxy-androstanes are active *per se* or more likely that oxidation to the 3-ketone in the comb is rapid and essentially complete.

It was of interest that we could not establish any difference in androgenic activity between testosterone and 17α-deutero-testosterone in the castrated rat by either the oral or subcutaneous route, nor could Bollinger and Wendler[4] increase the anti-inflammatory activity of hydrocortisone by conversion to the 11α-deutero analogue.

Table 2. INFLUENCE OF COMPOUNDS I, II AND III INUNCTED ON THE CHICK'S COMB

Material administered	Total dose (μgm.)	No. of chicks	Mean comb ratio* ± *S.E.*
0	0	19	0·37 ± 0·021
I	0·5	19	0·42 ± 0·020
	1·5	18	0·48 ± 0·030
	4·5	19	0·66 ± 0·033
	13·5	18	0·75 ± 0·041
II	0·5	19	0·46 ± 0·022
	1·5	20	0·58 ± 0·027
	4·5	20	0·66 ± 0·030
	13·5	20	0·78 ± 0·035
III	0·5	19	0·43 ± 0·019
	1·5	18	0·45 ± 0·022
	4·5	19	0·61 ± 0·026
	13·5	19	0·89 ± 0·058

$$* \text{ Comb ratio} = \frac{\text{mgm. of comb}}{\text{gm. of body-weight}}$$

This work was supported in part by grants from the U.S. Public Health Service, Nos. *A*-4044 and *CY*-2193.

HOWARD J. RINGOLD
SUMNER BURSTEIN
RALPH I. DORFMAN

Worcester Foundation for
Experimental Biology,
Shrewsbury,
Massachusetts.

---

[1] Talalay, P., *Physiol. Rev.*, **37**, 371 (1957).
[2] Wiberg, K. B., *Chem. Rev.*, **55**, 713 (1955).
[3] Rosemberg, E., Dorfman, A. S., and Dorfman, R. I., *Endocrinol.*, **67**, 116 (1960).
[4] Bollinger, F. W., and Wendler, N. L., *J. Org. Chem.*, **24**, 1139 (1959).

Separation of Linear from Branched Polysaccharides by Precipitation as Iodine Complexes

A MAJOR problem in studying polysaccharides is the isolation of individual polysaccharides from mixtures. In connexion with work on hemi-cellulose *B* it was observed that a solution of this polysaccharide in aqueous calcium chloride was partially precipitated by adding iodine–potassium iodide, and thus a separation of polymer types may have occurred.

Whistler and Lauterbach[1] have indicated that corn-cob hemi-cellulose (xylose 65·2 per cent, arabinose 13·3 per cent, glucuronic acid 9·8 per cent, galactose 2·9 per cent, glucose 8·6 per cent) contains three components: polymer I (xylose 89·4 per cent, arabinose 10·6 per cent, glucuronic acid 1 per cent), polymer II (not characterized, but probably a glucan), and polymer III (xylose 59·1 per cent, arabinose 21·9 per cent, glucuronic acid 11·3 per cent, galactose 7·6 per cent). A 200 mgm. sample of purified hemi-cellulose *B* prepared according to these authors was dissolved in 20 ml. of aqueous calcium chloride (specific gravity 1·3). On addition of 2 ml. of iodine solution (3 gm. iodine and 4 gm. potassium iodide in 100 ml. water) a dark blue complex precipitated which was separated by high-speed centrifugation (20,000*g*). The clear supernatant solution was decanted and the excess iodine destroyed by adding sodium thiosulphate. Pouring the solution into four volumes of ethanol resulted in precipitation of a polysaccharide consisting of uronic acid, galactose, arabinose and xylose residues, the galactose, arabinose and xylose being in the ratio of 1: 2·7: 7·9. This is essentially the same as the ratio 1: 2·9: 7·8 obtained by Whistler and Lauterbach for polymer III, a highly branched polysaccharide. The amount of precipitate available was too small to determine uronic acid by evolution of carbon dioxide.

The blue iodine complex decomposes when suspended in water. From it may be regained a polysaccharide consisting of glucose, a trace of uronic acid, arabinose and xylose residues. The ratio of arabinose/xylose is 1: 8·4, similar to the ratio found by Whistler and Lauterbach (1: 7·8) for polymer I, essentially a linear polysaccharide. The glucose in the iodine precipitate may represent a degraded cellulose. In this case, it appears that the linear polysaccharides are precipitated by iodine, and the branched ones remain in solution.

There are numerous references in the literature to reaction of iodine with hemi-celluloses to give blue complexes ('amyloid reaction'). This reaction is usually ascribed to the presence of glucose residues.

However, a purified, glucose-free hemi-cellulose *A*, as described by Whistler and me[2] can be partially dissolved in calcium chloride solution and gives a heavy blue-black precipitate with iodine.

It was of interest to see whether iodine precipitates other polysaccharides from a solution of calcium chloride. Ivory nut, mannan, which is described as a linear polymer[3], was quantitatively precipitated. Guaran, a chain of mannose units with galactose side-chains[4], and a highly branched mannan from bakers' yeast[5], remained in solution when treated with iodine as described. Therefore, it appears that reaction with iodine in a solution of calcium chloride discriminates between linear and branched polysaccharides and may be a useful means of separating and characterizing these materials.

I thank Prof. R. L. Whistler for providing a sample of pure guaran.

BLANCHE D. E. GAILLARD

Laboratory of Animal Physiology,
Agricultural University,
Wageningen.

[1] Whistler, R. L., and Lauterbach, G. E., *Arch. Biochem. Biophys.*, **77**, 62 (1958).

[2] Whistler, R. L., and Gaillard, B. D. E., *Arch. Biochem. Biophys.*, **93**, 332 (1961).

[3] Klages, F., *Ann.*, **509**, 159 (1934); **512**, 185 (1934).

[4] Ahmed, Z. F., and Whistler, R. L., *J. Amer. Chem. Soc.*, **72**, 2524 (1950).

[5] Haworth, W. N., Heath, R. K., and Peat, S., *J. Chem. Soc.*, 833 (1941).

Effect of 5-Fluorouracil on Hepatic Induced Increase of Tryptophan-Pyrrolase

THE competitive inhibition of tryptophan-pyrrolase, adaptive formation by ethionine[1] and preferential incorporation of valine-^{14}C into the enzyme protein after injection of the inducing agent[2] would indicate that this increase of enzymic activity is brought about through the synthesis of a new enzyme protein.

Poznanskaia[3], on the contrary, is of the opinion that such increase is a result of some activation of preformed proteins; while, according to Straub[4], the liver contains a large amount of inactive enzyme.

Recent investigations have demonstrated that purine and pyrimidine analogues are capable of inhibiting protein synthesis, by interfering with the metabolism of nucleic acid. It has been demonstrated in particular that induction of β-galactosidase in micro-organisms may be inhibited by either 8-azaguanine or[5,6] 5-fluorouracil.

Thus the use of these antimetabolites provides a valuable means of elucidating the mechanism of induced increase in liver tryptophan-pyrrolase.

So far, experimental researches in this direction yielded contradictory results.

In animals given an LD_{50} of 8-azaguanine and 6-mercaptopurine, Lee[7] found a net increase in tryptophan-pyrrolase; Feigelson, Dashman and Margolis[8], on the contrary, did not find any modification when non-toxic amounts of 8-azaguanine were used. Only recently, Kwam and Parks[9], treating adrenalectomized rats with 8-azaguanine, obtained a significant inhibition of glucose-6-phosphatase induction by hydrocortisone, and on tryptophan-pyrrolase induction by the substrate.

In our work we used 5-fluorouracil as antimetabolite because of the capacity of this compound of producing particular and noticeable inhibition of protein synthesis.

Table 1. EFFECT OF 5-FLUOROURACIL ON INDUCTION OF HEPATIC TRYPTOPHAN-PYRROLASE

Treatment	Group No.	No. of animals	μMoles kynurenine/gm. liver wet wt./hr.	*P*
Control	1	5	3·9 ± 1·6	
5-FU	2	4	5·3 ± 1·6	
Control + tr.	3	7	22·0 ± 2·6	0·01
5-FU + tr.	4	7	18·8 ± 4·5	
Control + tr.	5	9	23·6 ± 3·1	0·05
5-FU + tr.	6	9	18·9 ± 4·7	

The 5-fluorouracil was administered intraperitoneally in one dose (25 mgm./100 gm.) to groups 2 and 6; daily for 3 days to group 4.

Activity of tryptophan-pyrrolase was assayed 5 hr. after the L-tryptophan administration (100 mgm./100 gm.) in groups 3 and 4, and 4 hr. after in groups 5 and 6. Twenty hours before death, no food was given to the animals of all groups.

Table 2. EFFECT OF 5-FLUOROURACIL ON INDUCTION OF HEPATIC TRYPTOPHAN-PYRROLASE
(The 5-fluorouracil was administered during the decreasing phase of the substrate-induced tryptophan-pyrrolase curve)

Treatment	Group No.	No. of animals	μMoles kynurenine/gm. liver wet wt./hr.	*P*
Control + tr.	1	2	24·4 ± 1·0	
5-FU + tr. (FU 4 hr. after the 1 tr. injection)	2	2	19·1 ± 2·2	0·05
5-FU + tr. (FU 13 hr. after the 1 tr. injection)	3	2	20·9 ± 2·9	0·05

Tryptophan-pyrrolase activity was assayed 4 hr. after the second intraperitoneal administration of L-tryptophan (100 mgm./100 gm.). The second injection (100 mgm./100 gm.) was always made 17 hr. after the first administration, when the enzyme-level was returned to basal values.

5-fluorouracil appears to exert at least two distinguishable effects: it interferes with the normal synthesis of ribonucleic acid and inhibits the formation of thymidilic acid[10].

Male Sprague–Dawley albino rats weighing about 200 gm. were used for the experiments. The 5-fluorouracil was administered in one dose simultaneously with the tryptophan or daily for three days, the final injection being given together with the enzyme inducer.

Activity of tryptophan-pyrrolase was determined according to the method of Knox and Mehler.

Since the experiments of Feigelson, Feigelson and Fancher[12] showed an increased incorporation of inorganic phosphorus-32 into ribonucleic acid during the period following the maximal increase in tryptophan-pyrrolase, experiments were made, in which 5-fluorouracil was administered during that period and the enzyme formation was again induced afterwards.

As shown in Tables 1 and 2, 5-fluorouracil at the dosages used causes a limited but significant inhibition of enzyme induction, practically independent of the time of administration.

As the toxicity of 5-fluorouracil hinders the use of greater dosages, in order to obtain an increased inhibition we tried another experimental device.

Campbell and Kosterlitz[13], Munro and Clark[14], found that when rats are fed on a protein-free diet there is a breakdown of ribonucleic acid, which is resynthesized when the rats are again fed on a normal diet. It would therefore seem probable that, if rats were given a normal diet after a period of protein depletion, there would be a greater incorporation of 5-fluorouracil into ribonucleic acid with consequent greater inhibition in induction of tryptophan-pyrrolase, if this was really an *ex novo* protein synthesis controlled by ribonucleic acid.

The results in Table 3 demonstrate that this really happened. So far as the kinetic relationships between enzyme synthesis and ribonucleic acid metabolism are concerned, the results obtained do not reveal any modification in metabolism of ribonucleic acid either contemporary with or subsequent to the maximum increase in enzyme synthesis.

Table 3. EFFECT OF 5-FLUOROURACIL ON INDUCTION OF HEPATIC TRYPTOPHAN-PYRROLASE DURING PROTEIN REFEEDING

Treatment	No. of animal	Refeeding period days Rat's wt. (gm.)		µMoles kynurenine/gm. liver wet wt./hr.	*P*
		0	2		
Control + tr.	5	167	182	21·9 ± 4·1	0·001
5-FU + tr.	7	166	171	12·3 ± 2·8	

Rats fed on protein-free diet for 13 days were then given for 2 days a normal diet. During protein refeeding the second group daily received intraperitoneally 10 mgm./100 gm. of 5-fluorouracil.

Tryptophan-pyrrolase activity was assayed 5 hr. after the L-tryptophan injection (100 mgm./100 gm.). Food consumption was always the same for the two groups of rats.

Though the variability of response of single animals consequent on 5-fluorouracil stress makes advisable a more exact check, nevertheless these results agree with the observation that even marked changes in rate of synthesis of an enzyme would not necessarily require changes in synthesis of ribonucleic acid[15].

Furthermore, experiments of protein refeeding of rats previously depleted of protein would probably prove that inhibition of synthesis of tryptophan-pyrrolase occurs at the microsomal ribonucleic acid level.

Munro and Clark[14] found, in fact, that when the ribonucleic acid is reduced by feeding rats on a protein-free diet, loss of ribonucleic acid is essentially confined to the microsome fraction. When proteins are administered again, the microsomal ribonucleic acid is regenerated and in the presence of 5-fluorouracil we may well find the formation of a 'fraudulent' ribonucleic acid that hinders protein synthesis in general and largely inhibits synthesis of this specific protein tryptophan-pyrrolase.

5-fluorouracil was kindly given to us by F. Hoffmann-La Roche et Cie, Basle.

SANCIA GAETANI
M. ANTONIETTA SPADONI

Laboratorio di Biochimica,
Istituto Nazionale della Nutrizione,
Roma.

[1] Lee, N. D., and Williams, R. H., *Biochim. Biophys. Acta*, **9**, 698 (1952).
[2] Gros, P., and Coursaget, J., *Bull. Soc. Chim. Biol.*, **36**, 1569 (1954).
[3] Poznanskaia, A. A., *Biokhimiya*, **23**, 230 (1958).
[4] Straub, F. B., referred to by Poznanskaia, A. A., *Biokhimiya*, **23**, 230 (1958).
[5] Creaser, E., *Biochem. J.*, **64**, 539 (1956).
[6] Horowitz, J., Soukkonen, J., and Chargaff, E., *Biochim. Biophys. Acta*, **29**, 222 (1958).
[7] Lee, N. D., *Fed. Proc.*, **17**, 262 (1958).
[8] Feigelson, P., Dashman, T., and Margolis, F., *Arch. Biophys.*, **25**, 478 (1958).
[9] Kwam, D. C., and Parks, R. E., *J. Biol. Chem.*, **235**, 2893 (1960).
[10] Bosch, L., Harbars, E., and Heidelberger, C., *Cancer Res.*, **18**, 335 (1958).
[11] Knox, W. E., and Mehler, A. M., *Science*, **113**, 237 (1951).
[12] Feigelson, P., Feigelson, M., and Fancher, C., *Biochim. Biophys. Acta*, **32**, 133 (1959).
[13] Campbell, B. M., and Kosterlitz, H. W., *Biochim. Biophys. Acta*, **8**, 664 (1952).
[14] Munro, H. N., and Clark, C. M., *Proc. Nutr. Soc.*, **19**, 55 (1960).
[15] Roberts, R. B., McQuillen, K., and Roberts, I. Z., *Ann. Rev. Microb.*, **13**, 1 (1959).

Collagen Fractions in the Skin of New-Born and Adult Rats

SINCE the early works on the soluble fractions of collagen were published, two fundamental theories have been put forward claiming that probable precursors might be found either in the neutral salt-soluble collagen or in the acid-soluble collagen. At present Jackson[1], and Gross[2], seem to have modified the earlier approach on the ground that the various solutions used for extraction differ only in their capacity to split the cross-linkings between the fibre chains. This points to the conclusion that the earliest precursors are to be found in the solutions of the weakest ionic strength that still keep their extraction capacity.

Jackson[3] observed that a protein fraction soluble in distilled water, obtained by treatment of carrageenin granuloma with 0·2 *M* sodium chloride *p*H 7·4 and in which hydroxyproline was present, was greatly increased during the first days of the development of the granuloma, predominating even over the other soluble fractions. On the other hand, Green and Lowther[4] found very little hydroxyproline in the same fraction when compared with high levels of proline. They reported also that distilled water could extract large amounts of protein proline from the granuloma with very little hydroxyproline.

These experimental facts might be connected with certain theoretical considerations based on the possible origin of the collagen hydroxyproline from proline[4], which lead to the assumption that the earliest precursors might be low molecular weight proteins or peptides with little hydroxyproline, if any, and with a high content of proline[5].

In the course of a work on the variations in total collagen content and its various soluble fractions in rat skin from embryonic to adult stages, some comparative results were found. They confirm the presence of this water-soluble fraction, rich in proline, in normal skin. Jackson's fractionation method[3], with slight modifications, was applied together with extractions of fresh skin with distilled water. The fractions were purified but not as thoroughly as in the original method. In this way the following observations were made:

(*a*) As was expected, a considerable increase of 'total' proline (Pr) and hydroxyproline (HPr) soluble in hot water at 1 atm. pressure with age represented

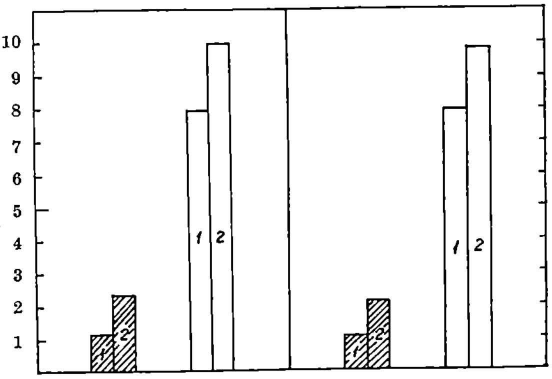


Fig. 1. Unfractionated rat skin. Left, 'total' proline and hydroxyproline; right, protein proline and hydroxyproline. Hatched areas, 22 one-day-old males (6·8 ± 0·4 gm.), 12·9 per cent dry defatted tissue; unshaded areas, 12 two-month-old males (2·8 ± 10 gm.), 26·8 per cent dry defatted tissue. 1 = HPr; 2 = Pr

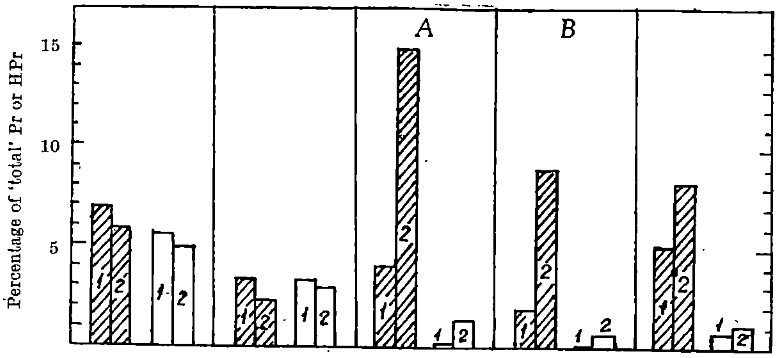


Fig. 2. Soluble collagens and water-soluble proteins. Hatched areas, one-day-old rats; unshaded areas, two-month-old rats. Left to right: neutral salt-soluble collagen (III); acid-soluble collagen (IV); water-soluble proteins (V); free amino-acids (VI). 1, HPr; 2, Pr

almost entirely by the protein fractions was found (Fig. 1). Multiplying the protein HPr content by 7·46 (ref. 6), 8·2 per cent and 58·6 per cent of collagen for the new-born and the adult rats respectively were obtained.

(*b*) Comparing both ages, no very significant differences between the acid- and neutral-soluble fraction percentages of 'total' Pr and HPr were found. The neutral salt-soluble collagen was slightly predominant over the acid-soluble collagen in new-born rats as well as in adult rats (Fig. 2).

(*c*) On the other hand a remarkable increase of the water-soluble fraction extracted with sodium chloride, *p*H 7·4, in 1-day-old rats was also observed. The most significant percentage of the soluble protein Pr corresponds to this fraction, being higher than that of the other soluble fractions (Table 1, V*A*). It showed a very high Pr/HPr ratio in both ages under study (Table 1).

Table 1. Pr/HPr Ratios of the Different Fractions

No.	Fraction	1 day-old rats	66-day-old rats
I	Total proline and hydroxyproline	1·99	1·26
II	Protein proline and hydroxyproline	1·90	1·24
III	Neutral salt-soluble collagen	1·80	1·05
IV	Acid-soluble collagen	1·30	1·10
V*A*	Water-soluble proteins extracted with sodium chloride *p*H 7·4	7·80	14·50
V*B*	Water-soluble proteins extracted with distilled water	8·50	13·50
VI	Free proline and hydroxyproline	3·30	1·50
VII	Insoluble collagen	1·15	1·15

Theoretical values of fibrillar collagen, 1·10–1·25.

(*d*) The distilled water also extracted a protein fraction with a high Pr/HPr quotient (Table 1, V*B*). In terms of Pr contents there is an almost four-fold increase in 1-day-old rats in comparison with 66-day-old rats, which is far greater than that corresponding to the water-soluble proteins extracted with sodium chloride *p*H 7·4 (Fig. 2). This is highly suggestive if we consider that the distilled water is the least effective agent in breaking up the fibres already laid down, extracting only the pre-existing material in soluble form.

(*e*) The 'total' Pr/HPr ratio in new-born rats is far greater than in adult rats due to the increase of the water-soluble fractions (Table 1). The same happens with the neutral salt-soluble collagen and in a smaller degree with the acid-soluble collagen. An attempt to investigate this ratio after an adequate purification process will be made in order to find out if differences in the chemical composition of these two soluble fractions could really exist.

(*f*) Free amino-acids, especially Pr, are increased in young rats (Fig. 2).

(*g*) The Pr/HPr ratio of insoluble collagen is the same in both ages, indicating no changes in fibrillar collagen composition (Table 1).

These preliminary observations are to be completed with the results obtained in other stages of development, together with the fractionation of water-soluble proteins. The data will be compared by labelling the various fractions of normal skin in connexion with autoradiographic and electron microscopical work in order to determine their biological significance.

We are grateful to Dr. G. Bourne for revising and criticizing the text.

Nestor Gonzalez Cadavid
Berta Denduchis
Roberto E. Mancini

Instituto de Anatomía General y Embriología,
Facultad de Ciencias Médicas,
and
Instituto de Química Biólogica,
Facultad de Farmacia y Bioquímica,
Buenos Aires,
Argentina.

[1] Jackson, D. S., and Bentley, J. P., *J. Biophys. Biochem. Cytol.*, **7**, 37 (1960).

[2] Gross, J., *Connective Tissue, Thrombosis and Arteriosclerosis* (Acad. Press, New York and London, 1959).

[3] Jackson, D. S., *Biochem. J.*, **65**, 277 (1957).

[4] Green, N. M., and Lowther, D. A., *Biochem. J.*, **71**, 55 (1959).

[5] Gould, B. S., and Woessner, J. F., *J. Biol. Chem.*, **226**, 289 (1957).

[6] Neumann, R. E., and Logan, M. A., *J. Biol. Chem.*, **186**, 549 (1950).

Inhibition of β-Glucuronidase by Oral Administration of D-Glucosaccharo-1,4-Lactone

Levvy[1] showed that D-glucosaccharo-1,4-lactone was a very powerful inhibitor for β-glucuronidase. Recently, Boyland *et al.*[2] suggested that the oral administration of D-glucosaccharo-1,4-lactone should be effective in the prevention of bladder cancer. It is probable that D-glucosaccharo-1,4-lactone, when administered *per os*, would, even though the lactone ring may partly be opened, yet more or less inhibit the tissue β-glucuronidase. In that event, as a result of inhibition of hydrolytic action of the enzyme, excretion of glucuronide compounds in urine would be increased. The present work has shown that the orally administered D-glucosaccharo-1,4-lactone actually caused an inhibition of liver β-glucuronidase.

D-glucosaccharo-1,4-lactone was administered orally to mice (*CF*-1 strain). At various time-intervals after administration, animals were stunned and killed and the liver immediately removed and homogenized in two volumes of isotonic potassium chloride solution with Potter–Elvehjem type of glass homogenizer. The homogenate was immediately run in the *p*H 5·0 (*M*/10 acetate buffer) incubation mixture[1] containing phenolphthalein glucuronide, and incubated for 1 hr. at 37° C. The activity of the β-glucuronidase was assayed by measuring the product of hydrolysis, phenolphthalein, by the method of Fishman[4].

Activities of mice liver β-glucuronidase measured 30 min. after oral administration of various doses of D-glucosaccharo-1,4-lactone are shown in Table 1.

Table 1. ACTIVITY OF MICE LIVER β-GLUCURONIDASE 30 MIN. AFTER ADMINISTRATION OF D-GLUCOSACCHARO-1,4-LACTONE

Dosage (mgm./kgm.)	Activity (per cent)*
Control	100
50	83·0
100	72·0
200	48·1
400	40·7
800	24·4

* Mean percentage of 5 mice.

As shown in Table 2, the enzyme was most strongly inhibited when tested 30 min. and 1 hr. after oral administration of D-glucosaccharo-1,4-lactone. After several hours, the enzyme activity was almost normal.

Table 2. ACTIVITY OF MICE LIVER β-GLUCURONIDASE AT VARIOUS TIME INTERVALS AFTER ORAL ADMINISTRATION OF D-GLUCOSACCHARO-1,4-LACTONE (400 MGM./KGM.)

Time after administration	Activity (per cent)*
Control	100
30 min.	40·8
1 hr.	37·3
2 hr.	81·6
4 hr.	94·9
6 hr.	87·4

* Mean percentage of 5 mice.

The enzyme was not strongly inhibited after administration of other sugar lactones such as glucuronolactone and gluconolactone (Table 3).

Table 3. ACTIVITY OF MICE LIVER β-GLUCURONIDASE 30 MIN. AFTER ORAL ADMINISTRATION OF SOME SUGAR LACTONES

Compound and dosage	Activity (per cent)*
Control	100
Glucuronolactone 1,000 mgm./kgm.	90·4
Gluconolactone 1,000 mgm/kgm.	95·3
Potassium saccharate 1,000 mgm./kgm.	85·8
D-Glucosaccharo-1,4-lactone 800 mgm./kgm.	24·4

* Mean percentage of 5 mice.

Similar results were obtained in experiments on the β-glucuronidase of rat liver and kidney, and in experiments in which *o*-aminophenol glucuronide was used as substrate instead of phenolphthalein glucuronide.

These results suggest that D-glucosaccharo-1,4-lactone would have some effect on the detoxication mechanism *in vivo*.

Karunairatnam and Levvy[3] reported that the administration of a large dose of saccharic acid to mice did not influence liver regeneration after damage or normal growth in infant animals.

S. AKAMATSU

Department of Biochemistry,
School of Medicine, University of Chiba,
Japan.

A. KIYOMOTO
S. HARIGAYA
S. OHSHIMA

Tokyo Research Laboratory,
Tanabe Seiyaku Co., Ltd.,
Saitama, Japan.

[1] Levvy, G. A., *Biochem. J.*, 52, 464 (1952).

[2] Boyland, E., Wallace, D. M., and Williams, D. C., *Brit. J. Cancer*, 11, 578 (1957).

[3] Karunairatnam, M. C., and Levvy, G. A., *Biochem. J.*, 44, 599 (1949).

[4] Fishman, W. H., *Methods in Enzymology*, 1, 263 (Academic Press Inc., 1955).

Incorporation of 5,10,15 ^{14}C-Farnesol Pyrophosphate into *Phycomyces* carotenoids

CELL-FREE extracts of *Phycomyces blakesleeanus* incorporate farnesol pyrophosphate labelled with carbon-14 into non-saponifiable lipids. Separation of these compounds on magnesium oxide-'Hyflo-Supercel' columns demonstrate that the carbon-14 is associated with crystalline β-carotene, purified ergosterol and fractions containing phytoene, phytofluene and lycopene. It has been reported several times that a great portion of the activity associated with the non-saponifiable compounds is lost on recrystallization[1]. As a consequence, the separated saturated fractions in this experiment are indicative only.

In the present experiment, 2-^{14}C-mevalonic acid and adenosine triphosphate were incubated with the soluble portions (30–60% ammonium sulphate saturation) of rat liver extracts to yield ^{14}C-farnesol pyrophosphate[2]. After separation and purification, the pyrophosphate was incubated with cell-free extract of *Phycomyces blakesleeanus* prepared as reported previously by Yokoyama *et al.*[3].

The *Phycomyces* incubation mixture contained adenosine triphosphate, reduced diphosphopyridine nucleotide, triphosphopyridine nucleotide, glucose-phosphate, manganine, sodium fluoride, and flavine adenosine dinucleotide. As a comparison, mould extracts of the same preparation were incubated with 2-^{14}C-mevalonic acid, and combinations of P-P farnesol and mevalonic acid. As the time of incubation was rather long (19 hr.) the preparations were checked before and after incubation for microbial contamination.

The β-carotene was isolated by chromatography and recrystallized to constant specific activity. The ergosterol was separated on similar chromatographic columns, eluted and precipitated as the digitonide. The digitonide was split with pyridine, and the ergosterol reprecipitated prior to counting. Unlabelled carrier was added to both compounds during purification.

The other compounds were collected as crude fractions after chromatography; they were then rechromatographed twice but not crystallized. Thus, they do not necessarily represent the minimum activity to be found in the components identified. The phytofluene is, in the circumstances of this experiment, relatively pure. The phytoene band, however, is likely to contain several high-counting contaminants. The lycopene band may contain some residual sterols.

The separated components were dissolved in toluene and sealed in counting vials. These were exposed to ultra-violet light (8–10 hr.) until completely bleached. Scintillating phosphors were added by injection through the vial caps, and the samples counted in a Packard 'Tri-Carb' counter. Appropriate controls were run to correct for quenching.

Table 1 gives the activities of the various fractions isolated, as well as the active materials added to the incubation medium.

It is apparent from Table 1 that farnesol pyrophosphate is more efficiently incorporated into β-carotene than is mevalonic acid. The fact that the efficiency of incorporation increases when a pool of 5 carbon fragments is supplied to the mixture indicates that the condensing unit is larger than 15. The general results of the experiment confirm the identity of precursors

Table 1
c.p.m.

Compound isolated	2-^{14}C-Mevalonic acid	^{14}C-Farnesol-P-P	^{14}C Farnesol-P-P inactive mevalonic acid
β-Carotene	8	69	131
Ergosterol	13	33	34
Phytoene	164	7,709	12,149
Phytofluene	26	735	1,485
Lycopene	24	222	112

in both sterol and carotenoid synthesis. Previous reports substantiate mevalonic acid as obligatory intermediate in the carotenoid synthesis. The findings indicated here would agree with this and would continue the identity through the C-15 level. Presumably the condensing unit for the formation of the C_{40} chain would be a C_{20} compound made from the condensation of farnesol pyrophosphate with an additional C-5 unit (possibly isopentenol pyrophosphate).

The ergosterol produced by the extract incorporates activity from both mevalonic acid and farnesol-P-P, but more efficiently from the latter. No increase in activity was shown when mevalonic acid was added, which would be as expected. The lack of stimulation in this case coupled with the increases noted in the carotenes would tend to confirm that the differentiation of the two pathways would occur at the C-15 to C-20 level.

As in most other reports on carotene synthesis, there is very high incorporation of activity in the phytoene fractions; the significance of these has not been established, since it has not yet been possible to crystallize phytoene or phytofluene. The activity is thought to reside in high-counting companions rather than in the carotenoids themselves.

This investigation was supported in part by a research grant from the National Institutes of Health.

H. YAMAMOTO
H. YOKOYAMA
K. SIMPSON
T. O. M. NAKAYAMA
C. O. CHICHESTER

Department of Food Science and Technology,
University of California,
Davis, California.

[1] Anderson, D. G., Norgard, D. W., and Porter, J. W., *Biochem. Biophys. Commun.*, **1**, 83 (1960).

[2] Goodman, D. W. S., and Pojak, G., *J. Lipid Res.*, **1**, 286 (1960).

[3] Yokoyama, H., Chichester, C. O., and Mackinney, G., *Nature*, **185**, 687 (1960).

Isolation and Fractionation of some Damselfly Pigments

THE imagines of *Ischnura elegans* are found in great numbers around some ditches and pools during the months of May–September. The females, especially, of this species exist in various colour forms, some of which experience changes in colour[1,2].

Two layers of granules situated immediately below the cuticle are concerned in the production of the different colours of the thorax[2]. The outer layer consists of small granules, while the inner layer consists of larger granules of varying sizes which are generally much darker in colour than the smaller ones, the different colours exhibited depending on the variation in thickness of the layers and the colour, distribution and density of the granules. The greatest numbers of both types of granules are present in the blue forms. The blue, being a structural colour based on the Tyndall effect, contrasts sharply with the orange and darker pigments of the granules themselves[2].

Ommochromes are found in a variety of insect eye cells and epidermal cells as granules responsible for pigmentation[3,4]. The isolation and structure of the ommochrome pigments, consisting of two major types, ommatines and ommines, have been extensively studied by Butenandt and his colleagues[4–7]. They are biosynthesized from tryptophan[6], through kynurenine and 3-hydroxykynurenine[4–6], and the latter compound is a characteristic constituent of acid hydrolysates of ommochromes, its presence being considered good evidence that the pigment is an ommochrome[4,5].

The granule pigments were isolated and their properties compared with those of samples of ommochrome pigments, namely rhodommatine and ommatine *D*, since ommochromes have been found as pigments (eye) of certain Odonata (including *Agrion mercuriale*[4]), and also with those of melanin prepared by enzymic synthesis, since the ommochromes and melanin have a common biosynthetic pathway as far as shikhimic acid.

One at least of the granule pigments is considered to belong to the ommochrome group, while evidence for the other is equivocal.

The extraction procedure followed that of Butenandt and his colleagues[4–7]. Three species of blue damselfly were extracted separately for comparison. The procedure was the same in each case.

The thoraxes of the damselflies, with wings removed (seventy *Ischnura elegans*—blue; twenty *Agrion pulchellum;* seventy *Enallagma cyathigerum*), were crushed in a small macerator (M.S.E.) with methanol (3 × 30 ml.) containing 2 per cent concentrated hydrochloric acid. The methanol was gassed with sulphur dioxide (3 min.) before use. The residue was centrifuged off between each extraction and each port-wine coloured extract was distilled under vacuum. The brownish-red residues, after distillation, were extracted with aqueous $M/15$ disodium hydrogen phosphate. The centrifuged extracts were green in colour. The brownish-red residues from this extraction were dissolved in concentrated hydrochloric acid (21 ml.) and an equal volume of distilled water was added. The mixtures were stored at −20° C. overnight and the precipitates were washed with acetone (5 × 5 ml.) and dried in air (yields: 2–10 mgm.).

In paper electrophoresis ($M/40$ sodium tetraborate solution: Whatman No. 54; 8 hr.; 12 V./cm.; 5 m.amp.) dinitrophenol-glycine and dinitrophenol-ethanolamine markers (fast and slow respectively) were used. The crude methanolic-hydrochloric acid extracts were found to contain three pigments distinguishable by paper electrophoresis: a faster moving mauve pigment, an orange pigment remaining with the slow marker, and a green pigment falling between the two. However, though the mauve pigment behaved in a similar fashion to rhodommatine, the latter appeared as a salmon-coloured pigment in the borate buffer. This mauve pigment was found to correspond to the red precipitate, while the orange pigment was found, together with the green pigment, to be concentrated in the $M/15$ disodium hydrogen phosphate extract. The latter pigment may have been chlorophyll.

The orange pigment was isolated by electrophoretic separation (Whatman 3*MM*; borate buffer), elution with methanolic hydrochloric acid (2 per cent) and distillation under vacuum.

The mauve pigment, isolated by a procedure for obtaining purified ommochromes, was found to be associated with protein (paper chromatography of acid hydrolysate; ninhydrin detection). When this pigment (2 mgm.) was hydrolysed with $6N$ hydrochloric acid (2 ml.) by refluxing for 10 hr., 3-hydroxykynurenine was found to be present (ascending paper chromatography; solvent, *n*-butanol/acetic acid/water: 12/3/5; Whatman No. 4 paper; dip detection by Ehrlich as well as 0·25 per cent potassium ferricyanide, followed by 0·25 per cent sodium dithionite).

Under the same conditions both the rhodommatine and ommatine *D* samples gave 3-hydroxykynurenine, as would be expected.

The orange pigment, similarly treated, gave equivocal responses to 3-hydroxykynurenine tests. (Even after alcoholic precipitation of a gum-like material, substances present in the hydrolysate of the orange pigment made chromatographic identification difficult.)

The mauve pigment behaves, on electrophoresis, in a similar fashion to rhodommatine and probably belongs to the ommochrome class: its solubility, its behaviour during isolation and the presence of 3-hydroxykynurenine in its acid hydrolysates are good evidence for this grouping. The character of the orange pigment, although originally suspected to be xanthommatine on the basis of colour, solubility and mode of isolation, remains in doubt.

Melanin, prepared by the action of mushroom polyphenoloxidase[8] (acetone precipitate) on tyrosine hydrochloride[9], gave no 3-hydroxykynurenine on acid hydrolysis and differed from the isolated granule pigments in appearance and solubility.

The large dark granules, in 5μ sections and in uncut integument, dissolve easily in methanolic hydrochloric acid (2 per cent) to give a reddish-brown solution. They do not appear to be soluble in $M/15$ disodium hydrogen phosphate, unlike the small orange-coloured granules in sections. These small granules do not pass into solution with phosphate buffer from intact integument even after the large ones have been removed with methanolic hydrochloric acid. This would imply that the matrix affects their solubility or dispersion. Medium-sized granules were present in damselflies where the blue colour was being formed, though they were not observed in older flies. One explanation is that these medium-sized granules are precursors of the large ones, another is that later they are obscured by the larger granules. Differential solution of the large (and possibly medium) granules from intact integument with methanolic hydrochloric acid yielded material which gave $6\ N$ hydrochloric acid hydrolysates containing 3-hydroxykynurenine. Solution of the remaining small granules + matrix in concentrated hydrochloric acid yielded material, the $6\ N$ hydrochloric acid hydrolysate of which gave a negative response to 3-hydroxykynurenine tests. These histochemical tests seem to link the large granules with the mauve pigment and the small orange ones with the orange pigment.

The mauve pigment isolated from *I. elegans*, *A. pulchellum* and *E. cythigerum* appears to belong to the group of Butenandt's ommochrome pigments and differs from melanin.

We thank Prof. F. W. R. Brambell for his interest and encouragement, Prof. W. C. Evans for his hospitality to one of us (P. M. L.), and Dr. A. B. Acton for his advice and suggestion of the problem. We are grateful to Prof. A. Butenandt, Munich, for the generous gift of rhodommatine, ommatine *D* and 3-hydroxykynurenine, and also to Dr. L. M. Henderson, Oklahoma State University, for his gift of 3-hydroxykynurenine. We are indebted to the Department of Scientific and Industrial Research for a postgraduate studentship (P. M. L.) and the University of Wales for an I.C.I. fellowship (C. B. C.).

C. B. COULSON *

Department of Biochemistry and Soil Science,
School of Agriculture,
University College of North Wales,
Bangor,
Caernarvonshire.

P. MARY LORD †

Department of Zoology,
University College of North Wales,
Bangor,
Caernarvonshire.

---

* Present address: Arthur D. Little Research Institute, Inveresk, Midlothian, Scotland.

† Present address: Department of Genetics, University of Sheffield.

[1] Acton, A. B. (unpublished work).
[2] Lord, P. M. (unpublished work).
[3] Kühn, A., *Naturwiss.*, **43**, 25 (1956).
[4] Butenandt, A., *A. Stoll Festschrift*, 869 (1957).
[5] Butenandt, A., *Naturwiss.*, **46**, 461 (1959).
[6] Butenandt, A., and Neubert, G., *Z. physiol. Chem.*, **301**, 109 (1955). Butenandt, A., and Rudiger, B., *ibid.*, **301**, 115 (1955).
[7] Butenandt, A., and Neubert, G., *Ann. Chem.*, **618**, 167 (1958). Linzen, B., *Z. physiol. Chem.*, **314**, 12 (1959).
[8] Freudenberg, K., and Richtzenhain, H., *Ber.*, **76**, 997 (1943). Freudenberg, K., *Progr. Chem. Org. Nat. Prod.*, **11**, 43 (1954).
[9] Dawson, C. R., and Magee, R. J., in *Methods in Enzymology*, **2**, edit. by Colowick, S. P., and Kaplan, N. O. (Academic Press, Inc., New York, 1955).

Lipids of the Common Pea (*Pisum sativum* L.) with Special Reference to the Occurrence of Galactolipids

IT has been firmly established that the lipids of most seeds consist almost entirely of triglycerides[1]. The lipids of leaves, on the other hand, are much more complex and contain considerable amounts of wax and phospholipids[1] in addition to a cold acetone-soluble fraction amounting to some 50–70 per cent of the total lipids[2]. This acetone-soluble fraction has been generally assumed to consist mainly of triglycerides along with lesser amounts of unsaponifiable matter and free fatty-acids. In the light of recent work, however, it appears that leaves contain not more than minor amounts of triglycerides, their place being taken by galactolipids[3,4]. The identification of galactolipids as the main lipid fraction involved in the early stages of photosynthesis suggests a relationship between the occurrence of chlorophyll and of galactolipids[5], and is in keeping with their presence in leaves and their apparent absence in seeds. In this regard it was thought instructive to examine the leaves, pods and seeds of the common pea (*Pisum sativum* L.) as the seeds in this instance contain relatively little oil, much of which is non-glyceridic in nature[6], and in addition the seeds contain some chlorophyll or chlorophyll-like pigments.

Using techniques previously described[2,4] it was found that the total lipids present in the leaves, pods and seeds taken at a stage when the pods were full and still green comprised respectively 10·3, 2·4 and 4·8 per cent of the tissues dried to constant weight at 100°. The corresponding values for the acetone-soluble fractions were 7·4, 1·3 and 1·8 per cent while those for the non-dialysable lipids from these fractions were respectively 4·4, 0·7 and 0·4 per cent.

Examination of the non-dialysable fractions (which include all the galactolipids present) showed that whereas in the leaves the values for galactose and glycerol indicated that the fraction consisted mainly of galactolipids, as previously found for other leaves, in the pods and seeds the galactose contents were considerably lower.

The relative contents of chlorophyll-like pigments, as measured by the blue colour of the acetone extracts using a Lovibond tintometer, indicated that the leaves contained much more of these pigments than did the seeds, and in general the amounts of galactolipids calculated from galactose contents followed approximately those of the chlorophyll-like pigments.

In agreement with Wagenknecht[6] we have been able to demonstrate that the acetone-soluble lipids of pea seeds are comprised largely of triglycerides. Whereas, however, Wagenknecht[6] considered that the sugars associated with this fraction are solely contaminants, our present work as well as earlier work[2–4] support the view that the sugars are combined in the lipid molecule.

The present results confirm that galactolipids are the main acetone-soluble lipids in leaves, but in addition indicate the presence of trace amounts of triglycerides in these tissues. Moreover, while triglycerides are the main component of seed lipids it is now shown that in some seeds, at least, traces of galactolipids may also be present. The work in this field is being continued.

S. Adhikari
F. B. Shorland
R. O. Weenink

Department of Scientific
and Industrial Research
Fats Research Laboratory,
Wellington,
New Zealand.

[1] Hilditch, T. P., *The Chemical Constitution of Natural Fats*, 3rd Ed. (Chapman and Hall, Ltd., London, 1956).

[2] Shorland, F. B., *J. Sci. Food and Agric.*, **12**, 39 (1961).

[3] Weenink, R. O., *N.Z. J. Sci.*, **2**, 273 (1959).

[4] Weenink, R. O., *J. Sci. Food and Agric.*, **12**, 34 (1961).

[5] Benson, A. A., Wiser, R., Ferrari, R. A., and Miller, J. A., *J. Amer. Chem. Soc.*, **80**, 4740 (1958).

[6] Wagenknecht, A. C., *J. Amer. Oil Chem. Soc.*, **34**, 509 (1957).

Isolation of a New Antioxidant from Oats

The antioxidant properties of oatmeal are well known[1] and have been ascribed to phospholipids[2]. Oats are also rich in tocopherols[3] which function as antioxidants. We now report the isolation of an active antioxidant from oats which belongs to neither of these classes.

Whole oat kernels were freed from most of their oil by soaking in light petroleum (boiling point below 40° C.) for several days. A crude preparation of the antioxidant was obtained by extracting the defatted kernels with diethyl ether. This was purified by precipitation from solution in diethyl ether by the addition of light petroleum followed by chromatography on columns of silicic acid.

The purified antioxidant is almost colourless, melting point 85° C., soluble in most organic solvents, but insoluble in light petroleum and water. It has the properties of a phenol, dissolves in aqueous alkali to give a bright yellow solution and gives a green colour with ferric chloride, which suggest the presence of a free catechol nucleus. It has a characteristic ultraviolet absorption spectrum with a principal maximum at 331 mμ (in ethanol), E(1 cm., 1 per cent), 440. Analysis of two specimens gave: C, 69·9, 69·7; H, 8·4, 8·9; OCH_3, 3·8, 4·1 per cent molecular weight by Barger's method, 700; no nitrogen or phosphorus was found. On alkaline hydrolysis caffeic acid, ferulic acid and a neutral substance were obtained apparently in equimolecular proportions.

The neutral component was crystallized from ethyl acetate giving colourless needles, melting point 105° (found: C, 76·9; H, 13·1; M, 290). It formed an acetate, melting point 70°, and on oxidation with chromium trioxide in acetic acid gave an acid melting point 114°. The infra-red absorption spectrum with bands at 1,716 cm.$^{-1}$ (C=O stretching) and at 3,300 and 1,055 cm.$^{-1}$ (OH stretching and bending respectively), for which we are indebted to Dr. M. J. Fishwick, of the Low Temperature Research Station, Cambridge, suggests that it may be a keto-alcohol.

Table 1. ANTIOXIDANT ACTIVITIES MEASURED IN ARBITRARY UNITS/MGM.*

Substance	Antioxidant activity (units/mgm.)
Purified oat antioxidant	30
Caffeic acid	90
Ferulic acid	14
Neutral component	0
Propyl gallate	30
Butylated hydroxytoluene	30

* The increase in induction period (hr.) when 100 mgm. of an oat oil (petroleum-soluble) was oxidized in oxygen in the presence of 1 mgm. of a test sample, was a measure in arbitrary units of the activity of the latter.

The results in Table 1 show the antioxidant activity of the new substance and its constituent parts as measured by a recording oxygen apparatus[4]. For comparison, the corresponding figures for the two synthetic antioxidants, *n*-propyl gallate and butylated hydroxytoluene, are also included. The results suggest that the activity of the antioxidant is largely related to its caffeic acid content.

Work is in progress to elucidate precisely the structure of this natural antioxidant and to determine the extent to which it is present in wheat and other cereals.

This investigation is being financed in part by the U.S. Department of Agriculture under the authority of U.S. Public Law 480, 83rd Congress.

D. G. H. Daniels
H. F. Martin

Research Association of British Flour Millers,
Cereals Research Station,
Old London Road,
St. Albans, Herts.

[1] Peters, F. N., and Musher, S., *Indust. Eng. Chem.*, **29**, 146 (1937).

[2] Dahle, C. D., and Nelson, D. H., *J. Dairy Sci.*, **24**, 29 (1941). Taufel, K., and Rothe, H., *Fette u. Seifen*, **51**, 100 (1944).

[3] Green, J., Marcinkiewicz, S., and Watt, P. R., *J. Sci. Food Agric.*, **6**, 274 (1955).

[4] Martin, H. F., *Chem. and Indust.*, 364 (1961).

Effect of Adrenalectomy on the Activity of Enyzmes of the Urea Cycle in Rat Liver

The procedures for the estimation of enzymes of the urea cycle reported by Brown and Cohen[1] have made it possible to study quantitatively the effect of hormones on the individual reactions of the urea cycle. These authors have recently shown that, in rat liver, the condensing enzyme of the arginine synthetase system is rate-limiting and will thus be a pacemaker for the whole system[2]. It is at such rate-

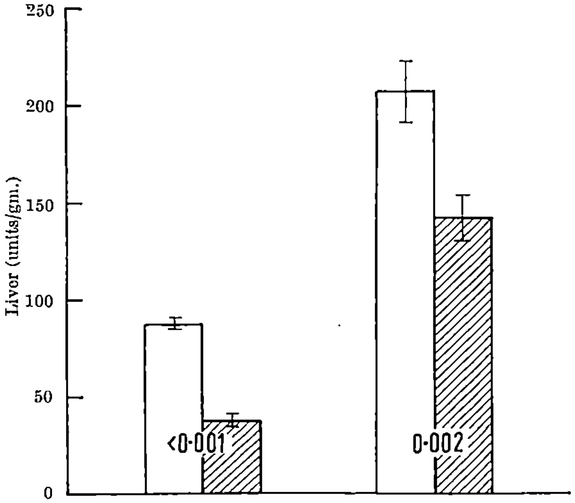


Fig. 1. Effect of adrenalectomy on the activity of rat liver arginine synthetase (left) and argininosuccinase (right). The open columns represent the mean values for 6 control rats, the hatched columns the mean values for 6 adrenalectomized rats. The vertical lines represent twice the standard error of the mean and the figures across adjacent columns are values for Fisher's *P*. The enzyme activities were determined 10 days after adrenalectomy by the method of Brown and Cohen (ref. 1). The control rats were pair-fed with the adrenalectomized rats which were maintained on 1 per cent sodium chloride in the drinking water. A unit of enzyme activity is defined as that amount which catalyses the production of 1 µmole urea/hr. under the assay conditions

limiting reactions that metabolic control might be exerted, and it therefore seemed of particular interest to measure the activity of this enzyme under conditions where there is a marked change in nitrogen metabolism and the production of urea.

The experiments of Long *et al.*[3] and many others (see Young[4]) have clearly established that adrenalectomy in rats causes a decreased nitrogen excretion and depressed gluconeogenesis from protein. Further, it has been shown that liver arginase is markedly depressed in adrenalectomized rats (Fraenkel-Conrat *et al.*[5], Folley and Greenbaum[6]). The experiments reported here are concerned with the effect of adrenalectomy on the activity of liver argininosuccinase and the arginine synthetase system as measured by the method of Brown and Cohen[1]. The arginine synthetase system is, in effect, a measure of the activity of the condensing enzyme forming argininosuccinate since the two succeeding enzymes, argininosuccinase and arginase, are present in excess. The results of these experiments are shown in Fig. 1.

Adrenalectomy caused a marked fall in the activity of the arginine synthetase system to a value less than half that of the pair-fed control rats. Liver argininosuccinase activity was also significantly reduced in adrenalectomized rats; but the magnitude of the effect was smaller—the enzyme activity was approximately 64 per cent of the control value. A similar pattern of change was found when the enzyme activities were expressed as total units in the liver and total units/100 gm. body-weight. The potential rate of urea synthesis may be calculated from the activity of the rate-limiting enzyme of the arginine synthetase system, and this is found to show a close correlation with the observed rate of urea production. Long *et al.*[3] found that the nitrogen excretion of adrenalectomized rats was approximately 34 per cent lower than that of control animals, a value confirmed in the present experiments.

Liver arginase was measured by the method of Folley and Greenbaum[7] and showed the usual marked depression in activity; the mean values for control and adrenalectomized rats are, respectively, 27×10^3 and 47×10^3 units/gm. liver. These results emphasize again the great disparity in the relative activities of different enzymes of the urea cycle.

Cohen *et al.* have already studied the effect of thyroxine on enzymes of the urea cycle in regenerating rat liver[8]. It would be interesting to know how the urea-cycle enzymes change under conditions where there is a negative or positive nitrogen balance as in rats treated with adrenal steroid or growth hormone, and this work will be extended to include such measurements.

I am indebted to Dr. R. G. Westall for a generous supply of argininosuccinate. This work was supported in part by a grant to the Medical School from the British Empire Cancer Campaign.

PATRICIA McLEAN

Courtauld Institute of Biochemistry,
Middlesex Hospital Medical School,
London, W.1.

[1] Brown, jun., G. W., and Cohen, P. P., *J. Biol. Chem.*, **234**, 1769 (1959).

[2] Brown, jun., G. W., and Cohen, P. P., *Biochem. J.*, **75**, 82 (1960).

[3] Long, C. N. H., Katzin, B., and Fry, E. G., *Endocrinol.*, **26**, 309 (1940).

[4] Young, F. G., *Ann. Rep. Chem. Soc.*, **42**, 214 (1945).

[5] Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.*, **147**, 99 (1943).

[6] Folley, S. J., and Greenbaum, A. L., *Biochem. J.*, **43**, 581 (1948).

[7] Folley, S. J., and Greenbaum, A. L., *Biochem. J.*, **43**, 537 (1948).

[8] Nazario, M., and Cohen, P. P., *Proc. Soc. Exp. Biol. Med.*, **106**, 492 (1961).

RADIOBIOLOGY

Decreased Urinary Excretion of 2-Aminoethanol by Irradiated Human Beings

PREVIOUS reports have described an increase in the urinary excretion of certain amino-acids by accidentally irradiated human beings[1] and by patients receiving radiation therapy[2]. This communication concerns the previously unreported finding of a decrease in urinary aminoethanol in human beings following total-body radiation.

Patients selected for radiation treatment were suffering from far-advanced neoplastic disease, resistant to conventional therapy. Total-body radiation was administered in a multiple-source cobalt-60 irradiator, which delivers a uniform air dose at rates up to 30 r./min.

Complete urine collections were obtained daily from each patient before and after irradiation. Aliquots were stored at −15° until they were analysed. Aminoethanol was determined by high-voltage electrophoresis on paper using a pyridine/acetic acid/water (1:10:89) buffer, *p*H 3·6, and a commercial apparatus[3]. (A potential of 30 V./cm. was applied for 90 min. at −3°.) By this method, aminoethanol is clearly separated from all other ninhydrin-reacting substances. Quantitative determination was by elution and colorimetric comparison with standards run simultaneously. Analysis for phosphorylaminoethanol was carried out in the same manner except that a pyridine–acetic acid buffer at *p*H 6·0 was used.

Among normal subjects and patients with various forms of cancer, the urinary excretion of aminoethanol, although variable, was never less than 10 mgm. a day. Following total-body radiation, the urinary content of aminoethanol decreased to undetectable concentrations, that is, less than 0·4 μgm./ml., in all six patients who were exposed to single doses in the range of 300–900 r. The results in one patient of this group are shown in Fig. 1. Aminoethanol virtually disappeared from the urine in this individual on the fourth and fifth days following irradiation. The other cases exhibited this effect for varying periods ranging from one to six days during the first week post-irradiation. The effect was more prolonged following higher doses. In all six cases aminoethanol re-appeared in the urine by the twelfth day.

In contrast, urinary aminoethanol was decreased but not absent following irradiation in one patient given 225 r. (single exposure) and in each of four patients who received multiple 100 r. exposures at two- to three-day intervals to a total dose of 500 r.

Changes in the excretion of aminoethanol were not observed in patients undergoing major surgery or in cases of body burns.

Phosphorylaminoethanol was not detected in the urine of any of the cases studied.

The mechanism responsible for the observed alteration in aminoethanol excretion following irradiation is open to speculation. Renal effects cannot be ruled out since analysis of plasma was not performed. A possible clue to the mechanism is offered by the finding of Kretchmar[4] that free serine disappeared from the urine of accidentally irradiated men. The metabolism of serine is closely related to that of aminoethanol. Both compounds are constituents of cephalins and plasmalogens. It is felt that the observations reported here may point to further investigations.

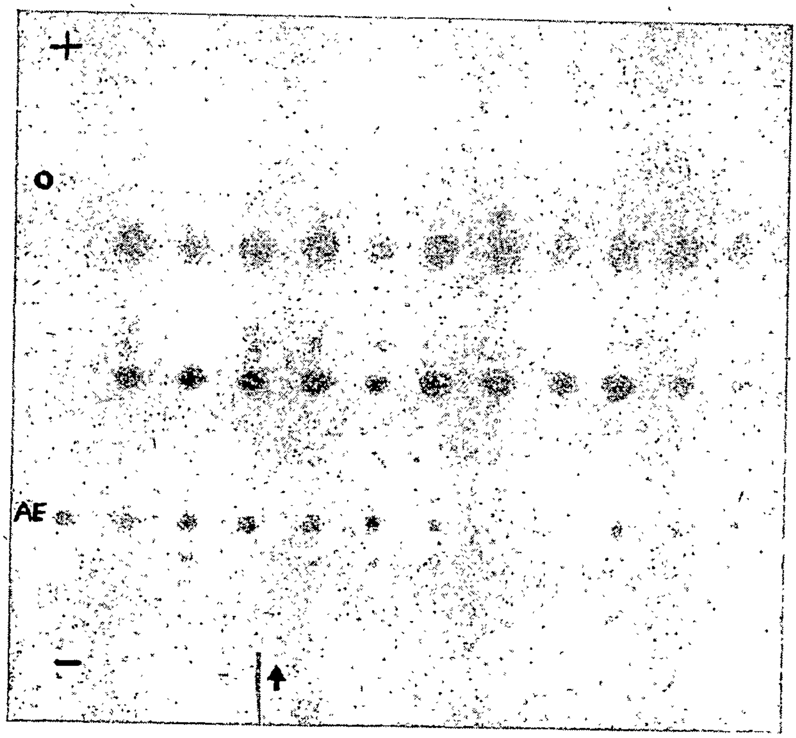


Fig. 1. Electropherogram, stained with ninhydrin, of samples of urine collected daily from a patient who received 300 r. (air dose) total-body radiation at the beginning of the fourth day (arrow). 15-μl. samples were applied in spots along line *O*. A standard (*AE*) containing 0·1 mgm. aminoethanol per ml. was run simultaneously at the far left. Note the virtual disappearance of aminoethanol in the urine excreted on the fourth and fifth days following irradiation

Ralph R. Cavalieri
M. T. Van Metre
W. I. Sivertsen

Nuclear Medicine Branch,
U.S. Naval Hospital and
Radiation Exposure Evaluation Laboratory,
National Naval Medical Center,
Bethesda,
Maryland.

[1] Katz, E. J., and Hasterlik, R. J., *J. Nat. Cancer Inst.*, 15, 1085 (1955).
[2] Cavalieri, R. R., Van Metre, M. T., Chambers, F. W., and King, E. R., *J. Nucl. Med.*, 1, 186 (1960).
[3] Weland, T., and Pfleiderer, G., *Angew. Chem.*, 67, 257 (1955).
[4] Kretchmar, A. L., *Nature*, 183, 1809 (1959).

Radiation Damage to Proteins

Physicochemical work shows that ionizing radiations cause fragmentation and aggregation of protein molecules[1–3]. When protein molecules are degraded there is a variety of products and also many amino-acids are degraded. However, the mechanisms by which these changes occur are still obscure. In our experiments, we define the radiation damage to cytochrome *c*, hæmoglobin, catalase, and egg albumin in terms of radiation-lability of amino-acids and loss of biological properties of the three hæmoproteins. The amino-acid pattern of scission products and insoluble protein aggregates was also determined.

If aggregates are formed by protein-protein cross-linkage or by denaturation of native molecules one would expect that the amino-acid pattern of insoluble aggregates would be similar to that of original protein. Also, if scission products arise by random cleavage at the most radiation-labile amino-acids into large polypeptide chains, then these scission products and the original protein should have similar amino-acid patterns except for destruction of the most radiation-labile amino-acids.

In our experiments, 0·1 per cent solutions of proteins were anaerobically exposed to 0·6–2-MeV. γ-irradiation; the dose-range varying from 10^5 to 5×10^7 rads[4]. Insoluble aggregates were separated by centrifuging and filtration. Soluble protein was precipitated using 5 per cent trichloroacetic acid (TCA) and thus separated from scission products. These three fractions were hydrolysed and amino-acids were determined by two-dimensional paper chromatography. Protein which did not receive any irradiation served as the control.

Cystine, methionine, histidine, phenylalanine, tyrosine, threonine and serine were most susceptible to radiation damage. Rank in terms of radiation-lability, however, varied with the protein. These amino-acids were degraded even at lower radiation doses ($< 10^6$) in contrast to other amino-acids, such as aspartic acid, glutamic acid, lysine and alanine, which were among the most stable. Loss of biological properties of the hæmoproteins cannot be attributed to destruction of any specific loci as the proteins are modified considerably and several amino-acids degraded by ionizing radiations. Although the mechanisms are different, ionizing radiation may be compared with active carbonyl-amine browning and lipid peroxidation reactions in which amino-acids are degraded. The same amino-acids are labile except for greater losses of lysine and arginine in the latter two reactions[5-7].

As a function of radiation dose, the survival of amino-acids in the TCA-precipitable protein declined, indicating degradation of original protein. The amino-acid pattern of scission products and/or insoluble aggregates was totally different from that of original protein or TCA-precipitable protein at a particular radiation dose. This suggested that denaturation or polymerization was not the only cause of formation of insoluble protein aggregates, and that hydrolysis induced by radiation was not the mechanism of fragmentation.

Radiation-lability of sulphur amino-acids in the proteins merits special attention. These amino-acids give rise to volatile products responsible for some undesirable flavour in irradiated foods and are a site for localization of radiation damage[8]. Fission of cystine and cysteine gives rise to hydrogen sulphide and alanine. Analogous to this reaction is the formation of CH_3SH and α-amino butyric acid from irradiation of methionine.

This research was supported by the Quartermaster Food and Container Institute for the Armed Forces.

U. S. Kumta
A. L. Tappel

University of California,
Davis, California.

[1] Fricke, H., Landmann, W., Leone, C., and Vincent, J., *J. Phys. Chem.*, **63**, 932 (1959).

[2] Koenig, V. L., Sowinski, R., Oharenko, L., Freling, V., and Wei, G. C., *Die Makromolekulare Chemie*, **42**, 79 (1960).

[3] Alexander, P., Hamilton, L. D. G., and Stacey, K. A., *Radiation Res.*, **12**, 510 (1960).

[4] Ambe, K. S., Kumta, U. S., and Tappel, A. L., *Radiation Res.* (in the press).

[5] Friedman, L., and Kline, O. L., *J. Biol. Chem.*, **184**, 599 (1950).

[6] Lea, C. H., and Hannan, R. S., *Biochim. Biophys. Acta*, **5**, 433 (1950).

[7] Lea, C. H., and Parr, L. J., *Brit. J. Nutrition*, **14**, 91 (1960).

[8] Gordy, W., *Symposium on Information Theory in Biology*, 241 (New York, 1958).

PHYSIOLOGY

Splitting of Serum Albumin during Electrophoresis on Paper

The existence in human and animal serum of protein constituents which migrate during electrophoresis at rates faster than that of albumin has been widely reported in the literature by investigators using moving boundary electrophoresis[1] and electrophoresis on paper[2-5] and in hydrolysed starch-gels[6,7]. Using *tris* buffer (*p*H 8·9), which consists of *tris*-(hydroxymethyl)aminomethane, ethylenediamine tetraacetic acid (EDTA) and boric acid, Aronsson and Grönwall[8] reported not only an enhanced separation of the protein constituents of human serum and the appearance of a pre-albumin component, but also a partial splitting of the albumin peak during electrophoresis in *tris*/boric acid buffers.

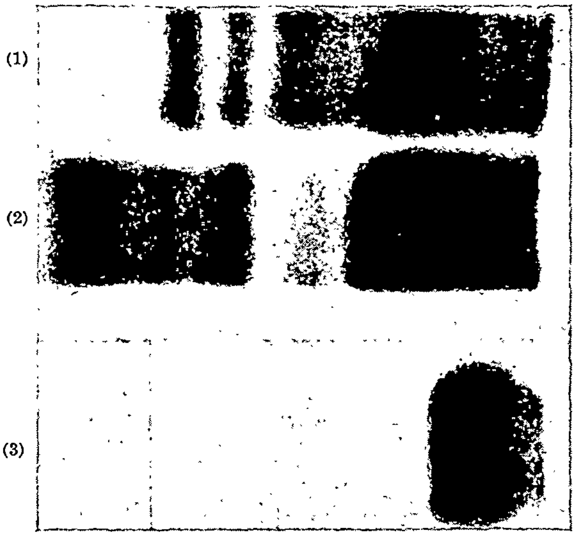


Fig. 1. The splitting during electrophoresis in *tris* buffer (*p*H 8·9) of (1) fœtal sheep serum albumin; (2) pregnant sheep serum albumin; and (3) crystalline bovine plasma albumin

In this Laboratory serum from maternal and fœtal sheep was examined at different stages of gestation by means of electrophoresis on paper using the *tris* buffer described by Aronsson and Grönwall[8]. Separation of the serum protein constituents was carried out in an air-tight 'Perspex' box, the buffer troughs of which were connected by gel bridges to silver/silver chloride electrodes immersed in saturated potassium chloride solution. The serum samples were applied to the paper after the current had been passed through the strip for at least 1 hr. Electrophoresis was continued for 18 hr. at room temperature, using a current density of 0·5 m.amp./cm. width of strip and a potential gradient across the paper of 3·2 V./cm. The paper strip was then dried at 115° C. for 15 min. and was stained with a bromocresol-green reagent. The stained paper was washed free of excess dye, and, after drying in air, was permanently blued by spraying with a 4 per cent solution of sodium carbonate.

In these conditions of electrophoresis a splitting of the serum albumin of fœtal, pregnant and normal adult sheep was observed. The degree of separation of the two components varied somewhat from one electrophoresis run to another, but nevertheless the two components always appeared as quite distinct entities. A third component was frequently detected lying between the two main components, but this material of intermediate mobility was not usually a well-defined band. It appeared to arise from a further splitting of the faster-moving of the two main components. In Fig. 1 are shown examples of the splitting of sheep serum albumin and the results of electrophoresis of crystalline bovine plasma albumin (Armour) under the same conditions, when this latter material showed the presence of three components. The electrophoretic patterns obtained with human serum showed a distinct band moving ahead of the main albumin component. Sheep serum albumin and crystalline bovine plasma albumin moved as single components during electrophoresis in veronal buffer, *p*H 8·6.

The splitting of sheep serum albumin reported is being investigated further.

I am indebted to the Medical Research Council of Ireland for the award of a fellowship.

J. V. McLoughlin

Protein Laboratory of the
Medical Research Council of Ireland,
Department of Physiology,
University College, Dublin.

[1] Deutch, H. F., and Goodloe, M. B., *J. Biol. Chem.*, **161**, 1 (1945).
[2] Aly, F. W., and Niederhellmann, K. H., *Klin. Wschr.*, **36**, 954 (1958).
[3] Cooper, D. R., *Nature*, **181**, 713 (1958).
[4] Kazala, S. A., and Nakayama, Y., *Chin. Chim. Acta*, **4**, 369 (1959).
[5] Ortega, M., *Nature*, **179**, 1086 (1957).
[6] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[7] Ashton, G. C., *Nature*, **179**, 824 (1957).
[8] Aronsson, T., and Grönwall, A., *Scand. J. Clin. Lab. Invest.*, **9**, 338 (1957).

Urinary Inhibitors of Hyaluronidase

In connexion with the recent discussion on the relationship between urinary hyaluronidase and diuresis between Ginetzinsky and Berlyne[1], some observations of ours may be of interest.

We have investigated hyaluronidase activity in urine, in serum, and in various kinds of tissue extracts by a somewhat modified viscometric method[2]. One essential feature of this method is that flow-time is read every 1 or 2 min. A plot of flow-time against reaction time gave, for most urine samples, approximately a straight line in contrast to the characteristic curved shape obtained with samples of pure enzyme.

We could reproduce the rectilinear curves by adding to the enzyme solution small quantities of various hyaluronidase inhibitors, for example, chondroitin sulphate, polyœstradiolphosphate ('Estradurin *R*', H. Lundbeck and Co., Copenhagen) and partly degraded hyaluronic acid.

Gibian[3] showed that the influence of inhibition in serum samples could be suppressed by diluting the samples. We have been able to confirm this observation[2] and have found that in human sera a dilution of about 1: 30 (that is, about 1: 200 in the reaction mixture) seems sufficient to eliminate the inhibition.

In Table 1 are given the results of similar dilution experiments with two normal adult night urine samples and it is seen that the inhibition is not with any certainty suppressed completely in even more dilute solution. Since the absolute activity in urine is much lower than in serum, it is impossible to get results with a dilution technique in anything but the most concentrated urine samples.

It will be necessary, therefore, to find a convenient way to separate enzyme and inhibitor in urine, and we have tried with some promise of success a 'Sephadex' filtration[4]. 4 ml. of a normal adult female night urine was poured on a 45 ml. 'Sephadex' *GR*-75 column and was washed through with acetate-buffer (pH 4·6). Hyaluronidase activity is found in the very early fractions, as shown in Fig. 1, where proteins of high molecular weight would be expected to occur. In similar filtration experiments on the same column with serum, tissue extract, and U.S.P. standard hyaluronidase, the activity was found in very nearly the same range of fractions, that is, in advance of hæmoglobin, for example. This was contrary to our expectations from prior knowledge of the molecular weight of hyaluronidase, and it seems to indicate an aggregation in this particular buffer milieu.

Table 1. Dilution Experiments with Two Night Urine Samples from Normal Adults

1	2		3		4	
	A	*B*	*A*	*B*	*A*	*B*
1 : 6	—	49·0	—	82	—	0·5
1 : 10	43·5	—	63	—	0·6	—
1 : 26	41·0	21·4	56	18	1·5	0·5
1 : 106	21·8	13·0	18	8	1·9	0·8
1 : 206	18·2	12·3	12	7	2·5	1·4
1 : 306	15·5	10·2	11	6	3·4	1·8
1 : 406	13·8	10·5	8	6	3·2	2·4

A, Female; *B*, male. 1, dilution of sample in reaction mixture; 2, hyaluronidase-like activity (*H*-factor activity) in arbitrary units (ref. 2); 3, *H*-factor activity converted to equivalent concentration of U.S.P. reference hyaluronidase standard in international units per litre of reaction mixture by means of a calibration curve (ref. 2); 4, *H*-factor activity expressed in international units per ml. of urine.

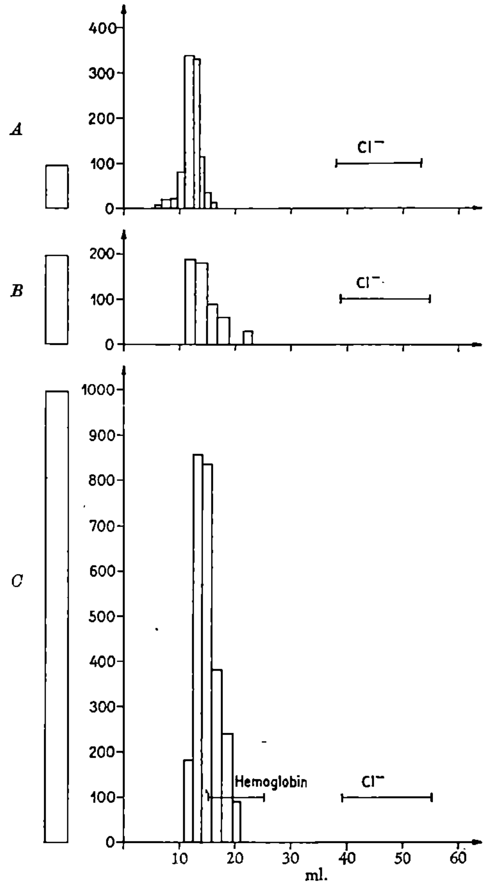


Fig. 1. 'Sephadex' filtration of 4 ml. of *A*, an undiluted night urine sample from a normal adult female; *B*, serum diluted 1 : 34 with acetic acid/acetate buffer pH 4·63, 0·07 M (from an adult normal female person); *C*, kidney cortex extract, diluted 1 : 34 with the same buffer (from a kidney removed *postmortem* from a male person with normal kidney function). Abscissa: Effluent from column in ml. Ordinate: Activity of *H*-factor expressed as international units U.S.P. reference hyaluronidase standard per litre of urine (undiluted) and of serum and kidney cortex extract (diluted 1 : 34). The columns to the left represent the activity recorded in a 4-ml. sample before the 'Sephadex' separation

The integrated activity of the active fractions corresponds to nearly 4 ml. of the most active fraction and is about 3·5 times the activity inferred from the untreated sample. Much of the inhibiting substances, therefore, must have gone into other fractions. In diluted serum the integrated activities of the fractions amounted to 1·4 times that of the directly measured activity and in kidney cortex extract to 1·2 times.

We feel that much of the work on hyaluronidase in urine under varying diuretic conditions will have to be done again in such circumstances that the enzyme is freed from inhibitors before the activity is measured. Until such material has been provided, further discussions of the interesting theories of Ginetzinsky would be unrewarding.

P. J. Knudsen
J. Koefoed

Centrallaboratoriet,
Kommunehospitalet,
and
Universitetets Fysisk-Kemiske Institut,
Copenhagen.

[1] Ginetzinsky, A. G., and Berlyne, G. M., *Nature*, **189**, 235 (1961).
[2] Knudsen, P. J., and Koefoed, J., *Scand. J. Clin. Lab. Invest.*, **13**, 4 (1961).
[3] Gibian, H., *Hoppe-Seyler's Z. physiol. Chem.*, **300**, 261 (1955).
[4] Porath, J., and Flodin, P., *Nature*, **183**, 1657 (1959).

Fructose and Lactic Acid Metabolism in the Perfused Liver of Premature Lambs

Recently, Andrews *et al.*[1] obtained evidence from perfusion studies of the inability of the liver of the fœtal sheep to utilize fructose. However, in lambs a removal of fructose from the perfusate occurred in preparations made from the fifth neonatal day onwards. It was of interest to ascertain whether premature birth could influence the time of onset of hepatic fructose metabolism.

The lambs were delivered from Welsh Mountain sheep by Cæsarean section under spinal anæsthesia. The umbilical cord was stretched to induce constriction of the vessels and ligated immediately. Respiratory movements began at once. After preliminary drainage of the nasopharynx, the animals were dried and stimulated generally by rough towelling: within 1–2 hr. all the lambs were capable of standing voluntarily. The animals were kept together in a pen at room temperature (15–20° C.) and maintained in good condition after birth on a 3–4 hr. feeding regimen of cow's milk administered via an 'Ingram' teat (Holborn Surgical Instrument Co., Ltd.).

Table 1 shows data on the conceptual age, age at the time of perfusion, milk consumption and body-weights. After the initial drop in body-weight, all the animals achieved or surpassed their weight at birth by the third or fourth day.

The lambs were anæsthetized with intravenous thiobarbitone and the perfusion of the liver was carried out by a technique similar to that reported in ref. 1, using fresh heparinized or stored defibrinated blood. In different preparations the perfusate volume varied from 320 to 380 ml. and the rate of flow was between 70 and 150 ml./min. The portal pressure was maintained at 11–15 cm. water. The liver weight ranged from 66 to 111 gm. Thermometers placed between the lobes of the liver registered 40–42° C. Fructose was determined by the method of Chinard *et al.*[2]; glucose by the method of Huggett and Nixon[3]; and lactic acid by the method in ref. 1.

Table 1. Data for the Premature Lambs at Cæsarian Section and at the Day of Liver Perfusion

Cæsarian section		Liver perfusion				
Conceptual age (days)	Body-weight (kgm.)	Conceptual age (days)	Age in excess of mean gestation period (days)	Neonatal age (days)	Body-weight (kgm.)	Ml. milk per gm. tissue deposited
139	2·76	147	−1	8	2·97	15·2
142	2·91	147	−1	5	3·16	18·0
142	2·08	148	0	6	2·56	9·2
141	3·19	148	0	7	4·20	7·5
142	2·77	151	3	9	4·11	9·2
142	2·51	155	7	13	4·40	7·8

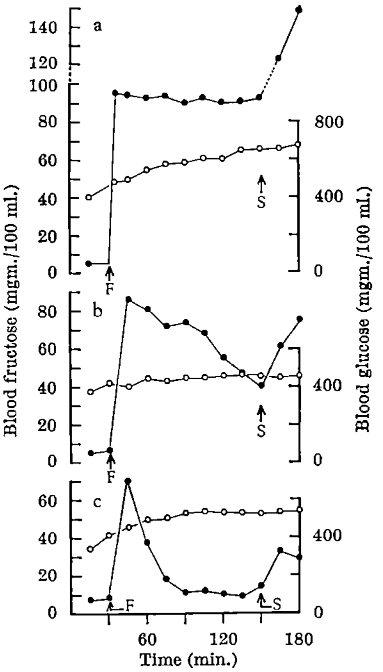


Fig. 1. Fructose and glucose concentration in the perfusion fluid of livers from prematurely delivered lambs. At *F*, 500 mgm. fructose and at *S*, 500 mgm. sorbitol added. Response : 6 days (*a*), 7 days (*b*) and 9 days (*c*) after Cæsarian section

Under the conditions of the experiments, there was no evidence of fructose utilization in premature lambs of 5 and 6 days neonatal age (conceptual age 147 and 148 days) over a period of 2 hr. (Fig. 1*a*), whereas it had been shown before that a rapid removal of fructose occurred by the fifth day after birth in lambs delivered normally at term (148 ± 2·6 (*S.D.*) days) (Young, G. B., personal communication). With premature lambs of 9 and 13 days neonatal age (conceptual age 151 and 155 days), however, a rapid uptake of fructose occurred with the fructose-levels returning to basal values after 60–75 min. (Fig. 1*c*). In premature lambs of 7 and 8 days neonatal age and 148 and 147 days conceptual age, respectively, a slow utilization of fructose was apparent (Fig. 1*b*). With all preparations the concentration of glucose in the perfusate increased; there was also prompt conversion of sorbitol to fructose, as was to be expected from previous results.

In five preparations the lactic acid was estimated and all showed an ability to take up lactic acid from the perfusate (Fig. 2). The rise in lactic acid concentration in the terminal perfusion period may have been due to deterioration of the preparation. However, the rise may be associated with the addition of sorbitol to the perfusate since the enzymic conversion of sorbitol to fructose involves the reduction of diphosphopyridine nucleotide (DPN) to reduced diphosphopyridine nucleotide (DPNH). It is, therefore, conceivable that the DPNH/DPN ratio may be affected in such a way as to bring about an increase in the rate of conversion of pyruvate to lactate such that lactic acid accumulates. The lactic acid-levels encountered were similar to those found before in perfused lamb livers and were considerably lower than those found in fœtal liver preparations (122–140 days conceptual age).

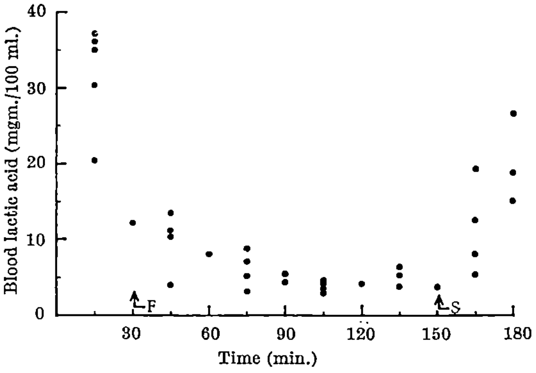


Fig. 2. Pooled values for the lactic acid concentration in the perfusate of livers obtained from prematurely delivered lambs. At *F*, 500-mgm. fructose and at *S*, 500 mgm. sorbitol were added to each of the five preparations

Hers[4] found that fructokinase was absent from the fœtal liver of the sheep, and therefore the onset of fructose uptake by the liver may well be due to the development of this enzyme system. The presence of a ketose reductase (sorbitol dehydrogenase) in the fœtal liver is in keeping with the conversion of sorbitol to fructose by these preparations. A number of other enzyme systems are known to develop at birth[5,6], but so far as is known the effect of premature delivery has not been investigated.

The results presented here suggest that conceptual age is an important factor in determining the onset of hepatic metabolism of fructose, but there may be some acceleration of the process by premature delivery.

This work was aided by the Medical Research Council and Wellcome Trust grants to Prof. A. St. G. Huggett.

W. H. H. Andrews
H. G. Britton
D. A. Nixon

Pharmacology and Physiology Departments,
St. Mary's Hospital Medical School,
Paddington, London.

---

[1] Andrews, W. H. H., Britton, H. G., Huggett, A. St. G., and Nixon, D. A., *J. Physiol.*, **153**, 199 (1960).

[2] Chinard, F. P., Danesino, V., Hartmann, W. L., Huggett, A. St. G., Paul, W., and Reynolds, S. R. M., *J. Physiol.*, **132**, 289 (1956).

[3] Huggett, A. St. G., and Nixon, D. A., *Lancet*, **273**, 368 (1957).

[4] Hers, H. G., *Biochem. J.*, **66**, 30P (1957).

[5] Jondorf, W. R., Maickel, R. P., and Brodie, B. B., *Biochem. Pharmacol.*, **1**, 352 (1959).

[6] Richter, D., *Brit. Med. Bull.*, **17**, 118 (1961).

Electrolyte Retention produced by the Antialdosterone *SC* 5233

Kagawa[1] reported that the spirolactone *SC* 5233 (3-(3-oxo-17β-hydroxy-4-androsten-17α-yl)propionic acid lactone) possessed weak cortexone-like activity in addition to its well-known antagonistic action towards cortexone and aldosterone. This weak cortexone-like (agonist) activity was estimated at 0·1–0·2 per cent that of cortexone. Reported here are some effects of *SC* 5233 on electrolyte excretion in normal and adrenalectomized rats which cannot be explained purely on a weak agonist activity.

In all experiments male Wistar rats were used the weights of which were approximately 120 gm. Bilateral adrenalectomy or a sham operation was performed under ether anæsthesia and the adrenalectomized animals were maintained on 1 per cent sodium chloride in the drinking water. On the day of the experiment the animals were uniformly hydrated with normal saline (25 ml./kgm.) and the drug injected beneath the skin; they were then placed in single cages suitable for the collection of urine. The response of the animals to *SC* 5233 (5 mgm./rat) was measured in experiment 1 on the first, fourth and eighth days after the operation. As the response of the animals was greatest on the eighth day, the animals in experiment 2 were dosed as in experiment 1, but the response was only measured on the eighth day after the operation. Similarly, in experiment 3 the animals were dosed with cortexone (10·0 μgm./rat) on the first, fourth and eighth days after operation, but urine was collected only on the eighth day.

Table 1. Comparison of the Effects of *SC* 5233 (5 mgm./rat) injected beneath the Skin on Electrolyte Excretion in Sham-Operated and Adrenalectomized Rats on the First, Fourth and Eighth Days after Operation

	Day	Sham-operated animals		Adrenalectomized animals	
		Controls	*SC* 5233 (5 mgm./rat)	Controls	*SC* 5233 (5 mgm./rat)
Percentage fluid load excretion	1	72·4 (4) ±15·2	87·9 (4) ±13·3	103·9 (4) ±15·5	80·1 (4) ±13·4
	4	93·0 +16·41	77·3 ±14·4	98·7 ±15·0	64·8 ±15·0
	8	101·9 ± 5·5	114·4 ±23·3	129·0 ±27·1	26·0 ±13·9
M.equiv. sodium/ 5 hr.	1	0·38 ± 0·08	0·42 ± 0·02	0·73 ± 0·14	0·51 ± 0·06
	4	1·12 ± 0·17	0·75 ± 0·09	0·74 ± 0·16	0·40 ± 0·02
	8	0·84 ± 0·17	0·79 ± 0·05	1·30 ± 0·19	0·36 ± 0·11
M.equiv. potassium/ 5 hr.	1	0·10 ± 0·03	0·11 ± 0·02	0·06 ± 0·01	0·05 ± 0·01
	4	0·30 ± 0·05	0·17 ± 0·02	0·10 ± 0·02	0·06 ± 0·02
	8	0·22 ± 0·04	0·12 ± 0·02	0·12 ± 0·01	0·04 ± 0·002
M.equiv. chlorine/ 5 hr.	1	0·39 ± 0·07	0·45 ± 0·03	0·58 ± 0·04	0·51 ± 0·07
	4	0·70 ± 0·04	0·66 ± 0·07	0·93 ± 0·11	0·56 ± 0·06
	8	0·88 ± 0·16	0·81 ± 0·06	1·29 ± 0·18	0·35 ± 0·10
*p*H	1	7·6 ± 0·07	8·0 ± 0·16	7·4 ± 0·09	7·6 ± 0·07
	4	8·3 ± 0·09	7·9 ± 0·09	7·4 ± 0·05	7·8 ± 0·07
	8	7·8 ± 0·03	7·9 ± 0·02	7·6 ± 0·07	7·9 ± 0·18

The results of experiment 1 are given in Table 1 and are illustrated in Fig. 1. In the graphical illustration the mean results for the three days of the animals treated with *SC* 5233 are expressed as a percentage of those obtained in the control animals. Injection beneath the skin of *SC* 5233 (5 mgm./rat) produced no consistent alteration in the fluid excretion of the sham-operated rats, nor was there any marked alteration in the excretion of sodium and chloride or *p*H of the urine. However, retention of potassium was observed on the fourth and eighth day in these animals. In the adrenalectomized animals *SC* 5233 produced a progressive decrease in the fluid, sodium, potassium, and chloride excretion and a rise in urinary *p*H. There was no alteration of the endogenous creatinine excretion induced by *SC* 5233 in either the sham-operated or adrenalectomized animals. The results of experiment 2 (Table 2*a* and *b*) were similar to those obtained in experiment 1. Analysis of the plasma did not show any marked alteration in the

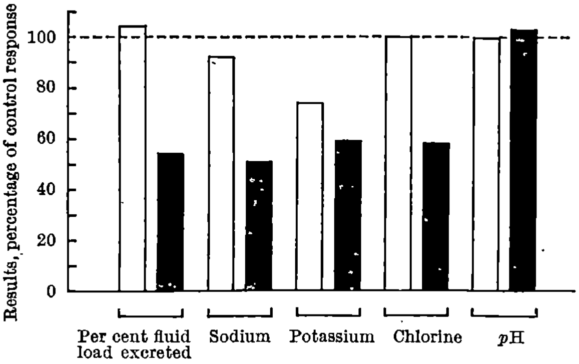


Fig. 1. Effect of the subcutaneous injection of *SC* 5233, 5 mgm./rat, on fluid, sodium, potassium and chloride excretion and urinary *p*H. Results are averages for the three experimental days and expressed as a percentage of the results obtained in the control animals, that is, 100 = control value. □, Sham-operated rats; ▨, adrenalectomized rats

sodium or potassium concentrations, although there was some suggestion of an increase in the plasma sodium concentration in both sham-operated and adrenalectomized animals. In experiment 3 (Table 3) injection beneath the skin of cortexone 10·0 μgm. per rat decreased sodium and increased potassium excretion; the adrenalectomized rats retained sodium and extruded potassium more than the sham-operated animals.

The experimental results show that *SC* 5233 does not possess a weak cortexone-like activity on repeated administration. Subcutaneous doses of cortexone and *SC* 5233, which produce similar degrees of sodium retention, produce opposite effects on potassium excretion. Cortexone will cause a rise in plasma potassium concentration, which has recently been shown to be an important factor in the mechanism of potassium extrusion produced by these substances[2]. The continued administration of *SC* 5233 did not produce an alteration in plasma potassium concentration. The fact that *SC* 5233 does not show a weak cortexone-like activity on potassium excretion, but does show this activity with regard to sodium excretion, would suggest that *SC* 5233 ought theoretically to be less complete in its anti-mineralocorticoid activity on potassium than on sodium excretion. There is clinical evidence that *SC* 5233 produces changes in sodium with little or no change in potassium excretion, both in the normal individual and in the Addisonian patient maintained on cortexone[3].

Table 2, *a* and *b*. COMPARISON OF THE EFFECTS OF *SC* 5233 (5 MGM./RAT) INJECTED BENEATH THE SKIN ON ELECTROLYTE EXCRETION AND PLASMA ELECTROLYTE-LEVELS IN SHAM-OPERATED AND ADRENALECTOMIZED RATS (EIGHTH DAY AFTER OPERATION)

	Mock-operated animals		Adrenalectomized animals	
	Control	*SC* 5233 (5 mgm./rat)	Control	*SC* 5233 (5 mgm./rat)
(*a*) Percentage fluid load excreted	110·6 (6) ±16·6	67·6 (6) ±12·2	71·7 (9) ±11·5 ..	51·8 (8) ± 6·8
Sodium ion excretion (m.equiv./5hr.)	0·58 ± 0·03	0·62 ± 0·05	0·54 ± 0·02	0·45 ± 0·44
Potassium ion excretion (m.equiv./hr.)	0·22 ± 0·02	0·21 ± 0·03	0·05 ± 0·01	0·03 ±0·003
(*b*) Plasma (sodium ion)(m.equiv./l.)	164·2 ± 2·1	169·3 ±3·2	159·5 (8) ± 3·5	164·1 (8) ± 3·5
Plasma (potassium ion) (m.equiv/l.)	6·08 ± 0·36	6·92 ± 0·49	8·11 ± 0·35	7·95 ± 0·42

Table 3. COMPARISON OF THE EFFECTS OF CORTEXONE (10·0 μGM./RAT) INJECTED BENEATH THE SKIN ON EXCRETION OF ELECTROLYTE IN SHAM-OPERATED AND ADRENALECTOMIZED RATS (EIGHTH DAY AFTER OPERATION)

	Mock-operated animals		Adrenalectomized animals	
	Controls	Cortexone (10 μgm./rat)	Controls	Cortexone (10 μgm./rat)
Percentage fluid load excreted	66·6 (6) ±11·5	41·5 (6) ±3·6	39·9 (5) ±4·8	45·6 (4) ±14·9
Sodium ion excretion (m.equiv. sodium/5 hr.)	0·62 ± 0·08	0·56 ±0·07	0·54 ±0·03	0·44 – 0·10
Potassium ion excretion (m.equiv. potassium/5 hr.)	0·25 ± 0·05	0·30 ±0·04	0·14 ±0·02	0·20 ± 0·04

Recently Mills *et al.*[4] have shown in the normal individual that 'Aldactone' will produce some measure of antagonism to the sodium-retaining properties of cortisol, but none towards its kaliuretic effect.

In these experiments the potassium retention, which is not attributable to any alteration in plasma potassium concentration, is most probably the result of an increase in the proximal tubular re-absorption of sodium. This would lead to a decrease in the amount of sodium available for the distal tubular exchange of sodium for potassium or hydrogen-ion. The fact that *SC* 5233 is effective in antagonizing the retention of sodium produced by cortexone and aldosterone would suggest tentatively that sodium-retaining steroids may exert part of their action on sodium excretion by an effect on the proximal tubule.

J. B. FARMER
B. PETCH
Pharmacological Research Department,
Pfizer Limited,
Sandwich, Kent.

---

[1] Kagawa, C. M., *Clinical Use of Aldosterone Antagonists*, edit. by Bartter, F. C., 33 (C. C. Thomas, Springfield, Illinois, 1960).

[2] Davey, M. J., and Lockett, M. F., *J. Physiol.*, **152**, 206 (1960).

[3] Liddle, G. W., in *Clinical Use of Aldosterone Antagonists*, edit. by Bartter, F. C., 33 (C. C. Thomas, Springfield, Illinois, 1960).

[4] Mills, J. N., Thomas, S., and Williamson, K. S., *J. Physiol.*, **155**, 18, P (1961).

Electron Transport System and Phosphoproteins of Mitochondria

IN previous papers[1] it was shown that phosphoprotein turnover and mitochondrial swelling went hand in hand, and that inhibition of one was associated with inhibition of the other. It was also shown that mitochondrial contraction and protein phosphorylation were closely associated.

In the present communication we give results which suggest strongly that phosphoproteins may accept phosphorus directly from some component of the oxidative phosphorylation system, and we show that the phosphoprotein fraction studied is identical with that studied by other workers[2,3] in ascites and brain.

Isolated mitochondria incubated in 0·125 *M* potassium chloride or 0·25 *M* sucrose containing inorganic phosphorus labelled with phosphorus-32 take up the tracer rapidly and incorporate it into adenosine triphosphate (ATP) and phosphoprotein. No other insoluble component is labelled. Table 1 shows the conditions for the experiment. Inorganic phosphorus was present at a concentration of 10^{-4} *M*, and swelling of the mitochondria was prevented by the inclusion of ethylenediamine tetraacetic acid (EDTA), both in the reaction medium and in the sucrose used for homogenizing and washing the particles. It can

Table 1. EFFECT OF DNP AND ANTIMYCIN *A* ON PHOSPHOPROTEIN TURNOVER

Exp. No.	Compound	Specific activity c.p.m./μmole phosphorus in presence of Nil	DNP (10^{-4} *M*)	Antimycin *A* (2 μgm./ml.)
1	Alkali-labile phosphorus	175,000	41,500	—
	ATP	1,100,000	1,080,000	—
2	Phosphorus-serine	1,860	430	—
	ATP	22,000,000	21,050,000	—
3	Phosphorus-serine	1,624	—	142
	ATP	21,800,000	—	18,000,000

Mitochondria equivalent to 600 mgm. liver were suspended in 2·0 ml. 0·125 *M* potassium chloride containing 0·02 *M* trihydroxymethylaminomethane hydrochloride buffer, *p*H 7·2. Phosphorus-32 was added as a solution giving 10^{-4} *M* phosphorus final concentration, with 2×10^6 c.p.m. in experiment 1 and 56×10^6 c.p.m. in experiments 2 and 3. The phosphorus–serine determinations were done as described by Heald (ref. 3), carrier phosphorus–serine being added before hydrolysis. The alkali-labile phosphorus was estimated as described by Judah (ref. 1). Temperature, 20°. Time, 30 min.

be seen from the results that 2:4-dinitrophenol (DNP) and 'Antimycin *A*' powerfully inhibit uptake of phosphorus-32 into phosphoprotein. This was measured both by determination of alkali-labile phosphorus and by determination of the radioactivity of phosphorylserine after partial acid hydrolysis and chromatography, as descibed by Heald[3], and by Kennedy and Smith[2]. There is no doubt as to the identity of the fraction inhibited. Other inhibitors such as cyanide, amytal, and azide give similar effects. It is to be noted that none of them affects the level and turnover of intramitochondrial ATP.

In further experiments, it was found that reduction of the electron transport chain by the addition of substrate and by anærobiosis also reduces phosphoprotein turnover (Table 2). This is in agreement with Wadkins and Lehninger's findings[4] that reduction of the carriers inhibits the ^{32}P-ATP exchange reaction. Though Table 2 shows that intramitochondrial ATP is unaffected by reduction of the carriers, the turnover of added ATP is inhibited in our systems, in confirmation of Wadkins and Lehninger[4].

As previously reported, several antihistamine drugs also inhibit phosphoprotein turnover, but have no effect on oxidative phosphorylation. In particular, the drug 'Benadryl' (diphenhydramine hydrochloride) inhibits phosphoprotein turnover by 60–80 per cent but has little effect on the ^{32}P-ATP exchange reaction or on oxidative phosphorylation. This would seem to rule out the possibility that phosphoproteins participate directly in the oxidative phosphorylation reaction. The participation of such compounds, though unlikely, is not ruled out by their low specific activity as compared to that of ATP. Rabinowitz and Lipmann[5] have shown that phosphorylation of ADP by labelled phosphoprotein gives rise to ATP of specific activity many times higher than that of the parent phosphoprotein. This indicates a considerable lack of homogeneity of the phosphorus groups in the latter.

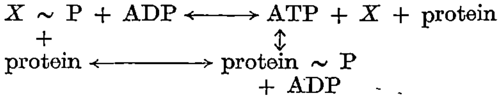
Table 2. EFFECT OF REDUCTION ON PHOSPHOPROTEIN RADIOACTIVITY

System	C.p.m. in phosphoprotein	Specific activity c.p.m./μmole phosphorus of mitochondrial ATP
Oxidized	2,300	$8{\cdot}1 \times 10^6$
Reduced	1,400	$8{\cdot}6 \times 10^6$

The system was as described in Table 1. Reduction of the mitochondrial system was effected by addition of potassium cyanide, 2×10^{-3} *M*, and glutamate, 5×10^{-3} *M*.

It is curious that turnover of mitochondrial ATP is unaffected by the inhibitors. The existence of ^{32}P-ATP exchanging systems insensitive to mitochondrial DNP, however, has been described by Chiga and Plaut[6].

A possible mechanism for the reaction of phosphoproteins is shown in the following reaction, in which they form part of a cyclical system:



$$\begin{array}{lcl} X \sim \mathrm{P} + \mathrm{ADP} & \longleftrightarrow & \mathrm{ATP} + X + \text{protein} \\ + & & \updownarrow \\ \text{protein} & \longleftrightarrow & \text{protein} \sim \mathrm{P} \\ & & + \mathrm{ADP} \end{array}$$

where $X \sim \mathrm{P}$ is an intermediate of the system.

This work was supported by grants from the Burroughs–Wellcome Fund, the Damon Runyon Fund and U.S. Public Health Service grant No. *C*–4534.

K. AHMED
J. D. JUDAH

The Wistar Institute,
36th Street at Spruce,
Philadelphia 4, Pa.

C. H. GALLAGHER*

The McMaster Institute,
Commonwealth Scientific and
Industrial Research Organization,
New South Wales.

[1] Judah, J. D., *Exp. Cell. Res.*, **19**, 404 (1960); *Nature*, **187**, 506 (1960); *Ciba Symp. Drugs and Enzymes* (in the press); *Biochim. Biophys. Acta* (in the press).
[2] Kennedy, E. P., and Smith, S. W., *J. Biol. Chem.*, **207**, 153 (1954).
[3] Heald, P. J., *Biochem. J.*, **68**, 580 (1958).
[4] Wadkins, C. L., and Lehninger, A. L., *J. Biol. Chem.*, **234**, 681 (1959).
[5] Rabinowitz, M., and Lipmann, F., *J. Biol. Chem.*, **235**, 1043 (1960).
[6] Chiga, M., and Plaut, G. W. E., *J. Biol. Chem.*, **234**, 3059 (1959).

Odour Receptors

WHILE there is evidence[1] that the odour of any compound is related to low-frequency molecular vibrations which give rise to infra-red absorptions in the 15–20μ region of the spectrum, and to Raman shifts below 600 cm.$^{-1}$, there is at present no information concerning the nature of the receptors of these energies. Yet it is quite clear that the olfactory epithelium must contain molecules capable of interacting with the odorant in the nasal space. Moreover, unless the interaction between odorants and receptors occurs within the epithelial cells, which is most unlikely, due to the rapidity of reception of odour and the tremendous variability in solubility of compounds with similar smells, the receptors must be located on the interface between the epithelial cells and the air cavity. Working on this basis, we have attempted to investigate the nature of compounds specifically located on this interface.

It has been known for many years[2] that the soft vascular mucous membranes of the olfactory areas are yellow, whereas non-olfactory areas are not. These yellow areas are known to contain carotenoids[3]. Considering the embryological similarity of the olfactory area and the retina, together with the known function of carotenoids and haplocarotenoids in vision[4], we have carried out experiments to test the hypothesis that the olfactory area contains protein-bound carotenoids which are the molecular receptors of odours.

The heads of freshly killed cows were opened to expose the olfactory regions. These were wiped with petroleum ether to remove excess mucus and melanin, and were then carefully scraped with a scalpel to collect the uppermost tissues. The material collected was pooled into a 2 per cent digitonin solution which was extracted with ether to remove free carotenoids. The absorption spectrum of both the digitonin solution and the ether extract was determined. The extract gave absorption peaks at a variety of wave-lengths in the visible and the ultra-violet regions. From an examination of the spectrum the presence of free α- and β-carotene and of vitamin A was deduced.

The digitonin solution was treated with acetone, which liberates carotenoids from combination with proteins. The mixture was shaken with ether and the ether fraction separated, dried *in vacuo*, and the resulting solution examined spectroscopically. The solution possessed absorption maxima at 445 and 475 mμ. The untreated digitonin solution possessed a weak absorption maximum at about 420 mμ and this was no longer detectable in the spectrum of the acetone-treated residue.

These results indicate that the olfactory epithelium contains free carotenoids and free vitamin A, and also protein-bound carotenoids the chromophore of which is removed by acetone treatment. By analogy with the protein-bound haplocarotenoids of the retina, the rhodopsins, it seems reasonable to assume that protein-bound carotenoids of the olfactory epithelium are the receptors of energy from olfactant molecules entering the nasal cavity. The mechanism of interaction is, of course, obscure, but the known ability of carotenoids to exist in numerous *cis–trans* forms[5], together with the knowledge that the only action of light on rhodopsins is to induce a geometrical isomerization[6], suggest that the exchange of energy from an olfactant molecule to an olfactory protein-bound carotenoid involves a similar molecular change.

This hypothesis suggests that, as animals are not able to synthesize carotenoids, an animal raised on a carotenoid-free diet will have impaired olfactory abilities.

Supporting evidence comes from clinical experience. One of us (R. B. D.) has treated a series of 56 patients with uncomplicated anosmia by intramuscular injection of large doses of vitamin A. Of these patients, 50 recovered olfaction in part or completely. This therapeutic effect may be ascribed to some interaction of carotenes and haplocarotenoids in the olfactory areas.

These experiments and clinical results will be published in more detail elsewhere.

We are grateful to the Wellington Meat Export Co., Ltd., for providing the animal materials and for helpful assistance.

M. H. Briggs

Department of Chemistry,
Victoria University of Wellington,
Wellington, New Zealand.

R. B. Duncan

403 D.I.C. Building,
Wellington, New Zealand.

[1] Wright, R. H., *Nature*, **173**, 831 (1954); *J. App. Chem.*, **4**, 611 (1954). Wright, R. H., and Serenius, E. S. F., *ibid.*, **4**, 615 (1954). Wright, R. H., Reid, C., and Evans, H. G. V., *Chem. and Indust.*, 973 (1956).

[2] Parker, G. H., *Smell, Taste, and Allied Senses in Vertebrates* (Lippincott, Philadelphia, 1922).

[3] Milas, N. A., Postman, W. M., and Heggie, R., *J. Amer. Chem. Soc.*, **61**, 1929 (1939).

[4] Wald, G., in *Comparative Biochemistry*, **1**, 311 (Academic Press, New York, 1960).

[5] Zechmeister, L., *Chem. Revs.*, **34**, 267 (1944).

[6] Hubbard, R., Brown, P. K., and Kropf, A., *Nature*, **183**, 442 (1959).

PHARMACOLOGY

Histidine Decarboxylase and Growth

Kahlson[1,2] has presented evidence of a relationship between activity of histidine decarboxylase and growth or wound-healing in rat tissues. In particular he has shown that the developing liver of the fœtal rat has much higher histidine decarboxylase activity than the normal liver of adults. Telford and West[3] have shown that the enzyme of the liver of the fœtal rat has an optimum *p*H of 6·5 compared with 8·0 for that of the adult. They have also found that the activity of histidine decarboxylase of adult rat liver is very low unless benzene is added to the incubation medium. The enzyme of the liver of the fœtal rat, on the other hand, is not activated by benzene. Low histidine decarboxylase activity in fœtal rabbits and guinea pigs has recently been reported (ref. 4).

We have carried out some experiments to determine the histidine decarboxylase activities of liver and kidneys of fœtal, young, and adult guinea pigs. Saline extracts (4·0 ml./gm. tissue) were prepared from the tissues in the usual way, and incubations were carried out as described in ref. 5. The reaction was stopped after 200 min., and the histamine produced was assayed on the atropinized guinea pig ileum.

The results, which are given in Table 1, show that the histidine decarboxylase activities are low in the liver and kidney of fœtal guinea pigs, and that they rise just before or just after birth to the adult-levels.

When incubations of fœtal guinea pig liver extracts were carried out in the presence of benzene, the histamine production was increased two- to four-fold, as occurs with adult tissues. The histidine decarboxylase activity of fœtal and adult guinea pig liver was measured over the range *p*H 8–10 in steps of 0·5 *p*H unit using *M*/20 borate buffer ; the maximum rate of histamine formation with both fœtal and adult liver occurred at *p*H 9.

All these results are in complete contrast to those observed with the rat.

We have also prepared extracts of regenerating rays of starfish (*Asterias rubens*), extracts of normal rays being used as controls. In no case was histidine decarboxylase activity detected.

Weiss and Matoltsy[6] reported that wounds made in the skin of chick embryos before the tenth day of incubation failed to heal, while those made in embryos after the twelth day of incubation healed normally. We have examined extracts of chick embryos for histidine decarboxylase activity. In each case whole embryos were used, but the disproportionately large eyes were omitted. Neither at the eighth nor at the seventeenth day of incubation was any histidine decarboxylase activity detectable over the *p*H range 6·4–8·2. No histidine decarboxylase was detected in the livers or kidneys of chicks one day after hatching.

Table 1. HISTIDINE DECARBOXYLASE ACTIVITIES IN LIVER AND KIDNEY OF FŒTAL, YOUNG AND ADULT GUINEA PIGS

	Animal weights (gm.)	Histamine (μgm./ml.) formed in incubation medium	
		Liver	Kidney
Fœtal guinea pigs	9·5–23	0·6–0·08	—
,, ,, ,,	48–68	0·1–0·2	0·1–0·4
Young ,, ,,	108–147	0·4–0·5	2·2–3·1
Adult ,, ,,	400–900	0·51 ± 0·21	2·6 ± 0·7

The results do not indicate any general relationship between histidine decarboxylase activity and growth.

We are grateful to Dr. J. D. B. MacDougall and Dr. A. J. Matty for the supply of eggs and starfish, respectively, and we acknowledge the award of research fellowships from Smith, Kline and French Laboratories (D. M.) and the Medical Research Council (J. D. R.).

D. Mackay *
Joan D. Reid
D. M. Shepherd

Department of Pharmacology and Therapeutics,
Queen's College,
University of St. Andrews,
Dundee.

---

* Present address: Department of Pharmacology, Leeds University School of Medicine, Leeds, 2.

[1] Kahlson, G., *Lancet*, i, 67 (1960).

[2] Kahlson, G., Nilsson, K., Rosengren, E., and Zederfeldt, B., *Lancet*, ii, 230 (1960).

[3] Telford, J. M., and West, G. B., *J. Physiol.*, **155**, 16P (1961).

[4] Kameswaran, L., Telford, J. M., and West, G. B., *J. Physiol.*, **157**, 23P (1961).

[5] Mackay, D., Riley, J. F., and Shepherd, D. M., *J. Pharm. Pharmacol.*, **13**, 257 (1961).

[6] Weiss, P., and Matoltsy, A. G., *Nature*, **180**, 854 (1957).

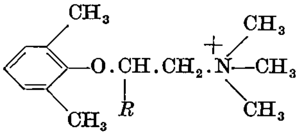
Adrenergic Neurone-blocking Agents related to Choline 2,6-Xylyl Ether Bromide (*TM* 10), Bretylium and Guanethidine

Several compounds have been shown to block transmission at peripheral adrenergic nerve terminals without antagonizing the effects of adrenaline and noradrenaline. They include the quaternary ammonium salts choline 2,6-xylyl-ether bromide (*TM* 10) (I)[1,2], bretylium (III)[3,4], and related substances[3,5–7]. The two compounds named have been shown to act by preventing the release of the adrenergic transmitters at the nerve endings[4,5]. Guanethidine (IV) also blocks peripheral adrenergic mechanisms. Its action, like that of reserpine but in contrast to that of bretylium[8], is associated with marked depletion of peripheral stores of catechol amines[9,10]. This depletion is accompanied by depression of the sympathomimetic responses to tyramine and amphetamine[11], which apparently act by releasing peripheral catechol amines[12]. Responses to tyramine and amphetamine are also affected by bretylium; but only when the drug has been administered for several weeks does the degree of depression equal that seen 24 hr. after guanethidine[13,14]. Whether the depletion of catechol amines is responsible for the adrenergic nerve blockade caused by either drug is uncertain. Depression of the responses of the nictitating membranes of cats to sympathetic nerve stimulation follows rapidly after intravenous injection of guanethidine, but depression of equivalent responses to tyramine is slight at this time and becomes prominent only after several hours[14]. Similarly, the depletion of catechol amines by guanethidine[10] is relatively slow in onset.

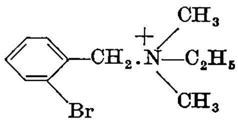
It is therefore possible that guanethidine may have a fundamentally similar action to that of the quaternary blocking agents. Compounds with either a guanidine or quaternary ammonium group are highly basic and would exist almost exclusively in the cationic form at physiological *p*H. These groups could have similar influences in governing pharmacological action.

To enable this possibility to be examined, guanidine derivatives such as V–VIII (for which patents are pending), which bear an obvious relationship to the *TM* 10 and bretylium series of drugs, were synthesized. The screening methods previously described[3,6] then showed that these compounds, too, are powerful adrenergic neurone-blocking agents.

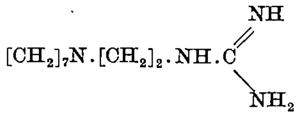
Among the most potent are *BW* 467*C*60 (V; R=H; $R^1=R^2=CH_3$) and *BW*392*C*60 (V; R=Cl; $R^1=R^2=CH_3$). They are about twice as active as guanethidine when tested by their relaxant action on the nictitating membranes of cats and are as effective by the oral route as they are parenterally. Their blocking action on peripheral adrenergic nerves is highly specific but is preceded by a brief sympathomimetic effect when high doses are given. Like bretylium[4] and guanethidine[11], they increase the pressor and nictitating membrane contracting effects of adrenaline and noradrenaline. *BW* 392*C*60 resembles bretylium rather than guanethidine in that it does not readily inhibit the responses of the nictitating membrane and blood pressure of cats to tyramine given 24 hr. later, and does not markedly lower the pressor amine contents of the spleens of cats even when 10 mgm./kgm. doses are injected daily for 6 months. In contrast, *BW* 467*C*60 resembles guanethidine by greatly depressing the responses to tyramine and amphetamine within 24 hr. after administration, and by greatly lowering the pressor amine content of the hearts and spleens of cats in experiments where the drug was injected daily for 6 months (shorter periods have not yet been investigated). However, under the conditions we have used so far, the inhibitory action of *BW* 467*C*60 on responses to tyramine was less than that shown by guanethidine in doses that produced similar degrees of adrenergic nerve blockade. The pharmacological properties of these two compounds



(I; R=H) *TM* 10
(II; R=CH_3)

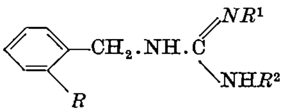


(III) Bretylium

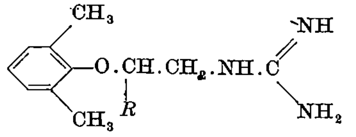


$$[CH_2]_7N.[CH_2]_2.NH.C\begin{array}{l}/\!\!/NH\\ \backslash NH_2\end{array}$$

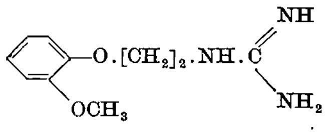
(IV) Guanethidine



(V; R=H, CH_3, Cl, Br, NO_2; R^1 and/or R^2=H, CH_3 or C_2H_5)



(VI); R=H)
(VII; R=CH_3)



(VIII)

are still being studied and will be published in detail elsewhere; but it is of general interest that they have already shown properties that appear to relate the effects of bretylium to those of guanethidine.

A particularly close analogy between quaternary ammonium blocking agents and related guanidine derivatives was found in the phenoxyethyl compounds. *TM* 10 (I) produces a number of powerful muscarine-like actions[1] that are shown to only a small degree by the β-methyl substituted derivatives (II)[7]. Similarly, in the guanidine series, adrenergic neurone-blocking and muscarine-like properties were shown by compound VI, but only the former effect was obvious with the substituted derivative VII. *N*-methyl substitution in these compounds has been accompanied by loss of adrenergic neurone-blocking activity.

A. L. A. Boura G. K. Ruffell
F. C. Copp M. F. Sim
A. F. Green E. Walton
H. F. Hodson
The Wellcome Research Laboratories,
Beckenham, Kent.
E. M. Grivsky
The Wellcome Research Laboratories,
Tuckahoe, New York, U.S.A.

[1] Hey, P., and Willey, G. L., *Brit. J. Pharmacol.*, **9**, 471 (1954).
[2] Bain, W. A., *Adrenergic Mechanisms*, 131, edit. Vane, J. R., Wolstenholme, G. E. W., and O'Connor, M. (Churchill, London, 1960).
[3] Boura, A. L. A., Copp, F. C., and Green, A. F., *Nature*, **184**, B.A. 70 (1959).
[4] Boura, A. L. A., and Green, A. F., *Brit. J. Pharmacol.*, **14**, 536 (1959)
[5] Exley, K. A., *Brit. J. Pharmacol.*, **12**, 297 (1957).
[6] Boura, A. L. A., Coker, G. G., Copp, F. C., Duncombe, W. G., Elphick, A. R., Green, A. F., and McCoubrey, A., *Nature*, **185**, 925 (1960).
[7] McLean, R. A., Geus, R. J., Mohrbacher, R. J., Mattis, P. A., and Ullyot, G. E., *J. Pharmacol.*, **129**, 11 (1960).
[8] McCoubrey, A., cited by Green, A. F. *Adrenergic Mechanisms*, 148, edit. Vane, J. R., Wolstenholme, G. E. W., and O'Connor, M. (Churchill, London, 1960).
[9] Sheppard, H., and Zimmerman, J., *Pharmacologist*, **1**, 69 (1959).
[10] Cass, R., Kuntzman, R., and Brodie, B. B., *Proc. Soc. Exp. Biol. Med.*, **103**, 871 (1960).
[11] Maxwell, R. A., Plummer, A. J., Povalski, H., and Schneider, F., *J. Pharmacol.*, **129**, 24 (1960).
[12] Burn, J. H., in *Adrenergic Mechanisms*, 326, edit. Vane, J. R., Wolstenholme, G. E. W., and O'Connor, M. (Churchill, London, 1960).
[13] Green, A. F., in *Adrenergic Mechanisms*, 148, edit. Vane, J. R., Wolstenholme, G. E. W., and O'Connor, M. (Churchill, London, 1960).
[14] Boura, A. L. A., and Green, A. F., *Brit. J. Pharmacol.* (in the press).

HÆMATOLOGY

Cytochemistry of *L*1210 Cells

A DIFFERENCE in the respiratory behaviour of sensitive and 6-mercaptopurine-resistant *L*1210 mouse leukæmia cells has been noted by me[1]. Sensitive cells incubated *in vitro* in a Warburg apparatus had a net average gas output of 1·3 (range 1·1–1·8) μμl./cell/hr., whereas the cells resistant to mercaptopurine had a net average gas output of 0·1 (range 0·5 to minus 0·15) μμl./cell/hr.

A possible interpretation of these results is that the sensitive cells have a much more active glycolytic metabolism than the cells resistant to mercaptopurine. Active glycolysis would cause the production of lactic acid, which, reacting with the bicarbonate in the medium, would liberate carbon dioxide and give a high net gas output. In order to obtain more information on this aspect of the metabolism of the two cell strains, cytochemical tests of their succinic and lactic dehydrogenase activities were carried out. Amethopterin-resistant cells were examined along with the mercaptopurine-resistant cells and those of a strain sensitive to both agents. Cytological and cytochemical investigations with some of the commonly used techniques were also carried out.

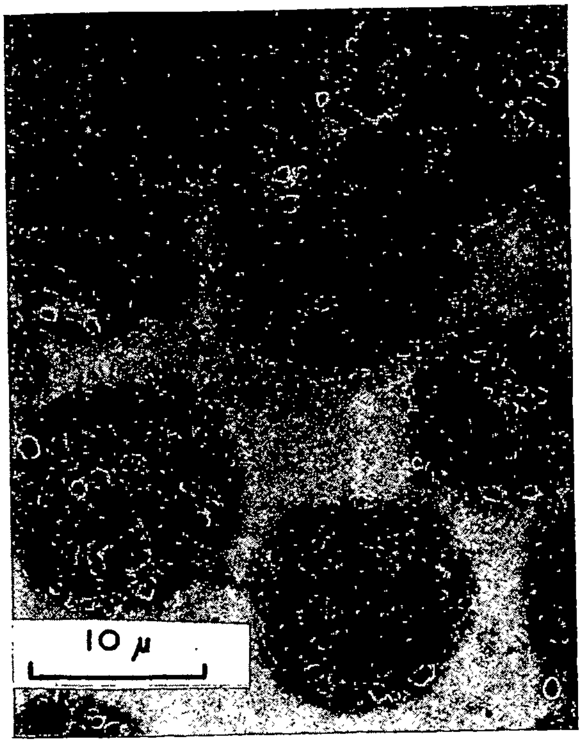


Fig. 1. Sensitive *L*1210 cells showing four-plus lactic dehydrogenase activity

Smears were made from the ascitic fluids of mice which had been inoculated intraperitoneally with one or other of the three cell strains. These were stained by May–Grünwald–Giemsa, periodic acid–Schiff, sudan black, alkaline phosphatase, peroxidase and Feulgen techniques using standard methods. Smears were stained for activity of succinic dehydrogenase using the technique in ref. 2 and for lactic dehydrogenase activity by a suitable modification of the same method.

Five separate specimens of the sensitive and amethopterin-resistant *L*1210 cells and four of the mercaptopurine-resistant *L*1210 cells were examined at intervals of at least a month. The results were consistent in each strain of cell. May–Grünwald–Giemsa stained smears showed no morphological differences between the three strains; each was composed of large pleomorphic leukæmic blast cells having mitotic indices ranging from three to twelve a thousand.

All three strains were negative with the periodic acid–Schiff, alkaline phosphatase and peroxidase stains. Vacuoles in the cytoplasm of all three stained a light grey by the sudan black technique; they did not have the intense positivity found in granulocytes. The Feulgen technique showed up the fine nuclear chromatin pattern and the nucleoli were prominent. Again no differences could be detected between the three cell strains.

The results of the dehydrogenase-staining techniques are given in Table 1. Each smear was assessed semi-quantitatively from one plus to four plus. In the one-plus smears an occasional cell contained a

Table 1. Dehydrogenase Activities of L1210 Cells

	Sensitive	Mercaptopurine-resistant	Amethopterin-resistant
Succinic dehydrogenase activity	+ + or +	+ or 0	+ or 0
Lactic dehydrogenase activity	+ + + + or + + +	+ or 0	+ or 0

few positively stained granules, whereas in the four-plus smears the majority of the cells showed many positive granules (Fig. 1). The sensitive cells were found to have a much greater lactic dehydrogenase activity than the resistant cells, a fact which lends support to the idea that they have a more active glycolytic metabolism. The former also had a greater succinic dehydrogenase activity, but the difference was less marked. The results suggest that sensitive cells may be generally more active in their respiration than the resistant cells.

The fact that sensitive and resistant L1210 cells have differences in respiratory metabolism which can be demonstrated cytochemically suggests that a similar examination of human leukæmic cells would be worth while. If cytochemical differences could be found between sensitive and resistant cells, they would be valuable for predicting the effect of therapeutic agents on human leukæmias.

I am grateful to Dr. L. Law for providing the resistant strains of L1210 cells, to Dr. S. R. M. Bushby and Miss M. Barnett for maintaining them and supplying me with inoculated mice, to Miss T. Minett for technical assistance and to the Lady Tata Trust for a grant-in-aid.

H. J. Woodliff

Department of Hæmatology,
Radcliffe Infirmary,
Oxford.

[1] Woodliff, H. J., *Nature*, 187, 156 (1960).

[2] Quaglino, D., and Hayhoe, F. G. J., *Nature*, 187, 85 (1960).

Hæmoglobin 'Bart's' and the Sickling Phenomenon

While examining the hæmoglobin of hydrops and erythroblastosis fœtalis, in an attempt to correlate fœtal disease with hæmoglobin 'Bart's', five consecutive cases (four in Indonesia and one in Malaya) were found in which fœtal hydrops and erythroblastosis were associated with this type of hæmoglobin[1,2]. Four of these cases were examined in detail, and the sickling phenomenon was noted in the abnormal erythrocytes; the amounts of the abnormal fast-moving hæmoglobin were very high, and formed by far the major component. A small amount of hæmoglobin H was detected in all four specimens; no hæmoglobin S was detected in any of them. Hæmoglobin 'Bart's' is now believed to be identical with the Fessas and Papaspyrou type of hæmoglobin[3–5].

In order to eliminate the possibility of false sickling[6], tests on the abnormal erythrocytes were carried out simultaneously with control tests of normal erythrocytes on the same slide. The abnormal erythrocytes, when left on a slide under a cover-glass, without sealing, or when left between two cover-glasses, were found to sickle in 1–1½ hr.; after 24 hr. most of these erythrocytes were sickled (Fig. 1). If the blood were stored under paraffin for 24 hr., sickling was not seen on immediate examination, but appeared after the slide had been left for about 1 hr.; thus it appears that de-oxygenation of the blood is not the cause of the sickling. If oxygen, nitrogen, or carbon monoxide were bubbled through the blood, the erythrocytes were found not to sickle immediately; but, if left for 1 hr. or more on the slide, sickle cells appeared. Sickling was found to occur at any point over the pH-range studied, 6·4–7·8. Clear solutions of the hæmoglobin prepared from these erythrocytes, when left to stand, were found to develop a precipitate; on examination this was found to be composed of fine granules.

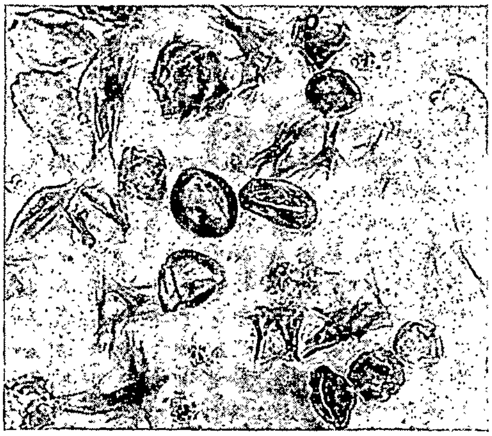


Fig. 1. Sickling of erythrocytes containing hæmoglobin 'Bart's' + H

The easy sickling of these cells seems to be provoked by a peculiarity of the 'Bart's' (= F and P) hæmoglobin. The influence of hæmoglobin H cannot be entirely ruled out, but this hæmoglobin by itself has never been reported to be associated with sickling.

While these investigations were in progress, Atwater *et al.* reported the sickling of erythrocytes containing another fast-moving hæmoglobin (I) in high concentration[7].

Since the completion of this communication, three further cases of severe hydrops fœtalis associated with a large amount of hæmoglobin 'Bart's' have been found. Careful examination of the red blood cells again revealed the sickling phenomenon in all three of them, while control studies were negative.

Lie-Injo Luan Eng

Institute for Medical Research,
Kuala Lumpur,
Malaya.

[1] Lie-Injo, Luan Eng, and Jo, Bwan Hie, *Nature*, 185, 689 (1960).

[2] Lie-Injo Luan Eng and Lie, H. G., *Acta Hæmat.* (in the press).

[3] Ager, J. A. M., and Lehmann, H., *Brit. Med. J.*, i, 929 (1958).

[4] Fessas, P., and Papaspyrou, A., *Science*, 126, 1119 (1957).

[5] Fessas, P., and Papaspyrou, A., *Brit. Med. J.*, ii, 886 (1959).

[6] Lie-Injo Luan Eng, *Doc. Med. Geogr. Trop.*, 5, 266 (1953).

[7] Atwater, J., Schwarts, I. R., Erslev, A., J. Montgomery, T. L., and Tocantine, L. M., *New Eng. J. Med.*, 263, 1215 (1960).

PATHOLOGY

Energy Pathways of Hepatoma No. 5123

Since the original experiments of Warburg with slices of tumour there has been intense investigation of, and discussion about, a specific tumour energy metabolism[1,2]. This concept, originally characterized by high rates of aerobic and anaerobic glycolysis, has been extended to include certain ancillary features: a moderate respiratory rate with minimal increase in respiration following the addition of succinate and other respiratory substrates[3], a respiratory quotient (*R.Q.*) between 0·75 and 0·90 signifying a mixed protein, fat, carbohydrate respiratory substrate[4], and a protein-synthesizing system which effectively incorporates amino-acid anaerobically if glucose is present[5]. Up to the present time the major exceptions to the concept of a specific tumour metabolism have been certain normal tissues (renal medulla, jejunal mucous membrane, myeloid bone marrow and retina) which share one or more of the characteristics of the tumour slice[2]. Occasional mouse tumours have been observed which display active anaerobic glycolysis without aerobic glycolysis[6], a finding which has been explained by the active respiratory rate and normal Pasteur effect of mouse tissues. The present communication is of particular interest because for the first time a rat tumour is described which displays very low rates of aerobic and anaerobic glycolysis.

Morphological and biological characteristics of the transplantable liver carcinoma No. 5123, induced in a Buffalo-strain rat by the injection of *N*-(2-fluorenyl)-phthalamic acid and carried by subcutaneous and intramuscular transfer, have been described by Morris *et al.*[7]. The levels of certain enzymes of deoxyribonucleic acid metabolism in this hepatoma were found to be similar to those of normal liver by Potter *et al.*[8].

Values of the conventional metabolic quotients (expressed in µl. oxygen or acid per mgm. dry tissue per hr.) for slices (35–40 mgm. dry weight) suspended in Krebs–Ringer bicarbonate or Ringer phosphate medium (total volume 4 ml.) and a gas phase of 95 per cent nitrogen/5 per cent carbon dioxide or of oxygen. An hour incubation at 37° was used with chemical determination of lactate by the method of Barker and Summerson[9].

Table 1. GLYCOLYSIS AND RESPIRATION OF SLICES OF HEPATOMA No. 5123*C* GENERATION 21_1

Quotients		Average duplicate values		
$Q_A^{N_2}$	(manometric)	1·3	1·4	1·7
$Q_{\text{Lactic}}^{N_2}$	(chemical)	1·0	1·0	0·9
$Q_{\text{Lactic}}^{O_2}$	(chemical)	0·6	0·4	0·5
Q_{O_2}		5·1	5·3	4·5
Q_{O_2}	(0·015 *M* glucose)	4·9	5·3	5·0
Q_{O_2}	(0·015 *M* glucose, 0·013 *M* succinate)	18·4	17·7	24·4

Very low rates of aerobic and anaerobic glycolysis were observed in the Morris hepatoma, rates which are lower than those usually reported for normal liver[10]. Hepatoma No. 5123 possesses a modest respiratory rate with a Q_{O_2} of 5·0. Two other features of this tumour which are distinctly unusual for neoplastic tissues are also reported in Table 1. These are the absence of a Crabtree effect and a respiratory rate in the presence of succinate which is more than three times the rate found in its absence. It has also been found that the mitochondrial fraction of the Morris tumour (prepared by centrifugal fractionation in isotonic sucrose[11]) contains 17 per cent of the total nitrogen content of the whole tumour. This value is similar to the 19 per cent value for the mitochondria of normal liver and should be contrasted with values of 2·4–3·9 per cent reported for the mitochondria of other transplantable rat tumours[11].

The data of Table 2 indicate that hepatoma No. 5123 is able to incorporate anaerobically very little amino-acid into protein even in the presence of glucose. This behaviour is similar again to normal liver, but quite different from the glycolysing neoplasms studied in the past which display active amino-acid incorporation in nitrogen if glucose is added[5].

Table 2. INCORPORATION OF ^{14}C-VALINE INTO HEPATOMA No. 5123*C* SLICES

Experimental conditions were similar to those of Table 1 except that 1·4 × 10^5 c.p.m. of valine-1-^{14}C (final concentration of 0·003 *M*) were added to each flask and the radioactivity of the purified proteins determined at the end of the experiment (ref. 5)

Conditions	Incorporation (c.p.m. per mgm. protein)		
Nitrogen (0·015 *M* glucose)	0·8	0·5	0·2
Oxygen	21·6	9·0	
Oxygen (0·015 *M* glucose)	19·4	12·9	10·6

The experiments reported here indicate that the Morris hepatoma possesses a primarily aerobic energy metabolism. This is evidenced by a large mitochondrial content, an excellent response of the respiratory rate to succinate addition, minimal anaerobic and aerobic glycolysis, absence of a Crabtree effect, and little glycolytically supported amino-acid incorporation. Its energy metabolism resembles adult liver, and is remarkable in the absence of the biochemical features which have previously been found to characterize the energy pathways of the tumour slice. The Morris hepatoma, though frequently lethal to the tumour-bearing animal, is a slow-growing and morphologically well-differentiated neoplasm. In the examination of the comparative biochemistry of tumours, this type of neoplasm may prove of more importance than the anaplastic, rapidly growing, glycolysing animal neoplasms which have been investigated in the past. The finding of additional tumours of this type will seriously weaken the hypothesis of a specific tumour energy metabolism.

We wish to thank Dr. Van R. Potter for his valuable suggestions concerning this work. Dean Burk and Mark Woods have also observed very low aerobic and anaerobic glycolysis in hepatoma No. 5123 (personal communication).

ALAN C. AISENBERG

Harvard Medical School,
Boston, Mass.

HAROLD P. MORRIS

National Cancer Institute,
Bethesda, Maryland.

[1] Warburg, O., *Science*, 123, 309 (1956). Weinhouse, S., *Science*, 124, 267 (1956).

[2] Aisenberg, A. C., *The Glycolysis and Respiration of Tumours* (Academic Press, New York, 1961).

[3] Elliott, K. A. C., Benoy, M. P., and Baker, Z., *Biochem. J.*, 29, 1937 (1935).

[4] Dickens, F., and Simer, F., *Biochem. J.*, 24, 1301 (1930).

[5] Quastel, J. H., and Bickis, I. J., *Nature*, 183, 281 (1959).

[6] Dickens, F., and Weil-Malherbe, H., *Cancer Res.*, 3, 73 (1943). Albert, Z., and Orlowski, M., *J. Nat. Cancer Inst.*, 25, 443 (1960).

[7] Morris, H. P., Sidransky, H., Wagner, B. P., and Dyer, H. M., *Cancer Res.*, 20, 1252 (1960).

[8] Potter, Van R., Pitot, H. C., Ono, T., and Morris, H. P., *Cancer Res.*, 20, 1255 (1960).

[9] Barker, J. B., and Summerson, W. H., *J. Biol. Chem.*, 138, 535 (1941).

[9] Burk, D., Behrens, O. K., and Sugiura, K., *Cancer Res.*, 1, 733 (1941)

[11] Aisenberg, A. C., *Cancer Res.*, 21, 295 (1961).

Inclusions of Mucopolysaccharide in the Lymphocytes of Patients with Gargoylism

GARGOYLISM is a syndrome which comprises a large number of abnormalities. The skeleton, in particular, is severely affected, with dwarfism, a large and deformed skull, a shortened neck and characteristic abnormalities of the thoracic and lumbar vertebræ. Deafness is often present, and the disease is also associated with enlargement of liver and spleen, with clouding of the cornea and with mental deficiency[1].

The condition is genetically determined and appears to be an inherited disorder of mucopolysaccharide

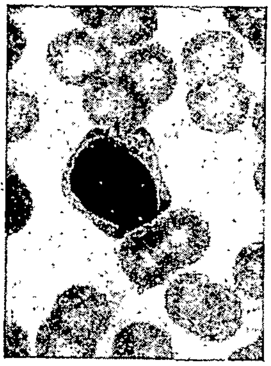


Fig. 1. Lymphocyte with cytoplasmic inclusions. Air-dried film, May–Grunwald–Giemsa

metabolism[2]. Large quantities of chondroitin sulphate have been isolated from the tissues and urine of patients[3], and deposits of acid mucopolysaccharide have been demonstrated histochemically in the liver and other tissues[2,4,5]. An earlier report[6] suggested that abnormal inclusions were present in the lymphocytes. This has now been confirmed in a total of eighteen patients, of whom fourteen were males and four were females.

Blood films were obtained from finger pricks and fixed in absolute methanol. The following stains were used: (1) May–Grunwald–Giemsa; (2) toluidine blue; (3) alcian blue in 2 *N* sulphuric acid (on three patients only).

In all patients some of the lymphocytes contained abnormal inclusions. In air-dried films stained with May–Grunwald–Giemsa they are usually in the form of granules of varying shapes (dots, commas or small rings; Fig. 1), stained purple; often each granule appears to be surrounded by a vacuole. Such inclusions are encountered in lymphocytes of all sizes, even in small ones containing only little cytoplasm. Sometimes one or two larger inclusions may be present instead of a cluster of small ones. The proportion of lymphocytes with inclusions varied between 5 and 50 per cent in different preparations. This may be connected with the fact that the inclusions are fairly soluble in aqueous solutions and dissolve readily in solutions of saline and of phosphate, leaving a vacuolated cytoplasm.

There is usually no difficulty in distinguishing the inclusions found in gargoylism from the azurophilic granules of normal lymphocytes. The inclusions are deeply stained and well defined and tend to occur in clusters, and each granule is typically surrounded by a well-defined vacuole, whereas the azurophilic granules are less well defined and tend to be scattered in the cytoplasm. With toluidine blue the difference is absolute, since the abnormal inclusions stain metachromatically (Fig. 2), while azurophilic granules are not stained at all. In films stained with alcian blue, bluish-green inclusions are present in some of the lymphocytes (Fig. 3).

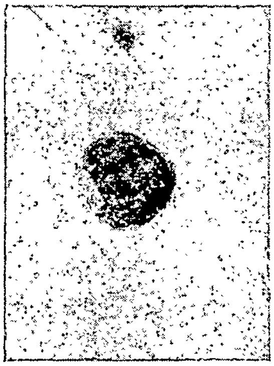


Fig. 2. Lymphocyte with cytoplasmic inclusions. Air-dried film, toluidine blue

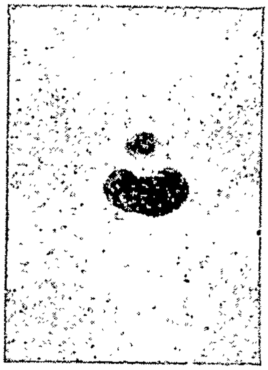


Fig. 3. Lymphocyte with large inclusion. Wet-fixed film, alcian blue and neutral red

The staining reactions of the inclusions with toluidine blue and with alcian blue, as well as their solubility, agree with the assumption that they are composed of acid mucopolysaccharide. There is a close parallel to the staining behaviour of the granules of the basophil polymorphonuclear leucocytes. The latter contain heparin, a sulphated mucopolysaccharide the cytochemical reactions of which are very similar to those of chondroitin sulphate.

The question whether the inclusions are secreted by the lymphocytes or are merely stored there cannot be answered at present, though secretion seems the more likely hypothesis. Lymphocytes containing mucopolysaccharides have so far been described in only one mammalian species, the guinea pig[7,8], and here the evidence suggests that the mucopolysaccharides are secreted.

The fact that, as a result of an inborn error in mucopolysaccharide metabolism, human lymphocytes contain inclusions of acid mucopolysaccharides, provides a simple diagnostic test for gargoylism. It may also throw light on the function of the lymphocyte, which, in spite of much literature devoted to it, is still unknown.

I am grateful to the following clinicians for permission to obtain films of blood from their patients: Dr. R. E. Bonham-Carter, Dr. Margaret Eastwood, Dr. H. R. Ferguson, Dr. O. P. Gray, Dr. B. H. Kirman, Dr. G. S. Mansell, Dr. David Morris, Dr. T. E. Oppé, Dr. B. W. Richards, Dr. Jean Smellie, Dr. D. H. R. Thomas and Dr. J. P. M. Tizard.

URSULA MITTWOCH *

Galton Laboratory,
University College,
London, W.C.1.

* External Scientific Staff, Medical Research Council.

[1] McKusick, V. A., *Heritable Disorders of Connective Tissue* (C. V. Mosby Co., St. Louis, 1960).

[2] Brante, G., *Scand. J. Clin. Lab. Invest.*, **4**, 43 (1952).

[3] Dorfman, A., *Pediatrics, N.Y.*, **22**, 576 (1958).

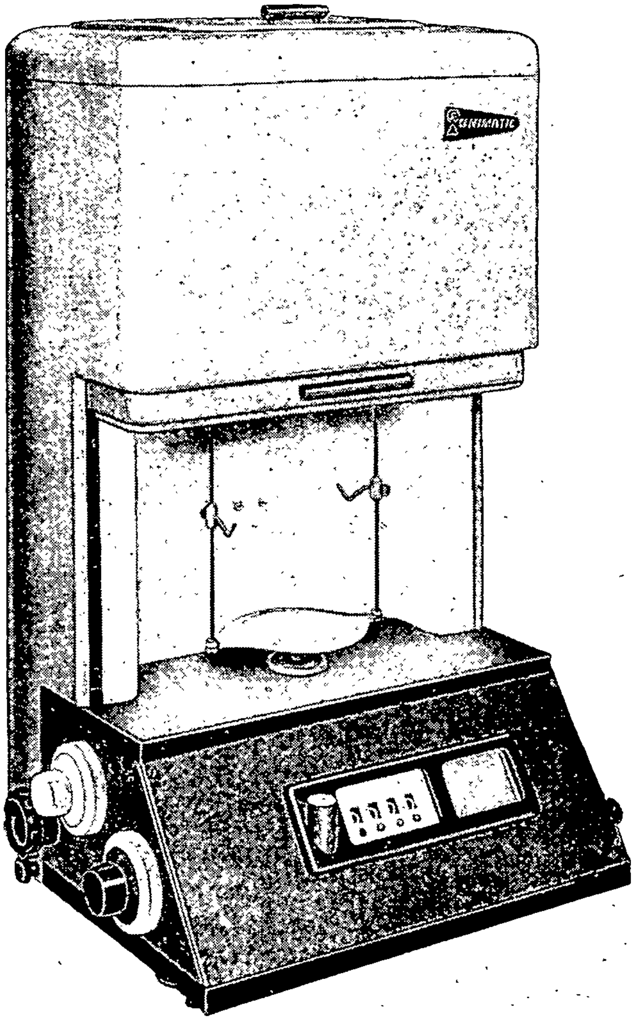
[4] Brante, G., *Cerebral Lipidoses*, edit. by Bogaert, L. van (Blackwell, Oxford, 1958).

[5] Haust, M. D., and Landing, B. H., *J. Histochem. Cytochem.*, **9**, 79 (1961).

[6] Mittwoch, U., *Brit. J. Hæmat.*, **5**, 365 (1959).

[7] Pearce, A. G. E., *J. Clin. Path.*, **2**, 81 (1949).

[8] Marshall, A. H. E., and Swettenham, K. V., *J. Anat.* (Lond.), **93**, 348 (1959).



CONSTANT LOAD WEIGHING BY SUBSTITUTION

As an alternative to the applied-load system of weighing, Stanton now offers the Unimatic single-pan balances—constructed on the constant-load principle of weighing by substitution. The compact, sturdy aluminium case enclosing the instrument is finished in green and black hammertone. Width 13", depth 16", height 20½", weight 32 lb. With the introduction of the Unimatic series, the Stanton range of precision balances now provides an unrivalled choice to meet every requirement.

UNIMATIC Balances	maintain their accuracy over the whole weighing range being designed on the now well known principle of weighing by substitution.
UNIMATIC Balances	are simple to use—even an inexperienced operator can learn how in a few minutes.
UNIMATIC Balances	are safe in use—their non-magnetic stainless steel weights are adjusted to within the new N.P.L. tolerances for analytical weights (errors less than 1 in 200,000). They are well protected within the balance case and their accuracy cannot vary.
UNIMATIC Balances	are fast—very fast in use. Their controls are conveniently positioned at bench level. Time and Motion study experts have approved this design which results in less fatigue and more weighings in less time—in fact less than 30 seconds for the weighing of an unknown object.

UNIMATIC
TRADE MARK

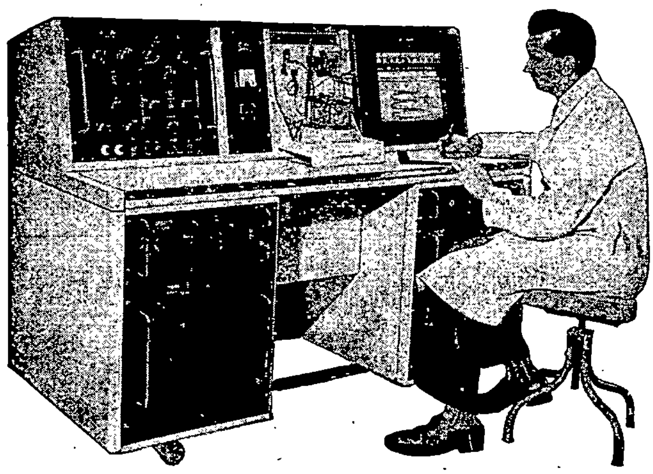
STANTON UNIMATIC BALANCES

Write for details to
STANTON INSTRUMENTS LTD. 119 Oxford Street, London, W.1
Telephone: Gerrard 7533 · Cables: Stanbal London

the best of both worlds—

The NEW and IMPROVED
MERVYN-HARWELL
SQUARE WAVE POLAROGRAPH

This is the instrument which, applying the famous Harwell team's principles, continues exclusively to supply the U.K.A.E.A.'s highest demands for the fastest, ultra-sensitive analysis, in its Research, Weapons and Production Divisions.



speed and sensitivity

SPEED	***SENSITIVITY***
Sampling rate exceeds 80 full analyses per day, even when deoxygenation might be necessary.	Determines traces at levels of 0.001% in sample weights of 20 mg. —and down to 10^{-9} molar.
for Industry	***for Research***

Write for further information mentioning 'Nature' to:

MERVYN INSTRUMENTS
WOKING · SURREY · ENGLAND · *WOKING 5200*

BIOLOGY

Inclusion of Parental Somatic Cells in Sponge Larvæ

THE common boring sponge *Cliona celata* (a marine siliceous type) is oviparous. This characteristic, exceptional among sponges, makes it particularly useful for embryological investigation.

When the fertilized eggs are ejected from a maternal osculum, each carries 20–35 small amœboid cells clinging to its surface. These contain numerous brownish-yellow cytoplasmic granules, and also retain some of the yellow picric acid stain of Bouin's fixative even after prolonged washing. These two points identify them with a type of small amœbocyte widespread in sections of the adult sponge, and conspicuous in the thin edges of reconstitution cultures[1]. They stain deeply if the eggs are immersed in dilute brilliant cresyl blue.

During cleavage, the amœboid cells move into the clefts between the blastomeres, apparently without themselves dividing. In sections of mature larvæ, they are found in the inner mass of amœboid cells and between it and the external flagellated epithelium. Though their cytoplasmic granules have almost disappeared, they are still recognizable by their stubborn retention of picric acid during histological preparation. They can be found in undiminished numbers throughout the 2-day larval life.

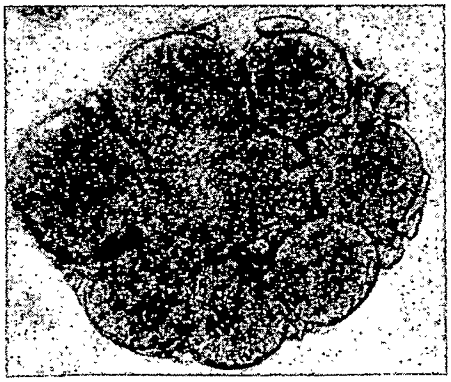


Fig. 1. Embryo of *Cliona celata* showing maternal cells on and among the blastomeres

Unfortunately, I have not been able to learn whether these maternal cells persist through metamorphosis, and become incorporated in the new young sponge, or (as seems more likely) merely serve as a food reserve for the larva. In either case, the larva of *Cliona* is a chimera, composed of parental somatic cells mixed with cells formed by cleavage of the zygote.

In 1920, Gatenby[2] mentioned that maternal cells from the nutrient capsule of the embryo sometimes enter the posterior mass of non-flagellated cells in the embryo of *Grantia compressa*, a calcareous sponge. This suggests that the chimeric structure of the larva may be common throughout the phylum Porifera.

FREDERICK E. WARBURTON

6171 Sherbrooke St., W.,
Montreal 28.

[1] Warburton, F. E., *Canad. J. Zool.*, **36**, 555 (1958).

[2] Gatenby, J. B., *J. Linn. Soc. Lond.*, **34**, 261 (1920).

Cyclical Infection of *Glossina morsitans* with Culture Forms of *Trypanosoma rhodesiense*

THE natural rate of salivary gland infection of tsetse flies with trypanosomes of the *brucei* sub-group is very low, being only about 0·25 per cent[1].

One of the main problems associated with the work on trypanosomiases of this sub-group is the difficulty research workers experience in obtaining adequate supplies of metacyclic trypanosomes for experimental purposes. No circumstances are known which will invariably lead to cyclical infection of the natural vector[1].

During 1959–60, one of us (the late R. M. G.) noticed that tsetse flies fed artificially on culture forms of *Trypanosoma rhodesiense* appeared more readily to develop gut infections than flies fed naturally on infected mammalian hosts. In view of the potential importance of this observation, a pilot experiment was planned in which a small number of *Glossina morsitans* were fed on culture forms of *Trypanosoma rhodesiense*, the tsetse flies afterwards being maintained for three weeks on heparinized human blood. One of these flies developed a patent gland infection.

The trypanosome strain used (*S.S.* 354) had been isolated from a human infection in 1959 and sent from Africa in deep freeze. Weinman[2] cultures were set up in January 1960 from the blood of mice previously inoculated with the human material. Since this date regular *in vitro* passages have been made, and in this experiment tsetse flies were fed on the 31st and 32nd sub-cultures.

The tsetse flies had hatched from puparia sent from East Africa, by the courtesy of the Director of the East African Trypanosomiasis Research Organization. They were given three feeds of the cultured trypanosomes. For each feed 0·2 ml. of the water of condensation from the culture containing approximately 2,000,000 trypanosomes was added to 10 ml. of fresh, heparinized human blood. This mixture, heated to 37° C., was offered to the tsetse flies through a specially prepared guinea pig skin membrane. Afterwards the same diluent without culture was fed twice-weekly to maintain the flies.

At time-intervals of 19–27 days after the first infective feed the tsetse flies were induced to probe on to a warmed glass slide[3], and Giemsa-stained drops of saliva were examined for metacyclic trypanosomes. One smear, from a fly first fed 27 days previously, showed typical metacyclic trypanosomes.

The whole group of experimental tsetse flies were then allowed to probe and to feed on an uninfected guinea pig which showed typical blood forms in peripheral wet blood films on the 9th day after the infective bite. Infection is persisting in this animal at a low level at the end of the fifth week after the infective bite. The trypanosomes are polymorphic.

It is hoped to confirm this finding in further experiments by showing that a significantly high proportion of tsetse flies will develop a salivary gland infection after feeding on trypanosome cultures, and that this can be cyclically transmitted by the arthropod vector.

(The late) R. M. GORDON
J. K. MILLER

University of Liverpool.

[1] Buxton, P. A., *The Natural History of Tsetse Flies* (Lewis, London, 1955).

[2] Weinman, D., *Ann. N.Y. Acad. Sci.*, **56**, 995 (1953).

[3] Burtt, E., *Ann. Trop. Med. Parasit.*, **40**, 141 (1946).

Thyroid Function in Active, Sleeping and Torpid Hummingbirds

HUMMINGBIRDS have been of interest to physiologists for many years due to their small size and high metabolic rate. When it became apparent that these birds could be maintained under laboratory conditions[1] and then studied in regard to various physiological parameters, it appeared of interest to examine the role of the thyroid gland in relation to metabolism. Diurnal torpidity has been demonstrated in two species of hummingbirds[2,3], and recent studies[1] on several other species have quantified the relationship of the active, sleeping and torpid metabolic states to ambient temperature. Although the causes and implications of the torpid state are incompletely understood, it was felt that perhaps the function of the thyroid gland might be related to the metabolic state. Seven hummingbirds that had been maintained under laboratory conditions for nine months were each injected beneath the skin with 1 μc. of carrier-free iodine-131 in a volume of 0·05 c.c. At selected intervals after administration of the isotope, the birds were killed by ether and the thyroid glands removed. The thyroid glands were counted with a probable error not exceeding 1 per cent in a well-type scintillation detector against suitable standards of the original iodine-131 solution. The time of injections was arranged so that the period of accumulation of iodine-131 occurred in birds that were either active, asleep or torpid.

During this period all birds were maintained at 25° C. with access to food and water. Those that were exposed to daylight and artificial light remained active, while those that were kept in the dark were either asleep or torpid. The sex, body-weight, species, and iodine uptake of the birds are given in Table 1.

Table 1

Thyroid uptake of iodine-131 (per cent)	Interval (hr.)	Condition	Genus and species	Sex	Body-weight (gm.)
9·1	2·0	awake	*Calypte costae*	male	3·6
15·7	4·0	awake	*Calypte costae*	male	3·5
25·6	6·0	awake	*Calypte costae*	male	3·6
24·7	6·0	asleep	*Calypte anna*	male	4·6
22·1	12·5	awake	*Selasphorus rufus*	male	3·3
8·6	6·0	torpid	*Calypte costae*	female	3·1
10·4	6·0	torpid	*Archilochus alexandri*	female	3·6

The shape of the iodine-131 thyroid uptake curve based on the four birds that were awake and active resembles the shape of similar curves described for birds and mammals[4]. The maximum uptake of approximately 25 per cent occurring between 6 and 12·5 hr. approximated the findings for other species of birds[5–8]. Although the metabolic studies cited[1] demonstrate a drop in metabolism in sleeping, non-torpid birds, the 6-hr. uptake of the one bird that was asleep, and not active, closely resembled the uptake of the active, awake birds. However, the 6-hr. uptake of the two birds in the torpid state was reduced significantly, when compared with the uptake of non-torpid birds in 6 hr. Perhaps the reduced uptake by torpid birds may be ascribed to the further reduced metabolic activity of the torpid state. A *t*-test comparing the uptake of the two torpid birds to the two non-torpid birds at the 6-hr. interval yields $P > 0{\cdot}91$. It is unlikely that either the sex, or the size, or species differences would influence the thyroid uptake of iodine-131 so drastically[6].

As pointed out by Matthews[9], the factors controlling torpidity are still unknown, but the abruptness of the decline in temperature and metabolic rate suggests that the central nervous system probably controls its initiation and hence the reduced thyroid uptake of iodine-131 observed in the torpid state is, in all probability, a reflexion of the lowered metabolism rather than a cause for the reduced metabolism of torpidity.

This work was supported in part by the U.S. National Science Foundation (Project *G*-9238) and an American Cancer Society Institutional grant (*IN*-40*B*).

C. J. SHELLABARGER
R. C. LASIEWSKI
G. E. HYNCIK

Medical School,
Department of Zoology,
University of Michigan,
Ann Arbor, Michigan.

[1] Lasiewski, R. C. (work to be published).

[2] Pearson, O. P., *Condor*, **52**, 145 (1950).

[3] Bartholomew, G. A., Howell, T. R., and Cade, T. J., *Condor*, **59**, 145 (1957).

[4] Pitt-Rivers, R., and Tata, J. R., *The Thyroid Hormones*, Chapter 7 (Pergamon Press, 1959).

[5] Vlijm, L., *Arch. Neerl. Zool.*, **21**, 467 (1958).

[6] Shellabarger, C. J., and Pitt-Rivers, R., *Nature*, **181**, 546 (1958).

[7] Kobayashi, H., and Gorbman, A., *Endocrinol.*, **66**, 795 (1960).

[8] Kobayashi, H., Gorbman, A., and Wolfson, A., *Endocrinol.*, **67**, 153 (1960).

[9] Matthews, L. H., *Brit. Med. Bull.*, **17**, 9 (1961).

Flimmer-flagellum of the Sponge

THERE is a considerable variation in the morphology of the flagella in the lower plant groups and in the flagellates. The flagellar structures have been used as systematic characters since the pioneering work of Petersen[1] and Vlk[2]. The following five types of flagella are recognized:

(1) One-sided flimmer flagella which have one row of hair-like appendages (called flimmer hairs or mastigonemata or, somewhat misleadingly, cilia). The flimmer hairs are about 3μ long, and the row extends along the entire length of the flagellum on its one side.

(2) Two-sided flimmer flagella which have two rows of flimmer hairs along the entire length of the flagellum.

(3) Whip flimmer flagella which have a demarcated thin end region devoid of flimmer hairs and a main region which possesses two rows of flimmer hairs. The flimmer hairs stand perpendicular to the flagellar axis.

(4) Whip flagella which are devoid of flimmer hairs but have a demarcated thin end region. These flagella are also called lash flagella.

(5) Flagella which lack flimmer hairs and also lack a demarcated end region visible in the light microscope.

These characterizations have been made from fixed specimens at high-magnification light microscopy after certain staining procedures. In some cases it has been possible to confirm the findings by dark-field microscopy on living flagella[2]. Manton[3] and Pitelka *et al.*[4] have been able to confirm and extend these observations by electron microscopy, mainly on whole mounts. Flimmer flagella have also become known under the names ciliated flagella and flagella of a tinsel-type structure.

The above classification is of interest mainly because the various groups of protozoans and plants are characterized by specific types of flagella. The flagellar ornaments can thus be used as systematic and phylogenetic characters. The euglenid flagellates have a one-sided flimmer flagellum; the flagellated cells of *Chrysomonadina*, Heterocontae, Phaeophyceae and Saprolegniaceae have one whip flagellum and one two-sided flimmer flagellum.

The whip flimmer flagellum has only been described from choano-flagellates (*Craspedomonadina*). It seems that sponges (Porifera) have never been examined in this respect. This is the more remarkable as prevalent hypotheses indicate that the ancestors of the sponges are to be sought in the choanoflagellate group[5,6].

When I examined several types of animal cilia and flagella in an attempt to find morphological differences that might be correlated to the different types of exhibited movement[7], it was found that the sponge choanocyte possessed a flimmer flagellum. An electron micrograph is published in that paper. The flimmer hairs of these flagella have a diameter of only 20 Å. and a length of 0·2–0·3μ. In a cross-section (∼ 400 Å.) of the flagellum there might be found a dozen flimmer hairs on each side of the flagellar axis. They extend laterally from the membrane limiting the flagellum, and are in the same plane as that which joins the two central filaments of the flagellum. All hairs do not arise at the same spot at the flagellar membrane, as seen in cross-section, and also in their further extension they are not fully parallel. They might therefore be difficult to discern. Although other electron microscopists have examined the sponges, both by means of sectioning techniques[8–10] and of whole-mount techniques[11], the flimmer flagellum of the choanocyte has not been described. It cannot be decided from the sections whether the flimmer flagellum of the sponge choanocyte is of type 2 or 3 in the above classification, that is, whether there is a smooth end region or not. The cross-sectioned choanocyte flagellum may or may not have flimmer hairs in a certain section, depending probably both on the level at which the flagellum has been cut, and on technical difficulties; it seems that the flimmer hairs are easily washed off the flagellum during the preparation technique. Flimmer hairs have not been observed on that part of the flagellum which is outside the collar.

The flagellar membrane might be thrown in folds or show two or more ridges. These protrusions are found without any relationship to the axial filaments or to the flimmer hairs, and are therefore believed to represent a preparation artefact.

The sponges that have been examined in this way are *Microciona* sp. (Demospongiae) and *Grantia compressa* (Calcarea), both from Marine Biological Laboratory, Woods Hole, Mass. They represent two classes of the Porifera.

This is the first demonstration of a flimmer flagellum in a multicellular animal. It is not likely that flimmer flagella will be found in higher metazoans, as flagella and cilia from a great many animals have been examined by electron microscopy without any evidence of the presence of flimmer hairs. The fact that the sponges do have flimmer flagella, possibly of the type called whip flimmer flagella, gives support to the hypothesis that the sponges are descendants of the choanoflagellates. It also supports the idea that the multicellular animals have a diphyletic origin.

This work was supported by a grant from the Swedish Natural Science Research Council.

BJÖRN A. AFZELIUS

Wenner-Gren Institute,
Stockholm.

[1] Petersen, J. B., *Botan. Tidskrift* (Copenhagen), **40**, 373 (1929).

[2] Vlk, W., *Arch. Protistenkunde*, **90**, 448 (1938).

[3] Manton, I., *Symp. Soc. Exp. Biol.*, **6**, 306 (1952).

[4] Pitelka, D. R., and Schooley, C. M., *Univ. Calif. (Berkeley) Publ. Zool.*, **61**, 79 (1955).

[5] Hollande, A., in Grassé, P.-P. (edit.), *Traité de Zoologie*, **1**, 579 (1952).

[6] de Saedeleer, H., *Ann. Soc. Roy. Zool. Belg.*, **60**, 16 (1929).

[7] Afzelius, B. A., in Goodwin, T. W., and Lindberg, O. (edit.), *Macromolecular Structure and Biological Function, Proc. First Symp. IUB/IUBS* (in the press).

[8] Fjerdingstad, E. J., *Z. Zellforsch.*, **53**, 645 (1961).

[9] Gatenby, J. B., and Tahmisian, T. N., *J. Roy. Micro. Soc.*, **77**, 100 (1958).

[10] Rasmont, R., *Ann. Sci. Nat. Zool.*, **12**, 253 (1959).

[11] Kilian, E. F., *Ber. Oberhessische Ges. Natur-Heilk., Giessen*, **27**, 85 (1954).

Effect of Ethylene Oxide on the Tapping of *Hevea brasiliensis*

IN recent years the importance of bacteria in limiting the effective yield of polyisoprene from *Hevea brasiliensis* has been described[1]. Further, the highly desirable stability and very low content of volatile fatty acids of sterile latex have been fully established[2]. Up to 1960, the apparatus for the extraction of sterile latex was costly, but recently I have used simple polythene 'hoods' to enclose the tapping panel, so that antibacterial atmospheres could be maintained during tapping.

The first gaseous sterilizer chosen was ethylene oxide (1,2-epoxyethane), which in preliminary trials proved an extremely effective bactericide for contaminated *Hevea* latex systems. Liquid ethylene oxide, direct from the cylinder, was placed in a small open cup beneath a polythene hood sealed to the tree immediately above the tapping panel. The lower edge of the polythene was then tied tightly to the tree below the tapping panel by a polythene strip. The lower seal was not entirely airtight, but merely limited the escape of gas from the hood—this proved sufficient. Ethylene oxide is a gas at Malayan ambient shade temperatures (about 28° C.) and thus liquid ethylene oxide, once exposed to atmospheric pressure, boils rapidly; 20 ml. evaporated in 16 min. under these experimental conditions. Preliminary sterilization of the enclosed space was carried out at 3.30 p.m. using 20 ml. of liquid ethylene oxide for the first two trees and 10 ml. for the third and fourth trees. The trees were tapped at 6.30 a.m. on the following morning, and 5 ml. of liquid ethylene oxide was introduced under the polythene hood at the time of tapping. During subsequent tappings of all trees, 5 ml. was used for preliminary sterilization and no ethylene oxide was introduced at the time of tapping. Latex was collected in a clean conical flask exposed to the atmosphere of ethylene oxide within the hood. A nearby control tree of similar girth, age and yield was observed throughout the experiments.

The effect on yield and flow was considerable. The increases in yield are shown in Fig. 1. Time of flow increased to some 27 hr. from 2½–4 hr. Latex from such treatments has remained liquid for up to 34 days at 15° C., provided it was not infected afterwards, and has been proved sterile (less than 1 viable bacterium/ml.) on three separate occasions throughout

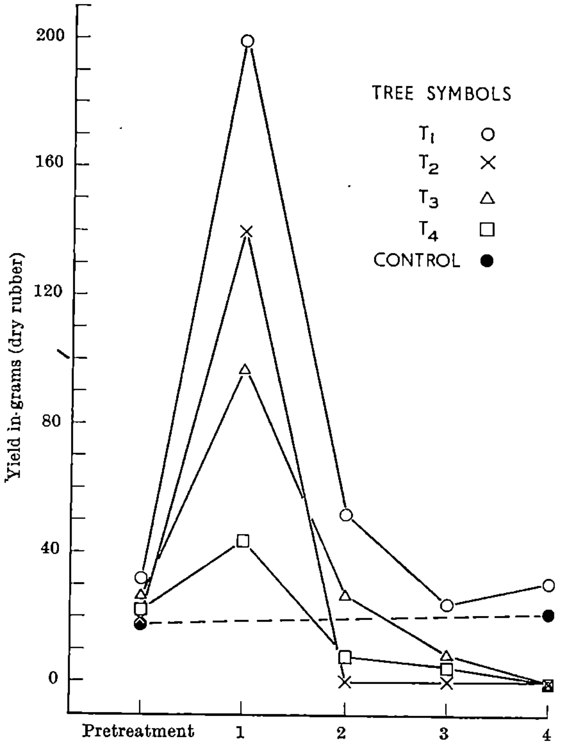


Fig. 1. Effect of ethylene oxide on yield

storage for 34 days. In contrast, normal (contaminated) latex from this area is fully coagulated after 20 hr. at 15° C. The effect of ethylene oxide was to drain latex almost completely from the entire area surrounding the tapping cut. Following this, successive tappings give decreasing yields of rubber until complete drying of the latex-bearing tissue is observed. One treatment with ethylene oxide may achieve this complete drainage, but on other trees up to four tappings may be observed. Complete drainage is followed by rapid necrosis of latex vessels and outer bark accompanied by fungal attack as a first stage.

Another interesting effect has been the bleeding of latex from old healed wounds following treatment of the tapping panel with ethylene oxide. Wounds healed and dry for at least three months and up to 2 ft. above the enclosed tapping cut (in some cases well outside the hood) have begun to bleed, the bleeding proceeding for several hours and in one case for two days. The simplest explanation of this bleeding seems to be a rapid translocation of ethylene oxide coupled with an interaction of ethylene oxide and wound tissue.

As flow following the first tapping with ethylene oxide exceeded 24 hr., it was possible to confirm the resurge of flow about 23–24 hr. after tapping. This had been observed for the sterile cabinet (ref. 1) using a combination of antibiotics and ultra-violet irradiation to achieve sterility and is similar to the resurge of bleeding sap of grape vines[3]. A further point of interest is that the pattern obtained on ultra-centrifuging shows no gross differences, after 24 hr. storage, from the normal pattern shown by fresh latex.

Whatever the fundamental mechanisms underlying this striking series of events, four major points emerge: ethylene oxide enables large volumes of sterile latex to be obtained in a simple way; latex sterilized with ethylene oxide remains stable; ethylene oxide effectively drains all latex from the immediate vicinity of the tapping panel; ethylene oxide induces bleeding from old wounds not deliberately treated with the gas.

D. H. TAYSUM

Rubber Research Institute of
Malaya,
Kuala Lumpur, Malaya.

[1] Taysum, D. H., *J. App. Bact.*, **20**, 188 (1957); Proc. Natural Rubber Research Conference, Kuala Lumpur, Malaya, 224 (1960).

[2] McMullen, A. I., *J. Rubb. Res. Inst. Malaya*, **13**, 129 (1951). Taysum, D. H., Proc. Natural Rubber Research Conference, Kuala Lumpur, Malaya, 834 (1960).

[3] Stoyev, K. D., Mamarov, P. T., and Benchev, T. B., *Fiziologiya Rasteni*, **6**, 408 (1959).

Uue of Glacial Acetic Acid for killing Parasitic Nematodes for Collection Purposes

MANY parasitic nematodes have a tendency to coil considerably when placed in water or cold-fixing fluids such as alcohol or formalin. Ideally, in order to facilitate the subsequent microscopic examination, nematodes should die in an extended position. This is best achieved by the use of 70 per cent alcohol or Looss' fluid (9 parts 70 per cent alcohol and 1 part glycerol), near boiling temperature, in which nematodes uncoil and become fixed rapidly. However, hot alcohol is rarely available during field work, and a simple alternative method for killing and fixing nematodes in an extended position is likely to be desired by other workers.

In the summer of 1960, parasitic nematodes from the proventricle of the fulmar (*Fulmarus glacialis*) were fixed in glacial acetic acid by mistake instead of cold 70 per cent alcohol, to which they were transferred a few minutes later. The fixed specimens were found to be remarkably straight, and the idea of using glacial acetic acid on purpose presented itself. More specimens from the fulmar were collected; some were fixed in cold 70 per cent alcohol, and others in glacial acetic acid. Those fixed in alcohol were found to be rather wavy and bent, while those treated with glacial acetic acid straightened almost immediately. After a few minutes, the majority of the latter were transferred to cold 70 per cent alcohol, while a few were allowed to remain in the glacial acetic acid. It has not been possible to make a specific determination of the specimens from the fulmar, but they belong to the superfamily Spiruroidea, sub-family Acuariinae, and appear to be generically related to *Seuratia* Skrjabin, 1916.

Several specimens of *Contracaecum aduncum* (Rudolphi, 1802), a common parasite of the digestive tract of the cod and other marine teleosts, have been treated with glacial acetic acid as described here. The specimens were found first to move vigorously, then they uncoiled and died in a matter of seconds. Small specimens were found to straighten more rapidly than larger ones. Larval ascarids, *Anisakis* sp. larva and *C. aduncum* larva, liberated from their capsules, have been treated with glacial acetic acid with equally good results. The nematodes were kept in the glacial acetic acid for up to 10 min., whereupon they were transferred to and stored in 70 per cent alcohol. Before fixation, the nematodes were washed in water in order to clean them from mucus and debris.

A mixture of three parts 70 per cent alcohol and one part glacial acetic acid did not kill as rapidly as the undiluted acid, nor did the specimens straighten so well in it. When the glacial acetic acid becomes diluted with water after repeated use, the killing effect is retarded and the specimens do not straighten so well in it. The acid should therefore be used undiluted. However, by adding various amounts of absolute alcohol to the glacial acetic acid, specimens of *C. aduncum* were killed even more rapidly, but they did not uncoil as well as in the glacial acetic acid alone. The advantage of adding absolute alcohol is that the freezing-point of the glacial acetic acid is lowered, thus avoiding solidification at low temperatures. A mixture of one part of glacial acetic acid and one part of absolute alcohol was found to remain fluid at $-32°$ C. This mixture may be useful for field work in extreme low temperatures, but at higher temperatures the amount of glacial acetic acid should be increased.

Admittedly, this simple method for killing nematodes seemed promising, but it remained to be seen whether or not the specimens had been damaged by this treatment. Specimens were cleared in the usual media, lactophenol and creosote, in which they cleared very rapidly. On microscopical examination, they were found to be in good condition. When cleared in lactophenol cuticular structures, such as lips, papillæ and striation were found to be very clearly visible. Internal organs were also seen clearly.

After having been kept in the glacial acetic acid for six months, some of the specimens from the fulmar were transferred directly to lactophenol and examined microscopically. The specimens were found to be in good condition.

It thus appears that glacial acetic acid with subsequent transfer to 70 per cent alcohol may be used for killing and fixing nematodes.

Acanthocephalans (*Echinorhynchus gadi*) and trematodes (*Hemiurus communis*) from the cod have also been treated with glacial acetic acid. Before killing in acid, the acanthocephalans had to be left for some time in fresh water in order to extrude their evertible hooked proboscés. No movement of the specimens was observed during fixing. They cleared quickly in lactophenol. The trematodes were washed in 1 per cent saline solution before fixing. Whole trematodes were found to stain fairly well in carmalum.

Manuals on microscope technique[1,2] do not recommend fixing in pure glacial acetic acid for histological work, although it is known to kill with the utmost rapidity. However, as nematodes rarely have to be sectioned, the method described may be useful, at least when it is imperative that they should die and remain extended. However, for histological work it is perhaps better to avoid prolonged stay in glacial acetic acid; the specimens should preferably be transferred to 70 per cent alcohol as soon as possible. It is possible that this method may be regarded as a two-step modification of Carnoy's.

Although nematodes in general are rather difficult to stain, transverse sections of *C. aduncum* killed in glacial acetic acid were stained in hæmalum for 1·5 hr., and overnight in eosin, with quite good results. Even the nuclei stained well. Carmalum or paracarmine alone stained poorly.

In conclusion, it is suggested that the use of glacial acetic acid, followed by transfer to 70 per cent alcohol, is satisfactory for fixing nematodes in an extended position. No adverse effect on cleared specimens was observed after this treatment. In addition, the anatomy may be studied satisfactorily in stained sections. However, it is not known what effect, if any, the glacial acetic acid treatment has on the finer histological or cytological details.

BJØRN BERLAND

Institute of Marine Research,
Directorate of Fisheries,
Bergen, Norway.

---

[1] Lee, B., *The Microtomist's Vade Mecum*, edit. by Gatenby, J. B., and Beams, H. W., eleventh ed. (London, 1950).

[2] Romeis, B., *Mikroskopische Technik*, fifteenth ed. (Munich, 1948).

Swarming of the Polychaete *Odontosyllis phosphorea* Moore, var. *Nanaimoensis* Berkeley, near Nanaimo, B.C.

In a communication to *Nature* some years ago[1] I mentioned that the annual concentration of the luminescent swarming forms of *O. phosphorea* in the channel at the entrance to Departure Bay during the summer and early autumn months could be partly attributed to the set of the currents around the islands in the vicinity. This channel has always been regarded as the classical locality from which these forms could be secured, but no effort to collect any had been made for many years until recently. Interest in them revived two years ago in connexion with work on bio-luminescence; diligent search was made for them in the channel throughout the summer of 1959, but not a single individual was seen. If present, they are easily detected at dusk by their brilliant flashes of light when the sexual products are discharged. Moreover, a dredging run through the channel failed to secure an example of the atokous bottom-living form.

The explanation is fairly clear. Since the worms were last collected there, a ferry service between Departure Bay and Vancouver has been established and boats of considerable draught ply many times daily through the channel. This creates sufficient disturbance to nullify entirely effects of currents and, apparently, to disperse the bottom dwellers, but this latter finding requires confirmation.

It is most unfortunate that this should have occurred, and I write regretfully to advise fellow workers interested in bio-luminescence that they can no longer rely with confidence on Departure Bay as an available source of swarming *Odontosyllis phosphorea*.

E. BERKELEY

Marine Biological Station,
Departure Bay,
Nanaimo, B.C.

---

[1] Berkeley, E., *Nature*, 136, 1029 (1935).

Light Rainfall and Plant Survival : Measurement of Stem Flow Run-off

Work on the effects of showers on droughted maize necessitated measurement of water run-off down the main stems. The following two collecting devices have proved simple to construct and reliable in use. They are attached directly to the stem 1–3 in. above the ground-surface after the leaf-bases ensheathing the first internode have been removed, and the stem surface dried.

In type 1 a rectangle of heavy-gauge polythene sheet (7 in. × twice the circumference of the stem) is wrapped around the stem in the form of a funnel, the upper circumference being 0·25–0·5 in. away from the stem surface. A small hole is cut in the sheeting near its lower edge and the flared end of a short length of stiff polythene pipe inserted and welded to the funnel wall while the flange is hot. The overlapping edges of the funnel are sealed with suitable plastic cement. The lower edge of the funnel is sealed to the stem in a similar manner and bound in place with wire. A length of flexible plastic tubing connects the exit pipe to a large storage tank sunk in the ground and shielded from the Sun.

In type 2, a large rubber bung with a hole in the centre slightly smaller than the plant stem is cut down one side, and clamped in position around the stem with wire. Any gap between stem and rubber is sealed with melted wax. This supports a rigid funnel made from a polystyrene specimen container of some 3 in. in diameter. The bottom of the container is removed and the remaining cylinder cut down one side with a hot wire. A piece of plastic tubing is sealed into the funnel near the lower edge to form an exit pipe similar to that of type 1. The split edge of the funnel is joined with polystyrene cement and the whole bedded down on to the rubber bung with wax. Melted wax is poured into the funnel to raise the level to the lower edge of the exit pipe which is connected to a sunken storage tank.

In view of the very large quantities of water obtained by the maize leaf catchment area from even very light showers, it is best to have large storage tanks available. These are emptied and the volume measured after each rainfall. Both funnels contain a dead volume of about 5 ml., but this amount is a negligible part of the total catch and represents 0·5–1·5 per cent of that obtained from mature maize plants in rains ranging from 0·3 to 0·1 in. The height of the funnels above ground ensures that they are free from splash effects in most light rains, while the closeness of fitting around the stem reduces the direct entry of rain to a minimum.

M. D. GWYNNE

Botany School,
University of Oxford.

J. GLOVER

East African Agriculture and
Forestry Research Organisation,
Kikuyu,
Kenya.

ENTOMOLOGY

Double and Single Spermathecæ in Fleas (Siphonaptera)

Two spermathecæ (or receptacula seminis) in female fleas which normally possess only one have been noted on various occasions[1]. In such abnormal specimens or monstrosities one spermatheca is usually smaller than the other. The best published description of this condition in the rat flea, *Xenopsylla cheopis* Roths. (overlooked by M. I. D. Sharma and G. C. Joshi[2]), is that by Smit[3], who figures a specimen in which both spermathecæ and their ducts are fully developed and of almost equal size. A very much rarer abnormality is recorded by Stark[4,5], who discovered a specimen of *Hystrichopsylla linsdalei* Holland (which normally has two spermathecæ) with three spermathecæ.

The spermatheca is single in the majority of insects, although in certain genera, for example, *Blaps* (Coleoptera), *Phlebotomus* and *Dacus* (Diptera), there are two, and in many of the Calyptratae, in *Culex* and the Tabanidae (Diptera) three[6]. In the Siphonaptera there is generally one spermatheca, but two occur in various genera scattered throughout the order. These genera are, on the whole, those in which various but well-marked primitive morphological features have been retained. It would appear likely that paired spermathecæ was the condition in the ancestors of the order. This view is enhanced by the fact that in all the genera in which there is a single spermatheca the blind duct presumed to be that of the missing organ is generally present in varying degrees of reduction. The position of this blind duct indicates that it is the left organ which has been lost. In a few scattered genera two spermathecæ are present, which are normally of unequal size, one being distinctly smaller than the other. Jordan[7] described this condition as a "link between single and double receptacula seminis". In the very scanty, badly preserved material available for study at that time it was not possible to say whether it was the right or left organ which was reduced. In two species recently examined, *Ctenoparia jordani* Smit[8] and *Atyphloceras shogakii* Jameson and Sakaguti[5], it is now clear that the smaller of the two spermathecæ is situated on the left side.

MIRIAM ROTHSCHILD

Ashton, Peterborough.

[1] Smit, F. G. A. M., *Tijdschr. Ent.*, **90**, 35 (1947).

[2] Sharma, M. I. D., and Joshi, G. C., *Nature*, **191**, 727 (1961).

[3] Smit, F. G. A. M., *Ent. Bericht.*, **12**, 436 (1949).

[4] Stark, H. E., *Pan-Pacific Entomol.*, **29**, 135 (1953).

[5] Hopkins, G. H. E., and Rothschild, Miriam, *Illustrated Catalogue of the Rothschild Collection of Fleas*, **3**, 9 (Fig. 7 by Smit, F. G. A. M.), 78, 95 (in the press).

[6] Imms, A. D., *A General Textbook of Entomology*, revised ninth ed., 187 (1957).

[7] Jordan, K., *Ectoparasites*, **1**, 127 (1921).

[8] Smit, F. G. A. M., *Trans. Roy. Entomol. Soc.*, **107**, 324 (1955).

Acaricidal Properties of Di-(*p*-chlorophenyl)-trifluoromethylcarbinol

Di-(*p*-chlorophenyl)-trifluoromethylcarbinol, $(Cl.C_6H_4)_2C(OH).CF_3$ [1,2], has several interesting biological properties: it is a synergist for DDT in the house-fly[1,3–5] and inhibits oviposition, on tarsal contact, in house-flies[6] and mosquitoes[7]. Since it is the fluorine analogue of two acaricides, namely DMC [di-(*p*-chlorophenyl)-methylcarbinol] and 'Kelthane' [di-(*p*-chlorophenyl)-trichloromethylcarbinol], it seemed interesting to assay also its acaricidal properties. The compound was tested against adult females of the red spider mite *Tetranychus telarius* L. on beans, by a method described recently[8], and was compared with the commercial acaricides 'Kelthane' and chlorobenzilate (ethyl *p,p'*-dichlorobenzilate). The activity of the fluorine compound was intermediate between those of 'Kelthane' and chlorobenzilate (Table 1).

It has been demonstrated in field trials on orange trees (A. S. Tahori and E. Swirski, personal communication) that di-(*p*-chlorophenyl)-trifluoromethylcarbinol did not affect the citrus rust mite (*Phyllocoptruta oleivora* Ashm.), but it was noted incidentally

Table 1. Toxicities for *T. telarius* (on Beans)

Compound		Mean 24 hr. mortality*								LC_{50}, p.p.m.
'Kelthane'	p.p.m.	10	15	20	25	30	50	80	100	29
	Mortality (per cent)	8	20	33	43	53	73·5	88·5	96	
Di-(*p*-chlorophenyl)-trifluoromethylcarbinol	p.p.m.	100	150	200	250	350	500	650	1,000	250
	Mortality (per cent)	13	21·5	39	50·5	61	82	89	100	
Chlorobenzilate	p.p.m.	250	300	400	500	700	1,000	1,500	—	450
	Mortality (per cent)	15·5	25	38	62	85·5	88·5	98	—	

* Mean mortalities were calculated from three series of experiments carried out with each compound.

that it caused high mortality among red spider mites on the branches infested with *T. telarius* and *Brevipalpus* sp.

Our thanks are due to Prof. E. D. Bergmann for a liberal sample of di-(*p*-chlorophenyl)-trifluoromethylcarbinol.

K. R. S. Ascher
R. Cwilich

Department of Toxicology,
National and University
Institute of Agriculture,
Beit-Dagan, Rehovot,
Israel.

Bergmann, E. D., Tahori, A. S., Kaluszyner, A., and Reuter, S., *Nature*, **176**, 266 (1955).

[2] Kaluszyner, A., Reuter, S., and Bergmann, E. D., *J. Amer. Chem. Soc.*, **77**, 4164 (1955).

[3] Tahori, A. S., *J. Econ. Ent.*, **48**, 638 (1955).

[4] Cohen, S., and Tahori, A. S., *J. Agric. Food Chem.*, **5**, 519 (1957).

[5] Reuter, S., Cohen, S., Mechoulam, R., Kaluszyner, A., and Tahori, A. S., *Riv. Parassitol.*, **17**, 125 (1958).

[6] Ascher, K. R. S., *Science*, **125**, 938 (1957).

[7] Ascher, K. R. S., *Riv. Malariol.*, **36**, 209 (1957); *Experientia*, **14**, 8 (1958).

[8] Ascher, K. R. S., and Cwilich, R., *Ktavim*, **10**, 159 (1960).

MICROBIOLOGY

Auxin Production by Species of *Arthrobacter*

The ability of fungi and bacteria to produce plant growth-promoting substances such as indole-3-acetic acid (IAA) has long been recognized[1–3]. Gruen[3] has recently reviewed the literature on auxin production by fungi. However, reports on bacterial synthesis are widely dispersed, and relatively few known species have been recorded as being able to produce these substances, although many unidentified cultures have been tested[1]. Of the known organisms, *Escherichia coli*, *Pseudomonas fluorescens*, *Agrobacterium tumefaciens*, *Rhizobium* sp. and *Azotobacter* sp. have received most attention[4–6], yet one of the largest groups of soil bacteria, the *Arthrobacter*, has not, to our knowledge, been examined.

Representative species of this genus from the culture collection of the Microbiology Research Institute were grown in a medium of the following composition (per litre): salt solutions *A* and *B* (ref. 7), 5 ml. each; ammonium nitrate, 0·5 gm.; yeast extract, vitamin-free casamino-acids, glucose, 1·0 gm. each; *p*H 6·8. The medium was dispensed in 100-ml. amounts in 300-ml. Erlenmeyer flasks, sterilized and inoculated from 24-hr. agar cultures. After 24 or 48 hr. on a rotary shaker at 26° C. the cells were removed by centrifugation, washed once, dried at 80° C. and weighed. The cell-free culture fluid was brought to *p*H 3·0 and extracted with ether; the extract was concentrated under vacuum at 35° C. and tested for auxin activity by means of a modified *Avena* coleoptile section test.

Table 1. Production of Auxin by Species of *Arthrobacter*

Species	Indole-3-acetic acid equivalents	
	µgm./l.	µgm./gm. cells
Arthrobacter globiformis	10	8
Arthrobacter pascens	8	6
Arthrobacter aurescens	9	7
*Arthrobacter tumescens**	8	11
*Arthrobacter citreus**	15	16
Arthrobacter atrocyaneous	15	15
Nocardia sp.	15	15

* 48-hr. cultures.

As indicated in Table 1, every *Arthrobacter* culture tested as well as a closely related organism of the genus *Nocardia*, which is also common in soil, showed activity. Furthermore, *A. globiformis* and *A. pascens* grown for 48 hr. in a completely synthetic medium (above medium without yeast extract and casamino-acids) produced 3·0 and 2·0 µgm. IAA equiv./l. or 8 and 4 µgm. equiv./gm. cells, respectively. Although its growth was poorer in the synthetic medium, *A. globiformis* produced as much active material per gram cells as it did in the complete medium. In further tests with this organism in the synthetic medium containing tryptophan (added aseptically) it was found that in 48 hr. 120 µgm. IAA equiv./l. or 750 µgm. equiv./gm. cells were produced. This tryptophan effect is well known[2,4].

The active substance produced in the three media (complete, synthetic and synthetic plus tryptophan) co-chromatographed with synthetic IAA in three solvent systems: propanol–ammonia–water, butanol–acetic acid–water and 0·05 *M* potassium dihydrogen phosphate. Segments of the chromatograms were also assayed by the *Avena* method. In every instance activity occurred in sections with an R_F value equivalent to pure IAA.

It is not possible at present to assess the importance of microbial synthesis of auxins in soil or at the root surface. A recent bacteriological study of the composition of the flora of wheat roots showed that *Arthrobacter* species constituted 22 per cent of the isolates (Rouatt, J. W., and Katznelson, H., unpublished results). In absolute numbers this represents 900 million organisms per gram oven-dry roots. To this number may be added approximately 2,000 million bacteria of the genus *Pseudomonas*, one representative of which was found in preliminary tests to produce auxin-like substances. Fungi, living on the surface of the roots[8], may also be expected to produce such compounds. Since it is well established that amino-acids are liberated by roots it is likely that active synthesis of auxins and related substances occurs at the surface of the roots. Kefford *et al.*[9] have reported that the liberation of tryptophan by legume roots permitted auxin synthesis by the legume bacteria when plant and organism were cultured together. Because of the intimate contact between microbial and plant cells at the immediate root surface it may also be expected that these substances would be absorbed directly by the root and would not be susceptible to alteration or destruction as those produced in the soil proper.

H. KATZNELSON
J. C. SIROIS

Microbiology Research Institute, and
Plant Research Institute,
Research Branch,
Canada Department of Agriculture, Ottawa.

[1] Roberts, J. L., and Roberts, E., *Soil Sci.*, **48**, 135 (1939).
[2] Brian, P. W., *Symp. Soc. Exp. Biol.*, **11**, 166 (1957).
[3] Gruen, H. E., *Ann. Rev. Plant Physiol.*, **10**, 405 (1959).
[4] Stowe, B. B., *Biochem. J.*, **61**, 9 (1955).
[5] Georgi, C. E., and Beguin, A. E., *Nature*, **143**, 25 (1939).
[6] Burger, K., and Bukatsch, F., *Zbl. Bakt.*, **111**, 1 (1958).
[7] Snell, E. E., and Strong, F. F., *Indust. Eng. Chem. Anal. Ed.*, **11**, 346 (1939).
[8] Peterson, E. A., *Canad. J. Microbiol.*, **4**, 257 (1958).
[9] Kefford, N. P., Brockwell, J., and Zwar, J. A., *Austral. J. Biol. Sci.*, **13**, 456 (1960).

Influence of Salts on the Uptake of Streptomycin by *Escherichia coli*

A PREVIOUS report from this laboratory[1] described the kinetics of uptake of streptomycin labelled with carbon-14 by *Escherichia coli W* growing in mineral-citrate-glucose medium *A*[2]. In the procedure used, the cells were separated from the medium on a membrane filter and washed with distilled water before determination of radioactivity. We now find that the uptake had been markedly enhanced by a step not reported previously: dilution of each sample with several volumes of distilled water immediately before filtration. (This predilution in water was considered trivial because the subsequent washing with water removed little of the radioactivity on the filter, although almost all of it could be removed by washing with medium *A*.) When this predilution step was omitted the radioactivity retained on the filter was lower by 50 to 80 per cent (Fig. 1), but there was still, as previously reported, an immediate 'primary' uptake and, after a lag, a gradually increasing 'secondary' uptake. (Szybalski and Mashima[3] have reported kinetics of streptomycin uptake which are difficult to compare with ours since their first sample was not taken until 1 hr. had elapsed and they measured the radioactivity retained after washing with non-radioactive streptomycin.)

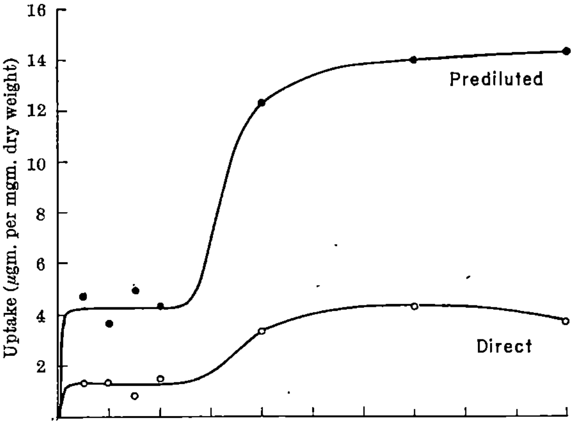


Fig. 1. Uptake of streptomycin by 'prediluted' and 'directly filtered' *E. coli W*

E. coli W was grown in medium *A* (ref. 2) supplemented with 0·5 per cent glucose at 37° C. on a Brunswick rotary shaker. Forty μgm. per ml. of streptomycin-^{14}C (30 c.p.m. per μgm.) was added to the exponentially growing culture when it reached a density of about 0·2 mgm. dry weight of cells per ml. (about 100 Klett units with No. 42 filter, or 4 × 10^8 cells per ml.). 'Directly filtered' samples were pipetted on to a membrane filter ('Millipore *HA*'); 'prediluted' samples were mixed with 4 vol. distilled water immediately before filtration. After washing the filters twice with 5 ml. of water and drying, the radioactivity was determined on a Nuclear-Chicago thin-window gas-flow counter. Sample sizes were chosen to yield 40–200 c.p.m. The data are corrected for cosmic background and for the blank values (about 4 counts per minute) obtained by filtering medium containing streptomycin-^{14}C but no cells

Table 1. TIME-COURSE OF THE PREDILUTION EFFECT

Time in diluting fluid (sec.)	Uptake (μgm. per mgm. dry weight) 5-min. exposure to streptomycin	40-min. exposure to streptomycin
No dilution	1·2	3·6
15	4·4	9·6
45	3·6	9·2
75	4·0	12·0
300	5·2	22·0

Cells were exposed to streptomycin-^{14}C as described for Fig. 1. At 5 and 40 min., 4-ml. samples were transferred rapidly to 16 ml. of distilled water. At the times indicated, 5-ml. portions of the diluted samples were filtered, washed and counted as described.

As Table 1 shows, the effect of predilution is rapid. With cells in either the primary (5 min.) or the secondary (40 min.) phase of streptomycin uptake, the enhancement occurred within 15 sec. of dilution and did not increase significantly during the next 30 sec. Longer periods in diluted medium further enhanced uptake, particularly with cells in the secondary phase, but this effect probably involved further action of the drug (the effectiveness of which is increased by low salt concentration[4]).

It seemed possible that, during the washing, directly filtered cells lost much of the already accumulated drug because of osmotic shock, whereas predilution moderated this effect. However, when cells were filtered directly but not washed, the radioactivity, contributed in part by the retained medium, was much less than that observed with prediluted and washed cells. The predilution step must therefore increase the amount of streptomycin taken up by the cells.

Conversely, it seemed possible that the effect might be due to damage to a permeability barrier by osmotic shock during predilution, increasing the accessibility of binding sites while external streptomycin was still available. However, in sucrose solution isosmotic with the growth medium the predilution effect occurred as readily as in water (Table 2). In contrast, not only did the effect fail to occur on dilution with medium *A*, but after it had occurred in distilled water it was immediately and completely reversed by the addition of 1/10 volume of 10-times concentrated medium *A*. It is therefore clear that one or more of the salts in the medium interferes with streptomycin uptake, and predilution decreases this interference.

Table 2. INFLUENCE OF VARIOUS SOLUTES ON THE PREDILUTION EFFECT

Solute added to diluting fluid	Final concentration (moles/l.)	Uptake (μgm. per mgm. dry weight)
No dilution (direct)		3·6
None (predilution in water)	1/5 medium *A*	9·2
Concentrated medium *A*	medium *A*	2·8
Sucrose	0·22	8·4
**K^+	*0·10	7·2
NH_4^+	*0·015	8·4
**Mg^{++}	*0·0004	4·8
Citrate	*0·0014	4·0
Phosphate	*0·062	2·8
Sulphate	*0·008	6·0

Samples were either diluted with fluid of the appropriate composition or (**) diluted with water and brought to the appropriate composition by the addition of one-tenth volume of concentrated salt solution. Values for the two methods were in close agreement when applied to the same sample. Counter ions were Na^+ and Cl^-, each without effect up to 0·1 *M*. The pH's of the samples after dilution were 6·9 (owing to the phosphate buffer of the medium); when the *p*H of samples diluted with water was varied from 5·6 to 7·7 there was little effect on uptake.

* Same concentration as in medium *A*.

ts of the medium are
At the concentrations
sphate, citrate, and Mg^{++}
n preventing or reversing
ndeed, the phosphate is
the low uptake observed in

ells to streptomycin in medium
s little as one volume of distilled
as much enhancement as predilu-
lumes. This unusual finding was
n experiment in which the concen-
dium *A* and streptomycin were inde-
ried over a wide range. The results
the amount of streptomycin taken up
creased markedly as the concentration of
A decreased.

It is evident that the uptake observed with prediluted cells represents a substantial amplification of the uptake that occurs in the growth medium, but this artefact does not affect the earlier conclusion concerning the existence of a primary and a secondary phase (Fig. 1). However, these observations do affect the interpretation of the secondary uptake. It is now clear that with prediluted samples this uptake represents largely molecules taken up rapidly during predilution rather than slowly during prolonged exposure to the drug. The gradual increase during the secondary phase therefore cannot be due solely to binding to sites made slowly accessible by slight damage to the membrane. The uptake curve must rather reflect a gradual increase in the number of freely accessible binding sites. The nature of these sites is under investigation.

This work has been supported by research grant *G*-9078 of the National Science Foundation. We would like to thank Merck, Sharp and Dohme for the gift of streptomycin-^{14}C.

PAUL H. PLOTZ*
DONALD T. DUBIN†
BERNARD D. DAVIS

Department of Bacteriology and
Immunology,
Harvard Medical School,
Boston 15,
Massachusetts.

---

* Post-sophomore Fellow of U.S. Public Health Service.

† Special Fellow of the National Institutes of Allergy and Infectious Diseases, U.S. Public Health Service.

[1] Anand, N., Davis, B. D., and Armitage, A. K., *Nature*, **185**, 23 (1960).
[2] Davis, B. D., and Mingioli, E. S., *J. Bacteriol.*, **60**, 17 (1950).
[3] Szybalski, W., and Mashima, S., *Biochem. Biophys. Res. Comm.*, 1, 249 (1959).
[4] Green, S. R., and Waksman, S. A., *Proc. Soc. Exp. Biol. and Med.*, **67**, 281 (1948).

CYTOLOGY

Deoxyribonucleic Acid Structures in the Nucleolus

PHASE-CONTRAST observation and electron microscopy indicate that the nucleolus is structurally heterogeneous consisting of a ground material in which is embedded a more compactly aggregated material generally described as filamentous (Estable and Sotelo[1] proposed for them the names of 'pars amorpha' and 'nucleolonema'). Nucleoli of plant and animal cells are seen at mitosis and meiosis associated with a particular chromosome or chromosomes—the so-called 'nucleolar organizer' chromosomes—at the site of a heterochromatic region. If a chromosomal deletion includes such a region or if a mutation occurs[2], nucleolar material is then produced at other 'loci' (the latent organizers).

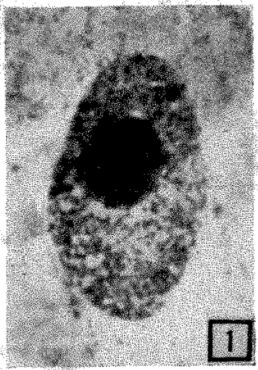


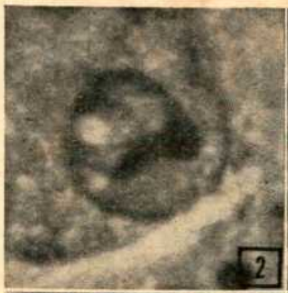
Fig. 1. Early prophase (acetocarmin, × *c*. 800)

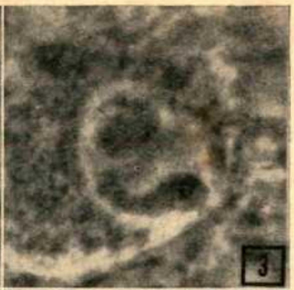
Nucleoli are described as being Feulgen-negative. However, Feulgen-positive granular and filamentous structures have been reported for different types of cell[3-5]. Estable and Sotelo[1,6], on the other hand, described a Feulgen-negative filamentous structure (nucleolonema). Such observations were based primarily on fixed and stained material without discriminatory nuclease digestion. No details are available on how the nucleolar structures were affected in those papers describing a loss of basophilia after treatment with ribonuclease (RNAse). Were RNA the only nucleic acid present in the nucleolus, under ideal experimental conditions only vestiges of RNA would be seen after digestion with RNAse. Granules and filaments of deoxyribonucleic acid (DNA) should be resistant to RNAse and thus become more easily visualized after RNAse digestion.

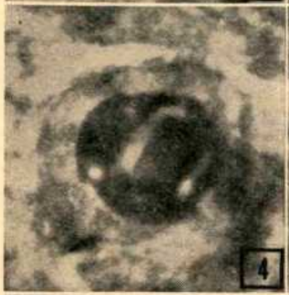
Nucleoli of some species of *Spirogyra* are specially suitable for cytological investigation because of their large size, 10–30μ (Fig. 1). Living cultures of two clonal populations (*Spirogyra* aff. *crassa* and *S.* aff. *lamellata*; identification tentative until fertilized material is obtained), grown under controlled laboratory conditions, were treated with RNAse and DNAse. Contact time with the enzyme was 2, 3, 4 or 5 hr. with RNAse and 2, 3 or 4 hr. with DNAse (the 5 hr. sample was discarded after a microscopic examination disclosed that the plastids were collapsing). To avoid distortions due to sectioning, the material was handled, stained and mounted in bulk. Appropriate controls for the Feulgen reaction, methyl-green-pyronin and acetocarmin techniques were used.

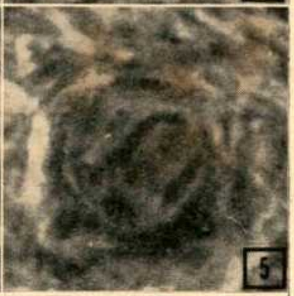
Images observed on stained material resembled those seen under phase-contrast on unstained material prior to any fixation or hydrolysis. Cells at interphase and early prophase treated with either RNAse or DNAse showed a selective enzymatic depolymerization restricted to certain nucleolar areas. If the ground substance and the embedded filamentous structure contain RNA, after RNAse digestion one would expect a homogeneous gradient of enzymatic action from the periphery towards the centre of the nucleolus and with increasing enzyme contact almost total digestion with the exception of nucleolar proteins.

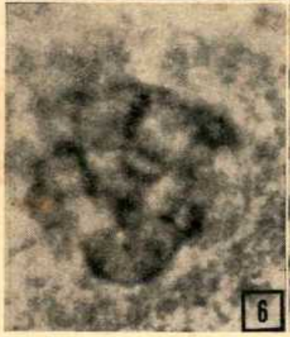
However, as can be clearly seen in Figs. 2 and 3, RNAse digestion was, in fact, restricted to definite nucleolar areas. Enzyme treatment left in the nucleolar material something like a continuous con-

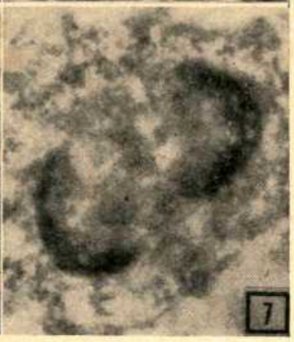












Figs. 2 and 3. RNAse treatment. Feulgen-positive structures. 2, Positive phase; 3, negative phase. (× *c.* 1,550)

Figs. 4 and 5. DNAse treatment. Empty 'tubule' on nucleolar mass. Undigested material Feulgen-negative. 4, Positive phase; 5, negative phase. (× *c.* 1,550)

Fig. 6. RNAse treatment. Early prophase. Chromonemata Feulgen-positive. (× *c.* 800)

Fig. 7. RNAse treatment. Middle prophase. Homologous nucleolar chromosomes. (× *c.* 1,300)

volved 'cave'. The spaces in between those occupied by the now digested material showed a Feulgen-positive and methyl-green positive filamentous structure made up of a double, thin chromonemata with small granules.

DNAse digestion also was incomplete and very selective. The empty spaces left in the nucleolar mass were considerably smaller in diameter; their limits very sharp and neat as if a convolved 'tubule' had been spirited away (Figs. 4 and 5). The ground-material unaffected by DNAse was Feulgen-negative and pyroninophilic. To eliminate the possibility of mistakenly identifying vacuoles for enzymatic digestion, all observations were carried out and recorded by both positive and negative phase-contrast.

An additional experiment was carried out under similar conditions with RNAse and DNAse applied sequentially. After this consecutive digestion for two hours for each nuclease, it was found that a 'ghost' of the nucleolus was left unstainable by either Feulgen or methyl-green-pyronin.

In 1950, Godward[7] described a similar nucleolar structure for *Spirogyra* which she interpreted as the expanded heterochr
region. The double ch
being inside the nucleolu
ments resembling the org
chromosomes in the karyo
my material the nucleolar RN
almost the entire nucleolar
chromosomes are interpreted as
In *Spirogyra* aff. *crassa* it was o
ing that two nucleolar chromosom
length in some cells (Fig. 7) and o
in other cells. In *S.* aff. *lamella*
nucleolar chromosomes were found (F
is therefore believed to occur in the
not in the zygotes.

When two nucleoli were found in teloph
phasic or prophasic cells, a small nucleo
always associated with the shorter chromosome and a larger nucleolus with the longer chromosomes. This would suggest that the material is actually synthesized at these chromosomes and not merely aggregated. The rate of RNA synthesis should thus accelerate at the time when RNA substance is rounding up into a nucleolus, and it should reach the peak some time between telophase and early prophase.

Experiments with RNA anti-metabolites, labelled RNA and DNA precursors, are being completed.

This work was supported by grant *DRG*-535 from the Damon Runyon Memorial Fund for Cancer Research.

I am indebted to Dr. Richard M. Klein for his criticisms.

ELSA H. J. O'DONNELL

New York Botanical Garden,
Bronx Park,
New York 58.

[1] Estable, C., and Sotelo, J. R., *Inst. Invest. Ciencias Biol.*, **1**, 105 (1951).
[2] Fischberg, M., and Wallace, H., *The Cell Nucleus*, edit. by Holmes, B., 30 (1960).
[3] Harris, H., *Biochem. J.*, **73**, 362 (1959).
[4] Lettre, R., *Symp. Fine Structure of Cells*, 141 (Noordhoff, Groningen, 1956).
[5] Lettre, R., *Tenth Congr. Cell. Biol.*, 1960 (in the press).
[6] Estable, C., and Sotelo, J. R., *Symp. Fine Structure of Cells*, 170 (Noordhoff, Groningen, 1956).
[7] Godward, M. B. E., *Ann. Bot.*, **14**, 39 (1950).

ANTHROPOLOGY

Inter-Individual Variability in Pigmentation

COLOUR of skin has always been and still is the most frequently used character in definition of races. The essential reason is that skin colour is considered as an inherited trait for which a hypothetical polygenic mechanism of transmission has been suggested (Davenport, Gates and Stern). It was almost the interracial differences that had caught the eye, but the possibility of using a spectrophotometer[1,2] has induced me to explore the inter-individual variability in one population. The essential conclusions of the examination of 143 Belgian male students and 177 female students with an *EEL* spectrophotometer at nine wave-lengths (430 mμ to 700 mμ) during April 1960 are reported here. Measurements were taken at the forehead and inner arm. Table 1 gives means and standard deviations. More detailed results will be published elsewhere[3].

(1) Percentages of reflectance are significantly different for arm and forehead in each of the male and female samples (except for ♀ at $\lambda = 700$ mμ).

Table 1

λ	Inner arm				Forehead			
	♂ $n=143$		♀ $n=177$		♂ $n=143$		♀ $n=177$	
(mμ)	Mean	*S.D.*	Mean	*S.D.*	Mean	*S.D.*	Mean	*S.D.*
430	37·71	4·83	36·50	3·98	27·87	3·63	32·44	3·53
470	44·73	5·01	43·48	3·54	32·53	3·53	37·26	3·66
490	46·74	4·76	45·31	3·53	34·22	3·71	39·05	3·60
520	46·71	4·30	45·65	3·57	33·68	3·90	39·13	4·20
550	44·77	3·96	44·57	3·62	31·56	4·00	37·27	3·74
580	48·87	3·84	48·77	3·34	36·68	4·46	41·86	3·86
600	59·52	3·75	58·50	3·44	51·07	3·92	55·50	3·35
680	65·72	3·15	64·28	2·92	60·67	3·75	63·50	3·95
700	67·27	2·90	65·88	2·47	62·48	3·81	65·66	3·60

(2) Males are darker at the forehead than females but lighter at the inner arm. This latter point does not agree with results collected by Edwards and Duntley[2] (on 3♂ and 3♀) nor with most of the results ollected by Garn *et al.*[4], at $\lambda = 420$ mμ only, on hildren of both sexes, female being lighter than es on the inner arm. However, two of the samples the last authors and just the samples which are the most comparable to mine with regard to age agree with my conclusion:

Garn *et al.*, 1956. Per cent reflectance at $\lambda = 420$ mμ						
	Range of age	n	Inner arm		Forehead	
			Mean	*S.D.*	Mean	*S.D.*
Mature nulliparæ	15–25	23	29·4	3·6	23·7	5·0
Young adult males	20–29	16	30·4	5·2	22·3	4·2

(3) Computation of coefficients of variation (4–13 per cent) demonstrates that their value depends on the wave-length considered and generally greater than similar coefficients for other biological traits (height: 3·60 per cent, weight: 10–12 per cent, pulse: 12·71 to 14·21 per cent).

A comparison with the same coefficient computed from the data published by Barnicot[5] for the forearm of Ibo (12–18·5 per cent) and Yoruba (11·3–21·5 per cent) reveals on the whole a more considerable variability for Negroes and a very different position of the maximum value of this coefficient: for male and female Whites it is a maximum at $\lambda = 430$ mμ, Negroes it is a maximum at $\lambda = 600$–680 mμ.

Table 2 gives correlation coefficients for each -length between percentage reflectance for inner and forehead. All are less than the weakest coefficient height-weight, for example.

Table 2

λ (mμ)	♂	♀
430	0·405 ± 0·070	0·466 ± 0·058
470	0·388 ± 0·071	0·449 ± 0·060
490	0·354 ± 0·073	0·462 ± 0·059
520	0·201 ± 0·080	0·401 ± 0·063
550	0·246 ± 0·078	0·280 ± 0·069
580	0·289 ± 0·076	0·293 ± 0·068
600	0·340 ± 0·074	0·411 ± 0·062
680	0·410 ± 0·069	0·461 ± 0·059
700	0·505 ± 0·062	0·373 ± 0·064

) Preliminary trials (comparison of means per-age reflectance of the skin for the different gories of eyes (scale of Martin–Saller) and hair ur (scale of Fischer–Saller), χ^2 tests applied to distribution of these latest categories charted on atter diagram ([per cent reflectance values at inner arm–per cent reflectance values at forehead] at each wave-length) seem to demonstrate that, in my samples, correlations between eyes or hair colour on one hand and skin pigmentation on the other are nil or extremely weak. This point will receive further consideration in the future: indeed, suggestive association in very different populations (and this is the important point) induced us to think of pleiotropic action of a gene. My results seem to demonstrate that if common genetic factors exist for pigmentation, they are few and minor in action, that lack of association within a geographical group in relation to association within sibships should make linked genes a more probable hypothesis.

This demonstrates once more the fundamental importance of genetics for anthropology and how spurious may be considerations evolved from an exclusively adaptative and geographical point of view[6].

I am indebted to M. Dillaerts, director of the Centre National de Calcul Mécanique, for his co-operation in computing the results.

ANDRÉ LEGUEBE

Laboratoire d'Anthropologie,
Institut Royal des Sciences Naturelles
de Belgique, Bruxelles.

[1] Weiner, J. S., *Man*, **51**, 152 (1951).
[2] Edwards, E. A., and Duntley, S. Q., *Amer. J. Anat.*, **65**, 1 (1939).
[3] Leguebe, A., *Bull. Inst. Roy. Sci. Nat. Belgique*, **37** (11), 1 (1961).
[4] Garn, S. M., Selby, S., and Crawford, M. R., *Amer. J. Phys. Anthrop.*, **14**, 101 (1956).
[5] Barnicot, N. A., *Hum. Biol.*, **30**, 150 (1958).
[6] Twiesselmann, F., *Volume Jubil. V. Van Straelen*, **2**, 1065 (1954).

PSYCHOLOGY

Stimulus-Response Compatibility in the Paired-Associate Learning of Nonsense Syllables

THE concept of stimulus-response (*S-R*) compatibility has been used mainly in the area of sensory-motor skills, where it was developed to explain why certain *S-R* pairs, said to conform to a population stereotype, were more easily learned than others[1,2].

The present experiment tests the hypothesis that language habits, determining *S-R* compatibility, affect the ease with which pairs can be learned. The relative compatibility of pairs of syllables was measured in the following way. A statistical subject is assumed who, given the last letter of the stimulus syllables, attempts to predict the first letter of the response syllable on the basis of the relative frequencies of the digrams in the English language[3]. The number of 'guesses' required is the measure of compatibility. For example, the compatibility score for the pair *QEM POG* is 6 'guesses', because *P* is the sixth most likely letter following *M* in English. If the pair is reversed, that is, *POG QEM*, its compatibility score is 22 'guesses', as there are 21 letters more likely than *Q*, following *G*.

Eight pairs of nonsense syllables were selected from Glaze's list[4], 2 pairs each of 0 per cent, 6·67 per cent, 13·33 per cent and 20 per cent associative value. The pairs were so chosen that in one order they were relatively compatible (mean score 10·8 'guesses'), but when reversed they became relatively incompatible (mean score 19·1 'guesses'). Two lists were made up; in list *A*, pairs 1–4 were in the compatible order and pairs 5–8 were incompatible, and in list *B* the order of each pair was reversed. Each list was learned by one of two groups of 15 naval ratings, using the anticipation method of paired-associate learning[4], with a presentation-rate of 3 sec. per item and an inter-trial interval of 15 sec. The order of occurrence of pairs was random within each trial. The subjects practised to a criterion of 3 successive errorless trials, or if this were not reached, for 20 trials.

Subjects' performances were scored in terms of the total error difference between pairs 1–4 and pairs 5–8.

Thus subjects learning list *A* would be expected to have lower scores than those learning list *B*. The mean score per subject for list *A* was − 0·13 and that for list *B* was + 12·67. A Mann–Whitney test[6] confirmed the predicted difference between the two groups ($P < 0{\cdot}01$, 1 tail). Of the 8 pairs used, 7 were learned more rapidly in the compatible order, indicating that the result was not due simply to the inadvertent selection of one or two atypical syllables.

It was concluded that *S-R* compatibility effects analogous to those shown in sensori-motor skills occur also in verbal learning.

A. D. BADDELEY

Medical Research Council
Applied Psychology Research Unit,
Cambridge.

---

[1] Fitts, P. M., and Deininger, R. L., *J. Exp. Psychol.*, **48**, 483 (1954).

[2] Alluisi, E. A., and Muller, P. F., *J. Exp. Psychol.*, **55**, 247 (1958).

[3] Baddeley, A. D., Conrad, R., and Thomson, W. E., *Nature*, **186**, 414 (1960).

[4] Glaze, J. A., *J. Genet. Psychol.*, **35**, 255 (1928).

[5] McGeoch, J. A., and Irion, A. L., *The Psychology of Human Learning* (Longmans Green, New York, 1952).

[6] Siegel, S., *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956).

Taste Thresholds and Food Dislikes

ALTHOUGH food likes and aversions are influenced by cultural, social and idiosyncratic variables[1], individual differences in the ability to taste certain classes of compounds may be one determinant of food rejections[2].

In the present work, forty-eight subjects checked food dislikes and aversions on an alphabetical listing of 118 foods previously tested on a college-age Ohio population[3]. Excluding foods unfamiliar or never sampled by each subject, the percentage of familiar foods disliked was used as the individual score. Scores ranged from 0–55 per cent ; the median was 10 per cent, as in the standard population.

Taste thresholds were determined for D-sucrose, sodium chloride and hydrochloric acid in forty-two subjects, and for quinine sulphate and 6-*n*-propylthiouracil[4] in all forty-eight subjects. The compounds were dissolved in double-distilled, copper-free water using twenty concentrations ranging from $3{\cdot}66 \times 10^{-7}$ *M* to $3{\cdot}84 \times 10^{-1}$ *M*, each numbered step representing a doubling of concentration. A preliminary training session, a later test session and a further retest session was used in all forty-eight cases.

Dislikes for foods could not be significantly related to a subject's sensitivity to, or thresholds of taste for, D-sucrose, sodium chloride, or hydrochloric acid. However, taste thresholds for 6-*n*-propylthiouracil and quinine, both bitter substances, show a relation to percentage of food dislikes. Two categories have been set apart, 0–10 per cent foods disliked (low) and 10–55 per cent foods disliked (high). Using the χ^2 test, the value of χ^2 was 3·0 for both quinine and 6-*n*-propylthiouracil, with low taste thresholds (high taste acuity) associated with a higher percentage of food aversions in both cases. On the basis of percentage of food aversions, analysis by the Mann–Whitney *U* test of goodness of fit[5] revealed a difference in taste thresholds, significant at the 1 per cent confidence level for the two categories.

The bimodal distribution of thresholds for 6-*n*-propylthiouracil partially accounts for our finding that the correlation between per cent of food dislikes and thresholds for 6-*n*-propylthiouracil is quite small.

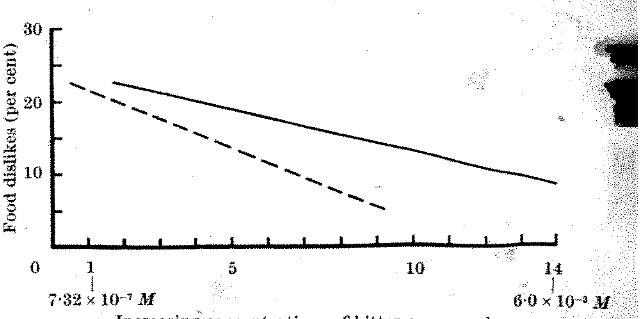


Fig. 1. Linear regression of the distribution of thresholds for 6-*n*-propylthiouracil and quinine related to percentage of foods disliked ($N = 48$). Numbers on the abscissa are concentrations of 6-*n*-propylthiouracil and quinine, each step representing a doubling of the concentration. — —, Quinine; ——, 6-*n*-propylthiouracil

Irrespective of this nearly zero correlation, however, the trend of the linear regression line of the percentage of food dislikes against thresholds of taste is similar to the regression line for quinine (Fig. 1). The equation of regression for 6-*n*-propylthiouracil is $Y = 23{\cdot}8 - 1{\cdot}14X$. The probability is 0·001 per cent that our sample came from a population with a slope of linear regression equal to zero.

The linear regression of percentage of food dislikes versus concentrations of quinine is $Y = 23{\cdot}167 - 2{\cdot}03X$. The probability that our sample came from a population of linear regression of zero is 0·001 using Student's *t* distribution.

The correlation between percentage food dislikes and quinine concentrations is +0·272. Although this accounts for only 7·5 per cent of the variance, it is significant at the 0·001 level of confidence.

While the source of food preferences and aversions is admittedly complex, the evidence indicates that taste-acute individuals have more food dislikes or that the number of foods disliked grows in proportion as the taste threshold for bitter compounds decreases. These findings bear on the adaptive significance [illegible] phenylthiocarbamide taste polymorphism[6], and t[illegible] indicate the need for item-analysis of food aversi[illegible] and attention to the mediating effect of cer[illegible] thyroxine precursors known to alter and to influe[illegible] thresholds of taste[7].

This work has been supported by the State of Ohio, Department of Mental Hygiene, and by grant No. *M*-2731 from the United States Public Health Service, National Institutes of Health. We are indebted to Dr. B. Pasamanick, Columbus, for helpful advice and assistance.

R. FISCHER
F. GRIFFIN
S. ENGLAND
S. M. GARN

Columbus Psychiatric Institute,
Research Division,
Ohio State University Health Centre,
Columbus, Ohio.
Fels Research Institute,
Yellow Springs, Ohio.

---

[1] Smith, W., Powell, E. K., and Ross, S., *J. Abnormal and Soc. Psych.*, **50**, 101 (1955); Eppright, E. S., *Iowa State Res. Bull.*, 376 (1950).

[2] Fischer, R., and Griffin, F., *Experientia*, **15**, 447 (1959).

[3] Garn, S. M., and Kagan, J. (unpublished work).

[4] Harris, H., and Kalmus, H., *Ann. Eugenics*, **15**, 24 (1949).

[5] Siegel, S., *Nonparametric Statistics* (McGraw-Hill, New York, 1956).

[6] Boyd, W. C., *Genetics and the Races of Man* (Little Brown, Boston, 1950).

[7] Fischer, R., and Griffin, F., *Experientia*, **17**, 36 (1961).

[8] Griffin, F., and Fischer, R., *Nature*, **187**, 417 (1960).

material make it possible by X-ray microscopic procedures to extract information about chemical composition and crystallographic structure of localized areas in a microscopic specimen. It is with these applications in mind that Drs. V. E. Cosslett and W. C. Nixon have composed their monograph *X-Ray Microscopy*, published in the series "Cambridge Monographs on Physics", by Cambridge University Press. In their presentation the authors have had excellent sources of material, such as those which have appeared in the proceedings of two international symposia on X-ray microscopy and X-ray micro-analysis held in Cambridge during 1956 and Stockholm 1959, respectively. A third symposium of this kind is planned at Stanford University, California, 1962. But besides a very careful recording of the literature in the field of X-ray microscopy, the book contains unpublished observations from the authors' own laboratory.

The techniques for the production of X-ray images by contact microradiography, projection X-ray microscopy, reflexion X-ray microscopy and ome other imaging procedures are well covered and ogether with the chapter on the production of X-rays a reader, both the physicist and the biologist, can get an advanced knowledge of the field.

The physics of X-ray microscopy differs from that of common X-ray physics, such as that used in X-ray diagnosis or X-ray therapy in that soft and very soft X-rays have to be used. At present, unfortunately, the numerical values of the X-ray constants, for example, the mass-absorption coefficients, for these soft X-rays, 1–50 Å., are not known with such a high precision as is desired, and in many cases no actual measurements are available at all and therefore only calculated values can be given. It is to be hoped that with the increasing use of X-ray micro-analytical procedures also the X-ray constants in the soft and very soft X-ray regions will be experimentally determined by precision measurements.

The monograph covers the three main forms of analytical applications of X-ray microscopy, namely, absorption micro-analysis, emission micro-analysis and micro-diffraction procedures. The techniques for specimen preparation are given both for inorganic and organic material, and an extensive presentation is devoted to X-ray microscopy of biological material.

It is clear that the possibilities of applying X-ray microscopic procedures to a variety of research problems now are fully established. Among the merits of these non-destructive techniques can be mentioned that it is possible to determine, for example, water and dry weight in a localized volume of a biological specimen less than one cubic micron in size. The quantities determined are in the order of 10^{-12}–10^{-15} gm., and the analytical error is in the order of 5 per cent. In the field of mineralogy and metallurgy the X-ray microscopic procedures, especially the scanning microprobe techniques for elementary analysis by emission, have proved to be most powerful tools. The same can be said about the micro-diffraction procedures; it has been possible to record diffraction patterns of crystals only about 1μ in diameter by such X-ray optical methods.

The field of X-ray microscopy and X-ray micro-analysis is rapidly expanding, and the book produced by Dr. Cosslett and Dr. Nixon is highly recommended all those who have problems in analysing very ll volumes of specimen, whether these workers are in mineralogy, metallurgy or in organic chem- ology or medicine.

ARNE ENGSTRÖM

MANUAL OF BACTERIA AND ACTINOMYCES

Diagnostik der Bakterien und Actinomyceten

Von Prof. N. A. Krassilnikov. Pp. xi + 813. (Jena: Gustav Fischer Verlag, 1959.) 59 D.M.

THE distinguished Soviet author of this manual of bacteria and actinomyces bases his classification on the following four criteria: (1) phylogenesis, and in particular his own view of this inspired guesswork; (2) morphological characteristics; (3) habitat; (4) variability under experimental conditions.

In accordance with his phylogenetic point of view there are four classes of micro-organisms, namely, Actinomycetes, Eubacteriae, Myxobacteriae and Spirochaetae, all of which have been elevated from Orders within Class II Schizomycetes of Bergey's *Classification* (seventh edition). The four criteria for classification have resulted in a far-reaching re-arrangement of orders, families and even species within the families, at the same time excluding conceptions such as sub-orders and tribes. The international principles of taxonomy are largely disregarded.

Keys are provided for the orders, families and species which make it possible to find and identify a particular species within this system if one knows what one is looking for. However, if doubt exists about some characteristic of a certain species this *Diagnostic of Bacteria and Actinomycetes* is of little help and even often misleading.

To quote some examples, the diphtheria bacillus can be found in the class of Actinomycetes, the order of Mycobacteriales, the family of Mycobacteriaceae and the genus *Mycobacterium*, and it figures as a species together with *M. phlei*, *M. tuberculosis* and *M. leprae*. Of the latter it is stated that cultures grow on blood agar plates, broth, serum and substrates containing glycerin. Disregarding the completely erroneous statement on the culture of *M. leprae*, it appears that the reasons for including corynebacteria in the genus *Mycobacterium* are: branching, which, according to the author, occurs rarely and only on certain media and under certain conditions of growth, and marked sensitivity to the antibiotic streptomycin. Organisms hitherto known within the separate genera *Escherichia*, *Salmonella*, *Shigella*, *Haemophilus*, *Pasteurella*, *Brucella*, *Bordetella*, *Klebsiella*, *Aerobacter*, *Proteus* appear merely as species of the genus *Bacterium* within the class of Eubacteria. To quote an instance of inadequacy, the *X* and *V* requirements of hæmophili are not mentioned though it is stated in the case of *Haemophilus influenzae* that growth is only obtained "in the presence of serum or animal tissues, and that additional growth factors are required". "*H. parainfluenzae* cannot be distinguished from *H. influenzae* as regards morphology, culture and physiology". In our present state of reasonably advanced knowledge the task of writing a reliable systematic of microbiology appears to be beyond the capabilities of any single person, which is probably the reason for such glaringly erroneous statements. For future editions Krassilnikov would do well to extend consultation of a few colleagues in a few fields of microbiology to collaboration with many experts in many fields. In mitigation the author's statement in the preface must be quoted that "the work is intended mainly for bacteriologists working in general, agricultural, soil, water and industrial microbiology". Yet, this statement does not make it any easier to diagnose, with the help of this manual, a strain belonging to, say, the hæmophili.

Krassilnikov would be perfectly justified in using his views on phylogenesis, morphology and habitat as a basis for his classification, but most experienced microbiologists in the West would think it quite wrong to make experimental variability and all that that term implies a criterion for classification. Thus the presence of an antibiotic in a culture may lead to bizarre forms which, if taken into account, will distort the criterion of morphology: and how, for example, is a freshly isolated strain of *M. tuberculosis* resistant to streptomycin to be placed in this scheme of things? In the last analysis the author's fourth criterion is the fundamental difficulty for a microbiologist with Western training. It appears that 'Michurinism' is responsible for this divergence of outlook in view of Krassilnikov's statement that external factors are capable of causing changes of the living substance of micro-organisms while denying that any such changes are governed by genes.

The book is well produced and printed, with 285 figures, including photographs of cultures, line drawings and photomicrographs, the latter of inferior standard. The translation from the Russian is competently done on the whole though quite a number of errors have crept in due to the transcription of the names of Western authors from the phonetic spelling in the Russian original text. There are 33 pages of indexes to literature and 53 to organisms.

K. ZINNEMANN

SATELLITE AND SPACE ORBITS

Space Trajectories

A Symposium sponsored by the American Astronautical Society, the Advanced Research Projects Agency, and Radiation Incorporated. Edited by the Technical Staff, Research Division, Radiation Incorporated. Pp. x + 298. (New York : Academic Press, Inc. ; London : Academic Press, Inc. (London), Ltd., 1960.) 12 dollars.

THIS volume is the record of the papers presented at the Symposium on Space Trajectories held in December 1959 and attended by representatives of various companies and institutions in the United States. The items vary widely in their quality and length. R. Hermann contributes a lengthy and excellent paper discussing re-entry trajectories and the problems of hypersonic flow. There is an admirable review of space manœuvres and rendezvous by A. R. Tanguay, and D. C. Hock supplements this with a useful survey of optimization problems, based largely on D. F. Lawden's work. Several papers deal with the detailed computations of satellite orbits, covering both the choice of analytical method and the numerical techniques : the phase of rampant individualism which at present characterizes this complex subject is reflected by the papers, in which, rather bewilderingly, almost every author advocates a different method. It will probably be some years before natural selection weeds out the weaker theories and methods. Perhaps the weakest method (despite its author's commendations) is that described on p. 266, in which the apogee height obtained, approximately 1,400 miles, is apparently in error by about 1,000 miles.

Volumes of individual papers are often marred b errors, inconsistencies and repetitions, and this boo has its share. The corporate wisdom of the edito (who sign themselves at the end of the preface "Radiation Incorporated") has not prevented fr quent mis-spellings of authors' names—Sir Haro Jeffreys enjoys the distinction of having his nam mis-spelt in two different ways—and there are man other irritating peccadilloes. For example, the legen on Fig. 2 on p. 217 is irrelevant and should have bee removed ; but Fig. 2 then becomes the same as Fig. on p. 83 and is superfluous. Also the introductor pep-talk, which has the curious title "Military Space Taking the High Ground", should have been prune of its nationalistic sentiments before being include in a technical book. To sum up, the book contai much to interest the specialist, but cannot be rec mended as a general introduction to the subject.

D. G. KING-HELE

RUMINANT DIGESTION AND NUTRITION

Digestive Physiology and Nutrition of the Ruminan

Proceedings of the University of Nottingham Sevent Easter School in Agricultural Science, 1960. Edite by Dr. D. Lewis. Pp. ix + 297. (London : Butterworth and Co. (Publishers), Ltd., 1961.) 50*s*.

THE importance of ruminants in agricultura economy has provided an effective stimul to the study of their nutrition for more than a centur Perhaps it was natural that the earlier work w concentrated rather on dietetics, with obvious pract cal applications, than on the physiological and bi chemical mechanisms governing the digestion an metabolism of feedstuffs. Nevertheless, it w recognized, long ago, that the most striking differen between ruminants and the familiar monogast animals was the capacity of the former to utilize cellulose as an energy source comparable in value wit starches and sugars. It is one of the most remarkabl features in evolution that only very rarely hav animals acquired the capacity to secrete cellulolyti enzymes—and this capacity does not seem to occ in any vertebrate. Cellulolysis in the digestive trac is essentially a function of microbial activity and, i the ruminant, provision has been made for this activit to take place in the so-called fore-stomach—a complex of three pouches developed from the cardiac region of the more primitive stomach. This development has led to extraordinarily complicated anatomical, physiological, microbiological and nutritional consequences. For more than a century, sporadic—and often brilliant—research contributions have been made in several facets of this field, but it was not until the 1930's that the subject started to receive the attention it merited ; since then interest has steadily accelerated. So rapid has been the development that a student must needs go to the original literature and to a handful of reviews for a survey of the field.

Especially welcome therefore is this recent publication. It is a collection of 23 short papers presented at the University of Nottingham's Seventh Easter School in Agricultural Science, held in 1960. In the space of some 270 pages it was obviously not poss to give a complete account of the present sta this subject, but a great deal of ground is co the 25 authors, all of whom have ma research contributions. Each chapter with a short list of selected references which will give ready access to the appropriate literature.

The work is conveniently divided into three sections. Under "Physiology of the Rumen" consideration is given to the movements and nervous control of the stomach, rumination, eructation, absorption and the flow of digesta. It is interesting to note that the phenomena associated with rumination, in spite of intensive, imaginative and ingenious research, still presents many unsolved problems. So too does the complex sequence involved in eructation—this latter function is especially important as its failure leads swiftly to the fatal consequence of hoven or bloat, a major disease in both dairy and beef cattle.

In the section "Metabolism in the Rumen" a brief outline of rumen microbiology is given, accounts of the fate of carbohydrates, proteins and lipids, and discussions of special techniques applicable to these fields. The microbiota of the rumen must surely be one of the most fascinating collections of organisms in a natural habitat ; the delicately poised system in the rumen, so easy of access for research, is potentially a most fertile source of material for the ecologist who is interested in the dynamics of natural microbial populations.

The third section, on "Ruminant Nutrition", makes no attempt to cover the conventional fields which are adequately treated in many text-books. Attention is focused on a few actively growing points, notably endocrine relations in metabolism and nutrition, and the synthesis of vitamins and microbial proteins in the rumen ; also, the recent advances in ruminant physiology have awakened renewed interest in energy utilization, to which two chapters are devoted.

In spite of the limitations inseparable from any compilation based on a series of conference papers, this book will prove valuable to all students of ruminant nutrition and will provide a good background for readers interested in the metabolic diseases of ruminants.

I. W. McDonald

TUMOUR METABOLISM

The Glycolysis and Respiration of Tumours

By Alan C. Aisenberg. Pp. xiii + 224. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 8 dollars.

THE present time seems quite appropriate for a dispassionate survey of our present-day knowledge and theorizing on the respiration and glycolysis of tumours. This subject begins with the original observations of Otto Warburg, made in 1923. The technical basis of these was important in itself: the introduction of the use of the thin slice of fresh tissue as experimental material and the development by Warburg of manometric methods based on those of Haldane and Barcroft; two of the most fruitful contributions to experimental biochemistry.

These are accepted. What is controversial concerns mainly the interpretation of these measurements in relation to the problem of cancer, and on this subject argument has tended to be hasty, intemperate and acrimonious, especially by the 'all-or-noth[illegible]ng' school, who will accept only a single key to [illegible]er, and will discard everything less universal. [illegible] other side of the picture, Warburg himself [illegible] more fervent disciples have sometimes [illegible]ush their case too far against the opposing weight of evidence, or at least to turn a blind eye to inconvenient experimental findings.

The primary beliefs of the Warburg school are briefly as follows: (*a*) in relation to tumour growth, and to the neoplastic process in general, energy-yielding metabolism is all-important; the rest is secondary. (*b*) While growing tissues, both embryonic and neoplastic, when maintained anaerobically have a high rate of glycolysis (that is, lactic acid formation from glucose), aerobically only the tumour tissues show strong glycolysis. (*c*) There is a disturbance in the respiration of tumours which is not only the cause of their aerobic glycolysis but also which is fundamental to the nature of cancer.

As Dr. Aisenberg clearly brings out in his very satisfactory review of the relevant factual information, it is quite easy to assemble data either in support of each of the above dogmas, or contrary to them. Indeed, of the 538 literature references cited, a fair proportion are regarded by their authors as providing decisive evidence one way or the other. The fact that honest and capable workers should find themselves so divided, even sometimes on the experimental findings themselves, is a good reason for the publication of this book, which presents an unbiased and reliable account of the work up to 1960 on the respiration and glycolysis of tumour tissue, and, where the comparison is available, on related normal tissues.

In spite of all the work summarized in this useful book, I am left with an opinion not essentially different from that which I put forward in *The Cancer Review* in 1931: "Perhaps it might even have been assumed to be improbable from the outset that such fine distinctions as exist between various types of malignant and non-malignant growths should be wholly explicable in terms of simple measurement of respiration and glycolysis". Surely the respiration of a tissue should not be assessed for this purpose solely in terms of its rate of oxygen uptake, when it is now very much clearer than when the above words were written that tissue respiration is merely the overall expression of an exceedingly complex chain of enzymic reactions, involving alternative substrates, alternative metabolic pathways, alternative co-enzymes, and alternative routes of energy utilization. Nor can the entity 'aerobic glycolysis' be conveniently divorced from the complex balance of enzymic activities and sub-cellular structure by means of which it is regulated. Finally, the control of synthetic reactions, especially of biosynthesis of protein and of nucleic acids, needs to be considered in relationship to what Krebs has called 'pacemaker' reactions, which, though all-important in regulation of cell metabolism, may constitute only a small part —though a determining one—in the overall crude picture of 'respiration and glycolysis'. The author of this carefully 'neutral' book might not perhaps be willing to go so far, but his well-balanced record of thirty-seven years of experiment, inference and controversy should assist towards a happy issue to this vexed question.

The book is generally excellently produced by the Academic Press, and is a publication of the Cancer Commission of Harvard University. Minor blemishes occur in the spelling of some names (Lipmann, p. 67; Stickland, p. 217); the date of Warburg's book, *Stoffwechsel der Tumoren* (p. 218), was 1926; "anaerobic glycolysis", attributed on p. 14, line 8, only to the very young embryo, should read "aerobic glycolysis".

F. Dickens

A Clinical Prospect of the Cancer Problem

By Prof. D. W. Smithers. (Monographs on Neoplastic Various Sites.) Pp. xv + 232. (Edinburgh and London: E. and S. Livingstone, Ltd., 1960.) 37*s.* 6*d.* net.

MORE than five years ago Prof. D. W. Smithers undertook to act as general editor of a series of monographs on neoplastic diseases at various sites, and four books in this series have already been published, dealing with cancer of the lung, bladder, rectum and œsophagus. Other volumes dealing with the prostate, brain, larynx and pharynx are also in preparation. The justification for this piece-meal way of approach to the cancer problem is derived from the fact that, from the clinical point of view, cancer of each organ of the body has its own particular characteristics, affecting ætiology, pathology and treatment.

Each volume in the series of monographs on neoplastic disease at various sites is edited by someone with special experience in this particular field, and this series of books is proving to be of exceptional value to those doctors who are engaged in the diagnosis and treatment of cancer of the different organs of the body selected for inclusion in the series. But to fill in the gaps another monograph was also obviously needed, one which would survey the cancer problem as a whole yet still preserve the clinical point of view. This necessary introductory volume has now been written by Prof. D. W. Smithers himself.

He has unique qualifications for this task. Not only is he the general editor of the whole series (and incidentally the originator of the idea) but also his work as director of the Radiotherapy Department of the Royal Marsden Hospital and Institute of Cancer Research has brought him into contact with clinicians and research worers concerned with every aspect of human cancer.

Prof. Smithers begins by saying that his book presents "a personal view of the cancer problem". It tends to be "speculative and philosophic" in character. It is certainly original, stimulating and provocative, and provides an admirable introduction to this useful series of monographs.

Cosmology

By Prof. H. Bondi. Second edition. (Cambridge Monographs on Physics.) Pp. viii + 182. (Cambridge: At the University Press, 1960.) 30*s.* net.

THE call for a new edition of this book is a tribute to the excellence of the book itself and also an indication of the vitality of its subject. Ever since the 'modern' work in cosmology was started by Einstein in 1917, or thereabouts, as soon as it has seemed to some workers that a stage had been reached when little more could be done, other workers have revitalized the subject by some major idea or discovery. Thus, for example, Prof. Bondi and his colleagues produced the ideas of continual creation and of steady-state cosmology in 1948, Walter Baade discovered the need for a far-reaching revision of the distance scale in 1952, and this year Prof. M. Ryle and his colleagues have published their new surveys of remote radio sources. To some of us it seems indeed that the subject still awaits the key discovery that may be expected to show the significance of all that has been done hitherto. Anyhow, anyone wanting to make a serious study of the subject at present, and probably for a long time to come, needs to know all that is contained in this book, and he is fortunate indeed in being given the material in such a clear, vigorous and stimulating form.

W. H. McCREA

The Use of Organic Reagents in Inorganic Analysis

By A. I. Busev and N. G. Polianskii. Translated from the Russian by J. T. Greaves. Pp. vi + 76. (London and New York: Pergamon Press, 1960.) 21*s.* net.

THIS translation from the original Russian has been produced, for reasons of celerity and economy, by non-letterpress setting and photolithography. The final result, although lacking the attractiveness of conventional printing, gives a quite legible text.

No booklet of seventy pages could possibly present a detailed practical description of the many applications of organic reagents in the analytical field. The present volume provides extended abstracts of the more important papers on the use of organic reagents, these abstracts being supported by a comprehensive bibliographical and reference section.

The first chapter of the book is devoted to general problems associated with the use of organic reagents for example, specificity, functional groups, masking reagents, effect on redox potentials, and on catalytic reactions, etc.

The determination of cationic elements is dealt with sequentially according to the group system of the Periodic Classification, the presentation being both lucid and informative. No mention is made of anion-reagent complexes.

This is a useful reference book for the practising analyst or researcher in analytical methods, and it also gives an interesting indication of the keen interest taken by Russian chemists in the work on the application of the newer organic reagents to quantitative analytical problems.

D. T. LEWIS

Die Cactaceae

Handbuch der Kakteenkunde. Von Curt Backeberg. Band 5: Cereoideae (Boreocactinae). Pp. xxxix–xliv + 2631–3544 + tafeln 212–255. (Jena: Gustav Fischer Verlag, 1961.) 107 D.M.

HERR BACKEBERG'S fifth volume concludes his vast systematic treatment of the Cactaceae with the remaining groups of the Cereoideae. A final supplementary volume with indexes is announced for next year. The present one is bigger than its predecessors because of the account of the important genus *Mamillaria,* which occupies more than 400 pages. The author recognizes 301 species and then adds descriptions of more than 100 "Wenig bekannte Arten", in alphabetical sequence, in accordance with the similar list in R. T. Craig's Handbook (1945). Herr Backeberg's adoption of the spelling *Mamillaria,* rather than the original *Mammillaria,* to be regarded as an orthographic error, will be hotly contested by many botanists, who will read his discussion in the lengthy footnote on p. 3091. Lewis and Short's Latin Dictionary certainly quotes *mammilla* as an incorrect form of *mamilla,* the diminutive of *mamma.* Th International Code of Botanical Nomencla however, retains the spelling *Mammillaria* list of *Nomina Generica Conservanda.*

N. Y.

SCIENCE AND THE CLASSICS*

By Prof. B. FARRINGTON

Formerly Professor of Classics, University College, Swansea

THE circumstances of our age seem to present us with a very real problem which may fittingly be discussed under the title of "Science and the Classics". In response to an international challenge we are engaged in expanding our educational services. The urgent need is for more scientists, more technologists, more engineers. This means a relative, if not an absolute, decline in the study of the humanities. Is this shift in the character of our education good or bad? Is there anything in the claim that the tudy of the humanities produced a more balanced ype of man? Is there in the scientific mode of training any tendency against which it is desirable to be on our guard?

I think that there is a tendency among scientists to suppose that the physical world alone is real, and that the very successful methods they have devised for dealing with it are applicable to every aspect of life. This, to my way of thinking, is a ngerous illusion. I am one with Schweitzer in lieving that "all progress in discovery and invention olves at last to a fatal result, unless we keep control over it by a corresponding progress in our pirituality"[1]. I am convinced, with him, that in the last resort, the practical can be realised only through the ethical". I fear that a preponderance of scientific elements in our education, and a decline in humanistic studies, may render us incapable of exercising effective control over our science of Nature.

Science, though it has its roots far in the past, is in the present acceptation of the term a recent growth. Four hundred years will cover its history; and the speed of its recent development gives it a still more modern aspect. But four hundred years of science have not put four thousand years of civilization out of date. There is a provincialism in time as well as in space. To think that everything has been changed in the past four hundred years is a mistake. The world did not begin with Galileo and Newton. All culture is not merely scientific culture; but all culture is a mode of thought, and man should try to think with the whole of his past. This is the specific business of the humanistic disciplines.

But I trust that no one will take me for an enemy of science. Twenty-five years ago I concluded a little book on the history of Greek science[2] with some words I should like to recall. "It seems impossible", I then wrote, "that a stable society should ever estab- h itself without the positive knowledge science has offer as a universal subject of education and the rality science imposes as an element in its law." I still stand by that to-day. But I did not believe n, and I do not believe now, that either the aims, the method, or the achievement of science give unds for hope that a stable society could ever be unded on science alone. The sciences are the reation of a specialized use of the mind. They are ot its only valid use. It is not true, as seems now be believed by many, that it is science alone which gs us into contact with 'reality'. The most portant aspects of reality for man are those that rn his own actions and aspirations, and these, for the most part, are not amenable to quantitative analysis; they elude the scrutiny of the exact sciences. Thought about them has not been radically altered by the discovery of the scientific method. Conceptual thought is an older and more necessary tool than the scientific method. Understanding is not always helped by the accumulation of information. Poverty of ideas is a more effectual bar to wise action than the absence of statistics. The intuitive powers of the mind, dependent more on sympathy and imagination than on exact information, open the door to the moral and æsthetic world, that is to say, to that aspect of reality ultimately decisive for our happiness. Neither technological nor sociological knowledge is of any use to us, as Shelley observed long ago, if we lack "the creative faculty to imagine what we know, and the generous impulse to act what we imagine".

Francis Bacon was of the same opinion. He never countenanced the illusion that scientific or technological progress in themselves could benefit mankind. They could only bring an increase of power, which could be used for good or evil according to the disposition of the will. His opinion is particularly worthy of attention since his advocacy played a decisive part in the technological revolution. He convinced his age both of the need for an immensely increased store of factual information, and of the dazzling potentialities of the science that could be raised on this foundation. But we should not pass over his fear lest "from the unlocking of the paths of sense and the enkindling of a greater light in nature any unbelief or darkness should arise in our minds to shut out the knowledge of the divine mysteries". Nor did he leave us in any doubt as to what was, in his eyes, the chief of the divine mysteries. "I would address", he writes, "one general admonition to all, that they consider what are the true ends of knowledge and that they seek it not either for pleasure of the mind, or for contention, or for superiority over others, or for profit, or fame, or power, or for any of these inferior things; but for the benefit and use of life; and that they perfect and govern it in charity." These essentials of which Bacon speaks, namely, "the true ends of knowledge", "the benefit and use of life", and the supreme duty of charity, are no part of a scientific training. The question to consider is whether the apprehension of these aspects of reality has, in fact, gained anything by the scientific revolution and whether it stands to gain anything in the future.

Bronowski, in his recently published *Science and Human Values*—a wise book, well-suited to our present needs—forcefully argues the case for a fundamental identity between the moral, æsthetic, and scientific imagination. The processes, he claims, by which we deepen and advance our understanding of the good, the beautiful, and the true, are essentially one. In each case men seek to find unity in variety, to connect things which previously lay apart. The creative flash in which the unity is perceived is in each case the same. I am sure there is some truth in this contention. Particularly do I appreciate his insistence that science is not the collecting of facts, but the creating of concepts. With an argument conducted at this level I have no quarrel. Nevertheless,

---

bstance of a lecture delivered at Hatfield Technical College ne 2.

I suspect that at the humdrum level of ordinary daily practice there remains an urgent problem which Bronowski's analysis ignores.

Sir Bernard Lovell recently said that, of all the scientists that have ever existed on Earth, 90 per cent are alive at the present moment. Think of the sudden expansion of scientific training that these figures imply. Tens of thousands, hundreds of thousands, of young men and women are now undergoing an initiation into scientific method. I welcome this. All my conscious life I have wished to see it. I believe, and hope, that it is destined to increase. But I am not convinced that such a transformation of human consciousness as this shift in education implies is unattended by the gravest threat. We have the prophetic fear of Bacon, we have the humble testimony of Darwin, that the necessary drudgery of science may dim the more imaginative powers of the mind on which lively apprehension of moral and æsthetic reality depends.

That this is so, I feel. Why it should be so is an obscure question on which I must now try, not very optimistically, to shed a little light. First, I should like to direct attention to the vast difference in time-scale between moral and æsthetic development on one hand and science on the other. No anthropologist that I have ever heard of claims to find among what we call primitive peoples the equivalent of a modern scientist. But anthropologists do profess to recognize in these simple societies philosophers, sages, leaders, law-givers, creators of admirable and viable patterns of social life. Prof. Ashley Montagu, for example, asserts that "in spite of our enormous technological advances we are spiritually, and as human beings, not the equals of the average Australian aboriginal or the average Eskimo—we are very definitely their inferiors"[3]. The reason for this early development of morality seems plain. If any human society at all is to exist, and such have existed for hundreds of thousands of years, its members must evolve or accept some rules of living together, some sense of community, some morality. This is true even of food-gatherers. Morality can be highly developed in technologically primitive societies.

The exploitation of the external environment, however, which is rudimentary science, has always introduced problems of social organization which swamp moral standards. Schweitzer's dictum that "all progress in discovery and invention evolves at last to a fatal result, unless we can manage to keep control over it by a corresponding progress in our spirituality", does not apply only to modern conditions, but also to all history. It was a commonplace with the philosophers of classical antiquity. Neither Platonist, nor Epicurean, nor Stoic would be surprised to find that the period of our greatest triumphs in science and technology coincides with the overwhelming of our humanity and sense of community. The gas chamber, the napalm bomb, the systematic employment of every refinement of torture are characteristics of a high material civilization. The bond of human fellowship is very old and very fragile. Morality has usually come off second best in its encounters with the social problems created by the increase of prosperity. The same appears to be true of man's æsthetic sensibility. It is an early starter and an easy victim. As the cave-paintings of primitive men remind us, hunters could create works of art revealing a power of visual imagination which eludes potters and farmers.

I have said that we should try to think with the whole of our past. One of the thoughts most strongly induced by this practice concerns the unevenness of progress. The most striking gains prove, on examination, to have involved serious loss. An example is alphabetic writing. This surely, one would think, is a wholly beneficent achievement of human ingenuity. It was, in fact, a necessary pre-condition of the emergence of large-scale organization, yet it wrought so decisive a change in human consciousness that it all but obliterated the memory, and certainly destroyed the understanding, of the age-old culture of pre-literate societies. The invention of the alphabet blinded men to the knowledge of some thousands of years of our human story. Much research and a great effort of the historical imagination were needed to restore to the twentieth century an appreciation of the oral cultures which writing destroyed. Yet so accomplished a stylist as Plato was near enough to the world of oral tradition to view the dissemination of the alphabet with a critical eye. He had two main fears. One, that writing would impair the memory. Has it not done so? "Without writing," notes Tom Harrisson, "memory is perfect, tradition exact." Secondly, Plato felt that a book was an inadequate substitute for a living teacher. We may note that the two greatest teachers in the European tradition, Socrates and Jesus, never wrote a book. In our own educational practice at its best, books are treated only as an adjunct to the spoken word.

But this is far from being the end of the matter. There are other penalties literacy exacts: sluggish powers of observation, inability to learn directly from observation, helplessness in the face of Nature. We learn to read and forget how to look and listen. Paul Radin speaks of "the distortion in our whole psychic life, and in our whole apperception of external realities, produced by the alphabet". Schweitzer reminds those charged with the education of simpler peoples that "civilization began with the handicrafts and agriculture, not with reading and writing". Our own Boy Scouts, Girl Guides, and other suc organizations usefully make up the deficiencies of an education too exclusively concentrated on books.

Furthermore, we should note, as particularly relevant to our subject, that the unique vitality a charm of ancient Greek literature arises from th fact that it remained for long much closer to the oral tradition than our modern manner of studying it suggests. Greek boys, like ours, studied grammar but grammar then had a much fuller meaning tha it has now. It meant, not so much learning to read, as to read aloud. A written text was then treated like a musical score. The grammar lesson included elocution. It taught the correct delivery for the various styles: epic, lyric, tragedy, comedy. It concerned itself also with pitch and intonation on which the beauty of composition was thought depend, and on strict observance of phrasin without which the full meaning was obscured. S long as Greek civilization lasted, in every city the founded round the Mediterranean, in the Black Se or the Near East, they preserved their cultur tradition by setting up schools where the boys learne to *recite* Homer, and theatres where adults *hea* great tragedies performed. As for silent readin it can only be said that the Greeks did not have word for it. So when Philip in the *Acts* encounte the Ethiopian eunuch he *heard* him reading prophet Esaias. Some five centuries later whe servants of St. Ambrose *saw* him reading the scrip and *heard* nothing, they took it for a miracle. Silent reading had begun. Literature was on the way to becoming voiceless, a matter of visual symbols which the eye is drilled to take in as quickly as possible. Mercifully, in this case, we manage to some extent to retain the advantages of both worlds. Mere information we take in quickly by the eye alone. Something of the glory of the spoken word as it existed in classical antiquity is preserved in the Church, in the theatre, and in our new medium of mass communication, broadcasting.

These are examples, chosen almost at random, to remind us of the ambivalent nature of social change. Technological advance sets society problems too difficult for its moral resources to handle. The acquisition of powers of abstraction weakens the visual imagination. The art of writing, which in one sense may be said to constitute the memory of mankind, in another sense destroys that memory by externalizing it. Why, then, should we wonder if it should appear that the development of the exact sciences damage the apprehension of all those aspects of reality that are not amenable to its method of analysis.

That this, in fact, is so, the history of ancient Greece strongly suggests. The Greeks were not yet a scientific people when, between the eighth and fourth centuries B.C., they created that literature which still to-day constitutes the most vital part of what we Europeans generally mean by the humanities. Before the end of the fourth century, epic, lyric, drama, history, and philosophy already existed in their most original and perfect examples. Nor is it true, as seems now sometimes to be supposed, that this literature was a sort of superfluous adornment of life, inessential, but adding grace and charm. This is by no means so. On the contrary, it was creative in the fullest sense of the word. It was a new mode of thought, an exploration of reality, and, indeed, the very substance of a new reality arising out of the old. Men then thought about themselves as they had learned how to do from Homer, Pindar, Aeschylus, Thucydides, and Plato. The minds, the characters, the passions of men, the destinies of individuals and of States had been portrayed by thinkers profoundly capable of reflexion on their national traditions and their personal experience, and gifted with the imagination to embody them in new art forms : and the intellectual adventure was carried farther still. Men were equipped with the concepts and the vocabulary to discuss intelligently the facts of society and politics. But not only that. They succeeded also in distinguishing between life itself and its representation in the various arts. They created not only a philosophy of life but also a philosophy of art. Aristotle, for example, freed the criticism of the drama from its entanglement with morals and politics, assigned it a distinct function and analysed its constituent elements in terms which are still the stock-in-trade of all critics. His pupil Aristoxenus did a similar service for music, distinguishing acoustics, which is properly concerned only with the physical antecedents of sound, from music itself in which the sounds are considered in their relation to one another in an integrated composition. Thus was created the humanist consciousness, a new awareness of social life through the instrumentality of literature and art. This, to my way of thinking, is still fundamental, and in no way replaceable by later modes of thought.

Then, after the fourth century, a change in the mental climate set in. Greek geometers set to work on the observational material accumulated by the Mesopotamian star-gazers, and the exact science of astronomy was born. It was discovered how to plot accurately the paths of the Sun, Moon, planets, and stars against a spherical background of which Earth was the centre. Thus was inaugurated a space-probe of a breath-taking kind, even if as yet it was accomplished only in the imagination of the mathematician. The new science had practical advantages for agriculture, navigation, and geodesy. It also adapted the religious consciousness to the new political conditions. Improved communications had begun to unify mankind. It was no longer expedient that different peoples should be divided by their local religious allegiances. The mathematical heavens became the chief object of religious awe. The countless anthropomorphic divinities of sky, sea and land were one by one identified with Sun, Moon, planets, and stars. All peoples were united in a common adoration of universal gods.

So far, so good. But it was not long before this stupendous astronomical conception took on an astrological complexion. When the Sun, who rules the day, and the Moon, who rules the night, had been identified with Apollo and Diana, that is with gods in human shape, when Venus, the goddess of love, and Mars, the god of war, when father Jupiter, strong in wisdom, and Mercury, guide of travellers and friend of thieves and business-men, had been installed as bright luminaries in the sky, was it to be supposed that this lessened their influence over the affairs of men ? Their influence, now that they were enthroned in the heavens, was stronger than ever. What is more, if only the correct technique could be devised, it was calculable. This technique the astronomers bit by bit perfected. It is a mistake to think of astrology as a popular superstition. It was a highly sophisticated way of misinterpreting the universe. Long before astrology was invented the people had their humbler ways of guessing what the future had in store. It was the astronomers, bending their mathematical brains to the task, who established the astrological technique. So vast, so complicated, so daring, so seductive a conception was the work of superior minds. The creators and defenders of this crippling superstition were the Hipparchuses and the Ptolemies. They had, in the language of Bronowski, brought together in one creative flash of insight two aspects of reality that had previously lain apart and fused them into one. It was to take something stronger than science to tear them apart again.[4]

Here, then, is a subject supremely worth pondering. The astrological view of the universe presented, in a degree unapproached by any other ancient system, the aspect of an exact science. Yet despite its scientific aspect and the fascination exercised by the exactness of its procedures, it represented a decline in culture, an impoverishment of personality, an enslavement of the will, a diminishment of man's moral and spiritual nature. Man had previously been understood in terms of what the poets, historians and philosophers had made of him—a being not exempt from weakness and villainy, but yet versatile, adventurous, resourceful, enterprising, adaptable, aspiring, and in great measure his own master. Now he had shrunk to being the puppet of the stars, whose relation to the powers that ruled his destiny could be represented in a mathematical diagram. No longer a wily much-enduring Ulysses, a magnanimous Hector, a Prometheus hurling defiance at the gods, a wise law-giver, a subtle politician, or a martyred philosopher, he had become a helpless lay-figure spread-eagled in a series of concentric circles in which revolved for ever those celestial gods whose influence, streaming down into every limb and organ of his body, determined both his physical and mental constitution and the pattern of his days.

It is often imagined that this harmful superstition was dissipated by the dawn of scientific knowledge and scientific thought. This reading of history is scarcely true. Its great strength was the scientific armature which supported its fantasies, and its opponents were humanists of one sort or another. Epicurus, for example, in antiquity, whose name is, indeed, inseparably associated with the atomism of Democritus, but as the one who asserted in the teeth of that deterministic creed the autonomy of the will. It was in the name of that same freedom of the will that St. Augustine fought astrology, drawing, I believe for the first time, a clear distinction between it and the genuine science of astronomy. At the Renaissance again it was essentially this question of freedom, presenting itself this time in the guise of the dignity of man, that brought a Pico della Mirandola into the ranks of the opponents of astrology. It is in this spirit too that Shakespeare makes his Cassius exclaim :

> "The fault, dear Brutus, is not in our stars
> But in ourselves that we are underlings."

Men had awakened from a sleep of some two thousand years in which they had allowed themselves to be imposed upon by the exaggerated claims of what was, in fact, a genuine advance in knowledge. Their awakening took the form of the resumption of those modes of thought which, though lacking the exactness of the new geometrical astronomy, were yet better adapted to handling those aspects of reality on which man's happiness ultimately depends. Astrology had given man confidence to believe that his understanding could reach out to embrace the stars ; but it was the stars that leaned down to hold him in their embrace. Astrology made man bold enough to declare himself a citizen of the cosmos ; but the tyrannical cosmos retorted that man was no citizen but a slave. It was only when the older humanistic culture had again asserted itself that man could take a more sober view of himself and resume his character of a humble lover of wisdom, neither boasting of his citizenship nor trembling as a slave.

This ancient example of the divergence between humanism and the exact sciences would not be worth dwelling upon were it not that it has something typical about it. Consider, for example, as a parallel from the modern world the history of Cartesianism. The contribution of Descartes to science (I can speak of it only at second-hand) was very great. He welded together Greek geometry and modern algebra, thus making a powerful instrument for the exact analysis of physical phenomena. But he was deficient both in æsthetic sensibility and historical sense, so that no warning voice held him back when it occurred to him to apply his mechanistic philosophy to problems of life and society. The results were disastrous. Regarding as but elegant trifling all that the senses, the imagination, and the memory had to offer, he trusted to the pure intellect alone and regarded the world of the analytic geometer as the sole reality. He arrived by this path at two major conclusions. The first was that the only existence he could be absolutely certain of (except as the result of a tedious argument) was his own. The second was that animals were only machines. He was devoted to vivisection and explained the cries of his tortured dogs as the crash of broken machinery.

From the first of these opinions, namely his solipsism, his friend Gassendi, who as a Christian defender of Epicureanism was doubly steeped in the humanist tradition, tried in vain to wean him. "You will say", he writes in a letter to Descartes, " 'I am mind alone' . . . But let us talk in earnest, and tell me frankly, Do you not derive the very sounds you utter in so saying from the society in which you have lived ? And, since the sounds you utter are derived from intercourse with other men, are not the meanings of these sounds derived from the same source ?" But Descartes could not take the point. Small wonder that Thomas Hobbes, who also knew him, was wont to say that "had Descartes kept himself wholly to geometry he had been the best geometer in the world, but that his head did not lie for philosophy".

As for the second of his pronouncements, that animals are machines, it is as good an example as one could wish of that reductionism which has been called the occupational disease of scientists. Descartes, because he had succeeded in arriving at exact knowledge of certain mechanical principles, was convinced that these principles must be equally valid in a wholly different sphere. For tens of thousands of years man had been in close relationship with the other animals, and his knowledge and understanding of them had not failed to profit thereby. It is certain that without his alliance with the dog man could never have held his own against the larger Carnivora. The dog was the fifth column through which man breached and conquered the animal kingdom. Similarly the ox and the ass, the camel and the elephant, helped him to exploit his physical environment, rendering essential service to agriculture and to trade. Where would the hunter and the shepherd have been without his dog, the trader without his pack-animal, the jungle-clearer without his elephant ?

Through an experience lasting over many thousands of years man had gained an insight into the character and mentality, as well as the physical capacities, of animals both wild and domestic. He appreciated both their resemblances to himself and their essential differences. The knowledge lacked quantitative precision, but it was both true and tested. What was more, it was knowledge of whole animals in their normal habitat. All this accumulation of wisdom was ignored by Descartes' unhistorical definition. Basing himself on his laboratory experience he brushed aside all the animal lore of the past with the curt remark that "the greatest of all the prejudices we have retained from our infancy is that of believing that the beasts think". But, long before Descartes and his experiments in vivisection, men had been capable of distinguishing between the mentality of man and that of animals, without losing the capacity to distinguish between animals and machines. Man had been defined by the Greeks as the rational animal *par excellence*, but to animals the Greek thinkers had allowed sense-perception and memory, and even a limited capacity for organizing experience. Now all this traditional folk-wisdom, together with all these careful philosophical distinctions, is cast aside by a man labouring under the delusion that the only way in which animal behaviour can be understood is to treat it as if it were a branch of mechanics. This is what meant when I gave the caution at the outset of tl article that we should not allow ourselves to be dazzled by our most recent achievements but should try to think with the whole of our past. The tradition of French thought which Descartes was brushing aside can be studied equally in the humanist Montaigne, the Huguenot Palissy, or the Catholic Richeome[5].

These remarks are not intended to belittle either the greatness or the humanity of Descartes. He was, as his biographer William Wallace says, "a type of that self-reliant, harsh, and abstract spirit of science to which erudition and all the heritage of the past seems but elegant and unworthy trifling". But he had also caught from Francis Bacon a warm faith in the humanitarian spirit of science. "I believed", he remarks with regard to his discoveries in natural philosophy, "that I could not keep them concealed without greatly sinning against the law which obliges us to procure, as much as lieth in us, the general good of all men. For they have shown me that it is possible to arrive at knowledge which is very useful to life, and that instead of the speculative philosophy which is taught in the schools, a practical philosophy may be found. By means of this, knowing the power and the action of fire, water, air, stars, heavens, and all the other bodies which environ us, as distinctly as we know the various trades and crafts of our artisans, we might in the same way be able to put them to all the uses to which they are proper, and thus make ourselves, as it were, masters and possessors of nature." No, Descartes did not lack humanity ; but this only makes the more significant that typical kind of scientific blindness which seeks the explanation of the phenomena of consciousness in terms of the physical sciences.

This blindness is not a phenomenon of the seventeenth century but is as alive and vigorous now as ever it was. A recent controversy in *The Observer* shows us Dr. N. S. Sutherland of the Institute of Experimental Psychology, Oxford, insisting that "the behaviour of animals and men can ultimately be explained in terms of the chemistry of the nervous system" ; and that it is arrogant to suggest that "because we have not yet found a full explanation in physical terms for some piece of behaviour, there cannot be one". Now behaviour is a big term. It is not covered by what can be observed in a laboratory under laboratory conditions. To a humanist human behaviour covers the whole field of human history from the appearance of man on Earth to the present day. For Vico, the great eighteenth-century opponent of Cartesianism, reality was not covered by physical reality. It included all man's customs and laws, all his forms of association and his institutions, all his arts and crafts, all literature and the fine arts, all politics and religion. In this field also knowledge is a possibility. But its methods, procedures, and modes of explanation are different from those of the natural sciences, and, perhaps, more difficult. To imagine that chemistry and physics can throw light on them is to make the mistake the old Greeks made before Aristoxenus explained to them that music was not the same as acoustics. It is to suppose that a musical instrument is real and music itself a subsidary and dependent phenomenon which will clear itself up without more ado once the physics of the instrument have been explored. It would be naïve indeed to suppose that with the intensification of scientific training on the scale now reached this sort of blindness will not spread. It is idle to hope that if we give young people the training of Descartes they will automatically be capable of thinking like Vico.

In my view it is impossible to be too deeply concerned about this problem. Within the span of my active life as a university teacher the balance tipped from humanistic to scientific and technological studies. There were sound reasons for the change, and it has been accompanied by solid advantages, by no means only on the material side of life. But the process has gone too far. A university senate used to be a majority of humanists with a sprinkling of scientists, now it is a body of scientists and technologists with a sprinkling of humanists. This change of character in the academic body has meant the emergence in university life of an absolute incomprehension of the older humanistic disciplines. They are felt to be an expendable luxury. I remember a heart-to-heart talk with a Fellow of the Royal Society in a position of great responsibility. He wanted to know why it was not possible to make a definitive translation of all the Greek and Roman classics and thus be done once for all with troublesome business of learning these dead languages. This man could understand the world of Descartes, and his distinction in this kind of knowledge had won high recognition. Of Vico's world he did not even know enough to know that he knew nothing. But we liked one another, and he was kind enough to put his helpful suggestion to me, politely, in the form of a question. However, it left me to chew sadly over a passage in one of Schweitzer's books. "To-day," he writes, "thought gets no help from science . . . The newest scientific knowledge may be allied with an entirely unreflecting view of the universe. . . Our age has discovered how to divorce knowledge from thought, with the result that we have, indeed, a science that is free, but hardly any science left which reflects."

A supreme illustration of the kind of science which divorces knowledge from thought, of a science which is indeed free but has lost the power to reflect, is supplied by the Kinsey Reports. In them an unprecedented effort has been made to bring to the masses a strictly scientific, morally neutral, factual survey of sex. Nor do I deny that these reports exemplify one of the virtues of science, absolute loyalty to the ascertained fact. I believe further that its statistics may in the long run contribute to some good end. But for Kinsey and his team sex is a purely physiological fact taken in its most material aspect because this alone is measurable. Science to be science must be exact. Sex, therefore, in these reports is divested of any specifically human quality. So understood, its analysis will present little difficulty to the experimental psychologists. The chemistry of the nervous system will come into its own and there should be little difficulty in achieving a full explanation in physical terms of this piece of behaviour. All that will be missing is the relevance of this analysis to man. It will set him a high standard of the sexual activity that may be achieved in the course of a life. But it will not answer any questions even about pleasure, still less about happiness, or love, or parenthood, or the family, or any other institution of human society ; for these things do not lend themselves to exact measurement and statistical treatment. To handle such matters as these the Cartesian conception of science must be supplemented by that of Vico[6].

I come back then, to my main point, namely, the insufficiency of the natural sciences as a key to the understanding of society. It is not difficult to see why this should be so. True, there is a sense in which man is an animal like any other, a product of biological evolution : and this fact inclines us to suppose that his behaviour should therefore be intelligible in purely biological terms. But this is not so. When man developed speech and conceptual thinking he cut the umbilical cord which bound him to mother Nature. He then ceased to inherit his specifically human behaviour genetically and began to hand on the tradition of culture by education. In this sense man is not simply a child of Nature. He is a kind of super-Nature. His animal inheritance is only a part of him. The other part of him is his cultural inheritance, which is the means whereby he decides what use to make of his animal nature. In this sense, a limited but very real sense, man is both free and creative. If we seek to explain him purely biologically we rob him of his freedom and his creativity. If we wish to rouse and instruct in him his capacity for free creativity we can only do this by increasing his understanding of what, in fact, man has accomplished in the brief span of human history. There is no key to this knowledge in its full force and significance other than the humanistic disciplines. For the humanist the pageant of history is as much part of reality as the non-human reality investigated by the natural sciences. For the future prospects of mankind it has the tremendous value that it gives him the sense of what man has done and therefore the courage to try to do better. Man in the biological sense is the end-product of a chain of accidents, the passive outcome of a process which he may come to understand but did not in fact control. But his human history is his own creation. In it he can study his mistakes and achievements. It is an infinitely difficult but infinitely rewarding pursuit, and anything that can be done to encourage it is not wasting time and money on frills but getting fully to grips with reality. Nor is there any other means by which man can learn to take his destiny into his hands.

The pitfalls, however, are endless. and there is no lack of false prophets to deceive the elect. Who, for example, would suspect that a biologist so cultivated in the widest sense as Bergson was should nevertheless lead us up the garden path ? But that is just what he did when he took his philosophy of the *élan vital* out of the biological field, in which it had originated, and used it in an attempt to explain some of the most fundamental problems of cultural history. In his *Creative Evolution* the philosophy of the *élan vital*, whether we accept it or not, was a useful hypothesis in the sense that it held together, without violently distorting their true nature, a great chain of biological observations. But Bergson did not stop there. Seduced by the success of his biological theory, he sought to unlock the problems of religion and morals with the same key. He imagined, absolutely gratuitously and without a particle of supporting evidence, that the conception of the *élan vital* was identical with the vision of the great mystics, and that this vision was the true source of progress in morals and religion. The identification is completely without historical foundation. But Bergson, though he could adopt a hare-brained notion, could not write other than brilliantly. The result is that his *Two Sources of Religion and Morality*, having already misled one generation of readers, has now, after a quarter of a century, emerged as a paper-back to mislead a still wider circle. It has, of course, the usual disabling effect of all attempts to explain the human in biological terms. For the *élan vital* is not under human control, and there is absolutely nothing for any follower of Bergson to do in the way of deepening the spirituality of the race except to wait for the unpredictable moment when the *élan vital* will inspire some mystic to utter a new oracle. But if we are ever to build Jerusalem we need some handier weapon which the average man can employ in what must be a co-operative enterprise[7].

By way of summing up let me add only this. I have not forgotten the general title of the series to which I have been privileged to contribute. We were. to discuss the "Common Ground shared by Technology, Science, and Art". Neither do I forget that it is a Technical College that has inaugurated the discussion and invited me to take part in it. This emboldens me to hope that in what I have attempted to do I shall not be misunderstood. I am very much of the opinion, expounded by Sir Eric Ashby in one of his books[8], that it is a function of technology to humanize science. For it is in its technological applications that science is forced to come out of the laboratory and consider the impact of its discoveries on the everyday lives of ordinary men and women. In such a liberal atmosphere I have ventured to interpret the words 'common ground' in my own way. I have hoped to show that it ought to be common ground between us that, in spite of the overwhelming need for science and technology in the world to-day, the humanities have a distinct function. I believe that there is a fundamental aspect of reality, not susceptible of analysis by scientific methods, the understanding of which is the proper business of the humanistic disciplines.

To this sphere of reality, the sphere of human history, the approach is not by way of mathematics and the experimental method. The path is through languages and the study of the masterpieces of art and literature. These disciplines should, in my opinion, even now and in spite of other claims be given vastly increased support. This support should, of course, include facilities for the study of living languages, the knowledge of which is essential in our contracting world. But it must also concern itself with the past, through the study of what are called, perhaps prematurely, the dead languages. For they may be dead, but they will not lie down. For those of us who are heirs of the Western European tradition Hebrew, Greek, and Latin obviously come first. But we must provide also for the languages of the older civilizations of Egypt and Mesopotamia, and for the languages of other continents and civilizations. It has long been recognized as the prerogative of man that he looks before and after. We may, I suggest, be certain that our forward vision will clear when we take the trouble to understand a bit more of our past.

---

[1] This and other quotations from Schweitzer are from his *Philosophy of Civilization*, Parts 1 and 2 (A. C. Black, 1923 and 1946).

[2] *Science in Antiquity* (Home University Library, Oxford University Press).

[3] For this quotation and that which soon follows from Paul Radin, see Coomaraswamy, A. K., *The Bugbear of Literacy* (Dobson, 1947), where there is much supporting material.

[4] For the Hellenistic origin of personal astrology see Neugebauer, O., *The Exact Sciences in Antiquity*, 163 (Oxford University Press, 1951). A recent work, Hadas, Moses, *Humanism* (Allen and Unwin, 1961), brilliantly illustrates the character of Greek culture before it succumbed to astrology. Cumont, Franz, *Astrology and Religion among the Greeks and Romans* (Dover Publications) is the classic analysis of the victory of astrology, a process oversimplified in my brief account.

[5] For Montaigne see the end of the essay on *Cruelty*; for Palissy various reflexions on animal intelligence quoted in Morley, Henry, *Palissy the Potter*, 2 (1855); for Richeome, H. Brémond's *Hist. Lit. du Sentiment religieux en France*, 1.

[6] Lionel Trilling's essay in *The Liberal Imagination* (Mercury Books, 1961) on the *Kinsey Report* has influenced me.

[7] Bergson's unintentional distortion of the historical reality is examined by Loisy, *Y A-t-il deux Sources de la Religion et de la Morale ?*

[8] Ashby, Sir Eric, *Technology and the Academics* (Macmillan and Co. Ltd.).

Appearing in September

Pulsating Combustion

The Collected Works of F. H. REYNST

Edited by **M. W. THRING,** *Professor of Fuel Technology and Chemical Engineering, University of Sheffield*

The fields of application dealt with in this book are mainly boilers and gas turbines (the latter in particular for aircraft propulsion). In the introduction Reynst's novel contributions to combustion science are enumerated and references to the papers describing the ideas are given. Professor Thring distinguishes eight important new contributions to combustion technology arising from Reynst's work which are developed more or less explicitly in the different papers.

310 *pages.* *Illustrated* £5 *net* ($15.00)

Theory of the Transmission and Processing of Information

A. G. VITUSHKIN *Translation Editor* **A. D. BOOTH,** *Birkbeck College, London*

An important research monograph dealing in a rigorous manner with the estimation of the information content of various problems in tabulation. Translated from the Russian it defines mathematically the concept of the complexity of a tabulation problem (the construction of tables for functions) by using concrete problems as examples.

Theory of the Transmission represents in clear and concise terms the latest Soviet thinking in this field and will be of special importance to all Western workers.

224 *pages.* £5 *net* ($15.00)

Method of Least Squares and Principles of the Theory of Observation

Yu. V. LINNIK

This important Russian translation work gives an account of the theory of the method of least squares with emphasis on the mathematico-statistical interpretation of the results obtained from the data by this method (which, of course, only has a meaning on the natural assumption that the errors of measurement can be regarded as random quantities). The basic schemes of calculation used in applying the method are also described.

372 *pages.* 84*s. net* ($12.50)

Fundamental Physical Theory and the Concept of Consciousness

JOHAN H. GREIDANUS, *Chief Engineer, Royal Netherlands Aircraft Factory "Fokker"*

This monograph presents an attempt to introduce the concept of consciousness explicitly into fundamental physical theory. The aim is to give this at present extra-physical concept an operationally effective physical status, and to clarify, at least in principle, the relations which exist between displays on the scene of consciousness and processes in its presumed material substrate.

98 *pages.* *Approx.* 42*s.*

Experimental Correlograms and Fourier Transforms

N. F. BARBER, *Dominion Physical Laboratory, New Zealand*

This authoritative volume reviews the great variety of electrical, mechanical or optical analogue devices that have been invented from time to time for creating further Fourier transforms correlograms and power spectra. The aim is to help the reader in devising a machine to suit his own particular purposes. Fourier theory, including the convolution theorem and the correlogram-power spectrum theory, is also developed. This is done from first principles and from a physical point of view to help the student gain a working knowledge of these ideas.

142 *pages* *Illustrated* 30*s. net* ($5.00)

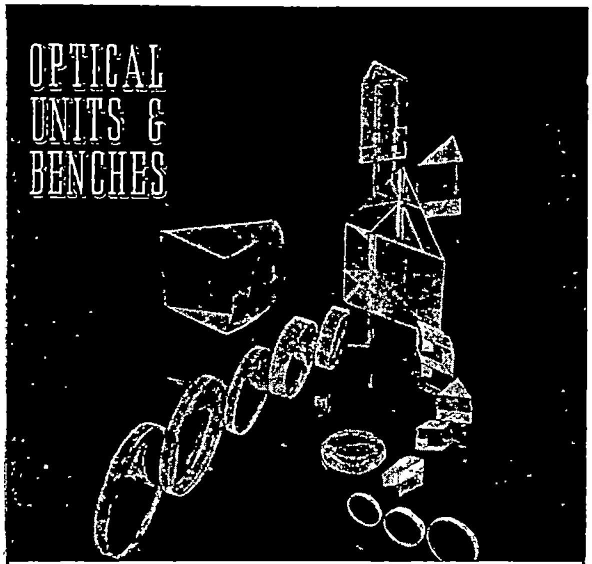
All books gladly sent on 14 days' approval without obligation

---

PERGAMON PRESS

OXFORD LONDON NEW YORK PARIS

Headington Hill Hall, Oxford. 4 & 5 *Fitzroy Square, London, W.*1. 122 *East 55th Street,*
New York 22, *N.Y. Gauthier-Villars,* 55 *Quai des Grands-Augustins, Paris* 6



OPTICAL UNITS & BENCHES

ESSENTIAL FOR RESEARCH AND EXPERIMENT

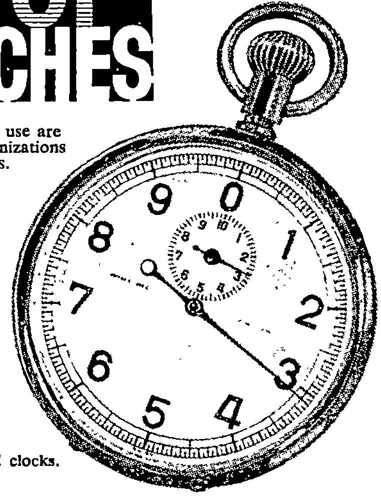
Full Catalogue from

R. & J. BECK Ltd.

69/71, Mortimer Street, London, W.1.

STOP WATCHES

for scientific and industrial use are supplied to industrial organizations and research establishments. Enquiries are welcomed.



Sectional catalogues and leaflets available:

Watches, stop watches and chronographs.

Recording apparatus.

Clocks, domestic and industrial.

Process timers and time interval meters.

Electrically controlled stop watches.

Repair Service

Watches, stop watches and clocks.

Marine chronometers.

Instruments.

Model 308 : 1/30th second escapement recording up to 10 minutes. NPL certification if required.

Sectional catalogue and leaflets available.

CAMERER CUSS

TIMING DIVISION

Makers of Good Clocks and Watches since 1788

at 54/56 NEW OXFORD ST., LONDON W.C.1
Tel : MUSeum 8968/9 *Service and Repairs: MUSeum 2255*
Our only other address: 91 Kingsway W.C.2

INTRODUCING WORTHINGTON RIBONUCLEASE A

Chromatographically Homogeneous
100 mg—$18.00
1 gram—$120.00

also

CRYSTALLINE RIBONUCLEASE

4X crystallized.

An old product with a new low price:

1 gram	$ 50.00
5 grams	225.00
10 grams	400.00

Quantity prices available on request.

For information write:

WORTHINGTON BIOCHEMICAL CORPORATION

FREEHOLD 19, NEW JERSEY

worthington

TRADITION, SCIENCE AND PRACTICE IN DIETETICS

THE third International Congress of Dietetics, organized by the British Dietetic Association and an International Committee of Dietitians, chaired by Miss D. F. Hollingsworth, of the Ministry of Agriculture, Fisheries and Food, was held at Church House, Westminster, London, during July 10–14, under the honorary presidency of Lord Boyd Orr. There were 916 registered members of the Congress from more than forty countries. The purposes of the programme were to place applied nutrition—or dietetics—in a historical setting and to show how dietitians are working throughout the world towards the better nourishment of all people. The programme was designed to allow time for discussion of scientific and of practical problems and for professional visits.

After the formal opening of the Congress by the Mayor of Westminster, Dr. Norman C. Wright, deputy director-general of the Food and Agriculture Organization of the United Nations, spoke on "International Collaboration in the Solution of Problems of Human Diet". He said that primitive man, in common with other animals, learned to procure food from his environment which provided the ingredients needed to ensure survival of the human race, but that with the development of a more complex civilization "this knowledge, acquired by trial and error rather than by scientific experiment, has been increasingly neglected". He traced the growth of international intervention in the problem of inadequate food supplies for the world's rapidly growing population, emphasizing "the vital role of women in ensuring the adequacy of family diets and of the urgent need to improve the status and education of women in . . . [the] less developed countries".

Mr. F. Le Gros Clark (London), in speaking of the "History of Human Diet", underlined the fact that "*Homo sapiens* is the only species whose dietary habits have had any history at all". He postulated that our primitive forebears developed preferences for a thoroughly mixed diet, of foods consumed mainly in their natural state, suited to the primitive mode of life and environment. He emphasized that under primitive conditions there would have been no need to consider the application of nutrition and no place for the dietitian. In his view, "the science of applied nutrition, and with it, the role of the dietitian, are the by-products of a 'civilized' mode of life" and that our task now is "to persuade men and women, whose elementary tastes and food inclinations were built into the species many centuries ago, to match their food habits to a completely changed mode of living".

Speakers from many parts of the world illustrated ways in which traditional food habits have proved to be scientifically sound: the most striking example was, perhaps, that provided by the traditional method in Central America of making tortillas by first soaking maize in lime water and thus making available bound nicotinic acid, a practice which doubtless explains the absence of pellagra in those countries. Many examples were also given of the dangers which follow the introduction of 'civilized' diets to primitive communities: for example, the increase in dental caries among the village children in Fiji and other South Pacific Islands which has followed the introduction to the diet of refined cereals and sugar. More than one speaker with public health experience in developing countries emphasized the prestige value in primitive communities of 'Western' foods and, in many places, the consequent neglect of locally grown foods, which are often of relatively greater nutritional value.

Then followed three and a half days of sessions for discussion and original communications on a variety of topics, including atherosclerosis, diet and ischæmic heart disease, and mother and child, with special reference to protein malnutrition. Other subjects discussed were dietetics and its history, therapeutic diets, feeding older people, survey work, teaching nutrition to nurses, school meals, catering problems and the education and occupations of dietitians.

The two sessions on various aspects of coronary heart disease provided ample opportunity for interchange of opinion and fact. Prof. J. J. Groen and Prof. G. Verdonk and their colleagues in the Netherlands and Belgium presented the results of their work on the influence of nutrition and ways of life on blood cholesterol and the prevalence of atherosclerosis and coronary heart disease among Trappist and Benedictine monks. They showed that the average blood cholesterol was higher among 168 Benedictine monks who lived on a 'Western' diet than among 180 Trappists who were vegetarian. There was more obesity among the Trappists than among the Benedictines, but no difference in prevalence of hypertension, myocardial infarcts, angina pectoris or electrocardiographic signs of ischæmic heart disease. Myocardial infarction appeared to be rarer among both Orders, but the other conditions mentioned occurred to much the same extent as in the general population of the Netherlands and Belgium. The conclusion reached was that the acute occlusion of a major coronary artery, leading to a macroscopic myocardial infarction, seemed to be related not to nutrition and low blood cholesterol, but to psychological factors in the Western ways of life against which monks were protected and that the nature of these factors required further investigation. Hypertension and diffuse ischæmic heart diseases were apparently not prevented by a frugal diet or by a monastic life.

Dr. J. Trémolières (Paris) discussed the many factors concerned in the study of diet and atherosclerosis. He emphasized the importance of obtaining more precise information on the diet, way of life and the cardiovascular condition of individuals. Prof. J. N. Morris and his colleagues from the Medical Research Council Social Medicine Research Unit presented the results of their attempts to devise a method of large-scale individual dietary surveys on middle-aged men, and Prof. G. Verdonk (Ghent) stressed the complexities of lipid metabolism and suggested that 15–20 different biochemical estimations are necessary to study the course of atherosclerosis. He also advanced the clinical opinion that to lower a patient's blood cholesterol-level is to increase his feeling of well-being. Dr. Trémolières concluded that myocardial infarction is more serious among the obese, the anxious, the smokers and those who appear to over-eat for their height and weight, and that a preventive regimen appears to be "une bonne hygiène de vie", without excessive anxiety, and a moderate diet maintaining the individual at slightly reduced weight. He suggested that not more than 35 per cent of the dietary calories should be derived from fats and that the diet should be "sans carence en acid gras essentiels, sans huiles de mais peroxydées et . . . sans trop de fritures". Dr. V. Socolovsky (Moscow) concluded his contribution on the same subject with the advice that one should "eat less and so live longer to eat more in the end". Prof. J. Yudkin (London), in opening the session on diet and cardiac ischæmia, emphasized the uncertainty of present knowledge. The only conclusions to be reached at the end were that more careful observation and painstaking research are required to solve this most complex problem and that, in the meantime, the only possible prescription is moderation of both life and diet.

The present state of our ignorance was also emphasized by Prof. B. S. Platt (London) when he introduced the session on the nutrition of the mother and her child. He then described recent progress in the experimental production of protein malnutrition in animals and new concepts on protein–calorie deficiency diseases, including marasmic kwashiorkor. Miss Margaret Cameron (New Zealand) described recent team work in Prof. Platt's unit to determine the protein values of foods, meals and mixtures as they are eaten by man. The protein value of a diet is affected by the quality of the protein, its concentration, the presence of certain other nutrients in the diet, for example, vitamin B_{12} or magnesium, and the energy value of the diet in relation to the energy needs of the person eating it. It is also affected by the physiological and pathological states of the individual; for example, it changes during growth or lactation and in some illnesses. She described the method of allowing for these factors in the calculation of the protein requirements of a growing child or a pregnant or lactating woman and illustrated ways in which the protein value of the customary diets may be calculated, and where necessary increased, by supplementing or substitution or by reduction of the amount of oil or sugar consumed. She emphasized the care needed to devise acceptable meals, in keeping with the tastes, religious beliefs and cultural patterns of the children and lactating mothers for whom they are intended.

At the final plenary session of the Congress, Mademoiselle Yvonne Serville (Paris) spoke of the means, indirect and direct, of conveying the findings of research on the science of nutrition to the consumer, emphasizing particularly the complexity of the science of nutrition and the need for some training in nutrition for all those concerned with food from production to consumption, and for teachers, particularly of home management and natural sciences.

This was a notable Congress with an admirable combination of discussions on science and practice, which itself was an illustration of the part dietitians can play in the interpretation of scientific findings in the interests of the consumer.

The Proceedings of the third International Congress of Dietetics, under the title *Tradition, Science and Practice in Dietetics*, have been published by Newman Books, Ltd. Copies may be obtained from the British Dietetic Association, 251 Brompton Road, London, S.W.3, or from Newman Books, Ltd., 68 Welbeck Street, London, W.1 (price £3 3*s*., postage paid).

D. F. HOLLINGSWORTH

BARIUM-140 IN NEW ZEALAND RAINWATER

By G. S. McNAUGHTON and R. N. WOODWARD

Department of Scientific and Industrial Research, Lower Hutt, New Zealand

SINCE the testing of nuclear weapons began, attempts have been made to use the resultant fission products as meteorological tracers. A major difficulty has been to know from which series of nuclear tests the radioactivity of any sample has come. One method which has been used to age fission products, and thus to determine in which series of tests they were formed, has been to measure the rate of decay of the gross beta or gamma activity of the sample, and to compare it with observed rates of decay of fission-product samples of known age. In recent years more precise information about sample ages has come from measuring ratios of specific isotopes such as barium-140/strontium-90 and cerium-144/strontium-90, and comparing these ratios with fission ratios for these isotopes formed in different types of fission reactions. An increase in the ratio of shorter- to longer-lived isotopes is indicative of fission products of more recent origin.

In 1958 we decided to try to obtain information relevant to the problem of global air circulation, particularly with respect to the exchange of Northern and Southern Hemisphere stratospheric air, by measuring isotope ratios in rain collected at Lower Hutt, New Zealand (41° S.). In November 1958 we began measuring 28-year strontium-90 and 12·8-day barium-140 in fortnightly accumulated rain samples, and by June 1959 we were determining these isotopes in each rainfall.

Some of the results in this programme have been recorded[1]. In Table 1 are shown the only positive barium-140 depositions obtained up to June 1961. The methods used are being reported elsewhere[2]. The strontium-90 and barium-140 depositions were calculated from the measured activities of their daughter products, 64-hr. yttrium-90 and 40-hr. lanthanum-140. The measured counts, using a low background beta counter with initially type GM-4 counters and later flow-counters, were never greater than 1 c.p.m. and although repeat lanthanum-140 determinations and barium-140 plus lanthanum-140 determinations were carried out, the activities were too small to identify accurately the presence of barium-140 by half-life determinations. It is considered that the samples quoted in Table 1 did contain barium-140. The errors quoted are due to the 90 per cent confidence-level of statistics of counting only.

Reference to measurements reported in ref. 1 will show, in addition to the positive barium-140 values recorded in Table 1, nine samples out of fifty-eight measured in 1959 which showed activities slightly greater than the statistical counting error of measurement. We now believe that these are not real barium-140 results. The first five during January–July were obtained at an early stage of the programme before the routine measurement techniques were completed, and it now appears this apparent activity was

Table 1. Positive Measurements of Barium-140 in New Zealand Rainwater, Lower Hutt, 41° S.: November 1958–June 1961

Sample No.	Dates when collected from to	Rain (in.)	Strontium-90 μc./square mile	Barium-140 μc./square mile at mid-collection	Barium-140 / Strontium-90	Probable source of barium-140	Per cent strontium-90 due to French test
	1958 (2 samples) November 7–December 4						
	1959 (58 samples) January 15–December 1						
	1960 (24 samples) February 13–May 31					1960	
B 044	February 13–February 19	0·2	28 ± 1	2·5 ± 1·2	0·1 ± 0·05	February 13	0·005
B 069	March 9–March 12	1·53	57 ± 1	5 ± 3	0·1 ± 0·06	February 13	0·02
B 072	March 12–March 16	0·23	17 ± 1	5 ± 1·5	0·3 ± 0·1	February 13	0·07
B 085	March 25–March 26	0·25	20 ± 1	3 ± 1·5	0·15 ± 0·1	February 13	0·06
B 122	May 1–May 3	0·13	19 ± 2	11 ± 4	0·6 ± 0·2	April 1	0·12
	1961 (13 samples) January 7–June 1						
C 024	January 24–January 31	0·08	21 ± 1	2·4 ± 1·3	0·12 ± 0·06	December 23	0·03

probably yttrium-90 not lanthanum-140. The other four apparent activities in September/October 1959 were due to the use of an incorrect reagent blank.

During early 1959 the accuracy of our barium-140 determinations was about ± 100 μc./square mile, but this was reduced to about 1–2 μc./square mile by 1961. From the limits of detection of barium-140, the measured values of strontium-90 deposited, and a value of 1480/1 for the barium-140/strontium-90 ratio[3] for the fast neutron fission of uranium-238, the maximum percentage deposition of strontium-90 due to the October 1958 test programme of the Soviet Union can be calculated. These values are compared in Table 2 with the maximum percentage strontium-90 deposited due to this series calculated from measurements of total beta activity which will be published shortly. The percentage contributions quoted here are maximum values; it has not been demonstrated that any strontium-90 is in fact due to this series. Table 2 shows that the percentage contribution of strontium-90 from the October 1958 series of the Soviet Union detectable by our methods increases as the barium-140 decays until after about March 1959 it was no longer possible to detect an influx of debris from this source. The limits of detection for this debris based on total beta activity measurements assumed all the activity came from the series of tests in the Soviet Union. However, age estimates based on rates of decay of the total beta activity of monthly rain samples suggest an influx of fresh debris from about March 1959 at least until our measurements ceased in June 1959. Although this fresher debris could originate in the Soviet Union, it is not possible to rule out entirely the last tests in the Pacific area. Results for total beta activity suggest that the October series of tests in the Soviet Union did not contribute more than 4 per cent of the total strontium-90 deposited at Lower Hutt from November 1958 until June 1959, assuming fast neutron fission of uranium-238.

Table 2. Maximum Percentage Contribution to Fall-out in New Zealand of the October 1958 Series of Tests in the Soviet Union

Sample collection	Calculated from	
	Barium-140/strontium-90	Total β/strontium-90
1958		
November	0·2	0·3
December	3	13
1959		
January	8	3
February	32	8
March	80	1·6
April		10
May		3
June		12

During 1960–61 six positive barium-140 results were found which we believe to be reliable. These results were obtained in the six-week period after the French test on February 13, 1960, and also approximately four weeks after each of the French tests on April 1 and December 23, 1960. If we assume that the fission/yield activity ratio of barium-140/strontium-90 for the fast fission of plutonium-239 is 1820/1 (calculated from ref. 3) then the French contribution to the measured strontium-90 deposited can be found. This is shown in Table 1 for individual rains and has never been found greater than 0·12 per cent. It seems reasonable that this amount of strontium-90 could have arrived at Lower Hutt through the troposphere from the Sahara tests.

[1] *U.S. Atomic Energy Commission HASL Reports*: **77**, 34 (1960); **84**, 62 (1960); **88**, 80 (1960); **95**, 86 (1960); **105**, 90 (1961).

[2] McNaughton, G. S., and Woodward, R. N., *New Zealand J. Sci.* (in the press).

[3] Katcoff, S., *Nucleonics*, **16** (4), 78 (1958).

OBITUARY

Prof. E. H. Neville

Eric Harold Neville, who died on August 21 aged seventy-two, was educated at William Ellis School where he came under the influence of Percy Nunn, who clearly stimulated in him an innate feeling for mathematics. In 1907 he went up as a senior scholar to Trinity College, Cambridge. It had then been announced that publication of the Mathematical Tripos lists in order of merit would cease with the lists for 1909; this challenged Neville to sit the examination a year earlier than normal, and he was placed second wrangler in 1909. Two years later he won the Smith's Prize and was elected into a fellowship at Trinity, which he held until 1917.

In 1919 he was appointed to the chair of mathematics in University College (now the University) of Reading, which he occupied until his retirement in 1954. He married Alice Farnfield in 1913, who predeceased him in 1956; they had one son who died in infancy.

At the beginning of 1914, while giving a course of lectures in India, he was charged by G. H. Hardy with the task of persuading the Indian mathematician Ramanujan to come to Cambridge. The account of this mission which he gave nearly thirty years later (*Nature*, 149, 292; 1942) is witness to the satisfaction he derived from the successful completion of this task.

His earliest work was in geometry. In *Prolegomena to Analytical Geometry* and *Multilinear Functions of Direction* he was clearing the ground for a treatment of the whole field of differential geometry based on secure logical foundations. Unfortunately, the breadth of his interests led him to undertake other tasks and the work was never completed.

The need to organize the teaching of undergraduates drew his attention to the unsatisfactory foundations of much basic mathematical instruction and he threw himself enthusiastically into the work of the Mathematical Association. With Percy Nunn he was largely responsible for the Association's first *Report on the Teaching of Geometry*. He became librarian of the Association in 1923 and in the course of thirty years he transformed the library from a small collection which could be accommodated in his own house to the valuable reference library for teachers now housed with the University of Leicester. He was president of the Mathematical Association in 1934. He was interested in the work of the British Association, principally in connexion with the Tables Committee, of which he was chairman during 1931–47. He compiled the *Table of the Farey Series of Order 1025* and the *Rectangular to Polar Conversion Tables*. His prefaces to these works contain much that is of interest to anyone contemplating the compilation or printing of mathematical tables. He was a member of the Council of the British Association and, in 1950, president of Section A. He also served on the Council and as vice-president of the London Mathematical Society.

Out of his concern for the improvement of the teaching of pure mathematics arose a number of notes on a wide variety of topics, characterized by freshness of point of view, elegance and economy of presentation. A more ambitious work directed towards the same end was his book on *Jacobian Elliptic Functions*, designed to restore the original beauty and symmetry to a field that had tended to become somewhat unwieldy.

Neville was a man of considerable moral and physical courage—he once rescued a child from the flooded Cam in midwinter. He was essentially a liberal humanist. His love and knowledge of literature matched his interest in the history of mathematics. He was a devoted bibliophile, and it is characteristic that his private library contained not only early editions of all the works of Lewis Carroll and H. G. Wells, but also what is believed to be the most complete set of works on elliptic functions.

R. RADO

NEWS and VIEWS

Textile Engineering at Leeds: Prof. P. Grosberg

ALTHOUGH the wool-textile industry is so highly mechanized, graduates in engineering have so far taken little interest in the construction and use of textile machinery, and have largely neglected the opportunities that all sections of the industry provide. There is here a serious threat to the future of the wool-textile industry of the United Kingdom, and the machinery-users showed commendable foresight in endowing a research chair of textile engineering at Leeds some twelve years ago. The first occupant of the chair, Prof. A. H. Nissan, left to take up an appointment in the United States, and he is now to be succeeded by Dr. P. Grosberg, who brings to the post qualifications in pure science, engineering and textile technology. Dr. Grosberg was born in Cape Town in 1925. After graduation in physics and mathematics, and in chemical engineering at the University of the Witwatersrand, he carried out research on thin film lubrication for the degrees of M.Sc.(Mech.Eng.) and Ph.D.(Eng.). On being appointed to the staff of the newly formed South African Wool Textile Research Institute in 1950, he was seconded to the Department of Textile Industries of the University of Leeds for a two-year course of advanced training in textile technology. After a period of some four years research on worsted drawing and spinning processes in South Africa, Dr. Grosberg returned to Leeds as an Imperial Chemical Industries Fellow in textile engineering, and he was appointed lecturer in textile engineering in 1956. His subsequent investigations, in which he has had the assistance of a number of Wool Textile Research Council Fellows, have already secured for him a reputation in such widely different fields as worsted drawing and spinning, warp-knitting, and drying processes. He is now to have the stimulating task of placing the subject of textile engineering on a sound foundation in the University, and of providing the textile industry with the engineers of which it stands in such great need.

The National Bureau of Standards: Dr. H. C. Allen

DR. HARRY C. ALLEN, jun., has been appointed chief of the Analytical and Inorganic Chemistry Division at the National Bureau of Standards, U.S. Department of Commerce, Washington, D.C. He will direct a broad programme aimed at developing methods for measuring the chemical properties, composition, and behaviour of substances; preparing standard reference materials; and making accurate measurements and data collections on chemical systems. Since 1954, Dr. Allen has been working at the Bureau on the analysis of high-resolution infrared spectra of gases, and the theory of molecular spectra. He spent the year 1959–60 in the Department of Theoretical Chemistry, University of Cambridge. Before becoming a staff member of the Bureau, Dr. Allen was an assistant professor of physics in Michigan State College. He has also been a consultant to the Bureau.

World Population Trends

THE 1960 edition of the *Demographic Yearbook* of the United Nations Organization has as its special feature an analysis of the evolution of the population of various countries, and includes a table showing the populations of each political area in every year since 1920 (Pp. xi+621. New York: United Nations; London: H.M.S.O., 1961). The first chapter entitled "How Well Do We Know the Present Size and Trend of the World's Population?" gives a valuable account of the methods used in preparing population statistics by the United Nations Organization. It is perhaps not always realized that approximately one-half of the world's population is now concentrated in four countries: China, India, the U.S.S.R. and the United States. The Organization assesses the accuracy of the 1953 census for China and suggests that the number of inhabitants of mainland China in that year was between 553 and 612 millions, and in mid-1959 between 605 and 697 millions. This is the population about which there is the maximum degree of uncertainty, India's population is known within 11 millions, and that of the two others within 3 millions. As regards the other areas of the world, it is believed that in most estimates there is a tendency to underestimate rather than overestimate populations, though some errors will no doubt be self-compensating between different territories. Their population is estimated as being between 1,431 and 1,476 millions; the four large areas have populations between 1,416 and 1,508 millions. The world's population in 1959 is believed to be between 2,853 and 2,964 millions, so it is evident that the uncertainty about China is the major factor in the range. The demographers of the United Nations Organization estimate that the annual rate of growth may lie between 1·5 and 2 per cent per annum, resulting in a total annual increase of 44–55 million persons. Tables in the *Yearbook* include data about the economically active and the illiterate population of different countries, as well as vital statistics dealing with births, marriages and deaths. The rudimentary data on expectation of life in African countries indicate the tremendous scope for mortality reduction that still exists in that continent.

Population Statistics in the United Kingdom

THE Registrar General's Statistical Return for the first quarter of 1961 records nearly 204,000 live births, the highest figure for the first (or any) quarter for more than ten years (No. 449. Pp. 28. London: H.M.S.O., 1961. 2*s*. 6*d*. net). Marriages, on the other hand, are fewer at 84,000, compared with 89,000 a year ago. There were 177,000 deaths, giving a relatively high crude death-rate of 15·7, but infant mortality continues to decline, and at 22·9 per thousand births is an all-time low for a first quarter. It will be interesting to see whether the declining trend in the number of marriages will continue, and be followed by a decline in births. The return contains, as is usual in the first quarter, estimates of the home population of administrative counties, boroughs and urban districts at June 30, 1960, together with vital statistics for these areas. In 1960 there were only 22 deaths from poliomyelitis: the lowest number since records began to be kept.

Technical Reports of the U.S. Armed Forces

SINCE the Second World War, the Office of Technical Services had been the agent in the Federal Government for the collection, reproduction and sale of reports of Government-sponsored research to firms and organizations not performing contract work for the Department of Defense. The Armed Services Technical Information Agency is the central agency of the Department of Defense, the mission of which is to provide for the efficient interchange of military research and development reports among Department of Defense agencies and their contractors. The number of technical reports of research and development sponsored by the Army, Navy, and Air Force which are made available to the scientific and industrial public is expected to double next year through an extension of the co-operative activities of the Office and the Agency. With the growing volume of Government-sponsored research, the extension of the co-operative work between the Office and the Agency will enable the Office to offer to the public in the next fiscal year 15,000–20,000 technical reports, prepared under military auspices. The Armed Services Technical Information Agency acquires and catalogues almost 30,000 military technical reports each year—about two-thirds of them unclassified. Under the new co-operative programme, the Agency will provide copies of the unclassified reports to the Office, together with catalogue information. A further use of work already carried out by the Agency will be made in the production of the Office's periodical *U.S. Government Research Reports*, which is used as an announcement medium to inform civilian scientists, engineers, and business men of new reports which are available. The unclassified portion of the Agency's *Technical Abstract Bulletin* which, so far, has been produced only for activities directly concerned with military research and development, will become a section of the *U.S. Government Research Reports*, which, from July 1961, will be published twice a month instead of once a month as in the past. Details of the new programme are outlined in the June–July issue of *Scientific Information Notes* (3, No. 3; June–July, 1961).

The National Lending Library

A RECENT issue of *N.L.L. News*, a news-sheet of the National Lending Library for Science and Technology, indicates broadly the types of literature now available from Boston Spa (No. 1; August 1961. Pp. 4. Boston Spa: National Lending Library for Science and Technology, Department of Scientific and Industrial Research, 1961). These include translations of Russian literature, including 'cover-to-cover' translations; Russian books; the 'PB' reports issued by the Office of Technical Services of the U.S. Department of Commerce; the published reports on German industry produced by the British Intelligence Objectives Sub-Committee, the Combined Intelligence Objectives Sub-Committee and the Field Information Agency Technical (U.S.A.); and certain frequently used periodicals in English which were on a list issued from Stanmore. Loan requests for English language serials and books in languages other than Russian should continue to be sent to the Science Museum Library. With the exceptions indicated above, the National Lending Library is not yet providing a general loan service for publications in the English language, but it is expected that in about twelve months time the man-power problems will have been solved and the transfer from the Science Museum Library completed. It is then proposed to make a full-scale survey of the National Lending Library's use, non-use, and of its failures to meet requests with the view of improving its services and promoting the greater use of scientific literature.

The Use of Scientific Literature

THE *Library Association Record* for August 1961 contains an article on "The Use of Scientific Literature", by B. C. Vickery, which is of some scientific interest. Mr. Vickery summarizes, with a useful bibliography and some account of the methods of study which have been used, the kinds of information sought by scientists, the extent and nature of their reading and the forms of document used. The survey shows that the quantitative contribution to the reading and learning of scientists made by formal bibliographical tools is equalled by the contributions of present-day scanning, cited references, and of oral recommendations. Mr. Vickery recognizes, too, the importance of oral communication, but he indicates rather than discusses the implications of such conclusions as are reached, and emphasizes the need for further research on ways of improving the present position. A second article in the same issue, by W. L. Emerson, discusses an approach to book selection in the sciences for public libraries.

The Geological Society of India

IN January 1959 the Geological Society of India was founded to promote the advancement of geological study and research throughout all India and to co-operate with similar institutions in other lands. One of the aims of the new Society is the publication of a journal, and of monographs and memoirs, dealing with special problems of Indian geology; and the first two volumes from this source have now appeared (*Journal of the Geological Society of India*, Vol. 1, 1959. Pp. 166+6 plates. Bangalore: Geological Society of India, 1959. Rs. 15). This periodical embraces the whole field of geological science, the first two issues comprising 25 short papers mostly of a high standard with many of world-wide interest. In the first presidential address, Dr. D. N. Wadia deals with "The Evolution of the Desert Belt of Asia", delineating briefly, but brilliantly, the overall geological problems of the vast arid tracts which stretch from the Red Sea to Mongolia. Within the past decade the number of geologists in India has increased greatly, and the potential importance of the geological contribution to national development there is becoming widely recognized. The Geological Society of India is likely to play a major part in maintaining the long-established tradition of sound work, and its publications will be warmly welcomed in geological libraries throughout the world.

Atomic Energy-levels in Crystals

Atomic Energy Levels in Crystals, by J. L. Prather, National Bureau of Standards Monograph No. 19, which was issued on February 24, presents a theoretical study of the sharp-line absorption spectra of crystals (National Bureau of Standards. Monograph No. 19. Pp. iv+84. Washington, D.C.: Government Printing Office, 1961. 60 cents). It is largely an exhaustive collection of the results of group theory that apply to the theory of atomic energy-levels in crystals, together with a detailed development of the general theory to permit application of the results to experimental problems. The discrete energy-levels observed within certain crystals are treated as being due to perturbations of the energy-levels of the free ion by an electrostatic field arising from the crystal lattice. The analytic procedures for determining the field from the charge configuration are given, and the resulting fields are classified according to their symmetry. After a general survey of group-theoretical ideas, the applicable groups are analysed in detail, and characters appropriate for both integral and half-integral angular momenta of the free ion are tabulated. The monograph contains eighteen tables and an extensive bibliography. The review on the general subject entitled "Group Theory and Crystal Field Theory", written by Dr. C. M. Herzfeld in collaboration with Prof. P. H. Meijer, which is to be published shortly by the National Bureau of Standards, Washington, treats the subject from the more abstract point of view of modern algebra and concentrates on the fundamental aspects of the theory, and will be a complementary work to that of the monograph.

Travelling-wave Tubes

THE travelling-wave tube is rapidly becoming established as an essential component in microwave links used for civil communications. It is a microwave amplifier that operates on the principle of a continuous interaction between the input signal and an electron beam as they travel together through the tube, and is particularly suitable for operation at frequencies greater than 1,000 Mc./s. It has a broad band-width and high gain. An outline of the construction, characteristics, operation and typical applications of the travelling-wave tube is given in the illustrated booklet, *Travelling Wave Tubes : Their Characteristics and Operation*, issued by the English Electric Valve Co., Ltd. (Pp. 40. Chelmsford: English Electric Valve Co., Ltd., 1961). Following a general description of the tube and its associated circuitry, explained by means of a simplified schematic diagram, the booklet discusses the various types of travelling-wave tubes now commercially available. They can be classified according to their functions into three major groups—power tubes, medium power or intermediate tubes, and low-noise tubes—and these can be subdivided, according to the type of *rf* connexion used, into either wave-guide or coaxial mounting types. All the travelling-wave tubes produced by the English Electric Valve Co., Ltd., require a longitudinal magnetic focusing field for maintaining the electron stream in a concentrated beam over the length of the tube. One section of the booklet deals with focus systems for the tubes, and another with the procedure to be followed in the installation of a tube into a circuit. In addition to the use of travelling-wave tubes as microwave amplifiers in both terminal and repeater stations in communication links, the low-noise tube is being increasingly used in the input stage of radar receivers as a pre-amplifier to the crystal mixer, and travelling-wave tubes may also be used as amplitude or phase modulators, frequency changers, stable oscillators or reflex amplifiers. The booklet is intended as an introduction only to the subject of travelling-wave amplification, and for those who want a more detailed treatment a bibliography of sixteen references is provided. The abridged data listed refer to the English Electric tubes and solenoids.

Lalor Grants and Awards for 1961

THE Lalor Foundation has announced the programme of awards for 1962, which it is offering for support of research on the fundamental biochemical and physiological mechanisms concerned with fertility and the early stages of reproduction in various forms of life. The objectives are to further the knowledge and understanding of the basic phenomena involved and to extend and develop the possibilities for effective regulation and control. The awards are open to all nationalities. The awards may range up to 8,000 dollars a year, depending on the scope and duration of the projects approved. Preference will be given to younger members of university and college faculties and staff with an upper age limit of forty-one years. The work may be carried on at the applicant's own institution or elsewhere. The Foundation will also grant post-doctorate summer or short-term research awards at the Marine Biological Laboratory at Woods Hole, Mass., or elsewhere, for appropriate projects in the fields specified. For these awards, the stipends will normally not exceed 1,000 dollars for a single man or a woman, 1,200 dollars for a married man working at his home institution, and 1,350 dollars for a married man with principal programme at another institution. Applications and inquiries should be sent to the Lalor Foundation, 4400 Lancaster Pike, Wilmington 5, Delaware, and applications must be received by January 15, 1962.

The Bowles Memorial Travel Scholarship

During 1962–63, £500 will be made available to enable a scholar to travel abroad to collect plants for introduction to British gardens, and/or to study practical horticulture in other countries. Applicants must be male, not more than twenty-eight years of age on January 1, 1962, and be holders of the National Diploma of Horticulture, or students or graduates in horticulture or botany at a British university, or past or present student gardeners at the University Botanic Garden, Cambridge, the Royal Botanic Garden, Edinburgh, the Royal Botanic Gardens, Kew, the Royal Horticultural Society's Gardens, Wisley, or other horticultural educational establishments. Applications should reach the Secretary, Royal Horticultural Society, Vincent Square, London, S.W.1, not later than November 20.

The Royal College of Science and Technology, Glasgow

An endowment of £800 has recently been made to the Royal College of Science and Technology, Glasgow, by Mrs. Hilda M. Beilby, of Darlington, Co. Durham, daughter-in-law of Sir George Beilby (who for sixteen years was chairman of the governing body) and grand-niece of Lord Kelvin. The endowment will be known as the Kelvin Prizes, and it will be used to provide awards in the subjects of natural philosophy and applied mathematics. Thus, in conjunction with the endowment made recently by Lord Fleck to commemorate the work of Sir George Beilby, the College is now able to offer awards in mathematics, natural philosophy, metallurgy and chemical technology.

University News: London

The following appointments have been announced: J. P. Quilliam, reader in pharmacology at St. Bartholomew's Hospital Medical College, to the chair of pharmacology tenable at that College; Dr. S. J. Taylor, lecturer in the Department of Pure Mathematics, University of Birmingham, to the University readership in mathematics tenable at Westfield College.

The title of professor of electrical engineering in the University of London has been conferred on Dr. J. H. Westcott, in respect of his post at the Imperial College of Science and Technology.

The following readerships, tenable at the Imperial College of Science and Technology, have been announced: Dr. J. R. Butler (pure geochemistry); Dr. C. B. Alcock (chemical metallurgy); Dr. J. N. Hunt (physical oceanography); Dr. J. A. Kitchener (mineral processing). Mr. J. C. G. Coleridge, lecturer in the University of Leeds, has been appointed as reader in physiology tenable at the Royal Free Hospital School of Medicine. Prof. J. N. Phillips Davies, professor of pathology at Makerere College, has been appointed as reader in morbid anatomy at the Postgraduate Medical School.

Sussex

The following appointments have recently been made: Dr. C. Eaborn, reader in physical-organic chemistry in the University of Leicester, to be professor of chemistry; Dr. R. J. Blin-Stoyle, Fellow and lecturer in physics at Wadham College, Oxford, and senior research officer in theoretical physics in the University of Oxford, to be professor of theoretical physics; Dr. K. F. Smith, Fellow of Sidney Sussex College, Cambridge, and lecturer in physics in the University of Cambridge, to be professor of experimental physics.

Announcements

The Department of Chemical Technology, Bradford Institute of Technology, is organizing a special short course on "Recent Developments in the Chemistry and Technology of Polymers" at the Institute during November 17–18. The fee for the course is £2 5*s*., and further information can be obtained from the Registrar, Institute of Technology, Bradford 7.

The Institute of Physics and the Physical Society announces a symposium on "High Energy Nuclear Physics", to be held in the Physics Department of the Imperial College of Science and Technology, during March 26–27, 1962. Further details can be obtained from the Administration Assistant of the Institute and Society, 47 Belgrave Square, London, S.W.1, during December 1961.

A symposium on "Redundancy Techniques for Computing Systems" will be held during February 6–7, 1962, in the Department of the Interior Auditorium, *C* Street, between 18th and 19th Street, N.W., Washington, D.C. The symposium is being sponsored by the Information Systems Branch, Office of Naval Research. Further information can be obtained from Miss Josephine Leno, Code 430*A*, Office of Naval Research, Washington 25, D.C.

An international symposium on "Enzymic Activity of the Central Nervous System" will take place in Gothenburg, Sweden, during June 18–21, 1962, under the auspices of the Neurochemical Commission of the World Federation of Neurology. Further information can be obtained from the secretaries: Dr. G. B. Ansell, Department of Experimental Psychiatry, Medical School, Birmingham 15, and Dr. A. Lowenthal, Institut Bunge, 59 Rue Philippe Williot, Berchem-Antwerp, Belgium.

Erratum. In the issue of *Nature* for September 16, p. 1142, it is announced that Dr. J. E. Stanworth is to succeed Mr. L. J. Davies. It was not made clear that Dr. Stanworth succeeds Mr. Davies as director of research of Associated Electrical Industries (Rugby), Ltd., Laboratories.

THE INTERNATIONAL MEASUREMENT CONFERENCE

THE second International Measurement Conference was held in the Engineering Societies Building, Budapest, during June 26–July 1. The host organization was again the Hungarian Scientific Society for Measurement and Automation, with Prof. György Striker as executive secretary.

The Conference was attended by some 550 delegates from twenty-two countries and 128 papers were presented in four, or sometimes five, simultaneous sessions. The four working languages of the Conference were English, French, German and Russian; but in fact no translation into French was available and for the first three days translation into English was practically non-existent. This breakdown in the translation service, coupled with the failure to provide reprints of the papers until the final day of the sessions, seriously reduced the value of the Conference.

The 128 papers were divided into eleven sections covering metrology and terminology; technology and organization in instrument manufacture, electronic measuring instruments, border questions related to measurement and automation, instruments and methods for measuring geometrical and mechanical quantities including geodetic measurements, instruments and methods for measuring thermal quantities, instruments and methods for physico-chemical measurements, instruments and methods for measuring electrical and magnetic quantities, instruments and measuring methods in radio engineering and instruments and methods for measuring radioactive radiation. Since papers from four different sections were presented concurrently, it was impossible for any one person with more than the most narrow field of research to attend all the papers in which he was interested.

The standard of the papers varied considerably from the specific to the most general survey and from new basic research to the re-statement of well-known principles: a reduction in number would have benefited all concerned.

Among the papers of general interest, that by T. Kemény (Hungary) described an automatic electronic potentiometer in which the conventional slide-wire had been replaced by a strain-gauge bridge. The four, all active, gauges of the bridge are attached in the standard configuration to a singly supported cantilever beam of low-energy-consuming elastic material. The free end of the beam is deflected by a cam driven through a gear train by the servo motor driving the pen carriage, until the out-of-balance voltage generated by the bridge is equal to the voltage being measured. Advantages claimed are no slide-wire maintenance or wear, high linearity and infinite resolution, and ease of generation of logarithmic and other non-linear scales merely by changing the cam. Other transducers, such as electromagnetic, capacitative and inductive, were suggested, but only the strain gauge bridge has been tried experimentally.

A method for measuring small capacities of about 100 pF., with an accuracy of 1 per cent, was presented by K. Tarnay (Hungary). The method is said to combine the advantages of both heterodyne and resonance methods. Essentially, the unknown capacity and a standard are switched, at mains frequency, alternately into an oscillatory circuit, the output being fed to a phase-sensitive rectifier and thence to a phase-sensitive voltmeter.

A paper by three Soviet authors dealt with a new instrument for the accurate measurement of power, current and voltage at frequencies up to 20 kc./s. A null method is employed in which the unknown alternating current or voltage is used to heat a resistance element, the temperature of which is monitored by a detector element consisting of a bank of some 35–40 thermocouples in series. The thermal e.m.f. is measured by means of a potentiometer and a direct-current voltage is then switched in in place of the unknown alternating current and is adjusted to give the same heating effect as measured by the potentiometer. The equivalent direct-current voltage is then measured by means of a second potentiometer of high accuracy. Good accuracy is obtained with power factors as low as 0·1 by using two heaters. The main source of error is said to be the reactance of the heater elements and shunts in the circuit, and this limits the frequency range. The overall error in comparison of alternating and direct current is less than 0·05–0·2 per cent, depending on range.

Prof. Gy. Striker (Hungary) discussed the possibilities of a long-range planning of instrumentation in a planned economy and suggested that the basic data required were the expected trend in capital investment in the respective industries and the index of instrumentation, that is, the ratio of instrument investment to capital investment and the expected changes in this index. Examples were given of how these factors may be fitted to equations to predict instrument requirements for a longer period.

An electronic statistical quality control apparatus which indicates average and variance directly was discussed by A. Ambrózi (Hungary). The method requires that the parameter to be controlled may be presented by means of a suitable transducer as a proportional alternating current. A batch of components is fed into the unit and the corresponding alternating-current signals are fed to a simple computer which then indicates the mean and the variance on meters, the chief advantage being the speed with which the required data can be obtained.

Two papers on measuring systems for flight testing of aircraft by J. K. Stotz and V. R. Boulton (United States) both stressed that main expenditure was the cost of data reduction which could take up to ¾ man-hr. per point. Strain gauge transducers are used wherever possible because of their inherent accuracy—0·1 per cent—and because they enable standardization of supply voltages and amplifiers as well as being easily calibrated by electrical simulation before and after a test. Recording is by magnetic tape either airborne or ground station, and overall system accuracies of 1–3 per cent may be obtained. Typical recorders have a band-width up to 90 kc./s. at a tape speed of 60 in./sec. Frequency multiplexing is used, up to 10 channels per tape track being common, while for low-frequency data, time division multiplexing at a rate of 900 samples/sec. is used. Such attention to the design of instrumentation and data handling equipment has reduced the data reduction cost by a factor of 4, produced 100 times as much data, which are four times more accurate, and are available for analysis in 1/15 of the time.

S. G. Rabinovitch, B. A. Seliber and V. I. Tchervyakova (U.S.S.R.) presented details of electrical measuring-instruments based on the photo-compensating amplifier. These included multi-range measuring units with a sensitivity up to 5×10^7 divisions per volt, panel amplifiers with 0·1 per cent accuracy and semi-automatic potentiometers, with an accuracy of 0·03 per cent, for instrument checking, etc.

B. Sternlicht (United States) described an indirect but extremely accurate method for measuring film thickness on bearings. He pointed out that there is a direct correlation between film thickness and temperature and he has used temperature-sensing elements for the measurement of the film thickness as well as the film temperature.

A novel technique for re-using resistance strain gauges was put forward by A. Bray (Italy). He pointed out that gauge factors cannot be given to an accuracy better than about ± 1·5 per cent, yet in many applications it is not possible to calibrate the gauge after it has been attached to the surface under test. The method entails using a second carrier of mica between the gauge and the test surface. The author used Eastman 910 cement, but it should be remembered that this is water-soluble. After the gauge has been used it may be recovered by splitting the mica. Experience has shown that the thickness of the mica must not be greater than 0·03 mm., and it must be about 6 mm. larger than the paper or foil base of the gauge. The mica will support 4,000 microstrain, and gauges have maintained a constant gauge-factor while being used and recovered four or five times.

T. M. Dauphinee presented the paper by Dauphinee, Kirby and Preston-Thomas (Canada) on the accurate measurement of temperature and depth in the ocean. Temperature was measured by means of a bifilar-wound copper-resistance thermometer cemented on to a thin-walled copper tube to give close thermal contact with the sea-water; accuracies of ± 0·01 per cent to depths of several kilometres have been achieved, as have accuracies of 0·2 per cent in depth measurement. Two methods were used for measuring depth, the sensing element of one being the bulb of a commercial mercury-in-glass regulator, change in depth being measured by the lowering of the control temperature that results from reduction in volume of the bulb due to the increase in pressure. The second method uses a water boiling-point apparatus with a thermocouple to measure the boiling temperature. An interesting planetary type of slip-ring was used to enable the cable to be wound on a drum winch. This consists of a copper stator and rotor, electrical connexion between the two being maintained by four hollow cylinders of thin spring bronze acting as planet wheels. The system was said to have thermal e.m.f.'s of less than 0·1 μV. and resistance variations of less than 0·01 ohm.

T. A. Perls (United States) described the use of pyroelectric transducers for heat-transfer measurements. A typical transducer of lead zirconate titanate 0·25 cm. in diameter and 0·05 cm. thick has a heat sensitivity of approximately 10 mV./W./cm.^{2} with a 1 M.ohm load. At present it is necessary to calibrate the transducers if accuracies to better than ± 20 per cent are required: a quartz lamp in a gold-plated reflector has proved satisfactory for this purpose, over the range 100–0·1 W./cm.^{2}. Advantages of pyro-electric transducers suggested include small size, low cost, good frequency response, high output, good linearity and ageing, and little sensitivity to temperature.

Dr. Cz. Graczyk (Poland) presented an account of a new method of measuring rate of flow of coal dust. The method is based on the application of a Venturi tube; two pressure drops are measured and it was shown that the dust concentration may be represented as a function of the ratio of the two pressure drops.

The measurement of dynamic flow by means of hot-wire anemometers of the constant-current and constant-temperature type were discussed by I. Kidron (Denmark), who pointed out the superiority of the constant-temperature type which will operate at frequencies several hundred times higher than a constant-current unit because of the reduced effect of the heat capacitance of the wire. The upper frequency limit increases with flow speed and is higher than 50 kc./s. at 100 m./sec. air speed. Kidron then discussed a newly developed hot-film transducer consisting of a thin platinum film fused to a glass support to give greater mechanical strength. The dynamic response is slowed by the heat capacitance of the glass support, but is still only 5 db. down at 50 kc./s. for 100 m./sec. air speed.

A novel bi-parametric displacement transducer with good frequency response and high sensitivity was described by Prof. N. Racoveanu (Rumania). The transducer consists of a magnetic core coil with a variable air-gap and also a condenser with variable plate-spacing. In use both coil air-gap and plate-spacing are varied together, modulating the frequency of a resonant circuit in a more linear manner than may be obtained by varying inductance or capacity alone. Designs with a plate condenser and a cylindrical condenser have been examined and a design with multiple concentric cylinders offers greatest linearity. Over the linear range of 2,000μ a sensitivity of 0·25 kc./μ is obtained in the frequency-range 370–870 kc./s.

A. Haas (Hungary), in his paper on the calibration of contact type surface thermometers, stressed the need for intimate contact between the detector and the surface under investigation and complete thermal-equilibrium between the detector, the surface and the surrounding atmosphere. The ultimate aim was 100 per cent thermal coupling, as was achieved by the immersion technique with the ordinary mercury-in-glass thermometer. He then discussed a detector consisting of a thermocouple junction set in a copper block which had been developed at his Research Institute, and which is capable of accurate results with only 60 per cent thermal coupling, and in spite of varying ambient temperature. Another surface pyrometer with an accuracy of 1 per cent was described; this consisted of two iron constantan couples separated by a layer of mica and connected in opposition across a sensitive galvanometer. A heating element above the second couple enables conduction and convection losses to be compensated. The instrument is calibrated against a third couple set into the calibrating surface. It was emphasized that during calibration it was necessary to ensure that thermal coupling should be the same as would be met in use, and thus for calibrating a surface pyrometer for use on rotating drying cylinders, a special calibrating surface was designed. This cylinder was oil heated and thermostatted, and its circumferential speed was set so that approximate thermal similarity with practical conditions may be achieved for a small area of the surface and the atmosphere surrounding the pyrometer. The cylinder has a Ward Leonard speed control and built-in thermocouples brought out via slip-rings so that the true calibration surface temperature is obtained.

Some ninety-five scientific institutions and manufacturing companies exhibited at the second International Instruments and Measurement Exhibition, which was held concurrently with the conference. The usefulness of the exhibition was reduced considerably by the lack of stand attendants and absence of literature on some stands. It was generally agreed by competent observers that nothing was shown by Eastern European countries which was in advance of equipment available in the West, and in many cases development in the former appeared to be 5–10 years behind the West. No Russian equipment was shown. Among Western exhibitors, emphasis was mainly on process instrumentation and control, particularly for the chemical industry, and on radiation counters of all types, while the Eastern countries exhibited more laboratory measuring equipment and in particular microwave equipment in the 8–10 cm. band for telephone and television uses.

Equipment exhibited by the Hungarian Measuring Instrument Research Institute included a six-channel transistorized amplifier for pressure pick-ups, strain gauges, thermistors, etc., with an input impedance of 10^{12} ohms and an output impedance of 1 ohm, with a frequency-range of direct current to 5 kc./s., and better than 5 per cent linearity. A transistorized servo amplifier measuring 6 × 6 × 20 cm. contained a two-stage pre-amplifier and a class *B* push–pull power amplifier stage, with an output capacity of 12 W. and working temperature range of -40–$+50°$ C. Other transistorized modular blocks of the same dimensions included current and voltage amplifiers, 400 c./s. RC oscillators, a direct-current amplifier with a gain of 100 and a non-contacting relay which was basically an inductive pick-off from a moving pointer unit, with a sensitivity of ± 0·5 mm. pointer movement and a switching capacity of 500 m.amp. at 50 V. supply.

A self-compensating ionization vacuum gauge on show was a transistorized miniaturized version of the Modion gauge and covered the range 10^{-8}–10^{-3} mm. in a single span. A differential β-ray thickness meter suitable for measuring coating weights of paper or impregnation weights of fabric had an accuracy of 1 per cent for an impregnant weight-range of 0·5 to 2 × the fabric basis weight. The time constant of the measuring unit was variable between 5 and 20 sec. Several other examples of β- and γ-radiation measuring equipment were on display.

The Czechoslovak Export Agency (KOVO of Prague) showed a long range of electronic equipment and accessories, including a high sensitivity direct-current oscilloscope with a band-width to 7 Mc./s. 3 db. down; alternating-current sensitivity 9 mV./cm., direct-current 25 mV./cm., input impedance 1 M.ohm at 30 pF. or 70 ohms direct current, time-base 5–500 kc./s., with provision for external input to *X* plates. A two-channel oscilloscope had a sensitivity of 10 mV./cm. and band-width to 1 Mc./s. with beam switching at 120 kc./s., and a two-gun oscilloscope with similar sensitivity had a frequency-range to 2 Mc./s. 3 db. down. The O.P.D. oscilloscope for slow-speed phenomena had a 25-cm. diameter, high-resolution tube. Maximum sensitivity was 100 mV./cm., a band-width of 0–6 kc./s., and a slowest time base sweep of 1·5 min. The 'Meopta' oscilloscope camera shown had an *f*/1·8 lens of 35-mm. focal length and held 6 m. of 35-mm. film. It is a continuous recording camera with a maximum film speed of 1,000 m./sec.

A semi-automatic valve tester with punched card setting up was shown also, and an electronic null point indicator with CRO indication for indicating alternating-current bridge balance.

The Polish Exterior Trading Co. for Electrical Technology, Warsaw, showed a limited range of equipment, somewhat out of date by Western standards. Also exhibited were chemical balances and thermographs, a phase-contrast microscope and a projection microscope, refractometers and *p*H meters, a disappearing-filament-type optical pyrometer and a twin-beam oscilloscope with a band-width of 10 c.p.s. to 5 Mc./s. A valve test meter looked about 1945 vintage. The sole electromagnetic flow meter exhibited was on the stand of Eckandt of Stuttgart, together with a proportional pneumatic controller, diaphragm operators, a twin-channel recorder and various Bourdon tube and diaphragm pressure units, and a smart-looking range of edge-on panel meters.

Precision slide-wire potentiometers were exhibited by Carl Zeiss of Jena. These were to *DIN R*5 standard with linearity of 1 per cent and a torque movement of 35 gm. cm. for the smaller sizes. Armoured and sealed versions were also available for use in basic heavy industries and in corrosive atmospheres. A precision instrument potentiometer requiring a torque of less than 2 gm. cm., and a small, low-inertia, magnetic clutch were also shown. A photomultiplier with a cathode diameter of 140 mm. and maximum gain of 10^7 was on display together with selenium cells with a spectral response said to be nearer the human eye than any other available, making it eminently suitable for colour matching, etc.

M. A. W., Messergate and Armaturenwerk, showed a self-balancing potentiometric recorder with a 250-mm. scale-length. Sensitivity was 0·1 per cent, reproducibility 0·5 per cent, response time better than 1·5 sec., minimum range 2 mV. f.s.d. suppressed zero available, models with 1, 3, 6 and 12 channels, chart speeds 0·2–3,600 mm./hr. This appeared to be the standard recorder on Eastern European equipment. A miniature recorder of the chopper-bar type was also shown and a thermocouple temperature-indicator/transmitter/controller of the lamp and pointer-vane type with 0–5 m.amp. transmission.

The most interesting pneumatic control equipment was to be found on the stand of G.S.T. (Automatic Temperature Control C.), Berlin. As well as high- and low-pressure pneumatic differential potential cells and a force-balance electropneumatic converter for thermocouple e.m.f.'s was a well-engineered three-term controller with push-button selection of hand or automatic control and balance, with a proportional band setting between 2 and 1,000 per cent, integral and derivative action times of 0·36 sec. to 200 min. and a sensitivity of better than 0·01 per cent. Also on display was a flow-metering unit which monitored the flow-rate of the main stream fluid and at any desired time interval would meter into it by means of a solenoid valve any desired proportion of a second liquid component. Fritz Hollige (Freiburg) demonstrated the 'Helcoscriptor' high-speed recorder available with 1–8 channels. All can be used with heated stylus on sensitized paper writing and are flat to 100 c./s. for 30-mm. recording width, or with cold stylus on carbon paper flat to 130 c./s. Sensitivities of 10 mm./V. input and paper speeds up to 200 mm./sec. are available.

Hottinger Measuring Techniques, Ltd., Darmstadt, showed a range of miniature inductive pick-offs suitable for carrier frequencies of 5 and 50 kc./s., the minimum range being 20 × 10^{-4} mm. f.s.d., and a maximum operating temperature of 90–120° C. according to type. Strain gauges with a minimum gauge-length of 3 mm., and which would withstand a maximum strain of 2 per cent up to 200° C., were also exhibited together with a strain gauge bridge torque-meter transmitting a maximum torque of 1,000 metre kiloponds, at 4,000 r.p.m. with a reproducibility of 0·5 per cent and an output of 1·5 mV./V. supply at maximum torque loading; silver/silver graphite slip-rings with up to 12 channels, with brushes that may be replaced during running, were capable of running at 6,000 r.p.m. continuously and 12,000 r.p.m. intermittently.

Krohne of Duisburg displayed their range of rotameter-type flow-meters with magnetic pick-off of float position to provide remote indication, and a float-type density meter with inductive pick-off which fits directly into the process stream line and is independent of fluid velocity; the most sensitive range is 0·99–1·01 gm./c.c. and the accuracy of this range is ± 1½ per cent. Heating or cooling coils and built-in temperature compensation are available. AB Lars Ljungberg, Sweden, demonstrated the 'Agnar' burette, a new and ingenious approach to precision metering, titrating and decanting. The burette has no tap, and is essentially a syringe in which the piston is positioned by means of a screw drive. Various syringe sizes are available, the 2 ml. size delivering 1 ml./rev. of the coarse knob and 12 μl./rev. of the fine knob. The drive is reversible, thus the burette is equally suitable for drawing off any volume from 1 μl. to 20 ml.

The Yokogawa Electric Works, Ltd., Tokyo, exhibited a range of direct-current precision measuring instruments including a good-looking miniature portable Wheatstone bridge. Panel meters were contemporary in style but appeared crudely manufactured and designed without regard to their function of precise indication of a measured quantity. Fully transistorized printed-circuit strip-chart recorders of about 4-in. and 8-in. chart-width had self-folding charts facilitating reading of back records. A three-term electronic controller was on view but no information about it was available.

A new instrument under development by DISA Electronik measures departures from constancy of the angular velocity of rotating equipment at speeds up to 100,000 r.p.m.

J. A. Place

ASSESSMENT OF PAIN IN MAN AND ANIMALS

A THREE-DAY symposium on the assessment of pain was organized by the Universities Federation for Animal Welfare (UFAW) in collaboration with Prof. C. A. Keele, presided over by Sir Russell Brain, and held in the Department of Pharmacology at the Middlesex Hospital Medical School during July 26–28. UFAW had been asked to arrange such a symposium by Dr. C. Radouco-Thomas, secretary of the Collegium Internationale Neuro-Psycho-Pharmacologicum in Geneva, who made many useful suggestions. In his presidential address, Sir Russell Brain referred to the usefulness of bringing together the peripheral and central aspects of the neurology of pain and the need for reconsidering views which at present are widely held on the subject; he said that UFAW's interest in the latter arose from the fact that pain sensations in many species appear to differ little from those experienced by man.

After Dr. K. D. Keele (London) had reviewed the history of theories of pain from primitive times, Prof. C. A. Keele (London) outlined a technique used by himself and his colleagues in which a blister is formed on the arm and the blister base is then stimulated in various ways, the resulting sensations being reported by the subject. The receptors appeared to be stratified in depth. The sensation at low levels of stimulation was not painful, and he proposed the term 'metæsthesia' for it under those conditions. Dr. Robert Smith (London) dealt with the quantitative relation between stimulus and sensation and described metæsthesia produced with potassium and polypeptide stimulations.

Prof. J. A. B. Gray (London) took the chair at a discussion of peripheral pain mechanisms. Dr. A. G. M. Weddell (Oxford), dealt with the structure of the nerve terminals in the skin as they appeared in electron and optical microscopy. Prof. Y. Zotterman (Stockholm) dealt with the specificity of cutaneous nerve fibres and the search at sub-microscopical level for the cause of this specificity. Dr. A. Iggo (Edinburgh) discussed the conduction in single *C* fibres of impulses initiated by noxious stimuli in the viscera, muscles and skin. Prof. H. Hensel (Marburg) showed a film record of afferent impulses in human single cutaneous nerve fibres. Dr. W. Koll (Göttingen) described the effects of morphine and other drugs on nociceptive spinal reflexes. An open discussion of the specificity of peripheral mechanisms followed.

Prof. D. Hill (London) presided over a session on central mechanisms of pain. Dr. D. R. Bowsher (Liverpool) and Prof. D. Albe-Fessard presented a joint paper showing that somatic sensations fall into two classes: discriminative sensations travelling by lemniscal pathways to the cortex via the thalamus and entailing a perception of location, and non-discriminative sensations travelling by extra-lemniscal pathways. Dr. G. Gordon (Oxford) described the electrophysiological approach to central mechanisms of somatic sensation. Dr. P. W. Nathan (London) dealt with traces of pain remaining in the nervous system, as in the case of phantom limbs. Dr. J. Hankinson (Newcastle upon Tyne) described the relief of pain by destruction of intracerebral structures under the guidance of stereoscopic radiography. In the ensuing discussion, Prof. J. Scherrer (Paris) and Dr. G. L. Kidd (Liverpool) suggested possible mechanisms of phantom pain, and Dr. M. R. A. Chance (Birmingham) directed attention to audiogenic seizures which do not arise in rodents unless these are deprived of escape routes.

Prof. O. L. Zangwill (Cambridge) took the chair for a session on the assessment of pain in man. Dr. H. K. Beecher (Boston, U.S.A.) discussed some working hypotheses and described his work on the effect of drugs on high-level intellectual performance.

He considered that data from experimental pain are often difficult to relate to clinical experience. Dr. J. D. Hardy (New Haven, Connecticut) described work on the pain threshold in relation to measurement of the intensity of human pain and the effects of analgesics. Dr. R. W. Houde (New York) said that an analysis of quantitative data on pathological and experimental pain in terms of the 'ridit' unit could establish a relation between these two different types of data. Dr. C. Wilson (Liverpool) discussed the factors affecting the placebo response, which may give rise to bias even in blind experiments. A general discussion of psychophysical correlation problems followed.

In a session devoted to the assessment of pain in animals, with Prof. C. A. Keele (London) in the chair, Dr. D. D. Bonnycastle (Jersey City), Dr. R. L. Kitchell (Minnesota), Dr. H. O. J. Collier (London), Dr. C. Radouco-Thomas (Geneva), Dr. K. Soehring (Hamburg), and Dr. J. Jacob (Paris) discussed the testing of analgesic drugs on animals. The final session, in which Prof. C. A. Keele again took the chair, was devoted to practical applications to animals used in research. In the unavoidable absence of Prof. A. Haddow, Dr. E. J. Delorme described several arrangements devised at the Chester Beatty Research Institute for the comfort of the animals used. Major C. W. Hume (UFAW, London) directed attention to the desirability of ensuring that experimentation on animals be restricted to scientifically qualified persons who recognize their responsibility towards the rodents and other animals used, the training of technicians and of beginners in research being particularly important in this respect. A discussion on legislation for the protection of laboratory animals followed.

A report comprising the full text of the papers with summaries of the ensuing discussions is to be published by UFAW and will, it is hoped, be available early in 1962.

SOCIAL STUDIES IN BRITAIN

IN the debate on science in the House of Commons on July 10, Mrs. Judith Hart urged the need for a social sciences research council, referring particularly to the contribution which social science could make in dealing with the special problems of the new towns, in urban research and development generally, in the National Health Service, and in the economic and social problems arising out of automation and industrial change in general. Mr. D. Freeth, Parliamentary Secretary for Science, did not deal with this subject in his reply in that debate, but it was raised afterwards by Mr. A. Albu in a debate on August 4. In the meantime, on July 18, Mr. Freeth had stated in answer to a specific question from Mrs. Hart that the Council for Scientific and Industrial Research is reviewing the support given by the Department in this field, and considering whether there is need to alter the present organization for encouraging and supporting these studies. Mr. Freeth said that the Minister for Science expects to receive the Council's views in the autumn, and on August 4 he added that the House would expect the Minister to await the Council's advice, though he was authorized to emphasize the Minister's great interest in the social sciences. The Minister would note very carefully the views expressed in the debate; Lord Hailsham believed that more work should be done in the universities and elsewhere in this field, and his continuing study would be directed to strengthening the social sciences in future.

The debate on August 4 was notable for the very full review of the whole position of the research effort in Britain in this field at present, and warm appreciation of Mr. Freeth's statement was expressed by Mr. F. Peart. Mr. Freeth began by referring to the report of the Clapham Committee in 1946, as a result of which the University Grants Committee made earmarked grants from 1947 until 1952 for the social sciences at a steadily increasing rate. A change in policy led to the discontinuance of earmarked grants after 1957, but at the present time the Department of Scientific and Industrial Research is making grants and awards totalling £140,000, the £40,000 mentioned by Mr. Albu covering grants to universities and colleges of advanced technology only. For this purpose, the Department has a special committee, and in addition the Industrial Grants Committee of the Department has made a 5-year grant to the National Institute of Industrial Psychology, depending on the amount of money raised for the purpose by the Institute. A list of investigations supported by the Human Sciences Committee of the Department is available, and although all these awards are related to industry they cover a wide field. Mr. Freeth cited as examples research at the Birmingham College of Advanced Technology into social factors affecting relations and individual performance in factories; at the University of Durham into worker–management relations in four industries in the north-east of England of differing techniques; at Brunel College, into the subjective nature of judgments made in the inspection and quality control of manufactured goods; at the University of Leicester into problems of the employment of married women in hosiery factories; and at the University of Oxford into plant-operating skills in relation to automation.

In this context Mr. Freeth referred to the dissemination of the results of such research; for example, the Ergonomics Conference held in September 1960 and the series of meetings organized to introduce the topics discussed to the research associations.

About £60,000 a year is being expended on research in this field at the Department's own laboratories, including, for example, the work at the Warren Springs Laboratory on ergonomics, such as the design and study of key-boards and a study in conjunction with the University of Manchester of secondary modern school-leavers. At the Building Research Station investigations are being made of the design and provision of flats for old people and the design of offices and hospitals. The Road Research Laboratory is continually examining the personal characteristics of road-users in relation to their behaviour on the road, and the National Physical Laboratory is investigating motor-vehicle noise, the effects of the light in a room on colour rendering, and readers for blind people, and the British Iron and Steel Research Association and the British Boot and Shoe and Allied Trades Research Association have human science and ergonomic sections.

The Medical Research Council is spending some £250,000 in this field, at its own research units and through grants for research undertaken in the universities. At Graylingwell Hospital, Chichester, the Council has a unit with a programme including the evaluation of a community mental health service to determine the social and clinical factors which favour admission of patients to hospital and those which favour treatment at home. The unit was also studying the effects on the family of caring for a member who is mentally ill. Work in the social sciences at the universities, however, must depend largely on the importance attached to it by the universities, and the extent of their resources, but it is also influenced by the scarcity of first-degree courses in sociology as such and the difficulty in attracting postgraduate students.

Mr. Freeth referred to considerable expenditure on research into such subjects as criminology and penology by the Home Office, though he did not indicate the amount. Lists of such researches were given in a written answer by the Minister of State at the Home Office on April 13, 1961. Other Departments finance work on such subjects as perception and reaction to extreme environmental conditions, and grants are also made by certain local education authorities. How far this should be done by central bodies and how much within the Departments is a difficult problem. Mr. Freeth made no specific reference to the Ministry of Education, although in opening the debate Mr. Albu had commented on the absence of such research by that Ministry, and had referred to a memorandum recently submitted to the Minister for Science by the Parliamentary and Scientific Committee.

The memorandum pointed out that the total expenditure on empirical research on education in Great Britain was less than £125,000 a year, or about 0·014 per cent of annual expenditure on education; and it pointed out that most university research education is carried out part-time either by staff or students for higher degrees, most of whom are practising teachers with full-time jobs. There are only ten research fellowships devoted to empirical research in education, and most of the research carried out by teachers and local education authorities loses its principal value because it has no skilled advice on such matters as experimental design, test construction and statistical analysis. The memorandum urged the establishment of an educational research council with a substantial budget and an independent chairman under the direction of a committee of the Privy Council and reporting to Parliament through the Lord President of the Council.

Mr. Freeth said that the work of the Social Survey of the Central Office of Information, on which some £129,000 is expended, also covers a wide field, including inquiries on behalf of Government economists and statisticians with a special interest in economic development, inquiries into health and social problems, and inquiries into the development of techniques for supplementing and completing existing official statistics and studying operational problems of public services.

There is also the considerable support to social science research given by the major charitable foundations, but Mr. Freeth thought that this source of support is likely to diminish in future. He believes that much more work in this field is needed in the universities and colleges of technology, and hopes that those interested in strengthening the study of the social sciences there will give evidence to the Robbins Committee.

In welcoming Mr. Freeth's reply, Mr. Peart expressed the opinion that a research council for the social sciences should be created, and hoped that the Government would act after considering the views of the Council for Scientific and Industrial Research and the recommendations of the Robbins Committee. Referring particularly to the need for more educational research to provide the basis of objective advice for decisions, he instanced the value of a proper survey into the way in which comprehensive schools are meeting educational needs and fitting into local environment, and of a proper social survey of university education, embracing the place of students, the nature and length of courses and the wastage of resources. It is as a stimulus to social research in the universities, to undergraduate and postgraduate training in this field and the provision of field observation groups and group behaviour studies in a wide field of human activity in industry as well as detailed socioclinical studies and surveys that Mr. Peart thinks that a social research council would help to supply the detailed information needed as a basis for sound decisions by the Government in matters which are often non-political.

B.B.C. ENGINEERING MONOGRAPHS

IN 1955, the British Broadcasting Corporation began the issue of a series of B.B.C. Engineering Monographs[*], describing technical work carried out by the Engineering Division of the Corporation in the development and operation of their sound and television broadcasting services (*Nature*, 177, 519; 1956). Nineteen of these monographs had been issued up to June 1958 (*Nature*, 182, 1490; 1958); and a further eighteen have been published in the following three years, up to June 1961. The authors of these monographs are usually members of the staff of the various departments of the Engineering Division of the Corporation, and collectively they show the progress of the development of the service of sound and television broadcasting through the Research, Designs and Planning and Installation Departments to the practical operation of the service transmitting stations.

The range of subjects dealt with is indeed wide. On the sound-broadcasting side, the problems of programme switching, control and monitoring have been described, together with details of the methods used to check the quality of very high frequency broadcasting. The various methods which can be used for stereophonic broadcasting have been surveyed and discussed with the view of appreciating the possibilities of this technique for domestic reception. In connexion with television, the subjects dealt with in recent monographs, range from the performance of optical lenses in the cameras, the general testing of cameras, and the processing of films, to the equipment of television studies and a new survey of experimental colour transmissions.

---

[*] B.B.C. Engineering Monograph No. 34: A Mobile Laboratory for U.H.F. and V.H.F. Television Surveys. E. W. Taylor and L. C. Munn. Pp. 16. No. 36: Some Aspects of Optical Lens Performance. Part 1: Secondary Flare in Lenses. By K. Hacking. Part 2: The Design of Lens Hoods. By W. N. Sproson. Pp. 19. No. 37: An Instrument for Measuring Television Signal-to-Noise Ratio. S. M. Edwardson. Pp. 23. (London: British Broadcasting Corporation, 1961.) 5*s*. each.

Progress common to both sound and vision broadcasting is reported in such subjects as the design of transmitting aerial systems and the general study of radio wave propagation. This work is essential in assessing the coverage likely to be attained from new transmitting stations, and particularly from those which will be required for extended services in the future. Some of the research work is carried out with mobile laboratories, while the operational engineers use mobile studios and control rooms for their outside sound and television services. The latest types of these mobile units are described in separate monographs.

Altogether these publications show that the B.B.C. is well aware of its responsibility to keep the technical side of its services up to date, and to take full advantage of progress and developments, whether these arise from within its own organization or from elsewhere in the fields of radio research and application.

THE LISTER INSTITUTE OF PREVENTIVE MEDICINE

THE report of the Governing Body of the Lister Institute of Preventive Medicine for 1961* again contains a detailed record of the numerous investigations carried out in the various departments.

In the Department of Microbiology, for example, the Guinness–Lister Unit continued its exploration of bacterial genetics, mainly of the *Salmonella* bacilli. The problems investigated include conjugation and gene-transfer initiated by the colchicine type of fertility factor and by a mutant of the classical fertility factor, *F* of Hayes; the effect of a colchicine factor on the resistance to ultra-violet irradiation; the genetics of resistance of *E. coli* to colchicines; the genic control of flagellation in a number of *Salmonella* mutants and of the production of different varieties of flagellar protein; and the chemistry of flagellar proteins and of the cell-wall polysaccharides.

The rates of bacterial multiplication in the infected mouse were studied with pathogenic *Salmonella* marked either with a non-replicating prophage or with an abortively transduced gene from another strain of *Salmonella*. The other purely microbiological investigation concerns the antigenic analysis of certain free-living protozoa.

In researches concerned with the immunology and pathology of infective diseases, the Institute's work includes infections by viruses, pleuropneumonia-like organisms, bacteria and protozoa.

The Trachoma Research Unit continued its epidemiological field study in The Gambia of trachoma in terms of virus and of bacilli of the *Haemophilus* group. Laboratory work at the Institute includes growth of trachoma and inclusion conjunctivitis virus in tissue culture, serological classification of the viruses, the vaccination of baboons against inclusion conjunctivitis and the serology of Gambian *Haemophilus* strains. In the smallpox-vaccine department, there is continued progress towards making vaccine from virus grown in tissue culture. The properties of measles virus were also studied.

As an indirect means of determining the significance of antibody to pleuropneumonia-like organisms in human subjects with abacterial urethritis and other kinds of genital infection, the study of the relation of pleuropneumonia-like antibody to the infecting agent and stage of infection in rats and mice was continued.

The immunological study of bacterial infections includes an analysis of the action of two types of protective antibody in mice infected with the whooping-cough bacillus; the biological activity of the *C* polysaccharide of *Streptomyces pyogenes*; and the process of refinement of antitoxin by proteolysis.

Further investigation has been made of the immunology of experimental trypanosomiasis in terms of soluble and bound immunizing antigen as well as of methods of bulk growth of the trypanosomes for antigenic analysis.

The refined serological methods devised to identify the animal source of food for blood-sucking insects provides valuable facts about the feeding-habits of tsetse flies in regions where these insects are vectors or possible vectors of disease.

In the Department of Biochemistry, the researches concerned were again with three kinds of substances, namely, the blood group substances, the cellular phospholipids and starches.

The investigations of the substances of the *ABO* blood-group system include analysis by improved methods of partial hydrolysis, determination of the amino-acid moiety of the substances and its relation to serological specificity, transformation of blood-group specificity by the action of selected enzymes and measurement of the molecular properties of the substances.

Work on the structure of phospholipids, their distribution in the tissues, cells and cellular particles, and their possible function in the cell includes analysis of the phospholipids of *Micrococcus lysodeikticus* of the pig kidney, lung and spleen and of mammalian plasmalogens.

The enzymes of plants, animals and bacteria synthesizing and degrading starch and glycogen were studied with regard to their specific effects on natural carbohydrates and on modified carbohydrates produced by chemical methods, including starches and various glucose phosphates. A disaccharide other than isomaltose was obtained from a dextran, and a new trisaccharide. Carbohydrates were synthesized for use as haptens in the immunochemical analysis of dextrans.

With human and animal plasma proteins, the Institute's work is concerned with the isolation, refinement, characterization, assay and, in some cases, clinical trial of the various biologically active proteins of human plasma.

A reliable assay of human antihæmophilic globulins was devised and improvements made in separation of stable preparations of the globulins from plasma.

Work on the isolation and characterization of biologically active proteins include those of plasmin, plasminogen, albumin, caeruloplasmin and Christmas factor.

---

* Lister Institute of Preventive Medicine. Report of the Governing Body, 1961. Pp. 29. (London: Lister Institute of Preventive Medicine, 1961.)

ISOELECTRONIC PRINCIPLE, THERMOCHEMISTRY, AND REACTIVITY OF RELATED FREE RADICALS

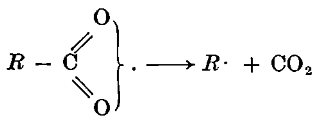
By Dr. PETER GRAY and J. C. J. THYNNE
School of Chemistry, The University, Leeds, 2

THE isoelectronic principle states that molecules containing identical numbers of valence electrons show many similarities in physical properties, molecular symmetry, structure and chemical reactivity. The principle was originally based on the striking physical resemblance between such isoelectronic molecules as nitrogen and carbon monoxide or nitrous oxide and carbon dioxide. It may also be applied to free radicals. Moreover, its range of usefulness is widened by the possibility of substitution of isoelectronic groups (such as —CH_2—, —NH— and —O—) for one another.

Recently, attention has been directed[1] to the usefulness of the isoelectronic principle in correlating the patterns of reactivity of two families of free radicals, namely, the alkyl radicals $R\text{CH}_2\cdot$ and the alkoxy radicals RO: For example, the parallelism between their decomposition reactions $R\text{CH}_2\dot{\text{C}}\text{H}_2 \rightarrow R\cdot + \text{C}_2\text{H}_4$ and $R\text{CH}_2\text{O}\cdot \rightarrow R\cdot + \text{CH}_2\text{O}$ has been discussed. In general it is to be expected, if two free radicals are isoelectronic, that they will exhibit similar patterns of reactivity. An important qualification should be made, however. It is well known that thermochemistry plays an especially important part in determining the behaviour and reactivity of free radicals. If a certain free radical may react by either of two paths, then their relative thermochemical requirements may be at least as important a consideration as a comparison with the behaviour of an isoelectronic analogue. When the isoelectronic principle and thermochemistry reinforce one another's influences, then prediction of the behaviour of the radical is probably safe. When they oppose one another, which will be dominant is not known.

Accordingly, in this article, we are concerned with the thermochemistry and the reactivity of two isoelectronic pairs of radicals: (i) the acyloxyl ($R\text{CO}_2\cdot$) and the azoxyalkyl radicals ($R\dot{\text{N}}_2\text{O}$), and (ii) the alkoxycarbonyl ($R\text{O}\dot{\text{C}}\text{O}$) and the alkoxyazo radicals ($R\text{ON}\dot{\text{N}}$). These radicals, it will be noticed, also form two isomeric pairs (iii) $R\dot{\text{C}}\text{O}_2$ and $R\text{O}\dot{\text{C}}\text{O}$, and (iv) $R\dot{\text{N}}_2\text{O}$ and $R\text{ON}\dot{\text{N}}$; so that comparison may be made not only between the behaviour of isoelectronic radicals but also between the behaviour of isomeric radicals.

(1) Acyloxyl and azoxyalkyl radicals, $R\dot{\text{C}}\text{O}_2$ and $R\dot{\text{N}}_2\text{O}$. Of these families of radicals the acyloxyl radicals are the most well known. They may conveniently be prepared[2] by the thermal decomposition of diacyl peroxides. The acyloxyl radicals readily decompose yielding carbon dioxide together with an alkyl radical (reaction 1), and it is this fact that has resulted in the frequent use[3,4] of diacetyl peroxide as a low-temperature source of methyl radicals:



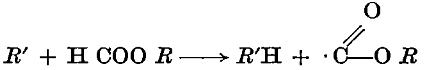
$$R-\text{C}\left.\begin{matrix}\nearrow\!\!\text{O}\\ \searrow\!\!\text{O}\end{matrix}\right\}\cdot \longrightarrow R\cdot + \text{CO}_2 \qquad (1)$$

Isoelectronic with the acyloxyl radicals are the isomeric pair of azoxyalkyl radicals $R\text{—N=}\underset{\substack{\downarrow\\ \text{O}}}{\text{N}}\cdot$ and $R\text{—}\underset{\substack{\downarrow\\ \text{O}}}{\text{N}}\text{=N}\cdot$. (These are related to the azoxyalkanes $R\text{N}_2\text{O}R$ in the same way as the acyloxyl radicals $R\text{CO}_2\cdot$ are related to the esters $R\text{CO}_2R$). Although aliphatic azoxy-compounds[5] have been prepared, their possible use as a source of radicals does not appear to have been much investigated, perhaps because of the hazards involved in their use.

Each of these families appears to have only one obvious mode of decomposition: acyloxyl radicals according to reaction 1, and the azoxyalkyl radicals according to reaction 2:

$$R\dot{\text{N}}_2\text{O} \longrightarrow R\cdot + \text{N}_2\text{O} \qquad (2)$$

(2) Alkoxycarbonyl and alkoxyazo radicals, $R\text{O}\dot{\text{C}}\text{O}$ and $R\text{ON}\dot{\text{N}}$. The alkoxycarbonyl radicals ($R\text{O}\dot{\text{C}}\text{O}$) have, until recently, been less well studied than the acyloxyl radicals although they are of considerable interest kinetically. They can be generated by the alkyl radical-induced decomposition of alkyl formates[6] (reaction 3), and this reaction has been shown[7] to be a good general source of thermalized alkyl radicals:



$$R' + \text{H COO}\,R \longrightarrow R'\text{H} + \cdot\overset{\text{O}\,\,}{\overset{/\!\!/}{\text{C}}}\text{—O}\,R \qquad (3)$$

Isoelectronic with these radicals are the alkoxyazo radicals $R\text{ON}\dot{\text{N}}$. These may be prepared by the thermal decomposition of hyponitrite esters[8,9] (reaction 4).

$$R\text{ON} = \text{NO}R \longrightarrow R\text{ON}\dot{\text{N}} + R\text{O}\cdot \qquad (4)$$

Comparison of the two isoelectronic families $R\text{O}\dot{\text{C}}\text{O}$ and $R\text{ON}\dot{\text{N}}$ is more subtle than that of the radicals $R\dot{\text{C}}\text{O}_2$ and $R\dot{\text{N}}_2\text{O}$, because, in principle, each may undergo either of two decompositions:

$$R\text{O}\dot{\text{C}}\text{O} \rightarrow R\text{O}\cdot + \text{CO} \qquad (5a)$$
$$\rightarrow R\cdot + \text{CO}_2 \qquad (5b)$$
$$R\text{ON}\dot{\text{N}} \rightarrow R\text{O}\cdot + \text{N}_2 \qquad (6a)$$
$$\rightarrow R\cdot + \text{N}_2\text{O} \qquad (6b)$$

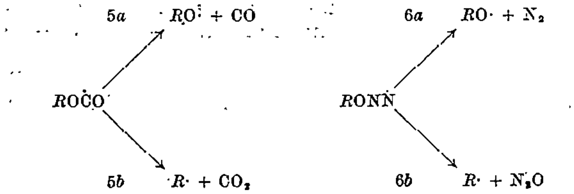
In fact, the observed decomposition behaviour is clear-cut and different in the two families. Alkoxycarbonyl radicals yield only alkyl radicals and carbon dioxide, for in none of the experiments[6,7,15] on the radicals $R\text{O}\dot{\text{C}}\text{O}$ ($R = \text{CH}_3$ to $R = \text{C}_4\text{H}_9$) is any product characteristic of alkoxy radicals found. Alkoxyazo radicals produce only alkoxy radicals and nitrogen. Neither in the decomposition of the lower alkyl hyponitrites[8] in the gas phase nor of benzyl hyponitrite[9] in solution is nitrous oxide or any product compatible with the formation of alkyl radicals found.

This striking difference is a departure from the behaviour expected on the basis of the isoelectronic

Table 1. ENERGY REQUIREMENTS OF ALTERNATIVE DECOMPOSITION PATHS FOR RELATED RADICALS $RO\dot{C}O$ AND $RO\dot{N}_2$

R	$\Delta H_{5b} - \Delta H_{5a}$ (kcal. mole^{-1})	$\Delta H_{6a} - \Delta H_{6b}$ (kcal. mole^{-1})
CH_3	35·7	51·5
C_2H_5	34·7	52·5
$n.C_3H_7$	32·7	54·5
$i.C_3H_7$	35·7	51·5
$n.C_4H_9$	31·6	56·5

$\Delta H_{5b} - \Delta H_{5a}$, enthalpy difference between reaction paths 5b and 5a; $\Delta H_{6a} - \Delta H_{6b}$, enthalpy difference between reaction paths 6a and 6b.



principle. Since it is essentially a kinetic difference it is helpful to consider the contribution of the Arrhenius factor A and the activation energy E to the velocity constant of decomposition. It is difficult to see any real basis for predicting different A factors for unimolecular decomposition of the radicals $RO\dot{C}O$ and $RON\dot{N}$, and it may be considered that the difference is attributable to difference in activation energies. In turn, the origin of the differences in activation energy may be sought in the thermochemistry of the radicals. Table 1 summarizes the differences in the energy requirements between the two decomposition paths for some members of each family of radicals. Thus in each family the energetically favoured path is followed. While carbon dioxide formation from the alkoxycarbonyl radicals needs some 35 kcal. less than carbon monoxide formation, nitrous oxide formation from the alkoxyazo radicals requires some 50 kcal. more for the production of nitrogen.

It is worthy of comment that the differences in energy requirements of the individual reaction paths of each radical can be evaluated without knowledge of the heats of formation of the individual radicals; thermochemical data for the reaction products are sufficient.

(3) Thermochemistry and reactivity of the acyloxyl and alkoxycarbonyl radicals. Although, for the purpose of comparison above, values of the heats of formation of the radicals $RO\dot{C}O$ are not needed, they are needed to make a comparison of the isomers $RO\dot{C}O$ and $R\dot{C}O_2$. Enthalpies of formation for some of the lower acyloxyl radicals are known[10] and these are tabulated (col. 2, Table 2). Values of enthalpies of formation may also be obtained for free radicals by group-energy terms[11], using a value of −39 kcal. for $H(CO_2.)$. These values also appear in col. 2. They have been used to calculate the enthalpy change of the decarboxylation of acyloxyl radicals, and these results appear in col. 3, Table 2.

No experimentally obtained thermochemical data exist for the alkoxycarbonyl radicals. Values for $\Delta H_f(RO\dot{C}O)$ which are unlikely to be seriously in error can be inferred from kinetic work on hydrogen abstraction by alkyl radicals for a series of aldehydes[13] ($RCH\dot{O}$) on one hand, and from various formate esters[14] ($RO.CHO$) on the other. In general, the values of the activation energies of hydrogen abstraction for both aldehydes and formates differ by little more than 1–2 kcal. mole^{-1} for members of both series, suggesting that the groups R and RO (which are not immediately adjacent to the point of radical attack) have little effect on the strength of the C—H bond being broken. If these bonds have similar strengths in the two families, then:

$$D(H - \overset{\overset{O}{\|}}{C}.CH_3) \simeq D(H) - \overset{\overset{O}{\|}}{C}OCH_3) \simeq 82 \text{ kcal.}$$

Since:

$$D(H - \overset{\overset{O}{\|}}{C}OCH_3) = \Delta H_f(H) + \Delta H_f(COOCH_3) - \Delta H_f(HCOOCH_3)$$

then a value of − 53·6 kcal. mole^{-1} may be calculated for the heat of formation of the methoxycarbonyl radical. Thermochemical data exist for few formates; however, it is still possible to obtain values for the heats for formation of the radicals from thermochemical data[15] for the acetate esters. In this case a similar assumption of equivalence of bond dissociation energies is made, this time comparing the appropriate bond-strengths of acetone and the acetates, using a 'medium' value for $CH_3 - COCH_3$, that is:

$$D(CH_3) - \overset{\overset{O}{\|}}{C}.CH_3) \simeq D(CH_3 - \overset{\overset{O}{\|}}{C}.OCH_3) \simeq 79 \text{ kcal.}$$

Using this bond energy, a value of − 51·7 kcal. mole^{-1} is obtained for $\Delta H_f(CH_3O\dot{C}O)$ which is in reasonable agreement with the value of − 53·6 kcal. obtained using methyl formate as the radical source. Values of ΔH_f for other alkoxycarbonyl radicals obtained in this manner appear in col. 4, Table 2, and using these values we may now make a direct comparison of the acyloxyl and alkoxycarbonyl radicals.

Assigning a value of − 18·5 kcal. to $H(\dot{C}O)$, then by using the usual group-energy terms, values of $\Delta H_f(RO\dot{C}O)$ may be calculated. These values are tabulated in col. 5, Table 2, and are in quite good agreement with the values obtained using the heats of formation of the acetate esters (col. 4).

Table 2. ENTHALPIES OF FORMATION AND BOND DISSOCIATION ENERGIES OF ACYLOXYL AND ALKOXYCARBONYL RADICALS
All values in kcal. mole^{-1}

R	$\Delta Hf\, RCO_2$	$D(R—CO_2)$	$\Delta Hf\, RO\dot{C}O$	$\Delta Hf\, RO\dot{C}O$	$D(RO—CO)$	$D(R—O\dot{C}O)$
CH_3	-45^a	−17·5	−51·7 (−53·6 from $HCOOCH_3$)	−55·8	24·8	−10·9
C_2H_5	-54^a	−15·5	−59·6	−60·8	24·7	−10·0
nC_3H_7	-60^a	−13	−65·8	−65·8	26·4	− 6·3
iC_3H_7	-60^b	−16·7	−68·9	−67·0	27·5	− 8·2
nC_4H_9	-64^b	−12·2	−71·9	−70·8	28·5	− 3·2
			c	d	e	e

a, Values reported by Jaffé, Prosen and Szwarc (ref. 10); b, calculated from group energy terms ($H(CO_2.)$ = −39 kcal.; c, obtained using $D[R—COOR]$ = 79 kcal., and using heats of formation of acetates (ref. 15); d, calculated from group energy terms $H(CO)$ = −18·5 kcal.; e, values of $\Delta Hf\,(RO\dot{C}O)$ taken from col. 4.

(4) Energetics of decarboxylation of $RO\dot{C}O$ and RCO_2. Since:

$$D(CH_3O - CO) = \Delta H_f(CH_3O) + \Delta H_f(CO) - \Delta H_f(CH_3O\dot{C}O)$$

and

$$D(CH_3 - OCO) = \Delta H_f(CH_3) + \Delta H_f(CO_2) - \Delta H_f(CH_3O\dot{C}O)$$

then, using a value of $-$ 51·7 kcal. for $\Delta H_f(CH_3O\dot{C}O)$, we obtain:

$$D(CH_3 - OCO) = -\ 10{\cdot}9 \text{ kcal. mole}^{-1}$$

and

$$D(CH_3O - CO) = 24{\cdot}8 \text{ kcal. mole}^{-1}.$$

Similar values for other alkoxycarbonyl radicals appear in columns 6 and 7 of Table 2. Decarboxylation of the $RO\dot{C}O$ radicals, although involving bond fission, is always exothermic (ΔH is negative): thus the alkoxycarbonyl radicals are thermodynamically unstable with respect to decarboxylation. This is the same rare behaviour that is observed in the acyloxyl radical where, for instance, $D(CH_3 - CO_2) = -\ 17{\cdot}5$ kcal. Throughout the two series, the alkoxycarbonyl radicals are thermodynamically less unstable than their isomers.

(5) Stability and reactivity of the $RO\dot{C}O$ and $RCO_2^{\cdot}$ radicals. There has been some controversy[16,17] about the kinetic stability of the acetyloxyl radical. The discussion has centred on whether the acetyloxyl radical survives long enough to react with a substrate and simultaneously decarboxylates, or whether the radicals are decarboxylated so that the substrate is attacked only by the methyl radicals produced. Recently, Herk and Szwarc[17] have concluded that, even in the liquid state, the reactions observed with the acetyl peroxide system are due to methyl radicals not to acetate radicals. No methyl acetate has been found in the gas-phase thermal decomposition of the peroxide, suggesting that there the acetyloxyl radical is not stable enough to associate with methyl radicals by:

$$CH_3CO_2\cdot + CH_3 \longrightarrow CH_3\ COO\ CH_3$$

Szwarc and Herk[12] consider that the decarboxylation of the acetyloxyl radical requires some small activation energy, about 1–2 kcal. mole^{-1}.

Less is known about the stability and reactivity of the alkoxycarbonyl radical. It appears from a consideration of the energetics of decarboxylation and from experimental observations[6,14,18] that the radical is unstable. Kebarle and Lossing[19], however, found traces of methyl acetate in a mass-spectrometric study of the mercury-photosensitized decomposition of methyl formate, presumably produced by the association of methyl with methoxycarbonyl radicals, that is:

$$CH_3^{\cdot} + CH_3OCO \longrightarrow CH_3\ COO\ CH_3$$

This apparent stability would suggest that the activation energy for decomposition of the methoxycarbonyl radical is perhaps somewhat greater than the value of 1–2 kcal. suggested for the acetyloxyl radical, a conclusion which would also concur with the relative bond strengths we have calculated for $CH_3CO_2\cdot$ and $CH_3O\dot{C}O$ ($-$ 17·5 kcal. and $-$ 10·9 kcal. respectively).

One of us (J. C. J. T.) would like to thank the Department of Scientific and Industrial Research for a research fellowship.

---

[1] Gray and Williams, *Chem. Rev.*, **59**, 239 (1959).
[2] Rembaum and Szwarc, *J. Amer. Chem. Soc.*, **76**, 5975 (1954).
[3] Kharasch and Gladslone, *J. Amer. Chem. Soc.*, **65**, 15 (1943).
[4] Gray and Rathbone, *Proc. Chem. Soc.*, 316 (1960).
[5] Langley, Lythgoe and Rayner, *J. Chem. Soc.*, 4191 (1952).
[6] Thynne, *Proc. Chem. Soc.*, 18 (1961).
[7] Thynne, *Proc. Chem. Soc.*, 68 (1961).
[8] Partington and Shah, *J. Chem. Soc.*, 2593 (1932).
[9] de Sousa and Ho, *Nature*, **186**, 776 (1960).
[10] Jaffé, Prosen and Szwarc, *J. Chem. Phys.*, **27**, 416 (1957).
[11] Franklin, *J. Chem. Phys.*, **21**, 2029 (1953).
[12] Szwarc and Herk, *J. Chem. Phys.*, **29**, 438 (1958).
[13] Birrell and Trotman-Dickenson, *J. Chem. Soc.*, 2059 (1960).
[14] Thynne (unpublished work, 1960).
[15] Schjanberg, *Z. physik. Chem.*, A, **172**, 197 (1935).
[16] Urry, abstract of a paper, Org. Symp. Denver (1950).
[17] Herk and Szwarc, *J. Amer. Chem. Soc.*, **82**, 3558 (1960).
[18] Ausloos, *Canad. J. Chem.*, **36**, 383 (1958).
[19] Kebarle and Lossing, *Canad. J. Chem.*, **37**, 389 (1959).

PATHS OF INTER-MOLECULAR ENERGY TRANSFER IN PHOTO-SENSITIZED OXIDATION-REDUCTION

By N. TAKEYAMA

Institute of Biophysics, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

IT has been recognized that there are two types of energy transfer of the electronic excitation from sensitizer to energy acceptor; one corresponds to the case of weak resonance coupling owing to the transitional dipole–dipole interaction between photo-excited sensitizer and unexcited acceptor, the other is the case of strong resonance coupling for which the effect of the first order of the same interaction as above is responsible.

The problem in this article is confined to the solution system of organic dyes. When S and A refer to the sensitizer and the energy acceptor, respectively, the types of energy transfer mentioned here are written in the manner of a chemical reaction as follows:

$$h\nu_s + \underbrace{S + A \rightarrow S^* + A \rightarrow S}_{+\text{reductant (or oxidant)}} + A^* \rightarrow A' \qquad \xrightarrow{\hspace{4em}} \text{redox process} \quad (a)$$

$$h\nu_s + \underbrace{S + A \rightarrow S^* + A \rightarrow [S \ldots A]^* \rightarrow S}_{+\text{reductant (or oxidant)}} + A' \qquad \xrightarrow{\hspace{4em}} \text{redox process} \quad (b)$$

where $h\nu_s$ denotes the energy of a photon absorbed by S, the molecular species accompanied with asterisk mean the photo-excited molecules in the singlet state, and A' stands for the metastable A in the triplet state. Here we have written the resonance state as $[S \ldots A]^*$. In other words, this state can be described as the resonance between the states (S^*, A) and (S, A^*). The case of (a) is called the weak resonance coupling, and the case of (b) is called the strong resonance coupling.

The following criteria, which were appointed by Simpson and Peterson[1] in the problem of the electronic spectra of molecular crystals, serve as an answer for

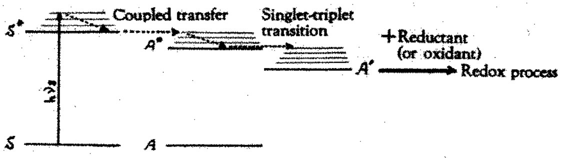


Fig. 1. Simplified diagram of sensitization in the case of weak resonance coupling

why either type (*a*) or (*b*) can be found in the mechanism of sensitization:

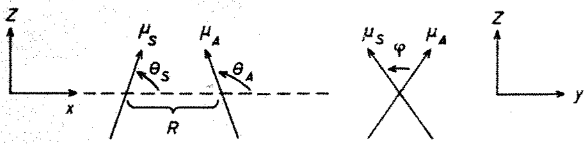
$2V/\Delta \ll 1$ for the case of weak coupling (*a'*)
$2V/\Delta \gg 1$ for the case of strong coupling (*b'*)

where V represents the so-called resonance coupling parameter, which is defined by the matrix element of the electronic dipole–dipole interaction, and Δ is the width of the band related to the electronic transition under consideration.

In the former case, it can be said that the energy transfer is achieved with the transition from the initial state $\psi(S^*)\psi(A)$ to the final state $\psi(S)\psi(A^*)$ owing to the dipole–dipole interaction, and the transfer probability has the dependence R^{-6} on the intermolecular distance R, where $\psi(S),\psi(S^*)$, and $\psi(A)$, $\psi(A^*)$ are the electronic wave functions of the unexcited and excited S and A, respectively. In the present problem, the resonance coupling parameter becomes:

$$V = -\frac{1}{\varkappa R^3}\int \psi(S^*)\psi(A)\mu_S\mu_A\psi(S)\psi(A^*)\mathrm{d}\tau_S\mathrm{d}\tau_A \times$$
$$(2\cos\theta_S\cos\theta_A - \sin\theta_S\sin\theta_A\cos\varphi) \qquad (c)$$

where $\varkappa$ is the dielectric constant of the medium, μ_S and μ_A are the dipole moments referring to S and A, and the angles of relative orientation of the dipoles are defined as:



If S and A are parallel in the resonance state, $\theta_S = \theta_A = \frac{\pi}{2}$ and $\varphi = 0$. Hence, V can be written as:

$$V = \mu_S'\mu_A'/\varkappa R^3 \qquad (d)$$

where μ_S' and μ_A' are the transitional dipole moments given by $\mu_S' = \int\psi(S^*)\mu_S\psi(S)\mathrm{d}\tau_S$ and $\mu_A' = \int\psi(A)\mu_A\psi(A^*)\mathrm{d}\tau_A$, respectively. It goes without saying that the transition probability of the energy transfer in weak resonance coupling is proportional directly to the square of equation (*c*) (ref. 2). The energy transfer from S to A is succeeded by the non-radiative transition from the excited singlet state to the triplet state due to the spin-orbit coupling in the acceptor molecule. The metastable molecular species of the produced triplet state take part in redox processes with a reductant or oxidant. This is the mechanism of photo-sensitized redox reaction in the case of sensitization due to the weak coupling. A simplified energy diagram of this mechanism is shown in Fig. 1.

The example can be found in the following system: in the ethanol solution of S = uranin, A = methylene blue and reductant = ascorbic acid or phenylhydrazine, the bleaching reaction of A photo-sensitized by S occurs with photo-excitation of S (ref. 3).

A characteristic property of the sensitization in the case of weak resonance coupling can be seen in the fact that the vibrational motion in the excited electronic state continues during an interval of time of $10^{-9} \sim 10^{-8}$ sec. (that is the life-time of fluorescence) according to the inequality (*a'*).

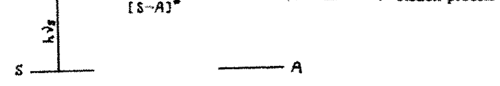
On the other hand, in the case of strong resonance coupling the resonance effect of the first order of interaction must be taken into account by taking the following linear combinations of both states $\psi(S^*)\psi(A)$ and $\psi(S)\psi(A^*)$ at a fixed molecular configuration:

$$\psi_+ = 2^{-1/2}\{\psi(S^*)\psi(A) + \psi(S)\psi(A^*)\} \qquad (e, \mathrm{i})$$
$$\psi_- = 2^{-1/2}\{\psi(S^*)\psi(A) - \psi(S)\psi(A^*)\} \qquad (e, \mathrm{ii})$$

because the electronic coupling occurs before the vibrational motion in the degenerate excited state at a reasonance configuration; this is the meaning of the inequality (*b'*). The resonance split of energy is given by the difference between the energy-levels of ψ_+ and ψ_-, or by $2|\mu_s'||\mu_A'|/\varkappa R^3$. Provided that $\vec{\mu}_s' = -\vec{\mu}_A'$, the lower energy state is ψ_+ and the higher one is ψ_-. Moreover, it is important that the dipole transition from ψ_+ to $\psi(S)\psi(A)$, in which $\psi(S)\psi(A)$ is the ground state free from interaction, is forbidden owing to vanishing the total transitional dipole moment.

Because the energy-level of the state ψ_+ is lower by $|\mu_s'|^2/\varkappa R^3$ than the level of the excited state of S without A, we can interpret the state ψ_+ as the excited state stabilized by the transitional dipole–dipole coupling with A. It can be expected that the transition probability from the stabilized singlet state ψ_+ to the lowest triplet state of A in the resonance complex is much greater than the transition probability from the excited singlet state of A only to the same triplet state, because the stabilizing energy, or $|\mu_s'|^2/\varkappa R^3$, is comparable with the energy of separation between the first excited singlet state and the lowest triplet state of A itself; in many cases of organic dyes the energy is a few thousand cm.$^{-1}$ (refs. 4 and 5). After the transition of A to the triplet state, the resonance complex $[S \ldots A]^*$ dissociates repulsively, owing to the vanishing of the transitional dipole moment. The metastable molecule A' in the triplet state proceeds to redox process with a reductant or oxidant. These processes are shown diagrammatically as Fig. 2.

Although it is not the example of sensitization in a narrow sense, an interesting example of the latter case can be found in the photo-reduction of some organic dyes in an aqueous solution of high polymer; in this reaction it is important to produce the metastable species of the dye due to the interaction between the photo-excited and unexcited dyes in the bound state with high polymer[6]. Another example is the reduction of crystal violet photo-sensitized by trypaflavine in the aqueous solution. As is well known, the triphenylmethane dyes cannot be photoreduced by ascorbic acid or some other reductant in



⟶, radiative transition; - - ⟶, radiationless transition

Fig. 2. Simplified diagram of sensitization in the case of strong resonance coupling

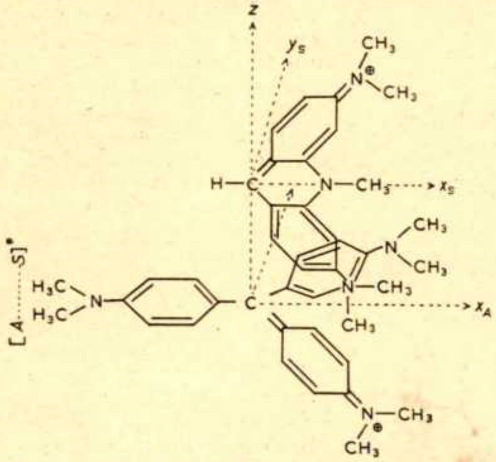


Fig. 3. A possible configuration in the resonance state of trypaflavine (*S*) and crystal violet (*A*)

free solution except in the presence of high polymer which contains the carboxyl groups. However, the photo-reduction of the dye with ascorbic acid occurs when trypaflavine is used as sensitizer[7]. The mechanism can be explained by sensitization of the latter case. In the resonance state in which the excited trypaflavine interacts with the unexcited crystal violet, the molecular plane surface of crystal violet will be conserved as a result of resonance stabilization. A possible molecular configuration in the resonance state is shown in Fig. 3. The excitation energy absorbed by crystal violet in the free system from trypaflavine, or even in the presence of trypaflavine, does not activate the redox reactivity, because the dissipative processes predominate over the intramolecular transition to the triplet state due to the deficiency of the stability of the excited singlet state of crystal violet.

The experimental facts show that the metastable crystal violet produced after the resonance stabilization in the sensitization takes part in the redox process with ascorbic acid, and the stabilization of the excited crystal violet results also from the resonance interaction with the unexcited crystal violet in the presence of high polymer.

[1] Simpson, W. T., and Peterson, D. L., *J. Chem. Phys.*, **26**, 588 (1957).
[2] Förster, Th., *Ann. Physik*, **2**, 55 (1948); *Disc. Farad. Soc.*, **27**, 7 (1959); *Radiation Res*, supp. 2, 326 (Academic Press, 1960).
[3] Tomita, G., and Takeyama, N., *Kagaku* (Tokyo), **27**, 629 (1957).
[4] Levison, G. S., Simpson, W. T., and Curtis, W., *J. Amer. Chem. Soc.*, **79**, 4314 (1957).
[5] McRae, E. G., and Kasha, M., *J. Chem. Phys.*, **28**, 721 (1958).
[6] Oster, G., and Bellin, J. S., *J. Amer. Chem. Soc.*, **79**, 294, 2461 (1957).
[7] Tomita, G., and Takeyama, N., *Kagaku* (Tokyo), **29**, 662 (1959).

ELECTRON MICROSCOPIC OBSERVATIONS OF STRUCTURE IN ULTRA-THIN SECTIONS OF VITRINITE IN COAL

By J. T. McCARTNEY, SABRI ERGUN and R. E. WALLINE

Pittsburgh Coal Research Center, Bureau of Mines, U.S. Department of the Interior, Pittsburgh, Pa.

MOST electron microscopic examinations of coal have been made on replicas of polished, etched, or natural fracture surfaces[1-4]. Faithful reproduction of structures in such replicas may be questioned, and resolution of detail is inferior to that which may be expected in direct observation of coal specimens. Consequently, the preparation of coal sections thin enough to transmit electrons has been an important objective. Recently, workers at the Centre d'Etudes et Recherches des Charbonnages de France[5] have succeeded in cutting ultra-thin sections of soft coals, using a microtome and a diamond knife. We have adopted their procedure and succeeded in cutting sections, 500–2000 Å. thick, of coals of various ranks and hardness. Success in preparing such sections has opened up a very promising field of study involving ultra-fine structures in coals such as preserved plant cell components, pores, and possibly structural units or crystallites.

Ultra-thin sections of coal are cut with a diamond knife mounted on a microtome designed for preparing sections for electron microscopy. The sections are cut from an 8–14 mesh particle embedded in a polymerized mixture of equal volumes of methyl and butyl methacrylates. They have an area of approximately 1 mm.^{2}. When cut the sections float out on a liquid surface from which they are picked up on specimen screens. A detailed description of the technique of preparing these sections will be presented elsewhere[6].

The limited number of electron microscopic observations of ultra-thin sections of vitrinites made thus far have revealed interesting structural details. Interpretation of these details has been approached

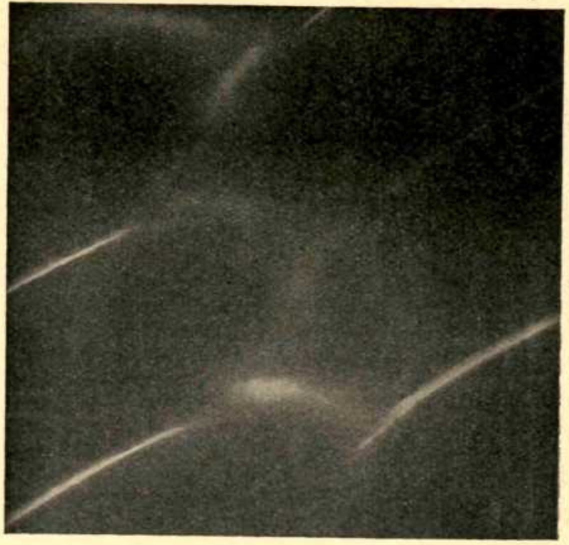


Fig. 1. Electron micrograph of structure in an ultra-thin section of a semi-anthracite (× *c*. 7,500)

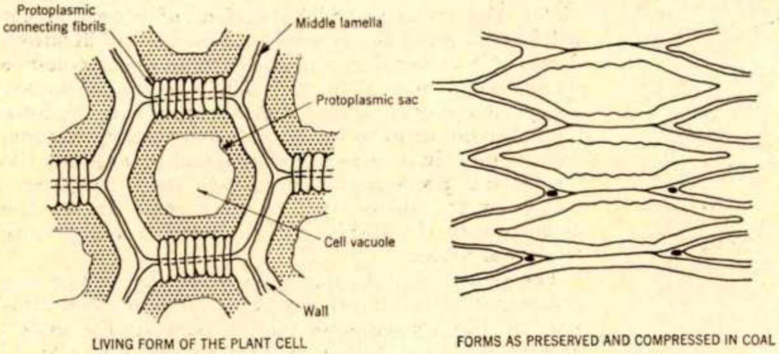

Protoplasmic connecting fibrils
Middle lamella
Protoplasmic sac
Cell vacuole
Wall
LIVING FORM OF THE PLANT CELL
FORMS AS PRESERVED AND COMPRESSED IN COAL

Fig. 2. Cell structure in the living plant cell and typical form of preservation in coal

cautiously because of possibilities of artefacts introduced in section preparation. One type of structure is shown in Fig. 1. This is an electron micrograph of a relatively thick section (≈ 2000 Å.) of a semi-anthracite. It shows outlines of areas resembling cross-sections of cells, with little internal detail visible. These areas have dimensions of about $3 \times 10\mu$. The manner in which such a cell structure might be preserved in coal is illustrated in Fig. 2[7]. Compression of the original plant cell form can result in the type of structure shown, to which the areas in Fig. 1 bear a striking resemblance. Such structures have been seen in electron micrographs of replicas of etched coal surfaces[1], but the dimensions were about $5 \times 50\mu$. Turner[8] has shown cells in anthracite averaging about $16 \times 33\mu$, and Raistrick and Marshall[9] have shown relatively uncompressed cells, in coals of unspecified rank, ranging upwards from 30μ in diameter. Thus, the areas of Fig. 1 seem to be considerably smaller than the normal cell sizes seen in coal. However, these differences in size are possible and the pattern is so well-defined that it probably represents an authentic structure. Structures of this kind appear to become prominent in the thicker ranges of these ultra-thin sections. This may be due to distortion of the structure because of compression in the thinner sections or to greater contrast in the thicker ones.

Another kind of structure similar to that of Fig. 1 is shown in Fig. 3. This was seen in a section of high-volatile *A* bituminous coal from the Pittsburgh bed. Here, too, there are some of the characteristics of a cell structure. These units average about $\frac{1}{2} \times 2\mu$, about a fifth as large as those in Fig. 1. Variations of this pattern also appear in several other coals. The appearance of this structure may have been determined largely by stresses operating during the cutting process. However, even if this were the case, the strains contributing to this pattern must have been conditioned by some repetitive inhomogeneity in the coal substance, for this pattern is not always obtained, it does not always cover the whole section, and it is not restricted to the middle or to the edges of the sections. It may be premature to accept the observed pattern as a definite structure; nevertheless, it could be regarded as an indication of some basic periodicity in the coal make-up.

Another type of structure seen in several vitrinites of different rank is shown in Fig. 4. This appears to be a very fine fibrillar structure with recurring units less than $0\cdot 1\mu$ in width. A fibrillar structure similar to, but coarser than, this has been seen in an ultra-violet light photomicrograph of a delignified cell wall of western hemlock[10]. It has been suggested that the cell walls of wood consist of interpenetrating systems of cellulose and lignin. Although coal is believed to contain little cellulose, it is possible that the fibrillar structure of wood is preserved in coal. However, this apparent structure may be an artefact introduced in the section during the cutting process. Its prevalence throughout the sections of this coal and several other coals favours this suspicion. An authentic interpretation must await further examination.

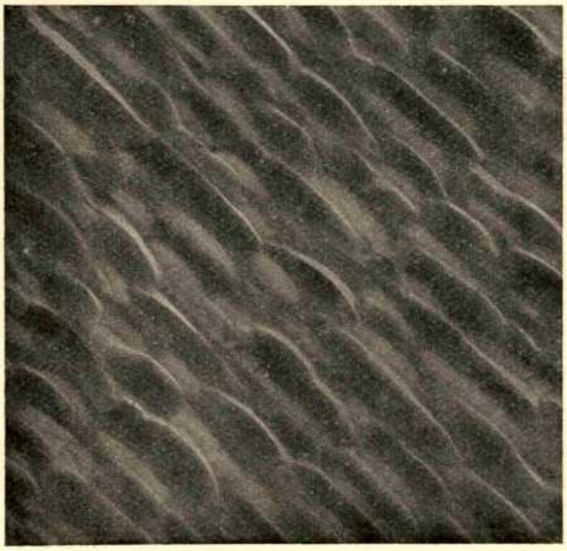


Fig. 3. Electron micrograph of structure in an ultra-thin section of a high-volatile *A* bituminous coal (× *c*. 7,500)

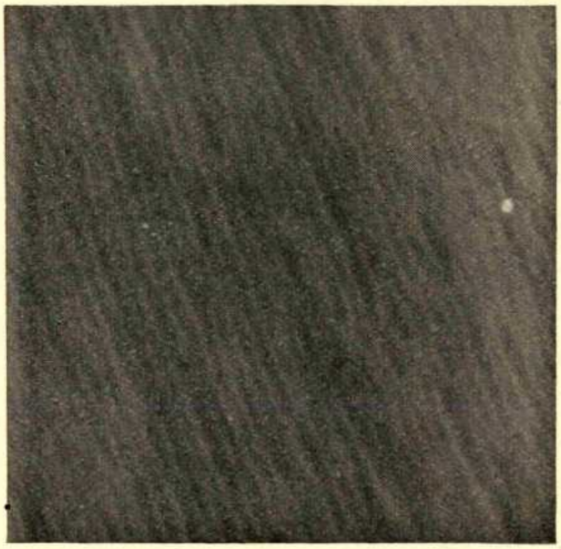


Fig. 4. Electron micrograph of structure in an ultra-thin section of a high-volatile *C* bituminous coal (× *c*. 22,500)

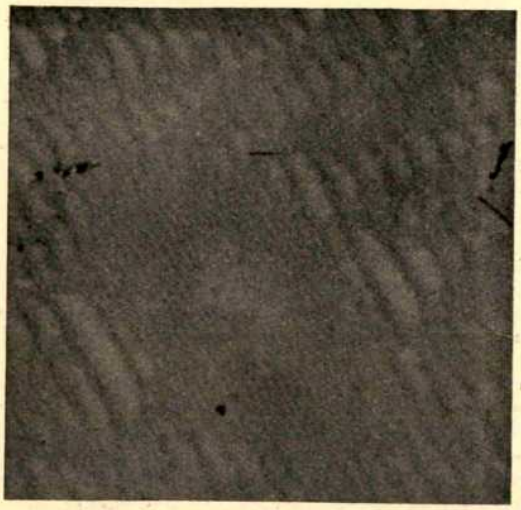


Fig. 5. Electron micrograph of structure in an ultra-thin section of an anthracite (×c. 7,500)

Fig. 5 shows an ultra-thin section of an anthracite. Three features are evident in this electron micrograph: an irregular pattern of thin areas surrounding an oval portion with a central thin area, and an overall background of striæ. The significance of the first two features with regard to coal structure is not readily evident, but it seems highly unlikely that the oval area and others similar to it could be preparation artefacts. They may be remnants of resin globules. The striæ, which persist throughout this section, are no doubt similar to those observed in Fig. 4 and, as discussed there, may or may not have structural significance.

It may be readily inferred from the foregoing discussions that interpretations of electron micrographs of these ultra-thin sections are far from conclusive. However, these preliminary results and comments are presented here as a stimulus to further studies of the physical fine structure of coals.

[1] McCartney, J. T., *Econ. Geol.*, **44**, 617 (1949).

[2] Walsman, B. A., Krivitzkii, M. D., and Krigman, F. E., *Doklady Akademie Nauk SSSR.*, **97**, 1031 (1954).

[3] Alpern, S., and Pregermain, S., *Bull. Micro. App.* (2), **6**, 16 (1956).

[4] Dutcher, R. R., and Spackman, W., *Bull. Geol. Soc. Amer.*, **71**, 1855, 1960.

[5] Pregermain, S., and Boyer, A. F. (private communication).

[6] McCartney, J. T., Walline, R. E., and Ergun, S., "Preparation of Ultrathin Sections of Coals", *U.S. Bur. Mines Rep. Invest.* (in the press).

[7] Hickling, G. (private communication).

[8] Turner, H. G., *Tech. Pub.* No. 234, *Amer. Inst. Min. Eng. Mg.* (1929).

[9] Raistrick, A., and Marshall, C. E., *The Nature and Origin of Coal and Coal Seams*, 184 (English Univ. Press, Ltd., Lond., 1939).

[10] Brown, H. P., Panshin, A. J., and Forsaith, C. C., *Textbook of Wood Technology*, 87 (McGraw-Hill Book Co., Inc., New York, 1949).

POISONING AND SIGN REVERSAL OF PERMSELECTIVE MEMBRANES

By Dr. FRANCIS de KŐRÖSY

Negev Institute for Arid Zone Research, Beer-Sheva, Israel

WHENEVER the counterions of a permselective membrane are held firmly to the fixed ions of the membrane, its conductivity decreases[1], and as a part of the fixed charge is neutralized the permselectivity of the membrane decreases also. The higher the electric charge of the counterions, the more firmly are they attached to the fixed sites. George, Rosenberg and Potter[2] even found that the transference number of Th^{+4} ions sank below 0·5—that is, the sign of permselectivity was reversed—when a cation-selective membrane was placed between two Th^{+4} solutions. In this experiment the Th^{+4} functioned both as 'poison' and as an indicator of 'poisoning'; it was the reduced mobility of these ions in the membrane that gave rise to low cationic transference.

I wanted to see whether the membrane could be poisoned to an extent that it became reversed even against ions which in themselves were not poisonous to it. Membranes were immersed between two solutions of potassium chloride of different concentration; here the mobility of K^+ and Cl^- was certainly the same. I then added equal amounts of 'poisoning' ions to both sides, so that there was no concentration gradient of these ions between the two sides. They served only to poison the membrane, while the potassium chloride indicated the degree of poisoning. As soon as the total charge of firmly bound counterions equals the sum total of the charge of fixed membrane-ions, the permselectivity of the membrane becomes zero, and, as the charge of bound counterions increases further, the sign of charge of the membrane changes and its permselectivity is reversed.

I worked with membranes made of parchment impregnated and cured with a phenolsulphonic acid-formaldehyde or with *N*-acetic acid-pyridinium-phenolformaldehyde resin. Permselectivity was generally determined by measuring the e.m.f. across the membrane between two potassium chloride solutions of different concentration which contained the same concentration of poison on both sides. In one experiment where permselectivity had been reversed by Th^{+4} ions I confirmed the sign reversal in a Hittorf transference-number experiment in $N/10$ potassium chloride (containing 0·008 N thorium chloride) and found a Cl^- transference number of 0·6 instead of nearly zero in pure potassium chloride.

Up to now I have found three ions which induce sign-reversal in these systems: Th^{+4}, $Fe(CN)_6^{-3}$ and $Fe(CN)_6^{-4}$. Equilibration of the systems was slow, ranging from about ½ hr. with $Fe(CN)_6^{-3}$ to about ½ day with Th^{+4}, being slower at low poison concentrations. It was reversible with the complex cyanides but not entirely reversible with Th^{+4}: though the sign of permselectivity changed back to normal in pure potassium chloride, the original e.m.f. value never returned.

Fig. 1 shows the poisoning curves of the $N/1 : N/2$, $N/10 : N/20$ and $N/100 : N/200$ potassium chloride systems with increasing amounts of $K_3[Fe(CN)_6]$. The points where the e.m.f. disappears are at $1{\cdot}5 \times 10^{-3}$, $1{\cdot}5 \times 10^{-2}$ and $1{\cdot}5 \times 10^{-1}$ poison normality respectively. This is exactly proportional to the three potassium chloride concentrations. The close agreement may be partly fortuitous, but it demonstrates that there is a competition between Cl^- and $Fe(CN)_6^{-3}$ for the active sites of the membrane according to the law of mass action. The membrane capacity was 0·55 m.equiv./gm. At the three respective concentrations

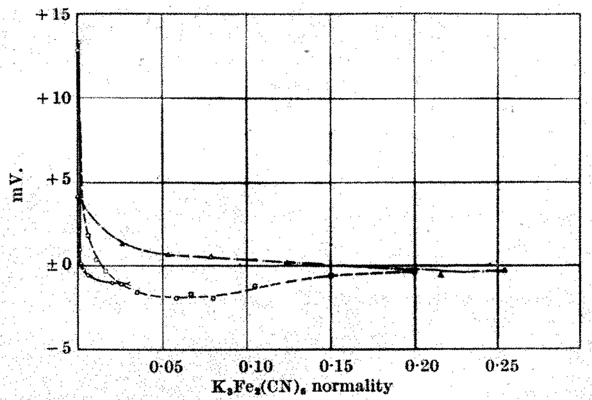


Fig. 1. Changes in e.m.f. due to adjusting the $K_3/Fe(CN)_6$ normality on both sides of an anion-selective membrane to the value given by the abscissa

Membranes between 0·010 *N* and 0·005 *N* (○), 0·10 *N* and 0·05 *N* (□) and 1·0 *N* and 0·5 *N* potassium chloride (△)

where the e.m.f. was zero, the membrane was blotted between filter paper and was found to contain 0·63, 0·57 and 0·47 m.equiv./gm. $Fe(CN)_6^{-3}$ respectively. It was not possible to distinguish between 'site-bound' and 'immigrant' counterions, but their concentration within the membrane was high and nearly the same whenever sign reversal occurred.

Permselectivity at high potassium chloride concentration is smaller than at low because of the Donnan equilibrium. The fact that the *N*/10 : *N*/20 curve shows a minimum of −2mV. at 5×10^{-2} poison normality may be due to the fact that progressive addition of the same amount of $K_3Fe(CN)_6$ to both sides decreases the ratio of ionic concentrations between the two sides and it is this ratio which determines the e.m.f. according to the Nernst equation. At high poison concentration the e.m.f. is swamped.

Increasing the potassium chloride concentration on both sides works competitively against poisoning according to Fig. 1. But a reversed e.m.f. may either grow or decrease if the potassium chloride concentration on the concentrated side is increased. It grows in the reversed direction if the poisoning ion is attached so firmly to the resin that only slight competition of potassium chloride occurs. Then the poisoning remains but the ratio of potassium chloride across the membrane increases, and hence the reversed e.m.f. increases also. Two such curves with Th^{+4} as poisoning ion are shown in Fig. 2. On the steeper curve, where the poison concentration and the potassium chloride ratio were both high, the considerable reversed e.m.f. of −19 mV. was attained.

The opposite effect is observed if the competitive influence of potassium chloride overrides its effect on the concentration ratio, as often with the complex cyanides. In such cases, the poison concentration in the membrane evidently decreases on the side towards the more concentrated potassium chloride as more potassium chloride is added, in spite of the fact that the concentration of the poison ion is the same on both sides. The resulting e.m.f. may hence decrease if it was reversed and even change back and increase in the normal direction.

It could be postulated that sign reversal is due to difference in the activity of poisoning ions on the two sides: though their concentration is the same, their activity is less in the more concentrated potassium chloride solution. This difference in activity could override the potassium chloride activities and be the cause of sign reversal. Calculations using the Debye–Hückel formula showed, however, that this hypothesis can be practically excluded for Th^{+4} poisoning and is very improbable with the complex cyanides. Difference in Th^{+4} activity would, for example, account only for a reversed e.m.f. of 0·4 mV. at a point of Fig. 2 where actually 15 mV. was observed. It is certain that the sign of the charge bound to the membrane has been reversed.

Al^{+3}, Fe^{+3}, Cu^{+2}, Ca^{+2}, Mg^{+2}, hexamethonium^{+2}, *N*,-*N*-tetramethyl-pyrazolidinium^{+2}, 1,2,3-*tris*-triethylammoniumethoxybenzene^{+3} ('Flaxedil'), HPO_4^{-2} and $O_3P.CH_2.PO_3^{-4}$ ions have been examined also on the same membranes in the same set-up. None of them was sufficiently active as poison to reverse the e.m.f. between *N*/10 : *N*/20 potassium chloride, though Al^{+3} seemed to reach this point within the limits of error (0·2 mV.). Fe^{+3} lowered the permselectivity without reaching zero e.m.f. The other three metal ions and the organic cations were scarcely active at all except that they swamped the e.m.f. by decreasing the ionic ratio. In the experiments of George *et al.*[2], however (that is, without potassium chloride), when only the active ions were present, a sign reversal was observed between *N*/1 : *N*/2 calcium chloride and *N*/1 : *N*/2 magnesium chloride solutions.

It is interesting to note that the three isolated single charges of 'Flaxedil' and the two double charges on the methanediphosphonic ion were not effective, though they gave the total charge of 3 and 4 respectively to their ions. This is probably due to the fact that the charge is spread out in space and not concentrated enough to be active in Coulomb attraction.

The physiological implications of this poisoning and the sign reversal effect are obvious. The small amount of 'poison' necessary to change the potential of, say, a

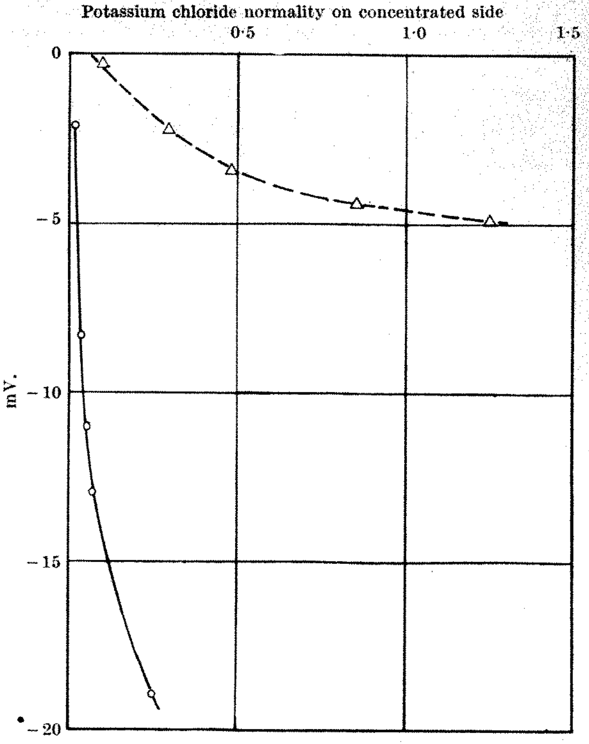


Fig. 2. △, Th^{+4} normality on both sides, 0·038 *N*, potassium chloride normality on dilute side, 0·06 *N*; ○, Th^{+4} normality on both sides, 0·079 *N*, potassium chloride normality on dilute side, 0·006 *N*

nerve ending might be generated by some physiological process and the short circuit could then well travel along the whole fibre. The active volume of such a physiological membrane is very small and the amount of poison necessary very small also, especially bearing in mind that specificity may increase its sensitivity compared with our crude artificial membranes.

I am indebted to Prof. F. Bergmann and to T.E.V.A., Ltd., Jerusalem, for the gift of hexamethonium salts, and to SPECIA S.A., Paris, for the gift of 'Flaxedil'.

[1] Juda, W., Marinsky, J. A., and Rosenberg, N. W., *Ann. Rev. Phys. Chem.*, **4**, 373 (1953). Spiegler, K. S., in Nachod-Schubert, *Ion Exchange Technology*, 133 (Academic Press, New York, 1956).

[2] Rosenberg, N. W., George, J. H. B., and Potter, W. D., *J. Electrochem. Soc.*, **104**, 111 (1957).

CONTROL OF SPECIFICITY IN HÆMOGLOBIN SYNTHESIS

By Dr. J. BISHOP*, Dr. G. FAVELUKES†, Prof. R. SCHWEET and Dr. E. RUSSELL‡

Department of Biochemistry, College of Medicine, University of Kentucky, Lexington

In recent years much attention has been focused on the control of specificity in protein synthesis. In general terms it is clear that the ultimate control of specificity resides in the genetic material of the cell. The mechanism whereby the information of the genetic material is translated into the unique amino-acid sequence of the finished protein molecule is generally believed to involve ribonucleic acid. The present article presents results which demonstrate that a particular cell structure, the ribosome, directly controls the amino-acid sequence of the protein which it synthesizes.

Recent work in this laboratory[1-3] has shown with some clarity that in the reticulocyte the site of synthesis of the hæmoglobin molecule is the ribosome. This, together with the large size of the ribosome[4], might suggest that the amino-acid sequence of the hæmoglobin molecule is determined by the structure of the ribosome. The possibility remains, however, that the amino-acid sequence is not determined by the ribosome alone, but by the ribosome acting in conjunction with other cytoplasmic factors. In this respect it is important to note that soluble cytoplasmic factors are known to be implicated in protein synthesis. A specific activating enzyme[5] and a specific soluble ribonucleic acid[5] are thought to be required for the incorporation into protein of each individual amino-acid. Furthermore, it has been suggested[5] that it is a correspondence between the structure of the soluble ribonucleic acid molecule to which the amino-acid is bound, and a specific structural site on the ribosome, which ensures that the amino-acid is incorporated into protein in the correct position. The question to be raised in the present article is whether or not these soluble factors, and in particular soluble ribonucleic acid, play a more direct part in the determination of the amino-acid sequence of hæmoglobin. This work was made possible by the demonstration[1,2,6] of the synthesis of soluble hæmoglobin in a reticulocyte cell-free system.

A first approach to the question was made using ribosomes and soluble fractions from reticulocytes of two species, rabbits and mice. The *C57BL/6J* inbred mouse strain shows a single hæmoglobin fraction by chromatography on 'Amberlite *CG*-50' type II resin with the sodium phosphate buffer number 2 of Allen *et al.*[7]. Under the same conditions rabbit hæmoglobin is strongly bound to the resin, but can be eluted with a two-fold concentration of the same buffer. Thus almost quantitative separation of these two hæmoglobins is possible.

Reticulocytosis was induced in rabbits as described elsewhere[1,3]. Mice were made reticulocytic by the subcutaneous injection of 0·1 ml. of 0·8 per cent phenylhydrazine daily for six days. Twice-washed ribosomes and supernatants were prepared from reticulocyte lysates as described elsewhere[1,3]. Rabbit ribosomes (40 mgm. dry weight) were incubated with 0·6 μmole of uniformly labelled ^{14}C-L-leucine (7 mc./mmole) in a total volume of 8·4 ml. under conditions described[1,3] except that isolated soluble ribonucleic acid and enzyme were replaced by a mixture of 30 mgm. each of the soluble supernatants of both species. After 40 min. of incubation the ribosomes were removed by centrifugation and a hæmoglobin fraction isolated[2] from the supernatant. On chromatography on *CG*-50, 61 per cent of the radioactivity recovered was seen to be associated with

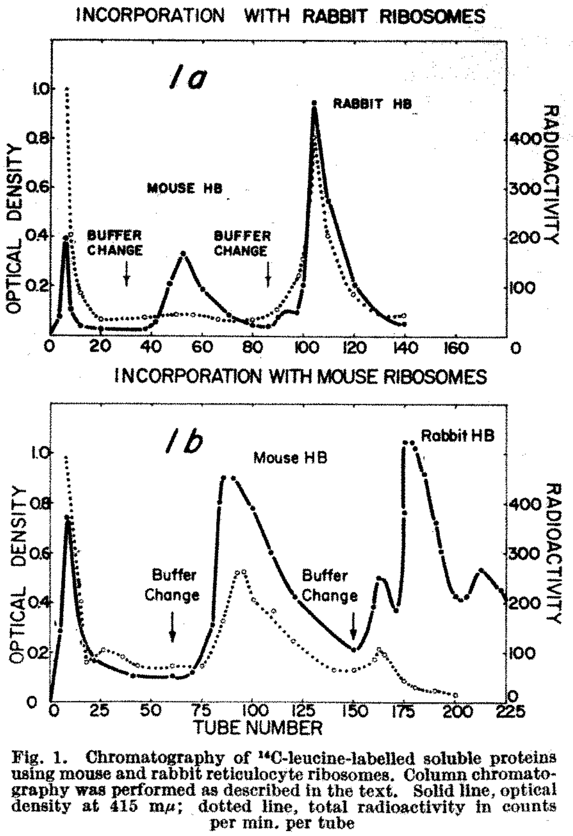


Fig. 1. Chromatography of ^{14}C-leucine-labelled soluble proteins using mouse and rabbit reticulocyte ribosomes. Column chromatography was performed as described in the text. Solid line, optical density at 415 mμ; dotted line, total radioactivity in counts per min. per tube

---

* Permanent address: Institute of Animal Genetics, West Mains Road, Edinburgh, Scotland.

† Fellow of the Consejo Nacional de Investigaciones Cientificas y Técnicas, Argentina. On leave of absence, Universidad Nacional de Córdoba, Córdoba, Argentina.

‡ Guggenheim Fellow at the California Institute of Technology. Permanent address: Jackson Memorial Laboratory, Bar Harbor, Maine.

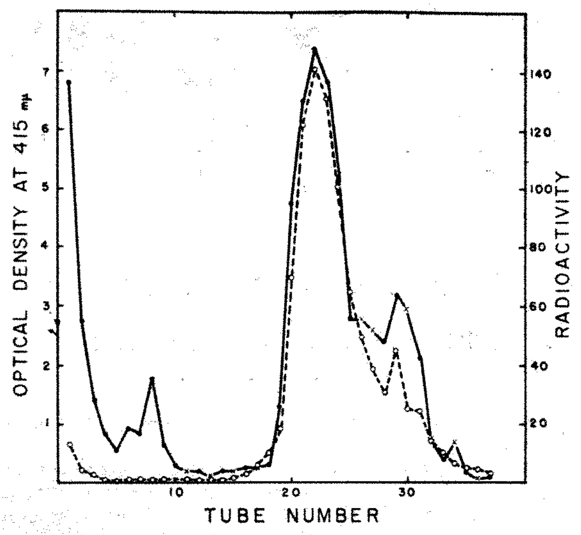


Fig. 2. Chromatography of soluble proteins after incubation of rabbit reticulocyte ribosomes with guinea pig liver ^{3}H-leucyl-ribonucleic acid. Dashed line, optical density at 415 mμ; solid line, total radioactivity in counts per min. per tube. Guinea pig liver soluble ribonucleic acid was labelled by incubation with ^{3}H-DL-leucine (150 mc./mmole) and 17 other unlabelled amino-acids, and isolated as previously described (ref. 8). Isolated ^{3}H-leucyl-ribonucleic acid (3·6 mgm.) was incubated at 37° with 18 mgm. of reticulocyte ribosomes and 42 mgm. of a reticulocyte enzyme fraction (ref. 9) plus $3 \cdot 3 \times 10^{-4}$ *M* guanosine triphosphate, 0·07 *M* potassium chloride, $3 \cdot 3 \times 10^{-3}$ *M* magnesium chloride, 0·02 *M* glutathione, and 0·033 *M tris* buffer, *p*H 7·5, in a volume of 9 ml. To increase the amount of soluble labelled protein, after 10 min. $3 \cdot 3 \times 10^{-4}$ *M* adenosine triphosphate, 0·01 *M* creatine phosphate and 0·34 mgm. of creatine kinase were added and incubation was continued for another 30 min. Ribosomes were removed by centrifugation, and the proteins precipitated with ammonium sulphate and chromatographed as described (ref. 2)

rabbit hæmoglobin, and only 5 per cent with mouse hæmoglobin (Fig. 1*a*). On the other hand, when the reciprocal experiment was performed (mouse ribosomes incubated with the mixed supernatants) 52 per cent of the radioactivity recovered was associated with the mouse hæmoglobin and only 3 per cent with rabbit hæmoglobin (Fig. 1*b*). Thus, in these experiments, the hæmoglobin synthesized was the specific hæmoglobin of the species which contributed the ribosomes. The small amount of labelled protein in the other hæmoglobin is believed to be an artefact, since in a control experiment, when labelled rabbit hæmoglobin was mixed and chromatographed with unlabelled mouse hæmoglobin, 3–4 per cent of the recovered radioactivity was found in the mouse hæmoglobin peak, while 65 per cent was found in the rabbit hæmoglobin fraction. In each experiment radioactive protein was also found in a fraction which did not partition between resin and buffer, but passed directly through the column. It is not possible to conclude from these experiments that soluble components, such as soluble ribonucleic acid, have no function in sequence determination. For example, since soluble ribonucleic acid from both species was always present (and, besides, ribosomes cannot be completely freed from soluble ribonucleic acid[3]), it is possible that soluble factors contain sequence information, but that ribosomes can react only with soluble factors from the same species. However, the experiments do demonstrate that the reticulocyte cell-free system synthesizes a specific protein, and that the ribosome must play an important part in the determination of the amino-acid sequence of this protein.

In order to preclude the participation of endogenous soluble ribonucleic acid, experiments were performed with rabbit reticulocyte ribosomes using labelled amino-acyl-ribonucleic acid rather than free ^{14}C-amino-acid as starting material. Guinea pig liver ^{3}H-leucyl-ribonucleic acid was used since it differed in both organ and species from rabbit reticulocyte soluble ribonucleic acid. A large-scale incubation of the ^{3}H-leucyl-ribonucleic acid and reticulocyte ribosomes was required to provide sufficient labelled soluble protein for chromatography (Fig. 2). The results show that much of the radioactivity was recovered with the hæmoglobin fraction and the labelling of protein fractions using this guinea pig liver amino-acyl-ribonucleic acid was similar to that obtained with rabbit reticulocyte soluble ribonucleic acid. These experiments demonstrate the formation of labelled rabbit hæmoglobin from unrelated amino-acyl-ribonucleic acid, indicating that the determination of amino-acid sequence is controlled by the ribosome. In a similar manner, labelled hæmoglobin has been isolated after incubation of *E. coli* ^{14}C-amino-acyl-ribonucleic acid and reticulocyte ribosomes (von Ehrenstein, G., and Lipmann, F., private communication).

It was previously shown[1] that starting with free amino-acids labelled with carbon-14, the ratios of various amino-acids incorporated corresponded to the composition of crude globin. This was taken as evidence that the incorporation represented hæmoglobin synthesis. The pattern of incorporation of different amino-acids should be a function of the ribosome if amino-acid sequence is determined by the ribosome. Ratios of amino-acids incorporated starting with labelled guinea pig liver amino-acyl-ribonucleic acid were therefore studied. In each experiment, the ribonucleic acid was labelled with two amino-acids. One was ^{3}H-leucine (150 mc./mmole) and the other was either ^{14}C-valine or isoleucine or lysine (uniformly labelled, 6–10 mc./mmole). Rabbit reticulocyte ribosomes treated with deoxycholate were prepared as previously described[9]. Guinea pig liver ribosomes treated with deoxycholate were prepared in the same manner, but with twice the concentration of deoxycholate. The isolated labelled amino-acyl-ribonucleic acid was incubated in the standard way[10] with either 2·5 mgm. of reticulocyte or 3·7 mgm. of liver ribosomes for 3 min. The reaction mixtures were precipitated by the addition of 10 ml. of acid acetone (6 ml. of 4 *N* hydrochloric acid per litre of acetone) and the protein was washed as previously described[1,3]. The dried powders were each dissolved in 1 ml. of hyamine base and counted in the Packard 'Tricarb' liquid scintillation counter using a naphthalene, xylene and dioxane mixture[9]. The amounts of ^{14}C- and ^{3}H-amino-acids incorporated in each tube were calculated by the method of differential equations[11] using known mixtures of the labelled amino-acids as standards. The relative amounts of the amino-acids transferred to protein in the presence of rabbit reticulocyte ribosomes (Table 1) did not resemble the composition of the amino-acyl-ribonucleic acid, but that of rabbit globin. Very similar results were obtained when the incorporation of labelled free amino-acids was examined in the complete[3] reticulocyte cell-free system. The results with leucine and isoleucine were quite different for guinea pig liver and reticulocyte ribosomes, six times more isoleucine being incorporated into protein by the liver ribosomes. Thus, the amounts of different amino-acids incorporated appear to be controlled by the ribosome, as part of the selection needed to build a specific peptide chain.

Table 1. TRANSFER OF AMINO-ACIDS FROM GUINEA PIG LIVER AMINO-ACYL-RIBONUCLEIC ACID (RNA) TO RIBOSOMES

Amino-acid	Composition of amino-acyl-RNA	Transferred from amino-acyl-RNA		Globin composition*	Incorporated from free amino-acids†
		G. pig liver ribosomes	Reticulocyte ribosomes		
Leucine	100	100	100	100	100
Isoleucine	45	45	7	11	9
Valine	29	—	63	77	77
Lysine	63	—	71	67	—

Results are expressed as moles of each amino-acid per 100 moles of leucine.

* Microbiological assay of crude rabbit globin (ref. 12).

† 40 min. incubation in the complete cell-free system (refs. 1 and 3), all components from reticulocytes.

From this it might be thought that rabbit reticulocyte amino-acyl-ribonucleic acid and guinea pig liver amino-acyl-ribonucleic acid are completely equivalent in their action. That this is not so was demonstrated by the following experiment. ^{3}H-leucyl-ribonucleic acid was prepared from guinea pig liver as above. ^{14}C-leucyl ribonucleic acid was prepared from isolated reticulocyte soluble ribonucleic acid. To overcome the interference which might be caused by the different relative amounts of amino-acids carried by the two types of soluble ribonucleic acid, by differences in the extent of labelling, or by other unknown differences between the preparations, the two leucyl-ribonucleic acids were mixed and then incubated with guinea pig liver or rabbit reticulocyte ribosomes. 0·2 mgm. of guinea pig liver amino-acyl-ribonucleic acid (0·41 mμmoles ^{3}H-leucine) and 0·4 mgm. of rabbit reticulocyte amino-acyl-ribonucleic acid (0·42 mμmoles ^{14}C-leucine) were incubated for 3 min. at 37° with either 2·5 mgm. of reticulocyte deoxycholate-ribosomes or 3·7 mgm. of guinea pig liver deoxycholate-ribosomes under conditions previously described[10]. The incubation mixtures were washed up and counted in the liquid scintillation counter as described here. Table 2 presents the results of two experiments, showing that more leucine was transferred to guinea pig liver ribosomes from guinea pig liver amino-acyl-ribonucleic acid (^{3}H-leucine) than from reticulocyte ribonucleic acid (^{14}C-leucine) and vice versa. It appears that while no function was found for amino-acyl-ribonucleic acid compounds in determining the nature of the protein synthesized, some species differences may exist in soluble ribonucleic acid structure which result in a greater rate of transfer with ribosomes of the same species.

All the results given here rest on the conclusion that labelling of hæmoglobin represents a limited synthesis of this protein. The evidence for this point of view has been discussed[2,6]. However, the final test of the synthesis of a specific protein is the demonstration that the amino-acids are incorporated in their correct locations in the polypeptide chain. This has not yet been shown in any cell-free system. The first stages of such an investigation have provided further evidence supporting the conclusions given here. Three types of hæmoglobin, each labelled with radioactive leucine, were prepared and 'fingerprinted' as described below and the distribution of the radioactive leucine in various peptides determined.

Table 2. TRANSFER OF LEUCINE FROM MIXED AMINO-ACYL-RIBONUCLEIC ACID (RNA)

Source of ribosomes	Amount of transfer		Ratio*
	Rabbit reticulocyte ^{14}C-leucyl-RNA mμmoles	G. pig liver ^{3}H-leucyl-RNA mμmoles	
Rabbit reticulocytes	0·047 0·049	0·043 0·041	1·12 1·21
Guinea pig liver	0·026 0·024	0·035 0·034	0·74 0·71

* The ratio is the amount transferred from reticulocyte leucyl-RNA divided by that from liver leucyl-RNA.

^{3}H-leucyl-ribonucleic acid was prepared from guinea pig liver using ^{3}H-leucine at a specific activity of 3,000 mc./mmole and 1·2 mgm. of this isolated amino-acyl-ribonucleic acid was incubated with 7·5 mgm. of reticulocyte ribosomes for a total of 40 min. The hæmoglobin fraction was isolated by ammonium sulphate fractionation as described here. A 'uniformly labelled' hæmoglobin was prepared from 2 ml. of intact rabbit reticulocytes incubated with 2 mgm. of DL-leucine-1-^{14}C (specific activity 10·3 mc./mmole) for 6 hr. under conditions previously described[2]. Equal amounts of the two hæmoglobins were mixed and digested with trypsin[13]. 1·25 mgm. of the digest was applied to a sheet of Whatman No. 3 mm. filter paper and fingerprinted by the technique of Katz *et al.*[14] except that butanol : pyridine : water (4 : 3 : 3) was substituted as the chromatographic solvent. The paper was sprayed lightly with 0·025 per cent ninhydrin in ethanol and developed for 10 min. at 80° in a water-saturated atmosphere. The peptide spots were cut out and eluted[15] and the eluates were dried down in 20 ml. counting vials. The material was counted in the liquid scintillation counter as described here but with the omission of hyamine from the counting mixture. Under these conditions tritium and carbon were counted with efficiencies of 10 and 90 per cent, respectively. A third hæmoglobin fraction was prepared by incubation of reticulocyte ribosomes for 20 min. with free ^{3}H-leucine (3,000 mc./mmole) as described elsewhere[1,3] and treated in an identical manner. The assignment of peptides to alpha and beta chains was based on separation of these chains on '*CG-50*' columns in an acid–urea gradient[16].

Table 3 shows the relative amounts of labelled leucine found in the peptides of the alpha (valyl–leucyl) chain of rabbit hæmoglobin, and in several regions of the fingerprints which did not show ninhydrin staining. Similar results were noted in the beta-chain peptides. It is seen that the pattern of labelling in the three types of labelled material is similar. The peptides which carry labelled leucine after the incubation of intact reticulocytes also carry labelled leucine from the cell-free labelling experiments, while the peptides which were not labelled in the whole cell (presumably due to the absence of leucine) were not labelled in either cell-free system. In addition, a number of blanks which did not show ninhydrin colour were counted and showed a low level of activity. These results offer convincing evidence for the synthesis of hæmoglobin from heterologous amino-acyl-ribonucleic acid when rabbit reticulocyte ribosomes are used, and also for the validity of previous conclusions which assumed the synthesis of hæmoglobin in the cell-free system. For example, the validity of amino-acyl-ribonucleic acid as an intermediate in hæmoglobin synthesis is established. It should be noted that the exact percentages of radioactivity in the ^{14}C-labelled peptides (used as internal controls of recovery) vary between the two fingerprints, probably due to variations in elution from the paper.

The reticulocyte ribosome, rather than the transfer-ribonucleic acid, therefore, is responsible for the correct assembly of the hæmoglobin molecule. Which component of the ribosome carries the information remains to be determined. Treatment of ribosomes with tiny amounts of ribonuclease which did not release any detectable nucleotide material[3,17] resulted

Table 3. RADIOACTIVITY OF PEPTIDES OF ALPHA CHAIN OF RABBIT HÆMOGLOBIN

Peptide No.	Fingerprint 1† Intact cells ^{14}C-leucine	Fingerprint 1† Cell-free, free ^{3}H-leucine	Fingerprint 2† Intact cells ^{14}C-leucine	Fingerprint 2† Cell-free, ^{3}H-leucyl-RNA
1	2·5	4·6	9·2	8·7
2	8·2	5·1	3·8	1·9
3	31·1	12·5	32·2	36·9
4	7·2	6·6	6·1	6·8
5	18·2	20·4	20·3	20·4
6 + 7	1·3	2·0	1·8	1·1
8	3·2	2·3	1·4	1·6
9	0	0	0	0
10	16·4	19·8	13·5	13·0
12	12·2	26·5	11·6	9·7
13	0	0	0	0
14	0	0	0	0
Blank 1*	0	0	0	0
Blank 2	0	0	0	0
Blank 3	0	0	0	0

Radioactivity is expressed as the percentage of the total radioactivity in all peptides. 0 denotes less than 0·1 per cent.

† Fingerprint 1 is the mean of two experiments, fingerprint 2 the mean of three.

* 12 blanks taken in close proximity to the 12 ninhydrin-stained regions showed a total of less than 9 per cent of the recovered radioactivity in each case.

in a total loss of incorporating ability. Thus, it is likely that it is the ribonucleic acid of the ribosome which is important, as originally suggested by Crick[18]. Ribosomal ribonucleic acid, however, may consist of many components, some or all of which may have functions in protein synthesis unrelated to sequence determination. At the moment, a considerable amount of interest centres on a particular type of ribonucleic acid having a base composition related to that of deoxyribonucleic acid[19]. The work reported here makes it possible to examine directly the components, and the structural aspects of these components, which control the assembly of amino-acids in the correct sequence of the specific protein.

Note added in proof. Recent work on the 'messenger' ribonucleic acid of *E. coli* suggests that it is destroyed and replaced continuously in bacterial ribosomes during protein synthesis[20]. The species of hæmoglobin synthesized by rabbit reticulocyte ribosomes has been changed by incubation with soluble components from other species[21], under different experimental conditions from those used here. Thus, while specificity information may be added from soluble components, this was not essential for hæmoglobin synthesis in the experiments reported here. It is suggested that specificity information is therefore more stable in reticulocyte than in bacterial ribosomes, and that rapid 'turnover' of this information may not be an obligatory feature of protein synthesis.

This investigation was aided by grants to Prof. R. Schweet from the National Science Foundation and the National Institutes of Health, and to Dr. E. Russell from the National Institutes of Health.

[1] Schweet, R., Lamfrom, H., and Allen, E., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1029 (1958).

[2] Bishop, J., Leahy, J., and Schweet, R., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1030 (1960).

[3] Allen, E., and Schweet, R., *J. Biol. Chem.* (in the press).

[4] Dintzis, H., Borsook, H., and Vinogrand, H., in *Microsomal Particles and Protein Synthesis*, Roberts, B., edit., 95 (New York, Pergamon Press, 1958).

[5] Hoagland, M. B., in *The Nucleic Acids*, **3**, Chargaff, E., and Davidson, J., edits., 349 (New York: Academic Press, 1960).

[6] Schweet, R., Bishop, J., and Morris, A., *Lab. Invest.* (in the press).

[7] Allen, D. W., Schroeder, W. A., and Balog, J., *J. Amer. Chem. Soc.*, **80**, 1628 (1958).

[8] Leahy, J., Glassman, E., and Schweet, R., *J. Biol. Chem.*, **235**, 3209 (1960).

[9] Bishop, J., Leahy, J., and Schweet, R. (in preparation).

[10] Bishop, J., and Schweet, R., *Biochim. Biophys. Acta*, **49**, 235 (1961)'

[11] Okita, G. T., Kabara, J. J., Richardson, F., and LeRoy, G. V., *Nucleonics*, **15**, 111 (1957).

[12] Ts'o, P., Bonner, J., and Dintzis, H. (unpublished results).

[13] Hill, R. L., Swenson, R. T., and Schwartz, H. C., *J. Biol. Chem.*, **235**, 3182 (1960).

[14] Katz, A. M., Dreyer, W. J., and Anfinsen, C. B., *J. Biol. Chem.*, **235**, 2897 (1959).

[15] Dintzis, H. M., *Proc. U.S. Nat. Acad. Sci.*, **47**, 247 (1961).

[16] Wilson, S., and Smith, D. B., *Canad. J. Biochem. Physiol.*, **37**, 405 (1959).

[17] Morris, A., and Schweet, R. (in preparation).

[18] Crick, F. H. C., *Symp. Soc. Exp. Biol.*, **12**, 138 (1958).

[19] Volkin, E., and Astrachan, L., *Virology*, **6**, 545 (1958). Nomura, M., Hall, B. D., and Spiegelman, S., *J. Mol. Biol.*, **2**, 306 (1960).

[20] Brenner, S., Jacob, F., and Meselson, M., *Nature*, **190**, 576 (1961). Gros, F., Hiatt, H., Gilbert, W., Kurland, C., Risebrough, R., and Watson, J., *ibid.*, **190**, 581 (1961).

[21] Kruh, J., Rosa, J., Dreyfus, J., and Schapira, G., *Biochim. Biophys. Acta*, **49**, 509 (1961). Lamfrom, H., *J. Mol. Biol.*, **3**, 241 (1961).

ANABOLIC AGENTS AND HISTAMINE METABOLISM

By DR. K. S. KIM

Department of Physiology, University of Malaya in Singapore

TESTOSTERONE propionate has been shown to reduce urinary histamine output in rats[1,2,3]. Using new anabolic steroids with low androgenic activity, an attempt has been made to determine whether the androgenic or anabolic effect of testosterone is related to histamine metabolism. Various anabolic agents were compared with testosterone propionate in their effects on the excretion of free histamine, potassium, sodium and total nitrogen in rat urine.

Female albino rats weighing 140–220 gm. were individually placed in metabolism cages and fed 'Complan' diet (Glaxo Laboratories), a blend of dried skimmed milk, arachis oil, calcium caseinate, maltodextrin, cane sugar, essential minerals and vitamins.

During the examination of urinary nitrogen, potassium and sodium, food intake was kept constant by giving either 8 gm. 'Complan' or 8 gm. 'Complan' plus 1 gm. glucose daily depending on body-weight. 8 gm. 'Complan' provides 398 mgm. nitrogen, 2·2 mmole potassium, 1·58 mmole sodium and 36 calories. Tap water containing 0·013 mmole/l. of potassium and 0·04 mmole/l. of sodium was given *ad lib.*

For a test of androgenic activity of testosterone and 'Durabolin' (Organon), castrated male rats weighing 285–385 gm. were used and were fed normal laboratory rat pellets *ad lib.*

Urine was collected in acid (1·5 ml. 1 *N* hydrochloric acid in each collection vessel) over a 24-hr. period. Collection funnels and metabolism cages were sprinkled with distilled water (30–60 ml.). The combined volume of urine and washing fluid was measured and filtered through Whatman paper No. 1. Aliquots of filtrate representing 2- to 4-hr. samples were taken and neutralized. Final volume was made up with Tyrode solution to an appropriate concentration of histamine for assay.

Urinary free histamine was estimated by the biological assay method described elsewhere[2] and expressed in terms of the base.

Total nitrogen was determined by the micro-Kjeldahl method and sodium and potassium by the EEL flame photometer model *A*.

Table 1. EFFECT OF 'DURABOLIN' AND TESTOSTERONE PROPIONATE ON URINARY HISTAMINE OUTPUT

Group	Av. body-wt. (gm.)	Dose (mgm.)	Average free histamine output/μgm./rat/day				Av. body-wt. gain (gm.)
			Control	Day after injection 4th	7th	11th	14th
Control	*(3) 163	—	32	36	43	44	20
'Durabolin'	(4) 180	1·25	31	13	2	3	38
Testosterone propionate	(3) 158	1·25	33	32	37	30	17

*No. of rats used.

Anabolic substances were all administered intramuscularly. Testosterone propionate ('Testoviron', Schering), 19-nor-androste-nolone phenylpropionate ('Durabolin', Organon) and 17α-methyl-17β-hydroxy-androsta-1, 4-diene-3-one ('Dianabol', Ciba) were used as steroid anabolic agents. The effect of growth hormone ('Somar-*A*', Armour) was also examined as an example of a different class of anabolic agents.

A preliminary examination showed that 'Durabolin', a powerful anabolic agent with low androgenic activity, reduced the output of free histamine (Table 1). These results indicated the existence of close relationship between anabolic mechanism and histamine metabolism. Therefore the effects of various anabolic agents on the excretion of free histamine were examined. The results are shown in Fig. 1.

'Durabolin' was the most potent substance in reducing urinary histamine output, and is followed by testosterone propionate. Although 1·25 mgm. testosterone propionate had no significant effect on the urinary histamine as shown earlier (Table 1), a larger dose (2·5 mgm.) produced a reduction (Fig. 1). Reduction of histamine output by 'Durabolin' lasted on the average for 40 days. 'Dianabol' was given in a dose of 1 mgm./rat/day for three consecutive days as it was not very effective in a single dose (1–2·5 mgm.). The effect of 'Dianabol' at this dose-level was similar to that produced by testosterone propionate (2·5 mgm.). Growth hormone was given to two normal females and to two ovariectomized females ; but since the effects observed were similar, both results were pooled to construct the figures shown in Figs. 1 and 2. Histamine excretion reached a minimum after the third injection and showed no further decrease after the fourth and the fifth injections. Neither continued injection for 7 days nor increased dose (2 mgm./day for 3 days) produced any further decrease. 'Heparin' (Leo) (2·5 mgm./day for 5 days) was also tested as it was reported to have an anabolic effect[5], but no significant effect was noted (Fig. 1).

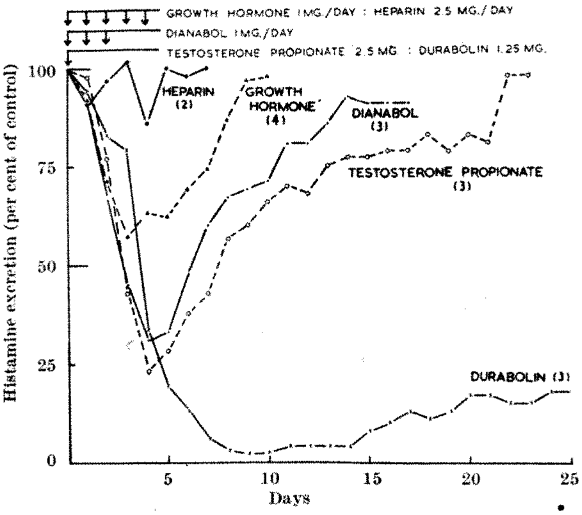


Fig. 1. Effect of anabolic agents on the urinary excretion of free histamine expressed as percentage of control-level (average value of daily histamine excretion during the last three days prior to the administration of drugs). The number in parentheses under the names of test substances indicates the number of rats used

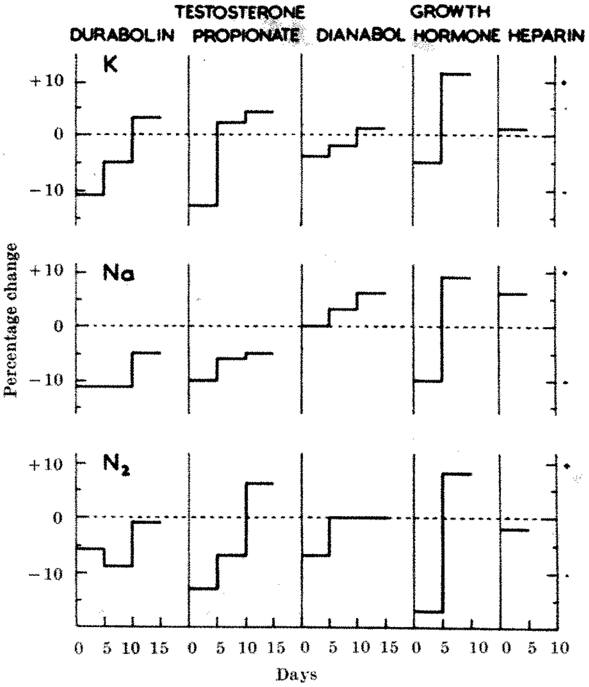


Fig. 2. Effect of anabolic agents on the excretion of urinary potassium, sodium and total nitrogen expressed as percentage change from the control-level. The investigation shown in Figs. 1 and 2 are concurrently carried out on the same rats

Anabolic substances are known to produce a retention of nitrogen, potassium and also sodium[4]. An attempt was therefore made during the investigation illustrated in Fig. 1 to examine whether the anabolic effect as reflected in the excretion of these substances is related to the potency in decreasing urinary histamine output. As shown in Fig. 2, 'Durabolin' showed a prolonged activity in retaining nitrogen, potassium and sodium, although testosterone propionate (2·5 mgm.) had a stronger effect during the first 5-day period. These effects were small with 'Dianabol'. Growth hormone produced a profound anabolic effect during its administration but showed rebound recovery over the control-level on discontinuation. 'Heparin' showed no significant effects indicating the absence of an anabolic effect.

Although the anabolic activity as reflected in the excretion of nitrogen, potassium and sodium appeared to be similar (Fig. 2), 'Durabolin' increased the body-weight more than testosterone propionate, as shown in Table 2. These rats, two in each group, were taken from the series illustrated in Fig. 1. This finding was further supported by the results shown in Table 1 although food intake was not controlled in this work. It should be noted that the absence of anabolic effect of testosterone propionate (1·25 mgm.) as reflected in gain in body-weight coincided with the absence of histamine reducing effect (Table 1).

Fifteen males, castrated 5 months previously, were divided into three groups—control, testosterone-treated and 'Durabolin'-treated. Testosterone- and

Table 2. EFFECT OF 'DURABOLIN' AND TESTOSTERONE PROPIONATE ON GAIN IN BODY-WEIGHT

	Dose (mgm.)	Daily food intake 'Complan' (gm.)	Initial body-weight (gm.)	Wt. gain in 26 days after injection (gm.)
Control	—	8	160	15
		8	165	10 Av. 12·5
'Durabolin'	1·25	8	165	30
		8	177	21 Av. 25·5
Testosterone propionate	2·5	8	175	12
		8	167	23 Av. 17·5

'Durabolin'-treated groups received two 1 mgm. doses of the respective drugs at an interval of 1 week, and the rats were killed one week after the last injection. As shown in Table 3, there was no appreciable effect on the weight of the kidneys; but significantly different effects were noted on the weight of the seminal vesicles. This result confirms the low androgenic effect of 'Durabolin' in comparison with testosterone propionate as reported by Overbeek and de Visser[6]. Therefore, it appears that the androgenic mechanism in the body is not related to histamine metabolism. Gain in body-weight was similar with 'Durabolin' and testosterone propionate.

In this work it is clearly shown that anabolic agents decrease output of urinary histamine. Evidence[2] has already been presented that testosterone propionate increases the capacity for histamine inactivation in rats. It is likely that 'Durabolin' and 'Dianabol' act by the same mechanism, the former more active and the latter slightly less active than testosterone propionate. An anabolic effect is believed to be produced by an increase in the activities of enzymes which synthesize protein. It is possible, therefore, that steroid anabolic agents may exert an effect on the output of urinary histamine through a potentiation of an enzyme system which inactivates histamine. Such an enzyme system might be common to both protein synthesis and histamine inactivation.

Table 3. EFFECT OF 'DURABOLIN' AND TESTOSTERONE PROPIONATE ON THE SEMINAL VESICLES AND THE KIDNEYS OF CASTRATED MALE RATS

Group	Initial body-wt. (gm.)	Wt. gain in 2 weeks (gm.)	Seminal vesicle (mgm.)	Kidney wt. (gm.)
Control	314 ± 10*	-14 ± 6	53 ± 17	2·09 ± 0·12
'Durabolin'	356 ± 6	25 ± 1·6	100 ± 9	2·16 ± 0·09
Testosterone propionate	356 ± 11	24 ± 5·1	237 ± 44	1·94 ± 0·04

*Standard error.

The anabolic effect produced by growth hormone appears to be different from that produced by steroid anabolic agents[7-10]. The anabolic effect of growth hormone showed a different time-course from the steroids tested, and produced an effect on histamine excretion (Fig. 2). This may be taken as further evidence that growth hormone and steroid anabolic agents have different sites of action. Histamine output has been shown to be affected by testosterone propionate more readily in ovariectomized rats than in normal female rats[2]. No such hypersensitivity was noted with growth hormone in ovariectomized rats.

Although the effect of 'Dianabol' on the excretion of urinary total nitrogen was much smaller than that produced by testosterone (Fig. 2), it was shown by others[11] to produce a gain in body-weight equal to that produced by testosterone propionate. Both 'Dianabol' and 'Durabolin' have been shown to have very low androgenic activities at the dose-level used in this work[6,11] (Table 3). It is reasonable to conclude that it is not the androgenic but the anabolic activity of steroid compounds that is related to histamine metabolism.

Heparin in a dose of 2·5 mgm./rat/day for 5 days failed to produce an anabolic effect (Fig. 2). It also failed to reduce histamine excretion in the urine (Fig. 1). Granitsas[5] reported the anabolic activity of heparin in normal rats. The discrepancy between the results of Granitsas and this study is not clear at present and needs further investigation.

My thanks are due to Prof. R. A. Kenney for his kind assistance in the preparation of this manuscript. 'Durabolin' (Organon), 'Dianabol' (Ciba) and 'Testoviron' (Schering) were kindly provided by local agents of the respective firms.

[1] Kim, K. S., *Amer. J. Physiol.*, **197**, 1258 (1959).
[2] Kim, K. S., *Amer. J. Physiol.* (in the press).
[3] Marshall, P. B., *Brit. J. Pharmacol.*, **16**, 50 (1961).
[4] Whitney, J. E., Bennett, L. L., and Li, C. H., *Proc. Soc. Exp. Biol. Med.*, **79**, 584 (1952).
[5] Granitsas, A. N., *Amer. J. Physiol.*, **199**, 729 (1960).
[6] Overbeek, G. A., and de Visser, J., *Acta endocrinol.*, **24**, 209 (1957).
[7] Kochakian, C. D., and Stettner, C. E., *Amer. J. Physiol.*, **155**, 255 (1948).
[8] Kochakian, C. D., and Stettner, C. E., *Amer. J. Physiol.*, **155**, 262 (1948).
[9] Kochakian, C. D., *Amer. J. Physiol.*, **160**, 66 (1950).
[10] Kochakian, C. D., *Proc. Soc. Exp. Biol. Med.*, **103**, 196 (1960).
[11] Desaulles, P. A., Kraehenbuehl, C., Schuler, W. ,and Bein, H. J., *Schweiz. med. Wschr.*, **89**, 1313 (1959).

STAINING OF SULPHATED MUCOPOLYSACCHARIDES

By DR. I. D. HEATH
Department of Anatomy, Queen's College, Dundee, University of St. Andrews

METHODS in use at present for detecting sulphated mucopolysaccharides consist of the exhibition of metachromasia with basic dyes, and a method using colloidal iron[1]. These methods tend to be rather unspecific, and nuclear staining tends to obscure the picture.

From the work of Stacey[2] it is apparent that sulphated mucopolysaccharides are found in mast cells (heparin), cartilage ground substance (chondroitin sulphuric acid) and some mucins. In addition, they have been isolated from other sites, for example, skin and cornea.

Previous work[3] has shown that nuclear fast red (Herzberg), when prepared as a 0·1 per cent stock solution in boiling 5 per cent aluminium sulphate solution which is diluted 1 to 2 with further 5 per cent aluminium sulphate solution for use, is a highly selective stain for mast cell granules, cartilage ground substance, and some mucins in tissue sections. This work has now been extended, the earlier conclusions having been confirmed.

Test slides of pure chemical substances dissolved in 5 per cent gelatin were prepared, fixed in formalin vapour overnight and stained 5–10 min. in the dye solution. The substances used included the sulphated polysaccharides; heparin, from ox, sheep and pig, fucoidin[4], and a sulphated polyglucose, and the unsulphated polysaccharides, dextrin, dextran and starch. In addition, other known constituents of mast cells, namely, 5-hydroxytryptamine, histamine and 5-hydroxytryptophan, were used. Only the sulphated polysaccharides and heparin stained (Fig. 1*c*).

Tissues from members of all classes of vertebrates and sites, fixed in a variety of fixatives, both aqueous and alcoholic, were stained, and it was found that only those tissues which contained sulphated mucopolysaccharides reacted, for example, media or aorta, although mast cells when present in the tissues did stain. Only sections fixed in acetic Zenker failed to react in this way.

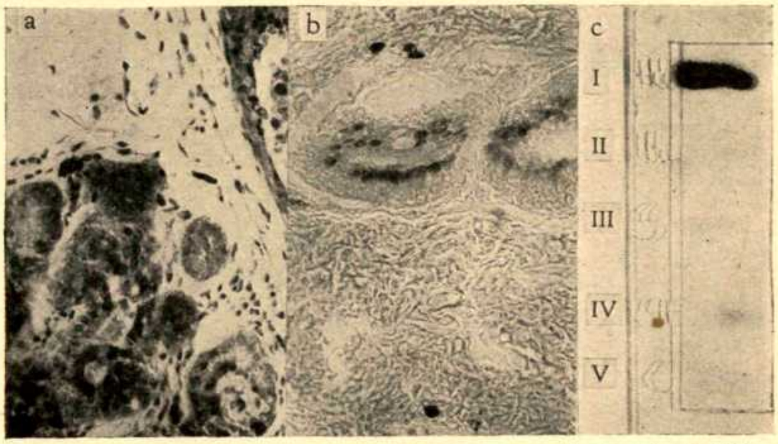
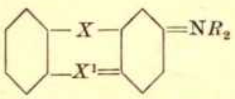


Fig. 1. (*a*) Ox trachea stained with 0·1 per cent aqueous methylene blue (×100). (*b*) An adjacent section stained with 0·1 per cent methylene blue in 5 per cent aluminium sulphate. Note only mucus and mast cells stain (×100). (*c*) Test slide of mast cell constituents in 5 per cent gelatin stained with 0·1 per cent nuclear fast red (Herzberg) in aluminium sulphate. I, heparin; II, histamine; III, serotonin; IV, 5-hydroxytryptophan; V, control gelatin blank

The stock solution of nuclear fast red in aluminium sulphate was buffered, using various buffers, to *p*H values between 0·5 and 7; at *p*H 7 a precipitate was formed. The *p*H did not affect the staining reaction.

These observations were extended to dyes of azo, quinone imine, phenyl methane, xanthine and phthalocyanine groups, in addition to several miscellaneous dyes, which were all prepared in the manner for nuclear fast red mentioned here. 0·1 per cent aqueous solutions were run in parallel as controls. In all, 56 different dyes were used, some of basic and some of acidic nature. The tissues used as test tissues were ox trachea and rat skin fixed in formol–dichromate (*p*H 6) and formol–calcium respectively. 6μ-sections were dewaxed, hydrated and stained for 5–30 min. in the dye solution, differentiated rapidly in 70 per cent alcohol, dehydrated in graded alcohols, cleared in xylene and mounted in polystyrene or Canada balsam.

With basic dyes prepared in aluminium sulphate solution, mast cell granules and cartilage ground substance stain deeply ortho- or metachromatically, depending on the dye used. Skin and mucin stain to a lesser extent and always orthochromatically (Fig. 1*b*). Those dyes which give the best results have the basic formula:



X = O, S, or N, X = C or N, R = CH_3 C_2H_5, etc.

The thiazines, toluidine blue and methylene blue, and the azines, neutral red and nuclear fast red, give the best results. Certain dyes give less specific reactions, staining, in addition, red blood cells, nuclei and all mucines. This is particularly noticeable with the rhodamines, but less with the phenyl methanes and dyes having large substituent groups attached to the benzene rings in the above formula, namely, cresyl fast violet, janus green. Triphenylmethane dyes tend to differentiate out rapidly and for that reason are not as satisfactory as the azines and thiazines. Basic dyes in aqueous solutions stain all the elements mentioned here, but nuclei and the cytoplasm of mucous cells stain too (Fig. 1*a*). Acidic dyes in aluminium sulphate solution stain all tissue elements deeply, including cartilage ground substance, but in aqueous solution the cartilage ground substance remains unstained, although the cell nuclei in the lacunæ stain.

In an attempt to find out what groups, if any, were specific for this reaction, sections containing sulphated mucopolysaccharides were methylated[5] and stained as mentioned. This prevented staining. Methylation has been reported to block various groups in tissue sections[5], including sulphate groups, and this seemed a possible explanation of its effect. This explanation was supported by the finding that sections containing unsulphated mucopolysaccharides, for example, umbilical cord, after sulphation[6] stained a uniform deep colour. It appears, therefore, that the sulphate groups are, in part at least, responsible for the reaction.

Equimolar concentrations of other aluminium salts, either as pure aluminium salts or as alums, were found to confer similar specificity of action on basic dyes, as did a number of salts of other metals. It appears from this that it is the cation which is responsible for the specificity of action, and by using a variety of different compounds it became apparent that only those cations which freely form co-ordination complexes confer specificity on the basic dyes.

It appears, therefore, that by preparing certain basic dyes in aluminium sulphate a highly specific stain for the detection of sulphated mucopolysaccharides in tissue sections is produced. The dye complex produced is probably a co-ordination compound of a suitable metallic cation and the dye, the reaction between the tissues and the complex being similar to mordanting in the textile dye industry.

I wish to thank Evans Medical Ltd., Bengers, Ltd., and Dr. D. M. Shepherd of the Pharmacology Department, Queen's College, Dundee, for supplying the heparins, dextran and sulphated polyglucose respectively.

[1] Hale, C. W., *Nature*, **157**, 802 (1946).
[2] Stacey, M., *Advances in Carbohydrate Chemistry*, **2**, 161 (1946).
[3] Coupland, R. E., and Heath, I. D., *J. Endocrinol.* **22**, 71 (1961).
[4] Percival, E. G., *Quart. Rev.* (*Chem. Soc.*). **3**, 369 (1949).
[5] Fisher, E. R., and Lillie, R. D., *J. Histochem. Cytochem.*, **2**, 81 (1954).
[6] Grillo, T. A. I., and Lewis, P. R., *J. Anat.* (Lond.) **93**, 581 (1959).

AN UNREACTIVE TYROSINE RESIDUE IN INSULIN AND THE EXCLUSIVE IODINATION OF THE *A* CHAIN

By DR. P. H. SPRINGELL*

National Institute for Medical Research, Mill Hill, London, N.W.7

IT has been known for some time that it is possible to introduce sufficient iodine into insulin to bring about disubstitution of all the tyrosine residues present[1]. More recently it has been observed that the *A* chain is preferentially iodinated, particularly at lower levels of iodination[2]. The present article describes iodination conditions under which one tyrosine residue of the *B* chain appears to be entirely unreactive and another sufficiently unreactive to facilitate exclusive iodination of the *A* chain.

Iodination of proteins, including insulin, has generally been carried out between *p*H 5 and 10 (refs. 3 and 5). Although there are reports of iodination under more acidic conditions[3,6], no detailed study has been described using insulin. It was found that iodination of insulin by the McFarlane method[5] proceeds almost as well at *p*H 1 as at higher *p*H values, providing only partial iodination is attempted (Fig. 1). Even with larger amounts of iodine no more than about 6 atoms of iodine/mole could be taken up in the first 5 min. of the reaction.

Examination of the distribution of iodine–131 was carried out after disulphide splitting by Swan's method[7], followed by high-voltage paper electrophoresis[8] and autoradiography (Figs. 2 and 3). The figures show that it is possible to iodinate the *A* chain almost exclusively at the 1 atom/mole level at *p*H 1 but not at *p*H 9 (Figs. 2*e* and 3*f*). When more iodine was used, the proportion found in the *B* chain gradually increased (Figs. 2*a–d* and 3*b–e*). If iodination is first carried out at *p*H 1 to the 6 atoms/mole level with inactive iodine and afterwards at *p*H 9 with 2 atoms labelled iodine/mole, then 75 per cent of the radioactivity is associated with the *B* chain (Fig. 3*a*).

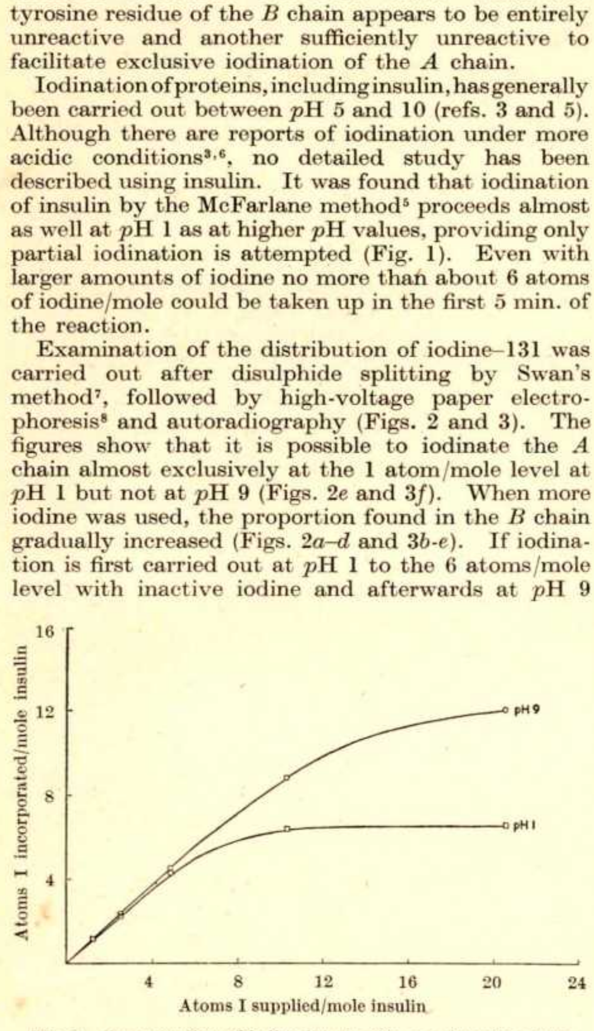


Fig. 1. Incorporation of iodine into insulin as a function of the amount of iodine supplied. Insulin (2 mgm. B.D.H. crystalline) was dissolved in 0·5 ml. 0·1 *N* hydrochloric acid. The following were added: 0·5 ml. buffer (containing per 100 ml.: □, *p*H 1–10 ml. 1 *N* hydrochloric acid + 3 ml. 2 *M* glycine + 12 ml. 2 *M* sodium chloride; ○, *p*H 9–1·3 ml. 2 *N* sodium hydroxide + 9 ml. 2 *M* glycine + 12 ml. 2 *M* sodium chloride), 0·5 ml. 0·1 *N* sodium hydroxide (in *p*H 9 experiments only), water to give a final volume of 4·5 ml. after iodine monochloride addition and iodine monochloride (3·3 μatoms I/ml.)[5] containing between 5 and 50μc. iodine-131 depending on requirements. After 5 min. reaction, 0·5 ml. 100 per cent trichloroacetic acid was added and the protein precipitated. The supernatant was withdrawn and the precipitate washed with 5 ml. 10 per cent trichloroacetic acid. Suitable aliquots of supernatants were counted. (Atoms I incorporated/mole insulin = atoms I supplied/mole insulin

$$\times \frac{\text{added activity} - \text{supernatant activity}}{\text{added activity}}\Big)$$

The uptake of 6 atoms of iodine could lead to the mono-substitution of the 4 tyrosine and 2 histidine residues, the di-substitution of only 3 tyrosine residues, or to di-substitution of some residues and mono-substitution of others. It is evident from the tyrosine and histidine distribution[9] that mono-substitution of all residues would result in an eventual predominance of iodine in the *B* chain. However, scanning for radioactivity in the appropriate regions (Fig. 3*b* and *c*) shows that this is not the case at *p*H 1. Also it is unlikely that histidine is involved since it is known to react much less readily with iodine than does tyrosine under a variety of conditions[10]. Analysis of enzymic digests of the split chains of insulin confirmed the presence of diiodotyrosine. Although monoiodotyrosine was also found, it is uncertain whether it represents a genuine product of iodination. Some of it is almost certainly formed by partial deiodination of the diiodo compound during proteolysis[11] and possibly also during the disulphide splitting reaction.

On the basis of the above it is concluded that *p*H 1 iodination at high iodine-levels probably results mainly in the di-substitution of 3 tyrosine residues, leaving one inaccessible at least for the first 5 min. of the reaction. Another tyrosine group is also iodinated less readily than the other two residues at low iodine-levels. Both these less reactive tyrosine residues are apparently located in the *B* chain.

Iodination of insulin at *p*H 1 brings about opalescence and eventually flocculation as more and more iodine is added. This could be interpreted as resulting from aggregation similar to that observed during formation of fibrous insulin[12]. One site of attach-

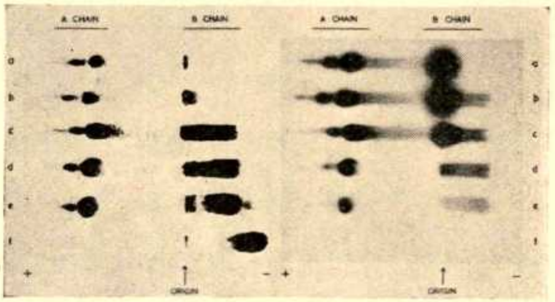


Fig. 2. Electrophoretograms of sulphite-split *p*H 9 iodinated insulin. The insulin precipitate (see legend Fig. 1) was washed with ether to remove trichloroacetic acid, dissolved in Swan's reagent[7], the mixed chains precipitated after the –SS– splitting and the *A* and *B* chains separated by paper electrophoresis (3,800 V., 2·5 hr.) in pyridine : acetic acid : urea as described by Dixon and Wardlaw (ref. 8). After drying and removal of excess urea, the paper was autoradiographed (right) and then sprayed with diazotized sulphanilic acid (Pauly) reagent (left). Amounts of iodine supplied and incorporated (expressed in atoms/mole) are as follows: (*a*) 20·6 and 12·1, (*b*) 10·3 and 8·9, (*c*) 4·8 and 4·5, (*d*) 2·5 and 2·3, (*e*) 1·2 and 1·1. Electrophoretogram (*f*) contains untreated insulin only. Minor components probably represent deamidated products. Because of the higher radioactive content, relatively less of the mixed chains was applied to spots (*a*) and (*b*), which accounts for the apparent reduction in the amount of Pauly-positive material

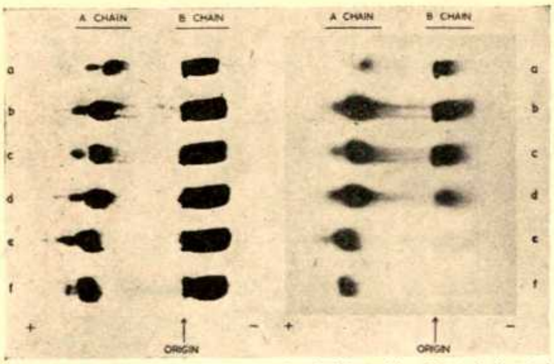


Fig. 3. Electrophoretograms of sulphite-split pH 1 iodinated insulin. The insulin was treated as described in the legend of Fig 2. Insulin in electrophoretogram (*a*) was first iodinated at pH 1 by addition of 20·6 atoms inactive I/mole and then at pH 9 by addition of 2·5 atoms I (containing iodine-131)/mole. At pH 9 2·3 atoms I/mole were shown to be incorporated by radio-assay; at pH 1 presumably 6·6 atoms I/mole were introduced. Amounts of iodine supplied and incorporated (expressed in atoms/mole are as follows: (*b*) 20·6 and 6·6, (*c*) 10·3 and 6·4, (*d*) 4·8 and 4·3, (*e*) 2·5 and 2·2, (*f*) 1·2 and 1·1. Left: After spraying with Pauly's reagent. Right: Autoradiograph

ment during association of insulin monomers may be in the vicinity of those portions of the *B* chains which contain the masked tyrosine residue. In this connexion it is of interest that Porter[13] gave a similar explanation for the observed masking of one of the *B* chain histidine residues.

Although earlier work seemed to indicate that all tyrosine groups of insulin were equally accessible[1,14,15], more recent work indicates that both tyrosyl residues in the *B* chain are hydrogen bonded[16] and that one tyrosyl group may be more strongly bonded than the other[17] within the 6,000 molecular weight unit. Since iodinations were carried out under pH conditions in which internal hydrogen bonding may no longer be taking place[16,18], the hypothesis involving inter- rather than intra-molecular factors is favoured.

Investigation of the masked tyrosine in the *B* chain is being directed towards establishing which of the tyrosyl residues is involved. Distribution of the iodine within the preferentially iodinated *A* chain is also being examined.

A Wellcome research travel grant is gratefully acknowledged. Thanks are also due to Dr. A. S. McFarlane for his interest in this work, Miss D. M. Parr for technical assistance, and Mr. C. D. Sutton for autoradiography.

* On leave from the Division of Protein Chemistry, Commonwealth Scientific and Industrial Research Organization, Parkville, Victoria, Australia.

[1] Harington, C. R., and Neuberger, A., *Biochem. J.*, **30**, 809 (1936).

[2] De Zoeten, L. W., Ph.D. thesis, Leyden (1959); *Rec. Trav. Chim. Pays-Bas* (in the press).

[3] Ramachandran, L. K., *Chem. Revs.*, **56**, 199 (1956).

[4] McFarlane, A. S., *Biochem. J.*, **62**, 135 (1956).

[5] McFarlane, A. S., *Nature*, **182**, 53 (1958).

[6] Ramachandran, L. K. (personal communication; Ph.D. thesis, University of Modias, 1953).

[7] Swan, J. M., *Nature*, **180**, 643 (1957).

[8] Dixon, G. H., and Wardlaw, A. C., *Nature*, **188**, 721 (1960).

[9] Ryle, A. P., Sanger, F., Smith, L. F., and Kitai, R., *Biochem. J.*, **60**, 541 (1955).

[10] Li, C. H., *J. Amer. Chem. Soc.*, **66**, 225 (1944).

[11] Kennedy, T. H., *Austral. J. Biol. Sci.*, **11**, 106 (1958).

[12] Waugh, D. F., *Advanc. Protein Chem.*, **9**, 369 (1954).

[13] Porter, R. R., *Biochem. J.*, **46**, 304 (1950).

[14] Crammer, J. L., and Neuberger, A., *Biochem. J.*, **37**, 302 (1943).

[15] Tanford, C., and Epstein, J., *J. Amer. Chem. Soc.*, **76**, 2163 (1954).

[16] Laskowski, M., Leach, S. J., and Scheraga, H. A., *J. Amer. Chem. Soc.*, **82**, 571 (1960).

[17] Inada, Y., *J. Biochem.* (*Japan*), **49**, 217 (1961).

[18] Laskowski, M., Widom, J. M., McFadden, M. L., and Scheraga H. A., *Biochim. Biophys. Acta*, **19**, 581 (1956).

RELATIONSHIP BETWEEN VELOCITY OF CARDIAC CONTRACTION AND SIZE OF THE HEART: A HYPOTHESIS

By DR. H. FEINBERG*

Cardiovascular Research Department, Medical Research Institute, Michael Reese Hospital and Medical Center, Chicago

A. V. HILL[1] suggested that "cardiac muscle may be able to change its intrinsic speed according to conditions . . . the heart, unlike the skeletal muscle, becomes intrinsically quicker when the frequency of its beat rises". Recently, experiments on cardiac muscle strips and papillary muscle have provided support for this suggestion[2,3]. These findings imply in regard to regulation of performance of the intact heart that cardiac muscle, unlike skeletal muscle, has no fixed length. This fact, considered with the experiments mentioned and the results of recent work on the effect of calcium ions infused into the intact heart of a dog[4], has suggested the following hypothesis.

For a given cardiac load (stroke volume and peripheral resistance), there is a relationship between the velocity of myocardial muscle fibre shortening and the momentary initial length of fibre or initial volume of the intact heart. Thus, an increased velocity of shortening of cardiac muscle fibres is associated with an increased extent of shortening and so leads to a decrease in the end-systolic volume of that beat and a decrease in the end-diastolic volume of the subsequent beat.

The cardiac contraction has been characterized as analogous to a skeletal muscle twitch[5]. Two separate loads for a given contraction must be considered: (1) the initial load, during the isovolumic phase of contraction, as defined by the end-diastolic volume; (2) the 'ejection' or after-load which operates during the auxotonic phase, and is defined by the volume of blood discharged and the aortic pressure during ejection, that is, the pressure-time curve between diastolic aortic pressure and end-systolic pressure. The cardiac contraction is a unique after-loaded performance since the after-load tends to disappear during ejection. This is due to the fact that tension of the wall in a hollow organ decreases proportionally to the cube root of the volume ejected, so that volume shrinkage may more than counteract rise in cavity pressure.

According to the working hypothesis, the final or contracted length, the end-systolic volume, is only a partial function of the initial length, the end-diastolic volume. The extent of shortening of cardiac muscle

* Established Investigator, American Heart Association.

must also be a function of the velocity of shortening for a given load, and the velocity of shortening can be related to the active state, which has been shown to be a discrete period in a muscle twitch. In the case of the heart, the active state would be partitioned between its two phases of contraction. Thus, if the initial load is achieved earlier in the contraction, the potential for further shortening is increased. At the same time, the ejection-after-load is decreased in the ensuing contraction. Thus, the potential for shortening in the heart is greater than would otherwise occur in an ordinary or simple after-loaded contraction.

Skeletal muscle contractile response to a load, the force of contraction, is customarily considered relative to the fixed or *in situ* length, since it has been shown that the maximum force or tension is attained at that length. On the other hand, end-diastolic cardiac volume, the equivalent of initial length of the myocardial fibres, may change from moment to moment in the same individual. There is really no fixed initial length of fibre in the heart. For example, it has been shown that an appreciable decrease in volume of the heart occurs when an individual shifts to the erect from the sitting or prone position[6].

The experiments of Abbott *et al.*[2] and of Sonnenblick and McCallum[3] have established that the velocity of contraction of cardiac muscle for a given after-load may be augmented : (1) by increasing the heart-rate ; (2) by increasing the initial length of the cardiac fibres ; (3) by the application of positive inotropic agents such as the catechol amines and calcium. The results have been interpreted as representing an increase in the intensity of the active state, since the increased velocity of shortening for a given load was shown to occur despite absence of change or even a decrease in the duration of the active state[2].

In relation to the present hypothesis, one of several factors leading to an increase in shortening velocity in the cardiac muscle strip will lead, in the intact heart, to : (1) an earlier attainment of the aortic diastolic pressure-level; (2) an increased velocity of shortening in relation to the ejection after-load ; (3) an increased amount of ejection and systolic muscle shortening, that is, a smaller end-systolic volume, and (4) a smaller end-diastolic volume in the subsequent beat.

Several findings may be cited as evidence for this hypothesis. Wiggers and Katz[7], in the open-chested animal, showed that increasing the aortic resistance at a constant stroke volume resulted in a transient augmentation in initial volume, followed by a subsequent decrease. Sarnoff *et al.*[8], using an *in vitro* preparation operated at constant coronary flow and stroke volume, confirmed this observation and termed it the 'homometric' regulation of the heart to distinguish it from the ordinary 'heterometric' mode. In Sarnoff's preparation the initial length of fibre, as measured with a strain gauge, increased at first when aortic resistance is augmented at fixed stroke input and then decreased. Consistent with the hypothesis presented, these findings are amenable to the interpretation that an increase in initial length of cardiac muscle will initiate an increase in the velocity of its contraction which, at constant stroke input, will result in a decrease in size of heart.

Infusion of catecholamines was shown by Wiggers and Katz[7] to result in a decrease in size of the heart. Recently[4] we found that calcium infusion into the open-chested anæsthetized dog heart operated at controlled cardiac input led to : (1) an increase in the rate of ventricular development of pressure ; (2) a decrease in end-systolic volume of the heart. Table 1 shows the results.

Table 1. EFFECT OF INFUSION OF CALCIUM IONS ON THE HEART OF THE DOG*

	$\Delta P/\Delta T$† mm./sec.	Stroke volume c.c./min.	ΔCircumference‡ per cent of initial
Control	433	7·8	
Calcium ions infused§	622	7·0	—21

* In the open-chested anæsthetized animal, the heart operated at constant cardiac input. (Mean of three animals.)

† Rate of rise in pressure of left ventricle.

‡ Change in left ventricular end-diastolic circumference as measured with a mercury-in-rubber gauge (ref. 10).

§ Continuous infusion of 10 per cent calcium gluconate.

Finally, Rushmer *et al.*[9] has found consistently that exercise in the intact unanæsthetized animal is associated with a relatively constant stroke volume and stroke work and that the increased cardiac output in exercise is due to an increase in heart-rate. Associated with these changes is a decrease in size of the heart. In one experiment it was shown that, if the response of heart-rate to exercise is tape-recorded and used to pace the heart in a resting unanæsthetized animal, a decrease in size of the heart ensued[9]. These findings would fit the hypothesis presented if they were interpreted to imply an increased velocity of shortening.

The cause of an increase in velocity of cardiac contraction in Rushmer's experiments might be due to an increase in heart-rate or an outpouring of catecholamines, or both. There has, as yet, been no unequivocal experimental demonstration, free of filling changes or of changing inotropic influences, that an increase in heart-rate will increase contraction velocity and decrease size of the heart.

The significance of an equilibrium between velocity of contraction, extent of shortening and the volume of the heart is far-reaching and provides an important supplement to the Starling mechanism for cardiac control. Thus, an increase in initial length, heart-rate, and the advent of positive inotropic influences are postulated as an effective counterbalance to factors that require greater initial length to sustain an augmented load. Those factors which initiate stretch of cardiac fibres are still not clearly understood. They may include : (1) metabolic factors, since cardiac work at smaller initial volume is less efficient ; (2) augmented input (diastolic) loads ; (3) negative inotropic factors. Finally, apparent or real changes in contractility and/or distensibility may be involved, both in increasing and decreasing the velocity of contraction and the extent of shortening.

I thank Dr. L. N. Katz for helping me.

[1] Hill, A. V., *Brit. Med. Bull.*, **12**, 165 (1956).

[2] Abbott, B. C., and Mommaerts, W. F. H. M., *J. Gen. Physiol.*, **42**, 533 (1959).

[3] Sonnenblick, E. H., and McCallum, Z. T., *Fed. Proc.*, **20**, 126 (1961).

[4] Feinberg, H., and Katz., L. N., *Fed. Proc.*, **20**, 101 (1961).

[5] Mommaerts, W. F. H. M., Abbott, B. C., and Whalen, W. J., *The Structure and Functions of Muscle*, edit. by G. Bourne (New York, Academic Press Inc., 1959).

[6] Rushmer, R. F., *Cardiovascular Dynamics* (W. B. Saunders Co., Phila., 1961).

[7] Wiggers, C. J., and Katz., L. N., *Amer. J. Physiol.*, **58**, 439 (1921-22).

[8] Sarnoff, S. J., Mitchell, J. H., Gilmore, J. P., and Remensnyder, J. P., *Circ. Res.*, **8**, 1077 (1960).

[9] Rushmer, R. F., Smith, O., and Franklin, D., *Circ. Res.*, **7**, 602 (1959).

[10] Hawthorne, E. W., *Circ. Res.*, **9**, 110 (1961).

ISOLATION AND PROPERTIES OF DEOXYRIBONUCLEIC ACID FROM MAMMALIAN SPERM

By Dr. E. BORENFREUND, E. FITT and Dr. A. BENDICH

Sloan-Kettering Institute for Cancer Research, and Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College, New York

MANY of the properties of the deoxyribonucleic acids (DNA) from numerous sources are well documented, yet relatively little is known of the DNA from mammalian spermatozoa despite their central role in the transmission of genetic determinants. Although DNA can be isolated easily from other mammalian cells, and from the sperm of fish and lower vertebrates, such isolation from mammalian spermatozoa has hitherto been extremely difficult, if not impossible[1]. This has probably stemmed from the inaccessibility of the DNA towards rigorous chemical and physical manipulations which appears due largely to the nearly impervious keratin-like membrane surrounding the sperm-head. The DNA of ram and boar spermatozoa, for example, remains insoluble and bound to a residual protein even after contact for 30 min. with 1·0 *N* sodium hydroxide[2]. Similarly the DNA of bovine sperm is inaccessible to extraction with 0·1 *N* sodium hydroxide[3].

In a brief report in 1950[4], a DNA isolated from defatted human sperm by a procedure involving extensive grinding followed by tryptic digestion was described. The physical properties of the product, however, suggested that considerable degradation had accompanied the isolation. When we applied this procedure to bull sperm it failed to yield DNA. The finding of a sulphur-rich, proteolytic enzyme-resistant keratin-like residue in ram and bull spermatozoa (refs. 5 and 6*a*, *b*) and their increased solubility in thioglycollate[3] suggested that prior rupture of protein-sulphur linkages might lead to an appropriate procedure for the isolation of DNA.

Accordingly, the effect of exposure of fresh mammalian sperm to three groups of agents which affect sulphur linkages was studied. These included oxidizing, mercaptide-forming and reducing agents. Use of only the reducing agents permitted the isolation of the DNA. Pretreatment of sperm with 2-mercaptoethanol, thioglycollic acid and British Anti-Lewisite (2,3-dimercaptopropane) at *p*H 7 was effective at a concentration of 0·25 *M*, and cysteine and sodium sulphite were less so. However, this treatment alone was not sufficient, and DNA was released into solution only after subsequent incubation with trypsin. Reversal of the sequence was ineffective.

Several samples of semen from healthy, highly fertile bulls and pooled samples from rabbits were obtained from the New York Animal Breeders' Corp., Ithaca, New York, through the kind co-operation of Dr. H. O. Dunn and Dr. R. H. Foote, of the Department of Animal Husbandry, Cornell University. Sperm-counts and motility-rates were determined prior to shipment of the freshly frozen specimens. Usually, 2–3 ejaculates from individual bulls (12–16 ml., about $1 \cdot 5 \times 10^9$ cells per ml.) were pooled.

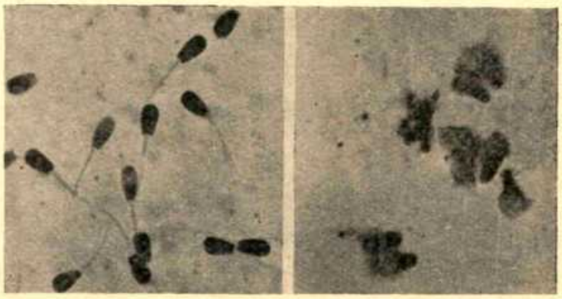


Fig. 1. (left), Bull spermatozoa before treatment. Unstained, unfixed (× 500); (right), bull spermatozoa after exposure to 0·25 *M* β-mercaptoethanol for 2 hr. Unstained, unfixed (× 500)

Table 1. PROPERTIES OF DNA FROM MAMMALIAN SPERM

	Bull 1	Bull 2	Bull 3	Bull 4	Rabbit	Human
Atomic N/P ratio	3·45	3·37	3·85	3·53	3·77	3·46
$S_{20,w}$	18·1	23·4	19·3	19·8	12·8	16·5
$M_s \times 10^{-6}$	4·5	8·2	4·9	5·4	1·8	4·0
(η), dl./gm.	49	53	45	47	19	40
$M\eta \times 10^{-6}$	5·0	5·7	5·2	5·1	2·3	4·4
T_m, °C.	90·0	87·0	88·0	87·0	88·0	89·0
Δ abs., %	37	36	33	35	33	30

We are indebted to Dr. J. MacLeod, of the Department of Anatomy of Cornell University, for several specimens of semen from normal, healthy medical students. The specimens were processed within ½ hr. of collection. Various methods of homogenization (Potter–Elvehjem, Virtis at 14,000 r.p.m., Mickel's vibrator) and attempts to rupture the sperm cell by freeze-thawing, treatment with deoxycholate or detergents, as well as extraction with phenol or strong salts, failed to release DNA from bull spermatozoa. Similarly, treatment with the enzymes lipase, chymotrypsin, trypsin and lysozyme, or exposure to 9 *M* urea, also failed to release the DNA.

A sperm pellet was obtained by centrifuging the semen for 20–30 min. at 8,000*g* in a refrigerated centrifuge in order to remove the seminal plasma, which is known to contain nucleases[7]. The sperm pellet was washed twice with 0·15 *M* sodium chloride –0·015 *M* sodium citrate and once with ethanol, followed by a 1 : 1 mixture of alcohol–ether to remove the lipoprotein from the capsule[8]. The washings were removed by centrifugation at 8,000*g* for 30 min. To about 100 mgm. of the lipid-free sample were added 20·0 ml. of 0·15 *M* sodium chloride –0·015 *M* sodium citrate containing 2 per cent 2-mercaptoethanol (0·25 *M*) and the mixture incubated for 2 hr. with gentle magnetic stirring in the cold. The effect of this treatment on the morphology of the spermatozoa is shown in Fig. 1. Trypsin (10 mgm.) was then added and the incubation continued for 1 hr. at room temperature. The sample was then centrifuged at 12,000*g* for 1 hr. The supernatant was kept, and the sediment was re-suspended in 10 ml. of saline-citrate containing 5 mgm. of trypsin and incubated for 1 hr. at room temperature and centrifuged. This enzyme step was usually repeated once more, to assure complete release of DNA. To the supernatants obtained after tryptic digestion were added 2 volumes of ethanol, and the nucleic acid fibres which precipitated were removed with a glass rod and redissolved in 0·01 *M* sodium chloride with gentle stirring in the cold room.

Table 2. BASE COMPOSITION OF DNA FROM MAMMALIAN SPERMATOZOA

Sperm	Hydrolysis	Bases in moles per cent				A/T	G/C	T/C	Pur/Pyr	$\frac{A+T}{G+C}$
		Adenine	Thymine	Guanine	Cytosine*					
Bull	Formic	28·7	28·2	21·6	21·6	1·02	1·00	1·30	1·02	1·31
Bull	$HClO_4$	28·9	26·3	22·8	22·1	1·09	1·03	1·19	1·06	1·23
Bull	HCl	25·9	29·4	22·9	21·7	0·99	1·05	1·35	0·96	1·24
Bull†	Formic	28·8	27·5	22·3	22·0	1·05	1·07	1·31	1·06	1·30
Human	Formic	30·5	28·9	19·9	20·6	1·05	0·96	1·40	1·01	1·47
Human‡	Formic	29·8	32·5	18·6	19·3	0·92	0·96	1·70	0·94	1·64

* Includes 5-methylcytosine.
† Data from Wyatt, G. R. (ref. 27), recalculated in moles per cent.
‡ Data from Chargaff, E., Zamenhof, S., and Green, C. (ref. 30); base composition is average of two determinations, recalculated in moles per cent.

After the first treatment with trypsin, the preparation was at times so viscous that efficient separation of DNA from the sediment by centrifugation was difficult. In those instances the sample was either further diluted with the saline-citrate or if separation was still not complete the entire mixture was precipitated with 2 volumes of ethanol. The fibres which formed were removed with a glass rod and redissolved in saline-citrate to which a fresh aliquot of enzyme had been added.

Further deproteinization was carried out by the procedure of Kay, Simmons and Dounce[9]. To the 0·01 *M* sodium chloride solution of the DNA, 0·1 volume of 4 per cent 'Duponol' (sodium dodecyl sulphate) in 45 per cent ethanol was added and the mixture stirred at 4° C. for 3 hr. The salt concentration was then raised to 1 *M*, and the stirring continued for 45 min. The mixture was centrifuged for one hour at 13,000*g*, and the DNA in the supernatant precipitated with two volumes of ethanol. In order to reduce the content of protein to less than 0·5 per cent, the level detectable by the method of Ramachandran[10], the preparation was subjected to four or five additional treatments with 'Duponol'. This procedure permits the isolation of about 96–99 per cent of the DNA present in the sperm. This was determined by extracting the remaining tissue sediments after the isolation of DNA with hot trichloracetic acid according to Schneider[11]. These extracts contained less than 4 per cent of the total DNA. DNA, determined by the Burton modification[12] of the Dische reaction, constitutes about 15 per cent of the alcohol–ether-extracted spermatozoa studied.

Phosphorus determinations were carried out acccording to King[13] and the Nessler reagent was used for nitrogen analysis. The atomic nitrogen to phosphorus ratios (N/P, Table 1) are well within the range found for mammalian DNA. Viscosities were determined at various concentrations between 15 and 70 μgm./ml. in 0·2 *M* sodium chloride with a capillary viscometer[14] at shear-rates of 158, 101 and 48 sec.$^{-1}$. Intrinsic viscosities were obtained by extrapolation to zero concentration and zero shear. Solutions containing 0·002 per cent DNA in 0·2 *M* sodium chloride were analysed at 59,780 r.p.m. (259,700*g*) (refs. 15 and 16) in a 'Spinco' model *E* ultracentrifuge equipped with ultra-violet optics, and average sedimentation coefficients, $S_{50\%}$, corrected to 20° C. and water were calculated[17]. With the exception of the DNA from rabbit sperm, which from the sedimentation and intrinsic viscosity values suggest a molecular weight considerably lower than those of the other specimens, the preparations from bull and human sperm showed intrinsic viscosities (η) between 40 and 50 dl./gm. and sedimentation coefficients of about 19–22 *S*. These values are consistent with average molecular weights (Ms and $M\eta$) of about $4–5 \times 10^6$ (ref. 18). Buoyant densities were determined by the method of Meselson, Stahl and Vinograd[19] in a density gradient formed by centrifugation (44,770 r.p.m.) of 8 *M* cæsium chloride solutions to which 2 μgm. of sperm DNA and 1·0 μgm. of a standard DNA–^{15}N from *Pseudomonas aeruginosa* with a density of 1·742 (kindly supplied by Dr. J. Marmur) were added. The buoyant density found for the DNA from bull and human spermatozoa was 1·701, which is about the same as that of DNA from other mammalian tissues[20].

Table 3. CHROMATOGRAPHIC FRACTIONATION OF BULL SPERM DNA*

Chromatographic region	Composition of eluant Molarity, NaCl	Molarity, NH_3	% of original	$S_{20 \cdot w}$
Original			100	20·8
1	0–0·5		41	17·7
2	2·0	0·1	16	20·0
3	2·0	0·1–1·0	10	
4	2·0	2·0	25	22·4
5	(0·5 *M* NaOH)		7	

* Bull sperm DNA (2·5 mgm. in 5·5 ml. of 0·01 *M* phosphate, *p*H 7·0) was applied to a 0·8 × 7·2 cm. column of 'Ecteola'–*SF*–1 and fractionated as described previously (ref. 31). Total recovery of DNA was 96 per cent. The fractions obtained in region 3 consisted of a low, ill-defined band. $S_{20 \cdot w}$ was determined on samples taken from the peak tubes in the regions indicated.

A special heating device attached to the Beckman *DU* spectrophotometer permitted determination of temperature-absorbancy curves according to Marmur and Doty[21]. The midpoint (T_m) of the rise of absorbancy at 260 mμ as a function of temperature averaged about 88° C., and the hyperchromic effect due to temperature, $\Delta_{abs.}$, averaged about 35 per cent. These data indicate a high degree of nativeness. This was supported by the fact that the preparations failed to react with formaldehyde; this is consistent with the behaviour of nucleic acid in the undenatured (double-stranded) form[22]. This is further supported by application of the procedure of Zamenhof *et al.*[23], in which transforming DNA is added to a tissue prior to extraction. When pneumococcal DNA active in transformation to streptomycin resistance was added to bull sperm, and the DNA was isolated by the procedure outlined here, the product still retained its transforming activity.

The values of T_m and buoyant density are consistent with a guanine plus cytosine content of about 43 per cent[20,21]. Base analyses were obtained by hydrolysis with 88 per cent formic acid[24], with 72 per cent perchloric acid[25] and with 6 *N* hydrochloric acid[26]. Wyatt's isopropanol-HCl solvent system[27] was used for paper chromatography. The ultra-violet-absorbing spots were eluted with 0·1 *N* hydrochloric acid and the concentrations of the individual bases determined with the Beckman *DU* spectrophotometer (Table 2)[28].

We feel that the base analyses of the DNA hydrolysed by formic acid are the most reliable. The low

British Flies: Empididae

J. E. COLLIN

The sixth volume—the third to be published—of a series on British Flies inaugurated by G. H. Verrall. This will be the standard work on the British Empididae. The volume is divided into three parts: Tachydrominae; Hybotinae and Empidinae (except *Hilara*); Empidinae (*Hilara* only) and Hemerodrominae. Parts I and II are available; Part III will be ready in October. A complete (clothbound) volume will be published subsequently.

Each part 30*s. net*
Complete volume, £5 *net*

Mechanisms in Biological Competition

EDITED BY F. L. MILTHORPE

The papers in the fifteenth Symposium of the Society for Experimental Biology consider the physical and chemical mechanisms of inter- and intra-specific competition and their consequences in plants and animals. This volume will be of interest to a wide range of biologists, including ecologists, agronomists, and many physiologists and geneticists.

50*s. net*

CAMBRIDGE UNIVERSITY PRESS

the world's
leading
international
booksellers

STECHERT-HAFNER INC.

Books on all subjects in all languages, new and out-of-print

Subscriptions handled for every journal

NEW YORK 31 EAST TENTH STREET, NEW YORK 3, N.Y.
LONDON STAR YARD, CAREY STREET, W.C.2
PARIS 16 RUE DE CONDÉ, PARIS VI^{E}
STUTTGART HÖLDERLINSTRASSE 38, STUTTGART

and agents throughout the world

INTERSCIENCE PUBLISHERS

Lectures in Theoretical Physics – Volume III

Edited by **W. E. BRITTIN, B. W. DOWNS** *and* **JOANNE DOWNS,** *Department of Physics, University of Colorado.*
The lectures delivered at the Third Annual Summer Institute for Theoretical Physics, University of Colorado, Boulder, Colorado, June to August, 1960.

Contents:
- Causality and Dispersion Relations
- Selected Topics in Theoretical Physics
- Statistical Mechanics in Irreversibility
- The Scattering of Electrons by Atoms
- Some Applications of the Generating Functional of the Molecular Distribution Functions
- Nonequilibrium Statistical Mechanics
- Quantum Theory of Collision Processes
- Canonical Commutation Relations in Field Theory and Functional Integration
- General Theory of Nonequilibrium Phenomena
- Martin-Schwinger Methods in the Many-Body Problem
- Green's Functions and the Quantum Theory of Fields

83/-

540 pages
106 figures
5 tables

Volume II *Edited by* **W. E. BRITTIN** *and* **B. W. DOWNS,** *University of Colorado.*
Lectures delivered at the Second Annual Summer Institute, 1959.
492 *pages,* 110 *figures,* 3 *tables,* 68/-

Volume I *Edited by* **W. E. BRITTIN** *and* **L. G. DUNHAM,** *University of Colorado.*
Lectures delivered at the First Annual Summer Institute, 1958.
421 *pages,* 36 *figures,* 4 *tables,* 45/-

Monographs and Texts in Physics and Astronomy

VOL. VII **THE ABUNDANCE OF THE ELEMENTS** *by* LAWRENCE H. ALLER, *The Observatory of the University of Michigan.*
A complete survey of the chemical composition of the sun, the stars, and gaseous nebulae in the neighbourhood of the sun, and the various objects in the solar system.
294 *pages,* 32 *figures,* 31 *tables,* August 1961, 75/-

VOL. VI **PHYSICS OF THE SOLAR CHROMOSPHERE** *by* RICHARD N. THOMAS, *Boulder Laboratories. National Bureau of Standards,* and R. GRANT ATHAY, *High Altitude Observatory, University of Colorado.*
432 *pages,* 67 *figures,* 61 *tables,* April 1961, 117/-

VOL. V **TECHNIQUES OF HIGH ENERGY PHYSICS** *edited by* DAVID M. RITSON, *Laboratory for Nuclear Science, Massachusetts Institute of Technology.*
552 *pages,* 182 *figures,* 52 *tables,* May 1961, 126/-

VOL. IV **FAST NEUTRON PHYSICS—Part 1** *edited by* J. B. MARION and J. L. FOWLER.
1002 *pages,* 303 *figures,* 91 *tables,* 1960, 218/-

VOL. III **INTRODUCTION TO THE THEORY OF QUANTIZED FIELDS** *by* N. N. BOGOLIUBOV and D. V. SHIRKOV. *Translated from the Russian by G. M. VOLKOFF.*
736 *pages,* 60 *figures,* 2 *tables,* 1959, 128/-

VOL. II **RADIATION EFFECTS IN SOLIDS** *by* G. J. DIENES and G. H. VINEYARD
234 *pages,* 47 *figures,* 16 *tables,* 1957, 50/-

VOL. I **FUNDAMENTAL CONSTANTS OF PHYSICS** *by* E. R. COHEN, K. M. CROWE and J. W. M. DuMOND.
299 *pages,* 31 *figures,* 37 *tables,* 1957, 58/-

Tracts on Physics and Astronomy

Provides nonspecialists with authoritative and relatively brief accounts of recent advances in the many specialized branches of physics and astronomy.
Available in both cloth and paper bound editions:
Volumes 1 to 6 — *Paper bound* 15/- *each, Cloth bound* 30/- *each.*
Volumes 7 to 11 — *Paper bound* 19/- *each, Cloth bound* 34/- *each.*

No. 11 **INTRODUCTION TO ELEMENTARY PARTICLE PHYSICS**—R. E. MARSHAK and E. C. G. SUDARSHAN
240 *pages,* 12 *tables,* May 1961.
No. 10 **GENERAL RELATIVITY AND GRAVITATIONAL WAVES**—J. WEBER
208 *pages,* 14 *figures,* May 1961.
No. 9 **INTRODUCTION TO CELESTIAL MECHANICS**—T. E. STERNE
218 *pages,* 7 *figures,* 4 *tables,* May 1961.
No. 8 **INTRODUCTION TO THE THEORY OF IONIZED GASES**—J. L. DELCROIX
162 *pages,* 39 *figures,* 8 *tables,* May 1961.
No. 7 **CRYOPHYSICS**—K. MENDELSSOHN
192 *pages,* 89 *figures,* 2 *tables,* 1960.
No. 6 **PHYSICS OF METEOR FLIGHT IN THE ATMOSPHERE**—E. J. ÖPIK
No. 5 **INTRODUCTION TO THE PHYSICS OF MANY-BODY SYSTEMS**—D. TER HAAR
No. 4 **MAGNETOHYDRODYNAMICS**—T. G. COWLING
No. 3 **PHYSICS OF THE FULLY IONIZED GASES**—L. SPITZER, Jr.
No. 2 **HIGH-ENERGY ACCELERATORS**—M. S. LIVINGSTON
No. 1 **NEUTRON OPTICS**—D. J. HUGHES

INTERSCIENCE PUBLISHERS LIMITED

NEW YORK AND LONDON

Extractive Metallurgy of Copper, Nickel and Cobalt

Edited by **PAUL QUENEAU,** *International Nickel Company of Canada.*
Based on an International Symposium, New York, 1960, sponsored by The Extractive Metallurgy Division of The Metallurgical Society, American Institute of Mining, Metallurgical and Petroleum Engineers (AIME). The papers which comprise this volume provide a good understanding of modern practice in the field; they are divided into five main sections plus an annotated bibliography.

169/-
664 pages
184 figures
95 tables

Sections

- General
- Fuel-fired Smelting and Converting
- Electric Furnace Smelting
- Atmospheric and Elevated Pressure Leaching
- Refining
- Annotated Bibliography

Powder Metallurgy

Edited by **WERNER LESZYNSKI,** *Schwarzkopf Development Corporation.*
The papers presented at the International Powder Metallurgy Conference, New York, 1960, sponsored by the Metal Powder Industries Federation and the Powder Metallurgy Committee of the Institute of Metals Division of The Metallurgical Society, American Institute of Mining, Metallurgical and Petroleum Engineers (AIME). The papers and discussions are grouped together under the following section headings.

188/-
864 pages
481 figures
115 tables

Sections

- Mechanism of Sintering
- Dispersion Strengthening
- Technology and Methods
- Materials and Applications
- Subject index

Metallurgical Society Conferences

Each volume in this series is the proceedings of a technical conference sponsored by The Metallurgical Society of AIME through its technical committees—affording the rapid and co-ordinated publication of scientific and technical information in a form most useful to metallurgists—to cover the entire field of metallurgy.

VOL. 10 **COLUMBIUM METALLURGY** (Bolton Landing, 1960)
Edited by **D. L. DOUGLASS,** *Vallecitos Atomic Laboratory, and* **F. W. KUNZ,** *Knolls Atomic Power Laboratory*
762 *pages*, 375 *figures*, 116 *tables*, September 1961, 196/-

VOL. 9 **RESPONSE OF METALS TO HIGH VELOCITY DEFORMATION** (Estes Park, 1960)
Edited by **P. G. SHEWMON,** *Carnegie Institute of Technology, and* **V. F. ZACKAY,** *Ford Scientific Laboratory*
504 *pages*, 255 *figures*, 39 *tables*, August 1961, 135/-

VOL. 8 **PHYSICAL CHEMISTRY OF PROCESS METALLURGY** (Pittsburgh 1959)
Edited by **GEORGE R. St. PIERRE,** *The Ohio State University*
Part II: 744 *pages*, 287 *figures*, 94 *tables*, June 1961, 188/-

VOL. 7 **Part I:** 658 *pages*, 240 *figures*, 76 *tables*, June 1961, 169/-

VOL. 6 **FLAT ROLLED PRODUCTS II: Semi-Finished and Finished** (Chicago 1960)
Edited by **E. W. EARHART,** *Lone Star Steel Co., and* **R. D. HINDSON,** *Steel Company of Canada*
160 *pages*, 73 *figures*, 23 *tables*, 1960, 30/-

VOL. 5 **PROPERTIES OF ELEMENTAL AND COMPOUND SEMICONDUCTORS** (Boston, 1959)
Edited by **H. C. GATOS,** *Massachusetts Institute of Technology*
352 *pages*, 172 *figures*, 32 *tables*, 1960, 64/-

VOL. 4 **PHYSICAL METALLURGY OF STRESS CORROSION FRACTURE** (Pittsburgh, 1959)
Edited by **T. H. RHODIN,** *Cornell University*
407 *pages*, 191 *figures*, 40 *tables*, 1960, 98/-

VOL. 3 **QUALITY REQUIREMENTS OF SUPER-DUTY STEELS** (Pittsburgh, 1958)
Edited by **R. W. LINDSAY,** *Crucible Steel Company*
319 *pages*, 107 *figures*, 66 *tables*, 1960, 64/-

VOL. 2 **REACTIVE METALS** (Buffalo, 1958)
Edited by **W. R. CLOUGH,** *Messrs Pratt & Whitney, Middletown, Conn.*
624 *pages*, 292 *figures*, 122 *tables*, 1960. 113/-

VOL. 1 **FLAT ROLLED PRODUCTS: Rolling and Treatment** (Chicago, 1959)
Edited by **T. E. DANCY** *and* **E. L. ROBINSON,** *Chicago*
148 *pages*, 82 *figures*, 7 *tables*, 1959, 30/-

In preparation:

VOL. 11 **REFRACTORY METALS AND ALLOYS** (Detroit, 1960)
Edited by **M. SEMCHYSHEN** *and* **J. J. HARWOOD**
636 *pages*, 324 *figures*, 63 *tables*, October 1961, 165/-

VOL. 12 **METALLURGY OF ELEMENTAL AND COMPOUND SEMICONDUCTORS** (Boston, 1960)
Edited by **RALPH O. GRUBEL**
506 *pages*, 198 *figures*, 26 *tables*, October 1961, 98/-

88-90 Chancery Lane, London, W.C.2

Absorption Spectra in the Ultraviolet and Visible Region

★

Edited by L. Lang

★

Publishing House of the
Academy of Science, BUDAPEST

"The aim of this volume and future supplements is to provide a unified presentation of UV absorption spectra from Hungarian laboratories, capable of continuous expansion as additional data become available, and readily comparable by means of tabulated $\log I_0/I$ values as well as the usual curves of $\log \epsilon$ vs wavelength."

From review in "British Bulletin of Spectroscopy."
See also review in "Nature", February 1961.

Vol. I (2nd Edition) with theoretical and technical introduction in German and English.
170 pp. text ; 414 diagrams & tables ; 1 supplement ;
Cloth. £6 17s. 6d.

Vol. II contains Hungarian and some Polish scientific results. 408 pp. of diagrams. Cloth. £6 0s. 0d.

Available from **Collet's**

44/45, MUSEUM STREET, LONDON, W.C.1

Journal of Station Biologique de Roscoff
QUARTERLY REVIEW

CAHIERS DE BIOLOGIE MARINE

published by : G. BACCI, C. BOCQUET, P. DRACH, M. FONTAINE, C. LEVI, I. G. POLJANSKY, M. PRENANT, B. SWEDMARK, G. TEISSIER.

The CAHIERS DE BIOLOGIE MARINE, open to authors of any nationality, are devoted to the study of marine organisms and their media.
They do not exclude any of the problems usually studied in a marine laboratory or on an oceanographic ship. Vols. 1 and 2 (1960 and 1961) include Memoranda and Notes set down under headings as varied as :

Study of marine media and their populations in all their oceanographic, ecological, and animal life aspects ;

reproduction and development cycles, the morphology and physiology of marine animals and plants ;

the biometry and genetics of the populations and their applications to systematics.

Scientific correspondence :
M. le Prof. G. Teissier,
Laboratoire de Zoologie,
Faculté des Sciences,
7 Quai St. Bernard, Paris V°.

Exclusive distributor and sole agent :
Interdoc-Périodiques,
12 rue Pierre Curie, Paris V°,
C.C.P. Paris II.188-05.

Subscription : 60 NF.
Single copy rates: 15 NF.

Books *ON PURE AND APPLIED SCIENCE*

LARGE STOCK ALWAYS AVAILABLE

Many of the books reviewed or mentioned in this journal are available from stock. Foreign books not in stock obtained to order with the least possible delay.

Catalogues on application. Please state interests

Scientific Lending Library

New books and latest editions available promptly
ANNUAL SUBSCRIPTION, FROM £2 - 5 - 0
Prospectus free on request

Second-hand Books

Large stock of recent editions

H. K. LEWIS & Co. Ltd.
LONDON: 136 GOWER STREET, W.C.1
Business hours—9 a.m. to 5.30 p.m., Saturday to 1 p.m.
Telephone: Euston 4282 (9 lines)

ACADEMIC PRESS New York and London

Now Complete *In 2 Volumes*

MECHANISMS IN RADIOBIOLOGY

Edited by **Maurice Errera** *Université Libre de Bruxelles*
and **Arne Forssberg** *Karolinska Institutet, Stockholm*

These two volumes cover the principles of radiobiology, including allied areas in radiation physics and radiation chemistry. The treatise presents a comprehensive treatment of the field, with major emphasis upon those subjects for which review articles are not readily available elsewhere.

Each author is actively engaged in research within the particular field of his contribution. The cooperation of these scientists has produced a work useful as a handbook for the student and as a reference work for the advanced research worker.

Volume 1, General Principles

August 1961, 534 pp., 114s. 6d.

Physical Principles of Radiation Action
BY F. HUTCHINSON *and* E. POLLARD,
Yale University

Target Theory and Radiation Effects on Biological Molecules
BY F. HUTCHINSON *and* E. POLLARD

Radiation Chemistry
BY EDWIN J. HART *and* ROBERT L. PLATZMAN,
Argonne National Laboratory

The Biochemical Lesion in Vivo and in Vitro
BY M. G. ORD *and* L. A. STOCKEN
University of Oxford

Cytological Effects
BY THEODORE N. TAHMISIAN,
Argonne National Laboratory

Effects on Subcellular Units and Free-Living Cells
BY TIKVAH ALPER
Hammersmith Hospital, London

Radiation Genetics
BY SHELDON WOLFF,
Oak Ridge National Laboratory

The Induction of Mutations as a Method in Plant Breeding
BY ÅKE GUSTAFSSON
Forest Research Institute, Stockholm

AUTHOR INDEX—SUBJECT INDEX

Volume 2, Multicellular Organisms

1960, 395 pp., 93s.

General Biology: Gametes, the Developing Embryo, and Cellular Differentiation
BY ROBERTS RUGH, *College of Physicians and Surgeons, Columbia University*

General Biology: The Adult Organism
BY CARL-JOHAN CLEMEDSON, *University of Göteborg, and* ARNE NELSON, *The Research Institute of National Defense, Stockholm*

Immunology
BY MILAN HAŠEK *and* ALENA LENGEROVÁ,
Institute of Biology, Czechoslovak Academy of Sciences, Prague

Mechanisms of Protective and Sensitizing Action
BY LORENTZ ELDJARN *and* ALEXANDER PIHL,
The Norwegian Radium Hospital, Oslo

Recovery and Therapy of the Irradiated Organism
BY D. W. VAN BEKKUM, *National Defense Research Council T.N.O., The Netherlands*

AUTHOR INDEX—SUBJECT INDEX.

Telephone : WHItehall 6631

111 FIFTH AVENUE, NEW YORK 3 17 OLD QUEEN STREET, LONDON, S.W. 1

IRRIGATION AND CLIMATE

New Aids to Engineering Planning and Development of Water Resources

H. Olivier, C.M.G., Ph.D., M.I.C.E., F.ASCE.

For engineers and agriculturists working on the preparation of water balance sheets and in the planning of new water resources. This book provides relatively simple methods for estimating the quantities and times of crop water requirements for different localities. It is based on the author's wide experience in this field.

100s. net

A DEGREE PHYSICS

C. J. Smith, Ph.D., M.Sc., A.R.C.S., F.Inst.P.

For students reading for the B.Sc. General Degree; treated from the experimental and theoretical outlook.

PART I. THE GENERAL PROPERTIES OF MATTER. Second Edition. 63s. net.

"There is a great need outside the universities for young graduates with a thorough working knowledge of the contents of this book . . . Dr. Smith's second edition contains a great deal of valuable physics and is an extremely competent piece of work." *Science Progress.*

PART III. OPTICS. 63s. net.

"The student who has worked through the text of this volume will have gained a very thorough grounding in optics." *Nature.*

PART V. ELECTRICITY AND MAGNETISM. Second Edition. 45s. net.

"It can be recommended without reservation. Throughout it shows the mark of the experienced teacher, who knows where stress should be laid and where difficulties lie for students." *Nature.*

NEWTONIAN SCIENCE

A. E. Bell, Ph.D., M.Sc.

"The author has already established himself as an effective commentator upon the science of the seventeenth century. Here we have a deeper study of the evolution of the physical science of the Newtonian era. . . . Mr. Bell's treatment is lucid and terse." *Technical Education.*

24s. net

EDWARD ARNOLD (PUBLISHERS) LTD.
41 Maddox Street, London, W.1

Back Numbers of Periodicals for Sale

AMERICAN JOURNAL OF BOTANY
Vols. 16–44 (lacks Vols. 27, 37, 40, 41). East Lansing, Michigan 1929–1959. **£140**

BEILSTEIN. HANDBUCH DER ORGANISCHEN CHEMIE
Main Work. 1st and 2nd Supplements. 3rd Suppl. to 2/pt. 2. Complete work in original edition as far as published to date. Price upon request.

BERICHTE UEBER DIE WISSENSCHAFTLICHE BIOLOGIE
Vols. 1–89 (lacks Vols. 57, 60–63, 71, 83). Berlin 1926–1954. **£440**
Single volumes and shorter runs available.

BIOCHIMICA ET BIOPHYSICA ACTA
Vols. 1–36 New York/Amsterdam 1947–1959. Mostly bound. Partly reprint. **£285**

BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE. REPORTS
1831–1939 all publ. With index 1861–1890. London. Bound.
With its continuation
Advancement of Science. Vols. 1–12. London 1939–1956. **£135**

BRITISH JOURNAL OF APPLIED PHYSICS
Vols. 1–10 with Supplements (lacks TPI to Vol. 10). London 1950–1960. **£55**

MATHEMATISCHE ZEITSCHRIFT
Vols. 1–72. Berlin 1918–1960. **£570**

VIRCHOW'S ARCHIV FUER PATHOLOGISCHE ANATOMIE
Vols. 1–318 (lacks 38 vols.) with Reg. 1–100 271–300. Approx. one-third bound. Berlin 1847–1950. **£1,440**
Single volumes and shorter runs available.

We hold in Oxford and New York a comprehensive stock of British, Continental and American Periodicals and frequently issue catalogues. Please send your lists of requirements.

I. R. MAXWELL and Co. Ltd.
Headington Hill Hall, Oxford
Tel. 64881. Cables: LEXICOM. Telex 83177.

Offices also at:
103, Fifth Avenue, New York 3, Tel. Chelsea 2–2100.
Kaiserstrasse 75, Frankfurt am Main. Tel. 33.74.61.
24 rue des Ecoles, Paris Ve. Tel. ODEon 30–00.

RECENT SCIENTIFIC and TECHNICAL BOOKS

Volumes marked with an asterisk (*) have been received at "Nature" Office

Mathematics

ALEXANDROFF, Paul. Elementary Concepts of Topology. Translated by Alan E. Farley. 8 in.×5¼ in. (20 cm.×13 cm.). Pp. 73. (New York : Dover Publications, Inc.; London: Constable and Co., Ltd., 1961.) 1 dollar ; 8*s*.*

BIRKOFF, Garrett, and WIGNER, Eugene P. (edited by). Nuclear Reactor Theory : Proceedings of the Eleventh Symposium in Applied Mathematics of the American Mathematical Society, April 23–25, 1959. 10¼ in.×7 in. (26 cm.×18 cm.). Pp. v+339. (Providence, R.I. : American Mathematical Society, 1961.) n.p.*

GEL'FAND, I. M. Lectures on Linear Algebra. Translated from the revised second Russian edition by A. Shenitzer. (Interscience Tracts in Pure and Applied Mathematics, Vol. 9.) 9 in.×6 in. (23 cm.×15·5 cm.). Pp. ix+185. (New York : Interscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1961.) 6 dollars ; 45*s*.*

KORN, Granino A., and KORN, Theresa M. Mathematical Handbook for Scientists and Engineers : Definitions, Theorems, and Formulas for Reference and Review. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xiv+943. (New York : McGraw-Hill Book Company, Inc. ; London : McGraw-Hill Publishing Company, Ltd., 1961.) 155*s*.*

LIUSTERNIK, L. A , and SOBOLEV, V. J. Elements of Functional Analysis. Translated from the Russian by Anthony E. Labarre, Jr., Herbert Izbicki and H. Ward Crowley. 9½ in. × 6¼ in. (24 cm.×16 cm.). Pp. ix+227. (New York : Frederick Ungar Publishing Company ; London : Constable and Co., Ltd., 1961.) 55*s*. net.*

MAXWELL, A. E. Analysing Qualitative Data. (Methuen's Monographs on Applied Probability and Statistics.) 7½ in.×5 in. (19 cm.×12·5 cm.). Pp. 163. (London : Methuen and Co., Ltd., New York : John Wiley and Sons, Inc., 1961.) 16*s*. net.*

MENNINGER, Karl. Mathematics in Your World. Translated by P. S. Morrell and J. E. Blamey. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. viii+244. (London : G. Bell and Sons, Ltd., 1961.) 21*s*. net.*

SEGRE, Beniamino. Lectures on Modern Geometry. (Consiglio Nazionale delle Ricerche—Monografie Matematiche, 7.) 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xv+479. (Roma : Edizioni Cremonese, 1961.) 7,000 lire.*

TRICOMI, Francesco Giacomo. Differential Equations. Translated by Elizabeth A. McHarg. 9¼ in. × 6¼ in. (23·5 cm. × 16 cm.). Pp. x+273. (London : Blackie and Son, Ltd., 1961.) 50*s*. net.*

ZHONGOLOVICH, I. D., and AMELIN, V. M. A Collection of Tables and Nomograms for the Processing of Observations Made on Artificial Earth Satellites. (Mathematical Tables, Vol. 12.) Issued by the Institute of Theoretical Astronomy, Academy of Sciences of the U.S.S.R. 11 in.× 8¼ in. (28 cm.×21 cm.). Pp. iv+188. (London and New York : Pergamon Press, 1961.) 100*s*. net.*

ZURMÜHL, R. Praktische Mathematik für Ingenieure und Physiker. Dritte verbesserte Auflage. 9¼ in.×6½ in. (23·5 cm.×16·5 cm.). Pp. xiv+548. (Berlin : Springer-Verlag, 1961.) 29.40 D.M.*

Physics

ABRAGAM, A. The Principles of Nuclear Magnetism. (International Series of Monographs on Physics.) 9½ in.×6½ in. (24 cm.×16·5 cm.). Pp. xvi+600+22 plates. (Oxford: Clarendon Press ; London : Oxford University Press, 1961.) 84*s*. net.*

BARRAUD, Jean. Principes de Radiocristallographie : Identification des Corps et Détermination de leur Structure par la Diffraction des Rayons X. 9½ in.×6¼ in. (24 cm.×16 cm.). Pp. xi+236. (Paris : Masson et Cie., 1960.) 42 N.F.*

BOHR, Niels. Physique Atomique et Connaissance Humaine. Traduit par Prof. Edmond Bauer et Dr. Roland Omnes. 9½ in.×6¼ in. (24 cm.× 16 cm.). Pp. ii+98. (Paris : Gauthier-Villars et Cie., 1961.) 15 N.F. ; 3·25 dollars.*

BROWN, R. C. A Textbook of Physics. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. xxi+1424+xxxvi. (London : Longmans, Green and Co., Ltd., 1961.) 60*s*.*

BROWN, Sanborn C., and CLARKE, Norman (edited by). International Education in Physics : Proceedings of the International Conference on Physics Education, Unesco House, Paris, July 18th–August 4th 1960. 9¼ in.× 6¼ in. (23·5 cm.×16 cm.). Pp. xvi+191. (Cambridge, Mass. : Technology Press of the Massachusetts Institute of Technology ; New York and London : John Wiley and Sons, Inc., 1960.) 36*s*.*

BRÜGEL, Werner. Physik und Technik der Ultrarotstrahlung. Zweite, wesentlich verbesserte und erweiterte Auflage. 9 in.×6 in. (23 cm.× 15·5 cm.). Pp. 448. (Hannover : Curt R. Vincentz Verlag, 1961.) 48 D.M.*

CORBEN, H. C., and STEHLE, Philip. Classical Mechanics. Second edition. 9 in.×6 in. (23 cm.×15·5 cm.). Pp. xi+389. (New York and London : John Wiley and Sons, Inc., 1960.) 96*s*.*

DEBRAINE, P. Unités de Mesure des Grandeurs Physiques. Deuxième édition. 8¼ in.×5¼ in. (21 cm.×13 cm.). Pp. xiii+186. (Paris : Dunod, 1961.) 24 N.F.*

DENISSE, J. F., et DELCROIX, J. L. Théorie des Ondes dans les Plasmas. (Monographies Dunod.) 6½ in.×4¼ in. (16·5 cm.×11 cm.). Pp. xiii+167. (Paris : Dunod, 1961.) 16 N.F.*

DIBDIN, F. J. H. Essentials of Light. (Essentials of Physics for General Certificate "A" Level Students.) 8½ in.×5½ in. (21·5 cm.×14 cm.). Pp. viii+240. (London : Cleaver-Hume Press, Ltd., 1961.) 15*s*.*

EINSTEIN, Albert, and INFELD, Leopold. The Evolution of Physics : The Growth of Ideas from Early Concepts to Relativity and Quanta. 8 in.×5¼ in. (20 cm.×13 cm.). Pp. xvi+302. (Cambridge : At the University Press, 1961.) 13*s*. 6*d*. net.*

ELTON, L. R. B. Nuclear Sizes. (Oxford Library of the Physical Sciences.) 8½ in.×5 in. (21·5 cm.×14 cm.). Pp. vii+115. (London : Oxford University Press, 1961.) 15*s*. net.*

FRADIN, A. Z. Microwave Antennas. Translated from the Russian by Morton Nadler. Translation edited by R. C. Glass. 8½ in.×5½ in. (21·5 cm. ×14 cm.). Pp. xii+668. (London and New York : Pergamon Press, 1961.) 105*s*. net.*

FRIEDLANDER, S. K., and TOPPER, Leonard (edited by). Turbulence : Classic Papers on Statistical Theory. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. ix+187. (New York : Interscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1961.) 6 dollars ; 45*s*.*

GRAHAM, G. M., and HALLETT, A. C. Hollis (edited by). Proceedings of the VIIth International Conference on Low Temperature Physics, University of Toronto, Canada, 29th August—3rd September 1960. 9¾ in.×6¾ in. (25 cm.×17 cm.). Pp. xviii+725. (Toronto : University of Toronto Press ; Amsterdam : North-Holland Publishing Company, 1961.) 114*s*.*

HAIS, Hermann A., and PENHUNE, John P. Case Studies in Electromagnetism. Problems with Solutions. Laboratory Experiments. 9 in.× 6 in. (23 cm.×15·5 cm.). Pp. xiii+336. (New York and London : John Wiley and Sons, Inc., 1960.) 52*s*.*

HEARMON, R. F. S. An Introduction to Applied Anisotropic Elasticity. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. viii+136. (London : Oxford University Press, 1961.) 35*s*. net.*

HINMAN, Frank. The Impact of the New Physics. 8¾ in.×5¾ in. (22 cm. × 15 cm.). Pp. vii+174. (New York : Philosophical Library, 1961.) 4·50 dollars.*

KITAIGORODSKII, A. I. The Theory of Crystal Structure Analysis. Translated from the Russian by David and Katherine Harker. 9¼ in.× 6¼ in. (23·5 cm.×16 cm.). Pp. xi+275. (New York : Consultants Bureau, Inc., 1961.) 12.50 dollars.*

LANDAHL, Marten T. Unsteady Transonic Flow. (International Series of Monographs in Aeronautics and Astronautics. Division II : Aerodynamics, Vol. 2.) 9¼ in.× 6 in. (23·5 cm.×15·5 cm.). Pp. x+134. (London and New York : Pergamon Press, 1961.) 60*s*. net.*

LANDSBERG, P. T. Thermodynamics, with Quantum Statistical Illustrations. (Monographs in Statistical Physics and Thermodynamics, Vol. 2.) 9 in.×6¼ in. (23 cm.×16 cm.). Pp. x+499. (New York : Interscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1961.) 109*s*.*

MARSHAK, R. E., and SUDARSHAN, E. C. G. Introduction to Elementary Particle Physics. (Interscience Tracts on Physics and Astronomy, Vol. 11.) 8¼ in.×5¼ in. (21 cm.×13 cm.). Pp. viii+231. (New York : Interscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1961.) Paper 2.50 dollars ; 19*s*. Cloth 4.50 dollars; 34*s*.*

MORRIS, Jr., A. C. A Physician's Introduction to Electronics : a Laboratory Manual. 8¾ in.×5½ in. (22 cm.×14 cm.). Pp. iv+43. (London and New York : Pergamon Press, 1961.) 15*s*. net*.

NOISE IN ELECTRONIC DEVICES : Papers based on material presented at a conference held by the Electronics Group of the Institute of Physics at the Services Electronics Research Laboratory, Baldock, Hertfordshire, 2–3 October, 1959. 9¾ in.×6 in. (25 cm.×15·5 cm.). Pp. 100. (London : Chapman and Hall, Ltd. ; New York : Reinhold Publishing Corporation, 1961. Published on behalf of the Institute of Physics and the Physical Society.) 35*s*. net.*

OREAR, Jay. Fundamental Physics. 8¼ in.×8¼ in. (21 cm.×21 cm.). Pp. xiii+381. (New York and London : John Wiley and Sons, Inc., 1961.) 54*s*.*

RICHARDSON, E. G. Dynamics of Real Fluids. Second edition. 8¾ in.× 5¾ in. (22 cm.×15 cm.). Pp. vii+231. (London : Edward Arnold (Publishers), Ltd., 1961.) 50*s*. net.*

RITSON, David M. (edited by). Techniques of High Energy Physics. (Interscience Monographs and Texts on Physics and Astronomy, Vol. 5.) 9¼ in. × 6¼ in. (23·5 cm.×16 cm.). Pp. xii+540. (New York : Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1961.) 16.75 dollars.*

ROLLMANN, Martin. Space and Condenser (Molecular Theory). 8¼ in.×5¾ in. (21 cm.×15 cm.). Pp. 68. (Zürich : Verlag Leemann, 1960.) 12 Swiss francs.*

ROMAN, Paul. Theory of Elementary Particles. Second improved and revised edition. (Series in Physics.) 9 in.×6¼ in. (23 cm.×16 cm.). Pp. xvi+580. (Amsterdam : North-Holland Publishing Company ; New York : Interscience Publishers, Inc., 1961.) 100*s*.*

SCHALL, W. E. X-rays : Their Origin, Dosage and Practical Application. Eighth edition. 9¾ in.×7¼ in. (25 cm.×18·5 cm.). Pp. xi+348+83 plates. (Bristol : John Wright and Sons, Ltd., 1961.) 50*s*.*

SCREATON, G. R. (edited by). Dispersion Relations : Scottish Universities' Summer School, 1960. 10 in.×6¼ in. (25·5 cm.×16 cm.). Pp. xiii+ 290. (Edinburgh and London : Oliver and Boyd, Ltd. ; New York : Interscience Publishers, Inc., 1961.) 55*s*. net.*

SNEDDON, I. N., and HILL, R. (edited by). Progress in Solid Mechanics, Vol. 2. 9 in.×6¼ in. (23 cm.×16 cm.). Pp. xi+331. (Amsterdam : North-Holland Publishing Company, 1961.) 70*s*.*

STRUTT, M., und VILBIG, F. (herausgegeben von). Fortschritte der Hochfrequenztechnik, Band 5. Geschäftsführende Redaktion : F. Rühmann. 9¼ in.×6½ in. (23·5 cm.×16·5 cm.). Pp. x+412. (Frankfurt am Main : Akademische Verlagsgesellschaft m.b.H., 1960.) n.p.*

SUITS, Guy, and WAY, Harold E. (edited by). The Collected Works of Irving Langmuir, with contributions in Memoriam including a Complete Bibliography of his Works. Vol. 3 : Thermionic Phenomena. Pp. xxviii+ 481. Vol. 5 : Plasma and Oscillations. Pp. xx+261. 9¾ in.×7 in. (25 cm.×18 cm.). (London and New York : Pergamon Press, 1961.) 100*s*. net per volume.*

TAYLOR. A. X-ray Metallography. 9 in.×6 in. (23 cm.×15·5 cm.). Pp. vii+993. (New York and London : John Wiley and Sons, Inc., 1961.) 216*s*.*

THE INTERNATIONAL DICTIONARY OF PHYSICS AND ELECTRONICS. Second edition. 10¼ in.×7¼ in. (26 cm.×18·5 cm.). Pp. iv+ 1355. (Princeton, N.J. : D. Van Nostrand Company, Inc. ; London : D. Van Nostrand Company Ltd., 1961.) 27.85 dollars ; 205*s*.*

TOLLMIEN, Walter, SCHLICHTING, Hermann, und GÖRTLET, Henry (herausgegeben von). Schriftleitung F. W. Riegels. Ludwig Prandtl: Gesammelte Abhandlungen zur Angewandten Mechanik, Hydro- und Aerodynamik. Erster Teil: Pp. xix+1–574. Zweiter Teil: Pp. xiii+575–1071. Dritter Teil: Pp. xiv+1071–1620. 9¼ in.×6½ in. (23·5 cm.×16·5 cm.). (Berlin: Springer-Verlag, 1961.) 273 D.M. Ganzleinen 288 D.M.*

Engineering

CHARLTON, T. M. Energy Principles in Applied Statics. 9¼ in.×6¼ in. (23 cm.×16 cm.). Pp. vi+112. (London: Blackie and Son, Ltd., 1959.) 25*s.* net.*

CLARRICOATES, P. J. B. Microwave Ferrites. 10 in.×6¼ in. (25·5 cm.×16 cm.). Pp. xi+260. (London: Chapman and Hall, Ltd., 1961.) 50*s.* net.*

COMRIE, J. (edited by). Civil Engineering Reference Book. Vol. 1: Pp. xiii+518. Vol. 2: Pp. xiii+527. Vol. 3: Pp. xiii+599. Vol. 4: Pp. xiii+392+lxii. 10 in.×6¼ in. (25·5 cm.×16 cm.). (London: Butterworth and Co. (Publishers), Ltd., 1961.) £17 17s. 0*d.* the four volumes.*

DIMENTBERG, F. M. Flexural Vibrations of Rotating Shafts. Translated from the Russian by the Production Engineering Research Association. 10 in.×6¾ in. (25·5 cm.×17 cm.). Pp. ix+243. (London: Butterworth and Co. (Publishers), Ltd., 1961.) 60*s.**

HURLEY, Richard B. Transistor Logic Circuits. 9 in.×6 in. (23 cm.×15·5 cm.). Pp. xvi+363. (New York and London: John Wiley and Sons, Inc., 1961.) 80*s.**

LAST, James. Natural Perspective. 10 in.×7½ in. (25·5 cm.×19 cm.). Pp. vi+66. (London: Sir Isaac Pitman and Sons, Ltd., 1961.) 21*s.* net.*

MEEROV, M. V. Introduction to the Dynamics of Automatic Regulating of Electrical Machines. Translated by J. S. Shapiro. 10 in.×6¼ in. (25·5 cm.×16 cm.). Pp. xxii+411. (London: Butterworth and Co. (Publishers), Ltd., 1961. Published in association with the Department of Scientific and Industrial Research.) 100*s.**

MOON, Parry, and SPENCER, Domina Eberle. Field Theory for Engineers. (The Van Nostrand Series in Electronics and Communications.) 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. ix+530. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1961.) 96*s.**

PALMER, R. G., and PLATT, A. Fast Reactors. (Nuclear Engineering Monographs, No. 12.) 8½ in.×5½ in. (21·5 cm.×14 cm.). Pp. x+93. (London: Temple Press, Ltd., 1961.) 12*s.* 6*d.* net.*

SCHÜTTE, Karl, und KAISER, Hans K. (herausgegeben von). Handbuch der Astronautic, Lieferung 1–6. Band 1. Heft 1: Pp. 1–32. Heft 2: Pp. 33–64. Heft 3: Pp. 65–96. Heft 4: Pp. 97–128. Heft 5: Pp. 129–160. Heft 6: Pp. 161–192. 10½ in.×7¾ in. (26·5 cm.×19·5 cm.). (Konstanz: Akademische Verlagsgesellschaft Athenaion, Dr. Albert Hacfleld, 1958 und 1960.) 1.50 dollars each part.*

THOMAS, W. Norman. Surveying. Fifth edition. 9¼ in.×6 in. (23·5 cm. × 15·5 cm.). Pp. viii+643. (London: Edward Arnold (Publishers), Ltd., 1961.) 40*s.* net.*

WOODS, L. C. The Theory of Subsonic Plane Flow. (Cambridge Aeronautical Series, Vol. 3.). 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xxii+594. (Cambridge: At the University Press, 1961.) 120*s.* net.*

Chemistry: Chemical Industry

AHRENS, L. H., and TAYLOR, S. R. Spectrochemical Analysis: a Treatise on the D–C Arc Analysis of Geological and Related Materials. Second edition. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xxiii+454. (London: Pergamon Press; Reading, Mass.: Addison-Wesley Publishing Company, Inc., 1961.) 105*s.* net.*

AYLWARD, G. H., and SYMES, F. S. (edited by). Approach to Chemistry: Lectures and Workshop Reports of the 1961 Summer School for Chemistry Teachers at the University of New South Wales. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. xvi+248. (Sydney: University of New South Wales, 1961.) 25*s.**

BAYER, Ernst. Gas Chromatography. (Elsevier Monographs: Chemistry Section.) 7½ in.×5¼ in. (19 cm.×13 cm.). Pp. xii+240. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1961.) 25*s.**

BLOCH, Konrad (edited by). Lipide Metabolism. 9 in.×6 in. (23 cm.×15·5 cm.). Pp. xiii+411. (New York and London: John Wiley and Sons, Inc., 1960.) 84*s.**

COPE, Arthur C., ADAMS, Roger, BLATT, A. H., BOEKELHEIDE, Virgil, CAIRNS, T. L., CURTIN, David Y., and NIEMANN, Carl (edited by). Organic Reactions, Vol. 11. 9 in.×6 in. (23 cm.×15·5 cm.). Pp. vii+501. (New York and London: John Wiley and Sons, Inc., 1960.) 96*s.**

DANIELS, Farrington, and ALBERTY, Robert A. Physical Chemistry. Second edition. 9¼ in.×6 in. (23·5 cm.×15·5 cm.). Pp. x+744. (New York and London: John Wiley and Sons, Inc., 1961.) 70*s.**

DANIELS, Farrington. Selected Studies in Chemical Kinetics. (Thirty-fifth Annual Priestley Lectures.) 10¾ in.×8½ in. (27 cm.×21·5 cm.). Pp. vi+123. (University Park, Penn.: Phi Lambda Upsilon and the Department of Chemistry, Pennsylvania State University, 1961.) 2.75 dollars.*

DURHAM, K. (edited by). Surface Activity and Detergency. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. xiii+250+14 plates. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1961.) 35*s.* net.*

FISCHER, Robert B. A Basic Course in the Theory and Practice of Quantitative Chemical Analysis. 9½ in.×6¼ in. (24 cm.×16 cm.). Pp. xi+501. (Philadelphia and London: W. B. Saunders Company, 1961.) 47*s.**

GIBBERELLINS: a Collection of papers comprising the Symposium on Gibberellins presented before the Division of Agricultural and Food Chemistry at the 138th National Meeting of the American Chemical Society, New York, N.Y., September 1960. (Advances in Chemistry Series, No. 28.) 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. vi+167. (Washington, D.C.: American Chemical Society, 1961.) 6 dollars.*

GRADDON, D. P. An Introduction to Co-ordination Chemistry. (International Series of Monographs on Inorganic Chemistry, Vol. 3.) 9 in.×6 in. (23 cm.×15·5 cm.). Pp. vii+111. (London and New York: Pergamon Press, 1961.) 25*s.* net.*

GREENSTEIN, Jesse P., and WINITZ, Milton. Chemistry of the Amino Acids. Vol. 1: Pp. xxi+1–760. Vol. 2: Pp. xi+761–1816. Vol. 3: Pp. xiii+1817–2872. (New York and London: John Wiley and Sons, Inc., 1961.) £40 the three volumes.*

HODGMAN, Charles D., WEAST, Robert C., and SELBY, Samuel M. (edited by). Handbook of Chemistry and Physics: a Ready Reference Book of Chemical and Physical Data. Forty-second edition. 7¼ in.×5 in. (18·5 cm.×12·5 cm.). Pp. xxv+3481. (Cleveland, Ohio: The Chemical Rubber Publishing Company; Oxford: Blackwell Scientific Publications, Ltd., 1960.) 12 dollars; 105*s.**

HODGMAN, Charles D., WEAST, Robert C., and SELBY, Samuel M. (edited by). Tables for Identification of Organic Compounds. Compiled by Prof. Max Frankel and Dr. Saul Patai, with the assistance of Robert Farkas-Kadmon and Dr. Albert Zilkha. (Supplement to "Handbook of Chemistry and Physics".) 9½ in.×6½ in. (24 cm.×16·5 cm.). Pp. vii+241. (Cleveland, Ohio: The Chemical Rubber Publishing Company; Oxford: Blackwell Scientific Publications, Ltd., 1960.) 7 dollars; 60*s.**

KOLTHOFF, I. M., and ELVING, Philip J. Assisted by SANDELL, Ernest B. Treatise on Analytical Chemistry. Part 2: Analytical Chemistry of the Elements, Vol. 3. 9½ in.×6½ in. (24 cm.×16·5 cm.). Pp. xvii+380. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1961.) 13.25 dollars; 100*s.**

MATHIEU, Jean, ALLAIS, André, et VALLS, Jacques. Avec la collaboration de POIRIER, P. Cahiers de Synthèse Organique: Tableaux d'Application. Vol. 6: Transpositions. Pp. v+418. Broché 110 N.F.; Cartonné toile 120 N.F. Vol. 7: Carbocyclisation Monomoléculaire. Pp. v+310. Broché 100 N.F.; Cartonné toile 110 N.F. Vol. 8: Carbocyclisation Polymoléculaire. Pp. v+234. Broché 75 N.F.; Cartonné toile 85 N.F. 8¾ in.×6 in. (22 cm.×15·5 cm.). (Paris: Masson et Cie., 1960 et 1961.)*

MODERN CHEMICAL PROCESSES: a Series of Articles describing Chemical Manufacturing Plants, Vol. 6. By the Editors of *Industrial and Engineering Chemistry*, in conjunction with the Technical Staffs of the Co-operating Organizations. 11¼ in.×8¼ in. (28·5 cm.×21 cm.). Pp. 126. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1961.) 48*s.* net.*

MOELWYN-HUGHES, E. A. Physical Chemistry. Second revised edition. 9¾ in.×6¼ in. (25 cm.×16 cm.). Pp. vii+1333. (London and New York: Pergamon Press, 1961.) 84*s.* net.*

MONK, C. B. Electrolytic Dissociation. (Physical Chemistry: a Series of Monographs, Vol. 8.) 9 in. × 6 in. (23 cm.×15·5 cm.). Pp. xii+320. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1961.) 60*s.**

MORRISON, G. A. Systematic Qualitative Analysis: an Introduction. 7½ in.×5 in. (19 cm.×12·5 cm.). Pp. ix+198. (London: Butterworth and Co. (Publishers), Ltd., 1961.) 25*s.**

NON-CONVERTIBLE COATINGS. (Paint Technology Manuals, Part 1.) 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. 326. (London: Chapman and Hall, Ltd., 1961. Published on behalf of the Oil and Colour Chemists' Association.) 30*s.* net.*

NYBURG, S. C. X-ray Analysis of Organic Structures. (Organic and Biological Chemistry: a Series of Monographs, Vol. 4.) 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xii+434. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 13 dollars; 93*s.**

PARKES, G. D. (revised and edited by). Mellor's Modern Inorganic Chemistry. New edition. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. xxii+1024+21 plates. (London: Longmans, Green and Co., Ltd., 1961.) 40*s.* net.*

RYABININ, Yu. N. Gases at High Densities and Temperatures. Translated from the Russian by H. K. Zienkiewicz. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. vii+52. (London and New York: Pergamon Press, 1961.) 25*s.* net.*

SOVIET RESEARCH ON ORGANO-PHOSPHORUS COMPOUNDS, 1949–1956. In English Translation. Part 1: Pp. viii+448. 50 dollars. Part 2: Pp. vii+449–890. 50 dollars. Part 3: Pp. iv+891–1208. 30 dollars. Complete collection 120 dollars. (Chemistry Collection Series.) 10¾ in.×8½ in. (27 cm.×21·5 cm.). (New York: Consultants Bureau, Inc., 1960.)

SOVIET RESEARCH IN PHARMACEUTICAL CHEMISTRY, 1956. In English Translation. Part 1: Pharmaceutical Chemistry. Pp. v+102. 12 dollars. Part 2: Medicinal Chemistry. Pp. vi+103–532. 45 dollars. Part 3: Pharmacognosy. Pp. v+533–603. 10 dollars. Complete collection 60 dollars. (Chemistry Collection Series.) 10¾ in.×8½ in. (27 cm.×21·5 cm.) (New York: Consultants Bureau, Inc., 1959.)*

WEISZ, Herbert. Microanalysis by the Ring Oven Technique. (International Series of Monographs on Analytical Chemistry, Vol. 1.) 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. 112. (London and New York: Pergamon Press, 1961.) 30*s.* net.*

Technology

CABELL, Randolph W., and PHILLIPS, Almarin. Problems in Basic Operations Research Methods for Management. 8½ in.×5½ in. (21·5 cm.×14 cm.). Pp. x+110. (New York and London: John Wiley and Sons, Inc., 1961.) 32*s.**

DUMMER, G. W. A., and ROBERTSON, Mackenzie (edited by). British Miniature Electronic Components and Assemblies Data Annual, 1961–62. 11¼ in.×8½ in. (28·5 cm.×21·5 cm.). Pp. xviii+479. (London and New York: Pergamon Press, 1961.) 15 dollars.*

HUKE, D. W. Introduction to Natural and Synthetic Rubbers. 8½ in.×5½ in. (21·5 cm.×14 cm.). Pp. 164+4 plates. (London: Hutchinson and Co. (Publishers), Ltd., 1961.) 25*s.* net.*

MEISSNER, C. Robert (edited by). Vacuum Technology Transactions: Proceedings of the Seventh National Symposium, October 12, 13, 14, Cleveland, Ohio. (American Vacuum Society, Inc.). 11¼ in.×8¾ in. (28·5 cm.×22 cm.). Pp. xix+427. (London and New York: Pergamon Press, 1961.) 147*s.* net.*

PICKARD, James K., WARREM, Frederick H., LOWE, William W., and MCLAIN, Stuart (edited by). Power Reactor Technology. (The Second Geneva Series on the Peaceful Uses of Atomic Energy.) 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. ix+417. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1961.) 84*s.* 6*d.**

PROCEEDINGS OF THE NATURAL RUBBER RESEARCH CONFERENCE, Kuala Lumpur, 1960. (Sponsored by the Rubber Research Institute of Malaya with the support of the Government of the Federation of Malaya and the Rubber Producers' Council of the Federation of Malaya.) 10 in.×7½ in. (25·5 cm.×19 cm.). Pp. x+986+xix. (Kuala Lumpur: Rubber Research Institute of Malaya, 1961.) n.p.*

WILLEY, E. L., TRIBE, Marion, D'AGAPEYEFF, A., GIBBENS, B. J., and CLARK, Michelle. Some Commercial Autocodes : a Comparative Study. (A.P.I.C. Studies in Data Processing, No. 1.) 9 in.× 6 in. (23 cm.× 15·5 cm.) Pp. vii+53. (London : Academic Press, Inc. (London), Ltd., New York : Academic Press, Inc., 1961. Published for the Automatic Programming Information Centre.) 15s.*

Astronomy

EDGEWORTH, K. E. The Earth, The Planets and the Stars : Their Birth and Evolution. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. xiii+193+9 plates. (London : Chapman and Hall, Ltd., 1961.) 25s. net.*

ERNST, Br., and DE VRIES, Tj. E. Atlas of the Universe. Translated by D. R. Welsh. Edited by Dr. H. E. Butler. 10¾ in.×7¾ in. (27 cm.× 19·5 cm.). Pp. 226 (94 plates). (London and Edinburgh : Thomas Nelson and Sons, Ltd., 1961.) 42s. net.*

LILLER, William (edited by). Space Astrophysics. (University of Michigan Institute of Science and Technology Series.) 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. viii+272. (New York : McGraw-Hill Book Company, Inc. ; London : McGraw-Hill Publishing Company, Ltd., 1961.) 77s. 6d.*

SHAPLEY, Harlow. Galaxies. Revised edition. 9½ in.×6¼ in. (24 cm.× 16 cm.). Pp. x+186. (Cambridge, Mass. : Harvard University Press ; London : Oxford University Press, 1961.) 40s. net.*

Geology : Mineralogy

CHALLINOR, John. A Dictionary of Geology. 8½ in.×5½ in. (21·5 cm.× 14 cm.). Pp. xv+235. (Cardiff : University of Wales Press, 1961.) 30s.*

MENDELSOHN, F. (edited by). The Geology of the Northern Rhodesian Copperbelt. 9½ in.×6¼ in. (24 cm.×16 cm.). Pp. xvi+523. (London : Macdonald and Co. (Publishers), Ltd., 1961.) 84s. net.*

NAIRN, A. E. M. (edited by). Descriptive Palaeoclimatology. 9 in.× 6 in. (23 cm.×15·5 cm.). Pp. xi+380. (New York : Interscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1961.) 11 dollars ; 80s.*

VON ENGELN, O. D. The Finger Lakes Region : Its Origin and Nature. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xi+156+29 plates. (Ithaca, New York : Cornell University Press ; London : Oxford University Press, 1961.) 36s. net.*

WINDER, C. G. Lexicon of Paleozoic Names in South-western Ontario. 10 in.×7 in. (25·5 cm.×18 cm.). Pp. vii+121. (Toronto : University of Toronto Press ; London : Oxford University Press, 1961.) 48s. net.*

General Biology : Natural History : Zoology : Botany

AUDUBON, Maria R. (edited by). Audubon and his Journals. With zoological and other notes by Elliott Coues. Vol. I : Pp. xiv+532. Vol. 2 : Pp. viii+554. 8 in.×5¼ in. (20 cm.×13 cm.). (New York : Dover Publications, Inc. ; London : Constable and Co., Ltd., 1960.) 2 dollars each volume.*

BASTIN, R. Physiologie Végétale. 9¾ in.×6¼ in. (25 cm.×16 cm.). Pp. 324. (Héverlé (Louvain) : R. Bastin, Institut Agronomique, Parc d'Arenberg, 1961.) 250 francs.*

BURTON, Milton, KIRBY-SMITH, J. S., and MAGEE, John L. (edited by). Comparative Effects of Radiation : Report of a Conference held in San Juan at the University of Puerto Rico, February 15–19, 1960. (Sponsored by the National Academy of Sciences—National Research Council.) 9 in.× 6 in. (23 cm.×15·5 cm.). Pp. xx+426. (New York and London : John Wiley and Sons, Inc., 1960.) 68s.*

BUSNEL, René-Guy, et GIBAN, Jacques. Colloque sur la Protection Acoustique des Cultures et Autres Moyens d'Effarouchement des Oiseaux : Compte-rendu des Réunions tenures au Laboratoire de Physiologie Acoustique de l'Institut National de la Recherche Agronomique a Jouy-en-Josas, les 26 et 27 Novembre 1958. 9½ in.×6½ in. (24 cm.×16·5 cm.). Pp. 246. (Paris : Institut National de la Recherche Agronomique, 1960.) 25 N.F.*

BUTCHER, Roger W. A New Illustrated British Flora. Part I : Lycopodiaceae to Salicaceae. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. viii+1016. (London : Leonard Hill (Books), Ltd., 1961.) 84s. net.*

CARLTON, William M. Laboratory Studies in General Botany. 11 in.× 8½ in. (28 cm.×21·5 cm.). Pp. vi+426. (New York : The Ronald Press Company, 1961.) 4.50 dollars.*

COLLIN, J. E. British Flies, Vol. 6 : Empididae. Part I : Tachydrominae. 9¾ in.×6 in. (25 cm.×15·5 cm.). Pp. viii+219. (Cambridge : At the University Press, 1961.) 30s. net. ; 6 dollars.*

DIMSDALE, Tim. Loch Ness Monster. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. viii+248+16 plates. (London : Routledge and Kegan Paul, Ltd., 1961.) 25s. net.*

DONCASTER, John P. Francis Walker's Aphides. 9¾ in.×7¼ in. (25 cm.×18·5 cm.). Pp. viii+165+8 plates. (London : British Museum (Natural History), 1961.) 60s.*

EALES, N. B. The Littoral Fauna of the British Isles : a Handbook for Collectors. Third edition. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. xvi+306. (Cambridge : At the University Press, 1961.) 35s. net.*

EAMES, Arthur J. Morphology of the Angiosperms. (McGraw-Hill Publications in the Botanical Sciences.) 9 in.×6 in. (23 cm.×15·5 cm.). Pp. xiii+518. (New York : McGraw-Hill Book Company, Inc. ; London : McGraw-Hill Publishing Company, Ltd., 1961.) 104s. 6d.*

EXELL, A. W., and WILD, H. (edited by). Flora Zambesiaca : Mozambique, Federation of Rhodesia and Nyasaland, Bechuanaland Protectorate, Vol. I, Part 2. 9¼ in.×6 in. (23·5 cm.×15·5 cm.). Pp. vi+337–581. (London : Crown Agents for Oversea Governments and Administrations, 1961. Published on behalf of the Governments of Portugal, The Federation of Rhodesia and Nyasaland, and the United Kingdom.) 25s.*

FRICK, George Frederick, and STEARNS, Raymond Phineas. Mark Catesby : The Colonial Audubon. 11 in.×8½ in. (28 cm.×21·5 cm.). Pp. x+137+14 plates. (Urbana, Ill. : University of Illinois Press, 1961.) 5 dollars.*

FROST, S. W. Insect Life and Insect Natural History. Second revised edition. 8¼ in.×5½ in. (21 cm.×14 cm.). Pp. viii+526. (New York : Dover Publications, Inc. ; London : Constable and Co., Ltd., 1959.) 2.25 dollars.*

GARDNER, Eldon J. Principles of Genetics. 9¾ in.×7¼ in. (25 cm.× 18·5 cm.). Pp. vii+366. (New York and London : John Wiley and Sons, Inc., 1960.) 60s.*

GRASSÉ, Pierre P. (Directeur). Encyclopédie Francaise, Tome IV : La Vie Fondement, Maintien, Reproduction. Vol. I : Pp. x+1–328+50+ planches 1–16. Vol. 2 : Pp. iii+329–710+50+planches 17–32. 12 in.× 10¼ in. (30·5 cm.×26 cm.). (Paris : Société Nouvelle de l'Encyclopédie Francaise, Librairie Larousse, 1960.) n.p.*

HARRIS, R. J. C. (edited by). Biological Approaches to Cancer Chemotherapy : a Symposium held at Louvain, June 1960, under the auspices of Unesco and the World Health Organization. 9¼ in.×6¼ in. (23·5 cm.× 16 cm.). Pp. x+431. (London : Academic Press, Inc. (London), Ltd. ; New York : Academic Press, Inc., 1961.) 96s.*

HERALD, Earl S. Living Fishes of the World. 11 in.×8¾ in. (28 cm.× 22 cm.). Pp. 304 (300 illustrations). (London : Hamish Hamilton, Ltd., 1961.) 63s. net.*

HOFER, H., SCHULTZ, A. H., und STARCK, D. (herausgegeben von). Primatologia : Handbuch der Primatenkunde, Vol. 2, Teil 1, Lieferung 3 : Volarhaut der Hände und Füsse. 10 in.× 6¾ in. (25·5 cm.×17 cm.). Pp. iv+326. (Basel und New York : S. Karger, 1961.) 75 Swiss francs.*

McELROY, William D., and GLASS, Bentley (edited by). A Symposium on Light and Life. (Sponsored by the McCollum-Pratt Institute of the Johns Hopkins University with support from the National Science Foundation.) 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xii+924. (Baltimore, Md. : The Johns Hopkins Press ; London : Oxford University Press, 1961.) 120s. net.*

MOLISCH, Hans. Anatomie der Pflanze. Siebente, neubearbeitete Auflage von Prof. Karl Höfler. 9¾ in.×6¾ in. (25 cm.×17 cm.). Pp. xii+172. (Jena : Gustav Fischer Verlag, 1961.) 14·20 D.M.*

NILSSON, Lennart, and JAGERSTEN, Gösta. Life in the Sea. 11 in.× 8½ in. (28 cm.×21·5 cm.). Pp. 184. (London : G. T. Foulis and Co., Ltd., 1961.) 50s.*

PARSONS, Frances Theodora. How to Know the Ferns : a Guide to the Names, Haunts and Habits of our Common Ferns. Second edition. 8 in.×5¼ in. (20 cm.×13 cm.). Pp. xiv+215+42 plates. (New York : Dover Publications, Inc. ; London : Constable and Co., Ltd., 1961.) 1.25 dollars.*

PAULET, Georges. l'Intoxication Cyanhydrique et son Traitement. 9½ in.×6½ in. (24 cm.×16·5 cm.). Pp. vii+114. (Paris : Masson et Cie., 1960.) 18 N.F.*

PAZOURKOVÁ, Z., and PAZOUREK, J. Rychlé Metody Botanicke Mikrotechniky. (Quick Methods of Botanical Microtechnics.) 9½ in.× 6¾ in. (24 cm.×17 cm.). Pp. 178. (Praha : Vydala Ceskoslovenská Akademie Zemedelskych ved ve Státnim Zemedelskem Nakladatelstvi, 1960.) Cena váz vyt 16·60 Kcs.*

PIRONE, Pascal P., DODGE, Bernard O., and RICKETT, Harold W. Diseases and Pests of Ornamental Plants. Third edition. 9¼ in.×6¼ in. (23·5 cm. × 16 cm.). Pp. x+776. (London : Constable and Co., Ltd., 1960.) 84s. net.*

POPHAM, Edward J. Some Aspects of Life in Fresh Water. Second edition. (The Scholarship Series in Biology.) 7½ in.×5 in. (19 cm.× 12·5 cm.). Pp. viii+127. (London : William Heinemann, Ltd., 1961.) 12s. 6d. net.*

SMITH, Arthur, and SHEARER, Vernon. Butterflies. (Puffin Picture Book, No. 115.) 7¼ in.×8¾ in. (18·5 cm.×22 cm.). Pp. 29. (Harmondsworth, Mddx. : Penguin Books, Ltd., 1961.) 7s. 6d.*

TCHERNINE, Odette. The Snowman and Company. 8¾ in.×5½ in. (22 cm.×14 cm.). Pp. 174+8 plates. (London : Robert Hale, Ltd., 1961.) 18s. net.*

TEMBROCK, Günter. Verhaltensforschung : Eine Einführung in die Tier-Ethologie. 9½ in.×6¾ in. (24 cm.×17 cm.). Pp. vii+371. (Jena : Gustav Fischer Verlag, 1961.) 41.60 D.M.*

VERONA, O., and BENEDEK, T. Iconographia Mycologica, IV. (*Mycopathologia et Mycologia Applicata*, Vol. XIV, Supplementum.) 9½ in.×6¼ in. (24 cm.×16 cm.). Plates A85–103 ; B24–37 ; C34–50. (Den Haag : Uitgeverij Dr. W. Junk, 1961.) n.p.

Agriculture : Horticulture : Forestry

CHOWDHURY, K. A., and GHOSH, S. S. With the assistance of RAO, K. Ramesh, and PURKAYASTHA, S. K. Indian Woods : Their Identification, Properties and Uses. Vol. I : Dilleniaceae to Elaeocarpaceae. 9¾ in.× 7¼ in. (25 cm.×18·5 cm.). Pp. liii+304+30 plates. (Delhi : Manager of Publications, 1958.) Rs. 25·50 nP. ; 39s.*

GRAY, Alec. Miniature Daffodils. Revised edition. 8¾ in.×5½ in. (22 cm.×14 cm.). Pp. 59+8 plates. (London : W. H. and L. Collingridge, Ltd. ; New York : Transatlantic Arts, Inc., 1961.) 21s. net.*

METCALF, R. L. (edited by). Advances in Pest Control Research, Vol. 4. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. vi+347. (New York : Interscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1961.) 12.50 dollars.*

MEYER, H. Arthur, RECKNAGEL, Arthur B., STEVENSON, Donald D., and BARTOO, Ronald A. Forest Management. Second edition. 9¼ in.× 6¼ in. (23·5 cm.×16 cm.). Pp. v+282. (New York : The Ronald Press Company, 1961.) 8.50 dollars.*

PROCEEDINGS OF THE EIGHTH INTERNATIONAL GRASSLAND CONGRESS held at the University of Reading, England, 11–21 July 1960. 10 in.×7½ in. (25·5 cm.×19 cm.). Pp. xxii+764. (Hurley, Near Maidenhead : British Grassland Society, c/o The Grassland Research Institute, 1961.) 80s.*

RUFFIN, Edmund. An Essay on Calcareous Manures. Edited by J. Carlyle Sitterson. (The John Harvard Library.) 8½ in.×5¾ in. (21·5 cm.× 15 cm.). Pp. xxxv+199. (Cambridge, Mass. : Belknap Press of Harvard University Press ; London : Oxford University Press, 1961.) 34s. net.*

RUSSELL, Sir E. John. Soil Conditions and Plant Growth. Ninth edition by Dr. E. Walter Russell. 9½ in.×6½ in. (24 cm.×16·5 cm.). Pp. xvi+688+38 plates. (London : Longmans, Green and Co., Ltd., 1961.) 63s. net.*

SALINITY PROBLEMS IN THE ARID ZONES : Proceedings of the Teheran Symposium. (Arid Zone Research, Vol. 14.) 10½ in.×8¼ in. (26·5 cm.×21 cm.). Pp. 395. (Paris : Unesco ; London : H.M. Stationery Office, 1961.) 28 N.F. ; 40s. 9d. ; 8 dollars.*

WALKDEN, G. B. Vegetables. (Amateur Gardening Picture Book, No. 12.) 8¼ in.×6 in. (21 cm.×15·5 cm.). Pp. 72. (London : W. H. and L. Collingridge, Ltd., 1961.) 7s. 6d. net.*

Biochemistry : Physiology

BARNETT, C. H., DAVIES, D. V., and MacCONAILL, M.A. Synovial Joints : Their Structure and Mechanics. 9 in.×6 in. (23 cm.×15·5 cm.). Pp. xi+304. (London : Longmans, Green and Co., Ltd., 1961.) 50s. net.*

BRANDLY, C. A., and JUNGHERR, E. L. (edited by). Advances in Veterinary Science, Vol. 6. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xi+382. (New York : Academic Press, Inc. ; London : Academic Press, Inc. (London), Ltd., 1960.) 12 dollars.

CHARLIER, R. Coronary Vasodilators. (International Series of Monographs on Pure and Applied Biology. Division : Modern Trends in Physiological Sciences, Vol. 10.) 9 in.×6 in. (23 cm.×15·5 cm.). Pp. xi+208. (London and New York : Pergamon Press, 1961.) 55s. net.*

CUTTING, Windsor (edited by). In association with DREISBACH, Robert H., and ELLIOTT, Henry W. Annual Review of Pharmacology, Vol. 1. 9 in.×6¼ in. (23 cm.×16 cm.). Pp. vii+479. (Palo Alto, Calif. : Annual Reviews, Inc., 1961.) 7 dollars.*

EDDS, Jr., M. V. (edited by). Macromolecular Complexes. (Sixth Annual Symposium publication of the Society of General Physiologists.) 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. vi+257. (New York : The Ronald Press Company, 1961.) 7 dollars.*

GARDNER, Lytt I. (edited by). Molecular Genetics and Human Disease. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xviii+297. (Springfield, Ill. : Charles C. Thomas ; Oxford : Blackwell Scientific Publications, 1961.) 92s.*

GSELL, O., et MAYER, R. L. (redactores). Antibiotica et Chemotherapia : Fortschritte—Advances—Progrès, Vol. 9. 9¾ in.×6¾ in. (25 cm.×17 cm.). Pp. iv+192. (Basel and New York : S. Karger, 1961.) 40 Swiss francs.*

GUNSALUS, I. C., and STANIER, Roger Y. (edited by). The Bacteria : a Treatise on Structure and Function. Vol. 2 : Metabolism. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xv+572. (New York : Academic Press, Inc. ; London : Academic Press, Inc. (London), Ltd., 1961.) 15 dollars.*

HALL, Victor E. (edited by). In association with FUHRMAN, Frederick A., and GIESE, Arthur C. Annual Review of Physiology, Vol. 23. 9 in.×6¼ in. (23 cm.×16 cm.). Pp. v+674. (Palo Alto, Calif. : Annual Reviews Inc. ; and the American Physiological Society, 1961. On sale by Annual Reviews, Inc.). 7 dollars.*

HARRIS, R. J. C. (edited by). Protein Biosynthesis : a Symposium held at Wassenaar, 29 August–2 September, 1960, under the auspices of Unesco and the Council for International Organizations of Medical Sciences. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xii+409. (London : Academic Press, Inc. (London), Ltd. ; New York : Academic Press, Inc., 1961.) 95s.*

JESSOP, William John Edward (edited by). Fearon's Introduction to Biochemistry. Fourth edition. 8¾ in.×6¼ in. (22 cm.×16 cm.). Pp. vi+473. (London : William Heinemann Medical Books, Ltd., 1961.) 30s. net.*

KLEMPARSKAYA, N. N., ALEKSEYEVA, O. G., PETROV, R. V., and SOSOVA, V. F. Problems of Infection, Immunity and Allergy in Acute Radiation Diseases. Translated from the Russian by Lydia Venters. Translation edited by R. Clarke. 10 in.×6¼ in. (25·5 cm.×16 cm.). Pp. viii+165. (London and New York : Pergamon Press, 1961.) 50s.*

KON, S. K., and COWIE, A. T. (edited by). Milk : The Mammary Gland and Its Secretion. Vol. 1 : Pp. xiii+515. 14 dollars. Vol. 2 : Pp. ix+423. 12 dollars. 9¼ in.×6¼ in. (23·5 cm.+16 cm.). (New York : Academic Press, Inc. ; London : Academic Press, Inc. (London), Ltd., 1961.)*

LARSON, P. S., HAAG, H. B., and SILVETTE, H. Tobacco : Experimental and Clinical Studies—a Comprehensive Account of the World Literature. 11¼ in.×8¾ in. (28·5 cm.×22 cm.). Pp. xii+932. (Baltimore, Md. : The Williams and Wilkins Company ; London : Baillière, Tindall and Cox, Ltd., 1961.) 160s.*

LAWRENCE, J. S. Assessment of the Activity of Disease. 10 in.×6¼ in. (25·5 cm.×16 cm.). Pp. viii+252. (London : H. K. Lewis and Co., Ltd., 1961.) 42s. net.

MÜNTZING, Arne. Genetic Research : a Survey of Methods and Main Results. 9¾ in.×7 in. (25 cm.×18 cm.). Pp. 345. (Stockholm : LTs Forlag, 1961.) n.p.*

MUSSEN, Paul H. (edited by). Handbook of Research Methods in Child Development. 9¼ in.×6¾ in. (23·5 cm.×17 cm.). Pp. x+1061. (New York and London: John Wiley and Sons, Inc., 1960.) 122s.*

PEETERS, H. (edited by). Protides of the Biological Fluids : Proceedings of the Eighth Colloquium, Bruges, 1960. 9½ in.×6¾ in. (24 cm.×17 cm.). Pp. x+356. (Amsterdam : Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1961.) 86s.*

RYTAND, David A. (edited by). In association with CREGER, William P. Annual Review of Medicine, Vol. 12. 9 in.×6 in. (23 cm.×15·5 cm.). Pp. vii+455. (Palo Alto, Calif. : Annual Reviews, Inc., 1961.) 7 dollars*.

SCHWARZACHER, H. G. (edited by). Histochemistry of Cholinesterase: Symposium, Basel, 1960. (Biblioteca Anatomica, Fasc. 2.) 9¾ in.×7 in. (25 cm.×18 cm.). Pp. vi+255. (Basel and New York : S. Karger, 1961.) 60 Swiss francs.*

SEELIGER, H. P. R. Listeriosis. 9½ in.×7 in. (24 cm.×18 cm.). Pp. xii+308. (Basel and New York : S. Karger, 1961.) 55 Swiss francs.*

SHERRINGTON, Sir Charles. The Integrative Action of the Nervous System. Second edition. (Yale paperbound No. Y-35.) 8 in.×5¼ in. (20 cm.×13 cm.). Pp. xx+413. (New Haven, Conn. : Yale University Press ; London : Oxford University Press, 1961.) 1.95 dollars ; 16s. net.*

STAROSTENKO, N. T. New Clinical and Experimental Data on Procaine. Authorized translation from the Russian. 10¾ in.×8¼ in. (27 cm.×21 cm.). Pp. iii+62. (New York : Consultants Bureau, Inc., 1961.) 12.50 dollars.*

STEWART, C. P., and STRENGERS, Th. (edited by). Symposium on Water and Electrolyte Metabolism, Amsterdam, 1960. 9½ in.×6¾ in. (24 cm.×17 cm.). Pp. v+202. (Amsterdam : Elsevier Publishing Company ; London : D. Van Nostrand Company, Ltd., 1961.) 38s.*

TEEVAN, Richard C., and BIRNEY, Robert C. (edited by). Color Vision : an Enduring Problem in Psychology—Selected Readings. (Insight Book, No. 4.) 7 in.×4½ in. (18 cm.×11 cm.). Pp. viii+214. (Princeton, N.J. : D. Van Nostrand Company, Inc. ; London : D. Van Nostrand Company, Ltd., 1961.) 1.46 dollars ; 11s. 6d.*

WALKER, W. (edited by). Thrombosis and Anticoagulant Therapy : Proceedings of a Symposium held in Queen's College, Dundee, 29 and 30 September, and 1 October 1960. 9½ in.×7¼ in. (24 cm.×18·5 cm.). Pp. 106. (St. Andrews : The University. Distributed by E. and S. Livingstone, Ltd.) 17s. 6d. net.*

Anthropology : Archaeology

AITKEN, M. J. Physics and Archaeology. 9 in.×6 in. (23 cm.×15·5 cm.). Pp. x+181 (28 plates). (New York : Insterscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1961.) 6 dollars ; 43s.*

CLARK, Grahame. World Prehistory : an Outline. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. xv+284+12 plates. (Cambridge : At the University Press, 1961.) 30s. net.*

GARN, Stanley M. Human Races. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xiv+137. (Springfield, Ill. : Charles C. Thomas ; Oxford : Blackwell Scientific Publications, 1961.) 44s.*

GRIMM, Hans. Einführung in die Anthropologie. 9½ in.×6¾ in. (24 cm.×17 cm.). Pp. xii+107. (Jena : Gustav Fischer Verlag, 1961.) 10.65 D.M.*

LÉVI-STRAUSS, Claude. A World on the Wane. Translated from the French by John Russell. 9¼ in.×6 in. (23·5 cm.×15·5 cm.). Pp. 404+63 plates. (London : Hutchinson and Co. (Publishers), Ltd., 1961.) 42s. net.*

Philosophy : Psychology

BIDERMAN, Albert D., and ZIMMER, Herbert (edited by). The Manipulation of Human Behaviour. 9 in.×6 in. (23 cm.×15·5 cm.). Pp. xii+323. (New York and London : John Wiley and Sons, Inc., 1961.) 64s.*

DAY, John Patrick. Inductive Probability. (International Library of Philosophy and Scientific Method.) 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. xvi+336. (London : Routledge and Kegan Paul, Ltd., 1961.) 40s. net.*

DOLLARD, John, DOOB, Leonard W., MILLER, Neal E., MOWRER, O. H., and SEARS, Robert R. Frustration and Aggression. (Yale paperbound No. Y-34.) 8 in.×5¼ in. (20 cm.×13 cm.). Pp. vii+209. (New Haven, Conn. : Yale University Press ; London : Oxford University Press, 1961.) 1.25 dollars ; 10s. net.*

DUIJKER, H. C. J., FRAISSE, P., MEILI, R., OLÉRON, P., et PAILLARD, J. Les Attitudes : Symposium de l'Association de Psychologie Scientifique de Langue Française, Bordeaux 1959. (Bibliothèque Scientifique Internationale. Sciences Humaines—Section Psychologie.) 9 in.×5½ in. (23 cm.×15 cm.). Pp. iii+191. (Paris : Presses Universitaires de France, 1961.) 10 N.F.*

HAMLYN, D. W. Sensation and Perception : a History of the Philosophy of Perception. (International Library of Philosophy and Scientific Method.) 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. xi+210. (London : Routledge and Kegan Paul, Ltd. ; New York : The Humanities Press, 1961.) 25s. net.*

LIFTON, Robert J. Thought Reform and the Psychology of Totalism : a Study of "Brainwashing" in China. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. x+510. (London : Victor Gollancz, Ltd., 1961.) 30s. net.*

LURIA, A. R. The Role of Speech in the Regulation of Normal and Abnormal Behaviour. Edited by Dr. J. Tizard. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. ix+100. (London and New York : Pergamon Press, 1961.) 50s. net.*

MARGENAU, Henry. Open Vistas : Philosophical Perspectives of Modern Sciences. 8¾ in.×5½ in. (22 cm.×14 cm.). Pp. x+256. (New Haven, Conn. : Yale University Press ; London : Oxford University Press, 1961.) 40s. net.*

MILLER, Benjamin F., and GOODE, Ruth. Man and His Body : The Wonders of the Human Mechanism. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. vi+375. (London : Victor Gollancz, Ltd., 1961.) 21s. net.*

PIAGET, Jean. Les Mécanismes Perceptifs : Modéles Probabilistes, Analyse Génetique, Relations avec l'Intelligence. (Bibliothèque Scientifique Internationale. Sciences Humaines—Section Psychologie.) 9 in.×5½ in. (23 cm.×15 cm.). Pp. ii+457. (Paris : Presses Universitaires de France, 1961.) 28 N.F.*

POWELL, Robert. Zen and Reality : an Approach to Sanity and Happiness on a Non-Sectarian Basis. 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. 140. (London : George Allen and Unwin, Ltd., 1961.) 21s. net.*

RUSSELL, Claire, and RUSSELL, W. M. S. Human Behaviour : a New Approach. 8¼ in.×5¾ in. (21 cm.×15 cm.). Pp. ix+532. (London : André Deutsch, Ltd., 1961.) 42s. net.*

SCHACHTER, Stanley. The Psychology of Affiliation : Experimental Studies of the Sources of Gregariousness. (Stanford Studies in Psychology.) 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. ix+141. (London : Tavistock Publications (1959), Ltd., 1961.) 30s. net.*

SHIPLEY, Thorne (edited by). Classics in Psychology. 9¼ in.×6¼ in. (23·5 cm.×16 cm.). Pp. xx+1342. (New York : Philosophical Library, 1961.) 20 dollars.*

SOLOMON, Philip, KUBZANSKY, Philip E., LEIDERMAN, P. Herbert, MENDELSON, Jack H., TRUMBULL, Richard, and WEXLER, Donald (edited by). Sensory Deprivation : a Symposium held at Harvard Medical School. 8½ in.×5½ in. (21·5 cm.×14 cm.). Pp. xviii+262. (Cambridge, Mass.: Harvard University Press ; London : Oxford University Press, 1961.) 46s. net.*

Miscellany

BALLS, Ronnie. Fish Capture : Being the Buckland Lectures for 1959. 7¼ in.×4¾ in. (18·5 cm.×12 cm.). Pp. 144+2 plates. (London : Edward Arnold (Publishers), Ltd., 1961.) 7s. 6d. net.*

BURGESS, Ernest W. (edited by). Aging in Western Societies. 9½ in.×6¾ in. (24 cm.×17 cm.). Pp. xvi+492. (Chicago : University of Chicago Press ; London : Cambridge University Press, 1960.) 60s. net.*

GOLDSMITH, Maurice. The Young Scientist's Companion : a Book of Information and Advice to all concerned with Science. 8¾ in.×5½ in. (22 cm.×14 cm.). Pp. 160+4 plates. (London : Souvenir Press, Ltd., 1961.) 15s. net.*

MARTINDALE, Don. The Nature and Types of Sociological Theory. (International Library of Sociology and Social Reconstruction.) 8¾ in.×5¾ in. (22 cm.×15 cm.). Pp. xiv+558. (London : Routledge and Kegan Paul, Ltd., 1961.) 35s. net.*

ROUCEK, Joseph S. (edited by). Sociology of Crime. 8½ in.×5¾ in. (21·5 cm.×15 cm.). Pp. vii+551. (New York : Philosophical Library, 1961.) 10 dollars.*

SMITH, A. H. The Place-Names of the West Riding of Yorkshire. Part 1 : Lower and Upper Strafforth and Staincross Wapentakes. Pp. xi+346. Part 2 : Osgoldscross and Agbrigg Wapentakes. Pp. xi+321. Part 3 : Morley Wapentake. Pp. xiii+278. 8¾ in.×5¾ in. (22 cm.×15 cm.). (Cambridge : At the University Press, 1961.) 35s. net each volume.*

Recent Pergamon Books in Chemistry & Astronomy

Printing Inks and Color Vol. I of Advances in Printing Science and Technology

Edited by W. H. BANKS, *PATRA, Leatherhead, England*

Under the Chairmanship of Professor A. C. Zettlemoyer, fifty-eight delegates attended the Fifth International Conference of Printing Research Institutes at Lehigh University, Bethlehem, Penn., U.S.A. Among the papers discussed are the influence of ink, paper and press on colour printing; some aspects of the chemistry and physics of lithographic printing; colour control and instrumentation for colour measurement; control of ink film thickness on the press; and studies of ink transfer in relation to the ink.

354 *pages* *illustrated* 80*s. net* ($12.50)

Physics and Chemistry of the Earth Vol. III

Editors : L. H. AHRENS, FRANK PRESS, KALERVO RANKAMA and S. K. RUNCORN

Each contributor to this valuable work of reference cites many sources and provides a good bibliography, and the papers include the origin of the elements (discussing types of nuclear reactions which may become involved during the formation of the elements), the distribution of the elements, silicate melt systems, and many other important topics.

464 *pages* £5 *net* ($15.00)

Gases in Light Metals

L. I. SOKOL'SKAYA

Firstly this book clarifies the problems of the reaction of gases with light metals and the methods for the determination of gases in aluminium and magnesium. The mechanism of the formation of defects due to gases in castings, and the influence of gases on the properties of castings are then examined and lastly the origins of the gases and the methods of preventing the formation of gaseous defects are indicated.

150 *pages* 60*s. net* ($9.50)

The Atlas of the Other Side of the Moon

Edited by N. P. BARABASHOV, A. A. MIKHAILOV, YU N. LIPSKII,
Academy of Sciences of the U.S.S.R.

When the Soviet Union placed the automatic interplanetary station Lunik III round the moon in 1959 the lunar surface invisible from the earth was photographed and pictures transmitted to the earth by radio. This volume is an exact and comprehensive translation of the Russian Atlas of the Far Side of the Moon and contains the best photographs of the moon's hidden surface, a catalogue of the formation thereon, a definitive map and a complete account of the methods used in studying the photographs.

141 *pages* 50*s. net* ($7.00)

Structure of the Moon's Surface

GILBERT FIELDER, *Fellow of the Royal Astronomical Society*

The aim of this book is to produce a largely unprejudiced reference text which brings lunar studies up-to-date. Cross-references may be found rapidly by the use of the List of Contents, the Sectional Numbering at the head of every page, and the Index. A small Chart, for identifying features discussed in the text, has been provided at the end of the book, which is abundantly illustrated with diagrams and plates of the Moon taken by the author at the Pic-du-Midi Observatory.

272 *pages* *numerous illustrations* 50*s. net* ($7.50)

Telomerization and New Synthetic Materials

R. KH. FREIDLINA and SH. A. KARAPETYAN, *Academy of Sciences, U.S.S.R.*

This is the first book devoted to an exposition of telomerization reactions. The result of intensive Russian research, this reaction makes it possible to obtain the intermediate products for the production of new synthetic fibres, plastics, perfumes and other industrial products from cheap starting materials—for example, from natural and industrial gases. A detailed description is given of the formation and properties of the new synthetic fibre 'enanth' which possesses more valuable properties than caprone and nylon.

112 *pages* 25*s. net* ($4.50)

Introduction to Petroleum Chemicals

Edited by H. STEINER

Contains a series of lectures on the petroleum industry given at the Manchester College of Science and Technology by British chemists and chemical engineers. This valuable work makes them available to a wider public and as a guide to further study there are extensive literature references.

208 *pages* *numerous illustrations* 50*s. net* ($8.00)

See also Page ccccxcix

Parasitology of Fishes

Edited by
V. A. DOGIEL, G. K. PETRUSHEVSKI and YU I. POLYANSKI

Translated by **Z. KABATA**

This book represents the first attempt at a comprehensive summing up of the problems of fish parasitology, both marine and fresh-water. It is based upon the many years of research by Professor Dogiel and his famous school of fish parasitology in the U.S.S.R., a country which offers within its frontiers an almost full range of the habitats existing on the earth.

It includes discussions on the important problems of parasitology: dependence of the fish parasite fauna on the internal and external environment, host-parasite relationship, specificity, physiology, and the life cycles of the fish parasites. It also contains the first-ever discussion of zoogeography of the fish parasite fauna.

The outstanding value of the book lies in the fact that it presents the principles of an entirely new approach to parasitological problems. **Price 84s. net**

O & B **OLIVER & BOYD**

Tweeddale Court, 14 High Street,
Edinburgh 1.

WHELDON & WESLEY, LTD.

Specialists for 100 years in

BIOLOGICAL & GEOLOGICAL BOOKS

Catalogues on Request

●

LYTTON LODGE, CODICOTE, Near Hitchin, Herts

Books Bought

BACK ISSUES OF JOURNALS IN
SCIENCE & THE HUMANITIES
ALWAYS REQUIRED BY:
WM. DAWSON & SONS, LTD.,
BACK ISSUES DEPT.,
16 WEST STREET,
FARNHAM, SURREY, ENGLAND.
TEL: FARNHAM 4664 CABLES: DAWBOOKS, FARNHAM

DILLON'S UNIVERSITY BOOKSHOP Ltd

All current scientific and technical textbooks available from stock.

1 MALET ST., LONDON, W.C.1. MUSeum 2128

To be published Autumn 1961

A comprehensive text for students and designers of optical instruments

Instrumental Optics

translated from
G. A. Boutry's Optique Instrumentale by R. Auerbach, B.Sc.

"Professor Boutry's book is of a special nature. Writing mainly for prospective instrument designers, the author describes the practical difficulties which they will have to overcome, and does not present a purely theoretical treatment as found in many instructional works. He first discusses the fundamental principles of geometrical optics, next shows what performance can be expected from a given system, and indicates the boundaries beyond which designers cannot hope to pass because of inherent imperfections of instruments and physiological characteristics of human vision. The physicist will find valuable guidance in the pages of this book to help him arrive at a correct interpretation of observations made with an instrument of a given specification."

From Armand de Gramont's Preface

Crown quarto, 560 pages, approx. 400 diagrams

Price £8 8s. (*postage extra*)

• HILGER & WATTS LTD., 98 ST. PANCRAS WAY, LONDON, NW1

Recent books in Aeronautics and Mathematics

Tables of Elementary Functions

M. WARMUS, *Nuclear Research Institute, Warsaw Academy of Sciences*

These tables comprise all the important elementary functions. Their characteristic feature is that the estimates of the errors of the values and of the approximate values obtained by linear interpolation are given on every page. Trigonometric functions are given both for degrees and for radians, and the need for conversion from degrees to radians is therefore eliminated. Tables are always given for functions, and their inverses, so that inverse interpolation is unnecessary.

565 *pages* £5 5*s. net* ($15.00)

Infinitistic Methods –

Proceedings of the Symposium on Foundations of Mathematics, Warsaw

At the Symposium were discussed works which use mathematical tools, commonly thought of as non-constructive, for example—inaccessible cardinals, infinitistic rules of proof, higher classes of number-theoretic and function-theoretic hierarchies of predicates, etc. All mathematicians will welcome this valuable work.

362 *pages* £5 *net* ($15.00)

High Altitude Aircraft Equipment

L. T. BYKOV, M. S. YEGOROV, P. V. TARASOV

This authoritative volume is invaluable as a comprehensive manual of basic instruction and information for designers, technologists, and technicians, and is intended as a textbook for higher technological institutes. The book is completely up-to-date for a general textbook, and it covers the developments common to Russia and the other industrialized countries.

445 *pages* *numerous illustrations* £5 *net* ($15.00)

Bio-Assay Techniques for Human Centrifuges and Physiological Effects of Acceleration

Edited by P. BERGERET, *Chairman of the Editorial Committee of the Aero-Space Medical Panel of AGARD*

This volume contains the 15 original contributions presented at the recent meetings of the AGARD Aerospace Medical Panel, including The Human Centrifuge and its Application to Pilot Selection—S. RUFF; A Review of the Physiological Effects of Angular Accelerations—W. H. JOHNSON and N. B. G. TAYLOR; Human Tolerance and Limitations to Acceleration—R. N. HEADLEY; Instrumentation and the Human Centrifuge—G. H. BYFORD; and Centrifuge Methods and Techniques in the U.S. Navy—F. K. SMITH.

173 *pages* *illustrated* 70*s. net* ($10.00)

Computing Methods and the Phase Problem in X-Ray Crystal Analysis

Vol. IV of International Tracts in Computer Science and Technology and their Application

Edited by RAY PEPINSKY, J. M. ROBERTSON and J. C. SPEAKMAN

This volume contains the proceedings of the University of Glasgow Symposium, edited by three leading authorities in this field. New and important contributions to two of the most important and fundamental aspects of X-ray crystal analysis—the phase problem, and computational methods—are discussed. Many of the papers are devoted to the programming of electronic digital computers to this task, and to the devising of better and more rapid refinement techniques.

326 *pages* 63*s. net* ($9.00)

Mathematical Methods for Engineers and Technologists

P. I. ROMANOVSKII

Translation Editor **T. KÖVARY,** *Department of Mathematics, Imperial College, London*

This invaluable book is intended for students of technical colleges and other higher technical education institutes who require a text-book for more advanced courses of mathematics on the following subjects: Fourier series and the Fourier integral; Analytic Field Theory; The Theory of Analytic Functions; Some Special Functions; Operational Calculus; and The Laplace Transforms. The volume is distinguished by the very high level of mathematical rigour, seldom found in this type of book. It fills a definite need and will be welcomed by all who require an intelligently written advanced course in mathematics.

267 *pages* 50*s. net* ($8.50)

All books gladly sent on 14 days' approval

PERGAMON PRESS

OXFORD LONDON NEW YORK PARIS

Headington Hill Hall, Oxford. 4 *&* 5 *Fitzroy Sq., London, W.*1. 122 *East* 55*th St., New York* 22, *N.Y.*

Lock PORTABLE

CONDUCTIVITY BRIDGE TYPE BC 1

This mains-operated instrument has a bridge frequency of 1500 c/s, and covers the very wide range of 0·05 to 1 × 10^6 micro mhos in seven ranges.
A portable fully transistorised bridge is also available. It covers the range of 0·5 to 1 × 10^6 micro mhos in six ranges.

Lock

NUCLEAR
CHEMICAL
ELECTRONIC

INSTRUMENTATION

A. M. LOCK & CO. LTD.
Prudential Buildings. 79 Union Street, Oldham, Lancs.
Telephone : MAIn 0333

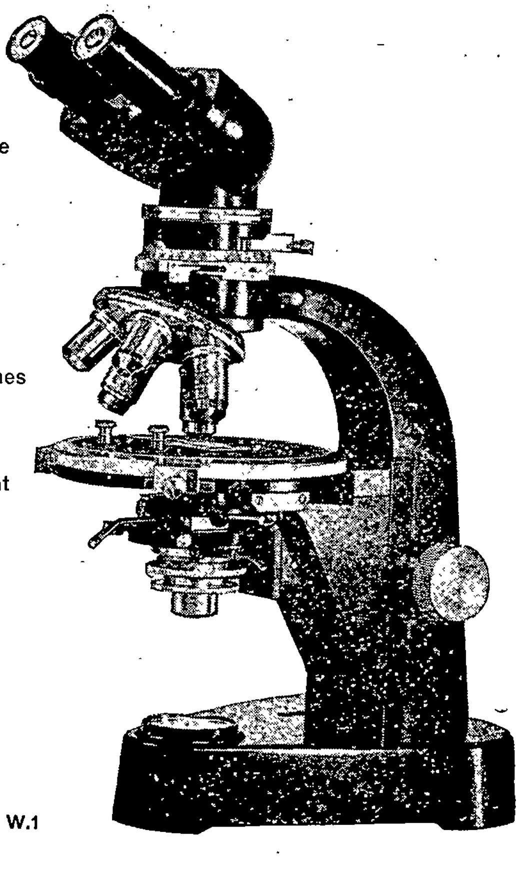
C.W. 7751

Leitz Polarizing Microscopes

for examinations in transmitted and incident light are of advanced design. The large research microscope DIALUX-Pol offers built-in illumination and unrestricted use of all accessories for the most specialised types of mineralogical work, including binocular conoscopic observations. Large precision rotating stage with 45° automatic arresting device. Supplementary equipment for the synchronous rotation of analyser and polarizer.

Also in the Leitz production range

Monocular and binocular microscopes for all branches of science and technology. LEITZ phase contrast equipment. Stereoscopic microscopes. Photomicrographic apparatus. Base sledge, rotary and freezing microtomes. Micro refractometers. Spectroscopes. LEICA 35 mm camera with supplementary equipment for scientific and technological photographic work. Heating Stages.



a microscope by Leitz a product of thoughtfulness and skill

E. LEITZ (INSTRUMENTS) LTD., 30 Mortimer St., London, W.1

THE WORLD METEOROLOGICAL ORGANIZATION

announces

PUBLICATIONS OF THE IGY METEOROLOGICAL DATA CENTRE

Meteorological Data of the International Geophysical Year (1957/1958) and International Geophysical Cooperation (1959)

Meteorological observations made during the IGY at some 4000 stations were entered on standard forms and sent to the IGY Meteorological Data Centre in the WMO Secretariat. Similar arrangements were made for some of the IGC–1959 observations. This unique collection of nearly nine million observations from all parts of the world constitutes an unbroken and comprehensive record of meteorological data for the 30 months July 1957 to December 1959. The IGY Meteorological Data Centre has been publishing these data in such a way that the various types of observations can be obtained separately for either the IGY or IGC periods or for both together. The original forms are reproduced by photographic methods on Microcards or in loose-leaf publications. With each of these publications a catalogue is supplied with information about the observing stations, their geographical coordinates, elevation and observation programmes.

MICROCARDS OF IGY METEOROLOGICAL DATA

This publication contains the surface and upper-air observations made at 3000 synoptic stations during the IGY. The original forms (about one million) on which the data were entered by the 110 participating countries are reproduced on Microcards of which over 15500 (about 92 per cent of the estimated total) are ready for distribution. This collection of meteorological data from the whole world will be invaluable for many kinds of meteorological and allied research.

The publication consists of four parts :

Part I :	Surface observations—Land Stations	2750 Microcards
Part II :	Surface observations—Sea Stations	2196 Microcards
Part III :	Radiosonde/rawinsonde observations	6317 Microcards
Part IV :	Upper-wind observations	about 5000 Microcards

Subscriptions to complete sets of these data are accepted at the price of US $5,990.00, post paid.

Partial sets may also be ordered for specified regions, periods or types of observations at the price of 50 cents per card of Part II, 40 cents per card of Parts I, III and IV.

MICROCARDS OF IGC (1959) AEROLOGICAL DATA

On 4000 Microcards radiosonde and rawinsonde observations made at over 550 stations are reproduced in a manner similar to that in which the corresponding observations were published in Part III of the IGY Microcards.

This record of the 1959 upper-air observations can be obtained in complete and partial sets.

The subscription price for one complete set is US $1,500.00, post paid. Partial sets of data for specified regions or periods may also be ordered at the price of 50 cents per Microcard.

IGY RADIATION DATA

The collection of over 13000 original forms on which measurements of the various components of the radiation balance were entered is now being published partly in photo-offset and partly on Microcards. This detailed record of radiation measurements made during the IGY at some 600 stations will be of great value to research workers interested in studies of the earth's radiation balance and for all those who are dealing with the application of radiation data to many practical problems in meteorology, agriculture, engineering and other fields.

Subscriptions to complete sets at US $200.00 per set.

Prices of partial sets as set out below :

IGC RADIATION DATA

Radiation measurements carried out in 1959 at some 600 stations will be issued by reproducing the original forms partly in photo-offset and partly on Microcards.

Subscriptions to complete sets of the IGC radiation data can be placed at the price of US $185.00 per set.

Prices of partial sets as set out below :

IGY Prices			*IGC Prices*
US $ 100.00	Part R–1 :	Daily total of radiation components on a horizontal surface and of duration of bright sunshine	US $ 80.00
US $ 9.00	Part R–2a :	Total radiation (sun+sky) on a horizontal surface : Monthly sums for each hour	US $ 9.00
US $ 3.00	Part R–2b :	Diffuse (sky) radiation on a horizontal surface : Monthly sums for each hour	US $ 3.00
US $ 52.00	Part R–3 :	Components of the radiation balance I : Daily sums for each hour	US $ 50.00
US $ 6.00	Part R–4 :	Components of the radiation balance II : Monthly sums for each hour	US $ 5.00
US $ 48.00	Part R–5 :	Instantaneous values of the intensity of direct solar radiation	US $ 45.00

IGY OZONE DATA

The complete record of atmospheric ozone measurements made during the IGY at 57 stations is now available in two loose-leaf publications, which contain the photo-offset reproduction of the original forms collected at the IGY MDC

IGC OZONE DATA

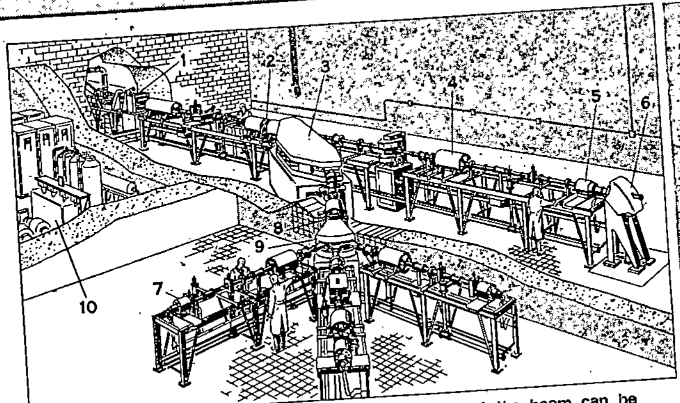
Measurements of atmospheric ozone carried out in 1959 will be issued in two loose-leaf publications containing photo-offset reproductions of the original forms.

IGY Prices			*IGC Prices*
US $ 36.00	Part 0–1 :	Total amount of ozone	US $ 24.00
US $ 3.00	Part 0–2 :	Umkehr observations on clear zenith sky	US $ 2.00

Inquiries should be addressed to : The Secretary General, WORLD METEOROLOGICAL ORGANIZATION,
IGY Meteorological Data Centre, Geneva, Switzerland

High Voltage Engineering Corporation...

"CHARGED PARTICLES"



Typical high power electron beam analyzing and deflecting system for 10 to 80-Mev, 30 kw linac

1. Linac
2. Positron Target
3. Deflecting Magnet System and Energy-Defining Slits
4. Focusing Lens
5. Neutron Target
6. Terminating Magnet
7. Targets (3)
8. Monoenergetic Gamma Target
9. Switching Magnet
10. Equipment Room

The main features of this analyzing system are: (a) the beam can be delivered to any of three different experimental areas, focused to a small spot or spread out over a large area to facilitate target cooling; (b) net energy dispersion of the beam after deflection is negligible. This means that maximum beam of the desired energy is available because particles of varying energies move along the same center line into target regions; (c) in the deflecting magnet system there is a large energy dispersion at a collimator, which selects the required energy spectrum with a precision of 0.1 per cent or better; (d) the part of the electron beam discarded at this collimator or elsewhere in the system produces x-rays, the major portion of which are directed away from experimental areas; (e) the system can be converted to handle positrons produced in a shower target close to the entrance of the deflecting magnet system.

High Power Linacs

In a recent discussion of *High Energy Linacs,* we indicated that the power of the new accelerators, plus better energy resolution opens up intriguing possibilities for the experimental physicist. One of these possibilities involves production of a monoenergetic gamma-ray beam, with adjustable energy, heretofore just a dream.

Positron annihilation in flight in a thin target produces such a gamma-ray beam. The linac electron beam is used to produce positrons in a wide energy spectrum in a shower target. These particles are energy-analyzed in a deflecting magnet before they impinge upon the annihilation target. Energy selection of the gamma rays is accomplished by varying the magnetic field of the deflecting magnet. The conversion factor is small, so it is essential to have an intense electron beam. It is not essential, however, to have a high degree of beam-energy homogeneity.

An experiment in which beam-energy homogeneity is important is the spectroscopic study of inelastically-scattered electrons from nuclei. Magnetic spectrometers for this kind of experiment can be built with a resolution of better than 0.1 per cent, and the electron beam must be analyzed to this accuracy before it impinges upon the scattering target.

Hot Electron Beams

The high accuracy and power called for in these experiments requires considerable beam-handling ingenuity. The problems may be illustrated: A typical high-energy linac will put 45 kw of high-energy electrons on a one-half centimeter square — this equals 180 kw/cm², enough to vaporize any known material in a few seconds. To make such a system fail-safe requires precisely-protected beam slits and electron scattering devices, and specially cooled vacuum chambers, in addition to the analyzing and collimating equipment. The situation is complicated by an x-ray background which prohibits use of organic materials for coil insulation and vacuum gasketing.

The beam analyzing and deflecting system is an increasingly complex and important part of the linac experimental facility. An inadequate system will greatly reduce the accelerator's research utility. Fortunately, High Voltage Engineering has had years of experience designing and building extremely accurate, magnetic analyzing systems to guaranteed specifications.

HIGH VOLTAGE ENGINEERING CORPORATION
BURLINGTON, MASSACHUSETTS, U.S.A.
●
APPLIED RADIATION CORPORATION
○
HIGH VOLTAGE ENGINEERING (EUROPA) N.V.

HIGH VOLTAGE SERVICING CO., LTD.
60 Carpenters Wood Dr.
Chorley Wood, Herts.

Table 4. AMINO-ACIDS RELEASED FROM BULL SPERM DNA*

Aspartic acid	Threonine	Serine	Glutamic acid	Alanine	Valine	Methionine	*Iso*leucine	Leucine	Lysine	Histidine
11·2	8·2	26·0	13·2	6·1	12·4	3·7	2·0	4·4	9·9	2·7

* Determined on a 27 mgm. sample in an automatic amino-acid analyser (ref. 33) after hydrolysis by refluxing with 6 *N* HCl for 24 hr. The glycine was not determined since purines break down under these conditions to give appreciable amounts of glycine.

The results are expressed as moles of amino-acids/100 moles of all amino-acids actually found. Phenylalanine and tyrosine were present as traces. Neither cysteine nor cystine was detected. Together, these amino-acids amount to about 0·1 per cent of the mass of the DNA sample. We are grateful to Dr. M. E. Balis for carrying out the analysis.

thymine value following perchloric acid hydrolysis and the low adenine after hydrochloric acid hydrolysis are consistent with the experience of others who have reported losses of these bases by these procedures[26,29]. The thymine to cytosine ratio (T/C) previously reported[30] for human sperm DNA is considerably higher than the present values (Table 2). The ratios of purines to pyrimidines (Pur/Pyr) are close to 1·0 as found for other mammalian tissues[1].

The DNA from bull spermatozoa was fractionated on a column of the anion exchanger 'Ecteola' using an elution system which yielded DNA in 5 chromatographic regions[31]. The sedimentation coefficients of the DNA in the peak tubes in each region were determined. The results, given in Table 3, show that bull sperm DNA, like that of calf thymus[32], consists of fractions which vary in molecular size and which can be separated on an 'Ecteola' column.

The requirement of the reducing agent used in the isolation of DNA from mammalian sperm suggests the presence of a special sulphur-containing linkage between the DNA (or a component attached thereto) in the sperm nucleus and the protein coat cleavage of which is necessary before the nucleic acid can be released. It is less likely that the protein coat consists of a highly cross-linked, rigid, disulphide-containing protein which merely engulfs the DNA, since release of the DNA should attend the disintegration of the coat, which is brought about by grinding, homogenization, etc.

Accordingly, the DNA isolated from bull sperm was hydrolysed with 6 *N* hydrochloric acid and examined for the presence of amino-acids[33]. The results (Table 4) indicate the presence of protein (or amino-acid) to the extent of about 0·1 per cent, with a remarkably high serine content. The relatively low content of basic amino-acids, the presence of many other amino-acids, and the absence of cystine have also been noted in highly purified DNA from human white cells, spleen and placenta[34]. It is noteworthy that the only sulphur-containing amino-acid detected was methionine, and this was present in small amounts (3·7 per cent). It is possible that sulphur-containing amino-acids may have been removed by the tryptic digestion step. The significance of the amino-acid content is being investigated since this might hold a clue to an understanding of the action of β-mercaptoethanol, which is needed for eventual release of the DNA. This pre-treatment is not required for release of DNA from the sperm of lower forms such as fish, invertebrates and birds, and such differences may therefore be a reflexion of evolutionary development.

These results with sperm DNA are of particular interest since the source material is not only devoid of RNA but consists, presumably, of a homogeneous cell population in contrast to the usual heterogeneous cell-types found in tissues. In addition, sperm is the only reasonably available mammalian haploid cell. Yet, obvious differences with the DNA of diploid tissues are not revealed by the properties given here.

The amino-acids (or protein) which are present in these as well as in so many other preparations described in the literature and which resist rigorous efforts to remove them may be co-valently linked to the DNA. Although these are present to only a small extent, they may play an influential part in the transmission and determination of heritable characteristics and in differentiation. Hence, a critical examination of the amino-acid composition of the DNA and a comparison of preparations from different tissues and sources may reveal an unsuspected basis of the biological activity of these polymers.

We thank Miss I. A. Wright for carrying out the ultracentrifuge runs. This investigation was supported by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (grant No. *CY*-3190), the Atomic Energy Commission (contract No. *AT* [30-1], 910), and the Health Research Council of the City of New York (Contract No. *U*-1096 II *A*).

[1] Chargaff, E., in *The Nucleic Acids*, **1**, 307 (E. Chargaff and J. N. Davidson, eds. Acad. Press, New York, 1955).

[2] Mayer, D. T., *Reproduction and Infertility*, 45 (Symposium, Michigan State University, 1955).

[3] Berry, R. E., and Mayer, D. T., *Exp. Cell Res.*, **18**, 398 (1959).

[4] Zamenhof, S., Shettles, L. B., and Chargaff, E., *Nature*, **165**, 756 (1950).

[5] Green, W. W., *Anat. Rec.*, **76**, 467 (1940).

[6] *a*, Zittle, C. A., and O'Dell, R. A., *J. Biol. Chem.*, **140**, 899 (1941). *b*, Zittle, C.A., and O'Dell, R.A., *J. Biol. Chem.*, **141**, 239 (1941).

[7] Mann, T., in *The Biochemistry of Semen* (John Wiley and Sons, Inc., New York, 1954).

[8] Dallam, R. D., and Thomas, L. E., *Nature*, **170**, 377 (1952).

[9] Kay, E. R. M., Simmons, N. S., and Dounce, A. L., *J. Amer. Chem. Soc.*, **74**, 1724 (1952).

[10] Ramachandran, L. K., and Fraenkel-Conrat, H., *Arch. Biochem. Biophys.*, **74**, 224 (1958).

[11] Schneider, W. C., *J. Biol. Chem.*, **161**, 293 (1945).

[12] Burton, K., *Biochem. J.*, **62**, 315 (1956).

[13] King, E. J., *Biochem. J.*, **26**, 292 (1932).

[14] Eigner, J., doctoral thesis, Harvard Univ., April (1960).

[15] Shooter, K. V., and Butler, J. A. V., *Trans. Faraday Soc.*, **401**, 734 (1956).

[16] Schumaker, V. N., and Schachman, H. K., *Biochim. Biophys. Acta*, **23**, 628 (1957).

[17] Rosenkranz, H. S., doctoral thesis, Graduate School of Medical Sciences, Cornell Univ. (Feb., 1959).

[18] Doty, P., McGill, B. B., and Rice, S. A., *Proc. U.S. Nat. Acad. Sci.*, **44**, 432 (1958).

[19] Meselson, M., Stahl, F. W., and Vinograd, J., *Proc. U.S. Nat. Acad. Sci.*, **43**, 581 (1957).

[20] Sueoka, N., *J. Mol. Biol.*, **3**, 31 (1961).

[21] Marmur, J., and Doty, P., *Nature*, **183**, 1427 (1959).

[22] Hoard, D. E., doctoral thesis, Univ. of California (1957).

[23] Zamenhof, S., Griboff, G., and Marullo, S., *Biochim. Biophys. Acta*, **13**, 459 (1954).

[24] Wyatt, G. R., and Cohen, S. S., *Biochem. J.*, **55**; 774 (1953).

[25] Marshak, A., and Vogel, H. G., *J. Biol. Chem.*, **189**, 597 (1951).

[26] Hershey, A. D., Dixon, J., and Chase, M., *J. Gen. Physiol.*, **36**, 777 (1953).

[27] Wyatt, G. R., *Biochem. J.*, **48**, 584 (1951).

[28] Bendich, A., in *Methods in Enzymology*, **3**, 715 (S. P. Colowick and N. D. Kaplan, eds., Acad. Press, New York, 1957).

[29] Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).

[30] Ghargaff, E., Zamenhof, S., and Green, C., *Nature*, **165**, 756 (1950).

[31] Bendich, A., Pahl, H. B., Korngold, G. C., Posenkranz, H. S., and Fresco, J. R., *J. Amer. Chem. Soc.*, **80**, 3949 (1958).

[32] Rosenkranz, H S., and Bendich, A., *J. Amer. Chem. Soc.*, **81**, 902 (1959).

[33] Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).

[34] Bianchi, P. A., and Shooter, K. V., *Biochem. J.*, **78**, 372 (1961).

SURFEN: A QUINOLINE WITH ONCOGENIC AND HEPARIN-NEUTRALIZING PROPERTIES

By DeWITT T. HUNTER, Jun. and JOSEPH M. HILL
J. K. and Susie L. Wadley Research Institute and Blood Bank, Dallas, Texas

SURFEN (*bis* - 2′ - methyl - 4 - amino - chinolyl - 6 - carbamid) has been described as an oral heparin antagonist more potent than protamine sulphate. Experiments were undertaken to ascertain its degree of inhibitory power on heparin *in vivo* and to determine its toxicity. Earlier personal communications have discounted untoward effects following oral administration to rats for 90 days (Dr. Jose Steinhardt, personal communication).

Eighteen mature Swiss mice were fed 'Purina' chow infiltrated with surfen. These animals were divided into groups 1 to 4, receiving a minimum intake of 60, 120, 150 and 300 mgm./kgm./day of surfen, respectively. Groups 1, 3, and 4 consisted of 3 animals each, while group 2 contained 9 animals. Groups 1, 3, and 4 received the diet for 80 days, after which time all animals were autopsied; those in group 2 were fed surfen for only 60 days. All animals were observed daily for evidence of toxic effects. Three untreated animals were autopsied as anatomical controls. The whole mouse colony served as physiological controls. Micro-coagulation times were performed on 3 groups of 2 rabbits given oral surfen before and after heparinization. Two untreated heparinized rabbits were used as coagulation controls.

Mice subjected to prolonged surfen ingestion at dose-levels slightly in excess of those thought to be of therapeutic value developed lymphosarcomata and lesions suggestive of nutritional deficiency (Table 1).

Table 1. MICROSCOPIC CHANGES OBSERVED IN CONTROL AND SURFEN-TREATED MICE

Microscopic observations	Group				Control
	1	2	3	4	
Surfen mgm./kgm./day	60	120	150	300	0
Animals/group	3	9	3	3	3
Lymphosarcoma, per cent animals with tumour	0	33	100	0	0
Per cent animals with lymphocytic infiltrates	67*	33†	0	0	0
Gastric mucosal hyperplasia					
Total thickness	450μ	500μ	500μ	300μ	400μ
Epithelial thickness	24μ	30μ	25μ	2μ	20μ
Polarity of nuclei	absent	absent	absent	absent	present
Pseudostratification	present	present	present	present	absent
Depot fat; lipocyte size	20μ	15μ	10μ	12μ	20μ
Lipid-containing cells/HPF	100	20	20	30	100
Distorted, giant hepatic cells/HPF	1	1	3	5	1
Colloid casts, distal tubules/LPF	1	0	1	0	0

* Two animals showed lymphocytic infiltrations of soft tissue and viscera similar to those seen in incipient mouse leukæmia or severe, generalized viral diseases. Although a virus would have affected the control and group 4 animals with equal probability, the absence of unequivocal sarcoma in group 1 necessitates interpretation as benign.

† Florid lymphocytic infiltrates were encountered in one additional animal. Prominent lymphocytic aggregates were evident in others. Absence of tumour masses necessitated that these cases be classified as 'lymphocytic infiltrates' rather than 'lymphosarcoma'.

The lymphosarcoma in one animal in group 3 became apparent after 55 days on the diet and accounted for the death of the animal on day 71. A second animal in this group showed the tumour at day 70 and the third sarcoma was discovered at autopsy. Those sarcomas in group 2 developed after day 80.

Evidence of dietary deficiency became apparent after only 10 days on surfen. Most marked gross lesions were seen in group 4 about 50 days through the experiment. At this time, the extremities, noses, and tails of all animals became eczematoid and œdematous. Two animals lost portions of their tails following necrosis of distal segments. Trauma was not a factor since physical activity was virtually nil at this time. These lesions partially regressed near the end of the experiment. Hepatic changes consisted of marked pleiomorphism of size and shape of parenchymal cells. Some disruption of lobular architecture was seen, but no necrosis was apparent to account for this. Nuclei were pycnotic and cytoplasm was deplete of glycogen. Severity of dietary deficiency closely paralleled dosage of surfen. Terminal coagulation times were normal.

Rabbits given 300 mgm./kgm. of surfen within 10 min. before, and at any time after, heparinization showed no change in coagulation times when compared with control heparinized animals. However, the feeding of surfen 4–6 hr. prior to heparinization resulted in a moderate depression of coagulation

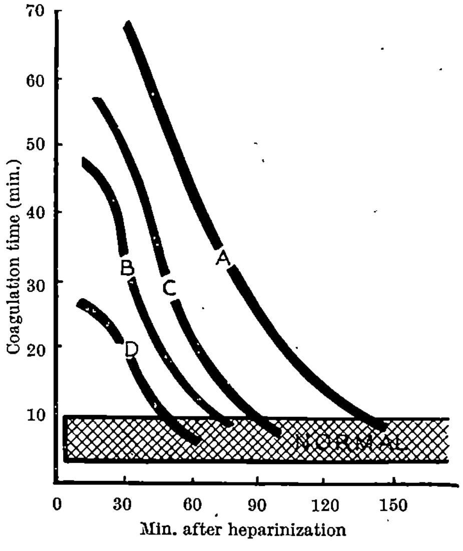


Fig. 1. *A*, Animals receiving 2 mgm./kgm./heparin intravenously. Curves of animals administered oral surfen 10 min. prior to, or at any time after, heparinization were identical with animals receiving no surfen. *B*, same as *A* except for heparinizing dose of 0·5 mgm./kgm. *C*, Animals receiving 2 mgm./kgm. heparin but pretreated with 300 mgm./kgm. surfen 4 to 6 hr. beforehand. *D*, Same as *C* except for heparinizing dose of 0·5 mgm./kgm.

times to about 50 per cent of that seen in controls (Fig. 1).

Although the series is small, presence of lymphosarcoma in a third of the animals in group 2 and all animals in group 3 strongly indicates that surfen is oncogenic at these dose-levels. Based on a spontaneous incidence of one per cent for group 2, p is calculated as 0·000080 and for group 3 as 0·000001.

Absence of sarcomata in group 4 may be explained by the suppressing effect oncogenic substances exert on neoplasia in high doses[4], and by the resistance of malnourished and/or vitamin-deficient animals to tumour development[3]. The findings, in 2 animals of group 1, and 3 animals of group 2, suggest that a prolonged test-period would result in increased tumour induction even at the low dosage in group 1. The oncogenic property of surfen is supported by its hyperplastic effect on gastro-intestinal mucosa. Tumours have not been observed before in this colony of Swiss mice. Although we accepted the manufacturer's chemical description of surfen, we did subject the substance to fractionation in an attempt to disclose oncogenic impurities. The product was homogeneous. Environmental factors would have probably induced neoplasia with equal frequency in the control colony and in group 1. The quinolines have not previously been incriminated in oncogenesis, except in the styryl complex[2], and indeed have displayed oncolytic properties[5].

The other changes observed indicate that surfen interferes with food and/or vitamin utilization. Similar changes are seen in vitamin-deficient animals but not in starved animals[1]. Although the diet was not enriched with vitamins, food intake was sufficient in all groups to maintain health in untreated animals. The liver pathology was suggestive neither of starvation nor of hepato-toxic effect.

Coagulation work performed using heparinized rabbits pre-treated with surfen indicated moderate heparin-neutralizing effects. Surfen is apparently slowly and imperfectly absorbed in the digestive system, and shows activity only if given several hours in advance of heparinization. In this respect, it is inferior to protamine sulphate.

[1] Boutwell, R. K., Pusch., H.P., and Chiang, B., *Proc. Soc. Exp. Biol. Med.*, **77**, 860 (1951).

[2] Browning, C. H., Gulbransen, R., and Neven, J. S. F., *J. Path. Bact.*, **42**, 155-59, 1936.

[3] Cerecedo, R. L., Lombardo, M.E., Reddy, D. V. N., and Travers, J. J., *Proc. Soc. Exp. Biol. Med.*, **80**, 648-52, 1952.

[4] Haddow, A., *J. Path. Bact.*, **47**, 567-79, 1938.

[5] Hughes, B., Bates, A. L., Bahner, C. T., and Lewis, M. P., *Proc. Exp. Biol. Med.*, **88**, 230-2, 1955.

NERVE ENDINGS IN THE MOLE'S SNOUT

By J. ARMSTRONG and DR. T. ANDREW QUILLIAM

Department of Anatomy, University College, London

CONCENTRATED in a small area of hairless skin at the tip of the snout of the near-blind mole are numerous cutaneous nerve endings. In all probability they play an important part in facilitating path-finding and feeding while the animal is either on or below the surface of the ground.

During waking hours the snout is constantly used in a probing fashion (accompanied by head movements) to explore the immediate environment, although the general direction of the animal's movements towards food or away from danger is presumably plotted beforehand in response to olfactory and vibratory sensations. A memory system must also be involved as even when traversing unaccustomed territory the animal may only deviate momentarily from its set course in order to circumvent local obstacles. Observations during life confirm that the snout region is markedly pink in colour and that it is also freely mobile. They also suggest that it is semi-erectile in character since during periods of hyperactivity or stress it assumes a redder hue and becomes more prominent (for example, as during obligatory swimming, when the animal progresses at a great speed and holds its snout well above the water-level). After death, the snout becomes paler and diminishes in size. However, it is not known whether such local circulatory changes could affect the character of responses from nearby nerve endings sensitive to external physical stimuli[7].

At present light-microscope work using *Talpa europaea* has confirmed the classical work of Eimer[4] that there are, in the snout region, many profusely innervated epidermal papillæ passing into the dermis (the so-called 'Eimer's organs'). The presence of encapsulated nerve endings near the deep aspect of Eimer's organs has been recorded by Ranvier[10], Bielschowsky[1] and Boeke[2]. More recently Cauna and Alberti[3] have denied the presence of encapsulated

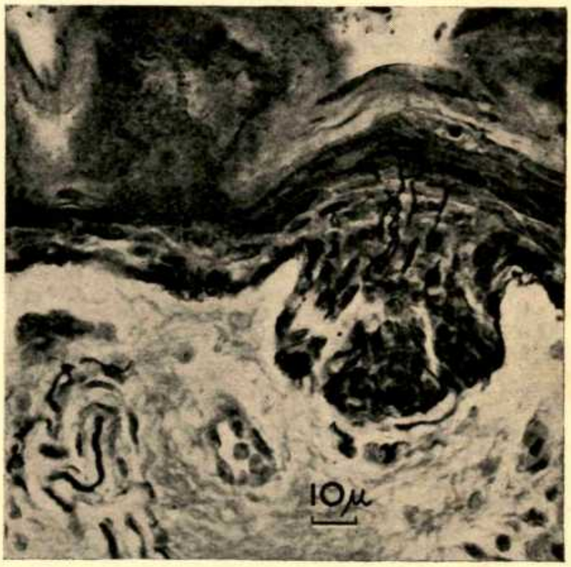


Fig. 1. Vertical section through the skin of the tip of the mole's snout. An Eimer's organ lies to the right. Part of the dermal plexus supplying it and some of the nerve 'terminals' within it can be seen. An encapsulated nerve ending of an unnamed variety lies to the left and intervening between these two structures is a blood sinus. (This material, together with that shown in Fig. 2, was stained by a modified Cajal method)

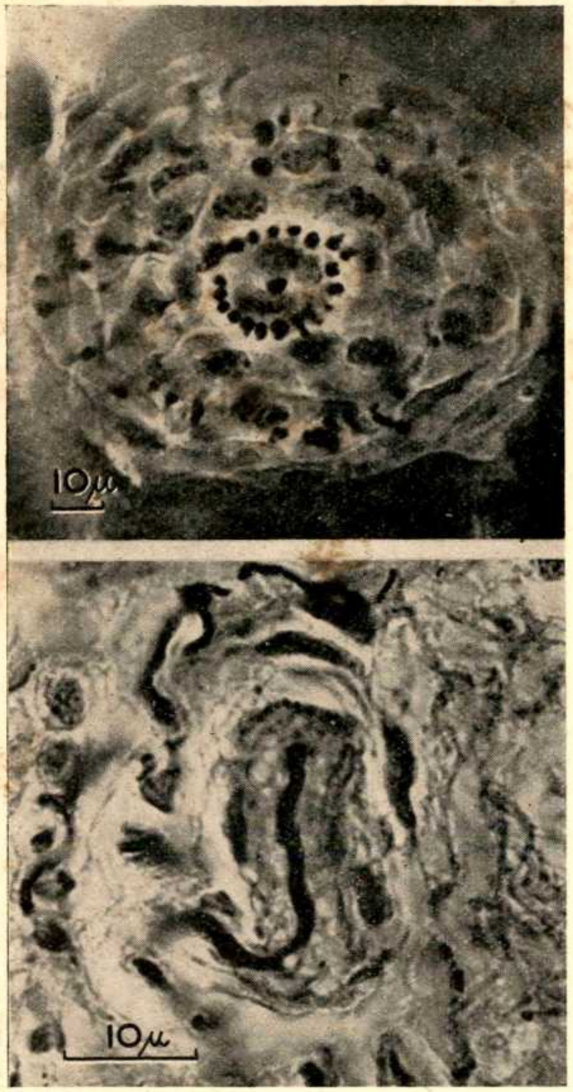


Fig. 2. Above, transverse section through an 'Eimer's organ' showing the axial nerve terminal closely related to paired cells of an inner core. Satellite nerve terminals are arranged circumferentially in a clear annular space. Lying peripherally are the epidermal cells that constitute the bulk of Eimer's organ.

Below, longitudinal section (high power) through an encapsulated nerve ending of an unnamed variety showing a single central axon surrounded by a comparatively simple core and an ill-defined capsule

nerve endings in this region, and have suggested, by implication, that the Eimer's organs are themselves multi-purpose transducers. The present work, together with unpublished electronmicroscopic findings (Quilliam), clearly demonstrate the presence of encapsulated nerve endings of an unnamed variety in the dermis and have considerably amplified the original descriptions and hand-drawn illustrations of them published 35–90 years ago[1,2,10].

The importance of the present findings lies in the fact that it is now no longer necessary to attach an extraordinary functional significance to the Eimer's organs, and in particular, to ascribe to them the unique ability of subserving a comprehensive spectrum of cutaneous sensory modalities.

Fig. 1 shows a vertical section through the skin of the tip of the mole's snout. Above can be seen the undulating profile of the epidermal surface and towards the right is an Eimer's organ in longitudinal section. A few of the fibres of a dermal nerve plexus supplying it from below are visible and a number of ascending nerve 'terminals' (or possibly 'pre-terminals') can be seen traversing it vertically on their way towards the more superficial layers of the epidermis. Located below and to the extreme left is an encapsulated nerve ending the long axis of which in the photomicrograph illustrated happens to be orientated vertically. It is separated from the Eimer's organ previously identified on the right by a blood sinus.

Fig. 2 (above) depicts a typical Eimer's organ in transverse section. A single nerve terminal, axially, is clearly seen to be surrounded by an array of circumferentially disposed satellite nerve terminals. The latter are situated in a clear annular zone that separates the outer core of epithelial cells from the paired cells of the central core which themselves closely invest the axial terminal. The unusually orderly pattern of these grouped nerve terminals may well form a satisfactory structural basis for the fine tactile discrimination that the mole practises during normal activity. Unfortunately, it has not yet proved possible to differentiate histologically between axial and satellite nerve terminals except in respect of diameter measurements and of their relative positions within an Eimer's organ. Consequently, it is not known whether any of the satellite terminals are collaterals of the dermal fibre which is in continuity with the axial terminal. In view of the many bundles of myelinated fibres present in the dermis it would appear likely that every Eimer's organ is innervated by several myelinated fibres. The relatively large number of satellite terminals in an Eimer's organ further suggests that at least some of the dermal fibres supplying it must branch prior to entry into its deep surface—presumably in a subjacent nerve plexus.

Fig. 2 (below) is an enlargement of the encapsulated nerve ending shown in Fig. 1. The capsule is rather poorly differentiated, and the core tissue surrounding the nerve terminal is somewhat scanty. However, nuclei can be made out in the latter on either side of the terminal (at 4 o'clock and 9 o'clock) and several small granules can also be distinguished (at 12 o'clock). The single nerve terminal is almost straight once it has entered the core and ends without visible structural modification. Since some of its morphological features suggest that it belongs to an unnamed type of ending not adequately described hitherto its fine structure will be described elsewhere (Quilliam).

The cellular organization of these encapsulated endings exhibits both certain similarities to and a number of divergences from the pattern typical of the Pacinian corpuscle in the cat. However, it may be tentatively assumed that, functionally, the former behaves like the latter in responding to high-frequency pressure changes[5,6,8].

[1] Bielschowsky, M., *Anat. Anz.*, **31**, 187 (1907) (Fig. 1).

[2] Boeke, J., *Z. mikr.-anat. Forsch.*, **4**, 448 (1926) (Figs. 29 and 30).

[3] Cauna, N., and Alberti, P., *Z. Zellforsch.*, **54**, 158 (1961).

[4] Eimer, Th., *Arch. mikr. Anat.*, **7**, 181 (1871) (Plate 17).

[5] Gray, J. A. B., *Twentieth Intern. Physiol. Congr.*, 59 (1956).

[6] Hubbard, S. J., *J. Physiol.*, **141**, 198 (1958).

[7] Kitchell, R. L., Campbell, B., Quilliam, T. A., and Larson, L. L., *Proc. Amer. Vet. Med. Assoc.*, **1**, 177. (92nd Annual Meeting, Minneapolis, 1955.)

[8] Loewenstein, W. R., *Sci. Amer.*, **203**, 98 (1960).

[9] Quilliam, T. A., and Sato, M., *J. Physiol.*, **129**, 167 (1955).

[10] Ranvier, L., *Quart. J. Micro. Sci.*, **20**, 456 (1880) (Plate 36, Fig. 2).

LETTERS TO THE EDITORS

RADIOASTRONOMY

Spectrum of Galactic Radio Emission

THE general radio emission from the Galaxy has now been accounted for as the synchrotron radiation of high-energy electrons in a magnetic field. An unsatisfactory aspect of this explanation is the relation between the observed radio spectral index α, where radio flux is proportional to (wave-length)$^{\alpha}$, and the expected energy spectrum of the cosmic-ray electrons, the number density of which should also vary exponentially according to (energy)$^{-(1+S)}$. In these circumstances the indices are related by $\alpha = S/2$.

It has been shown by Tunmer[1] that if the electrons are supposed to be generated by the collision of cosmic-ray protons with atomic nuclei, the value of S will depend on the predominating mechanism by which the electrons lose energy. Assuming a proton number density proportional to (energy)$^{-2\cdot5}$, the following values of S will be expected for the various loss mechanisms:

Synchrotron radiation	$S = 2{\cdot}5$
Bremsstrahlung radiation	$S = 1{\cdot}5$
Ionization losses	$S = 0{\cdot}5$

The measured value of $\alpha = 0{\cdot}37 \pm 0{\cdot}04$ for the frequency-range 38–178 Mc./s. (ref. 2) corresponds with a value $S = 0{\cdot}74 \pm 0{\cdot}08$. This value appears to indicate that losses are primarily governed by ionization; unfortunately, Tunmer's paper indicates that the only important loss for the electrons concerned with radiation in this frequency-range is the synchrotron radiation itself. Radiation at 100 Mc./s. is produced by electrons with energy of the order of 10^9 eV. in a field of 10^{-6} gauss. The actual conditions of emission cannot be far different from these, and it seems inescapable that a value of S near 2·5 should be found, corresponding to $\alpha = 1{\cdot}25$.

If the electrons are not formed throughout the energy spectrum, but only at higher energies than those concerned, afterwards falling through the range by synchrotron losses, then a radio spectral index of 0·5 would be found. Even making this extreme hypothesis of the life-history of the electrons, the expected value of α is still greater than that observed.

It is natural to suspect the experimental evidence, and indeed this is now being checked as rigorously as possible. However, some new observations at 408 Mc./s., soon to be published by Pauliny-Toth, support the low value of radio spectral index. The experiment reported by Molozzi, Franklin and Tyas[3], in which galactic radio noise at 3·8 Mc./s. was measured by a satellite-borne radio receiver, also lends some support. Here the measured field-strength E can be related to an effective black-body temperature T by:

$$T = \frac{3\lambda^2}{8\pi kB}\,\frac{E^2}{Z_0}\,\varepsilon^{1/2} \quad \text{(Hugill, in preparation)}$$

For the northern hemisphere, Molozzi's results give $E \simeq 0{\cdot}6$ μV./m., and it seems unlikely that this result is much affected by the ionosphere. Hence $T \simeq 1{\cdot}3 \times 10^6$ °K., while the value to be expected by extrapolating Costain's results from 38 Mc./s. to 3·8 Mc./s. using $\alpha = 0{\cdot}37$ is $2{\cdot}8 \times 10^6$ °K. The indication here is of an even lower value of α at the lower frequencies.

No theory has yet attempted to resolve this problem; the purpose of this communication is merely to point out a serious discrepancy between theory and experiment, which is not apparent from the papers by Tunmer[1] and Hoyle[4]. Further examination of the processes of loss and gain of energy by the electrons of cosmic-ray energies seems to be required.

F. GRAHAM SMITH

Mullard Radio Astronomy Observatory,
Cambridge.

[1] Tunmer, H., *Mon. Not. Roy. Astro. Soc.*, **119**, 184 (1959).
[2] Costain, C. H., *Mon. Not. Roy. Astro. Soc.*, **120**, 248 (1960).
[3] Molozzi, A. R., Franklin, C. A., and Tyas, J. P. I., *Nature*, **190**, 616 (1961).
[4] Hoyle, F., *Mon. Not. Roy. Astro. Soc.*, **120**, 338 (1960).

ASTROPHYSICS

Production of Neon in Stars

WE have recently made certain measurements of the properties of some levels in neon-20 which are related to the production of neon in stars. In particular we have shown that the 4·97-MeV. level has odd parity and $J = 2$. Consequently neon-20 cannot be formed through this level by thermonuclear reactions between helium and oxygen-16 as has been suggested to explain its production in stellar interiors after the hydrogen has been consumed[1,2]. The suggestion arose from the close proximity of the 4·97-MeV. level in neon-20 to the energy of oxygen-16 + α as shown in Fig. 1. For the level to be effective the parity would have to be $(-)^J$ where J is the total angular momentum.

The first evidence that the level has $J\pi = 2-$ arose from γ–γ correlations in the neon-20 $(pp'\gamma\gamma)$ reaction that established the total angular momentum as 2 (ref. 3) and the unexpectedly long life-time of $1{\cdot}9^{+3\cdot5}_{-1\cdot0} \times 10^{-12}$ sec. found by the Doppler shift technique in our experiments[4] using the carbon-12-(^{12}C $\alpha\gamma$)neon-20 reaction. The assignment of negative parity has now been established by our investigations (to be published) of neon-20$(pp'\gamma)$neon-20. In this reaction measurements of the linear polarization[5,6] of the 3·34-MeV. γ-ray by Compton scattering combined with the angular distribution of the γ-ray defined its electric or magnetic character and thereby demonstrated that the levels concerned at 4·97 MeV. and 1·63 MeV. have opposite parity. The parity of the 1·63-MeV. level is positive (ref. 7 and A. E. Litherland and E. Almqvist, D. A. Bromley, J. A. Kuehner and B. Whalen, to be published). A parity of $(-)^{J+1}$ for the 4·97-MeV. level has since been deduced (A. E. L., to be published) from measurements (E. Almqvist, D. A. Bromley, J. A. Kuehner and B. Whalen, to be published) of the α-particle angular distributions in the carbon-12(^{12}C α)neon-20 reaction, and $J\pi = 2-$ has been confirmed by α–γ correlations in this same reaction (H. E. G., A. E. L. and M. A. C., to be published).

The next available level for helium thermonuclear reactions is at 5·63-MeV. This can be formed by the α + oxygen-16 reaction (H. E. G., A. E. L. and

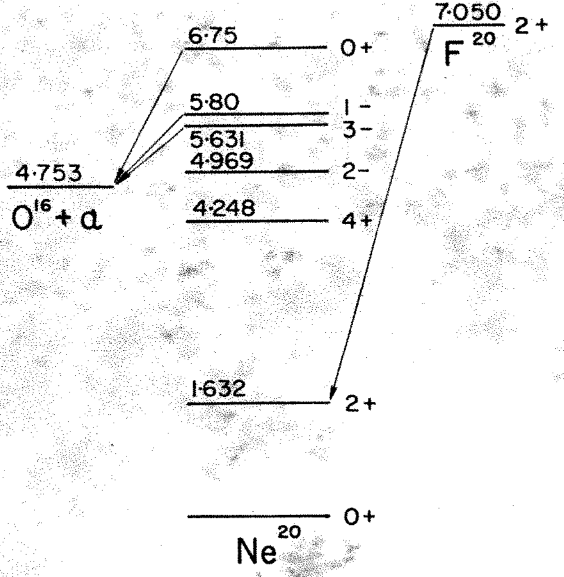


Fig. 1. Diagram for low-lying levels in neon-20

M. A. C. and J. A. Kuehner and E. Almqvist, to be published) and preliminary experiments (A. E. L. and H. E. G., unpublished) on the reaction at an α-particle energy corresponding to this level yield a value of $(2J + 1)\frac{\Gamma_\alpha \Gamma_\gamma}{\Gamma} = 0{\cdot}003 \pm 0{\cdot}002$ eV. The 5·63-MeV. level in neon has now been shown to have $J\pi = 3-$ (J. A. Kuehner, to be published) and $\Gamma_\gamma/\Gamma = 0{\cdot}07 \pm 0{\cdot}01$ (H. E. G., A. E. L. and M. A. C. and J. A. Kuehner and E. Almqvist, to be published). Cameron (private communication) has recently estimated that, under these conditions, neon-20 could be formed through the 5·63-MeV. level at temperatures above 5×10^8 °K. in supernova explosions. He has also pointed out the possibility that the strong neon lines observed in certain stars may be neon-22 formed in the reactions nitrogen-14($\alpha\gamma$)fluorine-18($\beta^+\gamma$) oxygen-18($\alpha\gamma$)neon-22.

The level at 5·80 MeV., which was first discovered in measurements of the sodium-23($p\alpha$)neon-20 reaction[8], has been shown to have $J\pi = 1-$ (J. A. Kuehner, to be published) and $\Gamma_\gamma/\Gamma = 0{\cdot}003 \pm 0{\cdot}003$ (J. A. Kuehner and E. Almqvist, to be published). It would be of great interest to measure $(2J+1)\frac{\Gamma_\alpha \Gamma_\gamma}{\Gamma}$ for this level in the oxygen-16($\alpha\gamma$)neon-20 reaction, since it may also contribute to neon formation in stars.

H. E. Gove
A. E. Litherland
M. A. Clark

Atomic Energy of Canada, Ltd.,
Chalk River, Ontario.

[1] Salpeter, E. E., *Phys. Rev.*, **107**, 516 (1957).

[2] Cameron, A. G. W., *Chalk River Rep. CRL*-41 (A.E.C.L. No. 454). (June 1957).

[3] Broude, C., and Gove, H. E., *Bull. Amer. Phys. Soc.*, Ser. II, **6**, 37 (1961).

[4] Clark, M. A., Gove, H. E., and Litherland, A. E., *Bull. Amer. Phys. Soc.*, Ser. II, **6**, 249 (1961); and to be published.

[5] Litherland, A. E., and Gove, H. E., *Canad. J. Phys.*, **39**, 471 (1961).

[6] McCallum, G. J. (to be published); for the appropriate formulæ see also Devons, S., and Goldfarb, L. J. B., *Handbuch der Physik*, **42**, 326 (Springer-Verlag, Berlin, 1957).

[7] Ajzenberg-Selove, F., and Lauritsen, T., *Nuclear Phys.*, **11**, 1 (1959).

[8] Adams, H. S., Fox, J. D., Heydenburg, N. P., and Temmer, G. M., *Bull. Amer. Phys. Soc.*, Ser. II, **6**, 250 (1961); and private communication.

SEISMOLOGY

Possible Method for increasing the Signal–Noise Ratio in the Detection of the First Motion of a Refracted *P*-Wave

In seismology a method has been developed of examining the focal mechanisms of earthquakes by studying the azimuthal variation of the first movements on seismograms[1]. In extending this method to differentiating between small earthquakes and explosions a basic experimental problem is the detection of the initial movement when its amplitude is of the order of only 10^{-7} cm. in the frequency-range 1–10 c.p.s., and is therefore comparable with the background microseismic noise-level at the quietest sites. The purpose of this communication is to suggest and illustrate a possible technique of increasing the signal : noise ratio.

It is generally considered that the majority of seismic noise consists of surface waves, a characteristic of which is that their amplitude decreases approximately exponentially with depth[2]. The first arrivals at a recording site at distance between 200 and 1,000 km. from a shallow event are P_n waves which have been refracted along the Mohorovičić discontinuity and emerge at the surface with an angle of incidence of about 50°. Allowing for surface reflexion their amplitude varies with depth by a factor of two. Thus, accepting the postulate that the noise is predominantly surface waves, it is immediately obvious that an increase in signal : noise ratio can be obtained by placing the detector deep undergound, typically a factor of 10 being gained at 3 c.p.s. for a depth of 4,000 ft. In this communication we are suggesting that a further improvement of signal : noise ratio can be obtained through the use of two detectors, one at the surface and the other at a depth of, say, a wave-length at the peak recording frequency. The signal at the surface detector is then regarded mainly as noise, and is subjected to a frequency dependent attenuation, higher frequencies being attenuated most, to give the noise signal theoretically expected at the lower detector. The two signals are now subtracted so that the noise is eliminated, and the required *P*-wave signal, slightly modified, remains.

To test the method, model seismic studies have been carried out. The model consists of a 1/16-in. sheet of 'Perspex' 48 in. × 12 in. (Fig. 1). The transmitter at the surface, T_1, generates a burst of 'white' noise, about 750 μsec. in duration, while the 'buried' source, T_2, transmits a 10-μsec. pulse. Two detectors are placed two-thirds of the way along the model, D_1 at the surface and D_2 at a depth of 1 in., corresponding to about one wave-length for a surface

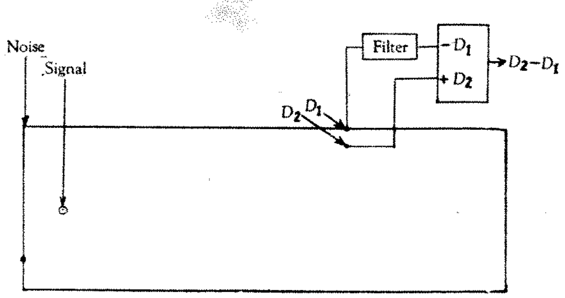
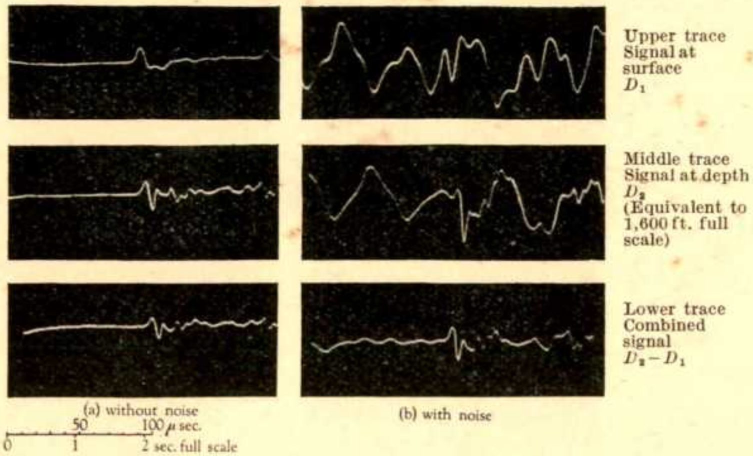


Fig. 1. Diagram of arrangement of transmitters and detectors on model



(a) without noise

(b) with noise

Fig. 2

wave with a frequency of 50 kc./sec. which is the dominant frequency of the *P*-wave. The timing of the two transmitted signals is such that the *P*-wave from T_2 arrives at the detectors about half-way through the burst of surface waves from T_1. The signal from D_1 is passed through a filter having a frequency attenuation closely resembling the theoretical surface wave attenuation at the depth of D_2, and this is then subtracted from the signal from D_2 at the appropriate relative gain. (To allow for frequency-dependent phase-changes in the filter, which are approximately linear with frequency, the two detectors are staggered horizontally by about ¾ in.) Typical records are shown in Fig. 2, the gain in signal : noise ratio being readily apparent.

The basic principles behind the method were developed in private discussions with Prof. J. Oliver of Columbia University and we are pleased to acknowledge his help.

F. A. Key
J. K. Wright
E. W. Carpenter
B. M. H. Stott

Atomic Weapons Research Establishment,
Foulness Island,
Southend-on-Sea,
Essex.

[1] Scheidegger, A. E., *Bull. Seism. Soc. Amer.*, **47**, 89 (1957).
[2] Kolsky, H., *Stress Waves in Solids* (Clarendon Press, Oxford, 1953).

PHYSICS

Effect of Ultra-Violet Irradiation on the Electrical Conductivity of Cathodes coated with Barium Oxide

It has been known for some years that irradiation with ultra-violet light can have an important effect on the photoelectric and thermionic properties of a cathode coated with barium oxide. Ultra-violet irradiation at room temperature causes an enhancement of photoelectric emission induced by illumination of the cathode by light of other wave-lengths[1,2], and a pronounced change in the spectral sensitivity curves for photoelectric emission[1-5] and photoconductivity[5] is observed after ultra-violet treatment. The enhancement is only observed at low temperatures and may be removed by heating the cathode to about 700° K. Ultra-violet irradiation may also cause an enhancement of thermionic emission[2]. It is the purpose of this communication to report the results of preliminary observations of the effect of ultra-violet irradiation on the dark electrical conductivity of cathodes coated with barium oxide, as distinct from its enhancing effect on photoconductivity[5].

The experimental tubes employed contained a tubular 'O'-nickel cathode base, provided with an insulated tungsten heater, and coated over a portion of its length with barium oxide. A tungsten thermocouple wire was welded internally to the nickel tube, allowing the measurement of cathode temperature. Embedded in the cathode coating was a helical probe of fine nickel wire. A cylindrical nickel anode with a small circular window pierced in it was mounted coaxially with the cathode. A side-arm attached to the glass envelope of the experimental tube bore a 'Pyrex'-to-quartz graded seal with a plane quartz window at the quartz end, aligned so that the cathode could be illuminated through the quartz window and the window in the anode. The barium oxide coating was prepared by the usual method of pyrolysis of a sprayed barium carbonate suspension. The tubes were submitted to the usual schedules of breakdown, outgassing and activation by drawing thermionic emission current, and after sealing off from the vacuum system an active barium getter was fired.

Measurement of the electrical conductance of the cathode sample could be made by applying small potentials (up to 100 mV.) between the probe and cathode base and measuring the conductivity current on a sensitive galvanometer. The conductivity current-voltage characteristic at any desired cathode temperature T °K. was traced and the conductance σ of the barium oxide sample at that temperature could be obtained by taking the gradient of the characteristic at zero applied potential. The curvature of the characteristic was slight, and only a small error was incurred in later stages of the work when, for the sake of speed of observation, the difference in galvanometer readings for $\pm$ 20 mV. applied between the probe and cathode base was taken as a measure of the conductance.

In determining the effect of ultra-violet irradiation on dark conductivity, the variation of conductance with temperature for the cathode under investigation was first determined before any ultra-violet treatment was applied. The conductivity of well-activated cathodes was sufficiently high to allow measurements to be commenced at a cathode temperature of about 800 °K. and continued down to room temperature. The results are best displayed by plotting $\log \sigma$ versus $1/T$, as shown in Fig. 1*a*. The conductivity plot shows the well-known Loosjes–Vink[6] pattern in which the two linear portions are interpreted as representing a conduction process in which free electron flight across the pores of the oxide coating predominates at high temperatures, and a process of bulk or surface semiconduction through or over the surface of the oxide crystallites operates at low temperatures. In the

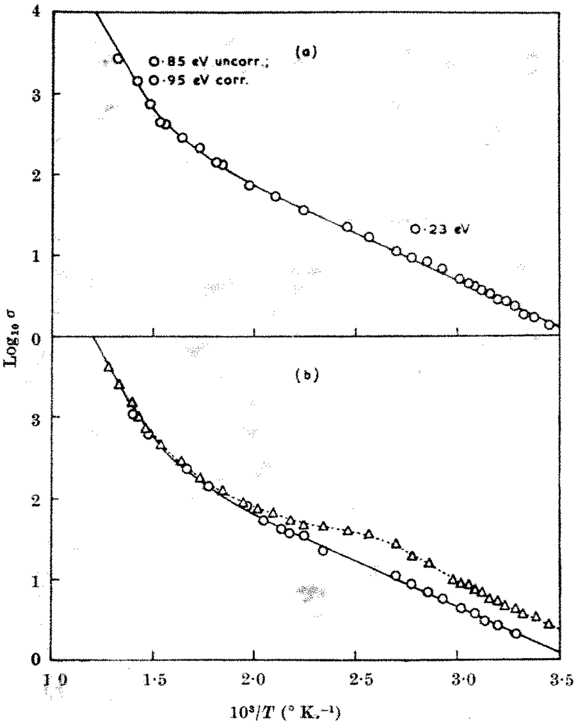


Fig. 1. Effect of ultra-violet irradiation on dark conductivity. *a*, Graph of log σ versus $1/T$ for an activated barium oxide coated cathode before ultra-violet irradiation; *b*, conductivity plots for the same cathode after ultra-violet irradiation: △, points plotted on warming cathode; ○, points plotted on cooling cathode. The conductance σ is expressed in arbitrary units

example given, the activation energy of the low-temperature process is 0·23 eV. and that of the high-temperature process is 0·95 eV., corrected for the parallel low-temperature component. On completion of the conductivity determination the cathode was irradiated through the quartz window with light from a high-pressure mercury arc, containing a high proportion of ultra-violet light, for 1 hr. The dark conductance of the cathode was then redetermined, starting at room temperature and heating the cathode. The effect of the ultra-violet irradiation was to increase the room-temperature dark conductance by a factor of about 2, and as the temperature was increased to about 370 °K. the enhancement increased slightly. From 370 °K. to about 550 °K. it decreased, and at temperatures above about 550 °K. the conductance was the same as that obtained at corresponding temperatures before irradiation. Heating was continued to about 800 °K., and the cathode was then cooled to room temperature. Conductance measurements taken during this process exactly reproduced the values obtained in the initial pre-irradiation determination. The results of this experiment are displayed in Fig. 1*b*; the curve obtained on warming the cathode immediately after irradiation is plotted as a broken line, and that taken on cooling as a continuous curve. For these plots separate current-voltage characteristics were not traced at each temperature, but the conductance was determined approximately, as indicated above. It was found that although the general form of this enhancement of dark conductivity is reproducible, the magnitude of the effect depends on a number of factors, such as the length of ultra-violet irradiation, the temperature of the cathode during irradiation, the period between the end of irradiation and the beginning of conductance measurements, and the rate of heating of the cathode. It was found that any enhancement could be completely removed by heating the cathode to about 600 °K. for a few minutes; on cooling, the conductance would return to the value obtained before irradiation.

This effect may be explained by postulating the existence of normally vacant donor-levels just below the conduction band in the energy-band model for barium oxide. Irradiation of the cathode sample with ultra-violet light may cause the excitation of electrons from the filled band and the occupation of some of these levels. Subsequent warming of the cathode in the dark in the course of conductivity determinations causes thermal excitation of electrons from these occupied levels to the conduction band, and an enhanced conductivity current is observed. Experiments on the enhancement of photo-electric emission by ultra-violet illumination[1-5] have led to the conclusion that such a band of normally unoccupied levels exists at about 2·5 eV. below vacuum potential. Although it is tempting to associate these levels with the enhancement of dark conductivity observed in the present work, there is, as yet, no direct evidence for the validity of such an association.

C. H. B. Mee

Physical Laboratory,
University of Southampton.

[1] McNary, B. D., *Phys. Rev.*, **81**, 631 (1951).

[2] McNary, B. D., Missouri Univ. Phys. Dep. Tech. Rep., No. 8 (1951).

[3] DeVore, H. B., and Dewdney, J., *Phys. Rev.*, **83**, 805 (1951).

[4] Noga, K., and Kawamura, S., *J. Phys. Soc. Japan*, **7**, 287 (1952).

[5] Sakamoto, M., *Vide*, **9**, 109 (1954).

[6] Loosjes, R., and Vink, H. J., *Philips Res. Rep.*, **4**, 449 (1949).

A Clear Instance of Rheopectic Flow

We have found an instance of rheopectic flow induced by shear in a rotational viscometer. The material exhibiting this rare type of non-Newtonian flow behaviour was a viscous suspension prepared by mixing kaolin clay, precipitated calcium carbonate, oxidized starch, a styrene–butadiene latex, sodium pyrophosphate and water. The total solids of the suspension amounted to 65 per cent.

Characterization of the material as rheopectic was based on shearing stress against time data obtained under conditions of constant shear-rate. A set of these data is presented in Fig. 1. The stress is seen to increase with time until it becomes measurably independent of it. It was ascertained that laminar flow prevailed during measurements.

It appears that this shear-thickening effect is due largely to the presence of calcium carbonate since, when it was replaced by an equal mass of clay, the flow behaviour of the suspension was that of a typically thixotropic material. The longest dimension of the pigment particles in the suspension was smaller than 2μ for the clay and 0·3μ for the calcium carbonate. Clay and calcium carbonate particles were mostly anisometric (length to width ratio for clay about 1·5; for calcium carbonate about 4·0) while the latex particles were essentially spheres (average diameter 0·2μ).

Earlier observations of the rheopectic phenomenon have been mostly visual, qualitative, and confined to certain types of colloidal systems, usually sols[1-5].

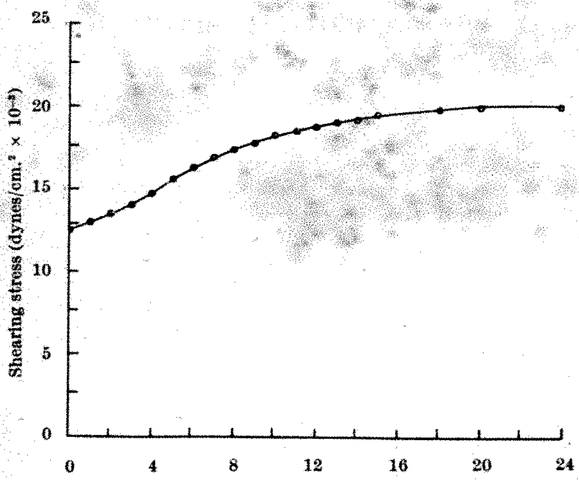


Fig. 1. Variation of shearing stress with time under conditions of constant shear-rate (1,850 sec.$^{-1}$) at 25 ± 0·2° C.

As a probable result of this, an assertion has been made recently that rheopexy is a flow phenomenon where structure build-up is brought about by small shearing-rates only[6], presumably less than about 10 sec.$^{-1}$. However, instances of rheopectic flow have been reported in the literature[7,8] although absolute values of shear-rate at which the phenomenon exhibits itself are not mentioned. Our observations demonstrate that rheopectic flow can develop at moderately high shear-rates (1,500–2,200 sec.$^{-1}$).

J. B. Yannas
R. N. Gonzalez

W. R. Grace and Co.,
Cambridge 40,
Massachusetts.

[1] Freundlich, H., and Juliusberger, F., *Trans. Farad. Soc.*, **31**, 920 (1935).
[2] Juliusberger, F., and Piquet, A., *Trans. Farad. Soc.*, **32**, 445 (1936).
[3] Hauser, E. A., and Reed, E. C., *J. Amer. Chem. Soc.*, **58**, 1822 (1936).
[4] Hartley, G. S., *Nature*, **142**, 161 (1938).
[5] Eliassaf, J., Silberberg, A., and Katchalsky, A., *Nature*, **176**, 1119 (1955).
[6] Wilkinson, W. L., *Non-Newtonian Fluids*, 9 (Pergamon Press, London, 1960).
[7] Crane, J., and Schiffer D., *J. Polymer Sci.*, **23**, 93 (1957).
[8] Weltmann, R. N., *Rheology*, **3** (6), 216 (Academic Press, New York, 1960).

METALLURGY

Scattering of Long Wave-length Neutrons by Irradiated Beryllium Oxide

Work on the scattering of long wave-length neutrons by irradiation-induced defects have been carried out by Antal, Weiss and Dienes for graphite[1] and by Antal and Goland for alumina[2]. The method depends on the fact that in materials of low neutron absorption cross-section, neutrons of wave-length greater than the Bragg cut-off are attenuated principally by inelastic scattering (for which the cross-section is a few hundred millibarns) and by the scattering from defects. Application of Babinet's principle shows that each isolated point defect introduces an amount of scattering equal to the free atom cross-section of the material.

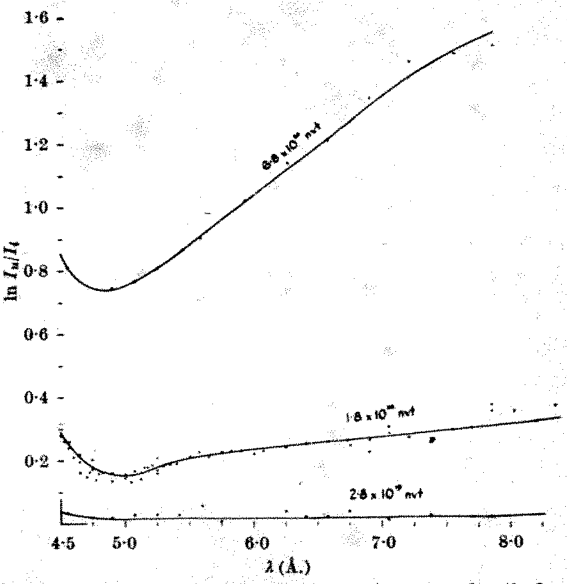


Fig. 1. Transmission ratios versus neutron wave-length for various neutron doses. Top curve, 6·8 × 10^{18} n.v.t.; middle curve, 1·8 × 10^{2} n.v.t.; ; bottom curve, 2·8 × 10^{19} n.v.t.

When the defects are randomly distributed a comparison of the attenuation by identical specimens before and after irradiation can give a direct measurement of the defect concentration. However, if the defects are clustered, interference occurs between the amplitudes of neutrons scattered from different defects within a cluster. In this case it is no longer possible to obtain an unambiguous estimate of the number of defects since the extra attenuation is dependent on wave-length, and the scattering is determined by the number of defects and the configuration of a cluster.

As part of an investigation of the behaviour of beryllium oxide under neutron irradiation, experiments have been carried out on specimens of this oxide irradiated to three different doses in *Hifar*.

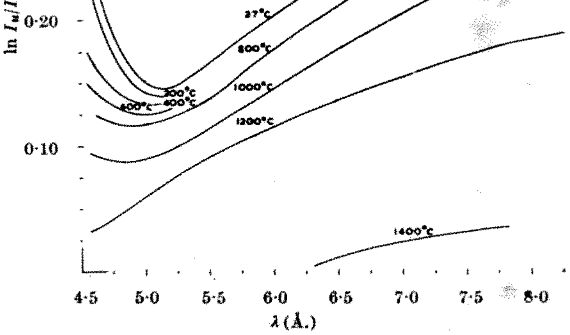


Fig. 2. Transmission ratios versus neutron wave-length after annealing at various temperatures

The neutron spectrometer used was similar to that described by Antal and Goland[2]. A mica monochromator was used with beryllium and beryllium oxide filters. The results are shown in Fig. 1 plotted as $\ln\left(\frac{I_u}{I_i}\right)$ versus wave-length, where I_u is the number of neutrons transmitted by 14 cm. of unirradiated beryllium oxide and I_i is the number transmitted by an identical specimen after irradiation. The doses quoted are the integrated doses over the fission neutron spectrum. It can be seen that the amount of extra scattering due to defects increases with neutron dose. For one set of specimens an annealing study was made by repeating the measurements after annealing the specimens at 200° C. intervals from 200–1,400° C. These results are shown in Fig. 2.

It can be seen that there are two distinct effects, a decrease in attenuation near the Bragg cut-off, which anneals steadily from 200° C. to 1,000° C., and an increase at longer wave-lengths, which anneals between 1,200° C. and 1,400° C.

The initial decrease in extra scattering near the cut-off wave-length could be explained if irradiation broadened the Bragg reflexions. However, this seems unlikely because the X-ray line broadening appears to be insufficient to account for the effect and this line broadening does not anneal until 1,400° C. A defect configuration which would explain the sharp decrease with wave-length is that proposed by Huntington[3] for copper, in which two interstitial atoms appear at each side of a vacant lattice site with an I–V spacing of less than 1 Å.

The theory developed by James[4] for the scattering of X-rays by polyatomic gases has been applied to the increase in scattering observed at long wave-lengths. A model which fits the observed dependence on wavelength is one in which the vacancies are clustered into close-packed groups containing 14 vacancies and the interstitials are randomly distributed. Values for the number of defects have been calculated on this model and these are shown in the Table 1.

Table 1

Specimen	Mean fission neutron dose (n.v.t.)	Irradiation temperature	Fraction of primary collisions	Fraction of displaced atoms	Displacements per primary collision
*X*22	$2\cdot8 \times 10^{19}$	70–75° C.	7×10^{-5}	$4\cdot5 \pm 4\cdot0 \times 10^{-4}$	1–13
*X*4	$1\cdot8 \times 10^{20}$	80–100° C.	$4\cdot5 \times 10^{-4}$	$4\cdot5 \pm 0\cdot4 \times 10^{-3}$	9–11
*X*39	$6\cdot8 \times 10^{20}$	80–100° C.	$1\cdot7 \times 10^{-3}$	$2\cdot2 \pm 0\cdot1 \times 10^{-3}$	12–14

The number of primary collisions was calculated by assuming a cross-section of 2·5 barns as the average of beryllium and oxygen atoms over the fission spectrum.

Further work on X-ray line broadening and other physical properties are being carried out in order to obtain more information on the nature of the defects.

T. M. Sabine
A. W. Pryor
B. S. Hickman

Australian Atomic Energy Commission
Research Establishment,
Lucas Heights,
New South Wales.

[1] Antal, J. J., Weiss, R. J., and Dienes, G. J., *Phys. Rev.*, **99**, 1081 (1955).

[2] Antal, J. J., and Goland, A. N., *Phys. Rev.*, **112**, 103 (1958).

[3] Huntington, H. B., *Phys. Rev.*, **91**, 1092 (1953).

[4] James, R. W., *The Optical Principles of the Diffraction of X-rays* (G. Bell and Sons, Ltd., 1958).

RADIATION CHEMISTRY

Radiation-induced Formation and Reactions of the Formyl Radical Ion

We have recently reported the formation of the CO_2^- radical ion and the related CO_2H radical by the action of ionizing radiation on aqueous solutions of carbon dioxide and bicarbonate[1].

It seemed of interest, therefore, to examine the corresponding reaction with carbon monoxide which should lead, in the first instance, to the CO^- radical ion and the COH radical. Some direct proof for the presence of CO^- in aqueous solutions has been obtained now from experiments in which oxygen-free aqueous solutions containing suitable organic solutes have been irradiated with ionizing radiations in the presence of carbon monoxide. It could be demonstrated that in certain cases the CO^- ion (or the COH radical respectively) could be combined with an organic radical with the formation of the corresponding formylated compound.

For example, the irradiation of aqueous solutions of methanol (10^{-1} *M*) in the presence of carbon monoxide (1 atm.) with Co-60 γ-rays ($1\cdot4 \times 10^{19}$ eV. ml.^{-1}) gave glycolaldehyde with a yield of $G = 0\cdot2$ (molecules/100 eV.) at $pH = 5\cdot4$. The glycolaldehyde was identified by the formation of a coloured derivative with the 2 : 3-diaminophenazine reagent in concentrated sulphuric acid[2].

It is again important that the reaction is carried out in deoxygenated solutions because otherwise the negative polarons $(H_2O)^-$, which are the reducing entities[3] produced by the action of radiation on water, may be scavenged to a greater or lesser extent by the molecular oxygen according to:

$$(H_2O)^- + O_2 \rightarrow O_2^- + H_2O$$

In general, this formylation process depends on the formation of a free radical from the organic solute by the action of the radiation-produced OH or (H_2O^+), for example, in the case of methanol:

$$CH_3.OH + OH \rightarrow CH_2.OH + H_2O \qquad (1)$$

and association of the organic free radical with the COH (CO^-) according to the reaction:

$$CH_2.OH + COH \rightarrow CH_2.OH.CHO \qquad (2)$$

the formyl radical being formed by the reduction of CO according to:

$$CO + (H_2O)^- \rightarrow CO^- + H_2O \qquad (3)$$

$$CO^- + H^+ \rightleftarrows COH \qquad (4)$$

In this way it should be possible to produce some ^{14}C-carbonyl compounds labelled in the CO group.

We wish to thank the Rockefeller Foundation for financial support; one of us (J. H.) thanks the Department of Scientific and Industrial Research for a maintenance grant.

John Holian
George Scholes
Joseph J. Weiss

Chemistry Department,
King's College
(University of Durham),
Newcastle upon Tyne.

[1] Scholes, G., Simic, M., and Weiss, J. J., *Nature*, **188**, 1019 (1960).

[2] Dechary, J. M., Kun, F., and Pitot, H. C., *Anal. Chem.*, **26**, 449 (1954).

[3] Weiss, J., *Nature*, **186**, 751 (1960).

A Reversible Photochromic Polymer

ULTRA-VIOLET irradiation of chromium, molybdenum or tungsten hexacarbonyls dissolved in donor solvents causes the substitution of one or more molecules of carbon monoxide by solvent. The products are yellow in colour[1].

Methyl methacrylate polymers which contain occluded metal hexacarbonyls behave similarly. On ultra-violet irradiation or on being placed in sunlight, the polymers assume a deep yellow colour when as little as 0·1 per cent of, for example, chromium hexacarbonyl is present. At room temperature, either in air or high vacuum, the colour fades over a period of about 4 hr. The colour vanishes rapidly on warming to 100° but is stable when the specimen is stored in liquid nitrogen.

We believe that, on irradiation, the chromium hexacarbonyl decomposes to give $Cr(CO)_5$ and carbon monoxide. The former molecule may exist as such in the coloured polymer or may be stabilized by interaction with neighbouring ester groups or occluded carbon–carbon double bonds. Since the hexacarbonyl is trapped in the polymer matrix, the carbon monoxide cannot escape even on pumping, and at room temperature it slowly returns to the metal, producing the original colourless hexacarbonyl. Heating to 100° relaxes the polymer matrix and facilitates the reversion to hexacarbonyl.

The infra-red spectrum of the yellow polymethyl methacrylate showed a carbonyl shift of about 40 cm.$^{-1}$ to higher wave-lengths compared with unirradiated samples. No electron spin resonance spectrum other than that normally associated with polymethyl methacrylate irradiated with ultra-violet could be detected. These facts are consistent with the presence of $Cr(CO)_5$ or its addition products.

A. G. MASSEY
L. E. ORGEL

Department of Theoretical Chemistry,
University Chemical Laboratory,
Lensfield Road,
Cambridge.

---

[1] Strohmeier, W., and Gerlach, Kl., *Z. Naturforsch.*, **15** b, 9 (1960).

CHEMISTRY

Fluoride-compensated Substitution of Bivalent Cations in the Magnetoplumbite Structure

$BaAl_{12}O_{19}$ has the structure of the mineral magnetoplumbite[1]. In the hexagonal unit cell, containing two formula units, the aluminium ions are distributed among five crystallographic positions. There are two tetrahedrally co-ordinated positions (4*f* in C_6/mmm) nine octahedrally co-ordinated (2*a*, 12*k*, 4*f*) and one ion surrounded by a trigonal bipyramid (2*b* in C_6/mmm) of oxide ions per formula unit.

The object of this work was to try to introduce bivalent cations, compensated by other foreign ions, in place of the Al^{3+} ions in the structure, in order to study the site preference of these ions in this structure. Cationic compensation (substitution of La^{3+} for Ba^{2+}) and anionic compensation (substitution of F^- for $O^=$) were both used. The latter method was first used for this structure by Frei *et al.*[2], who introduced fluoride into $BaFe_{12}O_{19}$ with concurrent reduction of some Fe^{3+} to Fe^{2+}.

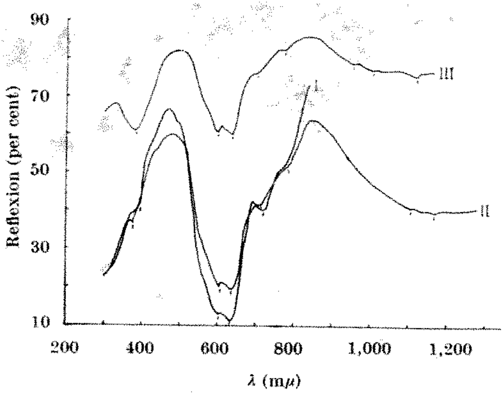


Fig. 1. Per cent reflexion versus wave-length, mμ (magnesium carbonate standard). I, $BaNi_{1·5}Al_{10·5}O_{17·5}F_{2·5}$; II, $BaNi_{2·5}Al_{9·5}O_{16·5}F_{2·5}$; III, $BaZn_2Ni_{0·5}Al_{9·5}O_{16·5}F_{2·5}$

Using cationic compensation, materials having the general formula $Ba_{1-x}La_xNi_xAl_{12-x}O_{19}$ were prepared. For samples where $x \leqq 0·2$, X-ray diffraction indicated the presence of only the magnetoplumbite phase. At values of $x \geqslant 0·3$ a mixture of $LaAlO_3$, Al_2O_3 and magnetoplumbite was found. The samples where $x \leqq 0·2$ were blue in colour; reflectance spectra, taken on pellets pressed from the powders, give the following absorption peaks:

cm.$^{-1}$	Intensity	Site
27,000	Very weak	Octahedral
17,700	Strong	Tetrahedral
16,400	Very strong	Tetrahedral
15,850	Very strong	Tetrahedral
13,950	Medium	Tetrahedral
12,900	Very weak	Tetrahedral

All these absorptions, except the first one, are in good agreement with the spectrum of Ni^{2+} in ZnO (ref. 3) and indicate that almost all the Ni^{2+} is occupying tetrahedral sites. The presence of a small amount of Ni^{2+} in octahedral sites is indicated by a weak absorption at 27,000 cm.$^{-1}$, which is in good agreement with the strongest absorption in $Ni(H_2O)_6^{2+}$ (ref. 3).

Due to the breakdown of the magnetoplumbite phase at $x \geqslant 0·3$, the second method of charge compensation was attempted in order to examine the site preferences as a function of concentration of the bivalent ion.

Materials having the general formula $BaM^{II}Al_{12-x}O_{19-x}F_x$ were prepared by firing mixtures of BaF_2, $BaCO_3$, MO, Al_2O_3 at 1,350–1,400° C. in an atmosphere of dry nitrogen gas.

The first system to be investigated was one in which $M^{II} = Ni^{2+}$ and $x = 0·1$, $0·2 \rightarrow 1$, 1·5, 2·0, 2·5. $BaNiZnAl_{10}O_{19}F_2$ and $BaNi_{0·5}Zn_2Al_{9·5}O_{16·5}F_{2·5}$ were also prepared, using AlF_3 where an extra source of fluoride was needed. X-ray diffraction showed that for all samples where $x \leqq 2·5$, only the hexagonal phase was present; where $M^{II} = Ni^{2+}$ and $x \geqq 1·0$ the *c*-axis was doubled. This effect did not appear where $M^{II} = Zn^{2+}$ or Co^{2+}. At values of $x > 2·5$, the hexagonal phase disappears and is replaced by a spinel phase, probably $Ni_{1+x}Al_{2-x}O_{4-x}F_x$, in the residue after leaching with hot concentrated hydrochloric acid.

The reflectance spectra of the materials the general formula of which is $BaNi_xAl_{12-x}O_{19-x}F_x$, where $x \leqq 2·0$, are identical with the spectra described previously. This indicates that, when substituted for up to 2 gm.-atoms of Al^{3+} (in $BaAl_{12}O_{19}$), Ni^{2+} strongly prefers tetrahedral sites (Fig. 1, curve I).

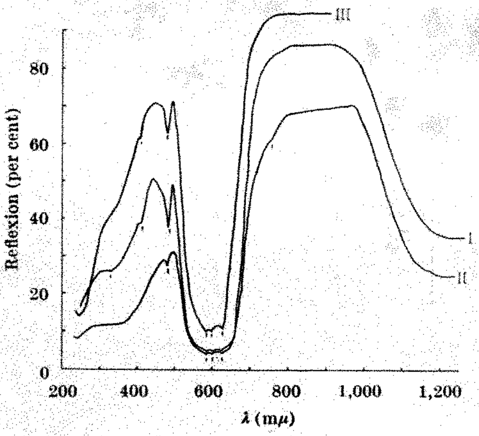


Fig. 2. Per cent reflexion versus wave-length, mμ (magnesium carbonate standard). I, $BaCo_2Al_{10}O_{17}F_2$; II, $BaCo_{2\cdot5}Al_{9\cdot5}O_{16\cdot5}F_{2\cdot5}$; III, $BaZn_2Co_{0\cdot5}Al_{9\cdot5}O_{16\cdot5}F_{2\cdot5}$

At $x = 2\cdot5$ (Fig. 1, curve II) a marked increase is observed in the absorption at 27,000 cm.$^{-1}$, indicating an increase in the amount of Ni^{2+} in octahedral sites. A weak absorption is found at 8,900 cm.$^{-1}$ which also indicates octahedral nickel. The increase in octahedral nickel is caused by the excess Ni^{2+} (greater than 2 gm.-atoms) which is forced to occupy the octahedral or trigonal bipyramid sites. The tetrahedral sites are saturated by the first 2 gm.-atoms of Ni^{2+}. All these samples were blue in colour. Fluoride analysis of $BaNiAl_{11}O_{18}F$ yielded a value of 2·2 per cent by weight of fluoride. The calculated value is 2·0 per cent.

The sample $BaZn_2Ni_{0\cdot5}Al_{9\cdot5}O_{16\cdot5}F_{2\cdot5}$ was prepared in order to see if Zn^{2+} (which has a strong tetrahedral site preference) could displace Ni^{2+} from the tetrahedral sites. Fig. 1, curve III, shows a large increase in the amount of octahedral Ni^{2+} with a corresponding decrease in the amount of tetrahedral Ni^{2+}. This sample had a light blue-green colour.

The next system to be investigated was one in which $M^{II} = Co^{2+}$ and $x = 0\cdot1$, $0\cdot4$, $1\cdot0$, $2\cdot0$, $2\cdot5$. $BaZnCoAl_{10}O_{17}F_2$ and $BaZn_2Co_{0\cdot5}Al_{9\cdot5}O_{16\cdot5}$ were also prepared. X-ray diffraction showed the presence of only one phase (magnetoplumbite). The intensities of the absorptions, of course, vary with composition.

The spectra of all these samples were identical except for that of $BaCo_{2\cdot5}Al_{9\cdot5}O_{16\cdot5}F_{2\cdot5}$. This sample showed a strong absorption in the near ultra-violet region which may be caused by cobalt in octahedral sites. The observed absorption maxima for these materials are:

cm.$^{-1}$	Intensity	Site
24,400	Medium	Tetrahedral
20,900	Medium	Tetrahedral
17,100	Very strong	Tetrahedral
16,800	Very strong	Tetrahedral
16,100	Very strong	Tetrahedral

These values are in fair agreement with the spectrum observed for Co^{2+} in ZnO (ref. 4) and they are in good agreement with the values obtained from the crystal field splittings as a function of *Dq* which were calculated by Low[5]. Curve III (Fig. 2) shows the spectrum of $BaZn_2Co_{0\cdot5}Al_{9\cdot5}O_{16\cdot5}F_{2\cdot5}$. The absorption spectra indicate that Zn^{2+} does not displace Co^{2+} from tetrahedral sites to any appreciable extent.

It has been shown that Ni^{2+} and Co^{2+}, compensated by F^-, have tetrahedral site preferences, in $BaAl_{12}O_{19}$, in all concentrations up to the saturation of the tetrahedral sites. The preference of Co^{2+} for tetrahedral sites has been shown to be greater than that of Ni^{2+}, as tetrahedral Ni^{2+} is displaced by Zn^{2+} and tetrahedral Co^{2+} is not. The tetrahedral site preference of Ni^{2+} (which would normally have an octahedral site preference) may be caused by a Jahn–Teller stabilization. This Jahn–Teller effect may also be the cause of the change of symmetry in the hexagonal unit cell which effected a doubling of the *c*-axis. This effect was not observed with Co^{2+}.

E. Banks
M. Robbins

Department of Chemistry,
Polytechnic Institute of Brooklyn,
Brooklyn 1,
New York.

---

[1] Adelsköld, V., *Arkiv. Kemi. Mineral Geol.*, **12A**, (1938).

[2] Frei, E. H., Schieber, M., and Shtrikman, S., *Phys. Rev.*, **118**, 657 (1960).

[3] McClure, D. S., *Solid State Phys.*, **9**, 400 (1959).

[4] Schmitz-DuMont, O., Brokopf, H., and Burkhardt, K., *Z. anorg. Allg. Chem.*, **295**, 7 (1958).

[5] Low, W., *Phys. Rev.*, **109**, 256 (1958).

Ultrasonic Velocity in Aqueous Silver Nitrate Solution

We have confirmed the recent report[1] that, in the case of aqueous solutions of silver nitrate, the ultrasonic velocity decreases with increasing concentration over the range of concentration investigated. However, a minimum velocity is observed at approximately 4 *M*; thereafter, increasing concentration up to saturation causes a monotonic increase in ultrasonic velocity, as is usual for aqueous electrolyte solutions. Part of the complete velocity/molarity curve is shown in Fig. 1; these results were obtained with a variable-path fixed frequency (263·3 kc./sec.) interferometer, at 25° C.

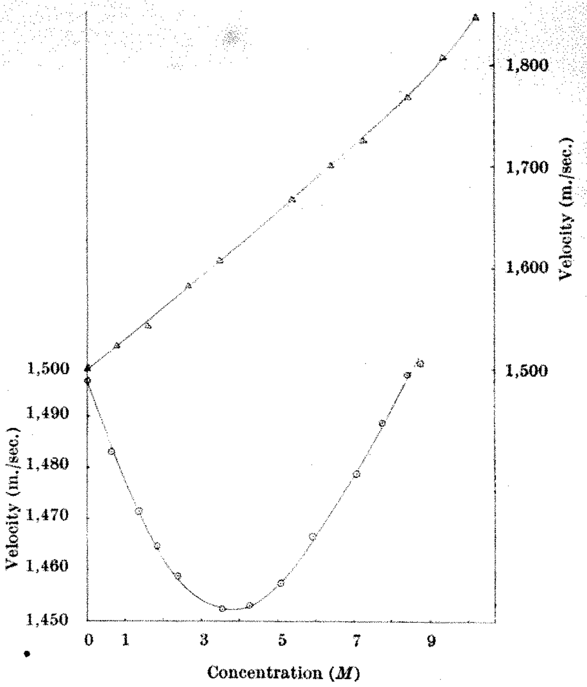


Fig. 1. Variation of ultrasonic velocity with concentration in aqueous solutions of electrolytes. ○, Silver nitrate; △, ammonium nitrate

Although the suggested explanations[2-4] in terms of heavy radicals or complex ions may be correct, it is clear that the parameters directly responsible for the minimum velocity are the density, and the adiabatic compressibility, β, of the solution. These are related by the equation:

$$v^2 = 1/\rho\beta$$

where v is the ultrasonic velocity. The variation in v with molarity may then be expressed:

$$dv/dc = -v/2 . (1/\rho . d\rho/dc + 1/\beta . d\beta/dc) \quad (1)$$

The terms $d\rho/dc$ and $d\beta/dc$ are of opposite sign; hence the relative magnitudes of the terms within brackets on the right-hand side of equation (1) may produce monotonic increase or decrease of v with concentration, or, as in the case of silver nitrate, a minimum velocity.

It is interesting that ammonium nitrate solutions, which show similar variations with molarity of electrical conductance and of mean-ion activity coefficient to those of silver nitrate, do not exhibit a minimum ultrasonic velocity; the curve for ammonium nitrate is included in Fig. 1. Presumably the ion-pairing in these solutions[5] does not always result in a velocity minimum.

D. S. Allam
W. H. Lee

Physical Chemistry Laboratories,
Battersea College of Technology,
London, S.W.11.

[1] Seshagri Rao, M. G., and Ramachandra, Rao B., *Nature*, **191**, 164 (1961).

[2] Barthel, R., *J. Acoust. Soc. Amer.*, **260**, 227 (1954).

[3] Balachandran, C. G., *Nature*, **187**, 136 (1960).

[4] Murty, M. S., and Murty, Bh. K., *J. Sci. Indust. Res.*, **17**, B, 216 (1958).

[5] Robinson, R. A., and Stokes, R. H., *Electrolytic Solutions*, second ed. (Butterworth, 1959).

Complexes of Vermiculite with Amino-Acids

Organic substances, either as neutral molecules or as cations, form 'sandwich' complexes with the expanding-lattice silicate minerals in which films of the organic substance a few Angstroms thick alternate with silicate layers 10 Å. thick. Recent work has shown that macroscopic vermiculite crystals complexed with butylammonium ions absorb large quantities of water and swell in the direction perpendicular to the plane of the silicate layers[1,2].

We have observed that macroscopic vermiculite crystals complexed with various amino-acids also swell anisotropically to many times their original size. Two apparently different swelling mechanisms are involved, depending on whether the amino-acid is present as the cation or as the dipolar ion; in both instances, swelling is confined to the direction perpendicular to the plane of the silicate layers (Fig. 1). In the jelly-like swollen crystals, the silicate layers remain effectively parallel to one another and, under optimum swelling conditions, may be some hundreds of Angstroms apart.

One type of swelling resembles that exhibited by the butylammonium–vermiculite complexes, and occurs after the amino-acid has been introduced as the interlayer cation replacing the magnesium or other inorganic cations present in the natural minerals. Treatment with 0·5 *M* aqueous solutions of the hydrochloride of the acid for two or three days at 80° C. is usually sufficient for complete exchange with crack-free crystals of dimensions 1 mm. × 1 mm. × 0·1 mm. The amount of amino-acid taken up is close to the cation exchange capacity of the minerals, that is, in the range 120–180 m.equiv./100 gm. Swelling develops when the amino-acid solution, in which the crystals are immersed, is diluted to a critical value which varies with the amino-acid and the vermiculite used. Swelling increases with decreasing solution concentration and is maximal at infinite dilution. The initiation of swelling is thought to coincide with 'hydration' of the interlayer amino-acid cations[2] and their dissociation from the negatively charged silicate surfaces, while subsequent swelling appears to be osmotically controlled. This type of swelling has been observed with ornithine, lysine and γ-aminobutyric acid.

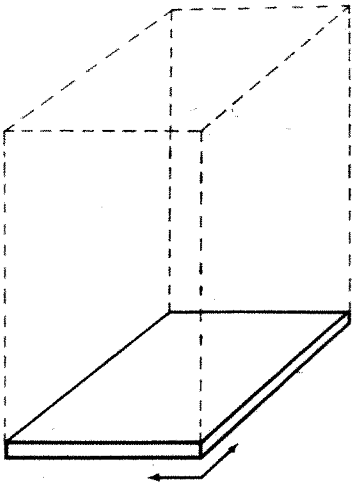


Fig. 1. Representation of the dimensional change in a vermiculite–amino-acid crystal before swelling (full line) and after swelling (dashed line). Silicate layer plane indicated by arrows

A second type of swelling occurs when natural vermiculite crystals are immersed in strong amino-acid solutions containing the acid in the dipolar form. In this type, replacement of the interlayer inorganic cations is inappreciable and swelling may take place at room temperature in a few minutes. Swelling develops only in solutions of sufficiently high concentration and increases directly with concentration. Considerable amounts of amino-acid are taken up into the interlayer regions of the mineral, the intracrystalline concentration being of the same order as that of the external solution. Here the dielectric constant of the immersion solution appears to be the controlling factor. The mechanism is thought to involve the masking of the charge on the interlayer cations so that the effective electrostatic attraction between them and the silicate layers is reduced. Swelling of this type has been observed with solutions of glycine, β-alanine, γ-aminobutyric acid and ε-aminocaproic acid.

G. F. Walker
W. G. Garrett

Chemical Research Laboratories,
Commonwealth Scientific and
Industrial Research Organization,
Melbourne.

[1] Walker, G. F., *Nature*, **187**, 312 (1960).

[2] Garrett, W. G., and Walker, G. F., *Proc. Ninth Nat. Clay Conf.*, Purdue, Nat. Acad. Sci., Nat. Res. Counc. (in the press).

Semiconductivity of Chlorpromazine

It has been shown in ref. 1 that chlorpromazine, the common name for phenothiazine-2-chloro-10-(3 dimethyl aminopropyl), is "a quite extraordinarily strong electron donor"; it is "the first substance ever found in which, in its ordinary stable state, the highest filled level corresponds to an antibonding orbital". It thus should be expected that chlorpromazine, also in view of its striking physiological activity, should exhibit some interesting electrical properties.

The parent compound, phenazin, has recently been shown[2] to be an intrinsic semiconductor with an activation energy of 2·1 eV., relating the conductivity σ to temperature T by:

$$\sigma = \sigma_0 \exp - E/2kT$$

We have examined the resistance of the purified substance, melting point 56·85° C., kindly supplied to us by Messrs. May and Baker, Ltd., Dagenham, Essex, England, in the form of glass-like slabs cast from the melt in a 'Perspex' former between roughened platinum electrodes. A thermistor bead was also cast into the slab, but located outside the field, to act as a thermometer. The whole apparatus was placed under thermostatical control. The electrical resistance was found to drift downwards for 20–30 min. after the application of an electric field. Once equilibrium was attained, very little further drift was observed during a period of 3 hr. A time-interval of 20–30 min. was therefore allowed between each reading. The voltage applied was 85 V. d.c.

Data of resistance against temperature were obtained on fifteen runs of heating and cooling cycles, referring to four different slabs. The temperature coefficient of the resistance R was found to reverse sign at 32° C., becoming negative at higher temperatures. A plot of log R against $1/T$ of this transition region is shown in Fig. 1, and a plot of the high temperature region between 34° and 80° C. is shown in Fig. 2.

Chlorpromazine thus appears to be an impurity semiconductor at low temperatures, and an intrinsic semiconductor at temperatures in excess of about 32° C. There is no significant change in either resistivity or value of E on fusion; E remains substantially constant at 2·1 eV. at temperatures between 34° and 80° C. The region of positive temperature coefficient connecting the impurity conductance with the intrinsic conductance regions is considered to be a régime where the concentrations of localized donor- or acceptor-levels nearly equal the concentration of charge carriers. The transition at 32° C. is believed to be associated with a crystallographic change, the substance existing in different modifications below and above that temperature.

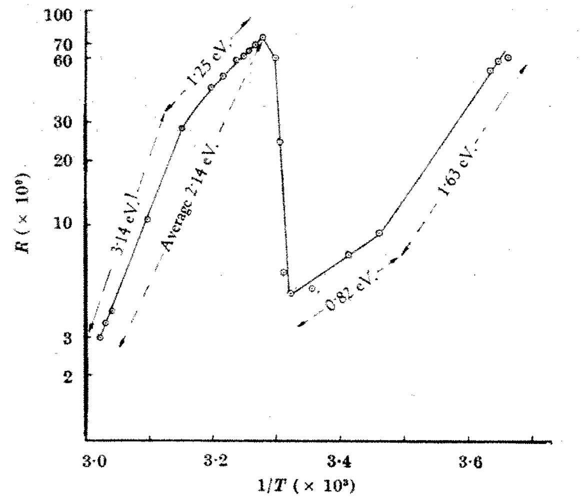


Fig. 1. The resistivity of chlorpromazine, plotted on a logarithmic scale against inverse temperature, showing the transition at 32° C.

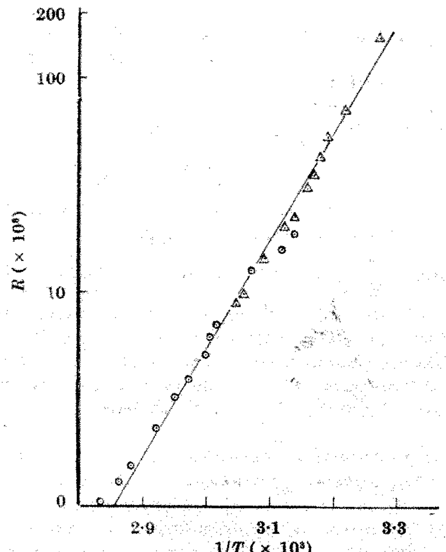


Fig. 2. Resistance of chlorpromazine above the transition. This plot covers the region between 34° and 80° C., the melting point being 56·85° C. There is no significant discontinuity on fusion, nor hysteresis between heating and cooling cycles. △, Heating cycle; ⊙, cooling cycle

There is no evidence for a hysteresis. The points referring to both the heating and the cooling cycle, shown in Fig. 2, are seen to fall on one single straight line.

The conductivity with direct current of chlorpromazine at the transition temperature of 32° C. was found to be $9{\cdot}2 \times 10^{-11}$ (ohm-cm.)$^{-1}$. The value of E found for chlorpromazine, namely, 2·1 eV., agrees with that reported[2] for phenazine; this conforms with ideas put forward[3] on the role of the delocalization of the π-electrons in a ring system. The phthalocyanines are also rather insensitive, so far as E is concerned, to modifications of the molecule which leave the ring structure substantially unaltered, as, for example, by the chelation of metal atoms[5].

Curves closely resembling that shown in Fig. 1 have been reported in ref. 4 for glassy, amorphous selenium. This element also exhibits no change in E on fusion.

Measurements of permittivity and of loss factor at audio- as well as at radio-frequency confirmed the existence of the transition at 32° C.

Direct proof of the existence of a crystallographical change in that region of temperature has been obtained by taking infra-red spectra at temperatures of about 25° C., 36° C. and 55° C.; these temperatures are approximate only, due to heating of the sample by radiation. While no change appeared between the spectra obtained at the two higher temperatures, pronounced differences were observed between those obtained at the two lower temperatures. The changes affect the side-chain as well as the ring system, and are consistent with a steric change in the alignment and the co-ordination of the molecules.

We wish to thank Messrs. May and Baker, Ltd., Dagenham, Essex, for the supply of chlorpromazine, and Dr. R. L. Werner of this Department for his assistance in the interpretation of the infra-red spectra.

F. GUTMANN
A. NETSCHEY

Department of Physical Chemistry,
University of New South Wales,
Broadway, Sydney.

[1] Karremann, G., Isenberg, I., and Szent-Györgyi, A., *Science*, **130**, 1191 (1959).

[2] Aftergut, S., and Brown, G. P., *Nature*, **189**, 827 (1961).

[3] Fley, D. D., *Nature*, **162**, 819 (1948); *Research*, **12**, 293 (1959).

[4] Eckardt, F., *Ann. Physik*, VI-**14**, 240 (1954).

[5] Gutmann, F., and Fielding, P. E., *J. Chem. Phys.*, **26**, 411 (1957).

Structure of δ-Plutonium

In a recent article[1] a modification to the normally accepted close-packed face-centred cubic structure of δ-plutonium was suggested. The purpose of this was to give a continuous gradation, with increasing temperature, in the amount of covalency in the various plutonium structures; from this was deduced a predicted value of the negative expansion coefficient of about the correct size.

The proposed modification consisted of slight displacements inwards of the face-centring atoms in alternate cells. This structure should give additional weak 'superlattice' lines on an X-ray diffraction pattern in addition to those normally observed. However, we could not detect these experimentally, although calculations showed that the X-ray diffractometer techniques used were amply sensitive. The absence of these lines does not lend credence to the proposed structure.

A full description of the experiments is to be published elsewhere.

J. A. C. MARPLES
J. A. LEE

Atomic Energy Research Establishment,
Harwell, Berks.

[1] Konobeevsky, S. T., and Chebotarev, N. T., *Atomnaya Energiya*, **10**, 1, 50 (1961).

Girard *T* Reagent for Carbonyls

The water-soluble properties of Girard *T* hydrazones make the reagent potentially effective for isolation of carbonyls from mixtures soluble in non-polar solvents. In work on the chemistry of rancidity[1], we have found the reagent useful in learning about the distribution of volatile and non-volatile carbonyls in rancid fat. This work indicated possible usefulness of the reagent for investigation of the complex carbonyl anatomy of autoxidized fats. Successful application depends on adaptation to quantitative isolation of very small amounts of carbonyls, and on devising means of controlling the reaction so as to isolate at will free carbonyl and bound or potential carbonyl.

This report deals with certain aspects of the methodology of the Girard reaction. In experiments with known amounts of monocarbonyls, 0·5–1·0 gm. of Girard *T* reagent was reacted in 20·0 ml. of purified anhydrous methanol or ethanol and shaken 3 hr. at room temperature, or refluxed for 1 hr. The reaction mixture was poured into 200 ml. of ice water, and then extracted with petroleum ether to remove non-carbonylic material. The aqueous phase contained the Girard *T* hydrazones. These were converted directly into 2,4-dinitrophenylhydrazones by treatment with 30 ml. of a saturated 2,4-dinitrophenylhydrazine solution in 2 *N* hydrochloric acid[2] and 190 ml. of water. After standing overnight at room temperature, or heating 1 hr. on the steam bath, the 2,4-dinitrophenylhydrazones were removed by successive extractions with carbon tetrachloride and benzene. Monocarbonyl hydrazones were separated by passage of extract residues through hydrated alumina[2]. Total amounts of these compounds were determined by measurement of absorbance at the wave-length of maximum absorption[3–6] in 100 ml. of carbon tetrachloride solution. In the case of mixtures, paper chromatographic methods are available for resolution, identification, and estimation of monocarbonyls[3–6].

Table 1. BLANK DETERMINATIONS

Girard *T* reagent	2,4-Dinitrophenylhydrazine		Ethanol	Tertiary butyl alcohol	No solvent
—	RT*	1 hr.	0·048	—	—
—	RT	24 hr.	0·130	0·024	—
—	Heated	1 hr.	0·480	—	—
0·5 gm. (unpurified) RT, 3 hr.	RT	24 hr.	—	0·250	—
1·0 gm. (unpurified) RT, 3 hr.	RT	24 hr.	0·850	0·518	—
0·5 gm. (purified) RT, 3 hr.	RT	24 hr.	0·338	0·050	—
0·5 gm. (purified) Reflux, 1 hr.	RT	24 hr.	0·478	0·120	—
0·5 gm. (purified) Reflux, 2 hr.	RT	24 hr.	0·900	—	—
0·5 gm. (purified) RT, 3 hr.	Heated	1 hr.	2·63	0·813	0·087
0·5 gm. (purified) RT, 3 hr.	Heated	1 hr.	—	0·310†	—

* Room temperature.
† Purified by treatment with 2,4-dinitrophenylhydrazine and hydrochloric acid.

The above procedure for the Girard reaction gave high blanks which seriously affected determinations of very small amounts of monocarbonyls. The Girard *T* reagent was found to contain hydrazone, which could be removed by several recrystallizations under mild conditions from ethanol. However, the greater part of the blank was found to result from a curious reaction between the primary alcohols used as solvents and both the Girard *T* and 2,4-dinitrophenylhydrazine. The amount of formaldehyde or acetaldehyde hydrazone formed was related directly to the severity of reaction conditions and independent of exposure to air. Apparently, this carbonyl formation is a dehydrogenation and analogous to the formation of osazones from α-keto alcohols. The reaction has been studied on a very large scale by Braude and Forbes[7], who determined that it resulted in a 5–25 per cent yield with primary and secondary alcohols containing two or more ethylenic or phenyl substituents in conjugation with the alcohol group. These investigators stated that derivatives were not formed with monophenyl, α,β unsaturated and saturated alcohols. This has been found not to be completely correct in the case of the two saturated primary alcohols used.

Methanol and ethanol have been the standard solvents used in the Girard *T* reaction since it was first reported[8]. Tertiary butyl alcohol is proposed as a relatively inert solvent for use in the presence of the Girard *T* reagent and 2,4-dinitrophenylhydrazine. The Girard *T* reagent has limited, but adequate, solubility in this alcohol, which is a much better solvent for fats than methanol or ethanol. Blanks found with ethanol and tertiary butyl alcohol are shown in Table 1. Ethanol blanks were substantial, and increased with vigour of conditions of reaction. Both Girard *T* and 2,4-dinitrophenylhydrazine are shown to act on ethanol. Purified butyl alcohol gave negligible blanks when the Girard *T* reagent was recrystallized, except when heated in the 2,4-dinitrophenylhydrazine step. Purification of tertiary butyl alcohol with 2,4-dinitrophenylhydrazine and hydrochloric acid reduced this blank somewhat. This may indicate the presence of a persistent impurity, possibly a ketal. In any event, heating in the 2,4-dinitrophenylhydrazine step was unnecessary for complete conversion.

The use of tertiary butyl alcohol in the Girard *T* reaction gave nearly quantitative results, under mildest conditions of reaction, with *n*-alkanals, *n*-alk-2-enals, and *n*-alk-2,4-dienals. Reaction of methyl ketones with the Girard *T* reagent was less complete (about 80 per cent).

The entire suitability of the Girard *T* reagent for study of total carbonyls in autoxidized fat remains to be determined. Little is known about its reaction with dicarbonyl compounds. In view of its dehydrogenation action on primary and secondary alcohols, the reagent might convert hydroxy compounds produced in the autoxidation into carbonyls. Direct reaction of Girard *T* reagent or 2,4-dinitrophenylhydrazine with fat autoxidation products could result in higher total carbonyl than actually present. There has been some indication that this may occur; such as continual removal of carbonyl on repeated treatments with Girard *T* reagent[1], but further study is necessary. It was considered that the information in this report might be of use to those interested in applications of the Girard *T* reagent.

A. M. Gaddis
Rex Ellis
G. T. Currie

Agricultural Research Service,
United States Department of Agriculture,
Beltsville, Maryland.

[1] Gaddis, A. M., Ellis, R., and Currie, G. T., *Food Res.*, **25**, 495 (1960).
[2] Gaddis, A. M., Ellis, R., and Currie, G. T., *Food Res.*, **24**, 283 (1959).
[3] Ellis, R., Gaddis, A. M., and Currie, G. T., *Anal. Chem.*, **30**, 475 (1958).
[4] Gaddis, A. M., and Ellis, R., *Anal. Chem.*, **31**, 870 (1959).
[5] Ellis, R., and Gaddis, A. M., *Anal. Chem.*, **31**, 1997 (1959).
[6] Gaddis, A. M., and Ellis, R., *Food Res.*, **24**, 392 (1959).
[7] Braude, E. A., and Forbes, W. F., *J. Chem. Soc.*, 1762 (1951).
[8] Girard, A., and Sandulesco, G., *Helv. Chim. Acta*, **19**, 1095 (1936).

BIOCHEMISTRY

Immunochemical Distinction between Insulins with Identical Amino-Acid Sequences

Virtually all human subjects treated with commercial mixtures of beef-pork insulin develop circulating insulin-binding antibodies that are readily detectable with insulin labelled with iodine-131 by means of paper or starch electrophoresis, paper chromatography or ultracentrifugation[1]. These antibodies react not only with the specific immunizing insulins but also with horse, sheep and human insulins[2], and with dog, monkey, rabbit and whale insulins (unpublished work) in greater or lesser degree. Harris, Sanger and Naughton[3] have shown that the amino-acid sequences of insulins from four different ungulate species (cattle, pig, horse and sheep) differ only in the 8–10 positions of the *A* chains. Although some human antisera react quite similarly with these four insulins, certain antisera can distinguish clearly among insulins from the different species, generally reacting much more strongly with beef and sheep insulins than with pork and horse insulins[2]. These differences in reaction of the ungulate insulins have been related[2] to the known differences in the 8–10 sequence of the *A*-chain or to possible differences in secondary or tertiary structure.

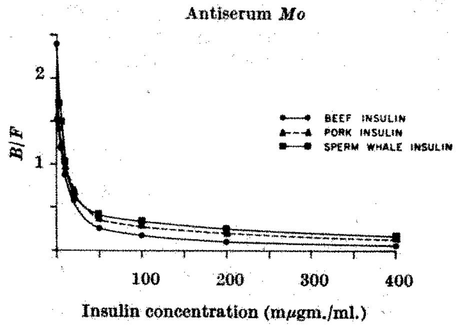


Fig. 1. Ratio of antibody-bound beef insulin-131 to free beef insulin-131 (*B/F*) as a function of the concentration of unlabelled crystalline insulins from various animal species. Human antiserum *Mo* at 1 : 10 dilution

Harris *et al.*[3] found the amino-acid sequences of a whale insulin sample to be identical with those of pork insulin. More recently, Ishihara, Saito, Ito and Fujino[4] established that these sequences are present in sperm whale insulin but differ slightly from those of sei-whale insulin.

The present communication describes experiments showing that certain insulin antisera can discriminate sharply between the competitive inhibition offered by pork and sperm whale insulins to the binding of beef insulin labelled with iodine-131 to combining sites on the antibody molecules.

Crystalline beef insulin labelled with iodine-131 was prepared by methods described previously[1]. Mixtures containing identical trace concentrations of beef insulin-^{131}I, different concentrations of unlabelled crystalline beef, pork or whale insulin and identical concentrations of antiserum (added last in all cases) were prepared at room temperature and allowed to stand for 4 days at 4° C., at the end of which time they were analysed by paper chromato-electrophoresis[1] for the percentage of insulin-^{131}I bound to antibody. Briefly, this determination depends on the adsorption of unbound ('free') insulin-^{131}I to the paper at the site of application[1,5] and the migration of antibody-bound insulin-^{131}I with the inter β-γ globulins[1,6]. The ratio of bound to free insulin-^{131}I is plotted as a function of the concentration of unlabelled insulin present in the mixtures.

Many antisera react quite similarly with crystalline beef, pork and sperm whale insulins. Antiserum from subject *Mo* (Fig. 1) is typical of this group of cases in which not more than about 1·5 times as much sperm insulin as pork insulin was required to reduce the ratio of bound to free insulin-^{131}I for beef insulin-^{131}I to any specified level. Similar results have been observed with antiserum from a guinea pig immunized with beef insulin. In contrast to this relative indifference to the species from which the insulin comes are the results obtained with antisera *Le* (Fig. 2, upper) and *Ge* (Fig. 2, lower), which discriminate sperm whale insulin sharply from pork insulin. More than 150 times as much sperm whale insulin as pork insulin was required to reduce the ratios of bound to free insulin-^{131}I (beef insulin) to

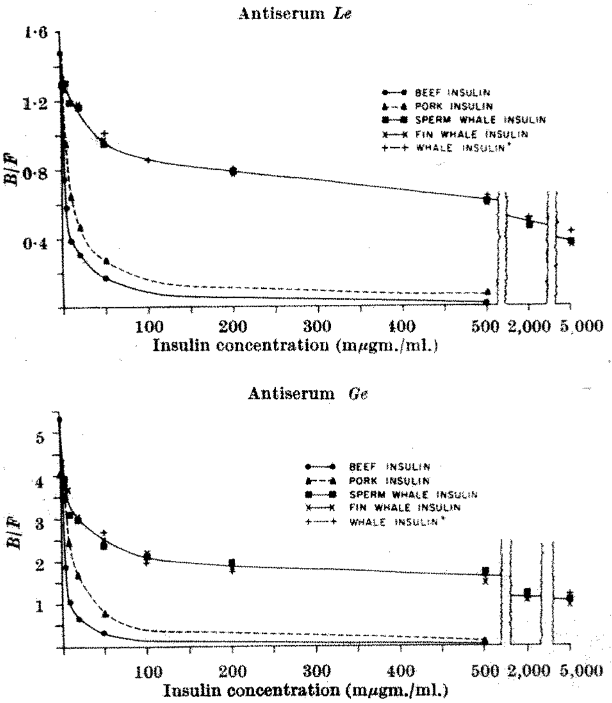


Fig. 2. Ratio of antibody-bound beef insulin-131 to free beef insulin-131 (*B*/*F*) as a function of the concentration of unlabelled crystalline insulins from various animal species. Upper: 1 : 60 dilution of antiserum *Le*; lower: 1 : 400 dilution of antiserum *Ge*. Whale insulin—unidentified as to species, see text

certain levels in *Le* and more than 100 times as much in *Ge*.

Experiments illustrated in Figs. 1 and 2 were carried out simultaneously with the same preparations and the results with antisera *Le* and *Ge* were confirmed repeatedly in several experiments with different preparations of insulin-^{131}I and different dilutions of antiserum and concentrations of insulin.

The differences observed between sperm whale and pork insulins in antisera *Le* and *Ge* can clearly not be attributed to any inherent inactivity or other anomaly of the sperm whale insulin crystals, in view of the essential similarity of reactions of the two insulins in many antisera as typified by antiserum *Mo* (Fig. 1). Furthermore, the procedure employed in purification of the insulin appears to be of no consequence, since three different preparations of beef insulin have been shown to react identically with an antiserum that clearly recognized species differences[2].

Fig. 2 also shows results with fin whale insulin and with a 'whale' insulin kindly supplied by Dr. A. M. Fisher, Connaught Laboratories, Toronto. The latter is not unequivocally identified as to species but may have been the same preparation analysed by Sanger and colleagues. The similarity of reactions of sperm whale and fin whale insulins suggests that these two insulins may have the same structure, but does not establish their identity with respect to amino-acid sequence since the immunochemical reaction does not always differentiate clearly between insulins with different sequences (cf. ref. 2 and Fig. 1). However, the marked discrimination between sperm whale and pork insulins by certain antisera is of considerable interest and would appear to provide the first evidence that two different proteins with identical amino-acid sequences (assuming the absence of any D-amino-acid configurations) may yet possess differences in secondary or tertiary structure. However, the observed species specificities entail differences in the standard free-energy change of binding of the order of only 3–4 k.cal. per mole, whereas the standard free-energy changes themselves are in the range of 8–12 k.cal. per mole[7]. Thus, a relatively small distinction in the structure of whale insulin at a critical site might well account for the lesser degree of complementarity to certain antibodies revealed by these investigations. If confirmed by more direct physico-chemical work, these findings can only mean that the overall protein structure is not determined by amino-acid sequence alone.

Finally, the possible significance of these observations to concepts of genetic transmission of inherited characteristics of protein synthesis is not to be overlooked. It would seem that, in addition to amino-acid sequence, the precise configuration of folding of the protein molecule is determined by the genetic apparatus.

We are indebted to Dr. O. Behrens, Lilly Research Laboratories, for supplies of crystalline beef and pork insulins and to Dr. T. Shibata, Taiyo Fishery Co., Yokosuka, Japan, through the kindness of Dr. Y. Ishihara, Hokkaido University, Japan, for supplies of crystalline sperm whale and fin whale insulins.

SOLOMON A. BERSON
ROSALYN S. YALOW

Radioisotope Service,
Veterans Administration Hospital,
Bronx, N.Y.

[1] Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, M. A., and Newerly, K., *J. Clin. Invest.*, **35**, 170 (1956).

[2] Berson, S. A., and Yalow, R. S., *J. Clin. Invest.*, **38**, 2017 (1959).

[3] Harris, J. I., Sanger, F., and Naughton, A., *Arch. Biochem. Biophys.*, **65**, 427 (1956).

[4] Ishihara, Y., Saito, T., Ito, Y., and Fujino, M., *Nature*, **181**, 1468 (1958).

[5] Kallee, E., *Z. Naturf.*, **7b**, 661 (1952).

[6] Berson, S. A., and Yalow, R. S., *J. Clin. Invest.*, **36**, 642 (1957).

[7] Berson, S. A., and Yalow, R. S., *J. Clin. Invest.*, **38**, 1996 (1959).

Biosynthesis of Hydrastine

LITTLE experimental work has been done on the biosynthesis of the phthalideisoquinoline, the benzylisoquinoline, and the protoberberine alkaloids. All these should be derived, according to biogenetic hypothesis, from two units of tyrosine[1] or two units of shikimic acid[2].

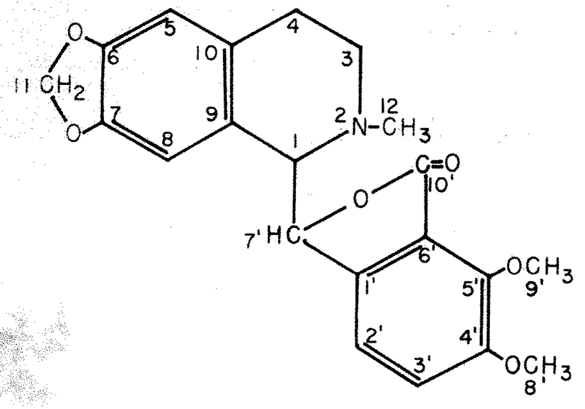
In extracts of *Berberis vulgaris* L. radioactivity from [2-^{14}C]-phenylalanine has been qualitatively detected by autoradiography in a chromatographic spot attributed to berberine[3]. In *Coptis japonica* Makino, DL-[2-^{14}C]-tyrosine gave rise to berberine containing radioactivity of unknown distribution in the nucleus of the alkaloid[4]. Activity from L-[U-^{14}C]-tyrosine was incorporated into papaverine, narcotine and narcotoline in *Papaver somniferum* L.[5]. The distribution of carbon-14 suggested that narcotoline was derived from two tyrosine units, but the evidence was indicative rather than conclusive. In the only detailed investigation to date Battersby and Harper[6] showed by unambiguous degradation of labelled papaverine obtained from DL-[2-^{14}C]-tyrosine in *Papaver somniferum* L. that two units of precursor were incorporated into the alkaloid without randomization.

We have examined the biosynthesis of hydrastine (I) in *Hydrastis canadensis* L. Mature plants were grown hydroponically in aerated nutrient solution[7]

Table 1

	Precursor		Duration of feeding (days)	Products			
				Hydrastine		Berberine	
	Total activity (counts/min.)	Specific activity (counts/min./mM)		Weight (gm.)	Specific activity (counts/min./mM)	Weight (gm.)	Specific activity (counts/min./mM)
DL-[2-^{14}C]-tyrosine	$8 \cdot 68 \times 10^7$	$5 \cdot 15 \times 10^8$	17	0·154	$5 \cdot 17 \times 10^4$	0·330	$12 \cdot 4 \times 10^4$
DL-[2-^{14}C]-tyrosine	$11 \cdot 0 \times 10^7$	$6 \cdot 55 \times 10^8$	8	0·128	$4 \cdot 47 \times 10^4$	0·172	$6 \cdot 52 \times 10^4$
DL-[2-^{14}C]-phenylalanine	$8 \cdot 87 \times 10^7$	$4 \cdot 18 \times 10^8$	8	0·405	$0 \cdot 06 \times 10^4$	0·472	$0 \cdot 42 \times 10^4$
D-[U-^{14}C]-glucose	$8 \cdot 75 \times 10^7$	$8 \cdot 75 \times 10^8$	17	0·506	$0 \cdot 41 \times 10^4$	0·612	$1 \cdot 27 \times 10^4$

containing DL-[2-^{14}C]-tyrosine. Hydrastine and berberine were extracted from the roots. Chemical and radioactive yields are recorded in Table 1. In parallel experiments DL-[2-^{14}C]-phenylalanine and D-[U-^{14}C]-glucose were administered. Radioactive incorporation of these into the alkaloids, also summarized in Table 1, was much less efficient. These results indicate that conversion of phenylalanine to tyrosine is minimal in *Hydrastis* (cf. ref. 8), and suggest that Wenkert's biogenetic hypothesis[2] is not valid in this case.



(I)

Hydrastine from plants grown with tyrosine-^{14}C for 17 days was degraded to locate the radioactivity, which was found, in accord with classical[1], but contrary to Wenkert's[2], biogenetic prediction, exclusively at carbon atoms 1 and 3. Oxidation gave inactive opianic acid (atoms 1′–10′) and active hydrastinine (atoms 1–12). The latter underwent Hofmann degradation to yield hydrastal (atoms 1, 3–11), which was oxidized to 6-vinylpiperonylic acid (atoms 1, 3–11). No loss of radioactivity occurred in these steps. Bromodecarboxylation, accompanied by modification of the vinyl group, gave radioactive 6-bromo-α-hydroxyhomopiperonylbromide (atoms 3–11) and radioactive carbon dioxide (atom 1). The latter was collected as barium carbonate, and then regenerated and converted to benzoic acid by treatment with phenylmagnesium bromide. The homopiperonylbromide derivative was oxidized to 6-bromopiperonylic acid (atoms 4–11), which contained little radioactivity. Radiocarbon was thus confined to carbons 1 and 3 of the hydrastine skeleton. The activities found in hydrastine and its degradation products are summarized in Table 2. The degradation sequence clearly demonstrates that tyrosine serves as a specific precursor of hydrastine, and that, as predicted, two tyrosine units participate in the biosynthesis of the alkaloid.

Table 2

Compound	Atoms of hydrastine	Specific activity (counts/min./mM)	Relative activity
Hydrastine		$5 \cdot 17 \times 10^4$	100
Hydrastinine	1–12	$5 \cdot 10 \times 10^4$	99
Opianic acid	1′–10′	$0 \cdot 04 \times 10^4$	< 1
Hydrastal	1, 3–11	$5 \cdot 16 \times 10^4$	100
6-Vinylpiperonylic acid	1, 3–11	$5 \cdot 02 \times 10^4$	97
6-Bromo-α-hydroxy-homopiperonyl bromide	3–11	$3 \cdot 20 \times 10^4$	62
Benzoic acid	1	$2 \cdot 00 \times 10^4$	39
6-Bromopiperonylic acid	4–11	$0 \cdot 14 \times 10^4$	< 3

The most interesting feature of these results, however, is the unequal labelling of carbon atoms 1 and 3 of hydrastine (relative activity 39 and 60 respectively), indicating differential utilization of radioactive, but not of chemical, precursor for the two segments of the alkaloid molecule. Such a possibility has never been considered, and it has hitherto always been tacitly assumed that biosynthetic utilization of two units of a single radioactive precursor to form the major portion of an end-product would inevitably be associated with equal labelling in the two segments of the latter. Reported work on papaverine[6] and morphine[9] has tended to support this assumption, which, nevertheless, is not generally valid. Equal incorporation of radioactivity into the two segments of such a 'dimeric' product is obligatory only if the 'doubling' step in the biosynthetic sequence is of the type $2A \rightarrow A'-A'$, that is, if two identical units of 'monomeric' precursor combine to yield a true dimer as a biosynthetic intermediate (direct doubling).

If the 'doubling' step were preceded by one or more dissimilar structural modifications of either or both 'monomer' units, such as $A \rightarrow X$, $A \rightarrow Y$: $X + Y \rightarrow X'-Y'$ (indirect doubling), then the ratio of radioactivities in the two segments of the product will depend on a number of factors. The most important of these are (*a*) the number of metabolic steps in each branch of the sequence prior to 'doubling', (*b*) the rate of each of these steps, (*c*) the size of the metabolic pool of each branch intermediate, (*d*) possible utilization of branch and 'dimeric' intermediates for other metabolic processes (leakage), (*e*) possible feed-in of branch intermediates from other, non-radioactive, precursors, and (*f*) the turn-over rate of the product. Equal incorporation into both segments of the product will be approached in a single-dose feeding experiment at infinite time, provided there is no leakage or feed-in of branch-precursors, no leakage of 'dimeric' intermediates, and provided the product continuously accumulates. Unless these restrictions hold, any ratio of radioactive incorporation in the two segments of the product, including a fortuitous ratio of one, is possible. The ratio will vary continuously with time, and its numerical value in any single experiment will depend on the length of time allowed to elapse between initial administration of the tracer and harvesting of the plant.

Although the major portions of each of the two segments of hydrastine (atoms 3–10, and 1, 1′–7′ respectively) are now shown to be derived from a tyrosine unit, the two fragments differ in oxygen substitution (atoms 11, and 8′–9′ respectively). In addition, a one-carbon unit (atom 10′) is required to complete the skeleton of the alkaloid. Any or all of these modifications of the tyrosine units may take place either before or after the 'doubling' step.

Work on the variation of the isotope ratio of the segments with time is proceeding and may throw further light on the dynamic aspects of alkaloid biosynthesis in *Hydrastis*. The systematic degradation of the radio-berberine, isolated from the present feeding experiments, will also contribute to the clarification of the problem.

J. R. Gear
I. D. Spenser

McMaster University,
Hamilton, Ontario.

[1] Robinson, R., in *The Structural Relations of Natural Products*, 78 (Clarendon Press, Oxford, 1955).

[2] Wenkert, E., *Experientia*, **15**, 165 (1959).

[3] Beal, J. L., and Ramstad, E., *Naturwiss.*, **47**, 206 (1960).

[4] Imaseki, I., Oneyama, R., and Tajima, M., *J. Pharm. Soc. Japan*, **80**, 1802 (1960).

[5] Kleinschmidt, G., and Mothes, K., *Z. Naturforsch.*, **14**, b, 52 (1959).

[6] Battersby, A. R., and Harper, B. J. T., *Proc. Chem. Soc.*, 152 (1959).

[7] Leete, E., Marion, L., and Spenser, I. D., *Canad. J. Chem.*, **32**, 1116 (1954).

[8] McCalla, D. R., and Neish, A. C., *Canad. J. Chem.*, **37**, 531 (1959).

[9] Battersby, A. R., and Harper, B. J. T., *Chem. and Indust.*, 364 (1958). Leete, E., *J. Amer. Chem. Soc.*, **81**, 3948 (1959). Battersby, A. R., Binks, R., and Le Count, D. J., *Proc. Chem. Soc.*, 287 (1960).

Ultracentrifugal Differences in Soybean Protein Composition

Soybeans vary widely in size, shape, colour, and chemical composition[1]. Their chemical composition is affected by varietal and environmental factors, and large differences in gross chemical composition are reported. For example, the protein content for 10 varieties over a 5-year period ranged from 36·6 to 53·2 per cent[2].

Little is known concerning the possible variation of the individual components within the protein fraction. The existence of such differences has been suggested on the basis of amino-acid analysis of various soybean protein preparations[3–6], but more direct evidence is desirable. Our results with ultracentrifugal analysis indicate more definitely that some soybean samples differ appreciably in protein composition.

Soybean proteins were extracted at room temperature from 3·0 gm. of dehulled, hexane-defatted flakes by stirring for 30 min. with 100 ml. of potassium phosphate–sodium chloride buffer, *p*H 7·6, $\mu = 0·5$, 0·01 *M* with respect to mercaptoethanol[7]. After centrifuging, the insoluble residue was re-extracted twice for 15 min. with 50-ml. portions of the same solvent. The three extracts were combined, concentrated by pervaporation, and equilibrated against the extraction buffer by dialysis prior to ultracentrifugal analysis.

Fig. 1*A* is the ultracentrifugal pattern for the unfractionated proteins of Clark soybeans grown in 1958 near Farmer City, Illinois. Four fractions are present with sedimentation constants of about 2, 7, 11, and 15*S* and the pattern resembles those recorded earlier[7,8]. Two other American varieties grown in Iowa gave similar patterns.

Fig. 1*B* is the ultracentrifuge pattern for the proteins from Hakuhou No. 1 soybeans grown in 1959 at Miyagi Experiment Station, Miyagi, Japan. (We are indebted to T. Watanabe, Food Research Institute, Tokyo, for supplying the samples of Japanese soybeans.) Again four sedimenting fractions are observed, but there are large differences in the percentages of the 7*S* and 11*S* fractions. Also, the 15*S* fraction is reduced in concentration and is less evident as compared with that fraction in the Clark variety. Patterns similar to Fig. 1*B* were obtained for two other soybean varieties grown at Miyagi. The distribution of components shown in Fig. 1*B* was unchanged when proteins from Japanese soybeans were extracted with water and analysed in the extraction buffer. Furthermore, the ratio of 7–11*S* fraction was not altered in the globulin fraction obtained by acidifying an aqueous extract of soybean meal to *p*H 4·5.

Extractability of soybean proteins depends on variety, age of beans, and possibly location of growth[9]. However, the differences between Clark and Hakuhou No. 1 soybeans cannot be due to differential extraction of the 7*S* or 11*S* fractions since more than 95 per cent of the nitrogenous components were extracted from each sample.

Similarity of the three soybean varieties grown in the same location in Japan tentatively suggests that the differences observed in Fig. 1 are caused by environmental factors.

Variations in protein composition have been detected in corn[10], wheat[11], and barley [12] by a number of methods, including isolation of individual fractions, electrophoresis, and ultracentrifugation. So far as we know, the data we report are the first direct evidence that similar variations occur in soybeans. Further

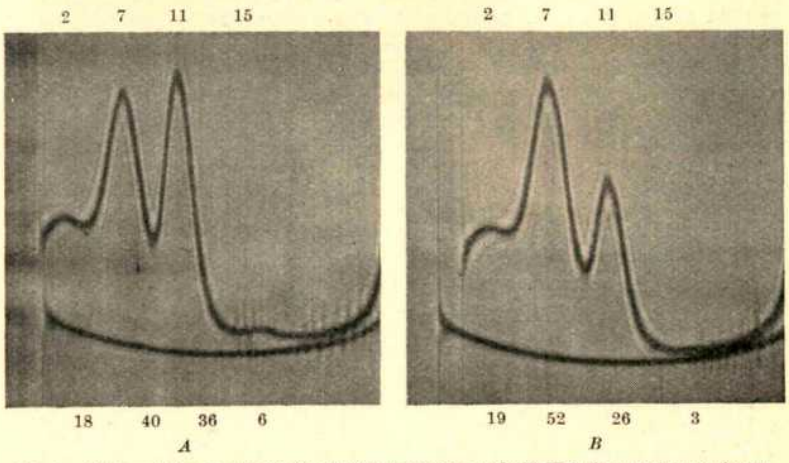


Fig. 1. Ultracentrifugal patterns for (*A*) Clark variety and (*B*) Hakuhou variety soybeans. Numbers across the top of the patterns give sedimentation constants in Svedberg units, across the bottom, the percentage composition. Compositions were determined by area analyses which are corrected for radial dilution. Solutions were analysed in a 30-mm. double-sector cell at 47,660 r.p.m. Protein concentrations ($N \times 6·25$) in gm./100 ml. were (*A*) 1·53 and (*B*) 1·24

work is under way with a number of Japanese and American varieties of soybeans to determine the effects of variety and location of growth on protein composition.

W. J. WOLF
G. E. BABCOCK
A. K. SMITH

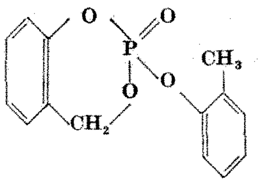
Northern Utilization Research and
Development Division,
Agricultural Research Service,
U.S. Department of Agriculture,
Peoria, Illinois.

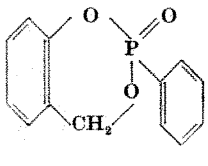
[1] Markley, K. S., ed., *Soybeans and Soybean Products*, **1** (Interscience Publishers, New York, 1950).
[2] Cartter, J. L., and Hopper, T. H., *U.S. Dept. Agric. Tech. Bull.* 787, 66 (1942).
[3] Csonka, F. A., and Jones, D. B., *J. Agric. Res.*, **46**, 51 (1933).
[4] Krober, O. A., *J. Agric. Food Chem.*, **4**, 254 (1956).
[5] Van Etten, C. H., Hubbard, J. E., Mallan, J. M., Smith, A. K., and Blessin, C. W., *J. Agric. Food Chem.*, **7**, 129 (1959).
[6] Belikov, I. F., and Tyuleneva, N. P., *Masloboino-Zhirovaya Prom.*, **25**, No. 10, 19 (1959).
[7] Wolf, W. J., and Briggs, D. R., *Arch. Biochem. Biophys.*, **85**, 186 (1959).
[8] Naismith, W. E. F., *Biochim. Biophys. Acta*, **16**, 203 (1955).
[9] Smith, A. K., Belter, P. A., and Johnsen, V. L., *J. Amer. Oil Chemists' Soc.*, **29**, 309 (1952).
[10] Bressani, R., and Mertz, E. T., *Cereal Chem.*, **35**, 227 (1958).
[11] Jones, R. W., Taylor, N. W., and Senti, F. R., *Arch. Biochem. Biophys.*, **84**, 363 (1959).
[12] Jensen, R., *Proc. European Brewery Convention Congr.*, Nice, 1953, 74.

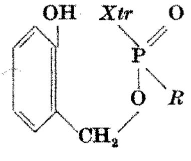
Biological Activity of a Tri-o-Cresyl Phosphate Metabolite

TRI-*o*-CRESYL PHOSPHATE (TOCP) is metabolized *in vitro* and *in vivo* to form potent esterase inhibitors[1–5]. The nature and biological activity of the metabolites were investigated.

Rats administered radiophosphorus-labelled TOCP were found to excrete radiolabelled di-aryl phosphates, mono-aryl phosphates and phosphoric acid in the urine. Three anti-esterase metabolites were present in the liver, intestine and fæces. Incubation of TOCP with rat liver microsomes fortified with reduced diphosphopyridine nucleotide also yielded three esterase inhibitors of similar chromatographic characteristics to those produced *in vivo*. The major esterase-inhibiting metabolite formed *in vivo* was isolated on silicic acid–'Celite' columns with benzene–ether mixtures. Examination of the infra-red spectrum and hydrolysis products suggested structure I for this metabolite. Reaction of *o*-cresylphosphoryl dichloride with *o*-hydroxybenzyl alcohol yielded 2-(*o*-cresyl)-4H-1,3,2-benzodioxaphosphoran-2-one, b.p. 159–161° C./0·09–0·1 mm. mercury, $n_D^{24.5^\circ} = 1{\cdot}5584$. This compound was identical with the major metabolite in respect to infra-red spectrum, chromatographic characteristics, and anti-esterase activity. One of the other esterase inhibitors appeared to be the *o*-hydroxymethyl derivative of I. The mechanism of esterase inhibition was investigated by reaction of chymotrypsin (*Xtr*) with I and a phenyl phosphonate analogue, II. Both appeared to phosphorylate by opening the cyclic phosphate structure at the P—O— aryl bond to yield III.


I


II



III

An ataxia and demyelination is induced in hens and certain other species by TOCP[6–9]. A similar ataxia in hens resulted from subcutaneous administration of 4–8 mgm./kgm. I, whereas 240 mgm./kgm. II failed to yield ataxia. Preliminary studies indicate a demyelination with I similar to that induced by TOCP. The high activity of I relative to that of TOCP in producing neurotoxicity in hens suggests that the anti-esterase metabolites rather than TOCP *per se* yield the ataxia. TOCP and other ataxia-producing phosphates are known to effect prolonged *in vivo* inhibition of the pseudocholinesterases of hen brain, spinal cord and sciatic nerve[10–13]. The function of this pseudocholinesterase which hydrolyses carboxylic esters of choline is unknown. Experiments with hens established that it also hydrolyses carboxylic esters of thiamine, that is, *O*-acetyl and *O*-propionyl thiamine, an observation consistent with previous reports on the substrate specificity of cholinesterases[14]. The demyelination in hens produced by TOCP is identical with that with certain other organophosphates, and similar lesions appear in hens on a thiamine-deficient diet[8]. Thiamine has been noted as a neuroactive material in peripheral nerves[15] and as a constituent of myelin in peripheral nerves of rabbits[16]. Inhibition of the pseudocholinesterase might result in an accumulation of carboxylic esters of thiamine to impair localized functions of the nerve requiring free thiamine or other thiamine derivatives.

In accordance with this hypothesis, thiamine and other compounds were tested extensively for their effect on the ataxia in hens from TOCP and/or I. Compounds tested alone or in conjunction with thiamine included *O*-acetyl thiamine, tocopherol and cortisone and their acetates, pilocarpine, saponin, and several aldoxime-type cholinesterase reactivators. With varying dosages and multiple administration of these materials before and after the organophosphate, no relief of the neurotoxic symptoms was evident.

TOCP potentiates the toxicity to mammals of malathion (*O,O*-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate)[4,17]. The intraperitoneal LD_{50} for mice was 150 mgm./kgm. for each of I and II. Simultaneous injection of malathion (LD_{50} alone of 1,500 mgm./kgm.) in a 10 : 1 ratio with the cyclic phosphates yielded LD_{50} values of about 1/45th those of either component alone. I and II were similar in this respect, and both yielded marked *in vivo* inhibition of the esterases of plasma and liver hydrolysing the carbethoxy groupings of malathion. The activity of these two compounds in malathion potentiation was greater than that of 120 other di-aryl and tri-aryl phosphates studied which lack the cyclic phosphate structure. The pattern of esterase inhibition in mice resulting from TOCP administration[4] also appeared with I, although the latter acted much more quickly and at a much lower dose.

Metabolic activation occurs with many tri-aryl phosphates containing *o*-methyl, *o*-ethyl, *o*-*n*-propyl and *p*-ethyl groupings[4,5]. *In vivo* hydroxylation of the *o*-methyl and subsequent cyclization to anti-esterase metabolites appears to account for the biological activity of TOCP and certain other tri-aryl phosphates containing *o*-methyl groupings. A similar mechanism involving α-hydroxylation and cyclization might explain the conversion of *o*-ethyl and *o*-*n*-propyl derivatives to anti-esterases. A different mechanism must be involved with tri-*p*-ethylphenyl phosphate.

Publication of this communication is approved by the Director of the Wisconsin Agricultural Experiment Station. The investigation was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation, and by grants from the U.S. Public Health Service, National Institutes of Health (contract *RG*-5304), the U.S. Army Chemical Research and Development Laboratories (grant No. *DA-CML*-18-108-61-*G*-6), and the U.S. Atomic Energy Commission (contract No. *AT* (11-1)-64, Project No. 14).

JOHN E. CASIDA
MORIFUSA ETO*
RONALD L. BARON

Department of Entomology,
University of Wisconsin,
Madison 6, Wisconsin.

* Permanent address: Department of Agricultural Chemistry, Kyushu University, Fukuoka, Japan.

[1] Aldridge, W. N., *Biochem. J.*, **56**, 185 (1954).
[2] Myers, D. K., Rebel, J. B. J., Veeger, C., Kemp, A., and Simons, E. G. L., *Nature*, **176**, 260 (1955).
[3] Davison, A. N., *Biochem. J.*, **61**, 203 (1955).
[4] Casida, J. E., *J. Biochem. Pharmacol.*, **5**, 332 (1961).
[5] Aldridge, W. N., and Barnes, J. M., *J. Biochem. Pharmacol.*, **6**, 177 (1961).
[6] Smith, M. I., and Lillie, R. D., *Arch. Neurol. Psychiat.* (Chicago), **26**, 976 (1931).
[7] Smith, M. I., Engel, E. W., and Stohlman, E. F., *Nat. Inst. Hlth. Bull.*, **160**, 1 (1932).
[8] Barnes, J. M., and Denz, F. A., *J. Path. Bact.*, **65**, 597 (1953).
[9] Geoffroy, H., Slomic, A., Benebadji, M., and Pascal, P., *World Neurology*, **1**, 294 (1960).
[10] Earl, C. J., and Thompson, R. H. S., *Brit. J. Pharmacol.*, **7**, 261, 685 (1952).
[11] Davison, A. N., *Brit. J. Pharmacol.*, **8**, 212 (1953).
[12] Thompson, R. H. S., *Chem. and Indust.*, 749 (1954).
[13] Witter, R. F., and Gaines, T. B., *Fed. Proc.*, **19**, 275 (1960).
[14] Augustinsson, K.-B., *Acta Physiol. Scand.*, **15**, Supp. 52, 1 (1948).
[15] Muralt, A. von, *Vitamins and Hormones*, **5**, 93 (1947).
[16] Sjöstrand, F., *Nature*, **157**, 698 (1946).
[17] Murphy, S. D., Anderson, R. L., and DuBois, K. P., *Proc. Soc. Exp. Biol.*, **100**, 483 (1959).

Isolation of Sterol Esters from Human Fæces

THE importance of obtaining more precise information regarding the sterol excretion pattern in human beings has recently become apparent and has been emphasized by Aylward[1]. The normal clinical laboratory techniques for stools, namely extraction of dried material or wet extraction coupled with saponification make impossible the isolation of any sterol esters which may be present. This difficulty in isolating (as distinct from estimating) sterol esters was indeed common to all lipid extracts until the methods developed by Börgstrom[2], and others, were applied by Fillerup and Mead[3] to extracts from animal tissues. Using silicic acid, they were able for the first time to separate cholesterol esters from triglycerides. We have applied a modification of this technique to the lipids of human fæces and have been able to isolate cholesterol ester fractions; and, using gas-liquid chromatography, have examined the fatty acids present in the esters.

Because the amount of sterol esters in human fæces is very small, it was necessary to extract large quantities of material. Accordingly, collections from five volunteers were arranged for periods of 6–9 days, and the extraction procedure was commenced immediately on the receipt of each sample. Preliminary experiments indicated that fæcal pigments interfered with the chromatographic separations, and it was therefore decided to extract the stools first with 0·9 per cent saline, a procedure which removes some of the bacterial lipids[4]. The solid obtained by centrifugation was then extracted successively by ethanol, ether and petroleum ether; precautions were taken to minimize oxidation by carrying out the procedures, wherever possible, under oxygen-free nitrogen.

Table 1. COMPONENTS OF PETROLEUM ETHER-SOLUBLE FRACTION FROM HUMAN FÆCES, CHROMATOGRAPHED ON SILICIC ACID (ref. 5)

Fraction	Case 1	Case 2	Case 3	Case 4	Case 5
Hydrocarbons and pigments	8·8	5·2	10·8	Nil	6·8
Sterol esters	3·5	7·1	2·0	1·0	13·0
Triglycerides	2·1	7·1	2·0	0·6	3·4
Sterols	77·1	76·7	65·4	93·6	62·0
Diglycerides, monoglycerides, phospholipids and pigment	8·5	3·9	19·8	4·8	14·7

In order to increase the proportion of sterol ester in the petroleum ether fraction to be chromatographed, the free fatty acids were first removed by washing with 1 per cent sodium carbonate solution; the extract remaining was dissolved in the minimum amount of hexane and fractionated on a column of Mallinckrodt silicic acid, the ratio of adsorbent: extract being kept at about 100 : 1·5. Pre-treatment of the silicic acid and the elution system followed Barron and Hanahan[5]. Positive oxygen-free nitrogen pressure was maintained, 25 ml. fractions were collected, and elution of the sterol esters was judged to be complete when a negative Liebermann–Burchard test was given. The solvent from the fractions was removed at 40° C. by means of a vacuum oven, the fractions were weighed, and the residues were dissolved in petroleum ether and combined according to the peaks shown when the weighed residues were graphed.

Neither free fatty acid nor free sterol was present in the sterol ester fraction, and the weight of the fraction agreed with the estimations of sterol ester made on the original petroleum ether-soluble fraction by a colorimetric method. Table 1 shows the amount of sterol esters isolated by this method, calculated as a percentage of the total material recovered.

The acids obtained by saponification of the sterol esters from extracts 1, 2 and 5 were methylated and submitted to gas-liquid chromatography, diethylene glycol succinate, ethylene glycol adipate, silicone oil, and dimethylchlorosilane being the stationary phases. The fatty acids present in the extract in the unesterified form, and those from the sterol ester fraction, were markedly similar in type and distribution and ranged from C12–C20 +, saturated and unsaturated.

We thank Mr. J. Piercy and Drs. Raymond Greene and D. S. Rideout of the New End Hospital for the facilities provided, and the Nuffield Foundation for a research grant (to P. W.).

FRANCIS AYLWARD
PAMELA WILLS

Department of Chemistry and Food Technology,
Borough Polytechnic,
London, S.E.1.

[1] Aylward, F., *Lancet*, ii, 852 (1958).
[2] Börgstrom, B., *Acta Physiol. Scand.*, **25**, 101 (1952).
[3] Fillerup, D., and Mead, J., *Proc. Soc. Exp. Biol. Med.*, **83**, 574 (1953).
[4] Hartman, L., Shorland, F. B., and Cleveley, B., *Biochem. J.*, **69**, 1 (1958).
[5] Barron, E. J., and Hanahan, D. J., *J. Biol. Chem.*, **231**, 493 (1958).

Effect of Ribonuclease and Deoxyribonuclease on Incorporation of Tritiated Pyrimidine-Nucleosides into Ribonucleic Acid and Deoxyribonucleic Acid in Human Cancer Cells (HeLa) in Culture

AUTORADIOGRAPHIC work in various laboratories on synthesis of nucleic acids in the intact cell has indicated that nuclear RNA is a precursor for cytoplasmic RNA, although different authors have placed different emphasis on the role of the nucleolus and chromatin portion[1–5]. It was suggested that RNA mediates incorporation of pyrimidine nucleotides into DNA, probably through an intermediate fraction[7,8]. In testing and extending these observations, the uptake of tritiated cytidine, uridine, thymidine and deoxyuridine into the various nucleotide fractions was examined for dependence on RNA-ase and DNA-ase, added to the cells in culture before, and at the same time as, exposure to the tritiated precursors.

Tritiated cytidine (specific activity 1 c./m.mole) and tritiated uridine (specific activity 0·62 c./m.mole) were used as common precursors for RNA and DNA at a concentration of 2 μc./ml. of medium. Tritiated thymidine (specific activity 1·9 c./m.mole) and tritiated deoxyuridine (specific activity 4·0 c./m.mole) as specific precursors for DNA at a concentration of 0·4 μc./ml. of medium. RNA-ase was obtained from the Nutritional Biochemicals Corporation in crystalline form, and DNA-ase from the California Corporation for Biochemical Research. Crystalline albumin was obtained from Armour Laboratories, and bovine serum from the Colorado Serum Co.

Leighton tube cultures of HeLa-S_3 cells, either with or without coverslips, containing about equal numbers of cells in the log phase of growth were used. Simultaneously with the addition of tritiated thymidine, or 90 min. before addition of tritiated thymidine, tritiated cytidine, tritiated deoxyuridine and tritiated uridine, the standard culture medium was changed to one supplemented with RNA-ase or DNA-ase (to make a final 0·1, 0·2 or 1 per cent solution), or one containing equivalent amounts of protein in the form of albumin or bovine serum, or one without supplement. The cultures in the various media were incubated at 37° for various lengths of time. At the end of the incubation period, the cells were washed, fixed and extracted differentially with perchloric acid to obtain the different nucleotide fractions (acid-soluble pool, RNA and DNA), or they were fixed with methanol and prepared for autoradiography with or without prior histochemical extraction of RNA with perchloric acid. Details of the methods used have been described in refs. 6 and 7.

Table 1 lists the total tritium activity of the separated nucleotide fractions, soluble pool, RNA and DNA, after 10 min. exposure to the tritiated precursors. The results show that the incorporation of tritiated thymidine and deoxyuridine is depressed in the HeLa cell by RNA-ase and to a smaller extent by DNA-ase. Tritiated cytidine and uridine incorporation into DNA is not inhibited and its incorporation into RNA is slightly enhanced by RNA-ase, in agreement with Ledoux's data on ascites tumour cells[9].

Similar results were obtained autoradiographically (grain counts) and will be reported later. The cells showed morphological changes when 0·2 per cent to 1 per cent RNA-ase was used, as a decrease in basophilic staining property in the cytoplasm as well as in the nucleoli, which often were distorted. The decreased basophilia might be explained in part by a complex formation of RNA with RNA-ase, thus preventing the basic stain from binding[10].

Incorporation of tritiated thymidine and tritiated deoxyuridine into the 'soluble pool' was inhibited by RNA-ase. Tritiated thymidine added to the cells simultaneously with the medium containing RNA-ase was more readily incorporated than when tritiated thymidine was given at 90 min. after addition of the medium containing RNA-ase to the cells. This in addition seems to indicate that the RNA-ase acts not primarily on the cell membrane but intracellularly[10]. The various effects reported for RNA-ase on cells might all be explained by an effect on RNA and not on enzymes, even if they are associated with microsomes[11], nor on energy-delivering reactions[10]. The RNA-ase used at present was tested in the fixed HeLa cell, and was shown to remove selectively RNA, leaving DNA intact.

If one assumes specificity of RNA-ase for RNA also in the living HeLa cell and despite the lack of further results on reactions in the soluble fraction, the inhibition of uptake of thymidine and deoxyuridine by RNA-ase in this work suggests that incorporation of those precursors into the nucleic acids in the HeLa cell may be mediated by, or depend on,

Table 1. TOTAL TRITIUM ACTIVITY OF NUCLEOTIDE FRACTIONS (SOLUBLE FRACTION, RNA AND DNA) IN COUNTS/MIN. AND AS PERCENTAGE OF CONTROL, AFTER 10 MIN. EXPOSURE OF THE CELLS TO DIFFERENT TRITIATED PYRIMIDINE NUCLEOSIDES IN REGULAR CULTURE MEDIUM AND IN MEDIUM CONTAINING RNA-ASE ,OF DNA-ASE, OR EQUIVALENT AMOUNTS OF PROTEIN AS BOVINE SERUM OR ALBUMIN. RESULTS ARE AVERAGES FROM DUPLICATE EXPERIMENTS

Precursor and time	Culture medium	Soluble pool (counts/min.)	Soluble pool Percentage control	RNA (counts/min.)	RNA Percentage control	DNA (counts/min.)	DNA Percentage control
Tritiated thymidine 90 min. after medium change	Control	468	100	—	—	1,053	100
	+ RNA-ase (0·2 per cent)	171	37	—	—	262	25
	+ DNA-ase (0·2 per cent)	317	68	—	—	594	56
Tritiated thymidine 90 min. after medium change	Control	1,836	100	—	—	4,718	100
	+ albumin	1,670	91	—	—	4,412	94
	+ serum	1,356	74	—	—	3,736	79
	+ RNA-ase (1·0 per cent)	189	10	—	—	408	9
Tritiated thymidine with medium change	Control	1,399	100	—	—	3,378	100
	+ albumin	1,266	90	—	—	2,449	72
	+ serum	1,278	91	—	—	2,520	75
	+ RNA-ase (1·0 per cent)	844	60	—	—	1,621	48
Tritiated deoxyuridine 90 min. after medium change	Control	210	100	17	100	110	100
	+ RNA-ase (o·2 per cent)	130	62	10	59	17	15
	+ DNA-ase (0·2 per cent)	92	44	15	88	51	46
Tritiated cytidine 90 min. after medium change	Control	27,771	100	14,172	100	526	100
	+ RNA-ase (0·2 per cent)	28,920	104	18,311	129	554	105
	+ DNA-ase (0·2 per cent)	30,131	108	14,830	105	476	90
Tritiated uridine 90 min. after medium change	Control	25,579	100	5,885	100	305	100
	+ RNA-ase (0·2 per cent)	29,623	116	8,046	137	334	109
	+ DNA-ase (0·2 per cent)	22,306	87	6,201	105	350	115

an RNA, probably in the soluble pool. RNA synthesis *de novo* apparently would not be dependent on this same RNA fraction, since tritiated cytidine and tritiated uridine incorporation was not depressed.

Since the labelling of DNA from tritiated cytidine and tritiated uridine was only slightly altered by RNA-ase and DNA-ase, the depression of the incorporation of tritiated thymidine and tritiated deoxyuridine apparently does not reflect a depression of the rate of DNA-synthesis.

This work was supported by the U.S. Atomic Energy Commission.

L. E. Feinendegen
V. P. Bond
R. M. Drew

Medical Research Centre,
Brookhaven National Laboratory,
Upton, New York.

[1] Goldstein, L., and Micou, J., *J. Biophys. Biochem. Cytol.*, **6**, 1 (1959).
[2] Woods, P. S., and Taylor, J. H., *Lab. Invest.*, **8**, 309 (1959).
[3] Fitzgerald, P. J., and Vinijchaikul, K., *Lab. Invest.*, **8**, 319 (1959).
[4] Amano, M., and Leblond, C. P., *Exp. Cell Res.*, **20**, 250 (1960).
[5] Feinendegen, L. E., in *Structure and Function of Genetic Elements, Brookhaven Symp. in Biology*, No. 12, 172 (1959).
[6] Feinendegen, L. E., Bond, V. P., Shreeve, W. W., and Painter, R. B., *Exp. Cell Res.*, **19**, 443 (1960).
[7] Feinendegen, L. E., Bond, V. P., and Painter, R. B., *Exp. Cell Res.*, **22**, 381 (1961).
[8] Feinendegen, L. E., Bond, V. P., and Hughes, W. L., presented at the first Intern. Congress of Histochemistry and Cytochemistry, Paris (1960).
[9] Ledoux, L., and Revell, S. H., *Biochim. Biophys. Acta*, **18**, 416 (1955).
[10] Brachet, J., *Biochem. Cytol.*, 264 (Academic Press, 1957).
[11] Novikoff, A. B., Ryan, J., and Pobder, E., *J. Histochem. Cytochem.*, **2**, 401 (1954).

Effect of Boric Acid on the Phosphoglucomutase of Pea Seeds

It has been suggested that a possible role of boron in higher plants is to activate the processes involved in the uptake and movement of sugars, and the manner of operation has been attributed to the facility with which boric acid forms complexes with the *cis*-hydroxyl groups of the sugar molecule. Dugger and Humphrey[1] suggest, as the result of assay of a comprehensive range of enzymes from pea seedlings, that boron inhibits sucrose synthesis from uridinetriphosphate, glucose-1-phosphate and fructose, and stabilizes the level of uridine diphosphoglucose as well as increasing the rate of its formation. They consider that boron inhibits starch phosphorylase, thus increasing the availability of glucose-1-phosphate for the formation of uridine diphosphoglucose. The transfer of the former through the normal glycolytic cycle is considered to be unaffected on the basis of assay of specific enzymes, including phosphoglucomutase. The phosphoglucomutase used was apparently prepared from muscle, and, in fact, it has been shown that low concentrations of boric acid specifically inhibit the phosphoglucomutase of pea seeds[2]. The enzyme can be purified by alcohol fractionation at −5° C. and obtained free of phosphorylase and phosphatases which utilize the substrate and products. The phosphoglucomutase activity is always associated with that of phosphohexoisomerase, but this enzyme has been found to be unaffected by boric acid. Fig. 1 shows the extent of the inhibition by a range of concentration in a preparation of this type. The fall in inorganic phosphorus + phosphorus released by hydrolysis at 100° C. in 1 *N* hydrochloric acid (free + 7′P) is used as a measure of formation of glucose-6-phosphate. When the purified system is used, no increase in inorganic phosphorus occurs during the time required for the system to reach equilibrium, and at this time chromatographic assay indicates the presence of only glucose-1-phosphate, glucose-6-phosphate and fructose-6-phosphate.

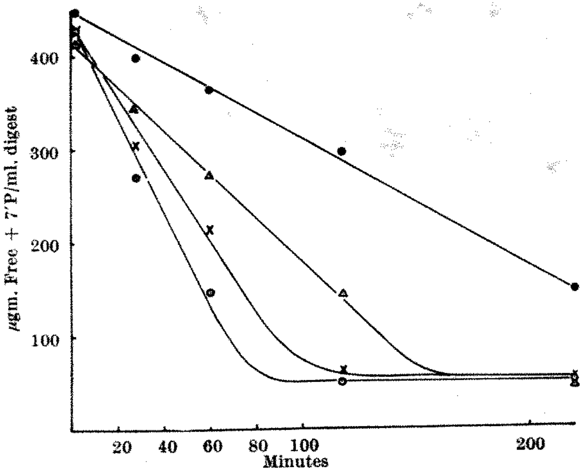


Fig. 1. Effect of boric acid on the activity of a purified phosphoglucomutase preparation from pea seeds. Digest components: glucose-1-phosphate, 13 μmoles/ml., magnesium chloride, 5 μmoles/ml.; glyoxaline/hydrochloric acid buffer *p*H 7·2, 20 μmoles/ml.; enzyme, 0·06 mgm. nitrogen/ml. Concentration of boric acid: 2×10^{-3} *M* (●); 5×10^{-4} *M* (Δ); 2×10^{-4} *M* (×); none (○)

The inhibition of phosphoglucomutase may be complex: for example, Fig. 2*a* shows that there may be an initial lag period, followed by an increase in rate though the final rate is still less than that in the control digests. This type of inhibition can best be explained on the assumption that the inhibitor is rendered ineffective by combination with one of the

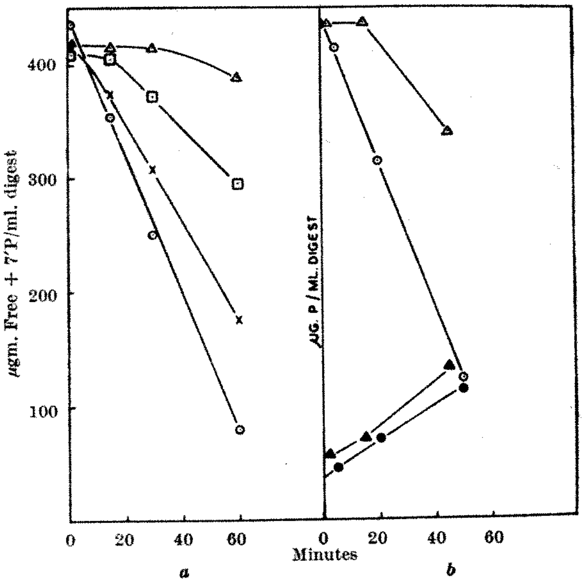


Fig. 2 (*a*) Effect of boric acid on the initial stage of utilization of glucose-1-phosphate by a purified phosphoglucomutase preparation. Main digest components as in Fig. 1. Concentration of boric acid: 5×10^{-3} *M* (Δ); 1×10^{-3} *M* (□); $2·5 \times 10^{-4}$ *M* (*X*); none (○). (*b*) Effect of boric acid on the utilization of glucose-1-phosphate by a crude extract of pea seeds (50 gm. dry seeds soaked and extracted with 100-ml. ice-cold 0·005 *M* neutral cysteine). Main digest components as in Fig. 1, enzyme extracts diluted four-fold. Concentration of boric acid: 1×10^{-3} *M* (▲ and Δ); none (● and ○). Open symbols (free + 7′P); filled symbols (free P)

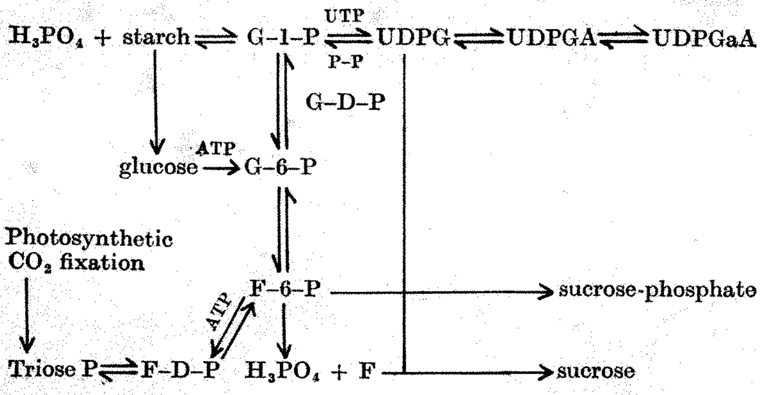


Fig. 3

products of the reaction. This enzyme system involves a low concentration of glucose-1,6-diphosphate as an essential component of the reaction, and on the basis of existing knowledge of the mechanism of action of the muscle enzyme it is likely that phosphorylation of the enzyme occurs; it is at this stage that boric acid possibly has its effect. The crude enzyme is also inhibited by boric acid under conditions in which the contaminating phosphorylase is unaffected as measured by increase in colour with iodine, and release of inorganic phosphate (Fig. 2*b*). The partially purified plant preparations contain appreciable quantities of inorganic orthophosphate as well as bound ketose, and the importance of these components is still under investigation.

The phosphoglucomutase activity is greatest at about *p*H 7·8, but similar percentage inhibitions occur with 0·002 *M* boric acid over a *p*H range of 5·6–8·0. At the *p*H normally used, namely, 7·2, 99 per cent of the added borate would exist as boric acid.

From these results it follows that if utilization of starch is blocked at the phosphoglucomutase stage, two immediate consequences are apparent:

(*a*) The formation of glucose-6-phosphate and hence fructose-6-phosphate and fructose can be prevented by boric acid, thus reducing the amount of fructose-6-phosphate and fructose available for the synthesis of sucrose. Since combined fructose is not normally stored in amounts comparable with glucose polysaccharides, it is reasonable to suppose that fructose arises primarily via the phosphorylated form by the normal glycolytic use of starch.

(*b*) More glucose-1-phosphate is available for synthesis of uridine diphosphoglucose, and in the absence of fructose acceptor it would either accumulate or be available for synthesis of other materials, for example galacturonic acid residues of the cell wall.

At this stage it would be premature to speculate further on the role of boron in higher plants. However, glucose-1-phosphate clearly occupies a key position in the utilization of hexoses, and the scheme of Fig. 3 indicates how the inhibition of phosphoglucomutase by boric acid could be used as a means of regulating closely the diversion of glucose-1-phosphate for different synthetic purposes. It is evident that the mechanism of action of the phosphoglucomutase of pea seeds is similar to that of the muscle enzyme which is unaffected by boric acid[1], so it is unlikely that the inhibiting action is brought about by the formation of complexes with one of the substrates. It is more reasonable to attribute the specific effect on the plant enzyme to the structure of the enzyme protein. The complex nature of the mechanism of action coupled with the type of inhibition shown in Fig. 2 makes kinetic studies difficult, and the elucidation of the type of inhibition involved is obviously necessary before an assessment of the importance of boric acid as a regulatory substance can be critically evaluated.

B. C. LOUGHMAN

Department of Agriculture,
University of Oxford.

[1] Dugger, jun., W. M., and Humphrey, T. E., *Plant Physiol.*, **35**, 523 (1960).

[2] Loughman, B. C., Ph.D. thesis, University of Cambridge (1953).

PHYSIOLOGY

Number and Arrangement of Osteons in Corresponding Regions of Homotypic Long Bones

TETRACYCLINES administered *in vivo* become incorporated into recently laid down rapidly calcifying bone-tissue; the labelled material can be detected by its fluorescence in ultra-violet light[1-5]. This technique represents a very valuable new tool in the investigation of bone growth and reconstruction.

The problem investigated here is whether it occurs, and what is the extent of structural symmetry, in various sectors and levels of the shaft cortex of homonymous long bones in the right and left side, in other words, in portions of osseous tissue which are largely submitted to equal mechanical stresses and undergo a series of parallel changes during bone growth and remodelling. In the present communication, results are reported on the distribution and number of new-forming Haversian systems (secondary or replacement osteons). This work is introductory to a study of the factors which govern bone

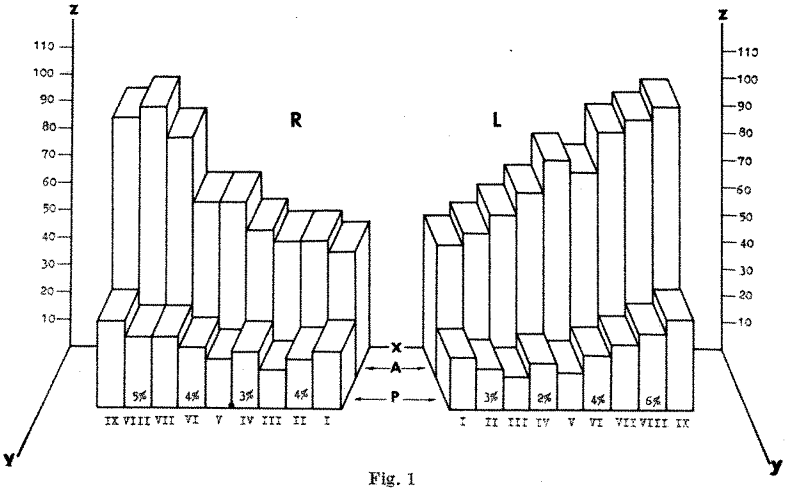


Fig. 1

reconstruction, and has also a practical meaning since the structure of the 'controlateral' bone is generally taken as the normal test of the bone submitted to experimental procedures.

Young growing and adult dogs were given *per os* demethylchlortetracycline ('Ledermycin') 30–40 mgm./kgm. body-weight for 3 days and killed 7 days later. Cross-sections of the entire diaphysis were taken from various long bones of both sides; care was taken to cut out the sections exactly at the same shaft-levels in each couple of homonymous bones. Generally, 5–15 planoparallel cross-sections 1 mm. apart, starting from the middle level of the diaphysis, were serially sawn and ground to a uniform thickness of about 60μ. Photomicrographs were made of each section in ultra-violet light (Ortholux Leitz microscope).

The distribution and number of the osteons labelled by a fluorescent ring were analysed in the photomicrographs of the shaft cross-sections, divided into four 90° quadrants. A rather close symmetrical correspondence in the arrangement of the newly formed osteons resulted in each of the four 90° quadrants of each couple of sections (right and left) at the same level. The 90° quadrants were further bisected and the distribution of the osteons analysed in each 45° sector of the left and right sections. Each 90° quadrant was divided also into an outer and an inner band by means of a line equidistant from the periosteal and the endosteal surfaces of the cortex. When comparing such sectors and zones in the right and left bone, a fairly close symmetry in the distribution of new-formed osteons was still apparent; such a correspondence was no longer found when comparing, on both sides, small areas of the sectors or zones.

The new-formed labelled osteons were counted in the corresponding 90° quadrants and 45° sectors of the right and left bone. The percentage differences in the number of labelled osteons between the right and left bone were as follows: 90° quadrants, max. 14·6 per cent—min. 0·87 per cent; 45° sectors, max. 28·5 per cent—min. 3·4 per cent. An analogous course in the variation of the number of the osteons was recorded in the corresponding 45° sectors of the two sides when studying the series of successive cross-sections from the middle shaft-level to the metaphysis.

An attempt was also made to assess the amount of bone tissue laid down in the osteons forming in corresponding sectors and levels of the right and left bone shafts during the period of tetracycline treatment. The labelled areas were cut out from enlargements (× 110) on photographic paper of constant known weight and their total weight computed as percentage of the weight of the entire area covered by bone tissue in the 45° sectors of the right and left side after removal from the photographs of the areas occupied by non-osseous material (for example, lumina of vascular canals and resorption cavities).

As an example, the results obtained from the analysis of an 18-mm. cylinder of the right and left tibial shaft in a 6-month-old dog are shown (Fig. 1). The number of labelled osteons in the left (L) and right (R) tibia is given as a function of the level of the section and of the position of the 45° sector. On the x axis are plotted the levels of the shaft cross-section in caudo-cranial order, commencing from the centre of the diaphysis (I, II, III, etc.); on the y axis are plotted A, the anterior, and P, the posterior 45° sectors of the 90° antero-medial quadrant; on the z axis are plotted the number of labelled osteons in each sector of every section. The per cent ratios between the amount of the new-formed labelled osseous tissue and all the bone material of the posterior sector P is reported only for four levels.

Altogether it seems that the differences in the distribution and number of Haversian systems (structures of the second order) forming during a given period of time in strictly corresponding regions of compacta of homotypic bones are not much more conspicuous than the differences in the shape and thickness of the cortex (structures of the first order) in the same regions.

G. Marotti

Institute of Human Anatomy,
University of Bari,
Policlinic, Viale Ennio, Bari.

[1] Milch, R. A., Rall, D. P., and Tobie, J. E., *J. Bone Joint Surg.* **40**, 897 (1958).
[2] Ghosez, J. P., *Arch. Biol.* (Liège), **70**, 169 (1959).
[3] Ponlot, R., *Le Radiocalcium dans l'étude des Os* (Ed. Arscia S. A., Bruxelles, 1960).
[4] Frost, H. M., and Villanueva, A. R., *Stain Tech.*, **35**, 135 (1960).
[5] Harris, W. H., *Nature*, **188**, 1038 (1960).

Effect of Adrenalectomy and Corticosteroids on Distribution of Radioactivity in Protein of Cell Fractions from Myocardial Slices

Adrenalectomy increases, and cortisone decreases, the incorporation of radioactivity from amino-acids labelled with carbon-14[1,2], and from amino-acid precursors[3], into the protein of isolated rat diaphragm. Implicit in these observations is the possibility that the adrenal steroids alter protein biosynthesis in muscle by an effect on the intracellular protein synthesizing machinery. The experiments to be described are part of an investigation of that possibility. Because of the relative ease with which myocardium is homogenized and fractionated that tissue was selected (in preference to skeletal muscle) for this work.

Adrenalectomy increased, and cortisone depressed, the incorporation of amino-acids into myocardial slices in a way, qualitatively and quantitatively, similar to their effects on skeletal muscle[1,3] (Table 1).

There is no reason to believe, then, that there is a fundamental difference in the effect of adrenal cortical hormone on protein biosynthesis in cardiac as compared with skeletal muscle, and myocardium would appear to be a suitable tissue in which to investigate the mechanism of the effect of corticosteroids on protein synthesis.

In an attempt to identify the locus at which corticosteroids influence protein biosynthesis, heart

Table 1. Effect of Adrenalectomy and Hydrocortisone on Incorporation of ^{14}C-Phenylalanine into Protein of Myocardial Slices

Experimental condition	Radioactivity incorporated into protein (counts/min./mgm.)
Intact	111 ± 9·5
Adrenalectomized	159 ± 6·7
Adrenalectomized, hydrocortisone, 0·4 mgm./day	123 ± 7·0
Intact, hydrocortisone, 4 mgm./day	77 ± 5·1

Male Sprague–Dawley rats of 200–300 gm. were used; adrenalectomy and hormone treatment were carried out as described before (refs. 1 and 3). Rats were decapitated, the hearts excised and sliced freehand on a cold glass surface; endocardial slices were discarded. Approximately 100–200 mgm. of tissue was incubated with 2 ml. Krebs–Henseleit bicarbonate medium, 1·5 mgm. glucose and 0·3 μc. 3-^{14}C-DL-phenylalanine (0·23 mM). Incubation was for 2 hr. at 37° C. in a Dubnoff shaker (152 cycles/min.); the gas phase was 95 per cent oxygen–5 per cent carbon dioxide. At the end of the incubation period the protein was isolated and its radioactivity determined (ref. 4). Each figure is the mean ± $S.E.$ of the mean of six observations. The effect of adrenalectomy ($P < 0·001$) and of 4 mgm./day of hydrocortisone ($P < 0·01$) are significant.

Table 2. DISTRIBUTION OF RADIOACTIVITY FROM ^{14}C-PHENYLALANINE IN PROTEIN OF CELL FRACTIONS FROM MYOCARDIAL SLICES: EFFECT OF ADRENALECTOMY AND CORTISONE (2 MGM./DAY)

Fraction	Condition	Radioactivity incorporated into protein (counts/min./mgm.)				
		0 min.	5 min.	15 min.	30 min.	60 min.
Slice	*I*	0	10	18	28	51
	A	0	8	28	53	97
	C	0	6	16	23	39
Cell debris	*I*	0	8	16	21	48
	A	0	10	24	46	79
	C	0	6	10	19	33
Mitochondria	*I*	1	5	15	31	40
	A	0	7	36	75	150
	C	1	6	12	28	32
Microsomes	*I*	0	8	23	45	80
	A	0	85	92	210	452
	C	0	6	13	28	70
Soluble	*I*	0	6	25	46	105
	A	0	17	54	116	242
	C	0	5	18	39	75

Approximately 500–700 mgm. of myocardial slices were incubated, for varying periods of time, in 5 ml. of Krebs–Henseleit medium containing 1·5 mgm./ml. of glucose and 0·1 μc./ml. of L-phenylalanine-U-^{14}C (0·01 m*M*). After incubation, two slices were removed for separate analysis and the remainder of the sample was fractionated at 0–4° by a modification of the method used by Zamecnik and Keller for liver (ref. 5). The tissue was minced in 0·35 *M* sucrose containing 0·01 *M* unlabelled DL-phenylalanine, homogenized at 1,200 r.p.m. for 10–15 sec. with a loose-fitting pestle (clearance 0·014–0·019 cm.) and for 20–30 sec. with a tight-fitting pestle (clearance 0·010–0·015 cm.). The homogenate was spun for 10 min. at 700*g* in a Servall *SS*-1 centrifuge. The supernatant was decanted and spun at 15,000*g* in a Spinco model *L* preparative ultracentrifuge for 30 min.; the 15,000*g* supernatant was decanted and spun again at 105,000*g*. The final soluble fraction (supernatant) was retained. The pellets obtained at 15,000 and 105,000*g* were rinsed and drained. Four fractions were obtained in this way: (1) at 700*g* a pellet consisting of unbroken cells, nuclei and myofibrils (hereafter referred to as 'debris'); (2) at 15,000*g* a fraction referred to as mitochondrial, although it is likely some small non-respiratory granules are also present; (3) at 105,000*g* the microsomes, and (4) a final soluble or supernatant fraction. Each figure is the average of two determinations. *I*, Intact; *A*, adrenalectomized; *C*, cortisone-treated.

slices were incubated with ^{14}C-phenylalanine and the distribution of radioactivity in the protein of cell fractions determined. Since the distribution of radioactivity was measured at a number of different time-intervals for intact, adrenalectomized and cortisone-treated animals (Table 2), of practical necessity, only duplicate observations could be made. For this reason the results must be considered to be preliminary.

After adrenalectomy the specific radioactivity of the protein in each of the fractions of heart muscle (debris, mitochondria, microsomes, soluble fraction) is increased (Table 2). The increase, after 1 hr. of incubation, is by far the greatest in the microsomal fraction (448 per cent as compared with 90 per cent in the slice itself). The magnitude of the effect of adrenalectomy on amino-acid incorporation into heart microsomes suggests the possibility that they are the intracellular site at which adrenalectomy acts to enhance protein biosynthesis. This would be analogous to the finding by Korner[6] that it is the liver microsomes, rather than the other participants in intracellular protein biosynthesis, which are stimulated by adrenalectomy. This suggestion—there is no direct evidence for an effect of hormones on microsomes in muscle—needs testing in an *in vitro* system.

Cortisone depressed amino-acid incorporation into each of the cellular fractions of myocardium. In contrast with the changes after adrenalectomy the decrease after cortisone was equal in each fraction (20–30 per cent), and not nearly so great. The distribution of radioactivity in the protein of the several fractions, then, offers no clue to the mode of intracellular action of cortisone in depressing protein biosynthesis; if anything, it suggests the most important effect of cortisone is not intracellular. On this score, there is evidence[7] that cortisone depresses amino-acid penetration into the muscle cell interior, an effect that accounts, at least in part, for the depression of protein biosynthesis; this effect need not be reflected in a selective depression of amino-acid incorporation into any specific cell fraction.

EDWARD I. WEINSHELBAUM
IRA G. WOOL
Department of Physiology,
University of Chicago.

[1] Wool, I. G., and Weinshelbaum, E. I., *Amer. J. Physiol.*, **197**, 1089 (1959).

[2] Manchester, K. L., Randle, P. J., and Young, F. G., *J. Endocrinol.*, **18**, 395 (1959).

[3] Wool, I. G., and Weinshelbaum, E. I., *Amer. J. Physiol.*, **198**, 360 (1960).

[4] Wool, I. G., and Krahl, M. E., *Amer. J. Physiol.*, **196**, 961 (1959).

[5] Zamecnik, P. C., and Keller, E. B., *J. Biol. Chem.*, **209**, 337 (1954).

[6] Korner, A., *J. Endocrinol.*, **21**, 177 (1960).

[7] Wool, I. G., *Amer. J. Physiol.*, **199**, 715 (1960).

Action of Plasmin on Cartilage Matrix *in vivo*

INTRAVENOUS injection of unactivated papain into young rabbits leads to collapse of their ears due to loss of matrix[1], reduction of the chondroitin sulphate in the ear cartilage[2,3] and the appearance of chondroitin sulphate in the blood and urine[3,4]. Activated papain does not produce this gross effect, and it has been suggested[5,6] that the active enzyme combines with inhibitors in the blood, whereas the inactive papain is activated by sulphydryl groups in cartilage itself and attacks the mucopolysaccharide/protein complex, releasing the chondroitin sulphate.

Lack and Rogers[7] reported the liberation of chondroitin sulphate from cartilage *in vitro* by the blood protease, plasmin. They suggested that the rapid chondrolysis which occurs in joint infections by streptococci and staphylococci might be due to kinases produced by these organisms activating plasminogen in the surrounding fluid. Astrup *et al.*[8] have shown that a diffusable activator of plasminogen is present in synovial membrane and articular cartilage and have postulated that this provides the mechanism for the removal of fibrin from joint cavities after trauma or hæmorrhage.

It now appears that if sufficient plasminogen reaches cartilage *in vivo* there is a sequence similar to that observed with papain. The cartilage matrix is depleted, chondroitin sulphate diffuses out and is afterwards excreted in the urine. As loss of matrix may be demonstrated following immersion of cartilage in plasminogen, it is presumed that the enzyme is activated *in situ* by tissue activator.

In the experiments summarized here we have injected rabbits with crude papain, rabbit plasminogen and human plasmin respectively. We also exposed ears, screened by asbestos from the rest of the body, to 10 min. infra-red radiation, giving 10 min. exposure to each side of the ear. The crude papain was provided by Messrs. A. M. Zimmerman (London), human plasmin by the Blood Products Division, Medical Research Council, and rabbit plasminogen was extracted from fresh rabbit serum by Kline's method[9]. Urines were collected in twenty-four hour periods and stored at −10° until assayed. Their content of acid mucopolysaccharide was measured by the method of Di Ferrante and Rich[10], the results being expressed in terms of the glucuronic acid content of the precipitated acid mucopolysaccharide fraction, as estimated by the method of Dische. Urine was collected for two days prior to injection and afterwards until the glucuronic acid content returned to the pre-injection level. The results obtained are summarized in Table 1.

Table 1. EXCRETION OF ACID MUCOPOLYSACCHARIDES IN RABBIT URINE

No.	Weight of rabbit (kgm.)	Treatment	Acid mucopolysaccharide excretion (mgm. glucuronic acid/24 hr.)		No. of days after treatment to reach excretion peak
			Pre-treatment level	Post-treatment peak	
1	1·5	Papain (3 ml., 2 per cent)	2·2	4·3	1
2	1·3	Rabbit plasminogen	1·3	3·0	5
3	0·8	Rabbit plasminogen	2·3	6·8	3
4	1·4	Rabbit plasminogen + local $CHCl_3$	1·5	4·3	1
5	1·5	Human plasmin	1·5	3·3	1
6	1·0	Infra-red to 1 ear	2·0	3·5	4
7	1·0	Infra-red to 2 ears	1·4	8·0	4

The ears were closely observed during these experiments and it was noted that papain produced greater collapse and also a greater tendency to hæmorrhage around the veins. A rise in the excretion of chondroitin sulphate was not always accompanied by a collapse of the ears: sometimes there was no more than a softening or curling of the tips.

We postulate that plasminogen, diffusing from the blood, is activated in cartilage and attacks the protein/chondroitin sulphate complex in a manner similar to papain; it appears that this may be achieved by increasing local vascular permeability, which allows plasminogen already in the blood to pass through to the matrix.

We gratefully acknowledge grants from the Nuffield Foundation for support to one of us (S. Y. A.) and from the Central Research Fund of the University of London for equipment.

C. H. LACK
A. J. ANDERSON
S. Y. ALI

Department of Pathology,
Institute of Orthopædics,
Royal National Orthopædic Hospital,
Stanmore, Middlesex.

[1] Thomas, L., *J. Exp. Med.*, **104**, 245 (1956).
[2] Tsaltas, T. T., *J. Exp. Med.*, **108**, 507 (1958).
[3] Grant, R. A., Hathorn, M., and Gillman, T., *Biochem. J.*, **76**, 412 (1960).
[4] Spicer, S. S., and Bryant, J. H., *Amer. J. Path.*, **34**, 61 (1958).
[5] McCluskey, R. T., and Thomas, L., *J. Exp. Med.*, **108**, 371 (1958).
[6] Potter, J. L., McCluskey, R. T., Weissmann, G., and Thomas. L., *Ann. N.Y. Acad. Sci.*, **86**, 929 (1960).
[7] Lack, C. H., and Rogers, H. J., *Nature*, **182**, 948 (1958).
[8] Astrup, T., and Sjølin, K.-E., *Proc. Soc. Exp. Biol. Med.*, **97**, 852 (1958).
[9] Kline, D., *J. Biol. Chem.*, **204**, 949 (1953).
[10] Di Ferrante, N., and Rich, C., *J. Lab. Clin. Med.*, **48**, 491 (1956).

Cholinergic Transmission in Fish Muscle

IT is known that the enzymes needed for the synthesis and the destruction of acetylcholine are present in fish muscle in large amounts[1,2], and that certain electric organs, probably derived from muscle, are sensitive to acetylcholine[3]. It is therefore usually taken for granted that neuromuscular transmission in fish occurs by a cholinergic mechanism, as in other vertebrates, but there is, in fact, little evidence that fish muscle can be activated by acetylcholine, or that acetylcholine is normally released by motor-nerve-endings in fish.

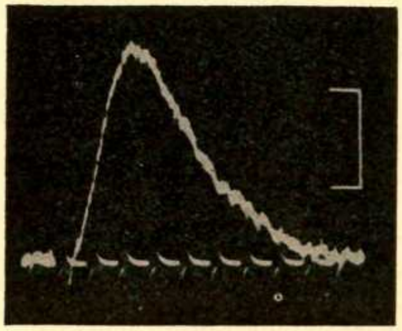


Fig. 1. Acetylcholine potential produced by a 1 msec. (3 *n* coulomb) pulse of outward current, recorded in deep lateral muscle of goldfish. Vertical scale: 1 mV.; horizontal scale, 10 msec.

Although muscle from the goldfish (*Cyprinus auratus*) is not markedly sensitive to acetylcholine injected into the bathing solution, acetylcholine applied from a micro-pipette can have a powerful depolarizing action. The membrane potential of fibres in the deep part of the lateral muscle, at the level of the visceral cavity, was recorded with a conventional glass micro-pipette filled with $3M$ potassium chloride, and acetylcholine was released from a similar micro-pipette filled with $3M$ acetylcholine chloride (Roche Products). When the acetylcholine micro-pipette was brought close to sensitive spots on the surface of the fibre, rapid depolarization could be produced by permitting acetylcholine to diffuse out of the tip; if sufficient acetylcholine was released, the fibre contracted. The effect was best seen by passing short pulses of outward current through the pipette. The resulting potential changes (Fig. 1) resembled normal junctional potentials observed in the same muscle during repetitive stimulation, but their time-courses were much slower (about ten times slower in the case of the potential shown in Fig. 1). The very sensitive regions were extremely sharply localized, as in other vertebrate muscle, and the maximum sensitivity was comparable with that of frog muscle (that is, about 1 mV. depolarization per *n* coulomb of charge delivered through the acetylcholine pipette). Similar observations were made on the deep lateral muscle of catfish (*Ameiurus nebulosus*).

Indirect evidence of acetylcholine-release in fish muscle is provided by the susceptibility of junctional potentials to the blocking action of tubocurarine (10^{-5} w/v) or to potentiation by eserine (10^{-5} w/v). In Fig. 2, eserine is shown to have caused a great increase in the amplitude of spontaneous miniature potentials recorded in a fibre of the deep lateral muscle with an intracellular micro-pipette.

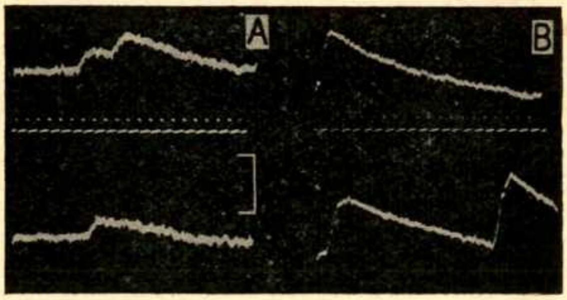


Fig. 2. Spontaneous potentials recorded in a fibre of deep lateral muscle of goldfish: (*A*) control; (*B*) 8 min. after adding eserine sulphate (10^{-5} w/v). Vertical scale, 1 mV.; horizontal scale, 1 msec.

These observations seem to give strong presumptive evidence of cholinergic transmission in at least some fish muscle.

K. Krnjević

Agricultural Research Council
Institute of Animal Physiology,
Babraham, Cambridge.

[1] Augustinsson, K. B., *Acta Physiol. Scand.*, **15**, Supp. 52 (1948).

[2] Cohen, M., *Arch. Biochem. Biophys.*, **60**, 284 (1956).

[3] Keynes, R. D., "Electric Organs". In *The Physiology of Fishes*, **2**, edit. by Brown, M. E. (Academic Press, New York).

Removal of Milk Constituents from the Mammary Gland of the Ewe

A number of experiments was conducted on the ewe to determine the role of lymph draining from the lactating mammary gland in the resolution of the milk constituents. Lymph flowed continuously for many weeks from a polyethylene catheter inserted into the main lymphatic duct draining the mammary gland. Initial investigations were concerned with the changes in composition of the lymph when the lamb was removed from its mother early in lactation. Plasma and lymph samples were collected at intervals for 7–14 days and the concentrations of free fatty acids, volatile fatty acids, cholesterol, phospholipid, total esterified fatty acids, protein, potassium, calcium and inorganic phosphate were measured. In addition, attempts were made to determine the presence of milk proteins in the lymph by the technique of specific precipitation in agar[1] and also by electrophoresis on cellulose acetate.

In three sheep out of six, the lymph, normally clear, became distinctly milky in appearance when the udder was very distended with milk. The milkiness persisted for as long as 2 days in one of the sheep and for only 4 hr. in another. The milky lymph contained increased concentrations of total esterified fatty acids, calcium, inorganic phosphate and potassium, and microscopic examination of the lymph under dark-ground illumination revealed the presence of particulate fat. Protein peculiar to milk was also identified in the milky lymph by the specific precipitation technique (Fig. 1). This could not be demonstrated electrophoretically. There was no evidence for the presence of this protein, nor significant change in the constituents measured in lymph which was not milky in character. More recent experiments demonstrated that a large proportion of human serum albumin containing iodine-131, which had been introduced into the gland sinus, was removed intact by way of the lymphatics. Under conditions in which the gland became very distended with milk, the radioactivity in the lymph rose to a high level and then decreased exponentially (Fig. 2). Lower levels of radio-activity were found in the lymph draining from mammary glands which did not become very distended with milk.

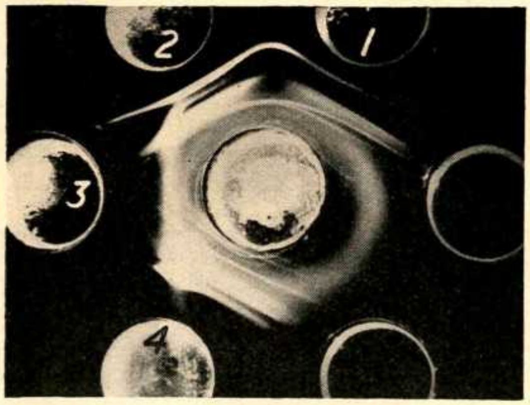


Fig. 1. Lines of precipitation between the central well containing ewe skim milk antiserum and the surrounding wells containing (1) non-milky lymph, (2) milky lymph, (3) diluted milk, and (4) wether's serum. A broad line of precipitation can be seen opposite the well containing milk. This line crosses all the precipitation lines opposite the well containing wether's serum and represents the precipitation between specific antibodies and protein in milk not found in plasma. This broad milk protein line is continuous with a line (often seen divided) opposite the well containing the milky lymph. No such line was found opposite the well containing the non-milky lymph

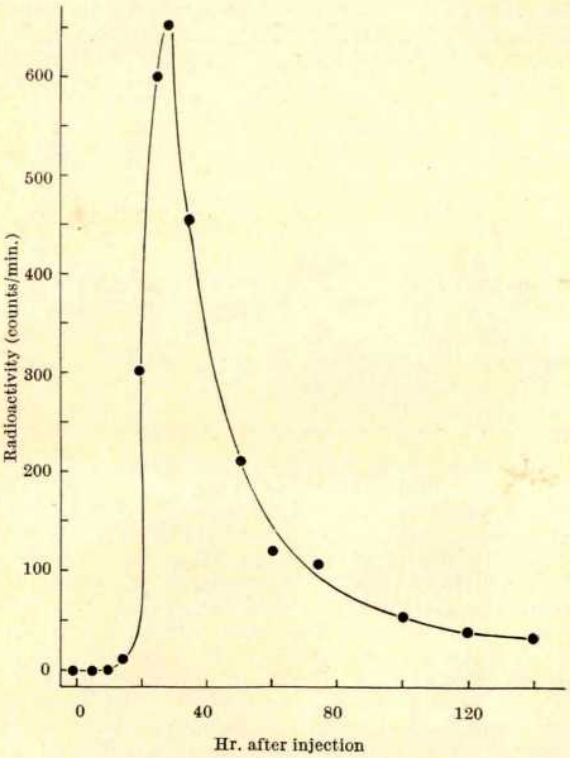


Fig. 2. Appearance of radioactivity in the lymph draining from a mammary gland following the introduction of human serum albumin labelled with iodine-131. Since the radioactivity was found in the trichloracetic acid precipitate it is reasonable to assume that the human serum albumin is being removed intact

These results indicate that fat and proteins of milk may be removed by the lymphatics of the mammary gland. The escape of milk from the gland when the udder is grossly engorged may be associated with the actual rupture of some acini or milk ducts. The finding that the lymph became rapidly milky following the insertion of a hypodermic needle deep into the mammary tissue may be significant in this regard. However, it appears that much of the secreted milk in the udder is removed by less-obvious means. Heyndrickx and Peeters[2], in their studies on the constituents of mammary lymph collected from freshly slaughtered cattle, point out that the lymph collected from the udder which had not been milked out prior to slaughter was excessively turbid, and analysis of this lymph showed that it contained milk constituents. The apparent ease of escape of milk into the lymph under these conditions may be associated with tissue anoxia or it may be a characteristic feature of the dairy cow and related to the higher rate of milk production.

A. K. LASCELLES

Department of Experimental Pathology,
Australian National University,
Canberra.

[1] Mansi, W., *J. Comp. Path.*, **67**, 297 (1957).

[2] Heyndrickx, G. V., and Peeters, G. J., *Biochem. J.*, **75**, 1 (1960).

HÆMATOLOGY

Recovery and *in vivo* Survival of Rabbit Erythrocytes

HUMAN and other mammalian red cells have been frozen and thawed with limited hæmolysis using a variety of cooling conditions and protective additives. Slow cooling in high concentrations (30 per cent) of glycerol[1], moderately rapid cooling in plasma containing sugars[2], and ultra-rapid cooling in low concentrations (6 per cent) of glucose[3] have allowed high recoveries (90–95 per cent) of red cells of which 80–90 per cent survive at least 24 hr. when transfused.

Polymeric solutes, including polyvinylpyrrolidone (PVP), dextran, polyethylene glycols, and albumin, afford effective protection for red cells as measured by *in vitro* recoveries[4]. Under conditions of rapid cooling and warming, polymers are the most efficient of all protective agents[5]. To our knowledge, however, only one investigator has reported *in vivo* survival studies on red cells frozen in the presence of polymers. Sloviter[6] observed poor viability (20 per cent) of rabbit and human red cells frozen with polyethylene glycols unless thawed cells were washed free of the polymer. Washing, however, lysed some 80 per cent of the initially recovered cells.

We have observed, in work described in this report, that rabbit blood containing a low concentration of PVP, a neutral, hydrophilic polymer, can be rapidly frozen and thawed with erythrocyte recoveries of greater than 90 per cent. Thawed blood was transfused directly, without removal of the polymer, and the red cells exhibited a 24-hr. survival averaging 93 per cent. Table 1 shows results obtained in four experiments using 24 separate blood specimens.

Rabbit blood was collected in acid-citrate glucose (3 vols. blood, 1 vol. ACD). Polyvinylpyrrolidone of average molecular weight 40,000 (*K*-30 PVP, Oxford Laboratories, San Francisco) in saline was added (4 vols. blood-ACD, 1 vol. PVP) to give a final concentration of 7 per cent in the mixture. Twenty to forty ml. of the mixtures was frozen rapidly in capped aluminium tubes of 17–33 mm. diameter by mechanical shaking in liquid nitrogen. After storage at − 170° C. for several days, samples were thawed by mechanical shaking in water at 45° C.

Recoveries of red cells were determined using hæmatocrits and colorimetric measurements of supernatant and total hæmoglobin as oxyhæmoglobin in 1 : 100 dilutions in aqueous sodium carbonate.

Aliquots of thawed blood were labelled with chromium-51 (ref. 7), (Abbott Laboratories, sterile sodium 'Rachromate') and an accurately measured volume was infused through an ear vein. Total infused radioactivity as red cells was measured on a separate aliquot. All chromium-51 measurements were made by scintillation counting using a Nuclear Chicago *DS*5–5 well counter. Blood samples were taken from the opposite ear into oxalate at 30–60 min. and again at 24 hr. after infusion. The percentage survival of red cells at each sample time was calculated using apparent red cell volumes previously determined on each rabbit by auto-transfusion of labelled unfrozen blood.

Table 1. RECOVERY AND SURVIVAL OF RED CELLS IN RABBIT BLOOD CONTAINING POLYVINYLPYRROLIDONE

Exp.	No. of samples	Container diameter (mm.)	Red cell recovery (per cent)	Red cell survival (per cent) 30–60 min.	Red cell survival (per cent) 24 hr.
1	6	17	92 ± 3	110 ± 15	98 ± 12
2	6	17	87 ± 6	97 ± 4	98 ± 6
3	6	17	93 ± 1	93 ± 16	89 ± 17
4	6	33	96 ± 1	97 ± 7	85 ± 7

In experiment 4, samples also were taken at 2–3 day intervals up to 12 days. Percentage survival at each sample time was corrected for labelled cells removed by previous sampling and chromium elution. Identical measurements were made on six additional rabbits which received unfrozen blood. Frozen cells were lost more rapidly during the first 48 hr.; subsequent rates of loss of frozen and control cells were equal (about 1·8 per cent per day). Approximately 85 per cent of the transfused frozen cells exhibited normal *in vivo* viability.

Results indicate that polyvinylpyrrolidone, long studied as a plasma expander, may prove useful as a protective agent in the low-temperature preservation of whole blood suitable for direct transfusion after thawing.

This work was supported under an office of Naval Research Contract No. *NONR*-3003(00).

G. F. DOEBBLER
R. G. BUCHHEIT
A. P. RINFRET

Research Laboratory,
Linde Company,
Division of Union Carbide Corporation,
Tonawanda, New York.

[1] Smith, A. V., *Lancet*, ii, 910 (1950). Sloviter, H. A., *ibid.*, i, 1350 (1950). Mollison, P. L., and Sloviter, H. A., *ibid.*, ii, 862 (1951).

[2] Florio, L., Stewart, P. H. M., and Mugrage, E. R., *J. Lab. Clin. Med.*, **28**, 1486 (1943). Strumia, M. M., Colwell, L. C., and Strumia, P. V., *Science*, **128**, 1002 (1958).

[3] Luyet, B. J., *Biodynamica*, **6**, 217 (1949). Meryman, H. T., and Kafig, E., *Proc. Soc. Exp. Biol. Med.*, **90**, 587 (1955).

[4] Bricka, M., and Bessis, M., *C.R. Soc. Biol., Paris*, **149**, 875 (1955). Glauser, S. C., and Talbot, T. R., *Amer. J. Med. Sci.*, **231**, 75 (1956). Steinbuch, M., and Quentin, M., *Vox Sang.*, **3**, 123 (1958).

[5] Doebbler, G. F., and Rinfret, A. P., *Fed. Proc.*, **18**, 564 (1959); **19**, 67 (1960).

[6] Sloviter, H. A., *Fed. Proc.*, **17**, 313 (1958).

[7] Sterling, K., and Cray, S. J., *J. Clin. Invest.*, **29**, 1614 (1950).

PATHOLOGY

An Iso-Antigen (γB^A) of Mouse γ-Globulin Present in Inbred Strains

HEREDITARY iso-antigens (allotypes) of rabbit γ-globulin were discovered in 1956 by Oudin[1], and independently in 1958 by Dubiski *et al.*[2]. Since then many publications have appeared reporting the serological, genetical and immunochemical characteristics of rabbit γ-globulins[3-6]. More recently, antigenically different types of serum globulins have been described in guinea pig[7], man[8] and baboon[9].

In the present experiments, two inbred strains (*BALB/c* and *C57BL*) of mice were used; the *BALB/c* mice were first immunized, by Dubiski's method[3], with *Proteus vulgaris* (*X* 19). *Proteus* cells coated with anti-*Proteus* sera were afterwards injected subcutaneously twice a week (15 times) into individuals of the *C57BL* strain. In this way, five pairs of mice were immunized with five individual sera (more particularly the γ-globulin selected by bacteria) of

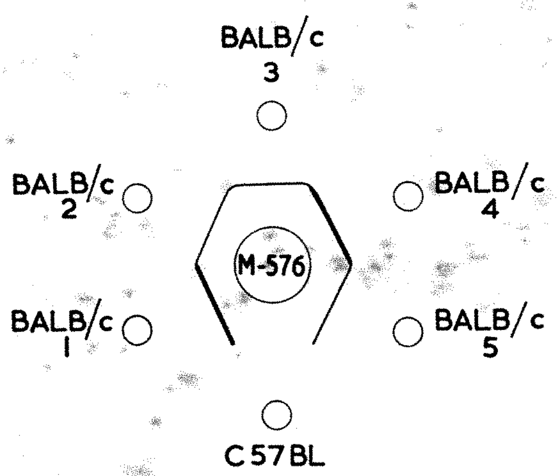


Fig. 1. Precipitation in 1·5 per cent agar gel between a *C57BL* mouse antiserum (*M*-576, in the central well) and sera of six different mice

the *BALB/c* strain. Each serum was therefore used for immunization of two mice. The animals were bled ten days after the last injection.

We tested the immune sera in 1·5 per cent agar gel against the individual *BALB/c* sera used for injections. Only six of ten mice produced precipitating antibodies. They showed an identity reaction varying only in strength. An example of this reaction is illustrated in Fig. 1.

Immunoelectrophoretic analysis showed that the antigen involved in the precipitation is mouse γ-globulin (Fig. 2).

For further investigations, the four strongest antisera were chosen and sera of several mouse strains tested. All four antisera reacted with all sera of individual mice of the strains: *BALB/c*, *C3H/He* and 'Champagne-Glaxo' (Table 1). None of the antisera was precipitated with serum of any *C57BL* mouse, in which strain the antibodies were produced. The experiments showed clearly that the *C57BL* mice lack a γ-globulin antigen, which we will call γB^A, present in each of the other strains of mice tested.

Snell[10] injected *BALB/c* mice with the sperm of *C57* mice and demonstrated an antigen which was absent in the *BALB/c* strain. S. Dubiski (unpublished results) found that rabbit γ-globulin antigens were present not only in the serum but also in the seminal plasma. It seems possible that the antigenic determinants shown by Snell and those described by us are common to serum γ-globulin and sperm and perhaps also seminal plasma.

Since the antisera produced in all *C57BL* mice reacted with sera of every mouse of the other strains, it would be possible in future to use pooled antisera to test a large number of mice belonging to different strains. Further immunization between various strains of mice is necessary to find how many hereditary γ-globulin antigens are present, and which of them are shared by particular strains. It would also be interesting to know if different γ-globulin antigens exist within an inbred strain.

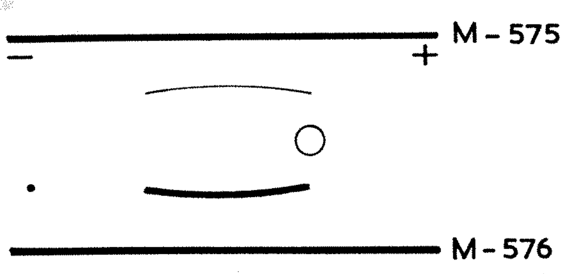


Fig. 2. Immunoelectrophoresis in 1·5 per cent agar gel of a *BALB/c* mouse serum (barbiturate buffer *p*H 8·6, 0·06 *M*, 10 V./cm. for 1 hr.). The antisera in the troughs were produced in *C57BL* mice

Table 1. PRECIPITATION TESTS OF MOUSE ANTI-MOUSE SERA

Mouse antisera (*C57BL* anti-*BALB/c*)	Mouse sera as antigens							
	C57BL		*BALB/c*		*C3H/He*		'Champagne-Glaxo'	
	+	–	+	–	+	–	+	–
M-571	0	25	25	0	25	0	20	0
M-572	0	25	25	0	25	0	20	0
M-575	0	25	25	0	25	0	20	0
M-576	0	25	25	0	25	0	20	0

The figures indicate the numbers of sera tested.
+, indicates a positive reaction; –, indicates a negative reaction.

We wish to thank the Wellcome Trust and the U.S. Public Health Service, National Heart Institute (fellowship No. *HF*-9538-C2), for their financial support.

A. KELUS
J. K. MOOR-JANKOWSKI

Department of Pathology,
University of Cambridge.

[1] Oudin, J., *C.R. Acad. Sci., Paris*,, **242**, 2489, 2606 (1956).

[2] Dubiski, S., Dudziak, Z., and Skalba, D., *Seventh Intern. Cong. Microbiol. Abst.*, 201 (Stockholm, 1958).

[3] Dubiski, S., Dudziak, Z., Skalba, D., and Dubiska, A., *Immunology*, **2**, 84 (1959).

[4] Dubiski, S., Skalba, D., Dubiska, A., and Kelus, A., *Nature*, **184**, 1811 (1959).

[5] Dray, S., and Young, G. O., *Science*, **129**, 1023 (1959); **131**, 738 (1960).

[6] Kelus, A., Marrack, J. R., and Richards, C. B., *Biochem. J.*, **76**, 13 P (1960); *Proc. Eighth Coll., Bruges*, 1960 (Elsevier, Amsterdam, 1961, p. 176).

[7] Benacerraf, B., and Gell, P. G. H., *Nature*, **189**, 586 (1961).

[8] Allison, A. C., and Blumberg, B. S., *Lancet*, i, 634 (1961).

[9] Kelus, A., and Moor-Jankowski, J. K., *Proc. Ninth Coll., Bruges*, 1961 (in the press).

[10] Snell, G. D., *Science*, **100**, 272 (1944).

Cell Transplants of Avian Lymphomatosis treated with Hydrogen Peroxide

LEUKOSIS is considered to be the most serious disease problem in chickens to-day. After Holman[1] reported his success in destroying implants of Walker 256 adenocarcinoma by replacing rats' drinking water with a dilute solution of hydrogen peroxide, it was decided to see if such treatment would alter the course of leukosis in chickens.

Fleischmann *et al.*[2] reported that survival-time of implanted rats was not lengthened by hydrogen peroxide treatment and hydrogen peroxide did not decrease the size of tumours induced by X-irradiation. Paoletti *et al.*[3] were unable to influence the growth of Ehrlich adenocarcinoma and Walker 256 adenocarcinoma in hydrogen peroxide-treated rats. Ghadially and Wiseman[4] found no difference in survival-time between rats treated with hydrogen peroxide and untreated rats which had been implanted with *RD* 3 sarcoma or Walker adenocarcinoma. Makins and Tanaka[5] prolonged the life of rats with ascites sarcoma to 20 days in contrast to 10 days for controls by intraperitoneal injection of 1 ml. of 2 per cent hydrogen peroxide. Hollcroft *et al.*[6] increased the effect of X-irradiation on regression of transplanted lymphosarcoma by intravenous injection of hydrogen peroxide immediately before X-irradiation, but hydrogen peroxide administered 5 min. before or after X-irradiation did not affect regression. Sugiura[7] was not able to demonstrate any favourable effect on transplanted tumours when the animals drank hydrogen peroxide but there was a moderate inhibitory effect on sarcoma 180 ascites tumour when large doses of hydrogen peroxide were given daily. Jeffree[8] reported no effect on survival-time between hydrogen peroxide-treated and untreated rats. Choraży *et al.*[9] reported no significant increase in survival-time between hydrogen peroxide-treated and untreated animals.

Approximately 4 million *RPL* 12 lymphomatosis tumour cells prepared and counted according to the method described by Mohos and Kidd[11] were injected intramuscularly into 20-day-old Leghorn cockerels. Three per cent hydrogen peroxide was given directly into the crop in one experiment. In the other, the chickens were given a 0·45 per cent solution in drinking water. All birds were kept in batteries and observed daily.

Seventy-six chickens were treated 3 times daily, beginning on the day of implantation, with 1 ml. of 3 per cent hydrogen peroxide directly into the crop. Fifty-two per cent died with lymphomatosis compared with 44 per cent deaths in the control group. A χ^2 test of significance between the treated and check groups resulted in a χ^2 value of 0·658, d.f. = 1, P = 0·40–0·50. The difference was not significant.

Nineteen chickens were not treated until tumours were readily palpable, after which time a 2-ml. dose was given 3 times daily. From the same group, 6 others were given 5 ml. 3 times daily. From the group given 2 ml., 10·5 per cent died, and from the 6, 50 per cent died. In the control group, 64 per cent of 25 died. A χ^2 test for differences between the 2 ml. dose and check group resulted in a χ^2 value of 10·761, d.f. = 1, P = 0·001.

The test indicated the need for further investigation of the effect of hydrogen peroxide. Therefore, a second experiment was initiated in which the dosage and method used by Holman[1] were followed. Hydrogen peroxide at 0·45 per cent level was given continuously in the drinking water to 78 chickens from the day of inoculation until nearly all were dead from tumours. In 78 others, treatment was started 5 days after inoculation when tumours were first palpable. Eighty-two controls were not treated. In the first treated group, 87·5 per cent died with tumours and in the second group, 83·3 per cent died. In the controls, 91·4 per cent died: $\chi^2 = 2·33$, d.f. = 2, $P = 0·30–0·40$.

Since treatment of the transplanted tumours in chickens was not encouraging, it was decided to see if hydrogen peroxide would destroy the cells by direct contact. Portions of tumours were macerated in a Ten Broeck grinder and mixed with 3 parts of 3 per cent hydrogen peroxide. Chickens were injected with 0·2 ml.; tumours developed and destroyed most of the chickens. Tumours were taken from chickens which had been treated with all dosage-levels of hydrogen peroxide and transplanted into other birds. All tumours grew readily and killed the chickens.

In neither instance had hydrogen peroxide altered the activity of the tumour in chickens. Tumours from treated chickens were examined histologically, but there was no difference between tumour cells from treated and untreated chickens.

Hydrogen peroxide was not effective in prolonging the life of chickens implanted with lymphomatosis tumour cells. In the one possible exception where only 10·5 per cent of 19 did die, the possibility of host-resistance cannot be ruled out since some chickens are known to have more resistance than others to this disease. Furthermore, the small number of birds in this group may not have been adequate for a good test.

V. L. Sanger

Department of Veterinary Science,
Ohio Agricultural Experiment Station,
Wooster, Ohio.

[1] Holman, R. A., *Nature*, **179**, 1033 (1957).

[2] Fleischmann, E., Werkgartner, H., and Karrer, K., *Nature*, **186**, 324 (1960).

[3] Paoletti, C., Truhaut, R., and Boiron, M., *Bull. Assoc. Française pour L'Etude du Cancer*, **43**, 756 (1959).

[4] Ghadially, F. N., and Wiseman, N., *Nature*, **181**, 1067 (1958).

[5] Makins, S., and Tanaka, T., *Gann* (Jap. J. Cancer Res.), **44**, 39 (1953).

[6] Hollcroft, J. W., Lorenz, E., and Matthews, M., *J. Nat. Cancer Inst.*, **12**, 751 (1952).

[7] Sugiura, K., *Nature*, **182**, 1310 (1958).

[8] Jeffree, G. M., *Nature*, **182**, 892 (1958).

[9] Choraży, M., Gettlich, A., Góral, L., Kałoczek, B., Molamka, E., Renar, B., and Szweda, A., *Nature*, **182**, 395 (1958). (From *Chem. Abstr.*, 4632).

[10] Burmester, B. R., Prickett, C. O., and Belding, T. C., *Cancer Res.*, **6**, 189 (1946).

[11] Mohos, S. C., and Kidd, J. G., *J. Exp. Med.*, **105**, 233 (1957).

IMMUNOLOGY

Serum Transferrins in the Genus *Macaca*: Species Distribution of Nineteen Phenotypes

In the genus *Macaca*, rhesus monkeys show a remarkable polymorphism of transferrin, the iron-binding protein, whereas cynomolgus monkeys (the Philippines variety) show only one molecular form of the protein[1,2]. Two-dimensional starch-gel immunoelectrophoresis[3] was used in previous work[1,2] to determine (with the aid of a uni-specific chicken anti-human transferrin serum) the number of transferrin components in the macaque sera. Then comparative one-dimensional starch-gel electrophoresis in the *tris* discontinuous buffer system was used to establish the subtle differences in the mobilities of transferrin, and fifteen phenotypes[2] due to various combinations of eight molecular forms of the protein were detected among rhesus monkeys. These results have now been confirmed by determinations based on the iron-binding property of transferrin. We have also adopted a provisional letter system of nomenclature which has simplified identification of the macaque transferrin phenotypes, thereby making it easier to discover new phenotypes. In this connexion a re-examination of the rhesus sera and an examination of sera from additional macaque species have now revealed nineteen transferrin phenotypes, sixteen being present in the rhesus group alone.

Fig. 1 presents the results of an experiment which involved: addition of iron-59 to rhesus sera representing the fifteen previously discovered phenotypes, analysis of these sera by vertical starch-gel electrophoresis in borate buffer[4], and then demonstration of the iron-binding components by autoradiography[5]. Again the fifteen phenotypes due to the different combinations of the eight molecular transferrins were observed. Each molecular form was assigned a letter, *A* designating the transferrin of slowest mobility and *H* that of fastest mobility. (The mobility increases from *A* to *H* in paper electrophoresis as well as in starch-gel electrophoresis and in the *tris* buffer as well as in the borate buffer.) In lettering the phenotypes it was assumed that when the transferrin genes were homozygous a double dose of a single molecular form of transferrin would be synthesized; but, when heterozygous, single doses of at least two molecular forms

Table 1. SPECIES DISTRIBUTION OF NINETEEN TRANSFERRIN PHENOTYPES IN THE GENUS *Macaca*

	No. of sera examined belonging to:																			
	AD	*BC*	*BD*	*B(D)G*	*BH*	*CC*	*CD*	*CE*	*CF*	*CG*	*CH*	*DD*	*DF*	*DG*	*FF*	*FG*	*GG*	H^1H^1	*HH*	Total
M. mulatta	2	2	1	1	1	*19*	12	6	2	5	5	*9*	4	4			*3*		1	77
M. irus												*95*								95
M. nemestrina							1			1		*4*	2	4	*1*	1	*3*			17
M. speciosa																		*10*		10

Note: The number of sera in each homozygous phenotype is in italics.

would be synthesized. Thus the phenotypes *CC*, *DD*, *GG*, and *HH* are thought to be controlled by homozygous combinations of genes and the other phenotypes by heterozygous combinations. As can be observed in the figure, transferrin *C*, *D*, *G* or *H* has a larger serum concentration in a 'homozygous' phenotype than in a 'heterozygous' phenotype.

The previous suggestion that in the genus *Macaca* there are sharp species differences in the degree of polymorphism of transferrin and in the frequencies of the transferrins[1] is substantiated by the results summarized in Tables 1 and 2. Sixteen phenotypes, four homozygous and twelve heterozygous, have now been recognized in rhesus monkeys (*M. mulatta*) from the Nepal border area of India (77 specimens from three different shipments). One of these phenotypes, a rare one, *BD*, is a new discovery. The transferrin having the highest frequency was *C*. In contrast to the extensive transferrin polymorphism of the rhesus monkeys, only the *D* transferrin has been found in cynomolgus monkeys (*M. irus*) from the Philippines (ninety-five specimens from four different shipments). The pig-tailed macaques (*M. nemestrina*) are again a polymorphic species. Although only seventeen specimens were examined, eight phenotypes (three homozygous and five heterozygous) were observed. Two of these phenotypes, *FF* and *FG*, are new discoveries. The most common phenotype in the rhesus group, *CC*, was not seen in the pig-tailed group. The *D* transferrin had the highest frequency in this group. The stump-tailed macaques (*M. speciosa*), like the Philippines cynomolgus monkeys, may prove to be transferrin homozygotes. The ten specimens examined contained only the H^1 transferrin. This transferrin, another new discovery, was not seen in the other species. H^1 moves faster in electrophoresis than *G*, but not quite as fast as *H*. The physico-chemical nature of the differences in the macaque transferrins and the evolutionary basis for the selection of the phenotypes have yet to be elucidated. In any attempt to do so, it would seem important to investigate the possible role of ecological and geographical factors on the distribution of the transferrin alleles in the genus *Macaca*. In this connexion, *Macaca speciosa* inhabits central India, which is supposed to be quite high in iron content.

Table 2. FREQUENCY OF MOLECULAR TRANSFERRINS

	Percentage of sera examined possessing:								
	A	*B*	*C*	*D*	*E*	*F*	*G*	H^1	*H*
M. mulatta	2·6	6·5	66·3	42·9	7·8	7·0	16·9		9·9
M. irus				100·0					
M. nemestrina			11·8	64·7		23·5	52·9		
M. speciosa								100·0	

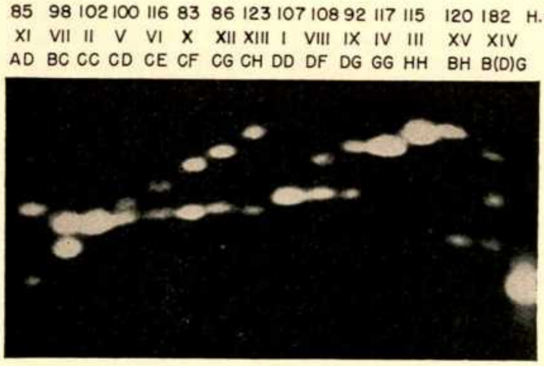

85 98 102 100 116 83 86 123 107 108 92 117 115 120 182 H.S.
XI VII II V VI X XII XIII I VIII IX IV III XV XIV
AD BC CC CD CE CF CG CH DD DF DG GG HH BH B(D)G

Fig. 1. The transferrins of rhesus sera, representing fifteen phenotypes, are compared in vertical starch-gel electrophoresis with the transferrin of normal human serum (far right). Note that the rhesus transferrins migrate faster than the human transferrin. At the top of the figure are the sample designations of the rhesus sera. Many of these samples were used in the original demonstration of rhesus transferrin phenotypes (ref. 1). The Roman numerals designate the phenotypes by the previous nomenclature (refs. 1 and 2). The letters, symbolizing the molecular transferrins, designate these phenotypes by the new nomenclature

We wish to thank Mr. W. Carey Parker for determining the iron-binding components of the rhesus sera by autoradiography and for suggesting the letter system of nomenclature for the rhesus transferrins. We also gratefully acknowledge the valuable help of Dr. P. E. Ayers of the Parke-Dale Unit, Parke Davis and Co., in providing the rhesus and cynomolgus sera, and Dr. R. C. Wolf of the Primate Laboratory, University of Wisconsin, in providing the pig-tailed macaque sera. The stump-tailed macaques, obtained from the Ferndale Pet Shop, were originally shipped from Calcutta, but were captured in central India. A collection of primate sera from Dr. John Buettner-Janusch is now under examination. His collection contains *Macara irus* specimens from Malaya. These specimens, in contrast to those from the Philippines, do show a transferrin polymorphism. This work is being supported by a grant (*G*–14152) from the National Science Foundation.

MORRIS GOODMAN
EMILY POULIK

Wayne State University College of Medicine
and the Lafayette Clinic,
Detroit, Michigan.

[1] Goodman, M., and Poulik, E., *Nature*, **190**, 171 (1961).
[2] Goodman, M., *Human Biology*, **33**, 131 (1961).
[3] Poulik, M. D., *J. Immunol.*, **82**, 502 (1959).
[4] Smithies, O., *Biochem. J.*, **71**, 585 (1959).
[5] Giblett, E. R., Hickman, C. G., and Smithies, O., *Nature*, **183**, 1589 (1959).

Products of Mild Acid and Alkaline Hydrolysis of *M*- and *N*-Blood Group Substances

THE preparations of *M*- and *N*-blood group substances obtained by phenol extraction of human erythrocyte stroma are mucoids[1]. They contain, on an average, 10 per cent of hexoses, 6·5 per cent of hexosamines, 1 per cent of fucose, 17 per cent of *N*-acetylneuraminic acid and 7·5 per cent of total nitrogen[1,2].

In an attempt to learn more about the carbohydrate structure of these mucoids, the products of their hydrolysis were studied. The conditions of hydrolysis were similar to those applied to bovine sub-maxillary gland mucoprotein by Gottschalk[3].

Acid hydrolysis: A solution of 15 mgm. of *M*- or *N*-substance in 2 ml. of 0·05 *N* sulphuric acid was heated at 80° for 40 min., afterwards cooled and neutralized with saturated barium hydroxide solution to *p*H 6. Barium sulphate sediment was centrifuged and the supernatant was dialysed exhaustively against distilled water at 2°. The dialysate was lyophilized.

The presence of *N*-acetylneuraminic acid in the dialysate was proved by characteristic reactions (diphenylamine and 2-thiobarbituric acid), paper chromatography in three solvent systems (I: *n*-butanol/*n*-propanol/0·1 *N* hydrochloric acid, 1: 2: 1 v/v; II: *n*-butanol/pyridine/water 6: 4: 3 v/v; III: pyridine/ethyl acetate/acetic acid/water, 5: 5: 1: 3 v/v) and by conversion into 2-carboxypyrrole[3]. After acid hydrolysis of *M*- and *N*-substances, 85–95 per cent of *N*-acetylneuraminic acid was split off and their group activity disappeared completely. In this process also small amounts of dialysable carbohydrate–amino-acid fragments were liberated.

Alkaline hydrolysis: 60 mgm. of *M*- or *N*-substance dissolved in 10 ml. of water was heated rapidly to 80° and saturated barium hydroxide solution was added to a final concentration of 0·02 *N*. The mixture was kept at 80° for 15 min., then cooled and acidified with 1 *N* sulphuric acid to *p*H 6. A further procedure was the same as that after acid hydrolysis.

By means of paper chromatographical analysis and characteristic reactions the following components were found in the dialysate: *N*-acetylneuraminic acid, small amounts of chromogen I, traces of *N*-acetylhexosamine, galactose (in some preparations) and two unknown compounds: C_1 and C_2. The last-named were separated from the other dialysable components by paper chromatography followed by elution of suitable strips of chromatograms; for the separation of C_1, the dialysate was subjected to chromatography in solvent system I, and for the isolation of C_2, in solvent system II.

Compound 1 (C_1): this was homogeneous in the three solvent systems used, gave a distinct colour with benzidine reagent, and a weak colour with direct Ehrlich reagent for sialic acids. Its R_F value was lower than that of galactose. On treatment with acid (0·05 *N* sulphuric acid, 80°, 40 min.) C_1 was split off into *N*-acetylneuraminic acid and galactose (chromatographical evidence). The presence of *N*-acetylneuraminic acid in C_1 was also stated by the methods described here (see acid hydrolysis). The secondary cysteine reaction of hexoses confirmed the presence of galactose in C_1. The amounts of *N*-acetylneuraminic acid and galactose were almost equimolecular.

C_1 was decomposed into *N*-acetylneuraminic acid and galactose after the action of influenza virus strain Lee at 37°.

Compound 2 (C_2): this was submitted to paper chromatography in two solvent systems: II and III gave one spot visible by direct Ehrlich reagent for sialic acids and by direct Ehrlich reagent in the cold (characteristic for chromogens which are formed on mild alkali treatment of *N*-acetylhexosamines[4]) and moved a little faster than *N*-acetylneuraminic acid. C_2, hydrolysed by 0·05 *N* sulphuric acid at 80° for 40 min., yielded only *N*-acetylneuraminic acid; the chromogen part of C_2 was destroyed during this hydrolysis. Chromogen I formed from *N*-acetylglucosamine was also fully destroyed under the same acid conditions. Moreover, *N*-acetylneuraminic acid in C_2 was identified by methods used here.

After the incubation of C_2 with influenza virus Lee at 37° *N*-acetylneuraminic acid and chromogen I (R_F value = 0·6 in solvent system II and positive direct Ehrlich reaction in the cold) were found.

Such disaccharides as *N*-acetylneuraminyl-galactose (C_1) and *N*-acetylneuraminyl-chromogen (C_2), obtained by mild alkaline hydrolysis of *M*- or *N*-substance, show that *N*-acetylneuraminic acid may combine with galactose or *N*-acetylhexosamine in this biologically active material. The linkage of *N*-acetylneuraminic acid in both disaccharides is α-ketosidic because it is labile in acid at elevated temperature and susceptible to virus α-neuraminidase. The mild alkaline hydrolysis of *M*- or *N*-substance repeated several times supplied the same products; it is possible, therefore, that these disaccharides form an essential part of the prosthetic group of *M*- and *N*-mucoids.

I wish to thank Prof. T. Baranowski for his interest and encouragement, and Prof. Zofia Skurska and Mgr. Maria Łobodzinska for preparation of influenza virus strain Lee.

ELŻBIETA ROMANOWSKA

Department of Biochemistry,
Ludwik Hirszfeld Institute of
Immunology and Experimental Therapy,
Wrocław, Poland.

[1] Baranowski, T., Lisowska, E., Morawiecki, A., Romanowska, E., and Strozecka, K., *Arch. Immunol. i Ter. Dośw.*, 7, 15 (1959).

[2] Romanowska, E., *Arch. Immunol. i Ter. Dośw.*, 7, 749 (1959); *Naturwissensch.*, **47**, 66 (1960).

[3] Gottschalk, A., and Graham, E. R. B., *Biochim. Biophys. Acta*, **34**, 380 (1959).

[4] Kuhn, R., and Krüger, G., *Chem. Ber.*, **89**, 1473 (1956); **90**, 264 (1957).

BIOLOGY

Thiabendazole: a Highly Effective Anthelmintic for Sheep

IN the past year, reports on several compounds with outstanding anthelmintic activity against the gastro-intestinal trichostrongyles and strongyles of sheep have been published. Among the anthelmintics tested were the organic phosphorus compound 4-tertiary-butyl-2-chlorophenyl methyl-methyl phosphoramidate[1], a pyridine derivative, 2-(β-methoxy-ethyl) pyridine[2], and one in which an organic phosphorus compound and phenothiazine are stated to act synergistically[3].

Very recently, 2-(4′-thiazolyl)-benzimidazole (thiabendazole) has been reported[4] to have outstanding anthelmintic activity against a wide range of nematode parasites in sheep, cattle and pigs, and some activity against nematodes in dogs.

When thiabendazole was tested in sheep in this Laboratory and in the field in Australia at a dose-rate of 50 mgm./kgm. body-weight, it exhibited a very high degree of anthelmintic activity against *Haemonchus contortus*, *Ostertagia* spp., *Trichostrongylus* spp., *Cooperia curticei*, *Nematodirus* spp., *Oesophagostomum venulosum*, *O. columbianum* and *Chabertia ovina*, but it was not effective against *Trichuris* spp.

In some experiments with penned sheep with a pure infection of either *H. contortus* or *T. colubriformis*, anthelmintic activity was assessed from the results of repeated worm egg counts, but in critical experiments with infections with parasites of the large bowel, the activity was assessed from the ratio of the number of worms passed in the fæces to the number of residual worms found at slaughter a week after dosing. Also, controlled experiments were conducted with penned sheep with naturally acquired mixed infections, an assessment of effectiveness being made by comparing at slaughter the worm counts in treated sheep and in control animals.

In field trials, anthelmintic activity was assessed from results obtained from repeated fæcal examinations, comprising worm egg counts and differentiation of the larvæ recovered from fæcal cultures. In some of the field trials it could be assumed that worm burdens would have been increasing just after the time of treatment, because in control sheep slaughtered at an appropriate time, immature worms were present in large numbers.

The results of all the trials indicated that the worm burdens had been reduced to extremely low levels by a single treatment with thiabendazole, and some treated sheep were found to be completely free from infection when slaughtered. In field trials most of the fæcal examinations made within the two weeks following treatment revealed no eggs, and cultures did not yield any larvæ. In these field trials, it was found that post-treatment egg counts either became constantly negative or remained very low, and it can therefore be assumed that any immature worms present at the time of treatment had also been destroyed.

Results from one controlled trial in penned sheep which harboured mixed infections, shown in Table 1, demonstrate the remarkable anthelmintic activity of the compound.

In one field trial with groups of 50 treated and control lambs, selected at random and held on pastures for fattening, the greater gain in weight in the group treated monthly with thiabendazole showed this drug to be a very effective anthelmintic, and the lambs gained weight at a very high and steady rate. In this trial, 36 per cent of the controls, 44 per cent of the group treated monthly with phenothiazine, and 68 per cent of those treated monthly with thiabendazole attained a weight of 70 lb. or more at 4½ months of age.

Table 1. NUMBERS OF ADULT WORMS IN SHEEP AFTER TREATMENT WITH THIABENDAZOLE, AND IN CONTROL SHEEP

Sheep	Abomasum			Small intestine		
	Haemonchus contortus	*Ostertagia* spp.	*Trichostrongylus axei*	*Trichostrongylus* spp.	*Nematodirus* spp.	*Cooperia curticei*
Controls						
1	6,000	500	400	3,000	300	3,600
2	1,000	400	200	2,700	650	1,400
3	12,000	3,000	1,200	4,600	1,200	400
4	10,000	1,100	—	3,000	1,100	700
5	3,060	2,000	170	3,000	1,100	1,000
Treated with thiabendazole						
6	—	—	—	—	—	—
7	—	—	—	—	—	—
8	—	—	—	—	300	—
9	—	—	—	—	—	—
10	—	—	—	—	—	—
11	—	—	—	—	—	—
12	—	—	—	—	—	—
13	—	—	—	80	80	—

— indicates the absence of worms in the aliquot taken for counting; this does not necessarily indicate that no worms were present, but that the total could not have exceeded 50.

In critical trials with *O. columbianum* and *O. venulosum* in Australia, Hebden[5] found that all worms were removed from sheep by treatment. His trials with other species gave results comparable with those recorded in this communication.

HUGH McL. GORDON

Division of Animal Health,
Commonwealth Scientific and
Industrial Research Organization,
McMaster Laboratory,
Glebe, New South Wales.

---

[1] Douglas, J. R., and Baker, N. F., *J. Amer. Vet. Med. Assoc.*, **135**, 567 (1959).

[2] Broome, A. W. J., and Greenhalgh, N., *Nature*, **189**, 59 (1961).

[3] Kingsbury, P. A., *Vet. Rec.*, **73**, 183 (1961).

[4] Brown, H. D., Matzuk, A. R., Ilves, Ivl. R., Peterson, L. H., Harris, S. A., Sarett, L. H., Egerton, J. R., Yakstis, J. J., Campbell, W. C., and Cuckler, A. C., *J. Amer. Chem. Soc.*, **83**, 1764 (1961).

[5] Hebden, S. P., *Aust. Vet. J.*, **37**, 264 (1961).

Growth Stimulation by High Indole-3-Acetic Acid Concentrations

It is well established that the curve depicting the extension growth of coleoptile sections as a function of indole-3-acetic acid concentration in the surrounding medium (the concentration curve) assumes the shape of an optimum curve. In the case of wheat coleoptile sections the curve ascends at a constant rate until the optimum is reached at approximately 10^{-5} *M* indole-3-acetic acid. The descent of the curve above the optimum is conditioned by the inhibiting effect of higher indole-3-acetic acid concentrations. This clear and distinct picture is found at least when an experimental period lasts 24 hr., while for shorter periods the course of the concentration curve is not so well known.

Foster *et al.*[1] found that the concentration curve is an optimum curve even for short-time experiments. They obtained time curves (curves representing extension growth for different concentrations of indole-3-acetic acid as a function of time), which were straight lines having slope coefficients increasing with indole-3-acetic acid concentrations until 10^{-5} *M*. At higher concentrations they still observed linear curves, but with slope coefficients decreasing with increasing concentration. In later investigations by Cleland[2] it is demonstrated, however, that time curves for different concentrations follow the same course for the first (2–4) hr. of growth. Only after a longer time-interval do the curves separate, showing the usual inhibiting effect of high indole-3-acetic acid concentrations. Working with 10-mm. coleoptile sections (wheat, variety Progress), I could not observe any significant separation of time curves for indole-3-acetic acid up to 10^{-3} *M* over a period of about 3 hr., thus confirming Cleland's results.

To examine more closely the non-appearance of an inhibiting effect of high auxin concentrations during the initial course of the time curves, a special technique was adopted. Attempts were made to let the indole-3-acetic acid act before the actual growth processes commenced. This principle has been introduced by Heyn[3]. The method employed was as follows. During a pre-treatment period (first period) the growth of the coleoptile sections was impeded by either a solution with sufficiently high osmotic pressure (sucrose or mannitol dissolved in McIlvaine's citric acid–phosphate buffer, *p*H 4·6 at 1/100) or by keeping the sections at low temperature (about 3° C.). During the first period treatment with indole-3-acetic

New Pergamon Journals in the field of Psychiatric Research

KORSAKOV JOURNAL OF NEUROLOGY & PSYCHIATRY

Scientific Translation Editor:
Dr. W. Ritchie Russell,
M.D., D.Sc., F.R.C.P.

This important and authoritative new journal will contain selected papers translated from the well-known Russian journal *Zhurnal nevropatologii i psykhiatrii imeni S. S. Korsakova,* with English abstracts of all original papers not published in full. This new English version, similar in scope to existing Western literature, will show the advance of Soviet knowledge of all aspects of the subject, and will be widely appreciated by all those who need to keep in touch with the world's neurological and psychiatric literature.

Quarterly. Subscription Rates:
£15. ($40.00) per annum
Single issues: £5 ($15.00)

Some of the Selected Papers to be Published in Early Issues

Cerebral Circulatory Insufficiency—N. K. BOGOLEPOV and L. O. BADALIAN. Experiments on the utilization of reflexes for the restoration of movement in hemiplegia, O. STARY, Z. MIRZHATSKI and M. BERANKOVA. The problem of the pathological change in the fibres and neuronal connexions of the brain in focal vascular disorders in man—T. S. MATVEYAVA. General principles of the use of choline-like drugs in neuropathology—N. N. ANOSOV. The significance of drug and experimental psychoses for the understanding of some general problems of psychiatry—V. M. BANSHCHIKOV and C. V. STOLIAROV. Changes in the peripheral part of the cutaneous analyser during acute radiation sickness—N. N. GARVEI. An objective method of studying sensory adaptation—P. A. BUGAYENKO. The electrical activity of the brain during the acute period of cerebral injuries—E. A. ZHIRMUNSKAIA and L. G. CHLENOV. Cerebral piezography in arteriosclerosis of the brain and in the sclerotic phase of hypertonic disease—E. S. GOLLAND. Chronic progressive malacia of the brain—Z. N. DRACHEVA. The significance of functional tests in electrocortigraphy of patients with brain tumours—B. S. OVNATANOV. Electroencephalographic changes due to deformation of structures within the brain stem at the level of the posterior cranial fossa—I. M. GIL'MAN. A comparative study of the cerebrospinal fluid of the IIIrd ventricle and of the lateral ventricles in cases of tumours or inflammatory processes of the brain—IA. M. PAVLONSKII. Sex differences in the nuclear structure of benign brain tumours—A. P. AVTSYN and S. M. SHIBAEVA.

JOURNAL OF PSYCHIATRIC RESEARCH

(A new, important and authoritative International Research Journal)

Editor-in-Chief:
Seymour S. Kety, M.D.

Associate Editors:
Joel Elkes *and* **David A. Hamburg**

Quarterly Subscription Rates: "A"
£7 0s. 0d. ($20.00) per annum
(For Libraries, Government Establishments and Research Laboratories)

Subscription Rates: "B"
£3 10s. 0d. ($10.00) per annum
(For private subscribers ordering direct from the publisher who certify the journal is for their personal use)

This journal has been founded as a contribution towards the growing need for research of high quality in the field of mental illness and mental health. It is hoped that the Journal may be useful in drawing together contributions from a variety of sources supporting a broad view of investigations in so far as they are relevant to psychiatric problems, tapping into a wide range of biological, psychological and social research.

Table of Contents of Vol. 1 No. 1

W. DINGMAN and M. B. SPORN: The incorporation of 8-azaguanine into rat brain RNA and its effect on maze learning by the rat: an inquiry into the biochemical basis of memory. V. G. LATIES: Modification of affect, social behaviour and performance by sleep deprivation. DAVID ROSENTHAL: Sex distribution and the severity of illness among samples of schizophrenic twins. ELWOOD H. LABROSSE, JAY D. MANN and SEYMOUR S. KETY: The physiological and psychological effects of intravenously administered epinephrine, and its metabolism in normal and schizophrenic men—III. Metabolism of 7-H^3—epinephrine as determined in studies in blood and urine. PHILIPPE V. CARDON, LOUIS SOKOLOFF, THOMAS S. VATES, E. V. EVARTS and SEYMOUR S. KETY: The physiological and psychological effect of intravenously administered epinephrine and its metabolism in normal and schizophrenic man. Effects on heart rate, blood pressure, blood glucose concentration and the electroencephalogram. WILLIAM POLLIN and SAMSON GOLDIN: Psychiatric observations. The physiological and psychological effects of intravenously administered epinephrine and its metabolism in normal and schizophrenic men—II.

Send for Free Specimen Copies of these New Journals

PERGAMON PRESS

OXFORD . LONDON . NEW YORK PARIS

Headington Hill Hall, Oxford. 4 & 5 *Fitzroy Sq., London, W.*1. 122 *East 55th St., New York* 22, *N.Y.*

ACADEMIC PRESS London and New York

A New International Journal

EXPERIMENTAL EYE RESEARCH

Edited by HUGH DAVSON, *University College, London*
and ENDRE A. BALAZS, *Retina Foundation, Boston*

Editorial Board:
JIN H. KINOSHITA
V. EVERETT KINSEY
DAVID MAURICE
LOUISE L. SLOAN
GEORGE K. SMELSER

Advisory Board:
ERNST BÁRÁNY
BERNARD BECKER
HANS GOLDMANN
RAGNAR GRANIT
STEPHEN W. KUFFLER
LORRIN A. RIGGS
W. A. H. RUSHTON
LUDWIG VON SALLMANN
PHILLIPS THYGESON

Volume 1, 1961/1962 (issued quarterly) 115s.

Experimental Eye Research will be available for the publication of the results of original research on the anatomy, physiology, biochemistry and biophysics of the eye. Contributions on such subjects as the production and circulation of ocular fluids, the fine structure, biochemistry, biophysics and metabolism of eye tissues, the photochemical processes related to visual function, and the neuromuscular mechanisms in the control of eye movements will be preferred.

Volume 1, Number 1, September 1961

Ultrastructure of Retinal Capillaries of the Rat
By ABBOTT T. KISSEN *and* J. M. B. BLOODWORTH, JR., *The Ohio State University*

The Rhodopsin Cycle in the Developing Vertebrate Retina. I. Relation of Rhodopsin Content, Electroretinogram and Rod Structure in the Rat
By SJOERD L. BONTING, LEO L. CARAVAGGIO *and* PETER GOURAS, *National Institutes of Health, Bethesda, Maryland*

Physicochemical Properties of Insoluble Corneal Collagen
By MOTOKAZU ITOI, *School of Medicine, University of Tokyo*

Studies on Corneal Polysaccharides. I. Separation
By ARVID ANSETH *and* TORVARD C. LAURENT, *Massachusetts Eye and Ear Infirmary, Boston*

The Utilization of Pyruvate and Its Conversion to Glutamate in Calf Lens
By JIN H. KINOSHITA *and* LORENZO O. MEROLA, *Harvard Medical School and Massachusetts Eye and Ear Infirmary, Boston*

Studies on Amino Acid Esterase Activity of Lens
By ABRAHAM SPECTOR, *Harvard Medical School and Massachusetts Eye and Ear Infirmary, Boston*

The Oily Layer of the Tear Film and Evaporation from the Corneal Surface
By SAIICHI MISHIMA *and* DAVID M. MAURICE, *Institute of Ophthalmology, London*

The Effect of Normal Evaporation on the Eye
By SAIICHI MISHIMA *and* DAVID M. MAURICE, *Institute of Ophthalmology, London*

Detection of the Electroretinogram in Retinitis Pigmentosa
By JOHN C. ARMINGTON, PETER GOURAS, DONALD I. TEPAS *and* RALPH GUNKEL, *National Institutes of Health, Bethesda, Maryland*

The Role of Polysaccharides in Corneal Swelling
By BENGT O. HEDBYS, *Massachusetts Eye and Ear Infirmary, Boston*

The Geometric Analysis of the Photopic Corneal ERG (I), ON and OFF Responses
By ROBERT H. PECKHAM *and* WILLIAM M. HART, *The Eye Research Foundation, of Bethesda, Bethesda, Maryland*

Telephone WHItehall 6631

17 OLD QUEEN STREET, LONDON, S.W. 1 111 FIFTH AVENUE, NEW YORK 3

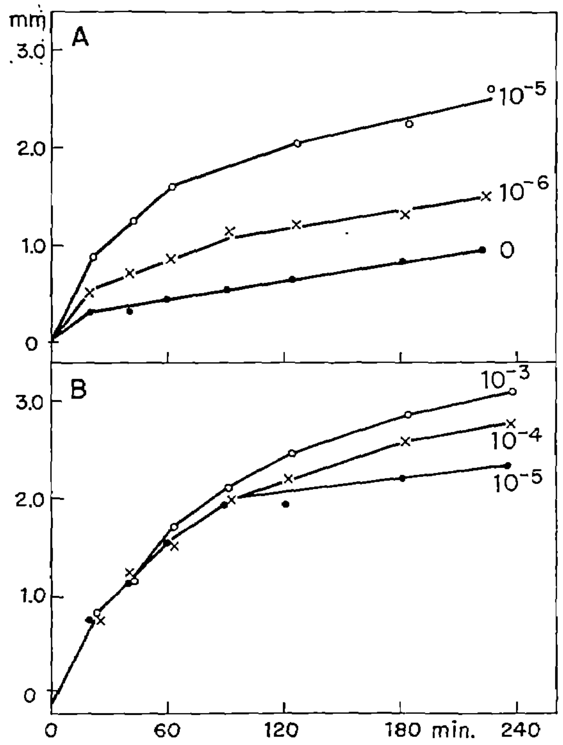


Fig. 1. Growth of wheat coleoptile sections in pure citric acid-phosphate buffer at 23° C. after pretreatment for 2 hr. in growth-impeding sucrose solutions (0·24 M) containing indole-3-acetic acid in molar concentrations as shown in the diagrams

acid was carried out with a concentration series ranging from 0–10^{-3} M. After a washing with buffer solution to remove any adhering sugar and indole-3-acetic acid the coleoptile sections were transferred to pure buffer and the growth period (second period) commenced. All experiments were carried out at 23° C. except for the pre-treatments performed in the cold.

A 2-hr. pre-treatment period in 0·24 M sucrose resulted in a second period of growth as shown in Fig. 1. For practical reasons, only treatment with three concentrations could be included in each experiment (*A* and *B*). The growth increases with increasing indole-3-acetic acid concentrations up to 10^{-3} M. Thus there has been a promoting action by high concentrations normally inducing inhibition when applied during the growth period proper. A repetition of the experiment with a 24-hr. growth period showed similar results.

Low temperature in the pre-treatment period had the same qualitative effect as impeding of growth with sucrose. When mannitol was used as osmotic medium the separation of the time curves for high concentrations could not be observed during the first 4 hr. of the second period, but it appeared distinctly after a period of growth of 24 hr. Prolonging sucrose or low-temperature pre-treatment from 2 to 4 hr. did not alter the results—an increase in growth with rising concentrations up to 10^{-3} M was still observed. This effect disappeared when the pre-treatment was extended to 17 hr. at low temperature. Some of these experiments showed, however, less growth with 10^{-3} M indole-3-acetic acid than with other concentrations. Complete growth cessation could not be achieved with this pre-treatment. Pre-treatment for 17 hr. in sucrose strongly retarded the growth in the second period.

Adding the auxin in the second period following a pre-treatment of 2 hr. in 0·24 M sucrose alone resulted after 20 hr. in a concentration curve with an optimum somewhat below 10^{-5} M indole-3-acetic acid. Without pre-treatment the optimum was at 10^{-5} M. A similar displacement of the optimum was observed by Schneider[4] in experiments with *Avena* coleoptile sections grown in combined sucrose–indole-3-acetic acid media.

Comparison of the concentration curves (20-hr. experiments) for addition of indole-3-acetic acid during the pre-treatment period, and for addition during the growth period, respectively, clearly showed a specific effect of indole-3-acetic acid in the pre-treatment period. The former procedure gave a concentration curve with a distinct minimum between 10^{-6} and 10^{-5} M indole-3-acetic acid while the latter showed a growth optimum in the same concentration interval.

A clear interaction has been demonstrated between indole-3-acetic acid added during the first and indole-3-acetic acid added during the second period. The occurrence of this interaction supports Cleland and Bonner's statement[5] that indole-3-acetic acid can be retained in the tissue from the first to the second period and give rise to a residual effect in the latter.

T. A. Truelsen

Laboratory of Plant Physiology,
Royal Veterinary and
Agricultural College, Copenhagen,
and
Danish Atomic Energy Commission's Research Establishment,
Risø.

[1] Foster, R. J., McRae, D. H., and Bonner, J., *Proc. U.S. Nat. Acad. Sci.*, 38, 1014 (1952).

[2] Cleland, R., *Plant Physiol.*, 35, Supp. 6 (1960).

[3] Heyn, A. N. J., *Rec. trav. bot. néerl.*, 28, 113 (1931).

[4] Schneider, C. L., *Amer. J. Bot.*, 25, 258 (1938).

[5] Cleland, R., and Bonner, J., *Plant Physiol.*, 31, 350 (1956).

Agar Disk Method for the Direct Observation of Nematode-trapping Fungi in the Soil

Attempts at the biological control of soil-borne eelworm pests have usually involved a process of green manuring or the addition of some organic amendment to the soil[1–3]. A method has been developed to obtain direct evidence of the effects of such treatments on the predaceous activity of indigenous populations of nematode-trapping fungi in the soil, such work being regarded as fundamental to the investigation of methods using these fungi as agents of biological control.

A weak maize-meal extract agar of the type used for the isolation of predaceous fungi from the soil was used[4]. Disks 1 cm. in diameter and about 3 mm. thick are cut from plates of this medium and placed on microscope slides, four disks usually being arranged in a row on each slide. Each slide is then buried singly in 15-cm. Petri dishes containing soil so that the disks lie about 1 cm. below the soil surface. At weekly intervals the slides are removed and replaced by others bearing fresh disks. On removal from the soil the disk surfaces are washed with a strong, fine jet of water from a wash-bottle. The disks adhere to the slide and can be examined immediately, unfixed and unstained, under the microscope.

During the decomposition of cabbage leaf tissue, glucose and sucrose, a diversity of nematode-destroying fungi (both trap-forming predators and endozoic parasites) appear in the predaceous phase on the disk surfaces. The disks are in effect used as micro-quadrats recording to some extent what is happening in that region of the soil in intimate contact with their surfaces.

The soil is used fresh after being passed through a 7-mm. mesh sieve, thus retaining its microflora and microfauna. Its moisture content is held constant throughout the course of any investigation. By varying the type and quantity of amendment added to the soil, and estimating the nematode population each time the disks are examined[5], the behaviour of the fungi under various conditions and their relationship with free-living nematodes can be studied. Usually four replicate dishes, each holding one slide with disks, are used for each treatment used in any experiment.

It is hoped to enlarge on the details of the relationships between nematode-trapping fungi, organic amendments and nematode populations in a later communication. The above work was carried out during the tenure of an Agricultural Research Council studentship.

Roderic C. Cooke

Department of Botany,
University of Birmingham.

[1] Linford, M. B., Yap, F., and Oliveira, J. M., *Soil Sci.*, **45**, 127 (1938).
[2] Duddington, C. L., Jones, F. G. W., and Moriarty, F., *Nematologica*, **1**, 345 (1956).
[3] Duddington, C. L., *The Friendly Fungi* (Faber and Faber, London, 1957).
[4] Duddington, C. L., *Trans. Brit. Mycol. Soc.*, **38**, 97 (1955).
[5] Peters, B. G., *Soil Zoology* (Butterworth, 1955).

Pneumodermopsis paucidens in the North Sea

Clione limacina Phipps is the only gymnosomatous pteropod normally found in the North Sea, but *Pneumodermopsis ciliata* Gegenbaur has been recorded occasionally from Shetland waters[1,2]. In 1960, however, *P. paucidens* Boas was recognized for the first time in the North Sea, where it appeared in collections taken with the small Plankton Indicator[3] and the Continuous Plankton Recorder[4], both sampling in the upper 10 m. It was first recorded in mid-June to the east of the Orkney Islands and, at about the same time, off the north-west coast of Ireland and to the south of Iceland. Thereafter it was found over a wide area (Fig. 1) until early December, with peak numbers of about 10 per m.[3] at the beginning of September. Previously this species has been recorded only from the North Atlantic and off the coast of Ireland[5].

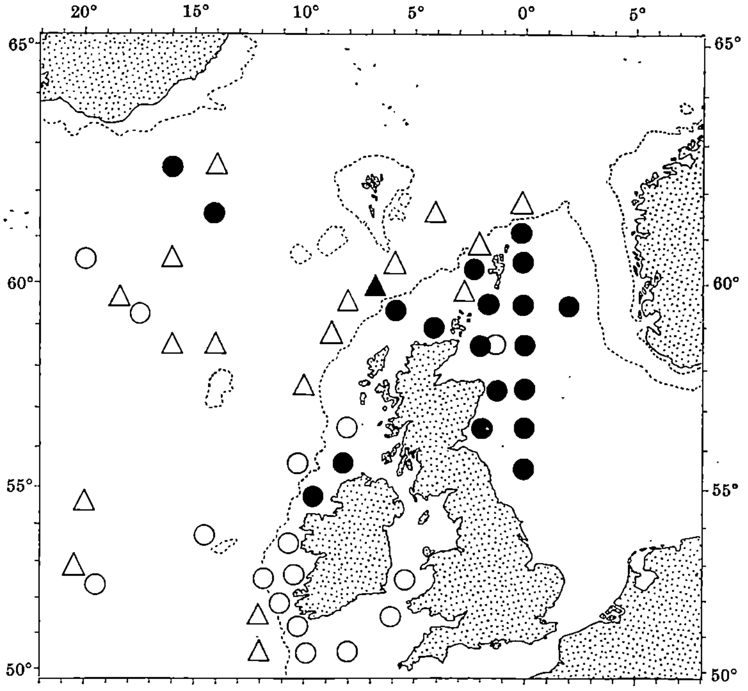


Fig. 1. Chart showing the location of *Pneumodermopsis paucidens* (●) and *P. ciliata* (▲) found in Recorder and Indicator collections in 1960. Previous records from Recorder and Indicator collections in 1956–59, and from the available literature (refs. 1, 2, 6 and 7), are shown as open symbols. Each symbol indicates the centre of a statistical rectangle (1° lat. by 2° long.) in which the species has occurred

Specimens of *Pneumodermopsis* have been re-examined from the collections taken during 1956–59 by Plankton Indicators, in the north-western North Sea, and by Plankton Recorders, throughout the area shown in Fig. 1. Of the total (including those found in 1960) some 700 were *paucidens;* only one of these was taken in the North Sea before 1960. All had the typical radula formula 2:1:2, six hooks and, when they were visible, the characteristic arrangement of the suckers on the median arm. Very few *P. ciliata* were found. These had a radula formula of 6:1:6, many hooks (30–40) and the distal pair of suckers of the median arm had the characteristic "spout at the margin"[6]. Both these species had a range in size of 1–5 mm.

Fig. 1 shows that in Atlantic coastal waters and in the northern North Sea *P. paucidens* was found, whereas in the waters to the west of the continental shelf, both *P. ciliata* and *paucidens* were present.

We are indebted to Dr. J. H. Fraser of the Marine Laboratory, Aberdeen, who confirmed the identification.

G. A. Cooper
D. C. T. Forsyth

Oceanographic Laboratory,
Craighall Road,
Edinburgh.

[1] Fraser, J. H., and Saville, A., *Ann. Biol.* (Copenhagen), **5**, 62 (1949).
[2] Fraser, J. H., *Ann. Biol.* (Copenhagen), **6**, 98 (1950).
[3] Glover, R. S., *Bull. Mar. Ecol.*, **5**, 1 (1957).
[4] John, D. E., and Brown, W. W., *Bull. Mar. Ecol.*, **5**, 44 (1958).
[5] Morton, J. E., *Fich. Ident. Zoopl.*, No. 79 (1957).
[6] Tesch, J. J., *Dana Rep.*, **36**, 32 (1950).
[7] Massy, A. L., *Sci. Proc. Roy. Dublin Soc.*, **15**, 223 (1917).

Chemical Character of the Pigments in a new Nitrogen-fixing Micro-organism

SOME time ago one of us (Gy. N.) published a communication[1] on a nitrogen-fixing micro-organism from the root nodules of *Lupinus luteus*. By analogy with leghæmoglobin, a red-coloured porphyrin pigment isolated by Virtanen[2] from root nodules of soya beans, Németh presented a working hypothesis of the hæmoglobin-like structure of his red pigment. Interpretation of ultra-violet spectroscopic measurements[1] seemed at the first glance to support this assumption. Furthermore, Matkovics and Tárnoki[3] reported to have isolated—whenever in impure state—a porphyrin methyl ester from root nodules of *Lupinus luteus*. As forecast by Németh[1] more extensive studies were still needed to support this assumption. We wish now to report on our ultra-violet absorption spectral examinations with pigments from strain *Pr-E*/3 of Németh, independently of the work by Schneider, and by Matkovics[6]. On the other hand, paper chromatographic investigation of the acidic extracts prepared from this micro-organism has also been carried out.

In order to separate pigments of different types and structure, before taking up spectra of the strain, *Pr-E*/3 cultivated on a bean agar when air-dry was powdered by quartz sand and, in turn, extracted with pyridine. This sample should contain practically all the pigments as well (Fig. 1, curve 1). Another sample was extracted with ether, the solvent removed very carefully in a nitrogen atmosphere and the residue dissolved in pyridine (Fig. 1, curve 2). A third sample was treated as the second one and then dissolved in *n*-hexane (Fig. 1, curve 3).

The substance which did not dissolve in ether was taken up in pyridine (Fig. 1, curve 4). For the sake of comparison an authentic bovine hæmoglobin dissolved in pyridine (Fig. 2) and paprika carotenoids were investigated spectroscopically in pyridine as well as in *n*-hexane (Fig. 3). Careful measurements of the solutions prepared as given above showed the following spectral results: the ethereal extract showed, when dissolved in pyridine, spectra very characteristic of carotenoids (Fig. 3); no comparison with pyridine-hæmochromogene can be made. The curve of the substance obtained after having been extracted by ether lacks any peak characteristic to carotenoids, but no trace of a pyridine-hæmochromogene could be detected either. The same holds true for the solution of the original pigment in pyridine.

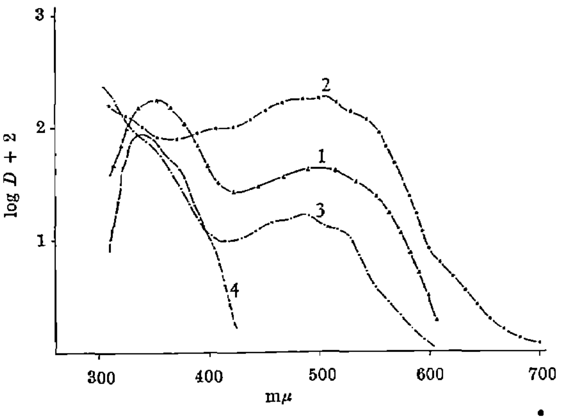


Fig. 1. 1, Strain *E*/3, pyridine extract; 2, strain *E*/3, ether extract, evaporated and dissolved in pyridine; 3, strain *E*/3, ether extract, evaporated and dissolved in *n*-hexane; 4, substance, insoluble in ether, extracted with pyridine

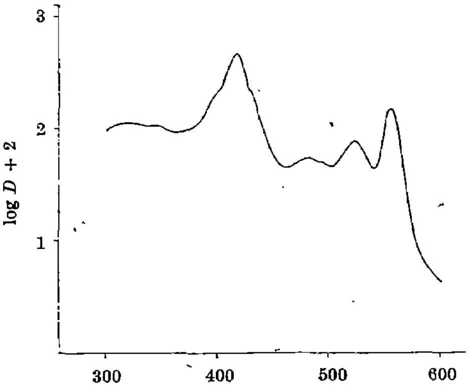


Fig. 2. Bovine hæmoglobin in pyridine

The spectra of the genuine pigment indicate absorption maxima in the range 340–360 mμ (Fig. 4). This can scarcely be ascribed to carotenoids, however; pyridine-hæmochromogene does not absorb in this spectral range either. Accordingly, the chromophore giving rise to this absorption still needs identification. The presence of carotenoids in yeasts is to be expected; for the micro-organism under consideration it has been recorded by others[6].

The red pigment of the strain *Pr-E*/3 could be dissolved quantitatively within a few minutes by distributing by 2 *N* hydrochloric acid and chloroform. The chloroform layer obviously contains fats and carotenoids while the aqueous one proteins of alkaline character and the products of their partial hydrolysis. The aqueous solution has been investigated chromatographically in order to separate amino-acids, porphyrins and flavonoid pigments. Details of this work will be published elsewhere. We shall refer only to the fact that according to the method of Bate-Smith[4] for flavonoids and anthocyanidines with Benedict's reagent a spot characteristic for flavonoids has been found in the case of *E*/3 strain pigment.

In conclusion, the strain *Pr-E*/3 cannot contain considerable amounts of any porphyrin pigments.

The mechanism of nitrogen fixation brought about by these micro-organisms must take quite a different course from that occurring in root nodules of soya beans caused by leghæmoglobin.

Whether carotenoids which are very widespread in micro-organisms, particularly in yeasts, may contribute in any way to nitrogen fixation requires more extensive investigation. Nevertheless, according to

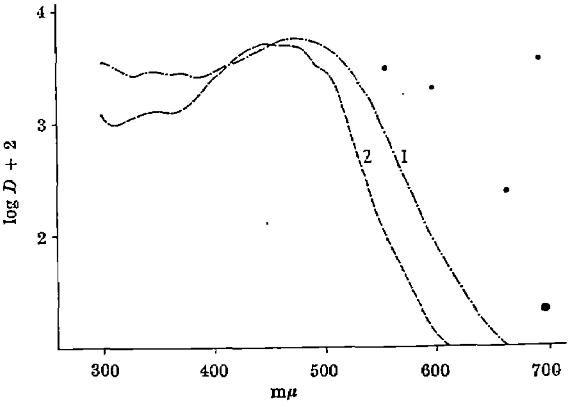


Fig. 3. 1, Paprika carotenoids dissolved in pyridine; 2, paprika carotenoids dissolved in *n*-hexane

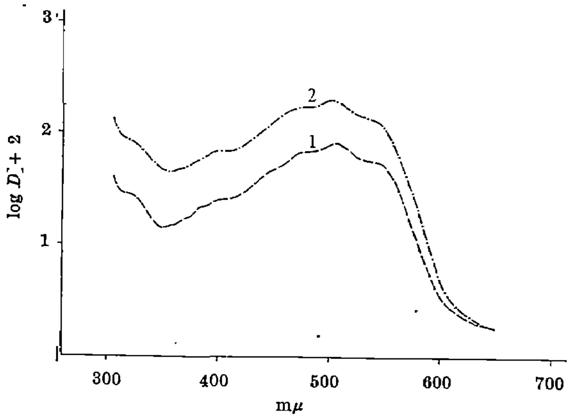


Fig. 4. 1, Pyridine extract of strain *E*/3 raised on a culture medium in distilled water; 2, ether extract of strain *E*/3 raised on a culture medium in distilled water, evaporated, then dissolved in pyridine

Cholnoky *et al.*[5], carotenoids play an important part in oxygen transport in higher plants. It cannot be excluded that oxygen transfer and nitrogen fixation in the yeasts are in some way connected in this case as they are with leghæmoglobin[2].

A more detailed investigation of the pigments of strains *Pr-E*/3 involving flavonoids awaits further investigation.

GY. NÉMETH

Agricultural Experimental Station,
Nyirtelek, Gyulatanya.

F. URESCH
G. FODOR

Stereochemical Research Laboratory
of the Academy of Science,
Budapest.

L. LÁNG

Institute of Physical Chemistry of
the Technical University,
Budapest.

[1] Németh, Gy., *Nature*, 183, 1460 (1959).
[2] Virtanen, A. I., *Acta Chem. Scand.*, 1, 90 (1947).
[3] Matkovics, B., Tárnoki, K., and Németh, Gy., *Acta Phys. Chem. Szeged*, 4, 69 (1958).
[4] Bate-Smith, E. C., *Biochim. Biophys. Acta*, 4, 427 (1950).
[5] Cholnoky, L., Györgyfy, K., Nagy, E., and Pánczél, M., *Acta Chim. Acad. Sci. Hung.*, 6, 143 (1955).
[6] Schneider, Gy., *et al.*, *Nature*, 186, 235 (1960).

Indigotin Content of Woad

BEFORE the general introduction of oriental indigo into Europe in the sixteenth century, woad, the product of the woad plant (*Isatis tinctoria*), was the only commercial source of a blue dye, and its production was the basis of a widespread and important industry. It was the result of a lengthy process involving the crushing of the leaves in specially designed mills, air drying the pulp obtained, grinding the dried material to a fine powder, wetting this, and allowing it to undergo spontaneous fermentation, during which it was kept moist and was frequently turned over. This last process took several weeks to complete, and the material became very hot while the fermentation was active. When cool, the mass was allowed to dry and, after re-grinding and sieving, constituted 'finished woad' ready for the dye-vat. The agricultural operations involved in growing the crop and details of the manufacturing process have been fully described by Hurry[1] and others.

It has long been recognized that the essential dye-stuff of woad was identical with that of indigo derived from various species of *Indigofera*, namely, indigotin, and that the former has a much lower indigotin content than the latter, but no estimate of their relative values based on chemical analysis has ever been made. In the absence of any possibility of obtaining a specimen of the medieval preparation it seemed of interest to grow some of the plant in order to determine its indigotin-yielding capacity and, from this, to arrive at a reasonable approximation to the relation.

In both cases indigotin is derived from a glucoside of indoxyl contained in the leaves. Beijerinck[2] has shown that the glucoside of woad is not identical with that of the species of *Indigofera*, but, since indoxyl results from the hydrolysis of both, the same methods of estimation apply. Methods of manufacture depend, in each case, on the activity of a glucosidase which comes into action when the temperature of the plant material is raised or the normal synthetic activity of the enzyme is reversed by some other means. In the case of indigo manufacture this was brought about by steeping the plant in water at about 90° F. for some hours. The solution of indoxyl thus obtained was then drawn off and oxidized by air, whereby a crude preparation of indigotin was precipitated. In the case of woad the necessary heat was produced in the fermentation process and the indigotin deposited in, or on, the finely divided vegetable material.

The glucosides are readily extracted without change if the operation is conducted in briskly boiling water. Indoxyl may be determined quantitatively in such extracts by hydrolysis with a mineral acid in the presence of an oxidizing agent, followed by sulphonation of the precipitated indigotin and titration of the disulphonate with permanganate. Rawson's ammonium persulphate method, as modified by Bergtheil and Briggs[3], was used for the determination in woad. Plants were grown from seed collected from stock descended in a continuous line from that of one of the few professional growers of woad who was still growing it in England in the early 'thirties of the present century. (Dyeing with woad alone had been abandoned long before this. The survival for so long of the cultivation of the plant on a small scale was due to a demand for it by indigo-dyers, who still used the organic fermentation process to reduce their indigo, since it was thought that its presence in the dye-vat led to the production of richer shades. The amount of indigotin contributed to the vat by the woad must have been negligible relative to that supplied by the indigo, but the finely divided vegetable matter involved doubtless facilitated the fermentation.) Only mature foliage was taken for analysis since neither very young nor very old leaves were used in the industry. Samples were taken at intervals throughout the summer. They showed an average indigotin-yielding potentiality of 0·05 per cent, and very little variation occurred between early July and late September. This is extremely low compared with the poorest *Indigofera* leaf, the analysis of which is recorded (0·54 per cent for *Indigofera sumatrana*). It is noteworthy, however, that in the case of woad there is a much higher proportion of mid-rib in the leaf than in those of indigo plants (roughly 50 per cent in the former as compared with 2 or 3 per cent in the latter) and this contains practically none of the glucoside. But even if the figure for woad leaf were doubled it would still remain very low by *Indigofera* standards.

Fresh woad leaf contains about 90 per cent water. If we assume that it was completely dry, and that the process of manufacture was fully efficient, the finished product would thus have contained about 0·5 per cent indigotin. The poorest-quality natural indigo which came on the market contained about 30 per cent indigotin, the best some 70 per cent. If we take the mean of these figures as standard we find that finished woad contained only about a hundredth part as much indigotin as average indigo.

It is difficult to understand how, with so little indigotin in the product, dyers were able to produce anything like as dark a shade of blue as we are accustomed to associate with indigo-dyed goods. If they did so, it must have necessitated repeated dippings with intervals for oxidation between them and constant renewals of the dye stocks in the vats. The amount of inert matter involved must have been enormous.

C. BERKELEY

Pacific Biological Station,
Nanaimo,
British Columbia.

---

[1] Hurry, J. B., *The Woad Plant and its Dye* (Oxf. Univ. Press, 1930).

[2] Beijerinck, M. W., *Nature*, **61**, 71, 331 (summaries) (1899–1900).

[3] Bergtheil, C., and Briggs, R. V., *J. Soc. Chem. Ind.*, **25**, 729 (1906).

A Poikilohydrous, Poikilochlorophyllous Angiosperm from Africa

THE sedge, *Trilepis pilosa* Boekl, a primary colonizer of rock surfaces on granitic inselbergs in West Africa, has been found to possess the property, most unusual in an angiosperm, of becoming dormant by losing most of its intracellular water during the dry season, with subsequent recovery on moistening. Plants with this property are found mainly among the lower groups (bacteria, fungi, algae, lichens and bryophytes); the word 'poikilohydrous' being first used by Walter[1] in this connexion. Some pteridophytes also exhibit this property: for example, Iljin[2] described drying out and recovery of the fern *Notochlaena marantae* R.Br., and I have found *Selaginella njam-njamensis* Hieron., which colonizes thin soil mats on West African inselbergs, and the epiphytic fern *Platycerium stemaria* (P. Beauv.) Desv., which is sometimes epiphytic on *Trilepis*, to be poikilohydrous.

Richards[3] remarked that the leaves of *Trilepis* last for one season and are green only during the wet months. He also remarked on the rapid development of new green leaves following the early rains. On the rock domes, however, the patches of *Trilepis* are seen to be of various shades of yellow and green following one of the infrequent dry-season showers. With the onset of the rainy season during April, a number of the old, apparently dead leaves nearest the apex of a plant become green. In the laboratory, detached leaves which were so dry as to be brittle and containing approximately 8 per cent water (normal green leaves contain about 56 per cent water), were found to become green and flexible within 24 hr. of moistening under normal conditions of illumination. Similarly the leaves of entire plants became green progressively from the base when the plants were watered; greening did not take place in plants watered and kept in the dark for up to a week; yet it occurred when these were returned to normal daylight conditions.

The colour of the adaxial side of dormant leaves is pale buttercup yellow ($5/_2$) (ref. 4), while the abaxial surface is palest yellow ochre (o$7/_3$). The dormant leaves may be distinguished by this coloration from the buff-coloured dead leaf blades (which are persistent near the stem apices until they decay during the rainy season), and the dead apical regions of the oldest of the dormant leaves. It must be emphasized that the plants appear 'completely dead' in the dry season, as Richards described them; the brittle straw-like texture of the leaves heightens the illusion that they are dead. On moistening, the yellow colours (which are due to carotenoid pigments in the 'chloroplasts') immediately brighten to a deep buttercup yellow ($5/_1$), gradually giving away to green: sulphur yellow ($1/_1$), citron green (7 $63/_1$) and fern green (o*8* $62/_2$) being successive colours recorded for a detached leaf floated on water for thirty hours. At maturity the leaves are a deep green colour, cf. lettuce green (*8* 61).

Dormant plants of *Trilepis* have been found to retain their viability for at least a year when stored in an air-conditioned room (at ambient temperatures between about 18° C. and 28° C., and relative humidity of about 45 per cent). In the dormant condition the protoplasm is less liable to high- and low-temperature injury, as would be expected from Levitt's[5] summary of work on the limits of high- and low-temperature hardiness. Green *Trilepis* plants rapidly cooled to − 10° C. and kept at this temperature overnight rotted on moistening, while similarly treated dormant plants became green in the normal manner. The lower limits of injury by heat appear to be much higher in the dormant plant than in plants in the hydrated condition; work on this aspect is still in progress. Single leaves will not become dormant—they dry green—and the apical tuft of leaves on a short exised stem may behave likewise: it appears that only with an intact plant or a long portion of stem is dormancy possible under normal conditions. The stem bears a thick 'armour' of dead leaf bases and fibres which loses water rather slowly; this slow drying out of the plant as a whole being possibly connected with the intracellular changes associated with dormancy.

Trilepis leaves have thus been found to last for more than a single rainy season. Their poikilohydrous nature, together with the water-absorbent armour of the stem, enables a rapid start of growth with production of new leaves to be made at the beginning of the wet season (an entire dry plant, in a heavy shower, takes up more than its own weight of water in less than 5 min.). Flowering and production of fruits take place very early in the rains; the nutlet germinates within 24 hr. of moistening, and the young plants thus have a maximum chance of becoming established in their rocky habitat before the onset of the dry season in November.

Trilepis is the second angiosperm found to be poikilohydrous, and closely resembles in its loss and gain of chlorophyll another member of the family Cyperaceæ, *Carex physodes* M. Bieb., described by Vassiljev[6] from the desert flora of Central Asia. Vassiljev mentions the brittleness of the brown, dried-out leaves in *C. physodes* which, as in *Trilepis*, is lost with moistening. The word 'poikilochlorophyllous' is now suggested to describe plants, such as these two angiosperms, which completely lose and regain their chlorophyll following natural variations in environmental conditions. Poikilohydrous members of the lower groups, including ferns (for example, *P. stemaria*), usually retain their green colour when dormant and may, in contrast, be described as homoiochlorophyllous.

Further work on the ecology of *Trilepis* and other poikilohydrous species from West Africa will be published elsewhere.

I wish to thank Prof. Richard Biebl, of Vienna, for kindly supplying me with references to literature on drought tolerance.

D. J. HAMBLER

Nigerian College of Arts,
Science and Technology,
Ibadan, Nigeria,

---

[1] Walter, H., *Ann. Rev. Plant Physiol.*, **6**, 239 (1955).

[2] Iljin, W. S., *Protoplasma*, **13**, 322 (1931).

[3] Richards, P. W., *J. Ecol.*, **45**, 563 (1957).

[4] Wilson, R. F., *Horticultural Colour Chart*, I and II (British Colour Council, 1938 and 1941).

[5] Levitt, J., "The Hardiness of Plants" in *Agronomy*, 6 (Academic Press, Inc., New York, 1956).

[6] Vassiljev, J. M., *Planta*, **14**, 225 (1931).

Enzymic Breakdown of Stigmatic Cuticula of Flowers

ENZYME preparations from different micro-organisms are able to break down the leaf cutin of different plants[1,2]. Furthermore, the incompatibility reaction in Cruciferae occurs on the surface of the stigma papillae: pollen tubes are unable to penetrate the cuticle of the stigma cells[3] after self-pollination. Therefore we tried to determine the activity of an enzyme preparation from *Penicillium spinulosum* Thom. on cutin preparations of several sources and especially on stigma cutin isolated from about 10,000 stigmata of black mustard by shaking with pectinase solution following the method in ref. 4, and treatment with ethanol. The results are listed in detail in Table 1. From this it is clear that cutin from mustard stigma is attacked more than material from the pistil of *Petunia* and much more than cutin from leaves and fruits. The breakdown is measured by titration of the enzymically liberated fatty acids following a new method[5]. The differences in enzymic decomposition of cutin should depend on a specific state or a special composition of the high-polymeric, reticular substrate. For the first time it has been possible to demonstrate the enzymic breakdown of stigma cutin.

Table 1

Object	Origin of cutin	Enzymically liberated ml. equivalent 0·01 normal fatty acids
Gasteria verricuosa (Mill.) Duval	Leaf epidermis	$3{\cdot}4 \times 10^{-3}$
Solanum lycopersicum L.	Fruit epidermis	$8{\cdot}2 \times 10^{-3}$
Petunia hybrida (Hook.) Vilm.	Pistil cuticula	$3{\cdot}5 \times 10^{-2}$
Brassica nigra (L.) Koch	Stigma cuticula	$5{\cdot}2 \times 10^{-2}$

Part of this work was aided by the Netherlands Organization for the Advancement of Pure Research (ZWO).

W. HEINEN
H. F. LINSKENS

Department of Botany,
University of Nijmegen,
The Netherlands.

---

[1] Heinen, W., and Linskens, H. F., *Naturwiss.*, **47**, 18 (1960).

[2] Heinen, W., *Acta Botanica Neerlandica*, **9**, 167 (1960).

[3] Christ, B., *Z. Bot.*, **47**, 88 (1959).

[4] Orgell, W. H., *Plant Physiol.*, **30**, 78 (1955).

[5] Heinen, W., *Acta Botanica Neerlandica*, **10**, 171 (1961).

MICROBIOLOGY

Mycobacteriophages isolated from Persons with Tuberculous and Non-tuberculous Conditions

IN a recent communication[1] certain effects of mycobacteriophages on acid-fast bacilli were described. They consisted of the lysis of phage-susceptible bacteria, and of biological changes in the phage-resistant elements of the bacterial population. The phage-resistant bacteria were lysogenic. They showed changes in morphology, in the pigmentation of their colonies, in their susceptibility to mycobacteriostatic drugs, in their antigenic composition and in their pathogenicity for laboratory animals.

These *in vitro* observations raised the question as to whether or not similar changes may occur *in vivo*. Any work along this line had to start by demonstrating the occurrence of mycobacteriophages in the human or animal body.

Bacteriophages active on acid-fast micro-organisms have been isolated so far only from soil, particularly fertilized soil[2-8]. Hnatko[2] attempted to isolate mycobacteriophages from specimens of sputum and of stool from tuberculous patients, but he could not detect any phage activity in his filtrates.

Fifty specimens of stool from hospital patients with pulmonary tuberculosis were examined for the presence of mycobacteriophages.

10-gm. amounts of the specimens were emulsified in 200 ml. of Proskauer and Beck synthetic medium modified by Youmans and Karlson[9]. To this suspension were added 1-ml. amounts of actively multiplying cultures from 12 test strains of mycobacteria. These test strains included pathogenic and saprophytic mycobacteria. They were chosen because they apparently were free of prophages, that is, they did not show spontaneous or ultra-violet irradiation-induced lysogeny. After 10 days incubation at 37° C., the suspensions were filtered on membrane filters and the filtrates were added to fresh culture media inoculated with the test strains. After five such passages, the filtrates were spot-tested to determine lytic activity on any of the test strains. The technique for this screening procedure was described by Froman *et al.*[3] and modified by Takeya and Yoshimura[6].

Table 1 shows the lytic activity of ten mycobacteriophages which were isolated from ten of the fifty specimens of stool that were included in this study.

It is interesting to note that only one mycobacteriophage, isolated repeatedly from specimens of stool from patient D.W., is active on pathogenic mycobacteria. While at the time the specimens were collected the sputum cultures of the other nine patients yielded typical *M. tuberculosis*, those of patient D.W. consisted of atypical chromogenic acid-fast bacteria.

Specimens of stool were then collected from 50 patients with pulmonary conditions of non-tuberculous ætiology. Table 2 shows the bacteriolytic spectrum of five mycobacteriophages isolated from this group.

Patient McL., from whom a mycobacteriophage with a large lytic spectrum, including the virulent human strain $H_{37}Rv$, was isolated, came to this hospital to undergo resection for bronchogenic carcinoma. No history of tuberculosis was given. Four phages were isolated from patients with a diagnosis of sarcoidosis. From eight other patients

Table 1. LYTIC ACTIVITY (*L*) OF TEN MYCOBACTERIOPHAGES ISOLATED FROM TUBERCULOUS PATIENTS

Mycobacteria	Bacteriophages from patients									
	S.G.	R.A.	L.C.	S.M.	W.O.	T.M.	B.R.	D.W.	L.A.	G.B.
Virulent human:										
$H_{37}Rv$	—	—	—	—	—	—	—	*L*	—	—
7101 *F*	—	—	—	—	—	—	—	*L*	—	—
Attenuated bovine:										
B.C.G.	—	—	—	—	—	—	—	*L*	—	—
Anonymous:										
Photochromogenic	—	—	—	—	—	—	—	*L*	—	—
Scotochromogenic	—	—	—	*L*	—	—	*L*	—	—	—
'Battey'	—	—	—	—	—	—	—	—	—	—
Fast-growing	—	*L*	—	—	—	—	—	—	*L*	*L*
Saprophytic:										
Phlei	*L*	—	*L*	*L*	*L*	—	—	—	*L*	—
Smegmatis	—	*L*	—	—	*L*	*L*	*L*	*L*	—	*L*
Fribergensis	*L*	*L*	*L*	*L*	—	—	—	*L*	—	—
Rabinowitschi	—	—	—	*L*	—	—	—	*L*	—	—
Stercoides	*L*	*L*	*L*	*L*	—	*L*	*L*	*L*	—	*L*

ith the same diagnosis no mycophages were isolated m stool specimens.

The presence of mycobacteriophages in the stool certain patients could either be temporary and cidental or it could be determined and maintained y infection with mycobacteria. In an attempt to pproach this question, the following experiment was ndertaken:

Five groups of 20 mice each (Rockefeller strain) ere inoculated intravenously with either of two rains of mycobacteria. Three groups of mice were oculated at the same time and by the same route ith filtrates of mycobacteriophages to which the cteria were susceptible, as follows:

Group 1 with *Mycobacterium smegmatis*.
Group 2 with *Mycobacterium smegmatis* and *Bacteriophage smegmatis* (courtesy of Dr. E. Vandra, Budapest).
Group 3 with the virulent human strain $H_{37}Rv$.
Group 4 with $H_{37}Rv$ and *Bacteriophage D*29 (courtesy of Dr. S. Froman, Olive View, California).
Group 5 with $H_{37}Rv$ and *Bacteriophage* D.W.

The amount of bacilli inoculated was 0·5 mgm. r mouse. The phage filtrates, from which 0·2 ml. re inoculated, contained 10^8 lytic particles per ml.

Specimens of stool were collected from each group mice and were examined for the presence of mycoacteriophages. *Mycobacteriophage smegmatis* (Group) was lost: no phage could be recovered from these animals 48 hr. or more after inoculation. Specimens of stool from Groups 4 and 5 contained phages up to nine weeks after infection. According to their plaque morphology and their activity on mycobacteria, these phages were identical with *Mycobacteriophages D*29 and D.W., respectively.

Table 2. LYTIC ACTIVITY (*L*) OF FIVE MYCOBACTERIOPHAGES ISOLATED FROM NON-TUBERCULOUS PATIENTS

Mycobacteria	Bacteriophages from patients				
	McL.	McC.	G.W.	R.C.	R.L.
Virulent human:					
$H_{37}Rv$	*L*	—	—	*L*	—
7101*F*	*L*	—	—	*L*	—
Attenuated bovine:					
B.C.G.	*L*	—	—	—	—
Anonymous:					
Photochromogenic	*L*	—	—	*L*	—
Scotochromogenic	—	—	—	—	—
'Battey'	*L*	—	*L*	—	—
Fast growing	—	*L*	—	—	—
Saprophytic:					
phlei	—	—	—	—	*L*
smegmatis	—	—	—	—	*L*
fribergensis	*L*	—	*L*	*L*	*L*
rabinowitschi	*L*	—	—	*L*	—
stercoides	*L*	—	*L*	*L*	*L*

When autopsies were performed on these animals nine weeks after infection, it was established that Groups 1 and 2, which were inoculated with *M. smegmatis*, did not show any gross pathology, whereas the mice inoculated with $H_{37}Rv$ showed lesions in kidneys and/or lungs.

The results of these animal experiments suggest that under certain conditions mice may remain infected with mycobacteriophages for long periods of time. One of the conditions appears to be the presence of multiplying mycobacteria in the tuberculous lesions.

I gratefully acknowledge the technical assistance given by Mrs. M. Liivak and Mrs. S. Dernuet.

E. MANKIEWICZ

Laboratories of the
Royal Edward Laurentian Hospital, Montreal,
and of McGill University.

[1] Mankiewicz, E., *J. Gen. Microbiol.*, **24**, 63 (1961).
[2] Hnatko, S. J., *Canad. J. Micro. Sci.*, **31**, 462 (1954).
[3] Froman, S., Will, D. W., and Bogen, E., *Amer. J. Pub. Health*, **44**, 1326 (1954).
[4] Will, D. W., Froman, S., Krasnow, J., and Bogen, E., *Amer. Rev. Tuberc. Pulm. Dis.*, **76**, 435 (1957).
[5] Takeya, K., Yoshimura, T., Yamaura, K., and Toda, T., *Amer. Rev. Resp. Dis.*, **80**, 543 (1959).
[6] Takeya, K., and Yoshimura, T., *J. Bacteriol.*, **74**, 540 (1957).
[7] Redmond, W. B., and Cater, J. C., *Amer. Rev. Resp. Dis.*, **82**, 781 (1960).
[8] Segawa, J., Takeya, K., and Sasaki, M., *Amer. Rev. Resp. Dis.*, **81**, 419 (1960).
[9] Youmans, G. P., and Karlson, A. G., *Amer. Rev. Tuberc.*, **55**, 529 (1947).

Release from the Feedback Inhibition controlling the Biosynthesis of Isoleucine

HAYASHI *et al.* have found that a large quantity of L-isoleucine was accumulated in the cultured medium of *B. subtilis* strain 14 when α-aminobutyric acid (designated as *AB*) was added to the medium containing glucose, peptone, urea and mineral salts[1]. The apparent conversion ratio of *AB* to accumulated isoleucine reached 70 per cent when 1 per cent of the former was added to the medium. In spite of the nature of *AB* as a possible precursor of isoleucine, incorporation of the added *AB*-1-^{14}C to produced isoleucine was observed only at the initial stage of the culture[2]. As the culture proceeded to the accumulation stage of isoleucine, the specific activity of isoleucine tended to be decreased, which means that isoleucine thus accumulated was derived from glucose. On the other hand, it is well recognized in yeast, *Neurospora* and *E. coli* that threonine is at first deaminated to α-ketobutyric acid, which then converts to isoleucine through several biosynthetic steps[3-8]. Further, it was depicted that biosynthesis of isoleucine was controlled by a feedback mechanism within the cells of *E. coli*[7,9].

We have examined the presence and the nature of threonine deaminase in *B. subtilis* strain 14, since it is one of the important enzymes in the most probable pathway of isoleucine biosynthesis, and in addition it was ascertained by Hayashi *et al.* that hydroxyacetyl butyric acid was converted to isoleucine by this strain[1].

An appreciable activity of threonine deaminase is detectable as compared with *E. coli*, and, indeed, specific activities (defined in the table) reached 2·0 to 3·0 in this strain compared with 9·0 in *E. coli* strain *K*12. Cells grown in the medium containing *AB* (*AB* cell) have usually lower activity as compared with those grown in the medium without *AB* (normal cell). Specific activities of the enzyme obtained from *AB* and normal cells, which were collected from 48-hr. cultures, ranged from 0·01 to 0·28 and from 0·13 to 0·63, respectively. In spite of this fact, which suggests that *AB* might repress or inhibit this enzyme through a certain mechanism, the enzyme activity was enough stimulated in either normal cells or *AB* cells when *AB* was added to the system of enzyme assay. In contrast, the enzyme activity was completely inhibited by the addition of 10^{-3} *M* isoleucine, as Umbarger pointed out a similar inhibition in *E. coli*[9]. An inhibition curve of sigmoid type was obtained between the rate of inhibition and the concentration of added isoleucine. As shown in Table 1, when isoleucine and *AB* were added simultaneously at various concentrations, the recovery from the isoleucine inhibition was observed according to the given concentrations of *AB*, where the inhibition curves by isoleucine shifted towards its higher concentrations. Leucine and valine, on the other hand, did not indicate any significant effect on the enzyme activity itself, but gave the similar effect as did *AB* on the inhibition by isoleucine.

Table 1. α-AMINOBUTYRIC ACID, LEUCINE AND VALINE AS RELEASER OF THE INHIBITION BY ISOLEUCINE OF THREONINE DEAMINASE

Concentration of α-aminobutyric acid (*M*)	0	10^{-3}	10^{-2}	10^{-1}
Concentration of isoleucine (*M*)				
0	100*	105	118	132
10^{-4}	30·1	37·0	57·9	82·1
5×10^{-4}	2·1	3·7	4·5	48·8
2×10^{-3}	0	0	0	4·2
Concentration of amino-acids (*M*)		0	Leucine 2×10^{-3}	Valine 2×10^{-3}
Concentration of isoleucine (*M*)				
0		100†	80·7	104
5×10^{-5}		57·0	72·8	77·6
10^{-4}		26·6	65·8	63·8

* Figures represent relative activities to specific activity of 2·4.
† Specific activity, 1·93.

The cells were grown for a period of 24 hr. at 30° C. on a reciprocal shaker, using a medium of following composition: glucose, 10 per cent; peptone, meat extracts, each 0·2 per cent; urea, 0·5 per cent; K_2HPO_4, 0·07 per cent; $MgSO_4.7H_2O$, 0·03 per cent; $FeCl_3.6H_2O$, 0·005 per cent. In the case of *E. coli*, 1 per cent $CaCO_3$ was added to the medium.

The cells were collected by centrifugation, washed twice with 0·1 *M* phosphate buffer (*p*H 7·5) and suspended in the same buffer.

Deaminase activity was assayed at 37° C. in a 1-ml. system containing 40 μmoles of DL-threonine, 100 μgm. of sodium deoxycholate, 0·03 ml. of toluene, 0·4 ml. of cell suspension which contained 2–5 mgm. of dry cells and 40 μmoles of phosphate buffer (*p*H 7·5), and appropriate volume of inhibitor and releaser. After 1 hr. incubation, the reaction was stopped by the addition of perchloric acid. The extent of deamination was followed by determining the keto-acid formed using the modified method of Friedemann and Haugen (ref. 10). Specific activities are expressed in μmoles of keto-acid formed per hr. per 1 mgm. of dry cell.

From the results obtained here, it is postulated that the negative feedback mechanism in the biosynthetic process of isoleucine may exist also in *B. subtilis* strain 14, and the interference with this feedback mechanism may occur in the presence of *AB*, leucine and valine, but only *AB* and not leucine or valine leads to isoleucine accumulation. Moyed and Friedman indicated that feedback mechanisms controlling the biosynthesis of tryptophan and histidine had been lost in such mutant strains of *E. coli* resistant to their analogues[11]. Further, Adelberg indicated that mutant strains of *E. coli* resistant to thienylalanine or *p*-fluorophenylalanine, an analogue of phenylalanine or tyrosine, excrete a large amount of phenylalanine or tyrosine[12,13]. Another example of metabolite excretion was indicated by us, in which a type of releaser from the feedback inhibition may cause the excretion of a metabolite, which is usually supplied moderately by the feedback mechanism for further synthesis. *AB* is not an analogue of isoleucine, nor does not lead a resistant mutant in the above-mentioned medium (unpublished work).

As to the control mechanism, such as the negative feedback loop which may control the rate of biosynthesis of metabolite, it might not be allowed to act in such a simple manner *in vivo* as conceived from the results obtained *in vitro*, since many agents interfering with this mechanism would have to exist within the cell as indicated above.

MASAYA HAYASHIBE *
TEIJIRO UEMURA

Laboratory of Microbiology,
Department of Agriculture,
Tohoku University,
Sendai.

* Present address: Oriental Yeast Industry Co., Shimuracho, Itabashiku, Tokyo.

[1] Hayashi, K., Watanabe, Y., and Shimura, K., *Amino Acids*, **1**, 89 (1959) (in Japanese).

[2] Saito, T., and Shimura, K., *Amino Acids*, **2**, 97 (1960) (in Japanese).

[3] Strassman, M., Thomas, A. J., Locke, L. A., and Weinhouse, S., *J. Amer. Chem. Soc.*, **76**, 4241 (1954).

[4] Adelberg, E. A., *J. Amer. Chem. Soc.*, **76**, 4241 (1954).

[5] Abelson, P. H., *J. Biol. Chem.*, **206**, 335 (1954).

[6] Umbarger, H. E., *Federation Proc.*, **15**, 374 (1956).

[7] Umbarger, H. E., and Brown, B., *J. Biol. Chem.*, **233**, 415 (1958).

[8] Umbarger, H. E., Brown, B., and Eyring, E., *J. Biol. Chem.*, **235**, 1425 (1960).

[9] Umbarger, H. E., *Science*, **123**, 848 (1956).

[10] Friedemann, T. E., and Haugen, G. E., *J. Biol. Chem.*, **147**, 415 (1943).

[11] Moyed, H. S., and Friedman, M., *Science*, **129**, 968 (1959).

[12] Adelberg, E. A., *J. Bacteriol.*, **76**, 326 (1958).

[13] Cohen, G. N., and Adelberg, E. A., *J. Bacteriol.*, **76**, 328 (1958).

Spore Dispersal in *Chaetomium globosum* (Kunze)

IN the genus *Chaetomium* the fruit-body, a perithecium, is ornamented with a large number of apical hairs which are either spirally coiled or dichotomously branched. The ascospores are released within the perithecium by breakdown of the ascal walls and the spores ooze out of the ostiole and either form a spore tendril or become entrapped in the apical hairs. Hitherto the mechanism of spore dispersal has been obscure, but recent experiments have shown that entire fruit-bodies as well as spores can be dispersed by falling water drops.

Target disks, 2 cm. in diameter, were cut from a three-week-old agar culture of *C. globosum* having approximately 150 perithecia per sq. cm. Disks were placed on glass slides and surrounded by small glass sheets. A single drop of water 5 mm. in diameter was allowed to fall from varying heights, up to 2 m., on to the centre of each target. This drop, on breaking up, cleared most of the perithecia from an area 0·5–1 cm. in diameter. The dispersed fruit-bodies were found scattered on the glass sheets either singly or in groups of up to twenty. The longest distance of dispersal recorded was 17 cm. The majority of perithecia were found in splash droplets, but others were dry and had obviously remained dry during dispersal. Other drops were found to contain ascospores and the maximum distance recorded for spore transport was 33 cm.

The ease with which perithecia can be dispersed by water is related to the very superficial position and loose anchorage of the fruit-body on the mycelium. The anchorage though loose is, however, resistant to wind. Experiments using a small wind-tunnel showed that with wind speeds up to 10 m./sec. the perithecia remained attached; the spores, however, were readily blown away from the spore masses trapped in the apical hairs.

The dispersal of perithecia in splash droplets and as dry fruit-bodies is probably related to the water-repellent nature of the fruit-body. Colonies of *Chaetomium* can be gently flooded with water without wetting the perithecia, the apical hairs retaining a bubble of air. The falling drop, however, has sufficient kinetic energy to break through the terminal hairs and to wet the perithecia at the point of impact. The unwetted perithecia are probably picked up as the impact drop breaks up into the splash droplets. These small drops would strike the perithecia laterally, the latter being carried on the surface of the drop from which they may be shed during the free flight.

This method of splash dispersal involving whole fruit-bodies appears to be unique in the fungi. Falling water drops act as the agent of dispersal in many bacteria and slime-spored fungi[1] and work by Gregory *et al.*[2] has established the basic mechanics involved. Comparable examples of the splash dispersal of larger units are few and are confined to the dispersal of the lenticular peridioles in the Nidulariales and the sclerotial balls of *Aleurodiscus minnsiae*[3].

P. A. Dixon

Birkbeck College,
London, W.C.1.

---

[1] Ingold, C. T., in *Plant Pathology*, edit. by Horsfall, J. G., and Dimond, A. G., 3, 137 (Academic Press, London, 1960).

[2] Gregory, P. H., Guthrie, E. J., and Bunce, Maureen E., *J. Gen. Microbiol.*, 20, 2, 328 (1959).

[3] Brodie, H. G., *Proc. Indiana Acad. Sci.*, 66, 65 (1957).

GENETICS

Fracture and Fusion of Coccid Chromosomes

Unlike gene mutations the expression of which may be subject to considerable modification, chromosomal changes will remain the same whether they occur as sporadic aberrations or become incorporated in the karyotype of a species. If the complements of two closely related species differ, spontaneous chromosomal mutations paralleling the karyotypic differences should be demonstrable in the same group of organisms.

In the Coccoidea, the haploid chromosome number ranges from 2 to 20 (ref. 1). Since the basic number for the superfamily is in the range of $n = 5$ to 7, both increase and decrease have occurred in the evolution of the karyotype. The chromosomes of the coccids lack localized centromeres and fragments perpetuate themselves during successive mitotic cycles[2]. Thus, simple breakage can provide the basis for increase in chromosome number unless a breakage-fusion-bridge cycle intervenes to eliminate the breakage products. Fragmentation with and without subsequent bridge formation has been previously reported[2]. When bridges are formed, either spontaneously[3] or after high-dosage irradiation[4], they are unmistakably identifiable in my preparations. Their absence in other circumstances indicates that fracture *per se* need not lead to a breakage-fusion-bridge cycle.

Related coccid species may vary in number by one or two chromosomes or by exact doubling. The latter changes, similar to polyploidy, are complex problems and will not be considered here, but parallels of structural aberration and karyotype change are now evident for the simple increases and decreases.

Spontaneous chromosome breakage occurs ubiquitously in the coccids[illegible]; there is [illegible] abundant availability of ruptured chromosomes for increase in number should chance or selection so dictate. Three cases are now known in which karyotypes of related species differ in the manner expected from fragmentation. On the basis of relative measurements, the karyotype of *Africaspis fici* with $n = 6$ chromosomes appears derivable by simple fragmentation from that of *A. chionaspisformis* with $n = 4$ (ref. 5). In this instance, the chromosomes of *A. fici* varied considerably more in size than those of its congener; however, size variation can be achieved through unequal translocation while breakage of the longest chromosome of a complement could lead to more nearly uniform sizes in the derived karyotype. A Mexican species with $n = 6$ is now known from the *Aspidiotus-Hemiberlesia* series and is the first departure, other than that of a highly atypical genus[6], from the basic diaspidid number, $n = 4$, among the numerous species of Aspidiotini examined (Fig. 1*A*). Among the endemic Hawaiian pseudococcids, the genus *Pedronia* is noteworthy for its association with ferns[6]. In two of the species studied cytologically, the basic number of the pseudococcids, $n = 5$, was found while in a third the number was 7 (Fig. 1*B*). In the last two examples, the increase of the haploid number by two required, on a minimal assumption, the rupture of two chromosomes to yield four breakage products. On the basis of size criterion, no breakage products can be identified in the Mexican species and only one or two in the *Pedronia* example; in both cases, therefore, breakage would have had to occur in the longer chromosomes of the antecedent karyotypes. Forms related to the Mexican species are as yet unknown, but the complements of the two *Pedronia* species with $n = 5$ show the expected size variation.

Fusion of the major portions of two chromosomes by means of a reciprocal translocation at sub-terminal points of breakage and loss of the small residual chromosome will reduce the chromosome number by one. The 'double-length' chromosome will be detectable in the karyotype unless its size is reduced afterwards by additional translocations or the original fusion involves two especially short chromo-

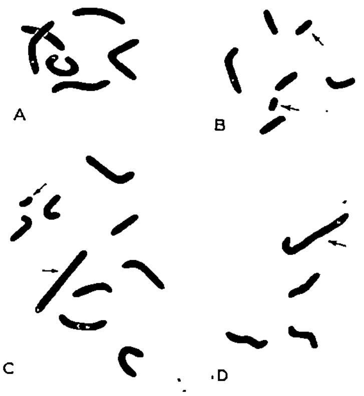


Fig. 1. Structural alterations of coccid chromosomes. *A*, Aspidiotine species, *Aspidotius-Hemiberlesia* complex, $n = 6$. *B*, *Pedronia cibotii*, $n = 7$; arrows, possible fragmentation products. *C*, Aberrant strain of parthenogenetic sp., *Howardia biclavis*, $2n = 10$; arrows, 'double-length' and residual chromosomes from unequal translocation. *D*, *Pseudococcus adonidum*, $n = 4$; arrow, 'double-length' chromosome

somes. A spontaneous change of this sort has been reported for an ovarian mosaic of an armoured scale [illegible] with a residual product undoubtedly too large for tolerable loss, has now been found in a collection of the parthenogenetic armoured scale, *Howardia biclavis* with $2n = 10$ (Fig. 1*C*); because öogenesis is here essentially mitotic, the occurrence in the sample of several individuals with the aberration is a reflection of the clonal type of reproduction. In the *Pseudoparlatoria* complex in North America, species have been recorded with $n = 4$, and one member of double length, and with $n = 5$ (ref. 5). There is no definitive evidence to show the direction in which the change occurred; although 4 is the basic diaspidid number, the 4-chromosome complement in question is highly distinctive and was most likely secondarily derived. A much simpler example is provided by *Pseudococcus adonidum* with $n = 4$; this, the only reduction yet known from the basic number, $n = 5$, of the pseudococcids, is accompanied by a double-length chromosome (Fig. 1*D*). In the armoured scale genera, *Protodiaspis* and *Ancepaspis*, species with $n = 4$ and $n = 3$ have been discovered, and in both cases a disproportionately long chromosome occurs in the species with the lower number. These two genera present an interesting phylogenetic problem.

The method of accounting for karyotypic changes on the basis of the evidence from spontaneous alterations in the same group seems to afford a better understanding of the evolutionary process than inferences from work with dissimilar organisms, no matter how conclusive they seem in themselves.

This work has been aided by a grant from the National Science Foundation.

SPENCER W. BROWN

Department of Genetics,
University of California,
Berkeley 4,
California.

[1] Hughes-Schrader, Sally, *Adv. Genetics*, **2**, 127 (1948).

[2] Hughes-Schrader, Sally, and Ris, H., *J. Exp. Zool.*, **87**, 429 (1941).

[3] Brown, S. W., *Nucleus*, **3**, 135 (1960).

[4] Chandra, H. S., *et al.* (unpublished work with *Planococcus citri* (Risso)).

[5] Brown, S. W., *J. Morph.*, **106**, 159 (1960).

[6] Beardsley, J. W., *Proc. Hawaiian Ent. Soc.*, **16**, 218 (1957).

Discriminant Analysis in Biometrical Genetics

SEVERAL notable attempts have been made to analyse and interpret the inheritance of polygenic characters in terms of simple Mendelian factors giving the familiar ratios in segregating generations[1,2]. More recently, Weber[3,4] proposed his so-named discriminant analysis technique, in which two or more component measurements of a character that may individually appear to be normally distributed are combined into a discriminant function which, in contrast, might assume generally a bimodal frequency distribution suggesting this linear compound measure to be simply inherited like qualitative traits. He illustrates this method for the cotyledon size i tomato by using a linear function of length/widt ratio (x_1) and area (x_2), $x = b_1x_1 + b_2x_2$, and showin x to have a bimodal frequency distribution in F_2 tha simulated a segregation ratio of 3: 1 in one case and o 9: 7 in the other. Apparently, Dr. Weber consider this method of analysing quantitative characters t be useful in general as he argues[3], "this total measurement of discriminant function has no dimensiona designation; it is without dimension in the physica sense, like the term red or white colour".

While this new analysis in biometrical genetics may have some appeal, for its empirical value in particular to those used to thinking only in terms of simple gene models and Mendelian ratios, it is, however, open t criticism on such grounds as the applicability of the method in general and its genetic implications. L statistical theory, it is well known that a linear function of several normal variables is also distribute normally, so that a bimodal distribution of the discriminant function such as that observed by Weber for the cotyledon size in tomato would seem to be very unlikely unless the sampling errors were rather large or the component characters were actually non-normal variates. In any event, this method might be applicable to some very rare examples merely fo expediency rather than as a real solution. Mor over, it seems quite inexplicable how several polygen systems governing the inheritance of various ind vidual components could be made to behave like on or two genetic units by merely considering the segregation together. This, in fact, appears contra to the usual approach of breaking down a comple character such as yield into its components that ma involve relatively simpler genetic situations[5]. Here, Dr. Weber perhaps argues that it is the total impression of a character which is under genetic control while the dimensional measures are artefacts from the viewpoint of genetic determination. Obviously, such an argument would seem to imply the non-existence of a polygenic mode of inheritance of many of the quantitative characters. Thus, it appears that a critical evaluation of this method of discriminant analysis is quite in order in view of an important issue on the polygenic inheritance being involved, and that many more examples need to be investigated before even its empirical usefulness is well demonstrated.

S. K. JAIN

Division of Botany,
Indian Agricultural Research Institute,
New Delhi.

[1] Powers, L., Locke, L. F., and Garrett, J. C., *U.S. Dep. Agric. Tech. Bull.*, No. 998, 1 (1950).

[2] Allard, R. W., *Brookhaven Symp. Biol.*, **9**, 69 (1956)

[3] Weber, E., *Genetics*, **44**, 1131 (1959).

[4] Weber, E., *Genetics*, **45**, 567 (1960).

[5] Smith, H. F., *Ann. Eugenics*, **7**, 240 (1936).

FORTHCOMING EVENTS

(Meeting marked with an asterisk (*) is open to the public)

Tuesday, October 3

Institution of Chemical Engineers, South-Eastern Branch (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. Calderbank: "The Origins and Purpose of Chemical Engineering" (Inaugural Lecture).

Tuesday, October 3—Thursday, October 12

Electronic Computer Exhibition (at Olympia, London).*

Wednesday, October 4

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 5 p.m.—Ir. A. R. Thatcher: "Some Results on Inventory Problems".

Institute of Petroleum (at 61 New Cavendish Street, London, V.1), at 5.30 p.m.—Mr. H. G. Priest: "The Packaging of Lubricants".

British Institution of Radio Engineers, London Section t the London School of Hygiene and Tropical Medicine, Keppel reet, Gower Street, London, W.C.1), at 6 p.m.—Mr. D. A. Drew: lethods Used for the Study of Vibration in Aero Engines".

Institution of Mechanical Engineers, Railway Engineering roup (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m. Dr. F. T. Barwell: "Some Speculations on the Future of Railway echanical Engineering".

Thursday, October 5

Institution of Electrical Engineers (at Savoy Place, London, V.C.2), at 5.30 p.m.—Mr. G. S. C. Lucas: Inaugural Address as resident.

Thursday, October 5—Friday, October 6

Biochemical Society (joint meeting with the British Bio- hysical Society, in the School of Pharmacy, University of London, runswick Square, London, W.C.1).

hursday, October 5

At 11 a.m.: "The Physical Chemistry of Phospholipids".

iday, October 6

At 10.30 a.m.: "Phospholipids and Sulpholipids".

Society for Analytical Chemistry (at the Institution of Mech- nical Engineers, Birdcage Walk, Westminster, London, S.W.1), at .30 p.m. on Thursday and 10.30 a.m. on Friday—Meeting on "Ad- ances in Analytical Chemistry—Selected Topics Originating from nalytical Requirements in the Field of Nuclear Technology".

Friday, October 6

Photoelectric Spectrometry Group (in the Department of hysics, Imperial College of Science and Technology, Prince Consort oad, London, S.W.7), at 2.30 p.m.—Annual General Meeting. 3.15 m.—Meeting on "Absorption Measurements at Wave-lengths Below 0 mμ".

Society of Chemical Industry, Fine Chemicals Group (in the llcome Building, Euston Road, London, N.W.1), at 2.45 p.m.— eeting on "Approaches to the Chemotherapy of Virus Diseases".

Institution of Electrical Engineers, Medical Electronics scussion Group (at Savoy Place, London, W.C.2), at 6 p.m.— cussion on "What is *p*H and Its Measurements ?" opened by Dr. Mattock and Mr. G. R. Taylor.

Saturday, October 7

Biochemical Society (at the Westminster Hospital Medical School, Horseferry Road, London, S.W.1), at 10 a.m.—Ordinary Meeting.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer (with a good honours degree in electrical engineering and preferably with special interests in electrical machinery) in Electrical Engineering—The Registrar, King's College (University of London), Strand, London, W.C.2 (October 6).

Demonstrator (with medical qualifications registrable in this country) in Anatomy—The Registrar, The University, Manchester 13, October 7).

Experimental Officer or Senior Experimental Officer, to ssume responsibility for the supervision and maintenance of electronic ontrol, computer and digital equipment associated with the radio elescope and other apparatus at Jodrell Bank—The Registrar, The University, Manchester 13 (October 7).

Lecturer and Administrative Officer (with a university degree in science and with good qualifications in physics, preferably with a knowledge of Welsh) in the Faculty of Education—The Registrar, University College, Cathays Park, Cardiff (October 7).

Assistant Chief Officer (with a degree in botany, agricultural botany or agriculture or equivalent qualifications, and preferably experience of seed improvement and staff administration) at the Official Seed Testing Station—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (October 9).

Assistant Lecturer or Lecturer in Genetics—The Registrar The University, Sheffield (October 14).

Lecturer or Assistant Lecturer in the Department of Pure Mathematics—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (October 14).

Research Fellows (2) (with an honours degree and three years postgraduate experience in a relevant field) in Seismology in the Department of Astronomy, for the investigation of local structure and local earthquakes, and the interpretation of dispersed seismic waves—The Secretary, The University, Edinburgh (October 14).

Lecturers or Assistant Lecturers (with a good honours degree or equivalent qualification, preferably with teaching and/or research experience) in Geology at the University, Kuala Lumpur, Malaya—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 15).

Assistant Lecturer (with a good honours degree in zoology and preferably a special interest in the arthropods) in Invertebrate Zoology—The Registrar, King's College (University of London), Strand, London, W.C.2 (October 16).

Experimental Officer or Senior Experimental Officer in the Department of Metallurgy, to operate, maintain and develop an electron beam micro-probe analyser—The Registrar, The University, Manchester 13 (October 16).

Senior Lecturer and a Lecturer in Mechanical Engineering at the University of Cape Town—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, October 23).

Lecturer or Senior Lecturer in Pure Mathematics at the University of New England, Armidale, N.S.W., Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 27).

Curator (with a qualification in zoology and preferably experience of a teaching museum) of the Zoology Museum—The Registrar, University College of North Wales, Bangor, North Wales (November 30).

Lecturer/Senior Lecturer (with a particular interest and qualifications in the philosophical and/or sociological aspects of education) in Education at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 30).

Lecturer in Pure Mathematics at the R.A.A.R. Academy, which is affiliated with the University of Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 31).

Lecturer (with an honours degree in mining engineering or equivalent, and considerable experience in metalliferous mining) in Metalliferous Mining—The Registrar and Secretary, King's College (University of Durham), Newcastle-upon-Tyne 1 (October 31).

Nuffield Research Fellow (preferably with an interest and experience in theoretical or experimental neutron physics)—in the Department of Physics—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (October 31).

Reader (with teaching and research experience and with published work in the field of economic statistics, preferably with a knowledge of econometrics) in Economic Statistics at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 31).

Food Technologist (preferably with a Ph.D. in food technology or postgraduate experience in this field) in the Fruit Research Division, D.S.I.R., Stoke, near Nelson, New Zealand, for the investigation of problems arising in the processing of fruits and vegetables, and the development of new products from these crops—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. B.13/18/2 (November 16).

Lecturer in Philosophy at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 18).

Lecturer in Education at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (November 20).

Plant Physiologist (with postgraduate experience), to undertake studies on the growth of the tobacco plant—The Director, Tobacco Research Board of Rhodesia and Nyasaland, P.O. Box 1909, Salisbury, Southern Rhodesia (November 30).

Analyst (B.Sc.(chemistry) and preferably G.R.I.C. or equivalent qualifications), for the operation and care of optical equipment including infra-red and nuclear magnetic resonance spectrometers—Mr. W. A. Wightman, School of Chemistry, The University, Leeds 2.

Assistant Experimental Officer (with a pass degree, preferably in zoology and chemistry) in the Unit for Biochemical Research on Fisheries' Problems—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Berks, quoting Ref. 61/24.

Assistant Lecturer in Chemistry—The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

Assistant Lecturer (with a good honours degree and preferably with research and/or industrial experience) in Physical Chemistry—The Academic Registrar, Hatfield College of Technology, Hatfield, Herts.

Editorial Assistant (with a degree in science and preferably editorial experience) for the *Biochemical Journal*—The Editorial Board, The *Biochemical Journal*, 133–135 Oxford Street, London, W.1.

Lecturer (B.Sc. (chemistry and biology) and preferably with a teaching diploma), in Nigeria to teach science subjects to pharmacy students—The Recruitment Secretary (Overseas), Northern Region Recruitment Agency, Nigeria House, 9 Northumberland Avenue, London, W.C.2.

Lecturer (graduate of a British university) in Physiology in the Department of Health Education—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

Physicist (with an honours degree and preferably with appropriate research experience), to take part in a programme or work concerning the physical properties of milk—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 61/27.

Research Assistant (preferably with research experience) in the Department of Genetics, to work in collaboration with Prof. J. A. Roper on nucleo-cytoplasmic interactions in fungi—The Registrar, The University, Sheffield.

Research Worker (with a qualification in chemistry and preferably previous experience in polymer chemistry) in the Isotope Unit, for work in the field of polymer reaction mechanisms—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

Senior Technician or Technician (with experience and training in electronics) in the Pharmacology Department—The Secretary, Royal Free Hospital Medical School, 8 Hunter Street, London, W.C.1.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

British Association for the Advancement of Science. Annual Report, 1960–61. Pp. 31. (London: British Association for the Advancement of Science, 1961.) [148

Ordnance Survey, Chessington. One-inch Map of Great Britain, Sheet No. 190: Truro and Falmouth. (National Grid—Seventh Series.) (Chessington, Surrey: Director General of the Ordnance Survey, 1961.) 5*s.* 6*d.* net, paper. [148

Committee of Privy Council for Medical Research. Report of the Medical Research Council for the year 1959–1960. Pp. viii+326. (Cmnd. 1422). (London: H. M. Stationery Office, 1961.) 15*s.* 6*d.* net. [148

Office of the Minister of Science; Ministry of Health; Department of Health for Scotland; Ministry of Health and Local Government for Northern Ireland. Code of Practice for the Display of Sources of Ionizing Radiations at Exhibitions. Pp. 6. (London: H.M. Stationery Office, 1961.) 6*s.* net. [148

Production Engineering Research Association of Great Britain. Report No. 49: Grinding Carbide Turning Tools—a Comparative Study of Methods. Pp. 14. (Melton Mowbray: Production Engineering Research Association, 1961.) [148

Central Sterile Supply. Edited by Brian Watkin. Pp. 58. (London: Macmillan and Co., Ltd., 1961. A *Nursing Times* publication.) 4*s.* 6*d.* [148

Privy Council. Medical Research Council Monitoring Report Series No. 2: Assay of Strontium-90 in Human Bone in the United Kingdom—Results for 1960, Part 1, with some further results for 1958 and 1959. Pp. ii+13. (London: H.M. Stationery Office, 1961.) 1*s.* 3*d.* net. [148

Ministry of Housing and Local Government. Fourth Progress Report of the Standing Technical Committee on Synthetic Detergents. Pp. 19. (London: H.M. Stationery Office, 1961.) 1*s.* 6*d.* net. [148

Department of Scientific and Industrial Research. Report of the Tropical Products Institute 1960: The Report of the Tropical Products Institute Committee, with the Report of the Director of the Tropical Products Institute. Pp. viii+40. (London: H.M. Stationery Office, 1961.) 3*s.* net. [148

Year Book of the Royal Society of Edinburgh 1961, (Session 1959–1960). Pp. 138. (Edinburgh: The Royal Society of Edinburgh, 1961.) 20*s.*; 3.50 dollars. [148

Ferranti, Ltd. A Review of Ferranti Activities. Pp. 24. (Hollinwood, Lancs.: Ferranti, Ltd., 1961.) [148

Royal Observatory Bulletins. No. 33: Radial Velocities Observed with the Mount Wilson 60-inch Telescope. By D. H. P. Jones and R. v.d.R. Woolley. Pp. E121–127. 2*s.* net. No. 39: Time Service 1960, October–December. Pp. B61–77. 3*s.* net. (London: H.M. Stationery Office, 1961.) [148

British Standards Institution. British Standard 1991: Recommendations for Letter Symbols, Signs and Abbreviations. Part 2: Chemical Engineering, Nuclear Science, and Applied Chemistry. Pp. 46. 10*s.* net. Part 3: Fluid Mechanics. Pp. 36. 7*s.* 6*d.* net. Part 4: Structures, Materials and Soil Mechanics. Pp. 50. 12*s.* 6*d.* net. Part 5: Applied Thermodynamics. Pp. 30. 7*s.* 6*d.* net. (London: British Standards Institution, 1961.) [148

The Nuclear Energy Industry in the United Kingdom. Second edition, revised. Pp. iv+67. (London: United Kingdom Atomic Energy Authority, 1961.) [148

The Kent Incorporated Society for Promoting Experiments in Horticulture. East Malling Research Station Annual Report, 1960, (Forty-eighth year), 1st October 1959 to 30th September 1960. Pp. xxiv+148. (East Malling, near Maidstone: East Malling Research Station, 1961.) 17*s.* 6*d.*; 2.50 dollars. [148

Other Countries

World Health Organization. Technical Report Series, No. 221: Scientific Meeting on Rehabilitation in Leprosy, Vellore, Madras State, India, 21–29 November 1960—Report. Pp. 37. (Geneva: World Health Organization; London: H.M. Stationery Office, 1961.) 2 Swiss francs; 3*s.* 6*d.*; 0.60 dollars. [148

Meddelanden från Oceanografiska Institutet i Göteborg. Nr. 29: Remarks on some Cenozoic Core Sequences from the Central Pacific, with a Discussion of the Role of Coccolithophorids and Foraminifera in Carbonate Deposition. By Eric Olausson. Pp. 35. Nr. 30: Optical Measurements in the Eastern North Atlantic ("Discovery II" Expedition of August and September 1959). By N. G. Jerlov. Pp. 40. (Göteborg: Elanders Boktryckeri Aktiebolag, 1961.) [148

Organization for European Economic Co-operation: Office f Scientific and Technical Personnel. Television for School Scienc Report on an O.E.E.C. Seminar, Ashridge (England), July 196 Pp. v+181. (Paris: Organization for European Economic Co-opera tion, 1961.)

American Society for Testing Materials. Special Technical Publica tion No. 297: A Five-Attribute System of Describing Visual Appearance. By Deane B. Judd. Pp. iv+15. (Philadelphia: American Society for Testing Materials, 1961.) 1 dollar. [148

Research Council of Alberta. Information Series No. 33: Alberta Subbituminous Coals as Drilling Fluid Thinners. By E. J. Jensen Pp. 25. (Edmonton: Research Council of Alberta, 1961.) [14

Colony of Mauritius. Annual Report of the Meteorological Department, 1960. Pp. ii+17. (Port Louis: Government Printer, 1961.) 25 cents. [148

United States Department of Agriculture. Index-Catalogue o Medical and Veterinary Zoology. Supplement 11: Authors A to Z. By Mildred A. Doss, Judith M. Humphrey and Dorothy B. Segal. Pp. i+409. (Washington, D.C.: Government Printing Office, 1961 1.50 dollars. [1

Smithsonian Institution, Washington. Uniform Regulations for t Army of the United States 1861. (Illustrated with Contempor Official War Department Photographs.) Pp. 61. (Publication 44 (Washington, D.C.: Smithsonian Institution, 1961.) [1

Smithsonian Miscellaneous Collections, Vol. 143, No. 1: Locom Mechanisms of Birds. By Frank A. Hartman. Pp. i+91. (Public 4460.) (Washington, D.C.: Smithsonian Institution, 1961.)

American Philosophical Society. A Catalogue of Instruments Models in the Possession of the American Philosophical Socie Compiled by Robert P. Multhauf, assisted by David Davies. I xi+80 (26 figures). 1.50 dollars. (Memoirs of the American Philosoph Society, Vol. 53.) A Catalogue of Portraits and other works of art the Possession of the American Philosophical Society. Pp. viii+1 (59 figures). 2.50 dollars. (Memoirs of the American Philosophica Society, Vol. 54.) (Philadelphia: American Philosophical Society, 1961.) [148

New Zealand: Department of Scientific and Industrial Researc New Zealand Geological Survey. Paleontological Bulletin No. 3 The Fossil Decapos Crustacea of New Zealand and the Evolution of th Order Decapoda. By Martin F. Glaessner. Pp. 79 (7 plates (Wellington: Government Printer, 1960.) 16*s.* 6*d.* [14

Forschungsberichte des Landes Nordrhein-Westfalen. Nr. 932: Di Anomale Diffusion in dem System Polystyrol-Toluol. Von Prof. Dr. Ernst Jenckel und Dr. Alfred Nogaj. Pp. 42. (Köln und Opladen: Westdeutscher Verlag, 1961.) 13.50 D.M. [148

Journal of the Geological Society of India, Vol. 1, 1959. Pp. 166+6 plates. (Bangalore: Geological Society of India, 1959.) Rs. 15. [158

Geological Survey of Uganda. Report No. 3: Explanation of th Geology of Sheet 69 (Lake Wamala). By Dr. R. J. Johnson. Pp. iii+35+3 plates+map. Shs. 15. Report No. 4: Explanation of the Geology of Sheet 76 (Buhwezu). By Dr. A. W. Reece. Pp. vi+73+7 plates+map. Shs. 20. (Entebbe: Government Printer, 1960 an 1961.) [152

United States Department of Interior: Geological Survey. Bulletin 1082–L: Tertiary Geology and Oil-Shale Resources of the Piceance Creek Basin between the Colorado and White Rivers, Northwestern Colorado. By John R. Donnell. Pp. v+835–891+plates 48–59. Bulletin 1091: Investigations of some Clay Deposits in Washington and Idaho. By John W. Hosterman, Vernon E. Scheid, Victor T. Allen and I. G. Sohn. Pp. iv+146+9 plates. (Washington, D.C.: Government Printing Office, 1961.) [159

United States Department of the Interior: Geological Survey. Bulletin 1146–A: Geophysical Abstracts No. 184, January–March, 1961. By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel and others. Pp. iii+170. 40 cents. Water-Supply Paper 1459–H: Microbiologic Factors in the Solution and Transport of Iron. By Eugene T. Oborn and John D. Hem. Pp. i+213–234. Water-Supply Paper 1535-E: Chemical Degradation on Opposite Flanks of the Wind River Range, Wyoming. By Charles H. Hembree and Frank H. Rainwater. Pp. iii+9. Water-Supply Paper 1586-A: Water Quality and Hydrology in the Fort Belvoir Area, Virginia, 1954–55. By C. N. Durfor. Pp. v+57. 25 cents. Professional Paper 353-C: Effect of Sediment Characteristics on Erosion and Deposition in Ephemeral-Stream Channels. Pp. iii+70. (Washington, D.C.: Government Printing Office, 1961.) [158

Annales de l'Institut de Physique du Globe de l'Université de Paris et du Bureau Central de Magnétisme Terrestre, Tome XXX. Pp. 190. (Paris: Institut de Physique du Globe de l'Université de Paris et du Bureau Central de Magnétisme Terrestre, 1960.) [158

Osservatorio Astrofisico du Arcetri, Firenze. Relazione Sull' attività dell Osservatorio per il 1960. Pp. 20. (Firenze: Osservato io Astrofisico di Arcetri, 1961.) [158

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 60–24: Tectonic Framework of Southern Yukon and Northwestern British Columbia. By H. Gabrielse and J. O. Wheeler. Pp. ii+37. (Ottawa: Queen's Printer, 1961.) 50 cents. [158

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office